Progress in Biological Control

Yulin Gao
Heikki M. T. Hokkanen
Ingeborg Menzler-Hokkanen *Editors*

Integrative Biological Control

Ecostacking for Enhanced Ecosystem Services

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Biological control of pests, weeds, and plant and animal diseases utilising their natural antagonists is a well-established but rapidly evolving field of applied ecology. Despite its documented applications and systematic development efforts for longer than a century, biological control still remains a grossly underexploited method of pest management. Its untapped potential represents the best hope to providing lasting, environmentally sound, and socially acceptable control of most problem pests in agriculture, and of invasive alien organisms threatening global biodiversity. Based on the overwhelmingly positive features of biological control, it is the prime candidate in the search for reducing dependency on chemical pesticides. Public demand for finding solutions based on biological control is the main driving force in the rapid developments in the various strategies of utilising natural enemies for controlling noxious organisms. This book series is intended to accelerate these developments through exploring the progress made within the various aspects of biological control, and via documenting these advances to the benefit of fellow scientists, students, public officials, and the public at large. Each of the books in this series is expected to provide a comprehensive, authoritative synthesis of the topic, likely to stand the test of time.

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Editors

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Editors
Yulin Gao
Chinese Academy of Agricultural Sciences
Institute of Plant Protection
Beijing, China

Ingeborg Menzler-Hokkanen
Department of Environmental
and Biological Sciences
University of Eastern Finland
Kuopio, Finland

Heikki M. T. Hokkanen
Department of Environmental
and Biological Sciences
University of Eastern Finland
Kuopio, Finland

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Foreword

Biological control is developing rapidly all over the world, based on progress in basic research, application technology, and product development. Environmental protection and green development have attracted great attention from all governments. The Chinese government has established "green growth and ecological development" as a great aim to build a future ecological civilization. Green, ecological, and healthy progress has been repeatedly declared as the main theme of global development.

In order to facilitate and to promote the development of biological control worldwide, the First International Congress of Biological Control was held in Beijing on May 14–16, 2018, co-hosted by the Chinese Academy of Agricultural Sciences (CAAS), International Organization for Biological Control (IOBC), and China Society of Plant Protection. This congress aimed to integrate all branches and approaches of biological control into a common overall strategy. Over 800 experts in the field of biological control, coming from more than 40 countries, attended the congress, and 12 prominent experts were invited to give the plenary lectures. In the congress, 160 academic reports, divided into 15 scientific sessions, were presented, and the following three critical aspects were discussed: (1) risk assessment of biological control and biosafety, the driving force of biological control; (2) key factors interacting with biological control, for instance, biodiversity, agroecosystem, climate change, human health, and social and economic development, and the "One Belt and One Road" idea; and (3) research on biological control products and technology, such as natural enemy insects, microbial pesticides, bioactive metabolites, and small molecules, biological control in IPM systems, transgenic technologies for biological control, and industrialization and market development of biological control. Based on the theme "Biological Control for a Healthy Planet," delegates had broad and deep communications and discussions concerning the latest development of basic research, the integration of products and technologies, producing great economic, social, and ecological benefits.

Based on this successful First International Congress of Biological Control in Beijing, we selected and invited 15 reports and combined them into this book titled *Integrative Biological Control* in the spirit of attempting to capture the spirit of the congress. The purpose of this book is to strengthen communication and cooperation between researchers, sharing and promoting the transformation and dissemination of scientific and technological achievements. In particular, the book provides a forum for integration of all aspects of biological control and opportunity for all subdisciplines within biological control to interact. We should plan and promote scientific and technological innovation with a global perspective, improve the capacity of original innovation and integrated innovation, and promote the formation of an international, scientific community through in-depth cooperation in the field of biological control.

Chinese Academy of Agricultural Sciences
Institute of Plant Protection, Beijing, China
Yulin Gao

Department of Environmental and
Biological Sciences
University of Eastern Finland, Kuopio, Finland
Ingeborg Menzler-Hokkanen

Department of Environmental and Biological Sciences
University of Eastern Finland, Kuopio, Finland
Heikki M. T. Hokkanen

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Improving the Efficacy of Biological Control by Ecostacking

Heikki M. T. Hokkanen and Ingeborg Menzler-Hokkanen

1.1 Introduction

Developing cropping systems, which ensure that functional biodiversity including biological control agents can deliver their full potential for the benefit of agricultural production, is of utmost urgency. We have proposed that maximising the benefits obtainable from ecosystem services in a cropping system can best be ensured by a comprehensive approach called "ecostacking" (Hokkanen 2017; Hokkanen and Menzler-Hokkanen 2018a). Stacking implies combining the beneficial services of functional biodiversity from all levels and types in an additive or synergistic manner. The various ecosystem service providers and their needs must be fully integrated in the cropping system, including agronomic practices. The ecosystem service providers include many types of organisms and their functions such as beneficial arthropods, vertebrates, microbes, and plants. Insects, mites and spiders function as predators and parasitoids for the control of pests, but also as seed feeders and other specialist herbivores for weed control, and as pollinators of plants. Furthermore, organisms involved in decomposition, nutrient cycling, soil aeration and mixing, etc., need to be considered. For example, vertebrates such as small mammals, bats, and birds function as seed feeders and predators of pests, while microbes provide critical ecosystem services including pest, disease, and weed control. This can be delivered directly as components of "suppressive soils" (see Hokkanen and Menzler-Hokkanen 2018a), as colonisers of plants as endophytes, or as epiphytic microbial flora. That certain soils are suppressive to plant pathogens, has been known for a long time (e.g. Hornby 1983; Schlatter et al. 2017), but soils suppressive to insect pests has not been considered so far (but see Hokkanen and

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H. M. T. Hokkanen (✉) · I. Menzler-Hokkanen
Department of Environmental and Biological Sciences, University of Eastern Finland, Kuopio, Finland
e-mail: heikki.hokkanen@uef.fi

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Menzler-Hokkanen 2017, 2018b). How endo- and epiphytic micro-organisms on plants can steer arthropod–plant interactions is currently under intensive study, but applications based on microbe-mediated bottom-up effects in crop protection have not yet reached practical applications (Shikano et al. 2017). How plants can provide ecosystem services related to pest, disease and weed management, includes the crop plants themselves (e.g., via the beneficial effects based on genotypic diversity, and the ability to activate induced resistance mechanisms), companion crops (e.g., trap crops, intercrops, undersown crops), and plants providing nectar and pollen resources to beneficial arthropods.

In order to decide, which techniques can best be used to support the most essential biological control agents (BCA) and their functions in each specific cropping situation, it is necessary to (i) determine the role of the BCA under real field conditions in providing the biocontrol services; (ii) determine the most reliable and cost-effective sources of BCA either from the surrounding off-crop habitats, or from sources within the crop; (iii) investigate the possibilities of enhancing functional BCA by engaging all possible levels of biodiversity in the cropping system (ranging from molecular and genetic levels to microbial, vegetation, and landscape level), and stacking these to optimise the effect; and to (iv) ensure that the agricultural system as a whole is supporting these processes for example by replacing toxic pesticides with biocontrol products and biorational pesticides, and by ensuring that the pesticides and other techniques, which still need to be used, do not harm the BCA.

As an example, we outline next the principles of ecostacking for a concrete case, as an inspiration to adopt similar approaches for other situations, and to illustrate how a complete biological control of a problem pest could be achieved in annual cropping systems by combining multiple elements of ecostacking. Depending on the specific cropping context, the number of these elements necessary for full control may vary and should be studied and optimised locally.

1.2 Principles of Ecostacking in the Context of Pollen Beetle Management

The pollen beetle *Brassicogethes aeneus* has been the most problematic pest of oilseed rape and turnip rape (“canola”) in Europe since decades. Locally and occasionally several other pest species may be more important, but the pollen beetle as a key pest has been more widespread, and a more constant threat to successful rapeseed production than any of the other species (e.g., Menzler-Hokkanen et al. 2006). Evolutionary adaptation to rapeseed growing practices, increasing the fitness of the pest, may have contributed to the pest status of the pollen beetle (Hokkanen 2000). The main control method against the pest has been, and continues to be, chemical control, mainly with synthetic pyrethroids over the last decades. Rapeseed fields are sprayed annually at least once, occasionally twice, and up to four times per season for pollen beetle control (Menzler-Hokkanen and Hokkanen 2018). Due to increasing resistance to synthetic pyrethroids in the pollen beetle (e.g., Tiilikainen and Hokkanen 2008), there is an urgent need to reduce the use of chemical pesticides, and to replace routine spraying with alternative control methods.

1.3 Landscape Level Processes

The pollen beetle overwinters as adult, and needs off-crop habitats for overwintering, selecting suitable sites within a range of several kilometers from the rapeseed fields (Müller 1941). The quality of the overwintering site undoubtedly affects the survival rate of the beetle, which can vary enormously. Hokkanen (1993) measured an overwintering survival rate of only 2–4% for a natural population in Finland, while later studies have found much higher survival rates (up to 20% in Northern Germany and 10–15% in Finland; Hokkanen unpublished). Furthermore, the presence or absence of early flowering plants at the overwintering site, offering pollen food to the beetles after emergence from hibernation, is expected to greatly influence pollen beetle survival. The beetles become active after temperatures rise to about +5 °C or above, but cannot fly away from the hibernation site until the temperature reaches about +12 °C (Mauchline et al. 2017). In the absence of pollen food they face starvation, unless they are able to fly away to early flowering spring plants such as dandelions. The beetles are strong fliers (up to 15 km measured, Taimr et al. 1967) and are able to optimize habitat use for their benefit within the broad landscape. How the spatio-temporal dynamics involved in pollen beetle management is affected by agricultural landscapes, needs further studies and is presently not well understood.

In contrast, the key natural enemies – hymenopteran parasitoids – are relatively little affected by landscape features. Main parasitoids overwinter as pupae in the soil of the rapeseed field and emerge in the spring at the time of start of bud formation in the crop. Landscape affects their capacity to find and parasitize the pollen beetle larvae at least in two ways: (i) depending on crop rotation patterns, the next year's rapeseed fields may be far away from the previous year's fields. In that case the landscape will affect the ability of the parasitoids to find the new fields of rapeseed, and increases mortality in transit; (ii) availability of floral resources to provide nectar nutrition to the parasitoids searching for the target habitat and the host larvae in the new rapeseed fields (Hatt et al. 2018).

Landscape quality has been shown to significantly affect the level of pollen beetle parasitism in rapeseed, and consequently the damage caused by the pest: parasitism increases linearly as the proportion of uncultivated land within 1.5 km of the rapeseed field increases, and the amount of damaged buds decreases linearly at the same time (Thies and Tscharntke 1999).

Studies in Finland revealed the existence of regional level processes that synchronise host-parasitoid dynamics over large geographical areas (Hokkanen 2006). Data on annual percent parasitism of the pollen beetle larvae by *Phradis morionellus* (Ichneumoniidae) were collected in Finland for 11 years (1985–1995) from 35 to 70 different locations (13 regions) covering the total area of rapeseed growing in Finland. Correlation analysis of the time-series data for the 13 regions revealed that the parasitoid dynamics follow a surprising pattern, being in synchrony over several large regions, but varying widely between these larger regions (more than 100 km in diameter). It has been suggested (Hokkanen 2006) that only edaphic and climatic factors can explain such regional patterns, and indeed, the boundaries of relief pattern types, and those of biotic zones in Southern Finland are similar to the approximate boundaries for the parasitism dynamics (Hokkanen 2006).

In the rapeseed growing system, improving ecosystem service provision in the target crop by optimizing provision of off-crop resources, involves the provision of habitat, food, and alternative hosts for crop pollinators and natural enemies of crop pests (c.f., Skellern and Cook 2018). Providing early-flowering resources near the target crop will attract and maintain pollinators to the crop vicinity before the focal crop flowering period, enabling better pollination. Late-flowering resources support pollinators after crop flowering, enabling pollinators to finish the season cycle. Likewise, early flowering resources support natural enemies of crop pests when resources are scarce, helping to build-up natural enemy populations in the crop early enough to prevent pest outbreaks, and late-flowering resources provide food, habitat and alternative prey species after mass-flowering crops have finished blooming, therefore promoting better survival of natural enemies for the next season.

1.4 Role of Field Margins and Trap Crops as Tools in Ecostacking

Off-crop elements can facilitate benefits for ecostacking purposes. There is an obvious connection between field margins, and specifically sown trap and nursery crops (the “botanical triad”, Shrestha et al. 2019), to the landscape-level processes discussed above. In addition, these off-crop elements can improve pollination and pest control, provide habitat for farmland biodiversity, and maximize use of space to increase overall crop productivity. Trap crops (Hokkanen et al. 1986; Hokkanen 1991; Shelton and Badenes-Perez 2006) can be used directly for pest management, and can facilitate optimally timed delivery of emerging biocontrol technologies such as entomovectoring with insect pathogens (Hokkanen and Menzler-Hokkanen 2007; Hokkanen et al. 2015) and RNAi-products (Hokkanen and Menzler-Hokkanen 2018c). Timing in this context is critical, and trap cropping in the spring as well as in the autumn needs to be considered. Autumn trap crops may be easier to implement (e.g., less competing flowering plants) and can be superior for example in delivering next generation biopesticides based on RNAi (Hokkanen and Menzler-Hokkanen 2018c). In the spring, turnip rapeseed can sometimes effectively be used as a trap crop for protecting oilseed rape from pollen beetle attack (Hokkanen 1989).

1.5 In-Field Botanical Diversity

Cropping systems based on designed ecological interventions such as mulches, or species or variety mixtures, have several advantages. These advantages include higher yield quality and stability, improved pest, weed and disease control, enhanced ecosystem services, and greater profitability (Balzan 2017). In general, variety mixtures can increase yield stability and suppress insect pests (Grettenberger and Tooker 2017).

Species or variety mixtures probably have never been tried or considered in the context of providing protection against the damage by the pollen beetle. Although there are distinct differences between pollen beetle preferences for different crucifer oilseed species (Ekbom and Borg 1996), this may not be useful for limiting pollen beetle damage in practice. Very little information is available concerning varietal preference (but see Herve et al. 2014; Herve and Cortesero 2016).

Possible impact on pest control in rapeseed of intercropping with various species of clover was studied in Finland (Perälä 1995). Strip intercropping with 50 cm broad strips of rapeseed alternating with 50 cm strips of four different species of clover, was compared with rapeseed monoculture. No differences were shown for the number of pollen beetle adults or larvae per plant, and no differences were observed in percent-parasitism by parasitic wasps, nor in the activity density of predatory ground beetles or spiders. The crop yields/ha (for the rapeseed strips in the intercrop-treatment) were equal, but the production of second generation pollen beetles/m^2 was significantly higher (more than double) in the monocrop than in the intercrop system (Perälä 1995; Hokkanen 2005). This was explained by the higher predator pressure on pollen beetles during pupation and adult emergence in the intercrop system.

Hokkanen and Menzler-Hokkanen (2017, 2018a) argue for the ecological benefits of growing white clover as an undersown crop in spring rapeseed in Finland. Based on several years of practical field data (unpublished) it is clear that white clover as undercrop can effectively suppress weeds in the rapeseed growing system. White clover provides additional benefits for soil processes including nitrogen fixation and increases essential functional biodiversity in the system (see next section).

1.6 Soil Biotic and Abiotic Properties

A key factor in determining the extent and impact of in-crop generation of biocontrol services is soil properties and management, along with soil cover management. Soil management and soil cover management are greatly affecting the soil and epigeial beneficial fauna (predatory mites, ground beetles, spiders, overwintering parasitoids, entomopathogenic nematodes, etc.) and flora (entomopathogenic fungi and other microbes). At the landscape level, abiotic factors such as soil type and texture via their differential impact on parasitoid dynamics, are suspected as the main drivers of the regional synchronisation of patterns in pollen beetle parasitism (Hokkanen 2006).

Hokkanen and Menzler-Hokkanen (2018a) proposed to develop the concept of "insect pest suppressive soils" and provided data on the occurrence and importance of soil-borne insect pathogens in pest population suppression. Agricultural fields usually harbour only low numbers of beneficial insect antagonists such as entomopathogenic nematodes (EPN) and fungi (EPF), so that their role in pest population dynamics currently is negligible. Simple improvements in field and crop management, however, can quickly increase the numbers of EPN and EPF to levels that will lower the peak pest population levels (Hokkanen and Menzler-Hokkanen 2018a).

Concerning specifically the known pathogens of main oilseed *Brassica* pests, only entomopathogenic nematodes (EPN) and fungi (EPF) are widely distributed (Hokkanen et al. 2003). The EPF usually infecting pollen beetles are facultative pathogens, widely distributed in soils throughout the world, and occurring throughout the *Brassica* oilseed crops. Several studies have been published concerning their distribution in America, Australia and Europe (e.g., St Leger et al. 1992; Roberts and St Leger 2004). Studies often report a general relationship of these pathogens with the soil type, preferences of habitat type, and other abiotic and biotic factors affecting their abundance and role as insect antagonists (Vänninen 1996). However, their reported natural abundances in agricultural fields have always been low (e.g., Vänninen et al. 1989).

Most thoroughly studied EPF for potential control of oilseed *Brassica* pests include *Beauveria bassiana* and *Metarhizium anisopliae*. Both taxa have repeatedly been shown to kill effectively the adults (Fig. 1.1) and larvae of the pollen beetle (e.g., Butt et al. 1994; Husberg and Hokkanen 2001; Hokkanen et al. unpublished). In the field, treatments with EPF have not reduced the new generation pollen beetle numbers, neither via soil treatment nor via fungal sprays (Hokkanen 1993). However, soil treatments with EPF have caused dramatic indirect effects via increased overwintering mortality. Soil treatment with *B. bassiana* reduced overwintering survival of beetles developed under unlimited food resources by 50% (from 14% to 7% survival), compared with the untreated control.

The weight (fat reserves) of the beetles is a crucial factor explaining overwintering mortality of *B. aeneus* (Hokkanen 1993). Soil treatment with *B. bassiana* lowered the weight of pollen beetles developed under unlimited food resources by 16%. This alone is likely to be enough to result in the measured reduction in survival by 50%. However, beetles developing under normal conditions (collected from the wild) and under shortage of autumn food sources (pollen plants), were before overwintering 46% lighter than the experimental reference beetles. This lighter weight resulted in only 3% survival over the winter (Hokkanen 1993). If we assume that all rapeseed fields would contain effective levels of EPF either naturally or via augmentation, the pollen beetle populations would be expected to crash completely during the overwintering. Survival might be reduced from the measured 3% to maybe around 1%.

Fig. 1.1 Pollen beetle *Brassicogethes aeneus* adult, killed by the entomopathogenic fungus *Metarhizium anisopliae*. (Photo credit: Heikki M. T. Hokkanen)

In contrast to the effect of EPF, direct mortality of pollen beetles in the field caused by entomopathogenic nematodes can be very high. Augmenting the field soil of rapeseed with *Steinernema feltiae* at the rates of 0.1–1.0 million infective juveniles (IJ) per m^2 resulted in 95% reduction in the number of emerging pollen beetle adults (Hokkanen 2008). This shows a very high potential of the field soil to be suppressive to the pollen beetle, provided that adequate levels of antagonists such as EPF and EPN are present in the soil (c.f., Menzler-Hokkanen and Hokkanen 2005).

Plant associated microbes and their metabolites have been shown to have the potential to significantly alter the above-ground insect community. Plant associated microbes can affect virtually every type of plant-plant, plant-pathogen, plant-beneficial organism and plant-herbivore interactions (Pineda et al. 2017). A number of fungal and bacterial strains that occur naturally as part of the root microbiome, or indeed are used for biocontrol (e.g. *Trichoderma* spp., *Bacillus* spp., mycorrhizal fungi, *Pochonia* spp. and *Paecilomyces* spp.), have an indirect effect against pests via the plant, by activating defense response pathways (e.g., Prieto et al. 2017). This induction can in some cases be more effective than the biocontrol agent itself and offers large but unexplored potential for ecostacking in the rapeseed cropping system.

1.7 The Role of Pesticides

Insecticide sprays to control peak population densities of the pollen beetle is routinely used in rapeseed cropping systems. In situations where the parasitoid occurrence and impact is low, as in winter oilseed rape in Germany, insecticide applications were not observed to affect significantly the parasitization of pollen beetle larvae by its ichneumonid parasitoids. This finding is regardless whether applied at the bud stage, at the beginning of flowering, or at full flowering (Brandes et al. 2018). In spring rapeseed, parasitoids often occur at much higher levels (e.g., Hokkanen et al. 1988; Veromann et al. 2009) and insecticide applications will affect parasitoids detrimentally if they take place close to flowering (Hokkanen et al. 1988). Spray window in Finland was determined to be before the accumulated day-degrees reach 320, normally at about mid-June. Insecticide treatments prior to that were considered to be safe to the parasitoids (Hokkanen et al. 1988).

An analysis of long-term patterns of pollen beetle parasitism in Finland revealed an interesting connection between the regularly occurring, severe crashes in percent-parasitism of the pollen beetle, and outbreaks of cereal aphids: parasitism levels always crash in years of cereal aphid outbreaks (Fig. 1.2; Hokkanen et al. unpublished). A strong negative correlation was found between pollen beetle parasitism level and the severity of cereal aphid attack, as well as between sales of the insecticide dimethoate (used to control aphids in cereals) and pollen beetle parasitism levels (Hokkanen et al. unpublished). It appears clear that in outbreak years of cereal aphids – about twice in a decade in Finland during the past several decades – the pollen beetle parasitoids are killed while the aphids are controlled with insecticide sprays. In crop rotations in Finland, spring oilseed crops are usually followed by spring cereals. Therefore, the pollen beetle parasitoids emerge in the following year from fields of spring cereals, usually exactly at the same time as when aphids colonise the fields and are controlled if needed (around mid-June till end of June). It would be best not to spray insecticides in those fields, where rapeseed was grown in the previous year, in order to maximize the biocontrol of pollen beetle by its effective parasitoids.

Simulation models (Hokkanen and Kaukoranta unpublished) show that parasitoids are capable of completely controlling the pollen beetles at levels much below economic thresholds. This is under the assumption that the crop management does not interfere and negate their impact. Crop spraying with insecticides and soil cultivation methods are the key factors in the current management, preventing effective natural control of the beetle by its parasitoids. Both of these can easily be modified to allow effective biological control (Hokkanen et al. 1988; Hokkanen 2008).

Insecticide treatments in general in the rapeseed ecosystem appear to have led to localized eradications of the specific EPF *Nosema meligethi*, previously classified as Protozoa (Lipa and Hokkanen 1992; Hokkanen and Lipa 1995). *Nosema meligethi* is an intracellular obligate parasite specific to the genus *Meligethes*. It is an ideal insect pathogen from a population dynamics point of view. *Nosema meligethi* causes chronical disease with lowered fecundity and lifespan, and high overwintering mortality, and is transmitted both horizontally and vertically (Hokkanen and Lipa 1995). Lipa and Hokkanen (1992) studied the occurrence of *N. meligethi* across Europe. Samples of *Meligethes* spp. from 12 countries in Eastern and Western Europe were inspected for the occurrence of infection. A total of 13,910 individual beetles were checked, and of these, 561 were found to be infected (4.03%). Infection was not detectable in samples from the UK, Germany, Sweden, or Switzerland. In contrast, the disease was found rather regularly in samples from Finland and from Eastern European countries. No obvious reason for this pattern of infection is known, but pesticide usage at or close to the sampling sites may play a role.

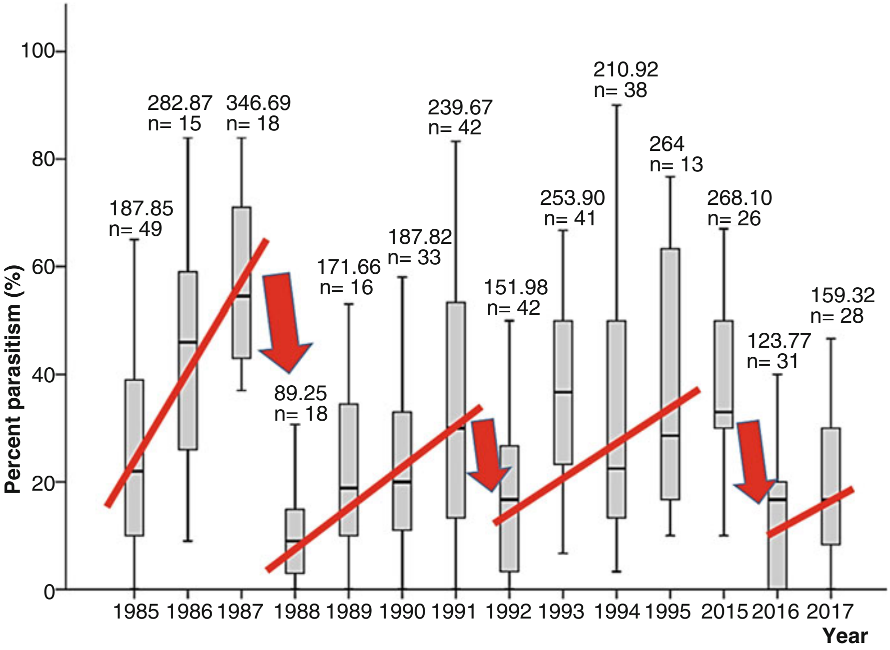

Fig. 1.2 Boxplot examining percent parasitism of pollen beetle in Finland in different years. Mean ranks and sample sizes are expressed above each whisker. Trend lines are fitted visually, arrows indicate parasitoid population crashes associated with chemical spraying of cereal aphids in years with severe aphid outbreaks. (Data from Hokkanen et al. (unpublished, in preparation))

Most samples from which *Nosema* was detected originated from areas where few pesticides are used.

Nosema infection was also shown to lower pollen beetle weights on the average by 13% – similar to the effect of soil treatment with *B. bassiana* – which is enough to explain the reported increases in the overwintering mortality of the beetles due to *Nosema* infection (Hokkanen and Lipa 1995).

1.8 Biopesticides and Entomovectoring

Only a few options are available as potential biopesticides for the control of the pollen beetle. Besides EPF and EPN, no known microbial pesticides are effective against this beetle. With EPF sprays in the field, good reduction of the pest has been obtained. For example, *M. anisopliae* treatment caused 75% mortality in pollen beetle larvae (Hokkanen 2008). Reduction of pollen beetle larvae on the inflorescences, however, may not be interesting from practical crop protection point of view, because the main damage to the crop has already been done by the adults.

Furthermore, even a high reduction in the size of the larval population is not likely to lower the population of the new generation emerging during the summer (Hokkanen 2000). *Metarhizium* sprays could possibly be used to kill adult pollen beetles in the spring via fungal sprays at the bud stage. The best option might be to grow and treat an early flowering trap crop (e.g., turnip rape) with the EPF.

Targeted, precision delivery of microbiological control agents such as EPF has been developed in the last two to three decades to improve practical and economic efficiency of biocontrol agent use. Usually, managed pollinating insects such as honey bees and bumble bees are employed as vectors; hence the term ‘entomovector technology’ (first used by Hokkanen and Menzler-Hokkanen 2007). The vectoring insects are loaded with the powdery biocontrol preparate at the exit from their hive, and while visiting the flowers of the target crop they deliver the biocontrol agent onto the crop inflorescences (Hokkanen et al. 2015). As pollen beetles feed in open flowers, they are good targets for entomovectoring, because rapeseed flowers are highly attractive also to pollinators.

Butt et al. (1998) report that honey bees foraging from a hive fitted with an inoculum dispenser at the entrance effectively delivered dry conidia of *M. anisopliae* to the flowers of oilseed rape in caged field plots. In both winter- and spring-sown rape experiments, a greater mortality of pollen beetles occurred in treated plots than in control plots. The mortality (61% on winter rape, 100% on spring rape) was greatest during peak flowering, when the feeding activity of both bees and beetles from the flowers was maximal. This situation provided optimal conditions for inoculum dissemination and infection. Conidial sporulation occurred on a significant proportion of the dead pollen beetles.

Carreck et al. (2007) show in a later study that *M. anisopliae*, disseminated to the flowering canopy of oilseed rape by honey bees, infect seed weevil adults and pollen beetle adults and larvae in the flowers. Subsequent mortality of adults of both species and the larvae of pollen beetle, following incubation in the laboratory was high, and always exceeded 70%. A high proportion of cadavers showed external conidiation, confirming infection by *M. anisopliae*.

Experience so far shows that bees are more efficient than conventional sprayers in delivering the EPF inoculum to the pest infested flowers (Butt and Copping 2000). Entomovectoring improves targeting and provides cost-effective control of pests (see also Hokkanen et al. 2015), and can be adopted for improving pest management in rapeseed.

Entomovectoring may be most appropriate in connection with an early-flowering trap crop (Hokkanen 1991). Field experiments have indicated excellent potential for employing *Brassica rapa* as a trap crop for *B. napus*, as it flowers clearly earlier and is highly attractive to rapeseed pests, as well as to bees and other pollinators (Hokkanen 1989).

1.9 Conclusion

The scientific community needs to develop, design and implement integrated systems for optimised provision of ecosystem services and use of plant protection tools, with focus on ecological, economic and social sustainability of the integrated system (Hokkanen 2015). To facilitate full integration, leading to integrative biological control, there is a need to find ways to support biological control in agroecosystems as much as possible, and to develop "bioinspired" plant protection products and tools, based on the use of natural products active against pests and pathogens, or that trigger plant defense responses. These products and tools are needed as successor technology to replace phased-out chemical pesticides.

This is why we need to increase our knowledge on stacking of the most promising and productive ecosystem services into an operational, profitable, and sustainable whole. In the specific case of the pollen beetle, we are confident that this problem pest can be brought under complete biological control by combining several of the ecostacking component techniques into one management system.

Disclaimer This chapter is largely a combined reproduction of two articles: "Ecostacking: maximising the benefits of ecosystem services" https://link.springer.com/article/10.1007%2Fs11829-017-9575-8 and "Developing ecostacking techniques for pollen beetle management in oilseed rape" https://link.springer.com/article/10.1007/s11829-018-9650-9.

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Chapter 2
The Joint Economic Impact Level (jEIL): A Decision Metric for Integrated Pest and Pollinator Management

Adam Flöhr, Johan A. Stenberg, and Paul A. Egan

2.1 Introduction

Injury from pests (including pest insects, weeds, and pathogens) and insect-provided pollination services are of large relevance to yield in the majority of the world's food crops (Klein et al. 2007; Oerke 2006). Various strategies are employed to manage these processes. For pests, Integrated Pest Management (IPM) incorporates a variety of cultural, mechanical, and biological control practices designed to supress pest populations in ways that are complementary, and which minimize the use of chemical pesticides (Stenberg 2017). For pollinators, managed bees are often used to boost crop pollination; in addition to agro-ecological practices (such as perennial flower strip plantings and the designation of no-till zones) designed to provision wild pollinators with forage and nesting resources (Garibaldi et al. 2017; Isaacs et al. 2017).

The effects of practices employed to manage pests and pollinators are not necessarily independent, however. Recent years have witnessed growing awareness of the widespread acute and chronic effects of pesticides on pollinators such as wild and managed bees (Godfray et al. 2014; Siviter et al. 2018). Furthermore, these effects can translate into impacts on crop pollination service (Brittain and Potts 2011; Stanley et al. 2015). Hence, owing to the potentially large and negative effects of pesticides on pollinators, up-take of more balanced pest control strategies – such as those inherent in an IPM approach – are increasingly demanded (Dicks

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A. Flöhr
Department of Biosystems and Technology, Swedish University of Agricultural Sciences, Alnarp, Sweden

J. A. Stenberg · P. A. Egan (✉)
Department of Plant Protection Biology, Swedish University of Agricultural Sciences, Alnarp, Sweden
e-mail: paul.egan@slu.se

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et al. 2016; Bartomeus and Dicks 2018). This explicit incorporation into IPM of the goal of protecting pollinators from the harmful effects of pesticides has been termed by Biddinger and Rajotte (2015) as *Integrated Pest and Pollinator Management* (IPPM), and was developed into an expanded framework by Egan et al. (2020).

Here, we introduce a decision metric for IPPM termed the *joint Economic Impact Level* (jEIL). Akin to the Economic Injury Level (EIL – Stern et al. 1959; Pedigo and Rice 2014) which underpins IPM decision making, the joint EIL can be used to weigh the economic rationale for pest and pollinator management in a coordinated manner. This metric is not biased towards either practice however, and its use is equally valid where one or both are of primary concern. In particular, the joint EIL can account for how pest and pollinator management practices may interact, such as where the implementation of one can influence the need for the other. For instance, is the benefit of pest control economically justified when a practice (e.g. insecticide use; eradication of flowering weeds) also results in an additional cost to enhance pollination service? Given the current field density of pests and pollinators in a crop, and their specific relation to yield, which management practice (if any) should be prioritised? The joint EIL can be used to tackle such considerations and scenarios, in providing a foundational basis for decision making in IPPM.

Conceptual development of the joint EIL requires substantial formulaic elaboration of the EIL, as documented in the following sections. The EIL traditionally defines the point (in pest population density units) at which a control intervention becomes economically justified – i.e. the point where economic benefit outweighs cost. Hence, action is usually taken once, and only if, pest levels pass an Action Threshold (AT – set for instance at 80% EIL) in order to prevent the EIL being reached. Although not without their drawbacks, pest EILs have successfully underpinned IPM decision making for decades (Pedigo et al. 1986). We posit that expanding this metric to support pollinator management – and, by extension, a more formalised decision making framework for IPPM – could similarly prove of large benefit.

Development of the joint EIL also requires harmonization of the diverse sets of terminology typical of the pest and pollinator literature. In contrast to pest control, the goal of pollinator management is somewhat inverted; in that it is primarily motivated towards avoidance of yield limitation (as opposed to yield loss), and the maintenance of pollinator populations above (as opposed to below) a certain threshold. Below an optimal density (or visitation rate) of pollinators, pollination deficits and impacts on yield quantity and quality become increasingly apparent in pollinator-dependant crops. Such thresholds are highly crop specific, however, as governed by the large gradient in pollinator reliance evident across crops (Klein et al. 2007). Hence, in respect of the differing yield influences of pests and pollinators, and their associated management goals, we here apply the more general term *impact* (within *joint Economic Impact Level*) to reflect its wider incorporation of yield injury and limitation.

In the following sections, we take a step-wise approach to documenting the rationale behind how the joint EIL is constructed and can be used. We start by recounting construction of the traditional EIL and introducing here a pollinator equivalent (PEIL – the *Pollinator Economic Impact Level*), before describing the means through which these univariate cases are incorporated within the joint EIL. We follow these theoretical sections with a practical worked example for strawberry, using empirical data to calculate a joint EIL in aid of management decision making. Finally, we discuss broader issues in relation to the joint EIL, including recommendations for its practical use, and possible future developments.

2.2 Derivation of EIL and Pollinator EIL

2.2.1 Pest Economic Injury Level with Constant Cost

We begin by deriving the classic EIL for pests. The basis of the EIL is a simple economic model in which economic loss due to pests and the cost of pest control depend on the pest density, denoted θ. The EIL is then given by the level of θ where the reduction of loss equals the cost of control. Terminology and symbology follows Pedigo et al. (1986) where possible. For a list of symbols used in the following sections, see the glossary included below.

Assuming economic loss depends linearly on the pest density, we have the loss function

$$L_{pe}(\theta) = \begin{cases} VD'\theta & \text{if } 0 \leq \theta \leq \frac{1}{D'} \\ V & \text{if } \theta > \frac{1}{D'} \end{cases}$$

where

V is the maximal value of crop per unit (e.g. value per hectar),

D' is the loss associated with an increase of one of the pest density, and

θ is the pest density per unit.

The loss function is constant for $\theta > 1/D'$, as loss is bounded by the maximal crop value V.

Under the assumption that the cost of pest control C_{pe} is a constant (i.e. cost does not depend on θ) in the interval $[0, V]$, the benefit of pest control is the reduction in loss minus the cost of control, i.e. $L_{pe}(\theta) - L_{pe}(\theta') - C_{pe}$, where θ and θ' are pest densities before and after control respectively, and C_{pe} is the cost of control per unit crop. The EIL is the smallest θ such that the benefit is greater than zero. Setting θ' to zero (i.e. assuming pest control removes the entire population) and solving for θ gives a basic expression for the EIL:

$$L_{pe}(\theta) - L_{pe}(0) - C_{pe} \geq 0 \iff$$

$$VD'\theta \geq C_{pe} \iff$$

$$\theta \geq \frac{C_{pe}}{VD'}.$$

Hence, pest control is economically beneficial if the pest density exceeds the EIL, which is given by the right-hand side of the final inequality.

This situation is illustrated in Fig. 2.1. The EIL is given by the projection of the intersection of the loss function and the cost of pest control. For pest densities below the EIL, the benefit of pest control is negative as the cost is higher than the current loss; for pest densities above the EIL, the loss exceeds the cost, so control measures are beneficial.

Before turning to the pollinator case, we look to relax the assumption that pest control is completely successful. Let K_{pe} denote the success proportion of pest control, so that e.g. $K_{pe} = 0.25$ would signify that pest control cuts the pest population by one fourth. The economic gain of pest control is then given by

$$L_{pe}(\theta) - L_{pe}((1 - K_{pe})\theta) - C_{pe},$$

i.e. the reduction in loss due to pests as the pest density moves from the current level θ to $(1 - K_{pe})\theta$, minus the cost of control. The EIL when taking the success of control into account is then derived as

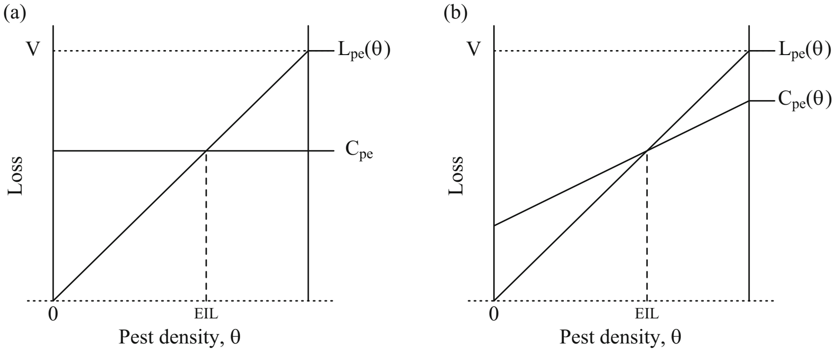

Fig. 2.1 Illustrations of the EIL for (**a**) the case of constant cost and (**b**) the case with pest density-dependent cost. The EIL is given by the projection of the intersection of the cost function, C_{pe} or $C_{pe}(\theta)$, and the loss function $L_{pe}(\theta)$. For densities below the EIL, the cost of pest control is greater than the current loss due to pests, meaning that pest control is economically unsound. For densities above the EIL, the current loss is greater than cost of control, meaning that there is an economical gain from pest control. The illustrations are under the assumption that pest control reduces the pest density to zero

$$L_{pe}(\theta) - L_{pe}((1 - K_{pe})\theta) - C_{pe} \geq 0 \iff$$

$$VD'\theta - VD'(1 - K_{pe})\theta - C_{pe} \geq 0 \iff$$

$$\theta \geq \frac{C_{pe}}{VD'K_{pe}}.$$

This derived EIL is equivalent to adjusting the cost of pest control by the success rate. Note that this derivation assumes $\theta \leq 1/D'$.

2.2.2 *Pollinator Economic Impact Level with Constant Cost*

We turn now to the case of pollinator management and derive a measure of economic impact in analog to the economic injury level for pests. As in the pest case, the total value of one unit of crop is denoted V. Following Gallai and Salles (2016), a proportion $\bar{D}$ (the pollinator dependence) of V is said to depend on pollinators, while the remaining part $(1 - \bar{D})V$ depends on other factors, e.g. self-pollination and pollination from insects other than the primary pollinator. We introduce an optimal density ϕ_{opt}, which is a level of pollinator density such that the marginal decrease in loss (i.e. pollinator limitation of yield) as the density increases is approximately zero. The maximal pollinator-dependent loss is $\bar{D}V$ and it occurs when the pollinator density (here denoted ϕ) is 0.

Assuming that loss depends linearly on the pollinator density ϕ now gives the loss function

$$L_{po}(\phi) = \begin{cases} \bar{D}V - \bar{D}V\frac{\phi}{\phi_{opt}} & \text{if } 0 \leq \phi \leq \phi_{opt} \\ 0 & \text{if } \phi > \phi_{opt}. \end{cases}$$

When the pollinator density is zero, the loss equals the value of one unit of crop times the pollinator dependence. The loss decreases linearly as the density increases and reaches zero at the optimal density ϕ_{opt}, after which there is no marginal effect of increased density.

As in the case for pest control we can introduce a cost of pollinator management C_{po} and construct a function for the benefit of management as the decrease in loss minus the cost: $L_{po}(\phi) - L_{po}(\phi') - C_{po}$. Under the assumption that pollinator management moves the pollinator density to, or above, the optimal density, we can derive a basic pollinator economic impact level (PEIL):

$$L_{po}(\phi) - L_{po}(\phi_{opt}) - C_{po} \geq 0 \iff$$

$$\bar{D}V - \bar{D}V\frac{\phi}{\phi_{opt}} - C_{po} \geq 0 \iff$$

$$\phi \le \phi_{opt}\frac{\bar{D}V - C_{po}}{\bar{D}V}.$$

Hence, the derivation shows that pollinator management is beneficial for observed pollinator densities below the PEIL, which is given by $\phi_{opt}(\bar{D}V - C_{po})/(\bar{D}V)$.

The basic pollinator EIL is illustrated in Fig. 2.2. The loss function is linear from $\bar{D}V$ at $\phi = 0$ to 0 at $\phi = \phi_{opt}$ and the cost is a constant at C_{po}. The PEIL is the projection of the intersection between cost and loss onto the ϕ axis. For ϕ below the PEIL, the loss is greater than the cost, and pollinator management is therefore economically beneficial.

A pollinator success rate can be included in the calculation by setting ϕ' to $\phi + K_{po}(\phi_{opt} - \phi)$. The rationale is that pollinator management reduces the distance to the optimal level ϕ_{opt} by a proportion given by the success rate. Simplifying and solving for ϕ gives a pollinator EIL under consideration of K_{po} as

$$L_{po}(\phi) - L_{po}(\phi + K_{po}(\phi_{opt} - \phi)) - C_{po} \ge 0 \iff$$

$$\bar{D}V - \bar{D}V\frac{\phi}{\phi_{opt}} - \left(\bar{D}V - \bar{D}V\frac{\phi + K_{po}(\phi_{opt} - \phi)}{\phi_{opt}}\right) - C_{po} \ge 0 \iff$$

$$\frac{\bar{D}V}{\phi_{opt}}K_{po}(\phi_{opt} - \phi) - C_{po} \ge 0 \iff$$

$$\phi \le \phi_{opt}\frac{\bar{D}VK_{po} - C_{po}}{\bar{D}VK_{po}}.$$

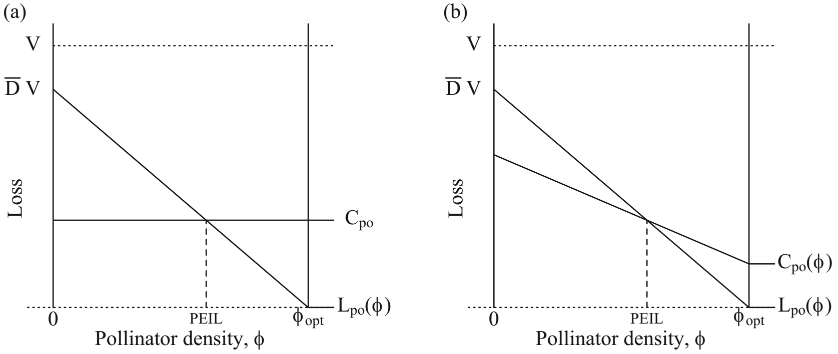

Fig. 2.2 Illustrations of the pollinator EIL for (**a**) the case of constant cost and (**b**) pollinator density-dependent cost. The PEIL is given by the projection of the intersection of the cost function, C_{po} or $C_{po}(\phi)$, and the loss function $L_{po}(\phi)$. For densities below the PEIL, the cost of pollinator management is greater than the loss due to a suboptimal pollinator density, while for densities above the PEIL, the loss is greater than cost, meaning there is an economical gain from pollinator management. The illustrations are under the assumption that pollinator management increases the pollinator density above the optimal pollinator density ϕ_{opt}

Hence, including the success rate K_{po} in the calculation gives a PEIL where the maximal value of the crop V is adjusted by the success rate.

2.2.3 *Pest Economic Injury Level with Linear Cost*

We move now to the slightly more general situation where the cost of pest control depends linearly on the pest density. The cost function is given by

$$C_{pe}(\theta) = C_{pe} + M_{pe}\theta,$$

where C_{pe} is a base cost and M_{pe} is a marginal cost interpretable as the increased cost associated with an increase of one in pest density. If M_{pe} is zero, the cost of pest control is constant and we return to the previously derived EIL for constant cost.

We set up a function for the benefit of control as the decrease in loss minus the cost:

$$L_{pe}(\theta) - L_{pe}(\theta') - C_{pe}(\theta).$$

Given the assumptions that control gives a decrease in the pest density to $(1-K_{pe})\theta$ and that cost is linear in θ, the benefit function is

$$VD'\theta - VD'(1-K_{pe})\theta - C_{pe} - \theta M_{pe},$$

where V, D', K_{pe} have the same meaning as in the derivation of the EIL with constant cost. Setting the benefit of control greater than zero and solving for the pest density θ gives

$$VD'\theta - VD'(1-K_{pe})\theta - C_{pe} - \theta M_{pe} \geq 0 \iff$$

$$\theta \geq \frac{C_{pe}}{VD'K_{pe} - M_{pe}},$$

where the last term $C_{pe}/(VD'K_{pe} - M_{pe})$ is the EIL for linear cost. This derivation is done under the assumption that θ is less than $1/D'$. If M_{pe} is zero, the EIL for linear cost reduces to the EIL for constant cost with a success rate K_{pe}.

The situation with density-dependent cost is illustrated in Fig. 2.1b. Both the loss function $L_{pe}(\theta)$ and the cost function $C_{pe}(\theta)$ increase with the pest density. The EIL is given by the value of θ where the lines intersect, as the benefit is less than zero below that EIL and greater than zero above. The point where the cost function cuts the y-axis is the base cost of pest control, i.e. $C_{pe}(0) = C_{pe}$.

Figure 2.1 also serves to illustrate two features of the model with density-dependent cost that are not present in the model with constant cost. Firstly, if the marginal cost is high in relation to the value V and the damage associated with an increase in pest density D', it is possible that the loss function and the cost function do not intersect. In that case the benefit of pest control will be negative for all values of θ and the calculated EIL will be negative or a division by zero. Secondly, since the loss function $L_{pe}(\theta)$ is bounded by V, the loss function and the cost function can intersect twice. This can be visualized by extending the functions to higher values of θ. Hence, the EIL has not only a lower limit to where pest control is beneficial, but also a higher limit.

2.2.4 Pollinator Economic Impact Level with Linear Cost

For the pollinator case, a cost function which depends linearly on the pollinator density ϕ can be defined as

$$C_{po}(\phi) = C_{po} + (\phi_{opt} - \phi)M_{po}.$$

Here, C_{po} is the baseline cost of pollinator management and M_{po} is the marginal increase of cost as the pollinator density decreases. For the calculations of the pollinator EIL, the baseline cost and the highest possible cost, attained at $\phi = 0$, are both assumed to be less than the maximal value of the crop times pollinator dependence ($\bar{D}V$).

As in previous cases, the PEIL is derived by forming a benefit function and solving for the pollinator density where the benefit of control is greater than zero. For the model with linear cost and a success rate K_{po} the benefit function is given by

$$L_{po}(\phi) - L_{po}(\phi + K_{po}(\phi_{opt} - \phi)) - C_{po}(\phi) =$$

$$\bar{D}V - \bar{D}V\frac{\phi}{\phi_{opt}} - \left(\bar{D}V - \bar{D}V\frac{\phi + K_{po}(\phi_{opt} - \phi)}{\phi_{opt}}\right) - C_{po} - (\phi_{opt} - \phi)M_{po}.$$

Setting an inequality where the benefit is greater than zero and solving for ϕ gives the EIL of the model:

$$\bar{D}V - \bar{D}V\frac{\phi}{\phi_{opt}} - \left(\bar{D}V - \bar{D}V\frac{\phi + K_{po}(\phi_{opt} - \phi)}{\phi_{opt}}\right) - C_{po} - (\phi_{opt} - \phi)M_{po} > 0 \iff$$

$$\phi < \phi_{opt}\frac{\bar{D}VK_{po} - C_{po} - \phi_{opt}M}{\bar{D}VK_{po} - \phi_{opt}M}.$$

The calculation uses the assumption that ϕ is between 0 and ϕ_{opt}. Note that the pollinator EIL of the model with linear cost equals the pollinator EIL (with success rate K_{po}) for constant cost if $M_{po} = 0$.

The situation with pollinator-dependent cost and $K_{po} = 1$ is illustrated in Fig. 2.2. Both cost and loss decrease with increasing pollinator density ϕ. In the point $\phi = \phi_{opt}$ the loss is zero and the cost is equal to the baseline cost C_{po}. The PEIL is given as the value of ϕ where the cost and the loss functions intersect, and pollinator management is beneficial for values of ϕ below the PEIL, i.e. the densities where the loss is greater than the cost.

2.2.5 *Non-linear Loss Functions*

The traditional EIL and the pollinator EIL presented here both rely on the assumption that economic loss depends linearly on pest or pollinator density. The justification of this assumption is twofold: the linear function is an approximation for most common functions used to model yield if one is interested in a small range of pest or pollinator density levels; and using linear loss results in simple expressions for the EIL. In this section we discuss some non-linear functions (variants of the sigmoid curve) which may be better approximations of the relation between insect density and yield. The discussion focuses on the case of pollinators.

We begin by looking at the connection between a function on yield and the loss function. Let $v(\phi)$ denote a measure of yield as a function of pollinator density. The sigmoid curve is given by

$$v(\phi) = t_1 + t_2(1 - \exp\{-t_3\phi^{t_4}\}).$$

Assuming t_3 and t_4 are both positive, the function is bounded from above by $t_1 + t_2$. Hence, $1 - v(\phi)/(t_1 + t_2)$ gives a proportional loss at the pollinator density ϕ, and multiplying that proportion by V gives a loss function per unit crop. Furthermore, the loss at $\phi = 0$ should equal $\bar{D}V$ (the value of the pollinator-dependent portion of the crop), the loss should decrease as ϕ increases, and be bounded from below by zero. These assumptions result in the following sigmoid loss function

$$L^s_{po}(\phi) = \bar{D}V \exp\{-t_3\phi^{t_4}\}.$$

The information loss of using a linear loss function depends on the in-going parameter values (t_3 and t_4 for the sigmoid loss function, and ϕ_{opt} for the linear loss function) and the range of densities taken into consideration. Figure 2.3 displays two examples of sigmoid loss functions and an approximated linear loss function. The parameter ϕ_{opt} is set to the smallest ϕ such that $L^s_{po}(\phi) < \bar{D}V/20$, i.e. the pollinator density where no more than 5% of the pollinator dependent crop value is lost. It is also possible to change the intercept at $\phi = 0$ in order to get a better linear approximation of the sigmoid loss function. The figures illustrate the possible differences between the non-linear and the linear loss function, and how two different sigmoid loss functions can give rise to similar linear approximations. This highlights how any application of the EIL or pollinator EIL should take into

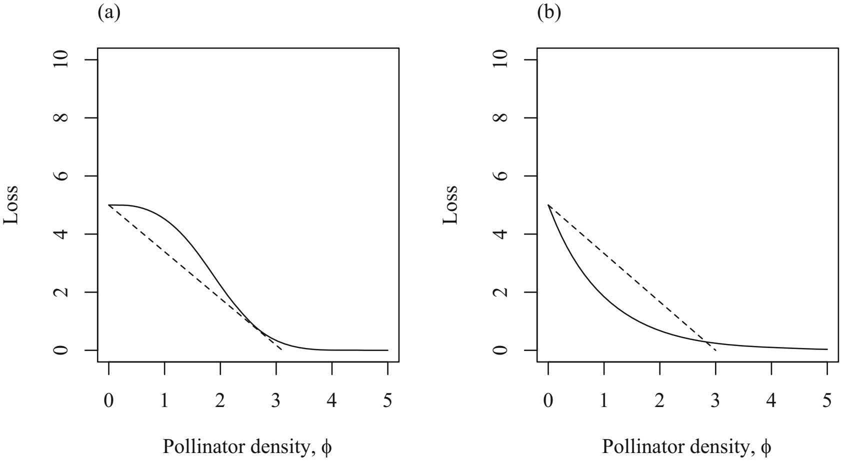

Fig. 2.3 Illustrations of non-linear ($L^s_{po}(\phi) = \bar{D}V \exp\{-t_3\phi^{t_4}\}$) and linear ($L_{po}(\phi) = \bar{D}V - \bar{D}V\phi/\phi_{opt}$) loss functions for (**a**) parameters $(t_3, t_4) = (0.1, 3)$ and (**b**) parameters $(t_3, t_4) = (1, 1)$. ϕ_{opt} set to the density level where the non-linear curve equals $\bar{D}V/20$. For (**a**) and (**b**), V and $\bar{D}$ equals 10 and 0.5

consideration whether the use of a linear or non-linear curve serves as the most suitable description of relation between insect density and yield.

2.2.6 A Joint EIL

So far we have only looked at the univariate cases, where the EIL of the pest density has been derived without taking the pollinator density into account, and vice versa. We turn now to the problem of finding a beneficial action in a situation where both pest control and pollinator management are available, and both pest and pollinator density affect the total loss. The reasoning parallels the univariate cases: we set up loss and cost functions of the densities, examine the reduction in loss following a specific action, and identify levels where that action is economically beneficial.

Given the univariate loss functions $L_{pe}(\theta)$ and $L_{po}(\phi)$, a natural joint loss function[1] is given by

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[1] We justify this loss function by help of an example. Say that the value of one unit of crop is four, that the current pest density is associated with a loss of one third of the crop, and that the current pollinator density is associated with a loss of one sixth of the crop. After accounting for the loss due to the low pollinator density, five sixths of the total value remains. Of this, one third is lost due to the pest density, leaving two thirds of five sixths, or ten parts of eighteen. The total loss is thereby the lost portion, eight parts of eighteen, times four (the maximal value). This reasoning corresponds to the joint loss function $L(\theta, \phi)$.

$$L(\theta,\phi) = V\left(1-\left(1-\frac{L_{pe}(\theta)}{V}\right)\left(1-\frac{L_{po}(\phi)}{V}\right)\right).$$

Note that the joint loss function reduces to a univariate case in the best case scenarios: $L(0,\phi) = L_{po}(\phi)$ and $L(\theta,\phi_{opt}) = L_{pe}(\theta)$. The joint loss function is in that sense an extension of the univariate cases. Inserting the expressions for the univariate loss functions L_{pe} and L_{po} gives

$$L(\theta,\phi) = \begin{cases} V(1-(1-D'\theta)(1-\bar{D}+\bar{D}\frac{\phi}{\phi_{opt}})) & \text{if } \theta \le \frac{1}{D'} \text{ and } \phi \le \phi_{opt} \\ V & \text{if } \theta > \frac{1}{D'} \\ VD'\theta & \text{if } \phi > \phi_{opt} \text{ and } \theta < \frac{1}{D'}. \end{cases}$$

The densities θ and ϕ are of course bounded from below by zero.

The joint loss function is illustrated in Fig. 2.4. The function is zero when the pest density θ is zero and the pollinator density ϕ is greater than the optimal level. For fixed ϕ, the function is linear in θ and vice versa. The function is bounded by the total value V, which can be seen in the constant section where θ is large.

In the univariate cases we set up a function for the benefit of control as the reduction in loss, minus the cost. This is not as straightforward in the joint case since there are multiple possible strategies and since control measures of one kind can have dynamic effects, i.e. pest control can affect the pollinator density and pollinator management can affect the pest density. In the following section we assume that applying both control measures is effectively similar to pest control followed by pollinator management, that the effect of pest control on pollinator

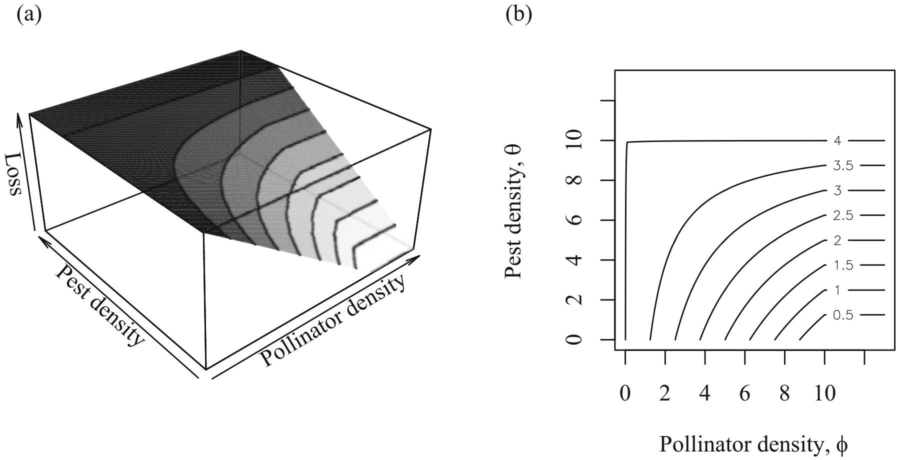


Fig. 2.4 Illustration of the joint loss function as a surface model and as contour lines for the model with parameters $(V, \bar{D}, D', \phi_{opt}) = (4, 1, 0.1, 10)$. Loss has a negative relation to pollinator density and a positive relation to pest density

density is a multiplicative factor J_{pe}, and that the effect of pollinator management on pest density is a multiplicative factor J_{po}. For example, $J_{pe} = 0.8$ would indicate a 20% decrease in the pollinator density after pest control, while $J_{po} = 1.1$ would indicate a 10% increase in the pest density after pollinator management.

In the joint model there are four available strategies: inaction (S_0), pest control (S_{pe}), pollinator management (S_{po}), and both ($S_{pe,po}$). The joint cost function is given by the unvariate cost function for strategies S_{pe} and S_{po}, and by the sum of the cost functions for strategy $S_{pe,po}$. In the latter case, the assumption that pest control affects the pollinator density ϕ means that the cost of pollinator management is calculated based on the density $J_{pe}\phi$, i.e. the pollinator density after applying pest control. These assumptions allow us to formalize a joint cost function as a function of the pest density, the pollinator density, and the chosen strategy:

$$C(\theta, \phi, S) = \begin{cases} 0 & \text{if } S = S_0 \\ C_{pe} + M_{pe}\theta & \text{if } S = S_{pe} \\ C_{po} + (\phi_{opt} - \phi)M_{po} & \text{if } S = S_{po} \\ C_{pe} + M_{pe}\theta + C_{po} + (\phi_{opt} - J_{pe}\phi)M_{po} & \text{if } S = S_{pe,po}. \end{cases}$$

Note that the term $J_{pe}\phi$ can exceed ϕ_{opt}.

The benefit of control can now be calculated as the reduction in loss minus the cost of control. This gives a function of the pest and pollinator densities and the choice of action:

$$L(\theta, \phi) - L(\theta', \phi') - C(\theta, \phi, S).$$

The densities after control measures (θ' and ϕ') depend on the type of control. In analog to the univariate cases, we assume that pest control and pollinator management have success rates K_{pe} and K_{po} respectively. Under these assumptions, pest control decreases the pest density from θ to $(1 - K_{pe})\theta$ and changes the pollinator density from ϕ to $J_{pe}\phi$. Pollinator management increases the pollinator density from ϕ to $\phi + K_{po}(\phi_{opt} - \phi)$, while the pest density changes from θ to $J_{po}\theta$. Pest control followed by pollinator management changes the pest density from θ to $J_{po}(1 - K_{pe})\theta$ and changes the pollinator density from ϕ to $J_{pe}\phi + K_{po}(\phi_{opt} - J_{pe}\phi)$, which can be slightly simplified to $(1 - K_{po})J_{pe}\phi + K_{po}\phi_{opt}$.

Putting the loss function, the cost function and the effects of control together gives the following expressions for the economic consequence of each possible action:

$$\begin{cases} 0 & \text{if } S = S_0 \\ L(\theta, \phi) - L((1 - K_{pe})\theta, J_{pe}\phi) - C(\theta, \phi, S_{pe}) & \text{if } S = S_{pe} \\ L(\theta, \phi) - L(J_{po}\theta, \phi + K_{po}(\phi_{opt} - \phi)) - C(\theta, \phi, S_{po}) & \text{if } S = S_{po} \\ L(\theta, \phi) - L(J_{po}(1 - K_{pe})\theta, (1 - K_{po})J_{pe}\phi + K_{po}\phi_{opt}) - C(\theta, \phi, S_{pe,po}) & \text{if } S = S_{pe,po} \end{cases}$$

In the univariate case we got a single expression for the economic benefit of control (since there was only one possible control measure), and an EIL could be calculated as the level where the benefit was greater than zero. In the joint case, all possible actions must be compared, and the straightforward calculation used in the univariate case is not possible.

Instead of calculating the analytical solution, we move on to present computational results. The computations are performed by setting up a grid of possible combinations of θ and ϕ for a specified set of ingoing parameter values, and calculating the economic consequence of each of the four strategies. Figure 2.5 illustrates the maximizing action for two different sets of ingoing parameter values.

Figure 2.5 (top) illustrates a symmetric situation where the marginal loss due to an increase in pest density equals the marginal loss due to a decrease in pollinator density, and the costs of pest control and pollinator management are equal. For cases where the pest density is low and the pollinator density high (the lower right corner of the plot), the most beneficial action is inaction. Pollinator management is the most beneficial action if both the pollinator and the pest densities are low (the lower left). Pest control is beneficial if both densities are high (top right). Both pest and pollinator management are the most beneficial action up to a point where the total cost of control measures exceed the value of the crop.

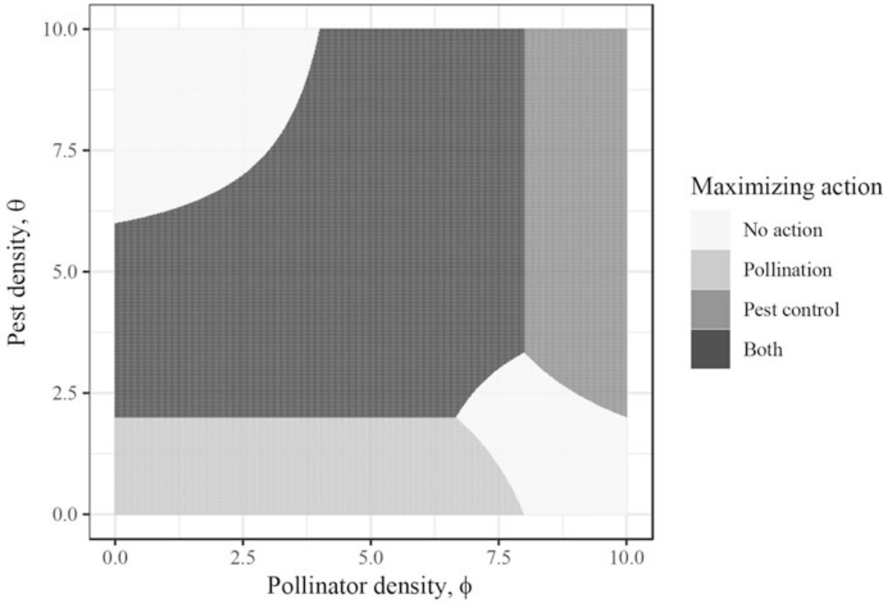
Figure 2.5 (bottom) illustrates the case where pest control is cheaper than pollinator management and less successful. The success proportion of pest control, K_{pe}, is set to 0.6, i.e. pest control reduces the pest population by 60%. We also assume pest control reduces the pollinator density by 20% ($J_{pe} = 0.8$). The resulting graph of maximizing action shows an irregular pattern. Pest control is the maximizing action in the most extreme case of high pollinator density and high pest density, while pollinator management and a combination of pest control and pollinator management are most beneficial for cases of low pollinator density and low pest density.

2.3 An Empirical Example

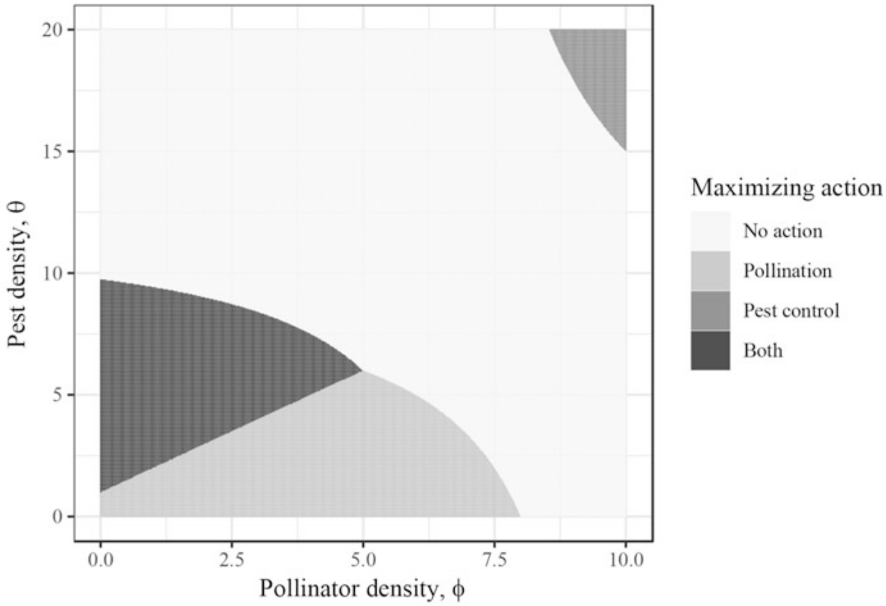
We give an empirical example of a joint EIL for strawberry based on published data. Data on pests is taken from Mailloux and Bostanian (1988), a study on tarnished plant bug. Data on pollinators is from Bartomeus et al. (2014).

Mailloux and Bostanian (1988) estimate the mean standardized weight (the weight measure is a percent of the weight if there is no pest damage) of strawberry as a non-linear function of the pest density, measured as number of nymphs per blossom cluster. We present the basic model with some altered terminology, in order to be consistent with previous sections. Note especially the meaning of V, which in the article is the value in kilos, but here is the total value in ha. The predicted standardized weight is modelled by

$$w(\theta) = \min(t_1 + t_2(1 - e^{t_3\theta}), 100),$$



V = 10, D' = 0.1, ϕ_{opt} = 10, C_{pe} = 1, M_{pe} = 0.5, C_{po} = 1, M_{po} = 0.5



V = 10, D' = 0.05, ϕ_{opt} = 10, C_{pe} = 0.1, M_{pe} = 0.2, C_{po} = 1, M_{po} = 0.5, K_{pe} = 0.6, J_{pe} = 0.8

Fig. 2.5 Illustration of the most economically beneficial action for combinations of pest and pollinator densities, for two different sets of parameters. Ingoing parameter values given in the graph. The top graph illustrates a symmetric situation where pest control and pollinator management are equally successful and beneficial. The bottom graph illustrates a situation where pest control has reduced success rate ($K_{pe} = 0.6$) and negative impact on the pollinator density ($J_{pe} = 0.8$)

where t_1, t_2 and t_3 are parameters estimated from data and θ is the pest density. The function is a sigmoid curve with $t_4 = 1$, bounded by 100. We will use the results pooled for the L'Assomption test site, for which the values of t_1, t_2 and t_3 are 110.649, −72.789 and −0.207. Given those parameter values, the weight is greater than zero for all possible values of θ.

The cost-benefit relation is formalized as

$$C_{pe} = K_{pe} V \frac{100 - w(\theta)}{100},$$

where the cost of control C_{pe} is set to 100, the success rate K_{pe} is 0.9 (or 90%), and the total value V is 10,000. The right-hand side of the the cost-benefit relation corresponds to what we have previously denoted $L_{pe}(\theta)$, the economic loss due to pests at the pest density level θ. Inserting the weight function into the cost-benefit relation and solving for the pest density θ gives an EIL of 0.85. In the original article an additional argument based on plant carrying capacity is used, giving a final EIL of 0.99. We will use the calculated value from the parametrized curve (i.e. 0.85), mainly for the direct comparison with the results for the joint EIL.

These results are visualized in Fig. 2.6. The loss function $L_{pe}(\theta)$ is the right-hand side of the cost-benefit relation and the horizontal line is the cost of control C_{pe}. The EIL is the projection of the intersection of the two curves.

Moving on, we look at the data of Bartomeus et al. (2014) and calculate a pollinator EIL. Here, the relation between mean berry weight and pollinator abundance (measured as total number of visits in a set time span) is given as a linear function. In order to calculate an EIL, we make the additional assumption that the goal mean berry weight is 15 grams. This results in a weight function given by

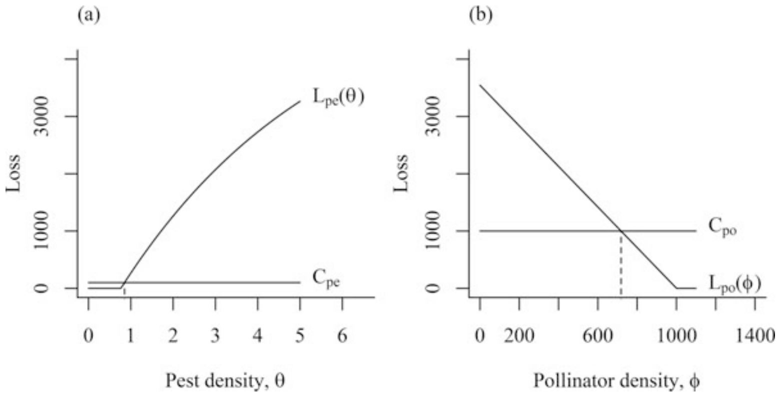


Fig. 2.6 Illustrations of the EIL and PEIL of the empirical examples. The loss function due to (**a**) pests and cost of pest control and the loss function due to (**b**) non-optimal pollination and cost of pollinator management. The EIL and pollinator EIL are given by the projection of the intersection of the loss function and the cost function

$$v(\phi) = \min(\beta_0 + \beta_1\phi, 15),$$

where β_0 and β_1 are parameters estimated from the data. Ordinary least squares estimation on the data for strawberry presented in the article gives the estimates $\beta_0 = 9.6827$ and $\beta_1 = 0.0053$.

We set up a cost-benefit relation as

$$C_{po} = V - V\frac{v(\phi)}{15}.$$

As in the pest example, the right-hand side corresponds to the loss function, $L_{po}(\phi)$. The value V is set to 10,000, taken from Mailloux and Bostanian (1988), and the cost of pollinator management C_{po} is set to 1000. Given these parameters, a pollinator EIL is calculated by solving the cost-benefit relation for ϕ, resulting in a value of 718.35.

Since the loss function is linear, it is possible to translate the empirical loss function given in terms of predicted weight to the loss function presented in the derivation of the pollinator EIL. If the pollinator dependence is given by $\bar{D} = 1 - \beta_0/15$ and the optimal pollinator density by $\phi_{opt} = 15 \cdot \bar{D}/\beta_1$, the loss function can be re-written

$$L_{po}(\phi) = V - V\frac{\beta_0 + \beta_1\phi}{15} = \bar{D}V - \bar{D}V\frac{\phi}{\phi_{opt}},$$

i.e. the same formulation of the loss function we have seen previously.

The pollinator EIL is illustrated in Fig. 2.6. The loss function decreases for ϕ less than about 1000. For greater ϕ the optimal mean berry weight of 15 is reached and the estimated loss is zero. The cost is a constant at the value of C_{po}. The pollinator EIL is given as the projection of the intersection between the cost and loss functions.

We can now set up a joint EIL by defining the joint loss function

$$L(\theta, \phi) = V\left(1 - \left(1 - \frac{L_{pe}(\theta)}{V}\right)\left(1 - \frac{L_{po}(\phi)}{V}\right)\right),$$

where V is 10,000, $L_{pe}(\theta)$ is the right-hand side of the cost-benefit relation for pest, and $L_{po}(\phi)$ is the right-hand side of the cost-benefit relation for pollinators. Based on this joint loss function, the reduced loss and cost of each of the four strategies (no action, pest control, pollinator management, and both) are calculated and compared. We assume pest control and pollinator management are completely effective in the sense that pest control brings the pest density to zero and that pollinator management brings the pollinator density above the optimal level (which is about 1000), and that there is no effect of pest control on the pollinator density or of pollinator management on the pest density.

The results of applying the joint EIL to the empirical data is given in Fig. 2.7 (top). The black lines signify the EIL and pollinator EIL for the univariate cases.

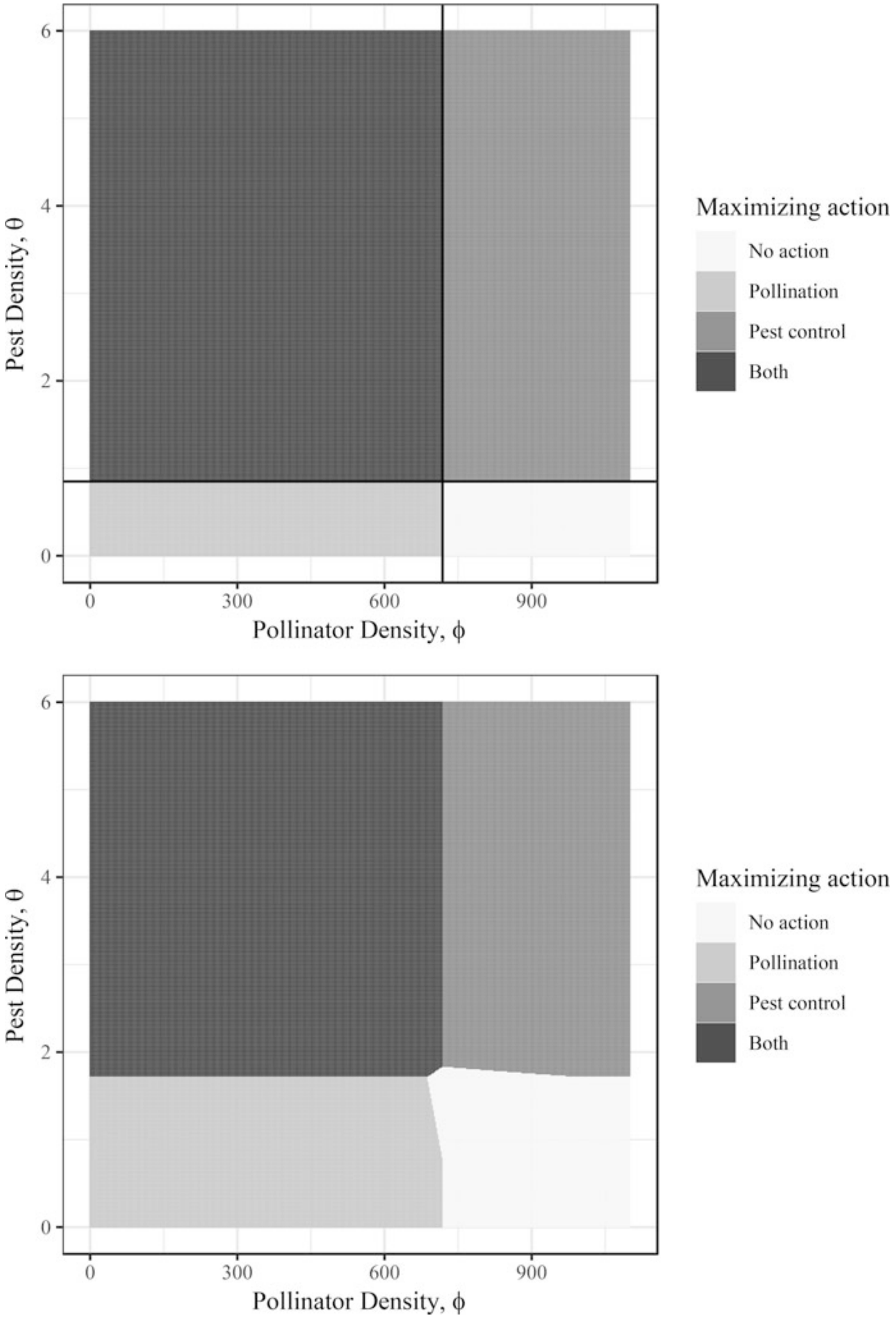


Fig. 2.7 Illustration of the most economically beneficial action for combinations of pest and pollinator densities for the strawberry empirical data. Cost of pest control is set to (top) 100 and (bottom) 1000. In the case of a low cost of pest control, the areas connected to each strategy are rectangular, indicating that the most beneficial decision according to the joint EIL is similar to applying the EIL and the pollinator EIL separately. In the case of a higher cost of control, the areas become more irregular and the suggested strategy according to the joint EIL may differ from the EIL or pollinator EIL

The four sections for the possible actions are almost completely rectangular, which indicates very small differences between applying the joint EIL and applying the EIL and the pollinator EIL independently. This is due to particular features in the loss functions, in particular the constant sections in the loss functions (see Fig. 2.6) and the low cost of pest control in relation to crop value. Figure 2.7 (bottom) shows the results of applying the joint EIL for the case where the cost of pest control is set to 1000. The increased cost of pest control leads to a higher EIL, which is reflected in the decreased area connected to pest control. Increasing the cost also reveals that the regions are not complectely rectangular, meaning that there is some difference between applying the joint EIL and applying the univariate EILs seperately.

2.4 Discussion

2.4.1 Flexibility of Use

The joint EIL can facilitate IPPM decision making in recommending which action (i.e. management of pest, pollinators, both, or neither) represents the most economically optimal solution, under the management practices considered. Key to this functionality is its broad flexibility, and in particular the possibility to parameterize the joint EIL in a variety of different ways, depending on the scenario at hand, and the information available. Furthermore, this decision metric can be adapted for use for any pest type and control strategy, regardless of whether interactions (interferences or synergisms) with or from pollinators are apparent.

Flexibility in how to treat the cost of management in calculating jEILs offers one particular advantage. Cost of management may be either fixed (density independent) or linearly related to the scale of intervention (density dependant). Fixed costs can hence better represent certain management practices (e.g. use of high-efficiency chemical pesticides), where a fixed level of intervention is usually effective at alleviating a problem (e.g. high or low pest densities) regardless of scale (Pimentel and Levitan 1986). In contrast, linear costs often typify other pest and pollinator management practices, such as the use of commercial biocontrol agents and pollinators, or the creation of flower strips (Garibaldi et al. 2016).

A further advantage to the joint EIL is its ability to accommodate a variety of curve types describing pest and pollinator yield-density relationships. Core dependency on this parameter also represents a potential challenge, however, in that such empirical relationships (at least for pollinators) are not widely known outside of the major crops. Lack of such information does not necessarily preclude the use of joint EILs however, where it is possible to reasonably approximate missing parameters. For instance, where the pollinator loss function is unknown for a given crop, evidence supports that a linear (as opposed to non-linear) relationship could in theory serve as a reasonable approximation (Morris et al. 2010). Likewise, the recommended stocking density of managed pollinators, available for many crops (Breeze et al. 2014; Delaplane et al. 2000), could in many cases serve to reasonably estimate optimal pollinator density. In addition, the point at which the yield-density curve intercepts the Loss axis (as in Figs. 2.2 and 2.3) describes the pollinator-dependency of the crop. Qualitative approximations of this parameter appear possible for a majority of pollinator-dependant crops (see Klein et al. 2007).

2.4.2 Dynamics

Similar to the traditional EIL, the joint EIL is best viewed as a dynamic metric; in that as input parameters change across the phenological development of a crop, so too will the calculated joint EIL. For instance, pest control measures are likely to be prioritised by the joint EIL in the early crop development stages, due to the detrimental impact of pest injury at this time, and the lower potential for pest control measures to impact pollinators outside of the flowering period. In such scenarios, the joint EIL will closely (if not precisely) equate to the EIL, and no disadvantage is incurred from its use when only pests are of concern. In contrast, as a crop approaches flowering, the joint EIL can increasingly weigh the potential need for pollinator management, as well as any interactive effects on – or from – pest control.

In order to precisely ascertain the benefit of pollinator management, careful consideration must also be afforded to setting the optimal pollinator level (i.e. the target density towards which management strives). Possibilities include whether to fix this level as a season-wide average, based on the general pollination needs of the crop, or if this level should more dynamically track the crop's pollination needs across the flowering season – e.g. as governed by changes in flower density.

2.4.3 Action Thresholds

In order to make the joint EIL applicable, Action Thresholds (ATs) are required for both pests (AT_{pe}) and pollinators (AT_{po}). Such ATs function much the same as the traditional AT, in that they define a density that should trigger an intervention in order to prevent the joint EIL being reached. Setting ATs for the joint EIL can be achieved by fixing AT_{pe} and AT_{po} within the *no action* margin of the jEIL analytical plot (e.g. Fig. 2.5). Traditionally, the AT is set relatively far away (20–80%) from the EIL – especially if there is a risk that a pest may vector even more harmful phytopathogens. The distance of the pest and pollinator AT from the three possible actions (pest control, pollinator management, or both) may hence differ; depending, for instance, on the lag time between a specific intervention and materialization of its effect.

2.4.4 Application in Practice

In practice, the joint EIL can be used by agricultural advisors, farmers, and other stakeholders as a tool to support decision making, or simply to weigh up or explore (using real, simulated, or projected data) the implications of specific scenarios or management strategies. However, crop-specific knowledge is no doubt fundamental to the parameterization of the joint EIL; a task which may be best fulfilled by agricultural advisors or other specialists. Similar to the EIL, calculation of the joint EIL relies heavily upon several economic parameters (e.g. the current market value of yield, input costs, etc.). Economic information of this sort is likely to fluctuate regularly, and hence must be kept up-to-date in order to best inform decision making.

Once basic parameterization of jEIL is complete for a crop, monitoring and input of pest and pollinator field densities are the only further requirement for its use. This step can be managed either by advisors or farmers directly. Regular monitoring of pest densities is an established routine in IPM (Pedigo et al. 1986). However, for pollinators, field monitoring (e.g. through focal observations or active or passive sampling) is relatively less common for the purpose of informing decision making. Rather, management still typically hinges on the calculation of managed bee stocking rates (Delaplane et al. 2000). Use of the joint EIL, as part of an adaptive management approach, in this sense calls for more active practices of pollinator monitoring.

The arithmetic complexity of the joint EIL without doubt poses a challenge to its practical use. To address this issue, a simple graphical interface for the calculation of jEILs has been developed (presently in beta version). This interface will provide users with both graphical-based input options in addition to quantitative and graphical outputs (such as depicted in Figs. 2.5 and 2.7). We hope that this interface, together with future conceptual development of the joint EIL, can stimulate uptake of this decision metric among the scientific and agricultural community.

Used Symbols

Symbol	
θ	The pest density
$L_{pe}(\theta)$	Economic loss as a function of pest density
V	Value of one crop unit
D'	Economic damage associated with on unit pest density
C_{pe}	Base cost of pest control
θ'	Pest density after pest control
K_{pe}	Success rate of pest control
$\bar{D}$	The proportion of the crop dependent on pollinators (*pollinator dependence*)

(continued)

ϕ	The pollinator density
ϕ_{opt}	The optimal pollinator density
$L_{po}(\phi)$	Economic loss as a function of pollinator density
C_{po}	Base cost of pollinator management
ϕ'	Pollinator density after pollinator management
K_{po}	Success rate of pollinator management
M_{pe}	Marginal cost of pest control
M_{po}	Marginal cost of pollinator management
$\upsilon(\phi)$	Yield as a function of pollinator density
$L^s_{po}(\phi)$	Economic loss as a sigmoid function of pollinator density
$L(\theta, \phi)$	Economic loss as a joint function of pest and pollinator densities
J_{pe}	The effect of pest control on pollinator density
J_{po}	The effect of pollinator management on pest density
S_0	The *no action* strategy
S_{pe}	The *pest control* strategy
S_{po}	The *pollinator management* strategy
$S_{pe,po}$	The *pest control and pollinator management* strategy
$C(\theta, \phi, S)$	Cost of control as a function of pest and pollinator densities, and strategy
AT_{pe}	Action threshold for pests
AT_{po}	Action threshold for pollinators

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Chapter 3
Night Warming Affecting Interspecific Interactions: Implications for Biological Control

Gang Ma, Cécile Le Lann, Joan van Baaren, and Chun-Sen Ma

3.1 Introduction

Previous research concerning temperature increase on species and interspecific interactions mainly focus on changes in temperature means (Traill et al. 2010; Smith 2011; Lloret et al. 2012; Flores-Mejia et al. 2017), with a rise in both daytime and nighttime temperatures or a rise just in daytime temperatures. However, it has been shown that the rise of global mean surface air temperatures has resulted from daily minimum temperatures increasing at a faster rate than daily maximum temperatures (Karl et al. 1993; Easterling et al. 1997; Caesar et al. 2006; Donat and Alexander 2012; IPCC 2014). Importantly, daytime and nighttime warming may lead to different thermal effects on organisms at almost all levels (Alward et al. 1999; Peng et al. 2013; Zhao et al. 2014; Speights et al. 2017; Barton and Schmitz 2018; Bai et al. 2019), suggesting that night warming may have differential effects on species and interspecific interactions from that of daytime warming. So far, however, only relatively few researches have investigated the effects of nighttime warming on insects and predator-prey interactions (Zhao et al. 2014; Miller et al. 2017; Barton and Schmitz 2018; Bai et al. 2019). In this chapter, we aim at giving existing examples of night warming on plants, insects and interspecific interactions to highlight the different effects of nighttime and daytime warming on species and ecosystems. This evidence emphasizes that ignoring the differential effects of asymmetrical warming may result in misleading or even inaccurate conclusions

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G. Ma · C.-S. Ma (✉)
State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China
e-mail: machunsen@caas.cn

C. Le Lann · J. van Baaren
UMR 6553 ECOBIO, Centre National de la Recherche Scientifique, Universite de Rennes I, Rennes, Cedex, France

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about the climate change on interspecific interactions and ecosystems, and thus have important implications for biological control and pest management in the context of ongoing climate warming.

3.2 Asymmetric Warming Between Daytime and Nighttime Temperatures

The global mean surface air temperature has risen substantially during the twentieth century (IPCC 2014). However, this increase has been found to be, at least partly, due to the differential changes in daily maximum and minimum temperatures (Karl et al. 1993; Easterling et al. 1997; Caesar et al. 2006; Donat and Alexander 2012). For example, by analyzing global monthly maximum and minimum temperatures and calculating anomalies from the mean of the base period of 1961–1985 in 5° by 5° latitude-longitude grid box, it was found that the global trend for the maximum temperature is +0.88 °C per decade, whereas the trend for the minimum temperature is +1.86 °C per decade (Easterling et al. 1997). By using a global dataset of daily gridded maximum and minimum temperatures, Donat and Alexander investigated changes in the probability density functions of both variables during 1951–1980 and 1981–2010. They found that the changes were greater for nighttime temperatures than for daytime temperatures: the global mean daily minimum temperature anomaly increased by 0.8 °C between the earlier and latter period, whereas the global mean daily maximum temperature anomaly increased by 0.6 °C (Donat and Alexander 2012).

The asymmetry increase between nighttime and daytime temperatures has resulted in a skewness of the probability density functions of both daily minimum and maximum temperatures towards the hotter part of the distribution, and has led to more daily temperature extreme events worldwide. For example, the distributions of both daily maximum and minimum temperatures have significantly shifted towards higher values in 1981–2010 compared to 1981–2010. These changes have the greatest impact on the extremes of the distribution of global daily temperatures and have led to more extreme daily temperatures since the middle of the twentieth century (Donat and Alexander 2012). Widespread significant changes in temperature extremes associated with warming, especially for those indices, are derived from daily minimum temperatures. Analysis of seasonal and annual indices of temperature extreme events for the period 1951–2003 showed a significant decrease in the annual occurrence of cold nights and a significant increase in the annual occurrence of warm nights for more than 70% of the global land area, and some regions have even experienced more than doubling of these indices (Alexander et al. 2006). Climate change also induces that the duration and severity of winter cold will decrease (Bradshaw and Holzapfel 2008). During the winter time, nights represent stressful moments for insects, and especially the frost nights. Yin and Sun (2018) showed that the number of non-freezing days (also meaning the number of nights with no frost event) predominantly display decreasing trends in the world, and especially in the Eurasian continent at middle to high latitudes.

3.3 Effects of Night Warming on Plants

3.3.1 Plant Phenology

The asymmetric warming of nighttime and daytime temperatures has been found to differently contribute to recent changes in plant phenology such as leaf onset and bud break (Hanes 2014; Piao et al. 2015; Fu et al. 2016; Rossi and Isabel 2017). The growing degree days, determined by the sum of daily average temperatures above a certain threshold value, is a common parameter widely used for the accumulation of heat needed to unfold leaves (Chuine 2000). Phenological models that use daily mean temperature often neglect the different responses of plants to daytime and nighttime asymmetric warming (Peng et al. 2013; Fu et al. 2014), and thus cannot accurately predict the recent advanced leaf onset (Piao et al. 2015). By using *in situ* observations of leaf unfolding dates in Europe and the United States, it was shown that the inter-annual anomalies of leaf unfolding dates during 1982–2011 are triggered by daytime, more than by nighttime temperatures (Piao et al. 2015). An increase of 1 °C in the daytime maximum temperature would advance leaf unfolding by 4.7 days in Europe, and by 4.3 days in the United States, more than the conventional temperature sensitivity estimated from daily average temperature. The triggering role of daytime maximum temperature, rather than the variables of nighttime minimum or daily average temperature, is also supported by analysis of the large-scale patterns of satellite-derived vegetation green-up in spring in the northern hemisphere (Piao et al. 2015). Although both daytime and nighttime warming significantly advanced leaf onset, the sensitivity to increased daytime and nighttime temperatures differed, so that the impact of daytime temperatures on leaf onset was approximately three times higher than that of nighttime temperatures (Fu et al. 2016). Similarly, the effects of an asymmetric warming on bud break in black spruce also showed that daytime warming is more effective than nighttime warming in advancing bud break (Rossi and Isabel 2017). Therefore, daytime and nighttime temperatures impact distinctly the heat requirement of growing degree days, and statistical and conceptual models of leaf onset should carefully distinguish which temperature should be used for modelling plant phenology (Piao et al. 2015). Current phenological models based on growing degree days would not accurately predict leaf onset in future climates because of the asymmetric warming of nighttime and daytime temperatures. These parameters should be incorporated into models of leaf unfolding to improve phenological predictions under climate change (Piao et al. 2015; Fu et al. 2016; Rossi and Isabel 2017).

3.3.2 Crop Yield and Vegetation Variables

The asymmetric warming of nighttime and daytime temperatures may have important effects on crop yield (Peng et al. 2004), and vegetation variables such as plant abundance, net primary production and vegetation greenness (Alward et al. 1999; Peng et al. 2013). On the one hand, when increased daily minimum temperature leads to longer growing seasons, net primary production and carbon sequestration may increase as a consequence. On the other hand, the opposite may also occur when increased daily minimum temperature leads to increased plant and microbial nocturnal respiration rates without a compensatory increase in photosynthesis (Alward et al. 1999). Analyzing relationships between rice yield and temperatures, using data from field experiments conducted at the International Rice Research Institute, revealed that the annual mean maximum and minimum temperatures have increased by 0.35 °C and 1.13 °C, respectively, for the period 1979–2003. Grain yield declined by 10% for each 1 °C increase in the growing-season minimum temperature in the dry season, whereas the effect of maximum temperature on crop yield was insignificant (Peng et al. 2004). Thus, increased nighttime temperature has led to decreased rice yields, and this pattern is also observed for other cultivated plants such as winter wheat and tomato (Jing et al. 2016). This effect of night warming on plant yield could be related to a decrease in biomass allocation to reproductive organs as most other plant morpho-physiological processes are hastened at high night temperatures, including leaf growth, photosynthesis and dark respiration (Jing et al. 2016). Furthermore, increased daily minimum temperature may also alter competitive interactions among C3 (cool-season) and C4 (warm-season) plants. For example, by using long-term data sets from the shortgrass steppe to identify correlations between daily minimum temperature and vegetation variables, Alward et al. (1999) demonstrated that the increased spring daily minimum temperature was correlated with the decreased net primary production by the dominant C4 grass, and with the increased abundance and production by exotic and native C3 forbs. An analysis of the interannual covariations of the satellite-derived normalized difference vegetation index (NDVI, an indicator of vegetation greenness) with daily maximum and minimum temperature over the Northern Hemisphere showed that the partial correlation between daily maximum temperature and NDVI is positive in most wet and cool ecosystems over boreal regions, but negative in dry temperate regions. In contrast, the partial correlation between daily minimum temperature and NDVI is negative in boreal regions, and exhibits a more complex pattern in dry temperate regions (Peng et al. 2013). Most of the current global carbon cycle models use daily mean temperature data as input, and neglect the asymmetrical response of terrestrial ecosystems to daytime versus night-time temperature anomalies. This may lead to divergent responses of natural ecosystems to climate warming.

3.4 Night Warming Impacts on Insects

3.4.1 Life History Traits and Behavior

Generally, insects often experience adverse thermal environments caused by daytime extreme high temperatures, but may recover or repair themselves during nighttime moderate temperatures (Roux et al. 2010; Zhao et al. 2014; Speights et al. 2017; Bai et al. 2019; Ma et al. 2018). However, the greater increase in nighttime temperature often leads to a warmer night on hot days especially in the summer (Zhao et al. 2014). On the one hand, high daytime temperatures usually have immediate and subsequent negative effects on insect life history traits (Ma et al. 2004a, b, 2015a, b; Roux et al. 2010; Gillespie et al. 2012; Sentis et al. 2013; Zhang et al. 2015a, b). On the other hand, warmer nighttime temperatures may further exacerbate the detrimental effects of hot days (Zhao et al. 2014; Speights et al. 2017; Bai et al. 2019). Although night warming on hot days is found to raise optimum temperature for development in the grain aphid, *Sitobion avenae*, it indeed reduces nymphal survival and depresses adult performance such as lifetime fecundity and longevity under daytime heat stress, and finally leads to a declined population growth (Zhao et al. 2014). Furthermore, thermal effects on development and survival differ between daytime and nighttime temperatures in a lady beetle species, *Propylea japonica*, and the extent of daytime heat stress and night-time recovery also affect development and heat tolerance of the lady beetle (Bai et al. 2019). This evidence suggests both independent and combined effects of daytime and nighttime temperatures on species thermal performances. Together, these findings may be helpful in predicting phenological changes in the aphids and the lady beetles as well as in predicting pest outbreaks and the resilience of natural biological control (Fig. 3.1).

The effect of night warming on insect performance is also found not to be simply temperature-dependent. There can be interactions between nighttime temperature and dietary allelochemicals (Yang and Stamp 1995), which suggests that it would be inaccurate to predict insect performance based on either allelochemical concentration of plants, or average air temperature without incorporating the interactions of allelochemicals and nighttime temperature. For example, when ignoring the temperature-rutin interaction for the entire larval stage of tobacco hornworm larvae, *Manduca sexta*, one would either underestimate or overestimate the developmental duration by several days (Yang and Stamp 1995), which would be costly when predicting their development for pest management. Furthermore, night warming may also affect insect behavior and activity patterns. For example, warmer nights may increase the take-off behavior of moths and lead to more dispersal activities (Battisti et al. 2006). If an insect species is active during the nocturnal phase, one could expect that night warming may affect important ecological interactions such as feeding, parasitism, and pollination, stronger than if the species is inactive at night (Miller et al. 2017; Speights et al. 2017; Barton and

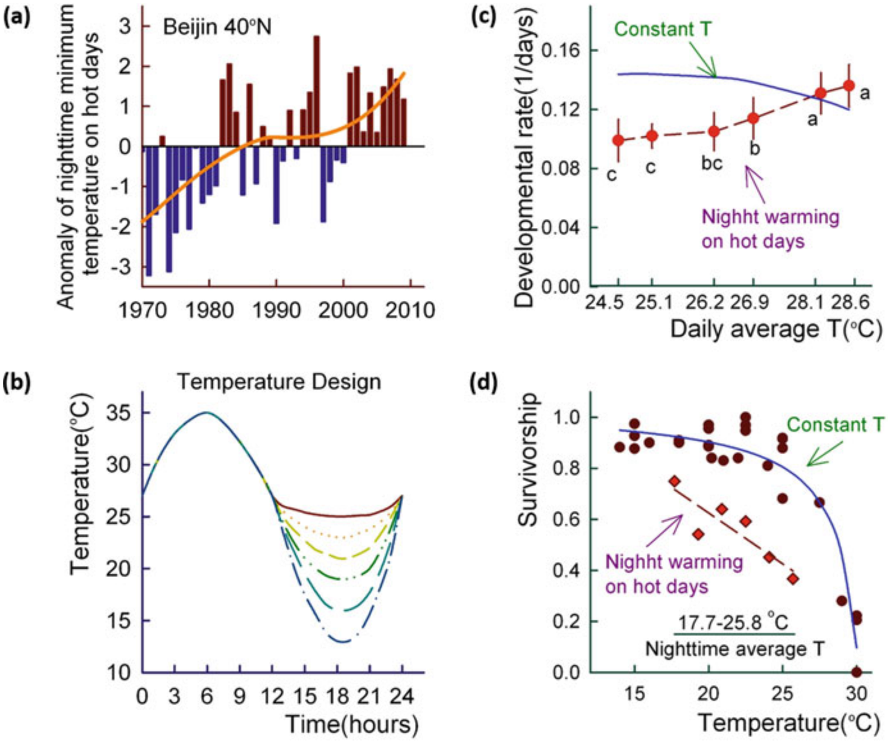

Fig. 3.1 Changes in nighttime temperature anomaly and the effects of nighttime warming on hot days on development and survival of the grain aphid *Sitobion avenae* (adapted from Zhao et al. 2014). (**a**) Anomaly (in °C) relative to 1971–2010 average value for the temperature variables of hot days (≥30 °C) during decimal wheat growth stages 30–90 in Beijing. (**b**) Temperature regimes designed for testing the effects of nighttime warming on hot days on life history traits of the grain aphid. (**c**) and (**d**) Effects of nighttime warming on hot days on the development and survival of the grain aphid

Schmitz 2018). However, night warming could also affect diurnal insects by altering their sleeping period (Tougeron and Abram 2017). For instance, sleep-deprived fruit flies due to high night temperatures present reduced courtship success and aggressive behavior during day time (Kayser et al. 2014, 2015). Furthermore, higher nighttime temperatures were shown to alter fitness components of a diurnal butterfly species resulting in a faster growth rate and a smaller pupal mass (Whitney-Johnson et al. 2005). Time-specific behavior and activity patterns of insects are likely to be affected by night warming, resulting in the alteration of both intraspecific and interspecific interactions (Speights et al. 2017).

3.4.2 Phenology and Microhabitat Use

Daytime and nighttime asymmetric warming may differently alter the temperature between soil and air microclimate and lead to differential phenological responses of insects with complex life history, e.g. using both above- and belowground habitats during their life cycle, to the asymmetrically diel warming. For example, the eggs of most grasshopper species live belowground and stay beneath the soil for a considerable time before hatching, while nymphal hoppers live aboveground and are exposed to air microclimate. Wu et al. (2012) found that although both daytime and nighttime warming can increase soil and air temperatures, nighttime warming appears to be more effective in increasing soil temperature than daytime warming whereas daytime warming is more effective in increasing air temperature than nighttime warming. As a result, nighttime warming is found to be more effective than daytime warming in advancing egg development. By contrast, daytime warming is found to be more effective than the nighttime warming in advancing nymph development (Wu et al. 2012). There are greater responses to warming in eggs than in other life stages. Therefore, the asymmetric warming would impose differential impacts on the temperature-dependent development of eggs and hoppers due to the different temperature changes in above- and belowground microclimates caused by the asymmetric warming between daytime and nighttime temperature change. These findings highlight the importance of considering the different effects of daytime and nighttime warming on the phenology of insects with complex life history, and have implications for predicting phonological responses of invertebrates to the diel asymmetric warming.

3.4.3 Distribution

Nighttime warming may lead to shifts in insect geographical distribution via influencing the temperature-dependent critical thresholds for dispersal behavior and/or overwintering or growing-season survival. During the growing-season, since night warming is found to have negative effects on aphid survival, reproduction and population growth rates, the distribution of the grain aphid *Sitobion avenae* is expected to contract in low latitude regions, where the ongoing climate change is predicted to increase nighttime temperatures across consecutive hot days (Zhao et al. 2014). The winter pine processionary moth (*Thaumetopoea pityocampa*) is found to rarely take off when nighttime temperatures are below a threshold, and there is a significant positive correlation between the mean night temperature and the flight activity of females (Battisti et al. 2006). During 1975–2002, warmer winters have led to a substantial but gradual expansion of its range. For most of this period, the upslope expansion has most likely occurred as a continuous process characterized by small increments and occasional temporary reversals. However, in the summer of 2003, an unusual, prolonged heat wave caused by extreme climatic anomalies, led to nights above the threshold temperature being over five times more frequent than usual, and considerably warmer than in an average year. As the consequence, *T. pityocampa* underwent an extraordinary expansion to high elevation pine stands in the Italian Alps, and its altitudinal range limit increased by one third of the total altitudinal expansion over the previous three decades. For the southern pine beetle *Dendroctonus frontalis,* it was predicted that an increase of 3 °C would lead to an approximate 178 km northward expansion in the occurrence of outbreaks, compared with historical times (Ungerer et al. 1999). As expected, the beetle outbreaks were rare or nonexistent at the north of the historical range between 1960 and the 1990s, because the average minimum winter air temperatures have increased by just over 3 °C since 1960. However, recent episodes of *D. frontalis* outbreaks in northern forests may have been allowed by a warming trend of 3.3 °C in the minimum winter air temperatures in the southeastern United States between 1960 and 2004 (Tran et al. 2007).

At the landscape scale, microclimate was shown to be different between habitats, and to influence the distribution of the species across habitats (Tougeron et al. 2016; Alford et al. 2018). During the winter, in mild winter areas where frost becomes more and more infrequent, the frost events occurring mostly during the night represent a major stress for ectotherms. In the western part of France, the landscape components include intensive simple landscapes (homogenous landscapes characterized by low diversity, large field sizes, and few semi-natural elements), and more complex traditional landscapes called “bocage” (fine grain heterogeneous landscapes characterized by a high diversity, high hedgerow density, small sizes, and the presence of grassland areas). Microclimate differs in these two types of landscapes. The temperature amplitude in complex landscapes is lower than in simple ones, whereas the complex landscapes had colder mean temperatures, with more relative humidity, and a lower wind speed. In the study of Alford et al. (2018) the frost events during the winter nights were four times more abundant in simple landscapes than in complex ones. Complex landscapes with a high hedgerow density provide a windbreak function, causing a reduction in local wind speed and retention of denser, cooler air, acting to lower the mean local temperatures, and raising the level of humidity. At the opposite, in simple landscapes, the daytime short radiations raise the mean daily local temperatures, but frost is more frequent during the nights. These differences between both types of landscapes were less significant during the spring due to the increase of vegetation growth across all landscape types, resulting in a buffering of temperatures. This disparity in winter microclimates between landscapes also influences the thermotolerance of insects, but differently between species and guilds. For the aphid species, Alford et al. (2018) observed that individuals of complex landscapes were more cold tolerant than those of simple landscapes. As aphids remain attached to their host plant most of their life, they may be more sensitive to mean temperatures than other insects. Indeed, for several other species of arthropods, as for the aphid parasitoids, it was shown that the extremes are considered to be of greater importance in determining thermal resistance (Tougeron et al. 2016). In some species, no effect of landscape was observed. For example, in the study of Tougeron et al. (2016), it was shown that the aphid predators (carabids) did not display different thermotolerance between landscapes, which could be explained by their ability to move between different microhabitats to escape large temperature variations (Damien 2018). The fact that the different species of a trophic web (aphids and different guilds of natural enemies) did not respond similarly to microhabitat and microclimate, indicates that climate warming could disrupt their relationships as well as the biocontrol ecosystem service provided by natural enemies. This is especially evident during the winter time, showing the great importance of cold stress on insect interactions.

3.5 Night Warming and Interspecific Interactions

3.5.1 Plant-Insect Interactions

Night warming may alter both bottom-up and top-down effects that influence plant-insect interactions. On the one hand, night warming may directly increase the temperature-dependent plant consumption by nocturnal insect herbivores, and thus result in stronger top-down effects. On the other hand, the nighttime versus daytime asymmetric warming is expected to affect carbon assimilation and consumption in plants. Photosynthesis mostly occurs during the daytime, and is more sensitive to daytime temperature, whereas plant respiration occurs throughout the day and is therefore influenced by both daytime and nighttime temperature (Peng et al. 2013). Although few studies have focused on the effects of nighttime warming on plant-insect interactions, any changes in plant responses to the asymmetric warming would likely lead to cascading indirect bottom-up effects on herbivores, pollinators, etc. (Speights et al. 2017). For example, flowers of some plants open during the night to maximize visits by their most efficient nocturnal pollinator (Young 2002). However, an asymmetric warming may lead to a decreased nectar volume (Mu et al. 2015), implying that plant responses to nighttime warming could alter plant-pollinator interactions. Additionally, plants and phytophagous insects feeding on them present different tolerance and resistance responses to the rise of temperatures (van Baaren et al. 2010), and temperatures on leaf surfaces are often 10–20 °C higher than those of ambient air (Calatayud et al. 2017). As nutrient allocation in plants vary during the day and night, and is under strong temperature influence (Jing et al. 2016), night warming may thus drastically impact the physiology of phytophagous insects and especially those feeding for long time periods, such as aphids (e.g. more than 14 h per day) (Kuhlman et al. 2013). Furthermore, among sap sucking insects, aphids were found to increase leaf surface temperatures by 2 °C (Cahon et al. 2018). This increase of temperature might be due to stomatal closure on the leaves of the plant resulting in a decreased transpiration rate in response to the presence or feeding activity of the phloem feeding insects (Cahon et al. 2018). Therefore, increased temperatures on insect feeding activity may also have negative feedback effects on plant physiology and on photosynthesis at the scale of the whole organism, but also in specific organs of plants such as leaves. This remains to be further investigated to better predict the effects of night warming on plant-phytophagous insect interactions.

3.5.2 Predator-Prey Interactions

Generally, insect predators often increase their foraging activity at higher temperatures (Mack and Smilowitz 1982; Xia et al. 1999; Rall et al. 2010; Rauf et al. 2013; Pepi et al. 2018). Some insect predator species show a nocturnal activity and night warming may thus lead to an increased prey consumption and in turn cause a declining population of insect herbivores. Daytime and nighttime asymmetric warming may also affect predator-prey interactions via insect behavioral thermoregulation caused by the differential thermal sensitivities between the prey and their predators. For example, daytime warming may force heat sensitive predators to seek thermal refuges in lower part of the plant canopy and away from their heat tolerant prey, which may result in an increased prey density. However, nighttime warming may not affect predator thermoregulatory behavior and instead leads to an increased activity in a spider thus causing the prey population to decrease (Barton and Schmitz 2018).

Furthermore, other components of climate change such as light pollution (artificial light at night) may interact with nighttime warming causing changes in predator-prey interactions. For example, two species of the most common ladybeetles, *Coccinella septempunctata* and *Coleomegilla maculate*, can exert strong top-down control and prevent the outbreaks of the pea aphid *Acyrthosiphon pisum* (Cardinale et al. 2003; Barton and Ives 2014). Although both species increase their prey consumption at higher temperatures and are found to be active at night, the former species uses visual cues to hunt, and thus is expected to increase their predation rates in the case of both night warming and light pollution. The latter species does not use visual cues to hunt, and thus is predicted to respond only to night warming. By investigating the combined effects of night warming and light pollution on pea aphids and the two predatory ladybeetle species, the predator using the visual cues was proven to reduce more efficiently aphid abundances under the combination of night warming and light pollution (Miller et al. 2017). These findings highlight the importance of multiple abiotic factors on species and the consequent predator-prey interactions.

3.5.3 Plant-Herbivore-Predator/Parasitoid Trophic Cascade

In natural communities and ecosystems, nighttime warming may lead to changes in the plant-herbivore-predator tri-trophic cascade by altering predator-prey interactions and indirectly affecting plant biomass and diversity. By comparing the effects of daytime and nighttime warming on a community comprising herbaceous plants, grasshopper herbivores and predatory spiders, Barton and Schmitz (2018) report different effects of daytime and nighttime warming on top-down control of plant diversity. They find that daytime warming may cause spiders to seek thermal refuges in lower parts of plants and thus away from grasshoppers, allowing grasshoppers to spend more time feeding on a competitively dominant plant species. By contrast, nighttime warming may have increased spider activity and caused grasshoppers to reduce feeding. As a consequence, daytime warming resulted in a suppression of the competitive dominant plant and in an increase of the diversity and evenness of the plant community, whereas nighttime warming had opposite effects (Barton and Schmitz 2018). In temperate climate areas (such as the Western part of France), warmer winters have modified the structure of aphid, parasitoid and predator guilds in cereal fields (Andrade et al. 2016; Tougeron et al. 2018; Damien et al. 2017; Damien 2018). The stressful conditions of the winter season for insects are cold temperatures, and especially frost events, which in the western part of France occur especially during the nights. The number of nights with frost has decreased during the last 30 years (Andrade et al. 2016). The consequence for the aphid guild is an increase of the number of species reproducing parthenogenetically during the wintertime: in the 1980s, *Sitobion avenae* and *Rhopalosiphum padi* were the only observed species, and since 2012, the cereal aphid *Metopolophium dirhodum* also reproduces during the winter. The general level of resources increases for the predators and parasitoids. The parasitoids *Aphidius avenae* and *A. ervi,* as well as hyperparasitoids, are now present in winter whereas two decades ago they were found only during the summer-spring period (Andrade et al. 2016; Tougeron et al. 2018). Damien (2018) showed that three groups of ground predator arthropods: spiders, carabids, and rove beetles are active during winter. Using directional traps in cereal fields and in semi-natural habitats like grassy margins closed to the fields, they showed activity flows of adults of these three groups both within and among the different habitats. Most of the species of these predatory arthropods are assumed to diapause during the winter. Therefore, it is likely – as shown for the aphids and parasitoids in the same areas – that the decreased numbers of frost events especially during the nights are responsible for the high degree of observed activity. These findings highlight the differential effects of daytime and nighttime warming on trophic cascades.

3.6 Implications for Biological Control

Climate warming is one of the most important components of global change affecting species, communities and ecosystems. It is predicted that climate warming would lead to an increase in crop losses due to insect pests (Deutsch et al. 2018), which highlights the significance of pest management as well as biological control in global food security under climate change. However, linking climate warming to the dynamics of interspecific interactions within natural communities and agroecosystems is a major challenge of the twenty-first century. Previous predictive models concerning the effects of climate warming on interspecific interactions are mainly based on changes in temperature means. However, daytime and nighttime warming have differential influences on species and interspecific interactions, emphasizing an urgent need for taking the diel asymmetric warming into account in accurately predicting the ecological consequences of natural climate change on population dynamics of insect pests and biological agents, as well as their interspecific interactions. Winter warming allows the populations of natural enemies to develop early in the season, with major effects on phytophagous insects (Damien et al. 2017), which could favor the biological control ecosystem service during the winter, but more importantly also in the following spring, in areas with mild winters.

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Part II
Biodiversity Enhancement for Improving Biological Control

Chapter 4
Perennial Flowering Strips for Conservation Biological Control of Insect Pests: From Picking and Mixing Flowers to Tailored Functional Diversity

Séverin Hatt, Frédéric Francis, Qingxuan Xu, Su Wang, and Naoya Osawa

4.1 Introduction

Increasing plant biodiversity in agroecosystems can enhance the delivery of ecosystem services, among them the natural regulation of insect pests, in agricultural systems using few chemical inputs (Hatt et al. 2018). In a meta-analysis, Letourneau et al. (2011) reported that increasing crop and non-crop plant diversity in agroecosystems participates in suppressing insect pests, favors their natural enemies, and reduces damage on crops. Specifically at the field scale, increasing plant diversity tends to enhance abundance of generalist predators, while it does not affect abundance of specialist pests (Dassou and Tixier 2016). At this local scale, plant diversity is managed by farmers, who can simultaneously cultivate multiple crop varieties or species to fight insect pests (Tooker and Frank 2012; Lopes et al. 2016). At field margins, semi-natural habitats can also be implemented and managed by farmers to conserve populations of predators and parasitoids that would control herbivores of adjacent crops (Holland et al. 2016).

S. Hatt (✉) · N. Osawa
Laboratory of Forest Ecology, Faculty of Agriculture, Kyoto University, Kyoto, Japan
e-mail: severin.hatt.77e@st.kyoto-u.ac.jp

F. Francis
Functional and Evolutionary Entomology, Gembloux Agro-Bio Tech, University of Liege, Liege, Gembloux, Belgium

Q. Xu · S. Wang
Laboratory of Applied Entomology Research, Institute of Plant and Environment Protection, Beijing Academy of Agricultural and Forestry Sciences, Beijing, China

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Wildflower strips (WFS) are one of these semi-natural habitats. They can be made of spontaneous vegetation or sown, composed by annual and/or perennial herbaceous flowering plants and may include a proportion of grass species (Haaland et al. 2011; Uyttenbroeck et al. 2016). In agricultural systems constrained by yearly crop rotations, sowing annual WFS may present the advantage to adapt the composition, size, and location of this non-crop feature to the changing crops. With respect to perennial WFS, they represent important overwintering sites for both flower-visiting and ground-dwelling natural enemies (Pfiffner and Luka 2000; Frank and Reichhart 2004; Sarthou et al. 2014; but see Sutter et al. 2018) which, by their proximity to crops, would be able to control the first colonizing pests. However, the challenge remains in composing perennial WFS that would attract and support the diversity of natural enemies preying and parasitizing the variety of pests that damage the adjacent successive rotating crops over the years (Fig. 4.1).

In this context, the present review aims at discussing ways to compose such perennial wildflower mixtures in the light of the most recent findings. A focus is made on how to attract and support flower-visiting natural enemies. First, it is recalled why a high abundance and diversity of natural enemies are required to enhance biological control of insect pests, justifying that they are the indexes often used to evaluate whether plant flowers, the functional traits, and the mixtures are valuable for conservation biological control. Second, the approach consisting of picking and mixing plant flowers known to attract and support populations of natural enemies is discussed. It has been the main approach over recent years to compose WFS and its efficiency in terms of natural enemy abundance and diversity enhancement, pest and damage suppression, and yield, is examined in the light of recent field-based studies. Third, in the context of an increasing interest for trait-based ecology in agricultural research (Martin and Isaac 2018), the approach comprising picking and mixing flower functional traits known to attract and support populations of natural enemies is considered. In particular, the hypothesis that a high functional diversity at the mixture level would enhance natural enemy abundance and diversity is addressed.

Fig. 4.1 Perennial wildflower strips sown in a field of the AgricultureIsLife experimental farm of Gembloux Agro-Bio Tech – University of Liege (Gembloux, Belgium). (**a**) Oilseed rape (*Brassica napus* L.) was cultivated in 2014, and (**b**) wheat (*Triticum aestivum* L.) in 2015. (Photo: S. Hatt)

4.2 Abundance and Diversity of Natural Enemies for Biological Control

4.2.1 Abundance

The abundance of natural enemies in semi-natural habitats adjacent to crops is often used as an indirect proxy to predict biological control of pests (Holland et al. 2016). In addition, measuring pest abundance in crops and natural enemy impacts (i.e., direct predation and parasitism) provides a realistic indication of pest control (Veres et al. 2013; Macfadyen et al. 2015a; Rusch et al. 2016). In many cases, the introduction of a semi-natural habitat such as WFS is considered positive in terms of biological control when it leads to a simultaneous increase of natural enemy abundance and decrease of pest density in adjacent crop fields (Tschumi et al. 2016b; Hatt et al. 2017; Toivonen et al. 2018). Although a high abundance of natural enemies in semi-natural habitats may not guarantee effective biological control in the adjacent crops (Cox et al. 2014), it is a prerequisite. Conservation biological control aims at developing high-quality habitats for natural enemies and thereafter their abundance is an indicator of that quality. For example in WFS, a high abundance of natural enemies that feed on flower nectar and pollen would notably indicate that the flower mixture provides a sufficient amount and a high quality of accessible food resource (Van Rijn and Wäckers 2016; Mansion-Vaquié et al. 2017). Several factors would then affect the ability of natural enemies to control pests: the spillover of natural enemies between semi-natural habitats and adjacent crops (Macfadyen et al. 2015b), the earlier – or at least simultaneous – occurrence of natural enemies as compared with the occurrence of pests (Alhmedi et al. 2009), the ratio pest/natural enemy (Thies et al. 2005), as well as the diversity of the natural enemy community.

4.2.2 Diversity

A high natural enemy richness (Letourneau et al. 2009) and evenness (Crowder et al. 2010) favor pest suppression in agroecosystems. Several processes, which have been repeatedly discussed (Tscharntke et al. 2007; Straub et al. 2008; Crowder and Jabbour 2014; Gurr et al. 2017; Jonsson et al. 2017), explain this trend. First, the sampling effect hypothesis predicts that a high natural enemy diversity increases the probability to have key natural enemy individuals that would greatly prey on or parasitize pests (Rouabah et al. 2014). Second, the species complementarity hypothesis forecasts that a high natural enemy diversity enhances the chance of a combined action of different natural enemies in the reduction of pest abundance (Dainese et al. 2017). Nevertheless, the additive and synergistic action of different species of natural enemies on pest regulation would still depend on their level of competition for the preys or hosts (i.e., the resource partitioning effect) as well as the ability of one natural enemy to favor the predation or parasitism of another one (i.e., the facilitation effect) (Roubinet et al. 2015). Moreover, when natural enemy diversity increases, competition and intra-guild predation may occur and weaken pest control (Finke and Denno 2004). In the end, the insurance hypothesis assumes that when the natural enemy diversity is high, a certain functional redundancy would balance the effects of competition and favor a stable pest control (Peralta et al. 2014).

Along with abundance, the diversity of natural enemies in semi-natural habitats and in adjacent crops is used as a predictor of biological control (Holland et al. 2016). The composition of these habitats often affects the richness (Sarthou et al. 2014) and functional diversity (Mansion-Vaquié et al. 2017) of natural enemy communities. Hence, the challenge remains in composing perennial habitats that would support complementary and functionally redundant species of natural enemies to control the diversity of pest species occurring on the various adjacent rotating crops.

4.3 Picking and Mixing of Suitable Flower Species

4.3.1 Screening of Flower Species That Attract and Benefit Natural Enemies

The pick and mix approach consists of identifying the flower species that attract and benefit natural enemies in order to constitute the mixtures to sow in fields towards biological control of pests (Wäckers and Van Rijn 2012) (Fig. 4.2). Attractive flower species are identified though laboratory-based experiments, by focusing on the attractiveness of the flower volatiles only (Adedipe and Park 2010; Nilsson et al. 2011; Belz et al. 2013), or by using the entire plant (Batista et al. 2017). In fields, attractive flower species are identified by trapping natural enemies in sown monospecific plots (Fiedler and Landis 2007a; Carrié et al. 2012) or nearby potted flowers (Sivinski et al. 2011). Flower visitation monitoring moreover allows assessment of the effective interaction between flowers and natural enemies (Colley and Luna 2000; Martínez-Uña et al. 2013). Additionally, consumption of nectar and pollen by natural enemies is established in the laboratory by weighing insects before and after flower exposure (Wäckers 2004; Nilsson et al. 2011), while biochemical tests performed on the gut content of dissected insects allow evaluation of the level of assimilated nutrients (Pinheiro et al. 2013). Insect frass can also be analysed to screen insect diets (Davidson and Evans 2010), and in fields, flowers can be marked with isotopes that are found back in trapped insects (Pollier et al. 2016) and consumed pollen can be identified with DNA metabarcoding (Lucas et al. 2018).

Feeding on nectar and pollen of flowering plants can benefit natural enemies by increasing their longevity and improving their fecundity (Lundgren 2009; Lu et al. 2014). The studies evaluating the increased longevity and improved fecundity of natural enemies feeding on flower resources have been generally conducted in

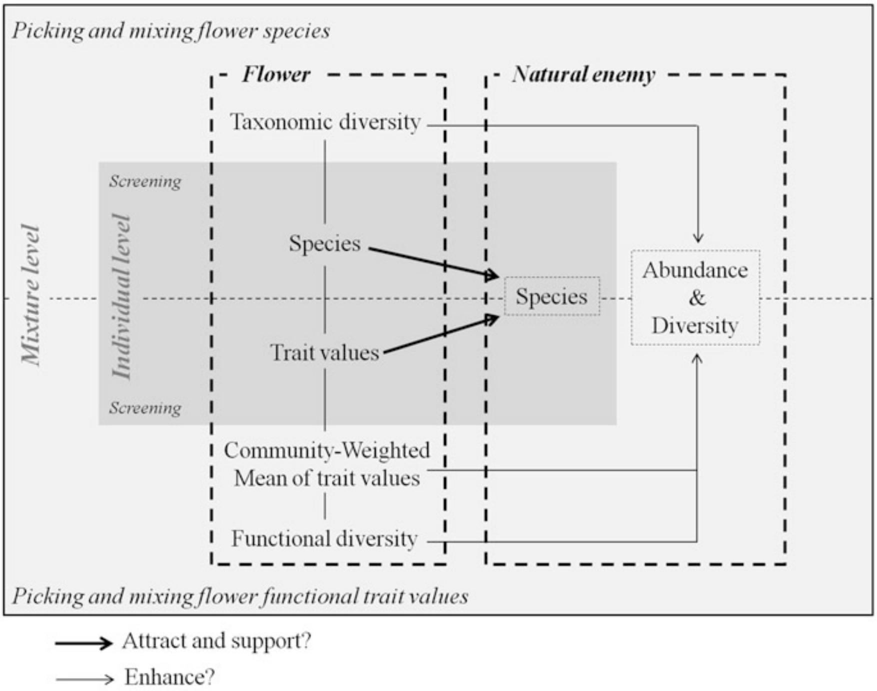

Fig. 4.2 Ways (i.e., picking and mixing flower species or flower functional trait values) to compose perennial wildflower strips to enhance pest natural enemy abundance and diversity for conservation biological control

the laboratory. Longevity is assessed by measuring the number of days a natural enemy species survives when feeding on a certain flower species (Winkler et al. 2009; Walton and Isaacs 2011; Furtado et al. 2016). Furthermore, fecundity is monitored by evaluating female egg load (i.e., the number of mature oocytes found in adult females) (Arnó et al. 2018) or by counting the number of oviposited eggs for predators (Resende et al. 2017) and the number of parasitized eggs and/or adults for parasitoids (Foti et al. 2017), and by assessing the proportion of females laying eggs (Laubertie et al. 2012).

4.3.2 *Mixing of Flower Species to Enhance Biological Control*

Perennial flower plants known to attract and benefit natural enemies can be sown in fields as monoculture strips (Balzan 2017) or mixtures (Hatt et al. 2017). The latter can be composed of purely perennial flowering plants (Blaauw and Isaacs 2015; Hatt et al. 2017) (Fig. 4.1) or a mixture of perennial and annual ones (Pfiffner et al.

2009; Tschumi et al. 2016a). The results of studies screening individual plants for natural enemy attraction and support (described above) are used to compose species-rich WFS sown in fields (Blaauw and Isaacs 2015; Toivonen et al. 2018) (Fig. 4.2). Some studies also use the flowers and mixtures that are already commonly sown for biodiversity conservation through agri-environmental schemes in Europe (Haenke et al. 2009; Tschumi et al. 2016a).

In crops adjacent to perennial WFS compared to crops without flowering borders, predatory hoverflies (Diptera: Syrphidae) are often found at a higher abundance, contributing to an increased control of pests, while inconsistent results were found across years and studies for other predators (e.g., ladybeetles (Coleoptera: Coccinellidae), lacewings (Neuroptera: Chrysopidae)), and parasitoids (Blaauw and Isaacs 2015; Hatt et al. 2017). In addition to monitoring insect abundance, sentinel cards are used to evaluate the effective predation and parasitism in crops. With such devices, a limited effect of WFS was found on the parasitism rate of cabbage moth (Lepidoptera: Noctuidae: *Mamestra brassicae*) eggs in cabbage fields (Pfiffner et al. 2009) and no difference between WFS and grass strips was reported on the predation of aphids (Hemiptera: Aphididae) in barley (*Hordeum vulgare* L.) crops (Toivonen et al. 2018). Moreover, in apple (*Malus domestica* Borkh.) orchards, eggs of *Ephestia kuehniella* (Lepidoptera: Pyralidae) were increasingly preyed on in trees adjacent to flowering strips compared to control trees (Campbell et al. 2017). Evaluating plant damage and crop yield also indicate the direct benefits of flowering margins. In wheat (*Triticum aestivum* L.) crops, Tschumi et al. (2016a) related the reduced abundance of eggs and larvae of cereal leaf beetles (Coleoptera: Chrysomelidae: *Oulema* sp.) at 5 m from WFS with lower damage on plants and higher yield, compared to control fields. Conversely in apple orchards, the presence of WFS did not affect either the abundance of aphids on trees nor fruit yield (Campbell et al. 2017).

From these past studies, it appears that inconsistent results were obtained regarding the effect of perennial WFS on natural enemy abundance and diversity in adjacent crops, pest control, crop damage, and yield. Several factors varied between these studies, notably the surrounding landscape that can affect processes at the field scale (Haenke et al. 2009; Woltz et al. 2012), the studied crop species, their pest species, but also the plants composing the WFS mixtures and the proportion of each flower species within mixtures. Better understanding regarding how pest natural enemies interact with the sown flowering plants would help to develop flower mixtures that consistently attract and support natural enemies and enhance biological control in adjacent crops. Considering flower functional traits would allow going beyond the taxonomic classification of flowering plants to study the mechanisms involved in the attractiveness and support of natural enemy populations in WFS.

4.4 Picking and Mixing of Suitable Flower Functional Trait Values

4.4.1 Screening of Functional Trait Values Involved in Natural Enemy–Flower Interactions

“A trait is any morphological, physiological or phenological feature measurable at the individual level, from the cell to the whole-organism level, without reference to the environment or any other level of organization” (Violle et al. 2007). Once a trait is involved in ecosystem processes, it is considered *functional* (Díaz and Cabido 2001) and *interaction traits* would be those traits specifically involved in arthropod–plant interactions (Gardarin et al. 2018). Every trait shows an array of values (e.g., the values of the trait “flower color” can be yellow, white, blue, red, or purple) but how do insects react to the various values of a given trait (Fig. 4.2)?

In laboratory conditions, artificial devices have been used to identify the values of some flower traits attracting natural enemies. Colored papers were used to identify that yellow attracts the ladybeetle *Harmonia axyridis* (Coleoptera: Coccinellidae) (Adedipe and Park 2010) and the hoverfly *Episyrphus balteatus* (Diptera: Syrphidae) (Sutherland et al. 1999). Fourteen isolated sugar types were separately provided to the parasitoid *Cotesia glomerata* (Hymenoptera: Braconicae) and it was shown that sucrose, glucose, and fructose are the sugar types that increase the most the parasitoid longevity (Wäckers 2001). Artificial flowers with various architectures were used to observe that the parasitoid *Edovum puttleri* (Hymenoptera: Eulophidae) is able to feed almost exclusively on nectar of flowers with exposed nectaries while *Pediobius foveolatus* (Hymenoptera: Eulophidae) can feed on nectar of various flower types (Patt et al. 1997). Artificial flowers were also used to show that the hoverfly *E. balteatus* prefers relatively small flowers with a rather high nectar concentration but does not respond to pollen concentration (Sutherland et al. 1999). In natural conditions, Koski and Ashman (2014) made artificial yellow flowers with various patterns of ultra-violet (UV) reflectance and identified that the flowers with a UV pattern increase the attraction rate of hoverflies compared to fully UV reflective or absorptive flowers.

Real flowers are used in laboratory studies notably to assess the attractiveness of flower volatiles to natural enemies and to monitor the attractive compounds and their concentration. For example, D-limonene and terpinolene were identified as major components of *Nepeta cataria* L. (Lamiaceae) volatiles and the combination of D-limonene and terpinolene (i.e., D-limonene at low concentration, but not terpilonene alone) attracts the ladybeetle *H. axyridis* (Song et al. 2017). Corolla width and depth are also measured and compared to the length of insect mouthparts or the size of insect heads to assess the theoretical accessibility of floral resources by insects (Nave et al. 2016; Van Rijn and Wäckers 2016). By coupling such measurements with survival experiments, Van Rijn and Wäckers (2016) identified that the hoverfly *E. balteatus* survives longer when exposed to flowers with a less-than-1.5 mm effective flower depth (i.e., the depth at which the nectar is available). Nectar is also extracted from flowers and the sugar content (i.e., the proportion of different types of sugars) is analyzed and related to the longevity of insects feeding directly on the flowers or on solutions equivalent to the nectar content. Such studies showed that sucrose-dominant nectar increases the longevity of the parasitoid *Microctonus hyperodae* (Hymenoptera: Braconidae) (Vattala et al. 2006), glucose- and fructose-dominant nectar increases the longevity of the parasitoids *Gonatocerus* sp. (Hymenoptera: Mymaridae) (Irvin et al. 2007), while the longevity of some other parasitoid species such as *Diadegma semiclausum* (Hymenoptera: Ichneumonidae) and *Dolichogenidea tasmanica* (Hymenoptera: Braconidae) is not affected by nectar sugar content (i.e., the sucrose/hexose ratio) (Tompkins et al. 2010).

In fields, flowers approached or visited by natural enemies are described through their traits to identify the natural enemy-attractive and -supportive trait values. By observing insects in mono-specific flower plots, it was reported that an increased floral size and a late peak bloom increase the abundance of natural enemies approaching flowers (Fiedler and Landis 2007b). Trapping insects near potted flowers showed that wide and deep corolla flowers enhance the abundance of parasitoids (Sivinski et al. 2011). Observations realized in naturally grown field margins composed of multiple flower species revealed that visitations of zoophagous hoverflies (i.e., mainly *E. balteatus* and *Sphaerophoria* spp.) declined with increasing flower depth (Van Rijn and Wäckers 2016).

In addition to considering the trait values of a given flower species, the Community-Weighted Mean (CWM) of trait values can be calculated at the flower mixture level to describe the functional composition of species-rich WFS (Fig. 4.2). The CWM is “the mean of [trait] values present in the community weighted by the relative abundance of taxa bearing each value” (Lavorel et al. 2008). Relating the CWM of seven flower functional traits with the abundance of natural enemies trapped in 15 flower mixtures showed that mixtures with a high cover of (i) yellow flowers and flowers with peripherical corolla parts reflecting UV attract and support pollen beetle (Coleoptera: Nitidulidae: *Meligethes* spp.) parasitoids, (ii) flowers with a UV pattern attract and support the lacewing *Chrysoperla carnae* (Neuroptera: Chrysopidae) and the ladybeetles *H. axyridis* and *Propylea quatuordecimpuctata* (Coleoptera: Coccinellidae), and (iii) flowers with open nectar attract and support the hoverflies *E. balteatus* and *Eupeodes corollae* (Diptera: Syrphidae) (Hatt et al. 2018; Hatt et al. 2019). Hatt et al. also showed that conversely, the mixtures with a high cover of violet and white flowers, flowers with totally hidden nectar and a UV pattern negatively affect the abundance of pollen beetle and weevil (Coleoptera: Curculionidae: *Ceutorhynchus* spp.,) parasitoids in WFS, as do mixtures with a high cover of flowers with a UV pattern with respect to the ladybeetle *Coccinella septempuctata* (Coleoptera: Coccinellidae).

4.4.2 Mixing of Flower Functional Trait Values to Enhance Biological Control

This accumulated knowledge on natural enemy-flower trait value interactions reveals that distinct natural enemies may respond to different values of a given trait. Hence, it has been hypothesized that increasing the functional diversity (i.e., the diversity of values for a selection of traits among the flower species composing a mixture) at the mixture level would enhance the abundance and diversity of insects in WFS (Fig. 4.2). At first with annual flowering plants, Balzan et al. (2014, 2016) compared three flower mixtures with a contrasted functional diversity based on floral morphological traits determining the accessibility of nectar. In these studies, the abundance and richness of flower-visiting natural enemies trapped in the WFS were not enhanced by the increasing mixture functional diversity. For perennial flowering plants, Hatt et al. (2017c) considered seven flower functional traits to compose four flower mixtures (plus a control grass-only mixture) with a contrasted functional diversity based on the Rao quadratic index (Botta-Dukát 2005). Similarly, the abundance and richness of flower-visiting predators trapped in the WFS were not enhanced with the increasing mixture functional diversity. These results suggest that increasing functional diversity *per se* is not the key to enhancing the abundance and diversity of natural enemies in WFS.

4.5 Perspectives: Towards a Tailored Functional Diversity

Instead of functional diversity *per se*, a *tailored functional diversity* could guide the composing of flower mixtures. Such mixtures would bear a diversity of functional trait values known to attract and support a high variety of natural enemies. Based on those studies assessing the effect of various trait values on natural enemy behavior (see above), a tailored mixture could comprise a diversity of flowers that, together, would bear (i) large and small corolla, (ii) with and without UV pattern but (iii) with high UV reflective peripherical parts, from which (iv) nectar – of various sugar compositions – and pollen are easily accessible, some of them being (v) yellow, and (vi) blooming at various periods of the year. Practically, depending on the rotating crops nearby WFS and the pest species to control, only the specific trait values known to benefit the targeted natural enemies of the pest species occurring on the crops rotating adjacent to the WFS could be assembled.

In this context, intensifying (i) the screening of functional traits of a diversity of flowers growing in various agro-ecological systems, and (ii) the identification of the trait values involved in flower-natural enemy interaction, is required. This approach will allow continuing the filling of plant trait databases such as the TRY (Kattge et al. 2011) and the BiolFlor (Kolz et al. 2002). As for trait values involved in flower-natural enemy interactions, a database gathering the increasing accumulated knowledge reviewed here and previously (Gardarin et al. 2018; Perovic et al. 2018) would be needed. In addition, several questions remain regarding the tailored flower mixtures: what should be the proportion of each key trait value in the mixture and the ratio of trait complementary/redundancy? Answering these queries would notably determine the flower species richness and the sowing density of each flower species composing the mixture. But how is it possible to guarantee that the sown functional diversity will be realized in the field since the competition between plants and the environmental conditions may affect the flower germination rate and development? Through time, management practices that must be applied to perennial semi-natural habitats can affect plant species richness and evenness, and hence the abundance and proportion of flower trait values, and in the end insect populations (De Cauwer et al. 2005; Blake et al. 2013). Thus, what would be the most appropriate management, notably the mowing time and frequency, to maintain the tailored functional diversity of the WFS? And last but not least: what would be the effect on pest suppression, damage reduction in the adjacent rotating crops, and the economic gain for farmers?

The benefits of WFS for insect conservation have been acknowledged (Haaland et al. 2011) and in some European countries such as Switzerland or Belgium, the sowing of WFS is financially supported through agri-environmental policies (Natagriwal 2014; Herzog et al. 2017). Improving the composition of WFS for conservation biological control as well as the delivery of other ecosystem services (e.g., pollination, Uyttenbroeck et al. 2017) while maintaining their positive effect on biodiversity conservation is the challenge to overcome.

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Chapter 5
Enhancement of Natural Control Ecosystem Services for Insect Pest Management by Manipulating Biodiversity in Rice-Based Ecosystems

Pingyang Zhu, Zhongxian Lu, Guihua Chen, and K. L. Heong

5.1 Introduction

Rice, one of the most important food crops for more than half of the world's population (FAO 2018; Yuan 2014), has been intensively cultivated in Asia since the Green revolution in the 1960s (Khush 1997) and has changed managed landscapes. The most important herbivore pests on rice plant are rice planthoppers, *Nilaparvata lugens* (Stål), *Sogatella furcifera* (Horváth) and *Laodelphax striatellus* (Fallen), rice stem borers, *Chilo suppressalis* (Walker), *Sesamia inferens* and *Scirpophaga incertulas* (Walker), and the rice leaf folder, *Cnaphalocrocis medinalis* Guenee (Lou et al. 2013). Rice is an annual crop grown one to three times a year, with high degree of disturbance from cultivation practices such as sowing, transplanting and harvesting (Heong et al. 2015). Intensive rice production with the primary goal of achieving high yield is characterized by applying large amounts of chemical fertilizers and pesticides, which can lead to a series of negative outcomes. Among the unwelcomed impacts are frequent pest outbreaks, which in turn trigger more pesticide use, negative impacts on the environment and non-target fauna, and threat to human health (Conway and Pretty 1991; Xu et al. 2017). Overuse of chemical pesticides has become a major obstacle to sustainable agricultural development,

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P. Zhu · Z. Lu (✉)
State Key Laboratory for Managing Biotic and Chemical Threats to the Quality and Safety of Agro-products, Zhejiang Academy of Agricultural Sciences, Hangzhou, China

G. Chen
Jinhua Plant Protection Station, Jinhua, China

International Rice Research Institute, Los Baños, Philippines

K. L. Heong
Institute of Insect Sciences, Zhejiang University, Hangzhou, China

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resulting in serious threats to the quality and safety of agricultural products, and ecological and environmental security (Xu et al. 2017).

In the last few years, research on the use of natural enemies to suppress crop pests and other non-chemical methods has increased. Significant progress has been made in the manipulation of biodiversity to enhance sustainable management of rice insect pests (Gurr et al. 2016; Horgan et al. 2017; Spangenberg et al. 2018). One of the first experiments and demonstration on using ecological engineering ecological engineering technology (EET) to manipulate biodiversity to enhance biological control and increasing environmental resistance to insect pests in rice was done in Jinhua, China in 2008. Ecological engineering is now applied to enhance the recovery of ecosystem services, and to reduce the use of insecticides. In 2014, ecological engineering has been recognized as a national recommendation by the Ministry of Agriculture and Rural Affairs (MARA) (MARA 2014). In this chapter we discuss the recent advances in using biodiversity manipulation for sustainable management of rice insect pests.

5.2 Conserving Natural Enemies by Manipulating Biodiversity

Manipulation of biodiversity through habitat management aims to suppress pest densities, usually by enhancing the impact of the natural enemy community, while altering vegetation patterns can also act directly on the herbivores (Gurr et al. 2017). Manipulation of biodiversity includes introducing plants that can provide shelter (Hossain et al. 2002), food (pollen, nectar) and other resources (Wäckers et al. 2007) to the arthropod natural enemies. These techniques have been examined and verified in other crop ecosystems (Wäckers et al. 2007; Jonsson et al. 2014; Gurr et al. 2016, 2017). To date, many habitat management measures have been well known in rice production, such as maintaining graminaceous plants (weeds) around rice fields, planting alternative plants, incorporating rice straw, and growing green manure crops after rice seasons, such as the Chinese milk vetch *Astragalus sinicus*. All these measures can provide native arthropod natural enemies with alternate hosts and shelter for overwintering, reducing natural enemy mortality from farming practices, and enhancing natural enemies' roles in the early crop stages. These practices together with restricting insecticide applications especially in the early crop stages form the foundation for natural control throughout the season (Chen et al. 2016; Huang et al. 2005).

5.2.1 Shelter for Natural Enemies

Insect pest management by natural enemies may be enhanced through growing green manure crops such as Chinese milk vetch *Astragalus sinicus* after each rice season. Maintaining graminaceous plants around rice fields and intercropping with *Zizania latifolia* are also applicable (Table 5.1). These plants can successfully provide arthropod natural enemies such as spiders, parasitoids and frogs with shelter during the winter periods, and when pesticides and herbicides are applied.

5.2.2 Alternative Hosts for Natural Enemies

Native natural enemies can be effective biological control agents. However, many highly host specific species typically exhibit delayed density dependence, and thus their populations tend to lag behind pest populations. The use of banker plant systems can provide alternative hosts before the crop season for natural enemies, and will thus enhance their development during the season (Zheng et al. 2017a). The banker plants not only can improve natural enemy survival and reproduction, but they can also build up natural enemy densities before pests occur. The use of banker plants is perhaps more economical than rearing and releasing of natural enemies, and might be more sustainable and less costly when the situation permits their use (Xiao et al. 2012; Zheng et al. 2017a). The integration of both natural enemy releases and banker plants is probably also feasible, and warrants further investigations. Two banker plant systems have been evaluated for rice production: the use of *Z. latifolia – S. procerus – Anagrus* spp. (ZSA), and *Leersia sayanuka – Nilaparvata muiri – Anagrus* spp. & *Typhus chinensis* (LNA&T) (Table 5.1).

The banker plant system *Leersia sayanuka – Nilaparvata muiri – Anagrus* spp. involves the planting of the grass species, *Leersia sayanuka*, adjacent to rice fields (Zheng et al. 2017a). *Leersia sayanuka* is the host plant of the planthopper *N. muiri*, which is not a rice pest as it is unable to complete its life history on rice. An egg parasitoid, *Anagrus nilaparvatae*, parasitizes the eggs of both BPH and *N. muiri*. Thus the establishment *Anagrus* spp. in the rice ecosystem with *L. sayanuka* is improved. Laboratory studies show that BPH was unable to complete its life cycle on *L. sayanuka*, and *N. muiri* could not complete its life cycle on rice. Thus, planting *L. sayanuka* poses no risk of it serving as an alternative host to the rice pest *N. lugens*. BPH densities in field studies were significantly lower in rice fields grown with the banker plant compared to control rice fields without (Zheng et al. 2017a). The banker plant system *Z. latifolia – S. procerus – Anagrus* spp. is an intercropping system of rice with the aquatic grassy vegetable *Z. latifolia* (Chinese wild rice). Green slender planthopper *Saccharosydne procerus* is main insect pest of *Z. latifolia* but does not feed on rice, and can share the egg parasitoid *Anagrus* spp. with rice plant- and leafhoppers in the rice field. So, the eggs of *S. procerus* in *Z. latifolia* can be the alternative host for *Anagrus* spp. in winter season, and the over wintering *Anagrus* spp. population can move from *Z. latifolia* fields to rice fields when rice planthoppers occur (Yu et al. 1999; Zheng et al. 1999).

Table 5.1 Details of measures to manipulate biodiversity for sustainable management of rice insect pests

Biodiversity manipulation in rice-based ecosystem	Target natural enemies	Target pests[a]	References
Conserving arthropod natural enemies			
Maintaining of graminaceous plants around rice fields	*Oligosita* spp. *Anagrus* spp.	RPHs, RLF, SSB	Zhu et al. (2015a)
Intercropping with *Zizania latifolia*	Spiders	Rice insect pests	Zheng et al. (2002)
Providing vegetable patches interspersed along rice bunds	Egg parasitoids	SSB	Vu et al. (2018)
Providing grass species as border crops	*Cyrtorhinus ividipennis*		Chandrasekar et al. (2017)
Alternative hosts for natural enemies			
Using the banker plant system:			
Z. latifolia –*Saccharosydne procerus* – *Anagrus* spp.	*Anagrus* spp.	RPHs	Yu et al. (1999) and Zheng et al. (1999)
Leersia sayanuka – *Nilaparvata muiri* – *Anagrus* spp. and *Typhus chinensis*	*Anagrus* spp. and *T. chinensis*	RPHs	Zheng et al. (2017a, b)
Floral resources for natural enemies			
Sesame (*Sesamum indicum*)	*A. optabilis* and *A. nilaparvatae*	RPHs	Zhu et al. (2013a)
	C. lividipennis	RPHs	Zhu et al. (2013b, 2014)
	Apanteles ruficrus	RLF	Zhu et al. (2015b)
	Cotesia chilonis	SSB	
	T. chilonis	SSB, RLF	
Soybean (*Glycine max*)	*A. nilaparvatae*	RPHs	Zheng et al. (2003)
Corn pollen	*A. nilaparvatae*	RPHs	Zheng et al. (2003)
Clover (*Oxalis triangularis*)	*T. chilonis*	SSB, RLF	Zhao et al. (2017)
Growing of trap plants		SSB, PSB, YRB	Chen et al. (2007), Zheng et al. (2009), Gao et al. (2011, 2015) and Lu et al. (2017)
Innudative release of natural enemies	*Trichogramma* species	SSB, RLF	Xu et al. (2017)

[a] RPHs rice planthoppers, *Nilaparvata lugens*, *Sogatella furcifera* and *Laodelphax striatellus*, BPH brown planthopper, *Nilaparvata lugens*, WBPH white-backed planthopper, *Sogatella furcifera*, SSB striped stem borer, *Chilo suppressalis*, PSB pink rice borer, *Sesamia inferen*, YRB yellow rice borer, *Scirpophaga incertulas*, RLF rice leaf folder, *Cnaphalocrocis medinalis*

5.2.3 Floral Resources for Natural Enemies

Strategic use of flowering plants to enhance plant biodiversity in a well-targeted manner can provide natural enemies with food sources to improve biological control. These non-crop habitats can provide appropriate resources to benefit natural enemies because of their proximity to the rice and high connectivity with non-rice vegetation (Gurr et al. 2011). Floral resources can enhance the effectiveness of natural enemies as control agents since they can prolong their longevity and increase their fecundity (Lu et al. 2014). The inclusion of suitable flowering plants in non-crop habitats could dramatically affect the activity of natural enemies. A growing number of studies have demonstrated that the abundance and fecundity of natural enemies are increased in the presence of floral resources in the ecosystem (Table 5.1).

In Vietnam, it has been demonstrated that the planting of a variety of nectar producing plants on rice field bunds significantly increases the number of parasitic wasps [mainly of the delphacid planthopper *Nilaparvata lugens*] (Lan et al. 2010). In China, growing sesame (which has a comparatively long flowering period) on bunds accompanied with insecticide reduction has been shown to effectively reduce planthoppers and to increase parasitic wasp numbers. The reduced pesticide use and effective pest control associated with the sesame planting can have significant economic and ecological benefits (Chen et al. 2016). Growing nectar plants around rice fields has been recommended as one of main agriculture techniques by the Chinese government from 2014 (MARA 2014).

5.3 Growing Trap Plants to Control Stem Borers

Rice striped stem borer (SSB) *Chilo suppressalis* prefer to lay eggs on the vetiver grass *Vetiveria zizanioides*, but the larvae cannot complete their life cycle on vetiver grass (Zheng et al. 2009). With this characteristic, planting vetiver grass around paddy fields in place such as bunds or roadsides, and along irrigation canals, can effectively attract stem borer adults to lay eggs and thus reduce invading female moths from multiplying, and thus populations of stem borers in the rice fields (Liang et al. 2015). In this management strategy the appropriate timing, plant density to be used, and the application of nitrogen fertilizer in vetiver can improve moth attraction ability (Chen et al. 2007; Lu et al. 2016). The best planting period was found to be from late March to early April in middle and downstream areas of the Yangtze River, before the establishment of the rice crop. The appropriate planting density was determined to be around 6–10% of rice field (Chen et al. 2007). The optimal planting pattern was in clusters, 3–5 m apart, and with a line spacing of 50–60 m (Zheng et al. 2017c). In addition, if the population of stem borers is too high on the vetiver grass, it is also feasible to kill eggs and small larvae of the stem borers manually, or with insecticides on vetiver to alleviate the hazards in the rice field (Chen et al. 2007; Xu et al. 2017). Insecticide sprays would not be needed in the rice crop, especially when densities of stem borers would be below the control thresholds (Table 5.1).

5.4 Inundative Release of Natural Enemies

Trichogramma species have been used extensively in biological control programs (Xiang and Zhang 2011; Consoli et al. 2010). They play important role in the management of Lepidopteran pests. Since the 1950s a lot of effort has focused on field release of *Trichogramma* adults in China (Xiang and Zhang 2011). There are, however, many constraints with large-scale applications, such as the selection of *Trichogramma* species, rejuvenation of culture stocks, and field application techniques (Wang 1978).

Recently applications of *Trichogramma* have gained renewed interest (Xu et al. 2017). *Trichogramma japonicum*, *Trichogramma chilonis*, *Trichogramma dendrolimi* and *Trichogramma ostriniae* are commonly found in rice fields (Guo et al. 2012). The devices and release technologies have developed in recent years, including a suitable device of releasing *Trichogramma* into rice fields (Zang et al. 2014). These devices for releasing *Trhichogramma* contain nectar food supplement, and apply unmanned aerial vehicles (UAV) (Xu et al. 2017). National Agricultural Technology Extension Service Center (NATESC) has carried out a series of *Trichogramma* releases, including *Trhichogramma* species selection, the interval of release times, application time, release density, and release height. These experiments contribute to improve technical standardization, and provide a foundation for application of *Trichogramma* in large-scale rice fields (Xu et al. 2017).

Often the *Trichogramma* parasitoids are reared in indoor facilities, using eggs of stored product pest species such as *Corcyra cephalonica* (Tian et al. 2017). The parasitoids that hatch from these cultures are then released to parasitize a “foreign” host such as stem borers or leaf folders with rather different egg appearances, sizes and locations. Most reports provide percentage of egg parasitism as the criterion of success (Li et al. 2018). However, it is difficult to tell if the increase in parasitism is due to native parasitoids, or the released ones. Since parasitoid releases work best when insecticides are not used, perhaps the absence of insecticides has contributed to the effectiveness of native parasitoids. Thus is important that egg parasitoid releases are coupled with withholding insecticide use, to ensure the success of this strategy.

5.5 Case Study of Sustainable Rice Pest Management by Manipulating Biodiversity in Jinhua, China

Zhejiang Academy of Agricultural Sciences (ZAAS) and the Jinhua Plant Protection Station (JPPS), China, in collaboration with the International Rice Research Institute (IRRI), initiated a pioneering attempt to manage rice insect pests by manipulating biodiversity in rice-based ecosystem in 2008. The demonstration site was located at Si Ping village, Jinhua city, with nearby mountains and high-quality water resources. The areas used for rice production had been impacted by intensive cultivation and overuse of chemical fertilizers and pesticides. The strategy, known as "Ecological Engineering", was to maintain graminaceous plants around the rice fields, intercropping zizania in some fields, inter-planting the nectar crop sesame on field bunds, planting vetiver grass on roadsides and along irrigation canals, and releases of *Trichogramma* spp. No insecticides were applied during the first 40 days after transplanting. The goal of the experimental and demonstration site was to reduce the use of chemical pesticides to conserve biological control agents, and by manipulating biodiversity to increase functional biodiversity and abundance of biological control agents. It must be emphasized that these two components have to be implemented together, and not one without the other.

5.5.1 Improved Biological Control

Field surveys show that the numbers of *Anagrus* spp. and invertebrate predators including damselflies in EET fields were over four times higher than those in the control fields (Chen et al. 2016). Frogs were also more abundant. *Fejervarya multistriata* was the dominant frog species, and its population density was well over 413 individuals per hectare in ecological engineering fields, while frog numbers in the control fields were only 107 per hectare (Kong et al. 2016). The control fields were sprayed several times with chemical pesticides (Chen et al. 2016). In addition, the number of egg parasitoids of planthoppers, *Oligosita* and *Anagrus* from common grassy flora around the EET fields, were significantly higher in the EET fields than in the control fields. The number of RPH egg parasitoids near the ridge was increased, while the population of RPH was reduced significantly by implementing the EET for rice pest management (Zhu et al. 2015a). A follow-up survey over 5 years showed that the numbers of predators such as Odonata and Tetragnathidae, as well as the larval parasitoids of RLF, in EET fields were significantly higher than those in the control fields (Zhu et al. 2017a). Furthermore, in the ecological engineering fields aquatic predators and 'neutral' insects in rice fields, were more abundant (Zhu et al. 2017b).

Applying the ecological engineering strategy for rice pest management can reduce dramatically insecticide applications. In the Si Ping fields no insecticides were used for rice planthopper control in 2009 and 2011, and the amount of insecticides used overall was reduced by more than 75% in the ecological engineering fields (Chen et al. 2016). In addition, insecticides were not used at all for the entire period from 2009 to 2014 in the ecological engineering fields, while insecticides were used about 5 times in each rice season in the control fields (Liu et al. 2014).

5.5.2 Increased Economic Benefits

With the application of ecological engineering technology (EET), pest populations have been kept at low levels throughout the rice growing season, and no yield losses have been found. The 75% reduction in insecticide use represents production cost savings, and together with reduction in labor costs amounted to savings of more than US $400 per hectare (Chen et al. 2016). In addition, rice from these ecological engineering fields were sold at a price of over 5 times higher than the market price from conventional production (Liu et al. 2014). Similar positive results were observed also in other sites in China, for example Xiaoshan, Lishui, Wenling and Wenzhou.

5.6 Conclusions and Perspectives

Rice cultivation can be traced back to more than 6000 years ago. The Green Revolution in the 1960s and 1970s aimed at meeting the increasing demand for food with the rapid world population growth (Lou et al. 2013). Rice production has since then increased with the use of high-yielding varieties, and extensive application of chemical pesticides and fertilizers. This intensive rice production system has greatly depended on high inputs of agrochemicals, resulting in a serious threat to the ecological safety, environmental health, and the sustainability of rice production (Heong 2009). It consequently has became essential to justify the high use of chemical pesticides, and to guarantee food safety by developing ecological pest management for the rice-based ecosystem. Rice productivity gains from insecticide use is now being questioned (Heong et al. 2015). New approaches are needed to create sustainable, healthy rice ecosystems that will have sufficiently high production with reduced or no insecticide use. The FAO in it 2012 publication, “Save and Grow” boldly declared that “Most tropical rice crops under intensification require NO insecticide use” (FAO 2012). Healthy rice ecosystems as far as possible avoid harmful insecticides are develop mechanisms to increase the functional biodiversity in the rice landscape to attract beneficial organisms (Westphal et al. 2015). In recent years, new principles and technologies have been introduced to develop sustainable pest management, and the new concept of “green plant protection” has been widely promoted and applied throughout China (Lu et al. 2012). This concept emphasizes the support and safeguards needed to obtain high-yield, good-quality and ecologically sustainable agricultural systems. Ecological control strategies are widely adopted for sustainable management of rice insect pests to reduce the use of chemical insecticides. As the Chinese government is attaching great importance to the development of sustainable agriculture, sustainable management for pest control will be developed further, and will become a more prevalent pest management strategy in the future.

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Chapter 6
Enhancement of Natural Control Function for Aphids by Intercropping and Infochemical Releasers in Wheat Ecosystem

Yong Liu, Jiahui Liu, Haibo Zhou, and Julian Chen

6.1 Introduction

Intercropping is a multiple cropping practice involving the growing of two or more crops in proximity. Andrews and Kassam (1976) categorized intercropping into four principle types based on the spatial and temporal overlap of plant species: (1) mixed intercropping – two or more crop species mixed with no distinct row arrangement; (2) row intercropping – two or more crops grown in separate alternate rows (when plant species are alternated within the same row, it is considered to be within-row intercropping); (3) strip intercropping – several rows of a crop (strip) alternated with several rows of one or more other crops; (4) relay intercropping – two or more crops grown in relay, but with the growth cycles overlapping to some degree. The most common goal of intercropping is to produce a greater yield on a given piece of land by making use of resources that would otherwise not be utilized by a single crop. Wheat-based intercropping systems have been extensively studied, and their utility for controlling pests was recently reviewed.

An infochemical, or semeiochemical (from Greek semeion meaning ‘signal’), is a generic term used for a chemical substance or mixture that carries a message for purpose of communication. Infochemical communication can be divided into two broad classes: communication between individuals of the same species (intraspecific), or communication between different species (interspecific) (Law and Regnier

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Y. Liu (✉) · J. Liu
College of Plant Protection, Shandong Agricultural University, Taian, Shandong, China
e-mail: liuyong@sdau.edu.cn

H. Zhou
Anhui Academy of Science and Technology, Hefei, Anhui, China

J. Chen
Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China

Y. Gao et al. (eds.), *Integrative Biological Control*, Progress in Biological Control 20, https://doi.org/10.1007/978-3-030-44838-7_6

1971). It is usually used in the field of chemical ecology to encompass pheromones, allomones, kairomones, attractants and repellents. Many insects, including parasitic insects, use allelochemicals, which are natural chemicals released by an organism that affect the behaviors of other individuals. Pheromones are intraspecific signals that aid in finding mates, food and habitat resources, warning of enemies, and avoiding competition. Interspecific signals known as allomones and kairomones have similar functions (Cardé and Willis 2008). The goals of using infochemicals in pest management are: (1) to monitor pest populations to determine if control is warranted; (2) to alter the behavior of the pest or its enemies to the detriment of the pest. In general, the advantages of using infochemicals are: (1) they have adverse effects only on the target pests; (2) they are relatively nontoxic and required in low amounts; (3) they are non-persistent and environmentally safe; (4) they appear difficult for insects to develop resistance against (Dicke et al. 1990).

Wheat aphids (Homoptera: Aphididae) including *Sitobion avenae*, *Schizaphis graminum*, *Rhopalosiphum padi* as well as *Metopolophium dirhodum*are, the dominant and destructive pests in wheat production regions of China, can cause heavy economic damage to wheat both as a phloem feeder, and as a vector of plant viruses (Liu et al. 2001; Zhou et al. 2013; Fan et al. 2014). Aphid populations are affected by a range of biotic and abiotic factors, and often fluctuate greatly from year to year.

Because of the urgent need for sustainable agriculture and for reducing reliance on pesticide use, increasing attention has been paid to conservation practices that seek to increase the biodiversity in agroecosystems. Biodiversity management brings long term benefits for sustainability of the farming system, providing an ecologically based approach in order to promote natural enemies and to enhance biological control in agricultural systems (Gurr et al. 2003; Hatt et al. 2015; Landis 2016). Numerous studies have evaluated the impact of plant diversification on pest and beneficial arthropod population dynamics in agro-ecosystems. Some evidence has been provided to display the positive effects from habitat manipulation techniques (e.g. intercropping, undersown non-host plants, field border planting) to ensure pest control (Balzan et al. 2014; Hatt et al. 2016; Lopes et al. 2016). Although mechanisms accounting for herbivores and natural enemy responses to plant diversification, in many instances, are not thoroughly tested, the rapidly expanding literature on biodiversity and agro-ecosystems is reviewed here with attention to the ways in which agricultural biodiversity may be increased to favour pest management. In particular, the contributions of plant diversification on the mechanisms influencing arthropod behaviour will be presented in relation to the conservation biological control strategy. Various potential options of habitat management and design that enhance functional biodiversity in crop fields have already been described (Zhou et al. 2009b).

At the same time, more studies on integrated pest management (IPM) also focus on ecological function of volatiles released by plants on herbivores and their natural enemies. The herbivore-induced plant volatiles (HIPVs) can repel herbivores and attract the herbivores' natural enemies (Ma et al. 2008; Hare 2011). They are also involved in rapid defense signaling (Heil and Ton 2008), which neighbouring plants can eavesdrop, inducing their own defenses, and changing of their volatile profiles (Ton et al. 2007).

In this context, the main objective of this review is to promote the functions of intercropping and infochemical releasers as IPM agents by developing alternative strategies for aphid control in wheat crops. Three different approaches have been adopted successfully: (1) intercropping as a habitat management tool to enhance biological control of aphids; (2) the potential use of plant volatiles for aphid control; (3) the combination of intercropping with semeiochemical releasers and its potential to control aphids in wheat fields.

6.2 Aphids and Beneficials

Ecologists have long sought explanations for fluctuations in the abundance of insects and other animals. Attention usually tends to focus on the role of natural enemies including predators, parasites, parasitoids and pathogens. The reason for the emphasis placed on natural enemies, particularly predators and parasitoids, is largely because, as Price (1975) wrote: ‘predation is certainly one of the most visible aspects of mortality’. This visibility stems first from the variety of natural enemies (particularly the many insect species of predators and parasitoids) and their obvious roles. It is therefore hardly surprising that the action of parasitic wasps, predatory ladybirds, and other natural enemies, has been observed by naturalists for over a hundred years. It is not the obtaining of food, but the serving as prey to other animals, which determines the average numbers of a species (Darwin 1866). In addition, the many adaptations of insects against attack by natural enemies, the successful cases of biological control, and population models have all given weight to the view that natural enemies have a dominant role in the population dynamics of insect herbivores.

Wheat, *Triticum aestivum,* is the most important food crop in the world. In China, wheat is the second largest crop after rice in cultivated area, and its yield accounts for one-fifth of China’s total grain output (Yang et al. 2014).There are more than 110 pests harmful to wheat in China, among which aphids are the most serious ones (Tang et al. 2013). There are four main aphid species, and several predatory and parasitic natural enemies in the wheat fields in China and several predatory and parasitic natural enemies in wheat fields (Table 6.1).

In temperate climates, aphids are among the most challenging insects to manage during the production of cereal grains and many other crops, because their populations can increase rapidly, and their feeding can damage both yield quantity and quality (Liu et al. 2001). In addition, aphids are the vector for many yield-reducing pathogens and viruses. For example, *S.avenae* damage affects 13 million hectares (ha) per year and causes of up to 40% wheat yield loss in China (Duan et al. 2006).

The English grain aphid *Sitobion avenae* (Hemiptera: Aphididae) is the most dominant pest species in the wheat fields. Its hosts cover wheat *T. aestivum*, sugar cane *Saccharum officinarum* (Poales: Poaceae), maize *Zea mays* (Poales: Poaceae),

Table 6.1 Wheat aphids and their natural enemies in China (Chi et al. 2014)

	Order	Family	Species
Aphids	Homoptera	Aphididae	*Sitobion avenae* (Fabricius)
			Rhopalosiphum padi (L.)
			Schizaphis graminum (Rondain)
			Metopolophium dirhodum (Walker)
Natural enemies	Coleoptera	Coccinellidae	*Coccinella septempunctata* (L.)
			Harmonia axyridis (Pallas)
			Propylea japonica (Thunberg)
	Hymenoptera	Braconidae	*Aphidius gifuensis* (Ashmaed)
			Aphidius avenae (Haliday)
			Ephedrus plagialor (Nces)
		Aphelinidae	*Aphelinus* sp.
	Neuroptera	Chrysopidae	*Chrysoperla sincia* (Tjeder)
			Chrysopa pallens (Rambur)
	Diptera	Syrphidae	*Episyrphus balteata* (De Geer)
			Eupeodes corollae (Fabricius)
			Syrphus ribesii (L.)

rice *Oryza sativa* (Poales: Poaceae) (Jiang and Wang 2007) and sorghum *Sorghum bicolor* (Poales: Poaceae) (Lu et al. 2009). *S. avenae* is mainly distributed in Asia, North America and Europe, and is widely distributed in China (Hu et al. 2012). It prefers living in a warm and humid environment, usually in wheatears and obverse side leaves (Zhang 2012). It is the most harmful to wheat from its heading stage to milk-ripe stage.

The bird cherry-oat aphid *Rhopalosiphum padi* (Hemiptera: Aphididae) sucks sap from wheat plants especially from wheat stems. Apart from wheat, corn, canna *Canna indica* (Zingiberales:Cannaceae) and plum blossom *Armeniaca mume* (Rosales:Rosaceae)as its secondary host in China, its primary host is Prunus padus (Rosales:Rosaceae) (Shang et al. 2017). It is often considered as a major pest in temperate cereal crops, and particularly in some Northern European countries. It prefers living in a humid environment, with photophobic distribution in the leaf sheath and the reverse side (Zhang 2012). It is the principal vector of Barley yellow dwarf virus (BYDV) (Wang 2010).

The greenbug *Schizaphis graminum* (Hemiptera: Aphididae) has as hosts wheat *T.aestivum* (Argandoña et al. 1981), barley *Hordeum vulgare* (Poales: Poaceae) (Hays et al. 1999), oats *Avena sativa* (Poales: Poaceae) (Zhao and Guo 2017), sorghum (Wu and Huang 2008), sedge, and other grasses in China. It prefers living in a dry environment and has a photophobic distribution on the plant. They are mostly distributed in the lower part of the plant and reverse side of the leaf (Zhang 2012). It is the most harmful in wheat seedling stage. Continued feeding leads to general yellowing and reddening, leaf and root death, and finally can lead to plant death. It is also the principal vector of many viruses – more than the aphid *R. padi*.

The rose grain aphid *Metopolophium dirhodum* (Hemiptera: Aphididae) has an almost cosmopolitan distribution, being found in most parts of the world where its secondary hosts are grown. Its primary host is rose *Rosa rugosa* (Rosaceae:Rosa) (Farrell and Stufkens 1988), and its secondary host is cereals such as wheat, barley, oats, and grasses (Weber 2010). It is an important vector of BYDV, which causes serious reductions in yields of affected crops.

Due to the biological characteristics of wheat aphids, the population structure of wheat aphids is different in wheat growing regions. For example, in the wheat regions of Huang-huaihai and the middle and lower Yangtze River, the main aphids are *S. avenae* and *R. padi*, while in the northwest wheat region the main aphids are *S. avenae* and *S. graminum* (Xu et al. 2011a, b).

There are many predatory and parasitic natural enemies of wheat aphids, such as ladybirds, syrphids, lacewings and parasitic wasps. The ladybird beteels (Coleoptera: Coccinellidae) in the wheat fields in China are *Harmonia axyridis*, *Coccinella septempunctata* and *Propylea japonica* (Wang et al. 2009, 2018; Cheng et al. 2020). Ladybirds feed on aphids, planthoppers, leafhoppers and lepidopterous eggs and larvae. Previous laboratory experiments have proven that the *H. axyridis* can effectively control wheat aphids, and the predation of *H. axyridis* increased with the density of aphids (Ji and Wang 2009). Field experiments show that the artificial release of *H. axyridis* could effectively control the aphids in a wheat field (Wu and Geng 2014). *P. japonica* has an obvious predatory effect on wheat aphids, and the maximum predation by one ladybird individual on wheat aphids was 75.19 aphids in one day (Ren and Liu 2006).

The main syrphids (Diptera: Syrphidae) in wheat fields in China are *Eupeodes corolla, Episyrphus balteata* and *Syrphus ribesii*. Syrphid larvae feed on aphids, preying on *S. avenae*, *R. padi*, *R. maidis* (Hemiptera: Aphididae), *Myzus persicae* (Hemiptera: Aphididae), etc. When the larva preys, it will raise its head to detect its surroundings with every step forward, and if there is no prey object, it will continue. When it finds a prey object, it will hook its mouth and absorb the body fluids. During the entire larval stage a syrphid can feed on about 400 aphids. The hoverfly adults are non-predatory, and feed on pollen and nectar.

The main lacewing (Neuroptera: Chrysopidae) species in cereal fields in China are *Chrysoperla sincia* and *Chrysopa pallens*. They feed on aphids, leafhoppers, and lepidopterous eggs and larvae.

The parasitic wasp (Hymenoptera: Braconidae) species usually found are *Aphidius gifuensis*, *A. avenae* and *Ephedrus plagialor*. The dominant species on wheat aphids are *A. gifuensis* and *A. avenae* (Liu et al. 2016). Field and laboratory experiments show that *A. avenae* only parasitizes *S. avenae*, while *A. gifuensis* can parasitize *S. avenae*, *S. graminum*, and *M. dirhodum* (Liu et al. 2016).

Wheat aphids can directly and indirectly damage the crop and cause yield loss. Besides being important vectors of viral diseases, honeydew, the aphid excretory product rich in sugars and amino acids, also provides ideal elements for the development of saprophytic fungi. These reduce plant transpiration and photosynthesis, further affecting growth and development of the plant and thus causing economic damage, and necessitating routine insecticide use.

To control aphid pests in wheat fields, systematic applications of pesticides are performed, leading to many well-known problems such as chemical residues and target pest resistance to the applied pesticides. Much attention has to be paid on sustainable agricultural production, and more studies on IPM by ecosystem services are needed. The application of intercropping, and the techniques of infochemical release, have been investigated to assess their impact on herbivores and their natural enemies in agroecosystems in an IPM context. Therefore, it is crucial to demonstrate the application of semiochemical releasers and/or intercropping in developing alternative strategies for pest control in the field.

6.3 Intercropping in Wheat Ecosystem

Many national and intergovernmental bodies have firmly decided that the future officially endorsed paradigm for crop protection will be IPM. For example, a European Union (EU) Directive (2009) has obliged all professional plant growers within the Union to apply the general principles of IPM since 2014. Most textbooks define IPM as a holistic ‘approach’ or ‘strategy’ to combat plant pests using all available methods, with minimal applications of chemical pesticides. The aim is not to eradicate pests, but to manage them, maintaining their populations below economically injurious levels (Stern et al. 1959; Kilgore et al. 1967). Putting this insight into practice would reduce not only the exposure of the farmers, consumers, and the environment to toxic compounds, but also problems caused by pesticide-resistant pests. However, despite the expressed enthusiasm for IPM, most cropping systems still depend on heavy use of chemical pesticides (Hokkanen 2015).

The understanding of the mechanisms by which diversification of habitat may favor pest management is important (Gurr et al. 2003). Some hypotheses have been put forward to explain why increasing biodiversity in agriculture can lead to suppression of specialist insects. The resource concentration hypothesis and the enemy hypothesis (Root 1973) are the ones quoted most frequently. The first one states that many phytophagous insects, especially those with a narrow host range, are more likely to find hosts that are concentrated. The enemy hypothesis might be further extended to predict that herbivore species diversity would be higher in complex habitats. By rapidly checking outbreaks in these environments, predators and parasitoids would prevent the potentially dominant herbivore species from monopolizing the available resources. Push-pull strategy involves the behavioural manipulation of insect pests and their natural enemies via the integration of stimuli that act to make the protected resource unattractive, or unsuitable, to the pests while luring them toward an attractive source from where the pests are subsequently removed (Pyke et al. 1987). This strategy in exploiting biodiversity has been studied and developed to manage cereal stem borers in maize-based farming systems in eastern and southern Africa (Khan et al. 1997). The concept was formalized and refined by Miller and Cowles (1990), who termed the strategy stimulo-deterrent diversion strategy (SDDS), based on their studies developing alternatives to insecticides for the control of the onion maggot *Delia antiqua* (Miller and Cowles 1990). During host plant finding, the searching insects land indiscriminately on green objects such as the leaves of host plants (appropriate landings) and non-host plants (inappropriate landings), but avoid landing on brown surfaces, such as soil (Finch and Collier 2010). Plant diversification can be beneficial in controlling pests via ‘top-down’ enhancement of natural enemy populations, while resource concentration and other ‘bottom-up’ effects are acting directly on pests (Gurr et al. 2003).

To avoid environmental pollution, health problems and species loss caused by the overuse of conventional synthetic pesticides, exploration of multi-function agricultural biodiversity to enhance pest management is an important research theme for sustainable agricultural systems (Gurr et al. 2003). Enhancing functional biodiversity in agroecosystems is a key ecological strategy to bring sustainability to production (Altieri 1999). In Latin America, farmers grow 70–90% of their beans with maize, potatoes and other crops. Maize is intercropped on 60% of the region’s maize-growing area (Francis and Dominguez 1986). In a detailed quantitative review, Andow (1991) found that although natural enemy densities tended to be greater in polycultures than in monocultures, only slightly more than half of the 287 herbivore species were consistently less abundant in polycultures. Despite such potential problems, there are many successful instances of using biodiversity in agroecosystems to promote natural enemies, to control pests and, in some cases at least, to reduce crop damage. An understanding of the mechanisms by which biodiversity may promote pest management is important in order to allow sustainable crop production.

Intercropping is one practice of spatial diversification, defined as the cultivation of at least two plant species simultaneously in the same field, without necessarily being sown and/or harvested at the same time (Lithourgidis et al. 2011b). Intercropping, the agronomic practice for the development of sustainable food production systems (Agegnehu et al. 2006; Eskandari and Ghanbari 2010), plays an important role in controlling pests, and in protecting beneficial insects relevant in an agroecosystem (Smith and Mcsorley 2000; Hassanali et al. 2008; Konar et al. 2010; Ram et al. 2010; Vaiyapuri et al. 2010).

Intercropping systems tend to produce higher yields compared with monocultures, and reduce the impact of agriculture on the environment. Specifically, intercropping may improve soil conservation, fertility and crop quality, while possibly reducing the incidence of weeds, diseases and insect pests (Lithourgidis et al. 2011a; Aziz et al. 2015; Bedoussac et al. 2015). Focusing on pests, as stated in the ‘resource concentration hypothesis’ (Root 1973), specialist herbivores are more likely to find their host plants when they are concentrated in dense or pure stands. Moreover, according to the ‘enemy hypothesis’ (Root 1973), the suppression of herbivores by their natural enemies (i.e. predators and parasitoids) is expected to be more efficient in diversified crop habitats than in simplified ones, as they may be more abundant in environments offering a greater diversity of prey/host species, and microhabitats to exploit.

Although the effect of intercropping on pests and natural enemies has largely been covered in the literature (Risch 1983; Andow 1991; Langellotto and Denno 2004; Ajr and Byrne 2010; Letourneau et al. 2011; Dassou and Tixier 2016), most comprehensive reviews are very general. As wheat is one of the most important crops worldwide, understanding the potential of wheat-based intercropping systems for biological control may be of crucial importance. We aim to give valuable information about the potential of intercropping as a tool to reduce insecticide use specifically in wheat production.

In the research and development of the ecological control technologies for wheat aphids, studies have reported on the utilization of wheat variety diversity, and wheat intercropping with different crops such as garlic *Bulbus allii* (Liliflorae: Liliaceae), oilseed rape *Brassica campestris* (Brassicaceae: Brassica), peas *Pisum sativum* (Rosales: Papilionaceae) and mung beans *Vigna radiate* (Rosales: Fabaceae) (Wang et al. 2008, 2009, 2011).

6.3.1 Wheat-Oilseed Rape and Wheat-Garlic Intercropping Patterns

Two intercropping patterns were performed in winter wheat fields in Shandong province China. In April 17–29, 2007 (Fig. 6.1), the number of *S. avenae* apterae in wheat-garlic intercropping field was significantly lower than that in wheat monoculture field, and in wheat-oilseed rape intercropping field. After May 5, the number of apterae per 100 wheat tillers in the different treatments was: wheat monoculture > wheat-garlic intercropping > wheat-oilseed rape intercropping. In the whole survey period, the average number of *S. avenae* apterae in wheat-garlic intercropping was highly significantly lower than wheat monoculture. It can be seen that wheat intercropping with oilseed rape or garlic can significantly reduce the population numbers of *S. avenae* apterae (Wang et al. 2008).

The population dynamics of *S. avenae* alatae in different intercropping patterns were all bimodal (Fig. 6.2). In the whole survey period, the average number of *S. avenae* alatae in wheat-garlic intercropping was significantly higher than in wheat monoculture and in wheat-oilseed rape intercropping. From April 17 to 20, the average number of *S. avenae* alatae in wheat-oilseed rape intercropping was significantly higher than that in wheat monoculture. However, from April 26 to May 2, the average number of *S. avenae* alatae in wheat monoculture was significantly higher than wheat-oilseed rape intercropping. On May 14, the number of *S. avenae* alatae in the three types reached the second peak, and the order in the average numbers was: Wheat-garlic intercropping > Wheat monoculture > Wheat-oilseed rape intercropping.

The population density of ladybeetles, and the ratio of ladybeetles to *S. avenae,* was highest in wheat-oilseed rape intercropping field (Fig. 6.3). Before May 2, the population density of aphid parasitoids in wheat-oilseed rape intercropping field was higher than that in wheat-garlic intercropping field, or in wheat monoculture field

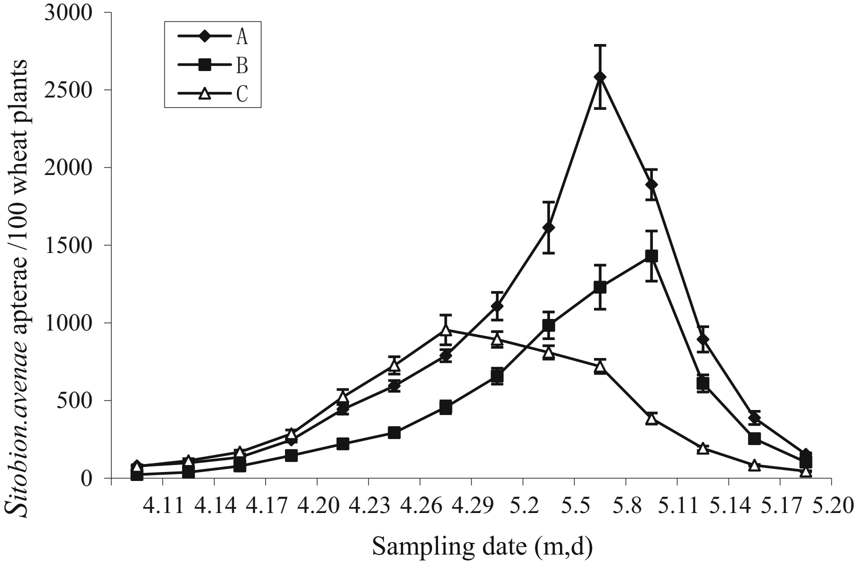

Fig. 6.1 Population dynamics of *Sitobion avenae* apterae in different intercropping patterns (mean ± SE) in 2007. A: Wheat monoculture; B: Wheat-garlic intercropping; C: Wheat-oilseed rape intercropping. (Wang et al. 2008)

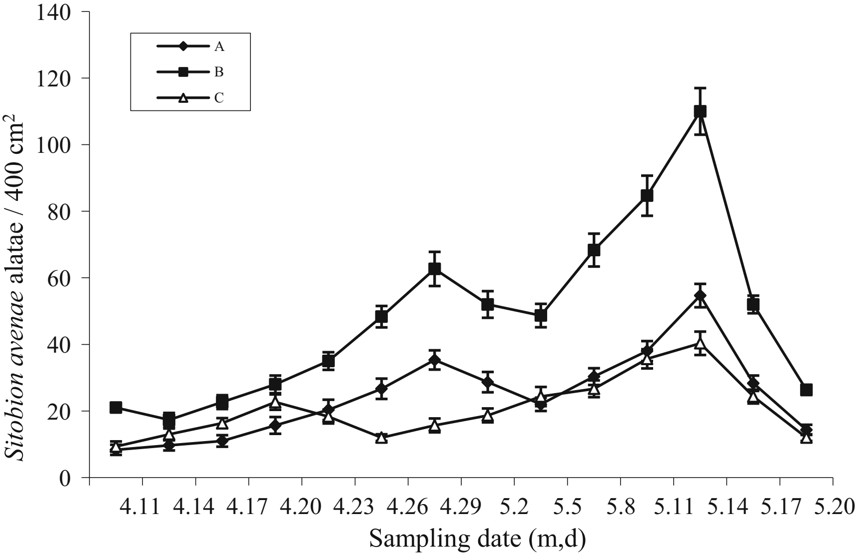

Fig. 6.2 Population dynamics of *Sitobion avenae* alatae in different intercropping patterns, as measured by direct counting on 400 cm^2 yellow cards in each plot in 2007. A: Wheat monoculture; B: Wheat-garlic intercropping; C: Wheat-oilseed rape intercropping. (Wang et al. 2008)

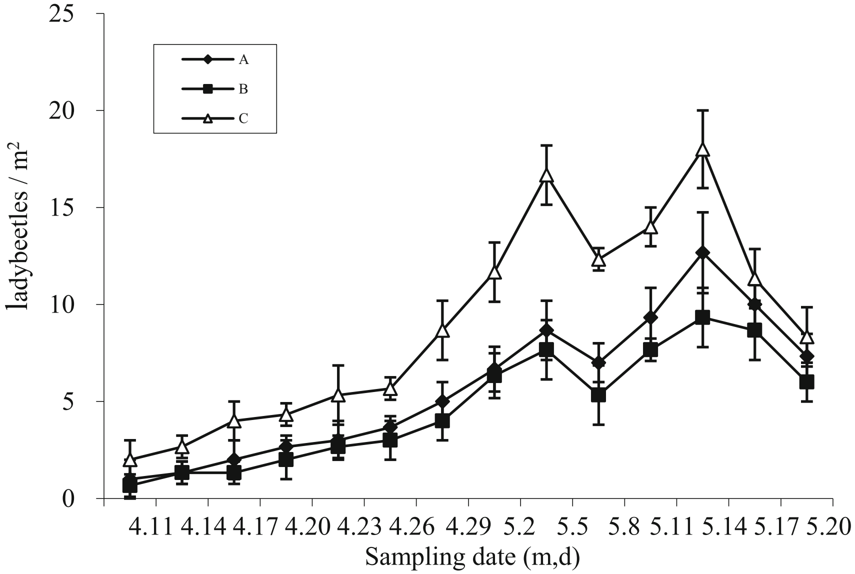

Fig. 6.3 Population dynamics of ladybeetles in different intercropping patterns, as measured by direct counting on 1 m^2 of wheat tillers in each plot in 2007. A: Wheat monoculture; B: Wheat-garlic intercropping; C: Wheat-oilseed rape intercropping. (Wang et al. 2008)

(Fig. 6.4). After May 5, the mummy rate of aphid parasitoids and the ratio of aphid parasitoids to *S. avenae* in wheat-oilseed rape intercropping field were significantly higher than those in the other two fields (Fig. 6.5). It was concluded that wheat-oilseed rape, or wheat-garlic intercropping, could control *S. avenae* by enhancing the biological functions in wheat fields.

6.3.2 Wheat-Pea Intercropping Patterns

Wheat-pea intercropping patterns were conducted in winter wheat fields in Heibei province China. The intercropping patterns of wheat and pea, by the proportions of planting row of wheat and pea in 2:2, 2:4, 2:6 and 2:8 (referred to as 2-2 intercropping, 4-2 intercropping, 6-2 intercropping and 8-2 intercropping, respectively) were planted, and the field cultivar monoculture of wheat was planted as the control.

The intercropping of wheat with pea significantly reduced the numbers of *S. avenae* apterae and *S. avenae* alatae in the field, so that the density of aphids between 2-2 and 8-2 was the lowest (Fig. 6.6).

Intercropping significantly increased the population richness of natural enemies. The natural enemy diversity indexes of 2-2, 4-2, 6-2, 8-2 and 8-2 were respectively

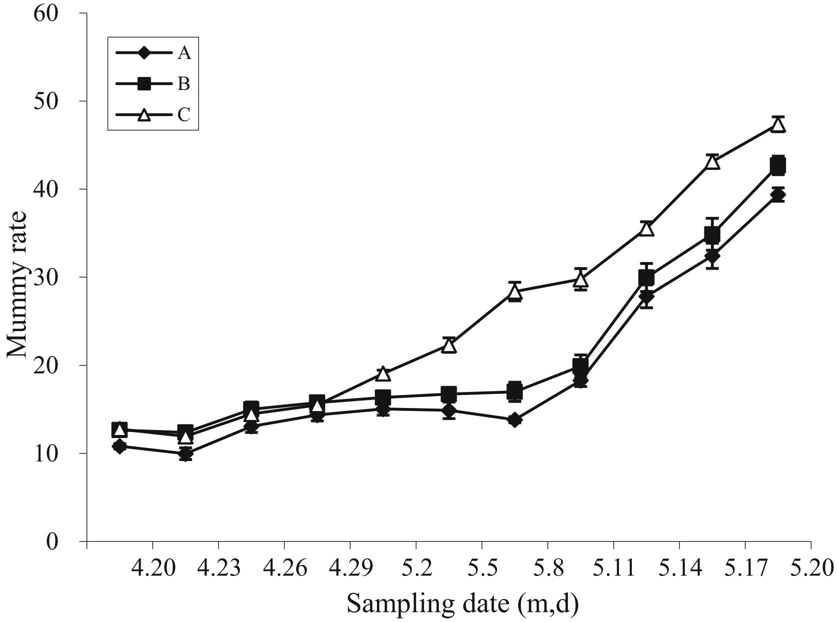

Fig. 6.4 Population dynamics of aphid parasitoids and mummy rate dynamics of *Sitobion avenae* (arcsin transformed) in different intercropping patterns. Aphid parasitoids were measured by direct counting of ten nets over 1 m^2 of wheat field in each plot in 2007. A: Wheat monoculture; B: Wheat-garlic intercropping; C: Wheat-oilseed rape intercropping (Wang et al. 2008)

3.0, 2.9, 3.0, 3.4 and 2.9, among which the value for intercropping with 8-2 was significantly higher than that for wheat. There was no significant difference among other intercropping treatments. Intercropping can increase crop yield (Table 6.2). In terms of yield per unit area of wheat, all intercropping treatments were significantly higher than that of wheat monoculture. Intercropping between 2-2 and 8-2 was significantly higher than 4-2 and 6-2.

The land equivalent ratio (LER) of intercropping between 2-2, 4-2, 6-2 and 8-2 are respectively 1.21, 1.15, 1.11 and 1.18, indicating that the intercropping between wheat and peas can clearly improve the land use rate, with increases from 11% to 21%. Therefore, the optimal model of wheat and peas in this study was 8-2 (Zhou et al. 2009a, 2013).

6.3.3 Wheat-Mung Bean Intercropping Patterns

Intercropping of wheat and mung bean was tested in winter wheat fields in Heibei province, China. It had a great influence on the number dynamics of *S. avenae.*

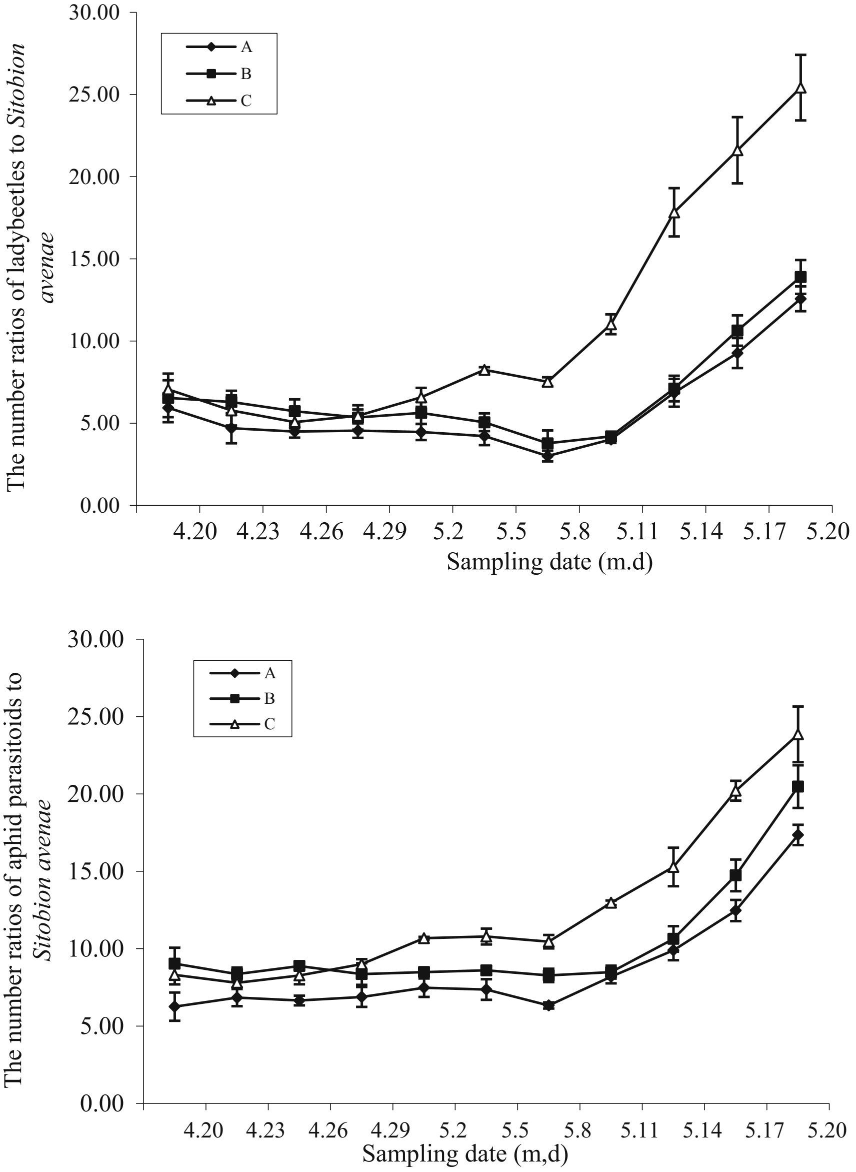

Fig. 6.5 Effects of different intercropping patterns on ratios of ladybeetles and aphid parasitoids to *Sitobion avenae* (arcsin transformed) in 2007. A: Wheat monoculture; B: Wheat-garlic intercropping; C: Wheat-oilseed rape intercropping. (Wang et al. 2008)

According to the total number of aphids, and peak aphid population during the whole survey period, compared with the control, was in the fields treated with the ratio of 12:4 (12 lines of wheat : 4 lines of mung bean)and 16 :4 (16 lines of wheat

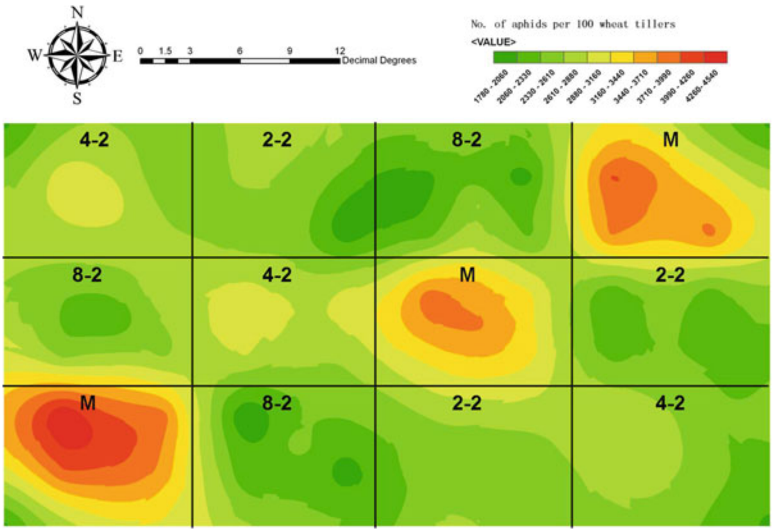

Fig. 6.6 GIS image of *Sitobion avenae* density estimates during their peak occurrence in 2008 (Zhou et al. 2009a; Fan et al. 2014). M = wheat monoculture; 2-2, 4-2 and 8-2 = different configurations of wheat-pea intercrops

Table 6.2 Crop yields (mean ± SE) and land equivalent ratio in different wheat-pea intercropping patterns

Intercropping pattern	Wheat yield (kg/ha)	Pea yield (kg/ha)	LER*
2-2 intercropping	6 940 ± 213 (3 239)aA	1 770 ± 171 (944)abA	1.187
4-2 intercropping	6 032 ± 190 (3 734) bB	1 822 ± 86 (694)abA	1.121
8-2 intercropping	6 197 ± 31 (4 695)bB	1 881 ± 87 (456)aA	1.148
Monoculture	5 448 ± 100cC	1 593 ± 136bA	

Data with small and capital letters indicate significant difference at $p < 0.05$, $p < 0.01$ level, respectively; the values in parentheses mean actual yields of crops per hectare

* land equivalent ratio

: 4 lines of mung bean), which significantly reduced *S. avenae* apterae populations. The number of *S. avenae* alatae was significantly reduced by row ratios of 12:6 and 12:4 (Xie et al. 2012).

The cluster index and Cassie index of aphids showed that the aggregation degree of intercropped wheat aphid was reduced. The number and species of natural enemies in intercropping fields increased. Intercropping also improved the wind speed and temperature in the wheat field to some extent, and reduced the relative humidity. It was found that the best intercropping ratio of wheat and mung bean was 16:4 (Xie et al. 2012).

6.3.4 Wheat-Wildflower Strips Intercropping

In Gembloux (Belgium), wildflower strips (WFS) were sown within wheat crops, in which pests (i.e. aphids), their predators (i.e. aphidophagous hoverflies, lacewings and ladybeetles) and parasitic wasps were monitored for 10 weeks from May through to July 2015, as indicators of ecosystem services for pest control. Aphids were significantly reduced, and adult hoverflies favoured in wheat between WFS, compared to wheat monoculture plots.

No significant differences were observed for adult lacewings, ladybeetles and parasitoids. In all treatments, very few lacewing and ladybeetle larvae were observed on wheat tillers. The abundance of hoverfly larvae was positively correlated with the aphid density on tillers between the WFS, showing that increasing food provision by multiplying habitats within fields, and not only along margins, can help supporting aphidophagous hoverflies in crops, enhancing the ecosystem services of biological pest control, this study shows that increasing both plant diversity and managing habitats for natural enemies may reduce aphid populations, hence insecticide use (Hatt et al. 2017).

6.4 Infochemical Releasers

In wheat production, both *S. avenae* and *R. padi* can cause economic damage, which necessitates routine insecticide use. Because of the urgent need for sustainable agricultural methods and reduced reliance on pesticide use, more integrated pest management studies are focusing on the ecological effects of volatiles released by plants on herbivores and their natural enemies (De Boer and Dicke 2004; James and Price 2004; James 2005; James and Grasswitz 2005; Yu et al. 2008; Lee 2010; Snoeren et al. 2010; Wang et al. 2011). Several studies on volatiles under natural conditions have demonstrated their applicability for enhancing natural enemy abundance on strawberry *Fragaria* × *ananassa* (Lee 2010), cotton *Gossypium* spp. (Yu et al. 2008), hops *Humulus lupulus* and grapes *Vitis vinifera* (James and Grasswitz 2005) and for reducing pest populations in wheat (Prinsloo et al. 2010) and barley (Ninkovic et al. 2015).

While attracting natural enemies of these herbivores (Turlings et al. 1990), volatiles emanating from herbivore-infested plants may also stimulate plant defense against herbivores, and serve as recognition cues between two or more individuals (Howard and Blomquist 2005). Dicke et al. (1990) presented the first convincing evidence that the active release of volatiles by herbivore-infested plants attracts natural enemies of the attackers (Dicke and Sabelis 1988; Dicke et al. 1990). Aphid behaviour is also affected by a density mechanism that is mediated by volatile compounds released at the feeding site, when their density exceeds a certain threshold (Ninkovic et al. 2015). A further study revealed that these volatiles could increase the sensitivity of aphids to disturbance, and promote mobility of nonsettled individuals (Pettersson and Quiroz 2011).

Because volatile compounds are a natural emission from plants, essential oils do not pose the toxicity problems of pesticides to animals and the environment (Park et al. 2010; Prinsloo et al. 2010). Plant infochemicals should be considered as potential, reliable infochemicals in relation to repelling pests and attracting natural enemies of these pests. Their long-distance effects and easy production and manipulation make these molecules very good candidates for use with crops by spraying, or by mixing with a slow-releasing carrier to repel insect feeding or ovipositing from host plants, and/or to guide them to non-hosts (Pickett et al. 1991).

Japanese termite *Reticulitermes speratus* (Isoptera: Rhinotermitidae) (Park and Shin 2005), sciarid fly *Lycoriella ingénue* (Diptera: Sciaridae)(Park et al. 2010)and pine wood nematode *Bursaphelenchus xylophilus* (Aphelenchida: Parasitaphelenchidae) (Park et al. 2005) were repelled by a garlic extract (GE), providing direct evidence that strongly aromatic crops such as garlic, can act as an olfactory camouflage by masking normal host-locating or feeding cues of insects, as well as of nematodes. (E)-β-Farnesene (EBF), an important volatile sesquiterpene that occurs widely in both plant and animal taxa, such as aphids (Francis et al. 2010) and peppermint *Mentha* × *piperita* (Crock et al. 1997), is an effective kairomone for ladybirds (Francis et al. 2004; Verheggen et al. 2007; Cui et al. 2012), lacewings (Zhu et al. 1999) and hoverflies (Raki et al. 2010). It is proven to be the main or only component of the aphid alarm pheromones for many pest aphids (Edwards et al. 1973; Pickett and Griffiths 1980; Raki et al. 2010; Vandermoten et al. 2012; Yu et al. 2012).

Herbivore induced plant volatiles (HIPVs), for example, (Z)-3-hexenol (Z3H), can directly affect the physiology and behavior of herbivores (Wei and Kang 2011). Z3H has been demonstrated to attract *Agrilus planipennis* (Coleoptera: Buprestidae) and the fruit moth *Cydia molesta* (Lepidoptera: Tortricidae) in the laboratory and in the field. Although it has been difficult to determine whether Z3H is an attractant or a repellent, accumulating evidence suggests that Z3H is an important plant-derived infochemical that can modulate the behavior of herbivorous insects, and that the release of Z3H induces defensive responses in plants against insect pests.

Extensive evidence implies that nearly all herbivorous insects and their natural enemies can perceive and positively respond to plant volatiles. Here we review how the essential oils of plant volatiles (EBF, GE and Z3H) were released in a wheat field to assess their potential for managing aphid populations, by reducing aphid abundance and promoting their natural enemies.

Infochemicals from plant essential oils were mixed with paraffin oil for slow release in field experiments on wheat to control the population density of cereal aphids, and to enhance their natural enemies. Zhou et al. (2016) showed that Z3H attracted *M. dirhodum* and *S. avenae*, the predominant species on wheat in Belgium, and that it may be a useful infochemical for aphid control by attracting aphids away from field plots. Release of EBF or a GE led to a signifcant decrease in the abundance of wheat aphids. The main natural enemies of cereal aphids found were lacewings (47.8%), hoverflies (39.4%), and ladybirds (12.8%). Ladybird abundance varied little before the end of the wheat-growing season. The results suggest that these chemicals conform the basis of a ‘push–pull’ strategy for aphid biological control by GE and EBF acting as a pest and beneficial insect pulling stimulus, and Z3H for aphid pulling (Zhou et al. 2016).

6.4.1 A Slow-Release Formulation of Methyl Salicylate and Its Application in Wheat Fields

Methyl salicylate (MeSA) is a herbivore-induced plant volatile that was reported to reduce *R. padi* colonization by altering plant metabolites, and the densities as well as the aggregation degree of *S.avenae* (Pickett and Glinwood 2007; Glinwood and Pettersson 2010; Wang et al. 2011; Dong et al. 2012a, b). A strongly repellent effect was found on *S. avenae* and on *R. padi* in laboratory tests (Guo and Liu 2005), while an attractive effect was observed on *C. sinica, C. septempunctata*, *H.axyridis*, *A. avenae* and *S. menthastri* (Han and Zhou 2004; Liu et al. 2005; Ma et al. 2008; Xie et al. 2014).

Since MeSA has a fast release rate in normal atmospheric temperatures, a slow-release formulation was needed for further field applications. Previous studies have confirmed that synthetic MeSA using a slow-release technique can effectively increase both the number and the residence time of recruited natural enemies. For instance, controlled-release sachets were used to apply synthetic MeSA in vineyards and hops. The result confirms that this method can effectively attract and hold some natural enemies (James and Price 2004).

Among the many slow-release formulations, an alginate bead carrier is one of the most efficient formulations (Heuskin et al. 2012a). Moreover, the United States Food and Drug Administration has defined it as ‘normally considered safe’ (George and Abraham 2006). The infochemical alginate beads were demonstrated to be an efficient slow-release system in biological control when formulated with E-β-carophyllene and E-β-Farnesene (Heuskin et al. 2012a, b).

6.4.1.1 Release Rate of MeSA Alginate Bead

The MeSA of the slow-release beads has a slow release rate at the beginning, and then reaches the maximum volatilizing rate. The maximum release rate of beads continue uniformly for approximately 15 days, while the release rate of pure MeSA (control) fades off in a week. There was still a small amount of MeSA emission after the last collection (from 15 to 25 days) (Fig. 6.7).

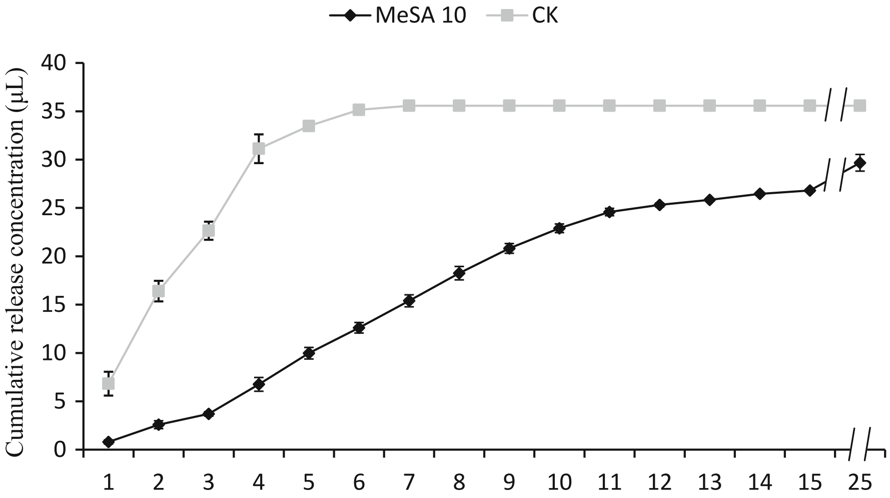

Fig. 6.7 Cumulative release concentration of methyl salicylate (MeSA) from 10 mL beads during 25 days in laboratory conditions. CK refers to pure MeSA as the control

6.4.1.2 Effects of Concentration and Distance

In the 2015 winter wheat field experiment in Shandong province, China, the released concentration of MeSA had a significant effect on the apterous *S. avenae*, alatae *S. avenae*, and *M. corollae*. However, the distance had no significant effect within 10 m of the release point. No interaction was found between concentration and distance (Wang et al. 2018).

6.4.2 Effect of MeSA Concentration on Abundance of S. avenae and M. corollae

Two different MeSA concentrations with beads were tested: MeSA 2.5 and 10 for 2.5 mL and 10 mL formulation rates of MeSA respectively, and control for releaser, free of MeSA. In the MeSA treatments, the apterous and alatae *S. avenae* tended to have similar abundances. Plots treated with MeSA 10 beads had lower abundance of *S. avenae* than MeSA 2.5, and the control plots. Both MeSA 2.5 beads and MeSA 10 beads could significantly reduce the abundance of apterous *S. avenae* on wheat plants. In addition, the MeSA 10 beads had a significant reduction effect on alatae *S. avenae* compared with MeSA 2.5 beads (Fig. 6.8).

The number of *M. corollae* in the yellow pan traps significantly varied according to the formulation concentration. The total numbers of *M. corollae* in treated plots with MeSA 2.5 beads and MeSA 10 beads throughout the experimental period were

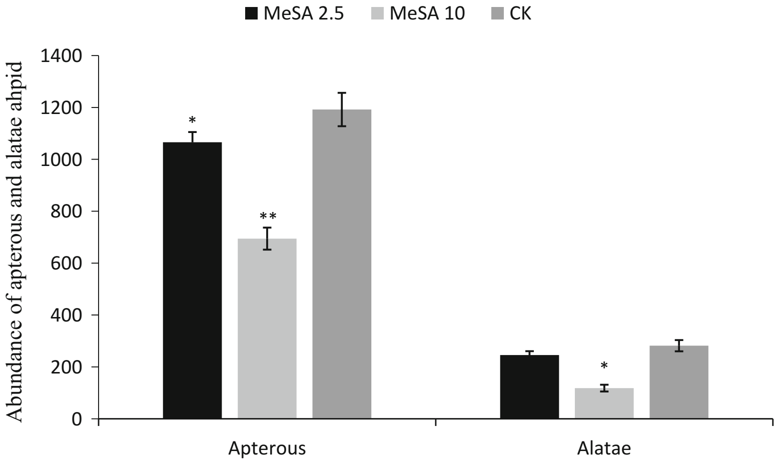

Fig. 6.8 Effects of methyl salicylate (MeSA) slow release formulations on apterous and alatae *Sitobion avenae* total abundance for the whole experimental duration in 2015 (Wang et al. 2018). MeSA 2.5 and 10 stand for 2.5 mL and 10 mL formulation rates of methyl salicylate, respectively, CK for control releaser free of MeSA. * and ** indicate significant differences ($p < 0.05$, $p < 0.01$, respectively)

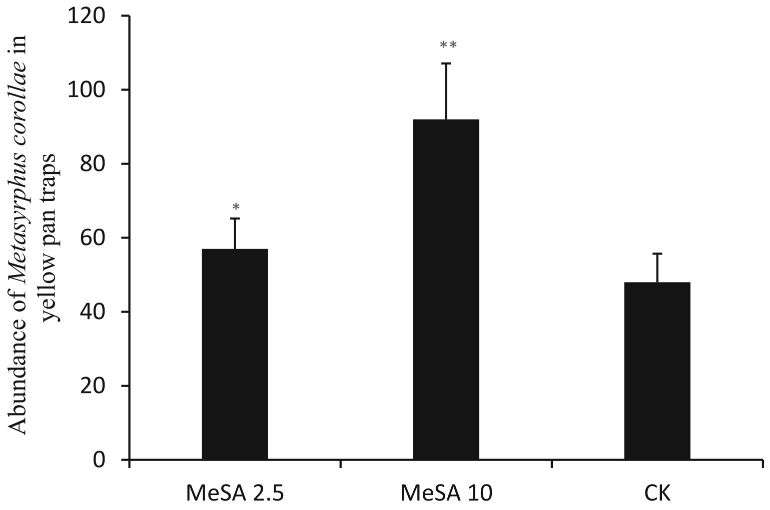

Fig. 6.9 Effects of methyl salicylate (MeSA) slow release formulations on *Metasyrphus corollae* total abundance in yellow pan traps for the whole experimental duration in 2015 (Wang et al. 2018). MeSA 2.5 and 10 for 2.5 mL and 10 mL formulation rates of methyl salicylate, respectively, CK for control releaser free of MeSA. * and ** indicate significant differences ($p < 0.05$, $p < 0.01$)

significantly higher than those observed in the control plots. Moreover, the MeSA 10 beads attracted more *M. corollae* than the MeSA 2.5 beads (Fig. 6.9).

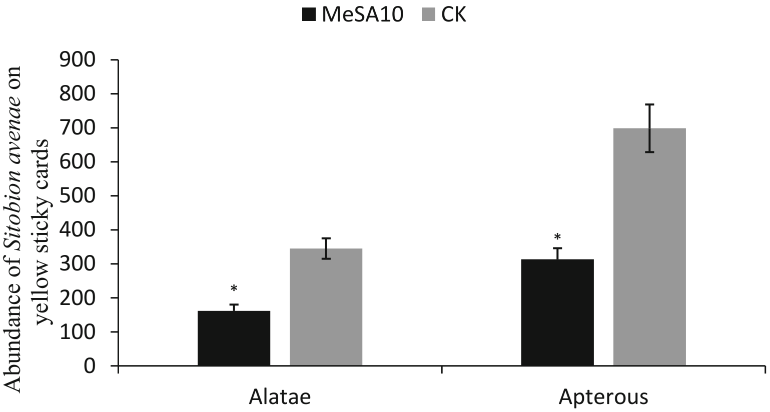

Fig. 6.10 Effects of methyl salicylate 10 mL slow release formulation (MeSA10) on apterous and alatae *Sitobion avenae* total abundance for the whole experimental duration in 2016 (Wang et al. 2018).* indicates significant differences ($p < 0.05$)

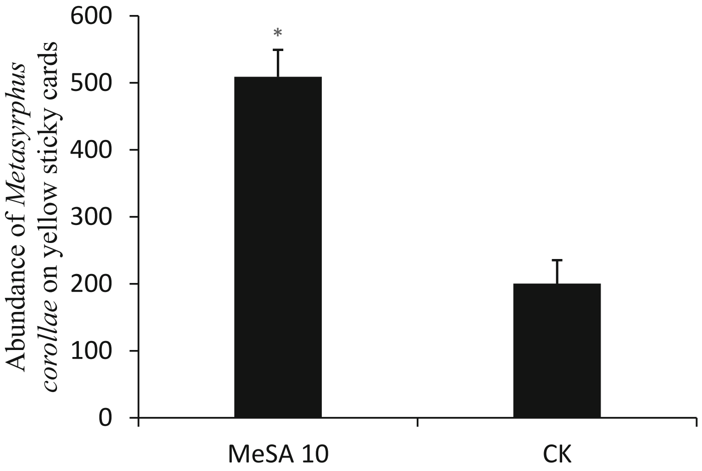

Fig. 6.11 Effects of methyl salicylate 10 mL slow release formulation (MeSA10) on *Metasyrphus corollae* total abundance on yellow sticky cards for the whole experimental duration in 2016 (Wang et al. 2018). CK for control releaser free of MeSA, * indicate significant difference ($p < 0.05$)

In the second year of the experiment, the population dynamics of apterous and alatae *S. avenae* treated with MeSA 10 beads were similar to the control. The cumulative numbers of alatae *S. avenae* on the yellow sticky cards and the apterous *S. avenae* on the 100 wheat plants in the plots treated with MeSA 10 beads were significantly lower than those in the control (Fig. 6.10).

The cumulative number of *M. corollae* on the yellow sticky cards was significantly higher than that in the control (Fig. 6.11).

6.5 Intercropping in Combination with Infochemical Releaser

Increasing plant diversity such as intercropping in wheat fields is as an alternative approach to control wheat aphids (Zehnder et al. 2007; Wang et al. 2008, 2009; Zhou et al. 2009a, 2013; Xie et al. 2014). Spatial diversification of cropping systems can disrupt the ability of pests to locate their host plant, increase pest mortality, or repel it (Poveda et al. 2008). Moreover, chance of pests to be suppressed by their natural enemies increase in diversified systems. Indeed, the development of natural enemies can be favoured by the higher diversity of prey, hosts and microhabitats found in complex systems.

Another tactic is to release infochemicals to attract natural enemies and/or to repel pests (Khan et al. 2008). Such infochemicals are numerous in the environment. Some are produced by plants, e.g. HIPVs when plants are attacked by herbivores (Kessler and Baldwin 2001), others by insects, e.g. sex or alarm pheromones (Vandermoten et al. 2012; Fassotte et al. 2016). Once identified as pest repellent and/or natural enemy attractant, they can be synthesised and released in fields for enhancing biological control (Rodriguez-Saona et al. 2011; Cui et al. 2012; Nakashima et al. 2016).

In China, intercropping is a common practice in wheat and rice fields (Knörzer et al. 2009). Of the about 50 studies conducted on wheat based intercropping systems for biological control, almost half have been conducted in China (Lopes et al. 2016). Moreover, they show that, overall, pests are reduced in intercropping systems, but their natural enemies are not necessarily enhanced, contradicting the enemy hypothesis of Root (1973). In order to attract natural enemies, two tactics should be considered. First, flowering plants can be introduced. Indeed, flowers can attract and support a diversity of natural enemies that need floral resources (i.e. pollen, nectar) at some of their developmental stages (Lu et al. 2014). Studies show that implementing wildflower strips (Hatt et al. 2015) or companion plants (Balmer et al. 2014) within crop fields can increase the presence of some natural enemies, and reduce pest density and damage. In intercropping systems, one of the combined crops can be a flowering species such as oilseed rape, known to be visited by insects such as hoverflies (Jauker and Wolters 2008). Second, intercropping can be combined with the release of infochemicals to attract natural enemies. Wang et al. (2011) reported a positive effect of wheat-oilseed rape intercropping, combined with the release of MeSA (i.e. a HIPV) on the suppression of the wheat aphid *S. avenae*, and on the increase of ladybeetles and parasitism rates. Fassotte et al. (2014) identified the sex pheromone of the ladybeetle *H. axyridis*, which is a main aphid predator native to China (Brown et al. 2011). The sex pheromone is emitted by females to attract males. In laboratory conditions, a synthetic blend composed of the identified compounds significantly attracted ladybeetle males (Fassotte et al. 2014). In field conditions, an experiment was conducted in Belgium to test the attractiveness of this infochemical, but the results were inconclusive because of a general lack of ladybeetles in the field (Fassotte et al. 2016).

6.5.1 Wheat-Oilseed Rape Intercropping with Infochemical Releaser

Wheat-oilseed rape intercropping in combination with a HIPV MeSA releaser is a successful example. Release technique of MeSA by a specific slow-release device: drop MeSA into a sponge-filled polyethylene box (6.5 cm in diameter, 4 cm in height, with four 2 cm^2 holes in the box) and fix it with a wooden rod at a height of 1 m above the ground. Place one device in the center of each plot, and apply MeSA at the rate of about 120 mg/m^2/7 days.

In an experiment in the wheat intercropping area, two rows of rapeseed were planted for every eight rows of wheat, with a spacing of 40 cm. The control effect of biological control index (BCI) on wheat aphid population was quantitatively evaluated. BCI refers to the ratio of aphid number differences between the last time of the experiment, and the first time of the investigation. The higher the BCI is, the stronger is the control effect. The integrated application of two technologies including intercropping (wheat-oilseed rape), and the active volatile MeSA continous release, has been carried out. In oilseed rape intercropping, in combination with MeSA treatment, the number of apterous *S. avenae* reached the peak about 12 days ahead of the wheat monoculture plots, but the peak value was significantly lower than that in the wheat monoculture plots (Dong et al. 2012a, b). The amount of apterous aphids on 100 wheat plants was hightest in wheat monoculture area, followed by intercropping area, MeSA treatment area, and lowest in intercropping combined with infochemical release. Intercropping with infochemical releaser plots had the highest abundance of ladybugs. The peak of parasitic wasps occurred about 10 days ahead of the wheat monoculture plots, which had an obvious control effect on aphids in the wheat filling period, and effectively inhibited the population growth of wingless aphids, and significantly reduced the aggregation degree of aphids with a tendency of uniform distribution. The biological control index was used to quantitatively evaluate the biological control of aphids. From the time of wheat heading to the grain-filling stage, the treatment integrating two factors could effectively inhibit the population growth of wingless aphids. Therefore, the integrated application of MeSA release and wheat–oilseed rape intercropping has great potential in improving aphid control and optimizing ustainable pest smanagement strategies (Fan et al. 2014).

In order to test the effectiveness of combining intercropping of wheat and oilseed rape with the release of MeSA, it has been reported that maximum and mean aphid densities are highest in wheat monocultures, significantly lower in intercropped plots and in MeSA plots, and lowest when intercropping and MeSA release were combined, which resulted in highest densities of predatory lady beetles and parasitoids. Importantly, grain yield and quality showed a similar pattern: they were highest for combined intercropped MeSA plots, intermediate in plots with either intercropping or MeSA alone, and lowest in monoculture control plots. The results suggest that combining these two tactics holds significant promise for improved management of aphid populations, and emphasize the need to integrate alternative pest control approaches to optimize sustainable insect pest management (Wang et al. 2011).

A field investigation on the spatial distribution of *S. avenae* and its main natural enemies under wheat-oilseed rape intercropping and MeSA application was conducted by Dong et al. (2012a, b).The result show that with the development of the wheat plant, an change from aggregation to uniform occurred in the spatial distribution of *S. avenae* in the intercropping and MeSA application. This was most obvious under the combination of these two practices.The spatial distribution of *S. avenae* natural enemies was in accordance with that of the aphid.These results can be used for improving sampling and forecasting of wheat aphids and their natural enemies in the field.

6.5.2 Wheat–Pea Intercropping with Infochemical Releasers

While intercropping alone may not enhance the activity of pest natural enemies, the use of infochemicals alone in monocultures may not be consistently successful, and may even negatively influence natural enemies in low pest density situations (Wang et al. 2011). Combining infochemicals with intercropping may bridge these problems. Particular attention has been paid to intercropping wheat with pea *Pisum sativum* as a way to reduce nitrogen inputs (Bedoussac and Justes 2010). Moreover, wheat–pea strip intercropping was reported to decrease pea aphid *Acyrthosiphon pisum* (Hemiptera:Aphididae) and *S. avenae* populations, when compared with pure stands, although aphid natural enemies were not particularly attracted (Ninkovic et al. 2003; Zhou et al. 2009a; Lopes et al. 2015).

In the study of Xu et al. (2017), a 2-year setup involving wheat–pea strip intercropping combined with the release of E-β-farnesene (EBF) or MeSA was tested as a push–pull strategy, to simultaneously repel aphids and to attract beneficials. Two types of slow-release formulations (i.e., oil and alginate beads) containing EBF or MeSA were deployed with the intercropping. The abundance of aphids was significantly decreased, while the numbers of hoverfly larvae and mummified aphids increased both on pea plants and on wheat tillers, by the release of oil-formulated EBF and MeSA. The proportion of parasitism of the aphids was also increased by treating both crops in both years. Releasing EBF through oil, rather than in alginate beads, proved significantly better for attracting natural enemies and for reducing aphids. Aphid numbers were negatively correlated with the density of hoverflies (both adults and larvae), and the number of mummies. These results show that combining intercropping wheat and peas with the release of EBF or MeSA formulated in oil, can significantly reduce aphid densities, and attract their natural enemies (Xu et al. 2017).

6.6 Food Web Analysis in Wheat Ecosystem with Focus on Wheat Aphids and Their Natural Enemies

Food webs can be used to describe trophic interactions, and to examine the potential for indirect interactions, such as apparent competition (Cohen et al. 1990; Polis and Winemiller 1996). Quantitative food webs consist of sets of binary links between ‘trophic species’ representing feeding interactions. Most early studies of food webs traced the presence or absence of trophic links between trophic species. On the other hand, some field research constructed food webs that include quantitative information on trophic interactions, and provide information on the potential for apparent competition.

Quantitative webs are used to assess the degree to which the community is divided into subunits that are isolated, or weakly connected, with the rest of the food web, and whose dynamics may be partly or wholly independent. Predator overlap graphs are used as a means to assess the potential for apparent competition (indirect interactions via natural enemies (Holt 1977; Holt and Lawton 1994), and to compare its importance at different trophic levels. An extension of predator overlap graphs has been developed to make use of the quantitative information available for the study community.

Chi et al. (2014) conducted a quantitative food web analysis of aphids and its natural enemies in wheat-oilseed rape, and wheat-garlic intercropping regimes (Fig. 6.12). The results show that the densities of *S. avenae* apterae were significantly lower in wheat-oilseed rape intercropped fields, and in wheat-garlic intercropped fields, than in wheat monocultures during almost the entire wheat filling stage. There were higher population densities of ladybeetles in the wheat-oil-seed rape intercropped fields. Population densities of aphid parasitoids were significantly higher in wheat-oil-seed rape intercropping systems during the wheat earing and flowering stages. In wheat-garlic intercropped fields, the numbers of *S.avenae* alatae was higher, but no effects were detected on the population densities of ladybeetles and aphid parasitoids. The quantitative food web analysis showed that, although the effectiveness of control by green lacewings and syrphid flies differed under the three different planting regimes, that by ladybeetles and parasitoids was almost the same (Table 6.3).

6.7 Prospective

Wheat aphids are typical hemipteran insects that suck vascular bundle juice. Although green control of wheat aphids is powerful and effective, its potential still need to be exploited far more. There are many new technologies that may help to develop and improve green control of wheat aphids in the future. We hope that this review could increase awareness of the available strategies for better control

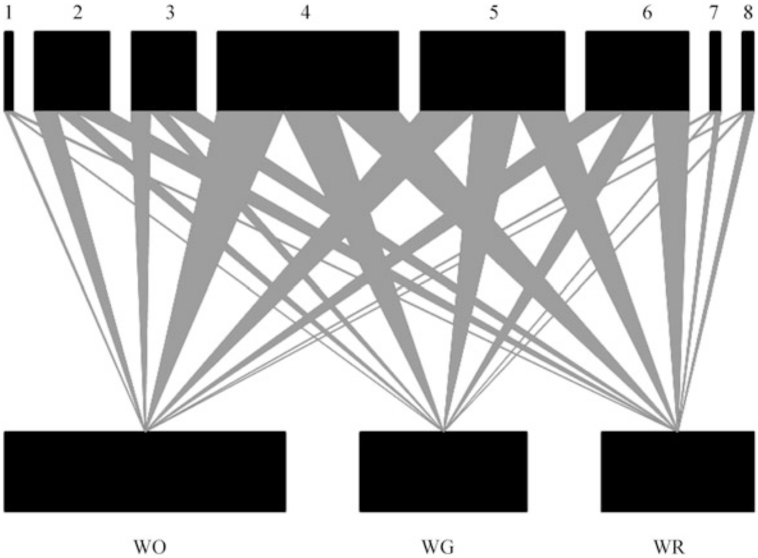

Fig. 6.12 Quantitive food web graphical presentation of the trophic interactions between *Sitobion avenae* and its natural enemies on different intercropping patterns (Chi et al. 2014). The top bars represent natural enemy abundance; the lower bars represent aphid abundance. The numbers above bars represent the codes of food web analysis (Table 6.3); WO, WG and WR represent wheat monoculture, wheat intercropping with garlic, and wheat intercropping with oilseed rape, respectively

of wheat aphids through intercropping and/or infochemical release, and inspire research for their further development and widespread use.

Previous studies show that wheat-based intercropping systems make it possible to reduce pest occurrence on crops. Management and technical issues are central for developing intercropping systems. Indeed, phenological and spatial constraints of crop species must be taken into account when selecting viable combinations. The flower stage and colour of the plant, and the quality and quantity of nectar and pollen, should be considered as the most important characteristics of a candidate crop for intercropping. Competition for resources (i.e. light, water, nutrients) (Thorsted et al. 2006), as well as allelopathic effects (Khan et al. 2002) may limit the possibilities, and determine whether the crop associations will work. Appropriate machines are also needed to sow, harvest and separate grains in mixed cropping (Lithourgidis et al. 2011a). However, the management of strip and relay intercropping systems may be facilitated, as two or more crops may be separately managed. Also, the size of the strips and the ratio between the associated crops can be adapted depending on the farmer's production objectives and agronomic constraints. Furthermore, intercropping patterns such as strip intercropping related to how many lines of wheat and other crops, and mixed intercropping associated with the area of the mixed crops, need to be clear depending on their effects on the aphids and other biological control agents, as well as yields. It also needs be

Table 6.3 *Sitobion avenae* and its natural enemies with their respective population densities in the quantitative food web analysis (Fig. 6.12). The numbers in brackets represent the codes used in the food web analysis.

Family	Species	Population density		
		WO	WG	WR
Coccinellidae	*C. septempunctata*(1)	2.7	1.7	3.8
	H. axyridis(2)	21.1	18.8	33.6
	P. japonica(3)	19.6	16.4	28.9
Braconidae	*A. gifuensis*(4)	49.7	41.7	49.3
	A. avenae(5)	40.6	36.1	35.7
Aphelinidae	*Aphelinus* sp.(6)	28.7	23.2	28.0
Chrysopidae(7)	*C.carnea*	3.2	1.8	5.6
	C. sinica			
Syrphidae(8)	*Z. balteata*	2.2	2.4	7.1
	S. ribesii			
Aphididae	*S. avenae*	6 392.1	3 784.2	3 411.9

WO, WG and WR represent wheat monoculture, wheat intercropping with garlic, and wheat intercropping with oilseed rape, respectively. The numbers in brackets represent the codes of food web analysis. The units of population density for ladybeetles and green lacewings are numbers per one square meter, and for parasitoids numbers of 10 nets in one square meter (Chi et al. 2014)

considered that the area surrounding the fields should preferably be diversified agroecosystems that sustain the successful overwintering of beneficials.

Infochemicals can manipulate the behavior of not only the aphids, but also their of natural enemies. Apart from some plant volatiles (e.g. Z3H) and HIPVs such as MeSA, as well as some insect pheromones including aphid alarm pheromone EBF, only few functional infochemicals have been screened, let alone put them into practice in agriculture for pest insect control. We suggest that intensive laboratory experiments and field tests should be made by researchers to find a larger number of potential molecules with high ecological functions that can be applied to optimize the conventional aphid control programs in the field.

Some shortcomings, such as short persistency period and low stability of some infochemicals, must be solved before they can be efficiently used for field applications. In order to promote the utilization efficacy of the behavioral manipulations, slow-release and stable formulations need to be manufactured, for example, alginate beads for MeSA release was used in wheat fields in China (Wang et al. 2018). The devices for infochemical release associated with monitoring should be delicately designed. They must meet the demands such as being practical in field, affordably priced, and highly efficient for volatile release.

Finally, entomologists, agronomist, ecologists, chemists, government officials, and farmers need to work together to develop and to demonstrate the new strategies and tactics to significantly reduce pesticide use on crops, and to increase incomes to satisfy the ecological, economic and social constraints on the farms.

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Chapter 7
Agricultural Biodiversity for Crop Disease and Pest Management

Chengyun Li, Jing Yang, Xiahong He, Shusheng Zhu, and Youyong Zhu

7.1 Introduction

The purpose of sustainable agriculture is to guarantee good crop quality and yields, while maintaining economical and environmentally friendly production modes that are beneficial to society and preserve resources for future generations. There are various strategies available for achieving such sustainable agriculture, with the simplest being those that seek to effectively manage and maintain biodiversity while minimizing any negative effects on affected ecosystems. Examples of such strategies include reductions in fertilizer use, minimizing the consumption of non-renewable resources, and preventing local environmental degradation. In practice, agricultural producers need to determine what strategies best fit with their local needs, in order to manage their local ecosystems in regard to three aspects: genetic diversity, species diversity, and ecological diversity.

There has been much discussion in recent years regarding the protection and utilization of biodiversity as a means of ensuring food safety, with a significant focus being placed on controlling diseases and managing insect pests, while maintaining biodiversity. Research in this area largely focuses on biodiversity from the perspective of ecological principles including genetic diversity, species diversity, and ecological diversity in order to investigate the interactions of crops and pathogens, and to elucidate the effects of agricultural biodiversity on diseases and insect pests. Much of this work has concluded that biodiversity has the potential to be an effective means of disease and pest management. This chapter will systematically elaborate on the key technologies and successful examples of the application of these principles of the three levels of biodiversity to disease and pest control, as developed at the Yunnan Agricultural University.

C. Li (✉) · J. Yang · X. He · S. Zhu · Y. Zhu
Yunnan Agricultural University, Kunming, Yunnan, China

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7.2 Genetic Diversity and the Control of Crop Diseases and Pests

Historically, many major instances of crop disease outbreaks or serious pest problems arose due to the large-scale cultivation of a single crop in a specific area, resulting in substantial economic losses. For example, the Irish potato famine in the 1840s resulted from a late blight pandemic caused by the large-scale cultivation of a single potato variety (Zeng 2005). Examples of serious economic losses caused by such large-scale single crop cultivation are numerous. As such, there are clear consequences of the widespread cultivation of a single plant variety, with the limited genetic diversity of many popular crops with good agronomic traits having lost important disease-resistance traits, resulting in a loss of genetic diversity in the agricultural production system.

Rice diseases are one of the main factors hindering high and stable rice yields, with the cultivation of disease-resistant varieties being the most economical and effective control measure. This approach has played an important role in the global rice disease control (Bonman et al. 1992; Mew 1991). However, as pathogens can rapidly evolve and undergo substantial variation, disease-resistance breeding cannot be an effective long-term strategy for controlling crop diseases. As such, chemical control is still the most commonly used method in production, but it can readily leave residues of concern on crops. Additionally, it further drives the deterioration of the local farmland ecology (Yamaguchi 2004), and can promote fungicide resistance in virulent races of the pathogen. Given such substantial negative impacts, chemical control is undesirable as a means of controlling rice diseases, necessitating the development of a safe, effective and sustainable crop disease management strategy.

Given the complexities of farmland ecology, the challenges of single crop species cultivation in large areas are becoming increasingly prominent. Taking rice genetic diversity for disease control as an example, and with the goal of ensuring high and stable yields of rice and food security, the author therefore proposes that disease resistance research should not only focus on a specific disease, but should also seek to explore and utilize the full range of diversity and disease resistance mechanisms, thus optimizing the allocation of genetic resources throughout the macro-ecosystems of rice fields. In order to make full use of the diversity of rice resistance resources, to increase the biodiversity of rice fields, to maintain ecological balance, and to achieve the goal of long-term disease control, it is important that the breeding of diverse rice varieties and the optimizing of crop cultivation should be carefully conducted.

7.2.1 Introduction for Rice Genetic Diversity Controlling Rice Blast Disease

Rational rotation of resistant rice varieties implies to utilize diversity of resistance genes in time, that is, novel resistant variety carrying different resistance genes

Fig. 7.1 Intercropping of two rice cultivars with different genetic background. Intercropping of high-stalk cultivar (one row) with dwarf culitivar (four rows). (Photo credit: Zhu et al. 2000)

replaces the varieties that were susceptible to the pathogen in the field. Many studies to date have assessed the efficacy of such rotation on controlling rice blast disease. For example, rational rotation of resistant rice varieties effectively controlled rice blast disease in Luxi, China (Wang et al. 1998). This rational approach to deploy resistant varieties in time and space can increase the diversity of resistance genes, and reduce the directional selection pressure placed on pathogens, thus reducing the risk of an epidemic disease outbreak. Researchers throughout the world are therefore aiming to increase the variety of available resistant cultivars, and to find new ways to apply the genetic diversity of such disease-resistant varieties to control rice diseases in a continuous fashion. Researchers at Yunnan Agricultural University carried out in-depth research on controlling rice blast disease by using rice genetic diversity (Fig. 7.1) (Zhu et al. 2000). By analyzing the genetic diversity structures of a large number of rice varieties, the optimum combination of different rice varieties was screened. These optimum combinations were deployed in the field, and to analyze the effects of combination of different varieties and different cultivating patterns on rice blast disease in an in-depth fashion. This research lasted for 9 years, Taking utilization and protection of genetic diversity of rice as the goal, the studies on co-evolution and molecular basis of rice varieties and pathogens were carried out, especially focusing on ecological functions and principles of rice genetic diversity to control the rice blast disease. By initially establishing the optimal parameters in a small-scale setting, and then expanding that to a larger format, the epidemic of rice blast was effectively controlled through the leveraging of genetic diversity. This approach was able to increase both the yield and the benefit per unit area, while the amount of pesticides used was decreased, and the in-situ protection was achieved. This successful conservation effort provides an example of the effective use and protection of biodiversity as a means of promoting food security.

7.2.2 Key Technologies for Rice Genetic Diversity to Control the Rice Blast Disease

Understanding the genetic differences among rice varieties is the basis for genetic breeding and is the primary goal of rational deployment of crops. It is important that genetic differences affecting rice plants at the morphological, cellular, biochemical, and molecular levels, are all considered, and further attention should be paid to the homologous sequences of identified disease-resistance genes. It is also important to consider the key agronomic characteristics of rice varieties of interest, such as plant type structure, plant height, growth period, and yield components. These parameters should all be considered in the context of a spatio-temporal deployment.

The main principles of techniques controlling rice genetic diversity include:

1. increasing the abundance of genetic diversity, reducing the directional selective pressure on pathogens, reducing the rate of formation and growth of dominant virulence races of pathogen, and prolonging the durability of disease-resistant varieties;
2. optimizing the arrangement and combination of planting groups, forming an effective three-dimensional plant community, enhancing ventilation and light transmission, reducing field humidity and dew area, and improving farmland micro-ecology in a manner conducive to crop but not pathogen growth;
3. enhancing physical barriers to pathogens, diluting localized concentrations of pathogens, and reducing the range and distance of pathogens transmission;
4. promoting complementary utilization of water, nutrients, light and other resources among different rice varieties, thereby enhancing plant growth, strength, and stress resistance.

Variety Combinations

Genetic difference parameters of varieties: Similar genetic distance (RGA technical parameter) among different resistant varieties cultivated in fields is less than 0.75.

Phenotypic parameters: short-stalk varieties and high-stalk varieties can be planted together, with high-stalk varieties being about 30 cm higher than that of short-stalk varieties; maturation rates should not differ by more than 10 days.

High-yielding and high-quality varieties: to meet demands for high-yield and high-quality varieties, planting of such varieties must be prioritized. At present, high-yield short-stalk hybrid rice is the main cultivar, and high-quality high-stalk local traditional cultivars are used as intercropping varieties.

Sowing and Raising Seedlings

Adjustment of sowing date: Sowing dates should be adjusted according to different growth periods of primary and intercropped varieties, with seedlings being sown in stages. Early-maturing varieties should be sown late, and late-maturing varieties should be sown early, resulting in similar maturation and harvest dates.

Raising seedling technologies: To cultivate strong seedlings and to improve the quality of seedlings, targeted breeding of suitable rice varieties is needed. For sowing and cultivation, the seedlings should be seeded sparsely.

Cultivation Patterns

Transplanting approaches: With regard to traditional strip planting, a row of high-yield and high-quality rice should be added in the middle of rows of high-yield and short-stalk varieties, every 4–6 rows (via throwing seedling transplanting, planting 1 row of high quality rice as seedlings every 1.2–1.5 m).

Field Management

Fertilizer and water management: According to conventional high-yield measures.

Disease and pest management: Carefully monitor diseases and pests; pesticides are not required for leaf blast; panicle blast disease is managed using fungicide, if necessary.

Harvest Management

Mechanical harvesting: The first harvest of high-stalk varieties is artificial, while the main dwarf varieties are harvested mechanically. Artificial harvest: according to the needs of farmers; different varieties can be harvested or harvested in a mixed fashion.

7.2.3 *Primary Economic Benefits of Controlling Rice Blast Disease Using Rice Genetic Diversity*

The technology of utilizing rice diversity to control the blast disease is remarkably effective in increasing crop yields. Since 2001, the results of such approaches have been applied in 11 provinces, including Yunnan and Sichuan. Statistics show that the incidence of rice blast disease in susceptible and high-quality rice varieties was less than 5% on average, while the effective control rate of rice blast disease was 81.1%–98.6% (Fig. 7.2). Lodging resistance was 100% through the use of this strategy, the yield per hectare increased by 630–1040 kg, and the average income per hectare increased by more than 1000 Yuan. As such, good economic, social, and ecological benefits were obtained via this approach (Zhu et al. 2000; Zhu 2007).

Applications of this approach in the Philippines, Indonesia, Thailand, Vietnam, Laos and other countries have shown rice blast disease control rates of 71–96%, with an average of 60.5% reduction in pesticide applications, lodging resistance rates of 95–100%, average yield increases of 600–1092 kg per hectare of high-quality

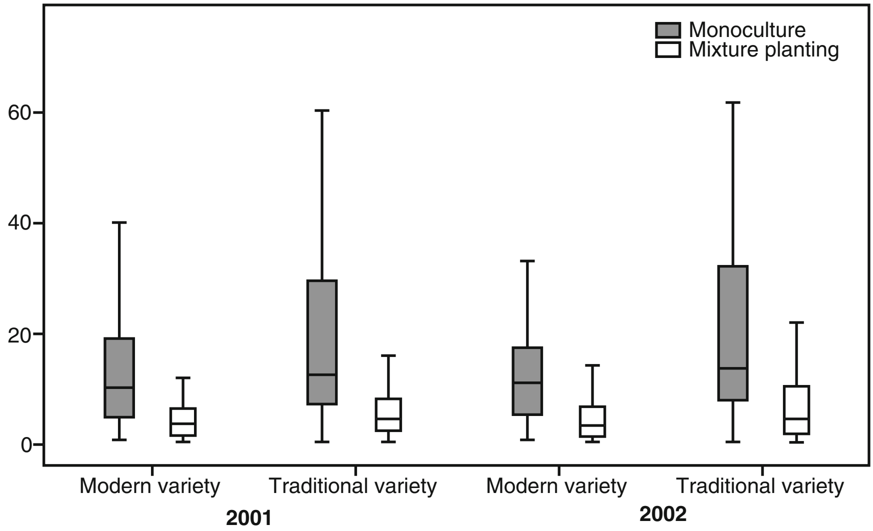

Fig. 7.2 Variation in the rice blast disease index using different cultivation methods in 2001 and 2002. The three lines of the rectangular frame represent the percentiles 25, 50, 75, highest line is the maximum value, and lowest line is the minimum value, line in the middle represents the median. (Figure adapted from Zhu 2007)

grain, and a cumulative production of more than 1.4 billion Yuan in socio-economic benefits.

7.3 Crop Species Diversity for Controlling Diseases and Pests

Intercropping, "growing two or more crops simultaneously in the same field", is a common agricultural practice in the tropics and in the developing countries of the world (Vandermeer 1989). Intercropping using different crop species is an effective means of increasing farmland diversity in organic agriculture settings, and it is an important approach to sustainable agriculture. Many cereal-legume intercropping patterns have been developed in Europe. In temperate regions, there is a long tradition for intercropping of clover-grass pastures for grazing and silage, and pea-barley mixture for silage (Hauggard-Nielsen and Jensen 2001). Further, faba bean (*Vicia faba L. sub. minor*) is cultivated in a two-year rotation with winter wheat (*Triticum durum L.*) for hay production (Pristeri et al. 2006). In addition to increasing productivity, intercropping of different species can also increase crop yields, organic matter content in soil, promote the circulation of organic matter, improve fertility and water use efficiency, reduce soil erosion, inhibit the occurrence of diseases and pests (Table 7.1), and increase the ecological services of a farmland system. This functional diversity thereby promotes ecological

Table 7.1 Examples of pest insect control by diversified intercropping

Crop combinations	Target pest insects
Maize-soybean	*Popillia japanica*
	Epilachna varivestis
	Helicoverpa armiger
	Stem borer
	Microtermes spp.
Maize-haricot bean intercrop	*Chilo partellu*
Maize-cowpea intercrop	*Chilo partellu*
	Chilo orichalcociliellus
	Sesamia calamistis
Maize-cassava intercrop	Stem borer
Maize-peanut intercrop	*Microtermes* spp.
Maize-molasses grass intercrop	*Chilo partellus*
	Busseola fusca
Sorghum-soybean intercrop	*Popillia japanica*
Sorghum-lablab intercrop	*Chilo partellus*
Sorghum-cowpea intercrop	*Chilo partellus*
	Megalurothrips sjostedti
Sugarcane-greengram companion cropping	Sugarcane borer
Sugarcane-sunnhemp companion cropping	Sugarcane borer
Sugarcane-maize intercrop	*Ceratovacuna lanigera*
Field bean-wheat intercrop	*Empoasca fabae*
	Aphis fabae
Field bean- *Ocimum basilicum* intercrop	*Aphis faba*
Broad bean-wheat intercrop	*Liriomyza huidobrensis*
Wheat-cotton relay intercrop	*Aphis gossypii*
Wheat-garlic intercrop	*Sitobion avenae*
Wheat-oilseed rape intercrop	*Sitobion avenae*
Wheat-wheat (varieties of different resistances) intercrop	*Sitobion avenae*
Cabbage-tomato intercrop	*Plutella xylostella*
Cabbage-sweet pepper intercrop	*Plutella xylostella*
Cabbage-peanut intercrop	*Plutella xylostella*
Cabbage-cowpea intercrop	*Plutella xylostella*
Broccoli-yellow sweet colver interplant	*Artogeia rapae*
	Hellula undalis
Cabbage-red clover intercrop	*Delia floralis*
Collard-bean intercrop	*Plutella xylostella*
Collard-onion intercrop	*Plutella xylostella*
Oat-clover intercrop	*Oscinella frit*
Carrot-onion mixed crop	*Psila rosae*
	Thrips tabaci
Tomato-celery intercrop	*Trialeurodes vaporariorum*
Cucumber-celery intercrop	*Trialeurodes vaporariorum*

(continued)

Table 7.1 (continued)

Crop combinations	Target pest insects
Cauliflower-tomato intercrop	*Lipaphis erysimi*
Pepper-sugarcane intercrop	*Phyllotreta striolata*
	Liriomyza huidobrensis
Zanthoxylum bungeanum-soybean intercrop	*Pseudaulacaspis pentagona*
Zanthoxylum bungeanum-potato intercrop	*Pseudaulacaspis pentagona*
Alfalfa-orchardgrass intercrop	*Hypera postica*
Alfalfa-smooth bromegrass intercrop	*Empoasca fabae*
	Empoasca fabae
Squash-maize-cowpea mixed crop	*Diaphania hyalinata*
Fennel-cotton with colored fibers intercrop	*Hyadaphis foeniculi*
Grape-tobacco intercrop	*Daktulosphaira vitifoliae*

Data adapted from Dong et al. (2016)

processes and farmland sustainability (Altieri 1999). Andow (1991) analyzed a large number of experiments involving 287 species of insects across different studies. A careful analysis of the population differences between monoculture and intercropping of crop species revealed that in intercropping systems, in 53% of situations the population density of natural enemies increased while it decreased in only 9% of instances. Vilich-Meller (1992) and others found that the intercropping of winter wheat with rye, barley, and oats offered better protection against fungal diseases than growing single crops. Lennartsson (1988) found that intercropping wheat with alfalfa could reduce the disease index of the soil-borne wheat take-all disease. Epidemiological simulations have shown that the effect of intercropping on disease control in different crops can be simply explained by the decreased chance of fungal spores contacting susceptible individuals in intercropping setting (Garrett and Mundt 1999). World Agroforestry Centre (2004) reported that a tree and maize intercropping system increases maize yields in sub-Saharan Africa (Fig. 7.3). The more genotypes were used in intercropping, the better effects were obtained. At the same time, changes in the microclimate are also important factors affecting the occurrence and development of diseases. Taking intercropping in rice and other crops as an example, we will next explore the principles, technologies, and successful examples of controlling rice diseases and insect pests via species diversity approaches. Li et al. (2009) report that some combinations increased crop yields for the same season between 33.2 and 84.7% and reached a land equivalent ratio (LER) of between 1.31 and 1.84. This approach can be easily applied in developing countries, which is crucial in face of dwindling arable land and increasing food demand (Table 7.2, Fig. 7.4).

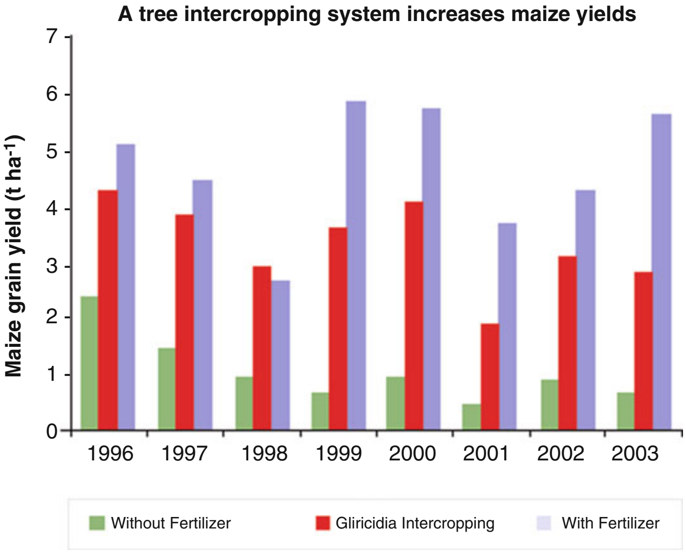

Fig. 7.3 Maize grain yield in a tree and maize intercropping system in sub-Saharan Africa. (Figure adapted from World Agroforestry Centre 2004)

7.3.1 Effects of Intercropping Different Crop Species on Natural Enemies

Crop species diversity can significantly increase the number of insect species that are natural enemies in a given area. For example, compared with carrot and onion monoculture systems, the natural predators of *Psila rosae* eggs were trapped more in fields with onion and carrot intercropping (Uvah and Coaker 1984). Similarly, sugarcane/maize intercropping could significantly increase the population density of predatory ladybirds (Zhang et al. 2011). A large number of parasitic wasps are easily hidden in fields with pumpkin and maize intercropping, and the number of parasitic wasps trapped in these fields was more than twice of that in a pumpkin monoculture setting (Letourneau 1983). Increasing the diversity of species in an intercropping setting can also significantly increase the predation rate of predatory natural enemies, and parasitism rate of parasitic natural enemies of pests. The rate of eggs and larvae of the melonworm moth *Diaphania hyalinat,* parasitized by parasitic wasps in pumpkin and maize intercropping fields reached 33% and 59% respectively, while the parasitism rates in pumpkin monoculture fields were 11% and 29% respectively (Letourneau 1983).

Table 7.2 Yield and monetary value for different crops

			Yield ± s. e. m (t/ha)		Crop value (US$ per ha)	
Crop	Variety	Plants m^{-2}	1st year	2nd year	1st year	2nd year
Tobacco MC	Yunyan-87	1.67	2.82±0.003	2.86±0.007	5829	5912
Maize MC	Huidan-4	5.35	6.94±0.003	6.99±0.017	1972	1986
Intercropping T+M		6.67	8.69	8.75	7477	7477
Tobacco IC	Yunyan-87	1.67	2.81±0.006	2.84±0.017	5808	5870
Maize IC	Huidan-4	5.00	5.88±0.004	5.91±0.017	1671	1679
Sugarcane MC	Xintaitan-2	9.62	105.87±0.851	105.23±0.256	2529	2514
Maize MC	Xundan-7	5.35	7.54±0.006	7.47±0.030	2142	2123
Intercropping Sc + M		13.45	110.35	111.67	3878	3878
Sugarcane IC	Xintaitan-2	9.45	105.58±0.575	106.95±0.409	2522	2555
Maize IC	Xundan-7	4.00	4.77±0.005	4.72±0.020	1355	1341
Potato MC	Hui-2	6.67	31.86±0.105	31.27±0.380	2058	2020
Maize MC	Huidan-4	5.35	7.17±0.022	7.13±0.026	2037	2026
Intercropping P + M		7.42	23.71	23.99	2687	2687
Potato IC	Hui-2	3.71	18.45**(115)**	18.75**(120)**	1192	1211
Maize IC	Huidan-4	3.71	5.26**(147)**	5.24**(147)**	1495	1489
Wheat MC	Yumai-3	277.36	5.31±0.013	5.32±0.016	1577	1580
Broad bean mc	Dabaidou	13.65	2.87±0.011	2.92±0.011	1389	1413
Intercropping W + Bb		280.05	6.27	6.28	2045	2045
Wheat IC	Yumai-3	277.36	5.29±0.020	5.31±0.017	1571	1577
Broad bean IC	Dabaidou	2.69	0.98±0.012	0.97±0.007	474	469

Note: "MC" = in monoculture, "IC" = in intercrop. Crop yield was determined by grain weight for rice, wheat and broad bean, dry leaf weight for tobacco, fresh stem and tuber weight for sugarcane and potato. Crop values are based on market prices of 2067.02 US$ per ton for tobacco, 284.15 US$ per ton for maize, 23.89 US$ per ton for sugarcane, 64.59 US$ per ton for potato, 296.98 US$ per ton for wheat, 483.97 US$ per ton for broad bean. Crop yield and value are for individual species within intercropping. Yields in tobacco-maize, sugarcane-maize and wheat-broad bean intercropping patterns showed higher productivity than monocrops. Yields of potato intercropped with maize, and maize intercropped with potato, compared with equal areas of monocrops are shown in **(bold)**. Statistical analyses: each survey plot was considered to be an experimental unit, and analyses were based on actual mean plot yields. Statistical analyses were conducted by software SPSS 13.0. One-tailed t-tests were used to determine if the yield differed significantly ($p \leq 0.05$). The table is adapted from Li et al. (2009)

7.3.2 *Ipomoea aquatica* Intercropping Patterns Utilize Species Diversity to Control Pests and Diseases

Intercropping *Ipomoea aquatica* can significantly reduce the incidence of rice blast, sheath blight, rice leaf roller, rust, and insect pests. Intercropping of two rice varieties significantly increased the rice biomass and tiller numbers, with no significant difference in plant height. Intercropping patterns can also improve rice

Fig. 7.4 Maize intercropping with potato, extended over large scale

grain size and amylose content, and intercropping of rice and *Ipomoea aquatica* can significantly improve land equivalent ratios and land use efficiency. Field experiments, pot simulation experiments, and laboratory bioassays assessing rice blast and sheath blight were carried out in rice-water chestnut, and rice-lotus root intercropping rotation systems, without the use of pesticides. Results of these studies show that intercropping allows rice to grow vigorously, with significant changes in rice agronomic characteristics and yield. The land equivalent (yield) ratio and seed equivalent ratio of rice yields were also significantly different in these studies, confirming that intercropping can effectively control rice diseases and insect pests. Specifically, water chestnut improved the resistance in rice to diseases, effectively preventing the spread of rice blast and sheath blight. Intercropping was also beneficial as a means of controlling common diseases and insect pests in fields of water spinach, where insect diversity was clearly increased in intercropping systems relative to monoculture settings, with a particular increase in the number of beneficial spiders.

7.3.3 Intercropping Patterns with Different Crop Species to Control Pests and Diseases

In order to exploit the principle of using a variety of crop combinations in strip communities to create a physical firewall-like barrier to disease spread, researchers conducted a study on potatoes, corn and tobacco in Banqiao, Xuanwei, Mailehongxi, Yuxi Hongta and Shiping Longpeng, from 2002 to 2006. The experiment was conducted using combinations of corn, sugarcane and corn, wheat, and broad beans, to control disease. The results of this study show that potato late blight disease indices decreased by 36.13%, maize big bang indices decreased by 16.75%,

Table 7.3 Summary of studies evaluating disease alteration due to intercropping[a]

Type of disease or pathogen	Total studies (number)	Disease response (percent of studies)				Unique intercrop-disease combinations (number)
		Reduced	None	Increased	All rxns[b]	
Fungi/oomycetes						
Leaf spots	61	75	18	5	2	40
Rusts	17	71	29	0	0	11
Powdery mildews	8	88	0	0	13	7
Rots/wilts	14	86	7	7	0	13
Foliar oomycetes	11	100	0	0	0	9
Total fungi/oomycetes	111	79	15	4	2	80
Bacteria	14	100	0	0	0	13
Viruses	39	72	13	13	3	31
Nematodes	35	37	37	14	11	29
Parasitc plants	7	100	0	0	0	5
Total all types	206	73	17	7	3	161

Note:
[a]Data adapted from Boudreau 2013
[b]All rxns: both increases and decreases in disease were reported

and tobacco late blight indices decreased by 36.13% and 16.75%, relative to controls. Rates of sugarcane yellow spot, wheat stripe, and rust also decreased by 52.71%, 32.55%, and 5.43%, respectively. This study therefore concluded that the reasonable planting of crops can reduce disease severity, although the specific planting patterns and species used will affect these reductions. For example, the disease index of potato and Tobacco Co-infected virus rates increased by 36.75% and 27.31%, respectively, resulting in a serious yield loss. Therefore, only through strict field trials can crops be safely planted and combined, in an efficient manner (Table 7.3).

7.4 Ecosystem Diversity as a Strategy for Controlling Crop Diseases and Insect Pests

Ecosystem diversity refers to the degree of ecological diversity in a region, considering a range of ecosystems within a given biosphere (such as forests or grasslands) that participate in a range of ecological and biological processes. Ecosystem diversity includes diversity in ecosystem composition and function, habitat and biological communities, and important ecological processes. Ecosystem diversity is related not only to habitat changes, but also to species diversity. Ecosystem diversity preservation directly affects species diversity and genetic diversity. Generally speaking, the more complex the composition and structure of an ecosystem is, the greater is the stability. Ecosystem diversity allows for more sustainable production of food, medicine, raw materials, and ornaments for human use. Such diversity also improves climate regulation, protects soil, enhances storage and circulation of the elements, maintains normal biological evolution, and allows for the absorption and degradation of pollutants. Given these advantages, protecting ecosystem diversity is a fundamental means of protecting biodiversity.

There are many ways to protect ecosystem diversity, such as *in situ* protection, biological control, protection of ecosystem integrity, ecological restoration, and reconstruction. Biological control can effectively reduce environmental pollution by controlling harmful organisms due to competitive relationships between organisms. Based on local habitat conditions, such a strategy is an effective means of rebuilding and restoring ecosystems and thereby protecting ecosystem diversity.

7.4.1 Ecological Effects of Weed Diversity and Landscape Configuration on Rice Pest Control

South China Agricultural University researched the potential of some crops and graminaceous weeds around rice fields to serve as hosts for the pest *Cnaphalocrocis medinalis*. They used rice (IR36, TKM 6), corn (*Zea mays*), sugar cane (*Saccharum officinarum*), *Lophatherum gracile*, *Paspaium distichum*, *Digitaria sanguinalis*, *Setaria viridis*, *Miscanthus sinicus*, *Echinochloa crus-galli* and other common graminaceous plants in southern China, as test materials, and studied the feeding of the rice leaf roller on these plants. The life table of an experimental population of rice leaf rollers feeding on these graminaceous plants was determined, and the influence of different tested plants on the growth, development and reproduction of the pest was analyzed. Further, the host range of rice leaf roller was defined, and the host selection mechanisms of rice leaf roller preliminarily explored. This provided a theoretical basis for the study of host control strategies and gave a reference basis for the agricultural control of the pest by using host control and facilitated an effective means for luring pests and protecting the crops by increasing the diversity of plants.

7.4.2 Protective Effects of Landscape Mosaic Patterns on Rice Germplasm Resources in Different Rice-Fish Systems

Based on Longxian Village, an important world agricultural cultural heritage site, the spatial distribution pattern of traditional rice varieties was studied, and a mosaic distribution of these traditional and hybrid rice varieties was found. The planting area of the traditional rice-fish systems in 25 natural villages (treating catchment areas as a unit) was investigated. It was found that the planting area of traditional rice-fish system was about 13%, which means that traditional rice-fish systems have an important protective role for traditional rice varieties. A comparative study on the performance of traditional glutinous rice, japonica rice, awn rice, and red rice in such rice-fish systems (focusing on yield, quality, occurrence of diseases, insect pests, and weeds) was conducted. It was found that the insect resistance of traditional local varieties was weaker than that of hybrid rice, but the quality of traditional local varieties was better, and the need for the application of chemical fertilizers and pesticides was lower. The yield of hybrid rice was not significantly different from that of traditional land varieties (Xie et al. 2011b).

At the same time, the performance of indigenous “field fish” in the rice-fish systems and pond systems, and the conservation effects of rice fields on indigenous “field fish” were also studied. The results show that, compared with the rice-fish system, the activity frequency of single-cultured “field fish” decreased (especially when water temperature was elevated), the color of fish became lighter, and the scales became harder. This means that the traditional rice-fish symbiosis system has a good protective effect on indigenous “field fish” (Xie et al. 2011a, b).

7.4.3 Chemical and Resistance Relationships Between Crops, Crops, Insects and Natural Enemies

As a sustainable and environmentally friendly model, genetic or species diversity as a basis for controlling diseases is increasingly being used to improve the ecological balance between different crops, between crops and pathogens, and as a means of controlling disease outbreaks. Previous studies on the mechanisms of genetic or species diversity in controlling disease have primarily focused on aerial airborne diseases. These have revealed that genetic diversity or species diversity can improve the diversity of species in the planting system and can further reduce the severity of disease occurrence by effectively diluting plant pathogens throughout the ecosystem, physically blocking disease transmission and improving the field microclimate. However, little is known about the mechanisms of controlling soil-borne diseases in multiple cropping systems.

In recent years, Yang et al. (2015) have systematically studied the chemical interactions between pathogens and crops, focusing on the relationship between crops and the soil-borne pathogen *Phytophthora*. A research platform for crop-pathogen interaction was established to explore the underlying chemical interaction mechanisms between pathogens and crops such as corn, rape, fennel, garlic, and onion. Based on these phenomena, an Attract-Kill -model was established (Yang et al. 2015).

It was found that the mycorrhizal network (mycelial bridge) of arbuscular mycorrhizal fungi among underground part of plants could also mediate communication between tomato plants. When a tomato plant is infected by diseases, or beset by insect pests, neighboring plants connected by a mycelial bridge could induce defenses and improve the resistance to the same pathogens. This underground communication is more reliable and concealed than volatile-mediated above-ground communication methods, allowing for transition over longer distances. This study provided a deeper understanding of the complex co-evolution of plants, mycorrhizal fungi, and pathogenic bacteria. In practice, the study on the mechanisms by which mycorrhizal fungi facilitate disease resistance is helpful, as it allows for screening efforts to identify high-efficiency mycorrhizal fungi, inoculate them into crops, induce disease resistance, improve the absorption of nutrients by plants, and promote the growth of crops (Ye et al. 2012).

Tobacco black shank disease is a very stubborn soil-borne disease, which is difficult to effectively control by conventional methods. It seriously threatens the healthy development of tobacco plantings in Yunnan Province. Ding et al. (2015) and Yang et al. (2015) demonstrated that rotation of oilseed rape and flue-cured tobacco could effectively control tobacco shank disease and improve the yield and quality of flue-cured tobacco, through 4 years of field experiments. They found that rape roots could absorb spores of pathogenic bacteria and release a large number of inhibitory compounds (such as phenylpropylthiazole, phenolic acid, and isocyanic acid). These inhibit the growth of pathogenic bacteria, and effectively reduce the number of pathogenic bacteria in the soil, and control the damage induced by black shank disease. Yuxi Tobacco Company has constructed a "flue-cured tobacco-rape-maize" diversified planting model based on these studies. They have carried out a large-scale demonstration leading to its popularization in tobacco producing areas, achieving good results with regard to disease control and yield increases. This study also provides a theoretical basis and technical support for the use of crop diversity to control other soil-borne diseases.

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Chapter 8
Conservation Biological Control in Organic Greenhouse Vegetables

Qingxuan Xu, Su Wang, Shu Li, and Séverin Hatt

8.1 Introduction

There is a worldwide trend promoting the development of organic agriculture to improve the sustainability of agriculture – that is, its environmental, social, and economic benefits (Crowder and Reganold 2015; Muneret et al. 2018). As organic agriculture has either entirely or largely avoided synthetic pesticides and inorganic fertilisers, it has shown many potential benefits, including improvements in biodiversity and associated ecosystem services (Mäder et al. 2002; Rusch et al. 2015; Seufert and Ramankutty 2017). A hierarchical meta-analysis notably showed that compared with conventional agriculture, organic agriculture increases species richness by approximately 30% on average (Tuck et al. 2014). In addition, consumption of organic food improves human health (Baudry et al. 2018). The content of secondary metabolites in organic products is approximately 12% higher than that in conventional food (Brandt et al. 2011).

Since the end of the 1990s, organic food standards have been introduced into China, and with the increase in domestic and export demand, organic agriculture has developed rapidly (Sheng et al. 2009). In particular, organic vegetable production plays an important role in improving farmers' income and peoples' quality of life, with China being one of the four major vegetable growing countries in the world (Willer and Lernoud 2016). Quality of greenhouse vegetables is a high

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Q. Xu · S. Wang (✉) · S. Li
Laboratory of Applied Entomology Research, Institute of Plant and Environment Protection, Beijing Academy of Agricultural and Forestry Sciences, Beijing, China

S. Hatt
Laboratory of Forest Ecology, Faculty of Agriculture, Kyoto University, Kyoto, Japan
e-mail: severin.hatt.77e@st.kyoto-u.ac.jp

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priority, hence recognition of the importance of management of pests and diseases is necessary, not only to ensure sufficient yields, but also to reach high cosmetic standards (Albajes et al. 2000). Natural enemies have been used to control arthropod pests biologically in greenhouse crops for decades (Yang et al. 2014; Zhang et al. 2015). However, due to high costs and sometimes low efficacy, together with societal criticism regarding risks of biological invasions (Messing and Brodeur 2018), fewer new arthropod natural enemies are being employed. Consequently, the current and future challenge of organic agriculture is to develop low-input farming practices with high economic efficiency (Porter et al. 2009; Messelink et al. 2014).

Conservation biological control (CBC) in greenhouses can be a viable alternative, both from an environmental and an economic perspective. The aim of CBC is to increase natural enemy resources by improving their habitat, thereby enhancing pest control (Ehler 1998; Begg et al. 2017). The effectiveness of natural enemies can be enhanced by providing alternative food, prey, hosts, oviposition sites, or shelters (Gurr et al. 2017). In addition, the effectiveness of natural enemies in controlling pests can be improved by using volatiles, adapting the greenhouse climate, avoiding pesticide side-effects, as well as food web disturbances (Hossain et al. 2002; Mathews et al. 2004). A meta-analysis showed that enemy richness increases top-down control of herbivores, and high diversity of natural enemy species is often associated with effective CBC (Letourneau et al. 2009). There are different ways to protect natural enemies of arthropod pests on greenhouse vegetables; but increasing flower resources is one of the most commonly used CBC strategies (Fu et al. 2014; Zhao et al. 2017).

This chapter focuses on the major pest species in greenhouses (especially in the context of China), and whether the management of habitats in or around greenhouses can provide diverse food sources and shelter for several important species of parasitoids and predators, which play an important role in suppressing pests on vegetables. We summarise the methods of CBC and their utilisation in greenhouses. Finally, future opportunities and challenges of CBC in organic greenhouse vegetable production are discussed.

8.2 Major Pest Species in Greenhouses

In greenhouses, growers are faced with a variety of arthropod pest species. Greenhouse aphids (Hemiptera: Aphididae), whiteflies (Hemiptera: Aleyrodidae), mites (Acari: Tetranychidae) and thrips (Thysanoptera: Thrips) are the main pests affecting vegetables (Tian 2000). The green peach aphid, *Myzus persicae* Sulzer, is a notorious pest. Feeding by *M. persicae* removes photoassimilates and transmits viruses, causing substantial loss of crop yields (Cao et al. 2016). The greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), and the sweet potato whitefly, *Bemisia tabaci* (Gennadius), cause direct damage, transmit plant viral diseases, and contaminate leaves and fruits with depositions of honeydew (Liu et al. 2007). *Tetranychus urticae* Koch, *Polyphagotarsonemus latu*s Banks, and *Tetranychus cinnabarinus* Boisduval are the most common spider mites in greenhouse vegetable crops in China. Because of their small size, quick reproduction, and short generation cycle, as well as their ability to colonise a wide range of host plants, they break out easily and can seriously affect yield and quality of vegetables (Cai et al. 2014). *Thrips flavus, Frankliniella occidentalis* and *Thrips tabaci* are the major native thrips on vegetables in China, causing substantial damage and economic losses on crops (Wu et al. 2018).

8.3 Plant Support System for Natural Enemies in Greenhouses

In greenhouses, non-crop plants can be introduced to enhance CBC. These are trap plants (that directly target pests), or banker plants (Fig. 8.1) that aim at sustaining natural enemies. We conducted a series of studies to understand the results of greenhouse experiments better, with the hope that it can be applied to organic vegetable greenhouses more effectively.

Fig. 8.1 Corn (*Zea mays* L.) strip as banker plant was sown in a greenhouse of the experimental farm of the Institute of Plant and Environment Protection, Beijing Academy of Agriculture and Forestry Sciences (Beijing, China)

8.3.1 Trap Plants

A trap plant attracts, transmits, and intercepts target pests away from a less-preferred main crop; thus, it is a bio-based alternative or supplement to chemical pesticides (Shelton and Badenes-Perez 2006). The concept of the trap plant is consistent with the ecological framework of habitat manipulation. As a principle, the trap plant must be more attractive to the pest as a food source than the main crop. In addition, it should limit the ability of the pest to develop, to avoid any risk of further colonisation of the adjacent main crop (Khan et al. 2006); what Veromann et al. (2014) called a 'fatal attraction' to a 'dead-end trap crop'.

In greenhouses with poinsettias (*Euphorbia pulcherrima* Willd. ex Koltz, Euphorbiaceae), eggplants (*Solanum melongena* L., Solanaceae) can help to control whiteflies, notably *T. vaporariorum* (Lee et al. 2009). In addition, eggplants can be used as a trap plant to attract *B. tabaci* adults and protect tomatoes (*Solanum lycopersicum* L., Solanaceae) (Choi et al. 2015). Introducing potted plants in greenhouses can be an easy-to-use practice to benefit from trap plants. Buitenhuis et al. (2007) showed that potted chrysanthemum, *Dendranthema grandiflora* (Tzvelev) (Asteraceae), significantly attracted the thrips *F. occidentalis,* reducing their abundance in adjacent cultivated crops.

8.3.2 Banker Plants

A banker plant aims at conserving and releasing natural enemies: it can directly or indirectly provide resources (such as food or hosts) during a specific period of time, conserve natural enemies before they migrate to the main crop when pest outbreak occurs, and provide food and hosts as well as habitat shelter when crops are harvested (Huang et al. 2011). Frank (2010) reviewed a body of studies spanning 32 years of research that investigated banker plant systems to support 19 natural enemy species targeting 11 pest species. Notably, he highlighted that, as an innovative approach of classical biological control methods, banker plant systems uniquely combine the advantages of both introducing and conserving natural enemies to provide sustainable control of targeted pests. Indeed, the release of natural enemies in classical biological control needs to provide a large number of insect individuals repeatedly, which is costly. The banker plant system can reduce the economic cost by utilising the space-time transfer characteristics of natural enemies, maintaining natural enemy populations and enhancing their diversity. Hence, the storage-and-release function is the biggest advantage of banker plants. Introduced within integrated pest management frameworks, a banker plant system allows the preventive control of pests. Therefore, the method is an interesting plant protection option for CBC in pest management programs.

Wang et al. (2016) developed a banker plant system for the biological control of vegetable aphids. Wheat (*Triticum aestivum* L., Poaceae), as a host plant of the

English grain aphid *Sitobion avenae* Fabricius, provides alternative hosts for rearing the parasitoid *Aphelinus asychis* Walker (Hymenoptera: Aphelinidae). *A. asychis* reared from *S. avenae* were allowed to parasitise second instar *M. persicae* nymphs feeding on chili pepper (*Capsicum annuum* L., Solanaceae) and cabbage (*Brassica oleracea* L., Brassicaceae). Barley (*Hordeum vulgare* L., Poaceae) banker plants infested by the bird cherry-oat aphid *Rhopalosiphum padi* (L.) allowed the aphid parasitoid *Aphidius colemani* Viereck (Hymenoptera: Braconidae) to significantly affect the growth of *M. persicae* populations on two spring flower crops grown in greenhouses: pansies (*Viola tricolor* var. *hortensis*, Violaceae) and Marguerite daisies (*Argyranthemum* hybrid, Asteraceae) (Van Driesche et al. 2008). Pineda and Marcos-García (2008) used sweet alyssum (*Lobularia maritima* L. Desv., Brassicaceae) and coriander (*Coriandrum sativum* L., Apiaceae) as flowering plants in sweet-pepper greenhouses to support predatory hoverflies (Diptera: Syrphidae). They reported that more hoverfly adults and larvae (especially *Eupeodes corollae* Fabricius, *Episyrphus balteatus* De Geer and *Sphaerophoria rueppellii* Wiedemann) were observed in the plots where flowers had been introduced, compared to control plots. Huang et al. (2014) found that castor (*Ricinus communis* L., Euphorbiaceae) is more tolerant to whitefly damage and environmental stresses than other host plants in Northwest China. Castor's large leaves could bear whiteflies and their parasitoids, and was considered as a potential banker plant to rear the whitefly *Trialeurodes ricini* (Misra). Compared to other potential host plants (eggplant, green bean, cotton), *T. ricini* was more attracted by castor—on which it performed better (higher survival rate, intermediate development time, higher development rate). It was suggested that *T. ricini* would be a good alternative host to the castor banker plant for a mass-rearing of whitefly parasitoids *Encarsia* spp. and *Eretmocerus* spp. (both Hymenoptera: Aphelinididae) to control *B. tabaci* or *T. vaporariorum* on vegetables in greenhouses (Huang et al. 2014). Against *B. tabaci*, papaya was also considered as a non-crop banker plant to rear the non-pest alternative host *Trialeurodes variabilis* (Quaintance) (Hemiptera: Aleyrodidae) and maintain the parasitoid *Encarsia sophia* (Girault & Dodd) (Hymenoptera: Aphilinidae) (Xiao et al. 2011a). Against the two spotted spider mite *T. urticae*, Xiao et al. (2011b) used corn (*Zea mays* L., Poaceae) plants to support the predatory gall midge *Feltiella acarisuga* (Diptera: Cecidomyiidae) in greenhouse vegetable production.

8.4 Plant Habitat Management Surrounding Greenhouses

Areas surrounding greenhouses should also be considered, in addition to in-greenhouse strategies. Indeed, naturally occurring natural enemies are able to migrate into greenhouses from plants managed outside (Fig. 8.2). Biodiversity and related ecosystem services are mainly due to natural habitats in agricultural landscapes—defined here as combinations of natural or semi-natural non-crop habitats, such as cropland boundaries, fallow land, grassland, woodland, wetlands, and forests (Haddad et al. 2015). Many natural enemies are influenced by non-

Fig. 8.2 Flower strips sown in greenhouse surroundings of the experimental farm of the Institute of Plant and Environment Protection, Beijing Academy of Agriculture and Forestry Sciences (Beijing, China)

crop habitat surrounding agricultural fields (Sarthou et al. 2014; Hatt et al. 2019). Understanding the impact of land use patterns outside greenhouses on natural enemies is essential to predict how landscapes alter biological control services. Natural enemies could become established, and their population numbers be enhanced, by providing additional resources from landscapes surrounding greenhouses, such as alternative food, prey, hosts, oviposition sites, or shelters (Andow 1991). The function of specific flowering plants can be monitored in various ways, for instance by assessing flower attractiveness, and their effect on natural enemy fitness traits in controlled conditions, and in the field (van Rijn and Wäckers 2015), or by analysing natural enemy gut content (Wratten et al. 2003) (for reviews: Hatt et al. 2018b.; Wäckers and van Rijn 2012). Local community structure and interactions have been shown to depend partly on landscape context. The presence of semi-natural habitats have been shown to enhance pest control in many systems (Landis et al. 2000; Thies et al. 2003; Schmidt et al. 2005; Bianchi et al. 2006; Morandin et al. 2014; Hatt et al. 2017), even though the presence of such diversity sometimes fails to support biological pest control (Tscharntke et al. 2016). Among the semi-natural elements of agricultural landscapes, wildflower strips sown at field edges, or within fields, provide potential habitats for the natural enemies of vegetable pests. Strips of flowering alyssum and coriander are commonly sown within lettuce crops in the Salinas Valley of California (USA), to support aphidophagous hoverflies against lettuce aphids (Smith and Chaney 2007). Marigolds *Calendula officinalis* (Asteraceae) sown adjacent to tomatoes can support Dicyphini (Hemiptera: Miridae) predators as well as parasitoids in order to reduce tomato leaf damage caused by Lepidopteran pests (Balzan 2017). Mixtures of wild flowers have also been considered, for instance against Lepidopteran pests of cabbage (Pfiffner et al. 2009). Such flower strips sown at the edge of greenhouses could also enhance natural enemies and potentially help to suppress pests on the inside, despite there being multiple ways to compose flower strips for CBC (see Chap. 4 by Hatt et al. in this book).

Indeed, the surrounding vegetation can contribute to the migration of natural enemies into greenhouses (Gerling et al. 2001). Moreover, it is acknowledged that multiple spatial scales should be considered simultaneously when designing CBC strategies (Begg et al. 2017; Hatt et al. 2018a). Hence, diversifying farms and landscapes may be crucial. Aviron et al. (2016) notably reported that both farming practices and the presence of semi-natural habitats in local landscapes participated in increasing the abundance of predatory mirids (*Macrolophus* sp. and *Dicyphus* sp.) in tomato fields. This suggests that transforming farms from conventional to organic production systems, adopting integrated plant management practices, and maintaining—or implementing—large areas of semi-natural habitats are needed to enhance CBC for horticultural crops. For example, strips of buckwheat (*Fagopyrum esculentum* Moench, Polygonaceae) are sown between vine rows in New Zealand to provide nectar and pollen for beneficial insects to enhance biological control of vine pests.

It can be concluded that diversified landscapes hold high potential for the conservation of biodiversity and sustaining pest control function. Still, there is a high variability of landscape effects on CBC. A meta-analysis of 132 studies conducted by Karp et al. (2018) found inconsistent effects of landscape composition on pest and enemy abundances, predation rates, crop damage, and yields. More work is needed in the future to help farmers understand when protecting habitats is a real win-win opportunity (Salliou and Barnaud 2017).

8.5 Perspectives and Conclusion

Most greenhouse vegetable production is labour and energy intensive; thus, high-level technology is needed to achieve adequate economic returns. The conservation methods described in this chapter are not only important for controlling pests that occur in conventional greenhouses, but also for organic greenhouses. Organic agriculture has great potential to develop low-cost, low-input, and locally available ecological technologies to produce food without harming the environment (Sandhu et al. 2010). Organic greenhouse systems offer the promising opportunity of success for pest biological control, and the lessons learned from these systems could contribute to the transformation of horticulture cropping production and consumption (Bellon and Penvern 2014).

There are still challenges in combating pest species that are not currently controlled by natural enemies, or in controlling pest species in crops where natural enemies are poorly established. For example, the susceptibility to host pathogens and viruses will pose a potential risk when using alternative plants in greenhouses or greenhouse surroundings (Cano et al. 2009). Besides, natural enemies must be abundant in greenhouses when pests occur on crops, because delayed colonisation of crops by predators or parasitoids will lead to unsuccessful control. The behaviour of natural enemies is partly guided by semiochemicals, and these volatile signals can be applied to attract them (Heuskin et al. 2012). Hence, combining semiochemical (e.g., E-β-farnesene, or methyl salicylate) release with other conservation practices may enhance CBC (Xu et al. 2018). Gonzalez et al. (2016) also suggested that insect pathogenic fungi, bacteria and viruses, and their possible direct and indirect effects on arthropod natural enemies might be combined with CBC. Future research directions—following the *ecostacking* approach developed in this book—could focus on the combined application of microbial and arthropod natural enemies in greenhouse production. We expect that this review will be especially useful in order to develop CBC strategies in organic vegetable systems further, where pest control is mainly dependent on biological control with natural enemies.

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Chapter 9
Landscape-Level Drivers of Biocontrol and a Case Study from Local to Regional Scale in China

Ouyang Fang, Men XingYuan, and Ge Feng

9.1 Introduction

Ecological regulation and biocontrol of pests are always important frontier areas in the science and technology for the prevention and management of insect pests. Knowing the ecological effects of agricultural landscape patterns on population dynamics of insect pests and of their natural enemies is fundamental for ecological regulation and management of insect pests under climate change.

In the past 10 years, our studies mainly focused on agricultural landscape ecosystem as the research system, aiming to explore the relationships among agricultural landscape pattern, crops, insect pests and their natural enemies. Based on the characteristic of agricultural landscapes in China, we define some concepts about agricultural landscape, and propose four keywords to describe its character. We will discuss the concept and category of ecological services by insects and their economic valuation. Our study on the ecological regulation and biocontrol of pests develops from local field level to regional agricultural landscape ecosystem level in China.

O. Fang (✉)
State Key Laboratory of Integrated Management of Pest and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing, China

Department of Environmental Science, Policy, and Management, College of Natural Resources and Berkeley Institute for Data Science, University of California, Berkeley, CA, USA
e-mail: ouyangf@ioz.ac.cn

M. XingYuan
Institute of Plant Protection, Shandong Academy of Agricultural Sciences, Jinan, China

G. Feng
State Key Laboratory of Integrated Management of Pest and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing, China

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9.2 Agricultural Landscape

Agricultural landscape is the geographic space made of farmland as main body, and the surrounding land cover or land use as background, such as grassland, forest, shrub, wetland, building area etc. (Ouyang and Ge 2011). The characteristic of agricultural landscape can be described with four keywords: composition, quantity, configuration, and scale. Composition implies the component type of agricultural landscape, or patch type, crop type. Quantity is component proportion of each component type, or patch size. Configuration signifies the shape structure of the patch, such as strip-type. Scale includes temporal and spatial scales (Ouyang and Ge 2011; Ouyang et al. 2016). Several landscape indices are listed in Table 9.1 to describe quantitative characters of agricultural landscape patterns (Wu 2000; Ouyang and Ge 2011).

9.3 Insect Ecological Services and Biocontrol

Insects, as one of the most important component in an ecosystem, play a great role in biodiversity because of their great quantity of individuals, biomass, species, and genes. Insects also play a significant role in maintaining ecosystem functions and ecological balance, and fulfilling human demands. Society has recognized the value of insect ecological services. Insect ecological services are the functional roles in ecosystem processes, benefiting humans with goods and services (Ouyang et al. 2013).

Insect ecological services are divided into four types: provisioning, regulating, cultural and support services (Ouyang et al. 2013). First, insect provisioning services are the products obtained from ecosystems and related to insects. This includes for example insects as food, and for medicine and industrial materials. Second, insect regulating services are the benefits obtained from the regulation of ecosystem processes related to insects. The ecological regulating services provided by insects involve biological control, pollination, seed dispersal and decomposition. Biological control is a particular type of pest control, where predatory/parasitoid insects suppress pest densities to relatively low levels. This type of ecologically based pest management infers that the “natural enemies” control the pest species. The types of natural enemies used for biological control vary depending on the type of pest. Third, cultural services supplied by insects are the nonmaterial benefits people obtain from ecosystems through spiritual enrichment, cognitive development, reflection, recreation, and aesthetic experiences, cultural services, and support services. Herbivores as the primary consumers transfer energy in ecosystems, and provide food for animals in next trophic level. Thus insects usually affect nutrient cycle in ecosystems directly and indirectly.

The economic value of insect ecological services can be divided into direct, indirect, option, and existence values. The economic value of regulating services

Table 9.1 Landscape indices describing characters of agricultural landscape pattern

Character	Landscape index	Abbreviation	Explaination
Composition	Patch type	PT	Qualitative index. Component type in agricultural landscape, or patch type, crop type
	Patch richness	PR	Number of patch types based on corps or plants in agricultural landscape
	Patch richness density	PRD	Patch richness divided by the total area of the landscape in agricultural landscape
Quantity	Number of patches	NP	Number of patches for each individual class in the agricultural landscape; different from patch richness
	Patch density	PD	Number of patches of the corresponding patch type divided by the total landscape area in the agricultural landscape; different from patch richness density
	Growing density	GD	Number of plants of crops per unit area in the agricultural landscape
	Total edge	TE	The sum of the length (m) of all edge segments involving the corresponding patch type in the agricultural landscape
	Edge density	TD	The sum of the length of all edge segments involving the corresponding patch type, divided by the total landscape area in the agricultural landscape
	Patch area	PA	The sum of the areas of all patches of the corresponding patch type or crop in the agricultural landscape
	Mean patch area	MPA	Average patch size of the corresponding patch or crop type in the agricultural landscape
	Largest patch index	LPI	The percent of the total landscape that is made up by the largest patch
	Shannon diversity index	SHDI	Measure of relative patch diversity
Configuration	Landscape shape index	LSI	The total landscape boundary and all edges within the boundary divided by the square root of the total landscape area (square metres) and adjusted by a constant (circular standard for vector layers, square standard for rasters)

(continued)

Table 9.1 (continued)

Character	Landscape index	Abbreviation	Explaination
	Mean shape index	MSI	Shape complexity. Sum of each patch's perimeter divided by the square root of patch area for each class or all patches, adjusted for circular standard (for polygons), or square standard (for rasters (grids)), divided by the number of patches
	Length width ratio	LWR	Patch length divided by the width of patch
Scale	Spatial extent	SE	Total area or the max space extent of agricultural landscape.
	Spatial granularity	SG	A characteristic length, area, or volume represented by a minimum discernible unit in the agricultural landscape, such as the area of a single plant, the size of a quadrate, pixel, or raster cell
	Temporal extent	TE	The time duration of agricultural landscape change in the research object
	Temporal granularity	TG	The frequency or interval of the occurrence (or sampling) of a phenomenon or event in the object of study, such as the sampling interval of biomass measured in the field

Wu (2000) and Ouyang and Ge (2011)

provided by insects in agriculture was evaluated in China in 2007, using agricultural production data from China. The value of natural pest pest control was estimated at 2621.00×10^8 yuan RMB, which corresponded to 9.09% of the total value of the crops serviced. The accurate valuation of insect ecological services is critical to establishing and preserving the ongoing development of insect-based resources and biodiversity in China (Ouyang et al. 2015b).

9.4 Case Study from Local to Regional Scale in China

9.4.1 Landscape-Level Drivers of Biocontrol at Local Scale

9.4.1.1 Farmland Landscape System Composed of Cotton, Maize, Aphids, and Predatory Beetles

An experimental landscape system composed of cotton and maize was designed and implemented by Ouyang Fang and Ge Feng for 3 years during 2008–2010. The study was conducted in a 1.2 ha field at the Langfang Experimental Station (39.53

°N, 116.70 °E) in the Hebei Province of China (Fig. 9.1). Cotton and maize are important crops and provide the main agricultural landscape in Northern China (Ge Feng 1995). The cotton aphid, *Aphis gossypii* (Glover), is a serious sucking pest of cotton that can cause substantial yield loss (Wu and Guo 2005). Especially since the 1990s, transgenic Bt (*Bacillus thuringiensis*) cotton has become an important tool for insect pest management of cotton worldwide (Huang et al. 2002; Horner et al. 2003; Wu et al. 2008). The decreased use of broad-spectrum pesticides for control of the cotton bollworm in Bt cotton fields has resulted in increases in non-target populations of sucking insects, such as mirids, in multiple crops in China (Men et al. 2005; Lu et al. 2010). Thus, Bt cotton is just one component to be considered in the overall management of insect pests in the diversified cropping systems common throughout China (Wu et al. 2008; Lu et al. 2010). Maize (*Zea*

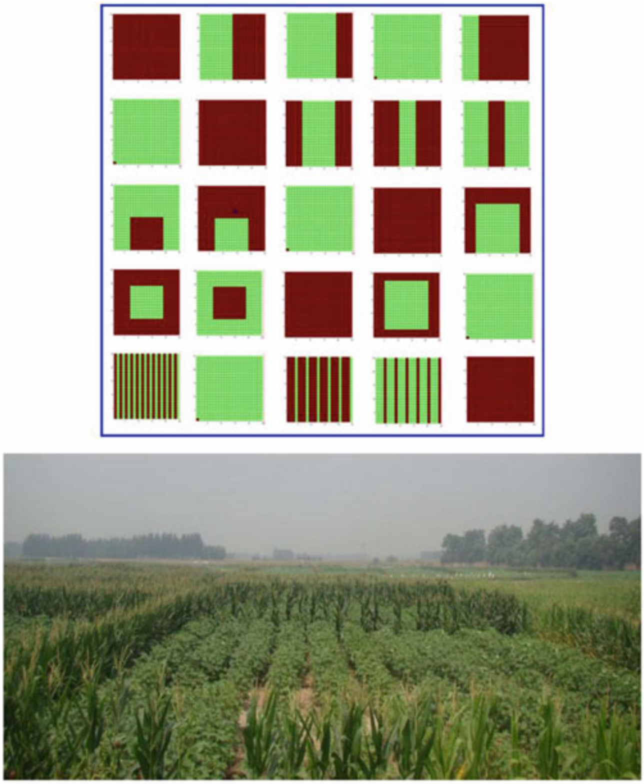

Fig. 9.1 Agricultural landscape system composed of cotton and maize. Upper figure is the spatial layout of the field experiment. The field was 90 m × 90 m, divided into 25 15 m × 15 m plots, each plot consisting of 24 rows and 50 columns. The spacing between neighboring plots was 3–4 m. Green and red areas in plot indicate the planting of cotton and maize. Lower figure is the field experiment planted with cotton and maize. (Photo by Ouyang Fang in 2010 at the Langfang Experiment Station (39.53 °N, 116.70 °E) in the Hebei Province of China)

mays L.), a C_4 plant, has been widely planted in China and occupied 29.9 million ha in 2008 (China 2009). Maize aphid, *Rhopalosiphum maidis* (Fitch), is a worldwide pest of maize. *Propylea japonica* is a prevalent mobile predator of aphids in maize and cotton, and moves between crops in agricultural systems (Ge Feng 1995; Liu et al. 2004; Gao et al. 2010). Much research on its predation on aphids in cotton has been reported (Ge Feng 1995; Liu et al. 2004; Gao et al. 2010). However, the factors affecting intercrop movement and foraging behavior of *Propylea japonica* between cotton and maize remain to be elucidated from a landscape perspective.

Cotton and maize were the main crops in this station and accounted for a total area of ~35 ha. An experimental model system was planed to study the explicit process of natural enemies in the agricultural landscape system, and to define responses in population density of *P. japonic*a adults to spatial variation. Plot treatments of spatial variation were based on patch area and spatial arrangements of cotton and maize. The experimental design in this study refer to the research described in the article of Crist et al. (2006). The experimental plot was 90 m × 90 m, divided into 25 15 m × 15 m plots, each plot consisting of 24 rows and 50 plants along a row. A 3–4 m gap was left between the plots to buffer influence from arthropods in the neighboring plots (Men et al. 2003). All vegetation between the plots was removed, when necessary, to minimize effects from the surrounding environment (Ouyang et al. 2012).

In the current study, field investigation and stable carbon isotope ratio analysis ($^{13}C/^{12}C$) from complementary laboratory and field samples between 2008–2010 were used to examine the process of oviposition preference, crop colonization and subsequent feeding by the predatory beetle, *P. japonica* in agricultural landscapes composed of cotton and maize. Our results suggest that integrative analysis of stable carbon isotope ratio can be regarded as a useful method for quantifying and tracing prey origins, proportions of diets, and feeding periods of natural enemies (Ouyang et al. 2014, 2015a). Our results can provide quantifying techniques for habitat management of natural enemies. In this agricultural landscape system, "landscape" was defined in a general sense, as a spatially heterogeneous area (Turner 1989) that is scaled relevant to the process or organism under investigation (Wiens 1989).

The effect of landscape structure on predator–prey interactions in red clover was studied in an experimental model landscape system (With et al. 2002). Our objectives were to: (1) establish the oviposition preference of *P. japonica* at the patch and landscape levels within agricultural landscapes composed of cotton and maize; (2) identify the preferred crop patches of *P. japonica* adult populations to inhabit in agricultural landscapes composed of cotton and maize, and define responses in population density of *P. japonica* adults to spatial variation; (3) and to determine the feeding behavior of *P. japonica* in multiple crop landscapes composed of cotton and maize.

9.4.1.2 Oviposition Preference of Predatory Beetle *P. japonica*

Egg abundance of *P. japonica* was different between maize and cotton patches in the field landscape plots in 2008–2010 (Fig. 9.2a–c). Densities of accumulative *P. japonica* eggs in cotton patches were greater than those in maize patches during all the sample dates in 2008 (Fig. 9.3d, $F = 4.7949$, $p = 0.0348$) and 2009 (Fig. 9.2e, $F = 18.7710$, $p = 0.0001$), while there were no significant differences in densities of accumulative *P. japonica* eggs between maize and cotton patches in 2010 (Fig. 9.2 F, $F = 0.9634$, $p = 0.3327$). The relationship between densities of *P. japonica* eggs and larvae, and densities of aphids in agricultural landscapes can be described by a linear regression model (Table 9.2). Linear regression analysis revealed that egg densities of *P. japonica* were positively correlated with aphid densities in agricultural landscapes in 2008–2010 (Table 9.2A). Similar results were found for regression analysis that larval densities of *P. japonica* were also positively correlated with aphid densities in agricultural landscapes in 2008–2010 (Table 9.2B).

9.4.1.3 Habitat Selection of Predatory Beetle *P. japonica* Adults

Repeated measures analysis indicated that *P. japonica* adult densities were significantly affected by crop type in the field landscape plots composed of cotton and maize in 2008 (Table 9.2A), 2009 (Table 9.2B) and 2010 (Table 9.2C). *P. japonica* adult densities established in maize patches were significantly greater than those in cotton patches during most of the sampling dates from 2008–2010 (Fig. 9.3a–c). Densities of accumulative *P. japonica* adults in maize patches were significantly greater than those in cotton patches during all sample dates in 2008 (Fig. 9.3 D, $F = 133.8022$, $P < 0.0001$), 2009 (Fig. 9.3e, $F = 6.7601$, $P = 0.0137$) and 2010 (Fig. 9.3f, $F = 161.9278$, $P < 0.0001$). Linear regression analysis revealed that adult densities of *P. japonica* were positively correlated with aphid densities in agricultural landscapes in 2008–2010 (Table 9.2C).

9.4.1.4 Feeding Behavior and Movement of *P. japonica* in Multiple Crop Landscapes Composed of Cotton and Maize

Our results from this 3-year study found that *P. japonica* adults actively search host plants for aphids before ovipositing, regardless of the composition of the agricultural landscape as evidenced by *P. japonica* egg densities being positively correlated with aphid densities rather than host plant. The data indicate that the predatory beetle seeks out high prey densities before ovipositing; presumably this is to ensure there is enough food for their offspring. Densities of *P. japonica* adults in maize patches were significantly greater than those in cotton patches during most of sampling dates in the agricultural systems. Adults of *P. japonica* apparently prefered to inhabit maize patches, even when prey was scarce in maize and abundant in cotton. *P. japonica* adults in maize were not significantly positively correlated with aphids in

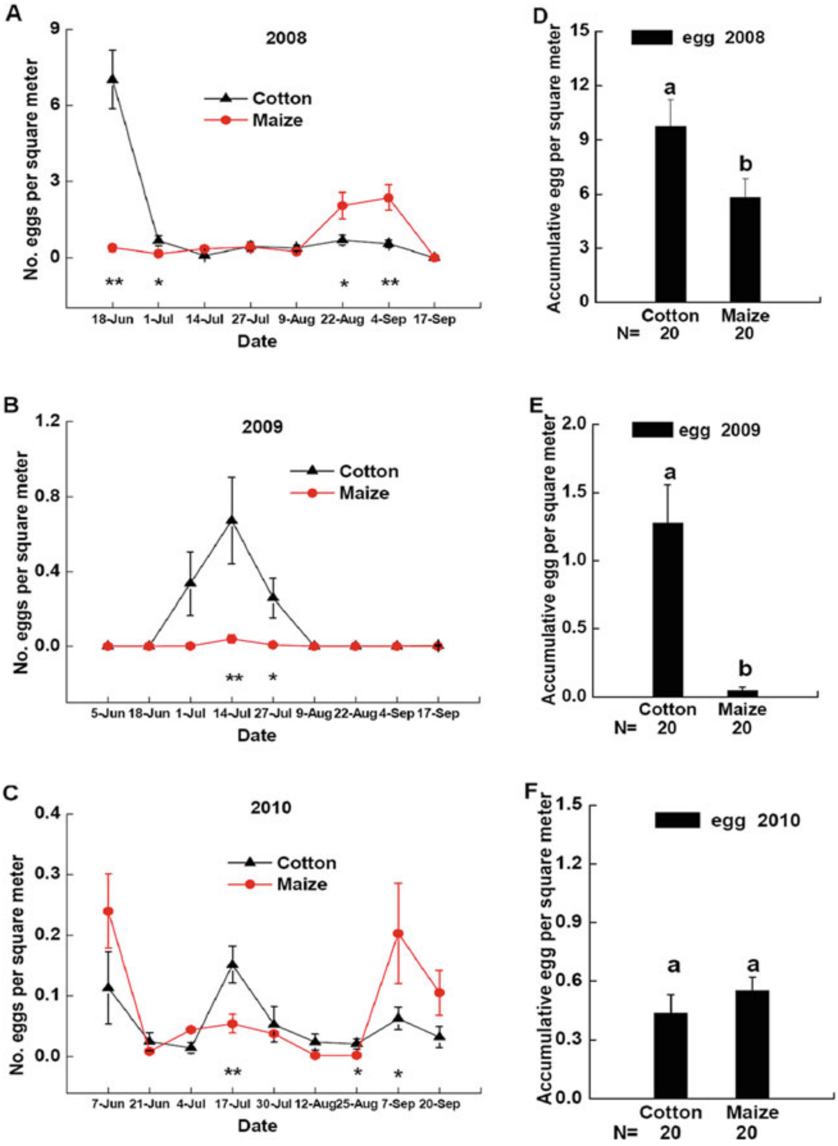

Fig. 9.2 Dynamics of *P. japonica* eggs. Densities of *P. japonica* eggs in cotton patches (black triangle) and maize patches (red circle) in field landscape plots in 2008 (**a**), 2009 (**b**), and 2010 (**c**). * Significant differences between densities of *P. japonica* eggs in cotton patches and maize patches at $p < 0.05$. ** Significant differences between densities of *P. japonica* eggs in cotton patches and maize patches at $p < 0.01$. Densities of accumulative *P. japonica* eggs in cotton patches and maize patches at all sample dates of field landscape plots in 2008 (**d**), 2009 (**e**) or 2010 (**f**). Different lowercase letters above the bars indicate significant differences in densities of accumulative *P. japonica* eggs in cotton patches and maize patches at $p < 0.05$. Data are presented per square meter of crop plants (mean ± SE) with separate field landscape plots used as replicates. Sample size of cotton patch and maize patch are both 20. N indicates the size of samples tested

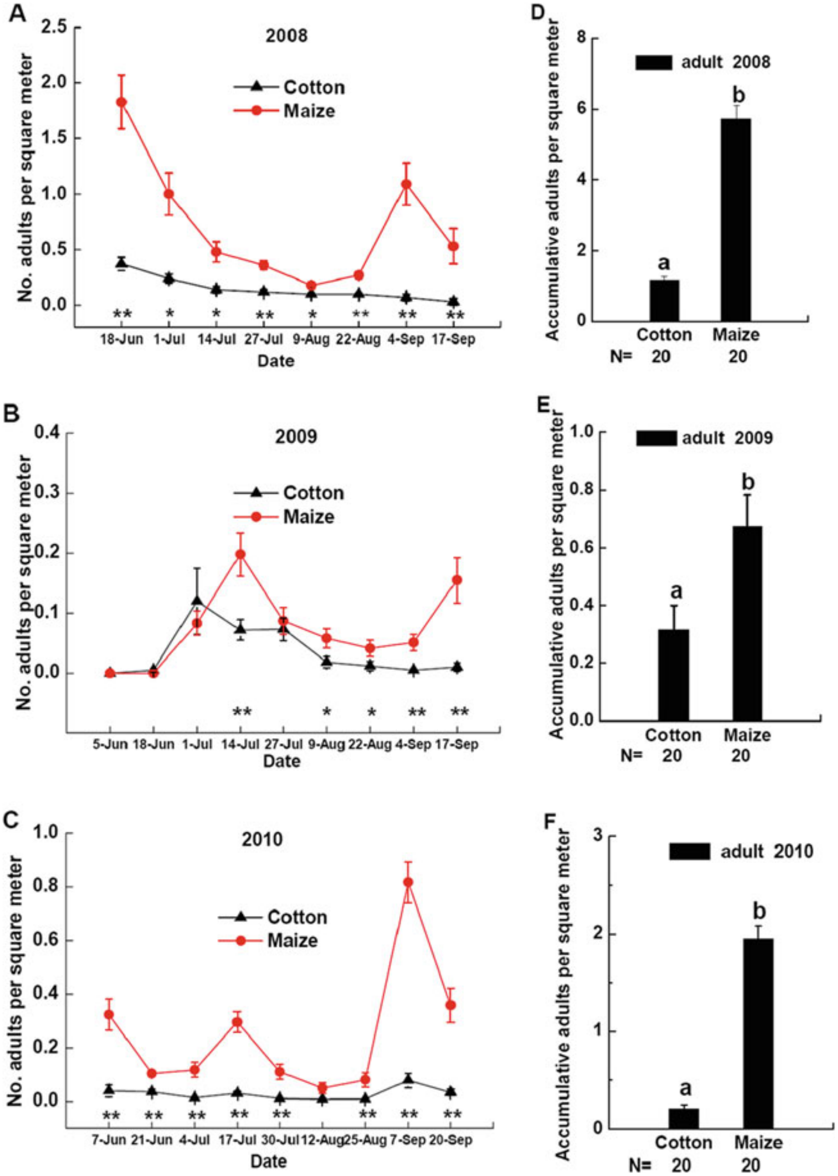

Fig. 9.3 Dynamics of *P. japonica* adults. Densities of *P. japonica* adults in cotton patches (black triangle) and maize patches (red circle) in field landscape plots in 2008 (**a**), 2009 (**b**), and 2010 (**c**). * Significant differences between densities of *P. japonica* adults in cotton patches and maize patches at $p < 0.05$. ** Significant differences between densities of *P. japonica* adults in cotton patches and maize patches at $p < 0.01$. Densities of accumulative *P. japonica* adults in cotton patches and maize patches at all sample dates of field landscape plots in 2008 (**d**), 2009 (**e**) or 2010 (**f**). Different lowercase letters above the bars indicate significant differences in densities of accumulative *P. japonica* adults in cotton patches and maize patches at $p < 0.05$. Data are presented as adults per square meter of crop plants (mean ± SE) with separate field landscape plots used as replicates. Sample size of cotton patch and maize patch are both 20. N indicates the size of samples tested

Table 9.2 Relationship between *P. japonica* eggs, larvae and adults, and aphids in landscape plots during 2008, 2009 and 2010[a]

No.	*P. japonica*	Year	Linear model[a]	R^2	F	DF	P
A	Eggs	2008	$y = 0.8770x-2.5687$	0.7028	14.1871	1,7	0.0093
		2009	$y = 0.0504x-0.0769$	0.8845	53.6286	1,7	0.0002
		2010	$y = 0.0270x-0.0042$	0.7212	18.1032	1,7	0.0038
B	Larvae	2008	$y = 0.3758x-1.1201$	0.7320	16.3876	1,8	0.0067
		2009	$y = 0.0322x-0.0287$	0.8305	34.3064	1,8	0.0006
		2010	$y = 0.0231x-0.0161$	0.6335	12.0990	1,8	0.0103
C	Adults	2008	$y = 0.2856x-0.7356$	0.9743	227.2308	1,8	<0.0001
		2009	$y = 0.0185x + 0.0027$	0.7367	19.5874	1,8	0.0031
		2010	$y = 0.0519x + 0.0040$	0.5388	8.1776	1,8	0.0243

[a] x is the aphid density data, which was log-transformed ($\ln(n + 1)$) for analysis. y is density of *P. japonica* eggs, larvae and adults

maize, whereas they were significantly positively correlated with aphids in cotton. In the landscape plots, *P. japonica* adults were significantly positively correlated with aphids. The results imply that *P. japonica* adults preferentially inhabit maize patches but that they will transfer from cotton patches to forage on cotton aphids in agricultural systems consisting of both transgenic cotton and maize crops. Maize may serve as a better habitat or shelter for the predatory beetle from adverse conditions in agricultural landscapes composed of transgenic cotton and maize.

In conclusion, besides serving as a refuge from selection pressure for adaptation to transgenic cotton varieties that produce a toxin from the bacterium *Bacillus thuringiensis* for *Helicoverpa zea* (Gould et al. 2002), maize can provide a favorable habitat for natural enemies capable of controlling cotton aphids on transgenic Bt cotton in an agricultural system composed of cotton and maize. In many parts of the world, transgenic crops such as Bt crops have come to dominate agricultural landscapes, which has often led to non-target insect pests becoming the key pests within cotton and many other non-Bt host crops (Men et al. 2005; Lu et al. 2010). Recent studies report that the decrease in use of insecticide sprays associated with Bt crops could enhance biocontrol services, and found evidence that the predators might provide additional biocontrol services spilling over from Bt cotton fields onto neighbouring crops (maize, peanut and soybean) (Lu et al. 2012). Our results clearly indicate that *P. japonica* adults lay their eggs on aphid infested host plants in large agricultural fields, and preferentially inhabit maize patches, but move to cotton patches to feed on aphids, which suggests that habitat management with a suitable proportion and spatial arrangement of cotton and maize may be an alternative planting pattern to enhance biological control in order to meet the challenge of managing non-target pest densities in Bt cotton. Maize benefits predators and provide potential to enhance biological control for non-target pests in transgenic cotton in the field, while further work is needed to determine how to maintain and enhance biological control for insect pests in larger regions for a long time in the agricultural landscape systerm.

9.4.1.5 Farmland Landscape System Composed of Multiple Crops, Insect Pest and Natural Enemy

An experimental landscape system composed of multiple crops was designed and implemented by Men Xinyuan, Ouyang Fang, and Ge Feng for 4 years from 2007 to 2010 in Yishui county, Linyi City, Shandong Province, China (35°48′05″N, 118°37′11″E). Twenty primary crop species commonly grown in North China were selected, including *Gossypium* spp., *Zea mays* L., *Triticum aestivum* L., *Glycine max* (L.) Merr., *Solanum lycopersicum* L., *Brassica oleracea* L., *Setaria italica* (L.) Beauv., *Sorghum bicolor* (L.) Moench, *Lolium perenne* L., *Vigna angularis* (Willd.) Ohwi et Ohashi, *Arachis hypogaea* L., *Vigna radiata* (L.) R. Wilczek, *Medicago sativa* L., *Solanum melongena* L., *Apium graveolens* L., *Trifolium repens* L., *Helianthus annuus* L., *Phaseolus vulgaris* L., *Brassica napus* L., and *Sesamum indicum* L. Five plant richness levels (1, 2, 4, 8, and 16) were designed, and we randomly selected 1, 2, 4, 8, and 16 species to achieve the five crop species richness levels. Every treatment was replicated 10 times. Fifty 9 m × 9 m plots were used, located 1 m apart; the entire experimental site covered 70 m × 150 m (Fig. 9.4). For a given plot, the crops were distributed in a matrix of 22 rows and 22 columns.

The Huang-Huai-Hai Plain in North China is one of China's most important grain producing regions. In the last few decades, population growth and changes in agricultural management and planting patterns have caused landscape simplification and a loss of biodiversity in the region's agro-ecosystems (Parker and Macnally 2002; With and Pavuk 2011; Huang et al. 2013; Trichard et al. 2013). In particular, the rapid expansion of crop monocultures has led to serious outbreaks of agricultural pests, and loss of biological control agents. Crop species richness facilitates biological control through the use of natural enemies of plant pest species in annual arable systems. However, agricultural intensification has led to high levels of landscape simplification and a reduction of habitat heterogeneity. In the present experiment, we

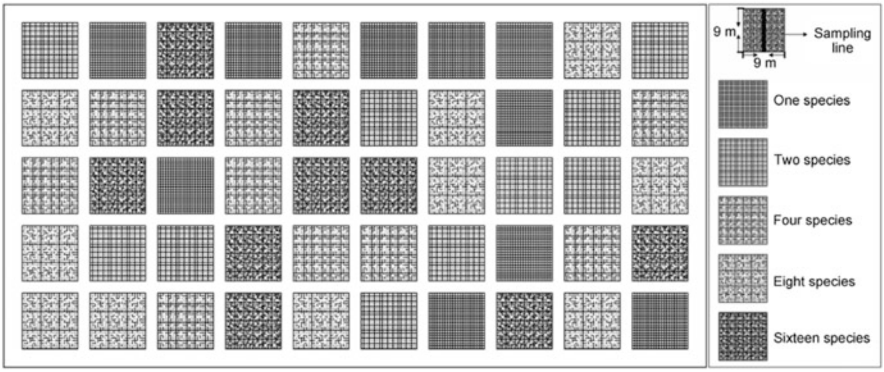

Fig. 9.4 Crop arrangement and composition in an experimental landscape system composed of multiple crops

studied the relationship between crop species richness and the biomass of pests and natural enemies based on a microlandscape. Two hypotheses were tested: (1) based on the resource concentration hypothesis, high crop species richness suppresses pest populations, whereas decreased crop species richness will result in increases in pest species populations (Hamback and Englund 2005); (2) based on the natural enemy hypothesis, high crop species richness would supply abundant resources and refuge for natural enemies, which would indirectly improve the biological control service they provide and suppress pest populations (Tscharntke et al. 2008). The aim of this experiment was to achieve multiple ecological services, and to supply a theoretical basis for potential pest management (Werling and Gratton 2008).

9.4.1.6 Effects of Crop Species Richness on Pest and Natural Enemy Biomass

Crop species richness had major effects on the biomass of pests and natural enemies (Fig. 9.5), with the pest biomass increasing with increasing crop species richness. However, the differences were not significant (Fig. 9.5a, $F1.593 = 1.464$, $p = 0.227$). In addition, the natural enemy biomass also increased with increasing crop species richness, although the differences were not significant (Fig. 9.5b, $F1.593 = 0.682$, $p = 0.409$). The mid-range value of the pest and natural enemy biomass peaked when the crop species richness was at a maximum ($N = 16$); the mid-range value of biomass was 0.12 g/22 plants and 0.04 g/22 plants, respectively. When the crop species richness was at a minimum ($N = 1$), the mid-range value of pest and natural enemy biomass was also low (pest, 0.04 g/22 plants; natural enemy, 0.03 g/22 plants).

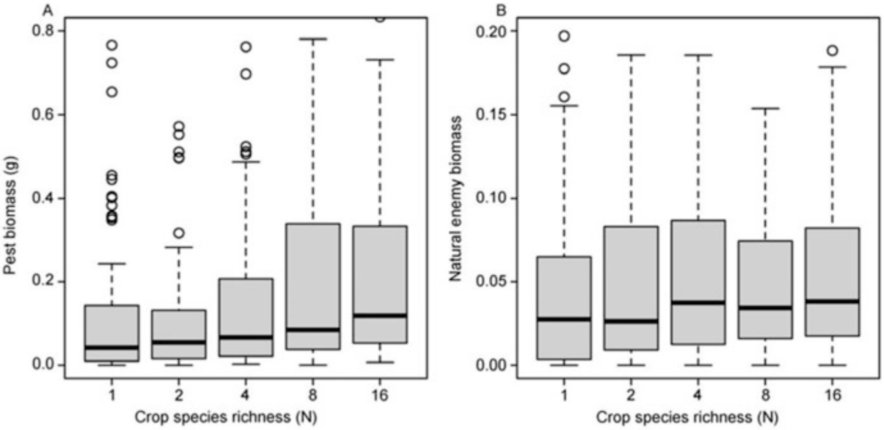

Fig. 9.5 Relationship between crop species richness and insect biomass. (**a**) Crop richness and pest biomass. (**b**) Crop richness and natural enemy biomass

9.4.1.7 Relationship at the Tri-trophic Level (Crop-Pest-Natural Enemy)

The relationship at the tri-trophic level was analyzed by generalized additive models. The effects of sampling time, pest biomass, and crop biomass on the natural enemy biomass were also analyzed (Fig. 9.6). The sampling time had significant effects on the biomass of natural enemies (Fig. 9.6a, $F1.000 = 4.007$, $p = 0.046$). Although the crop biomass had no significant effect on the natural enemy biomass (Fig. 9.6e, $F1.044 = 0.574$, $p = 0.462$), the pest biomass did have significant effects (Fig. 9.6c, $F8.846 = 15.130$, $p < 0.001$). The sampling time had no significant effect on the pest biomass (Fig. 9.6b, $F2.092 = 1.908$, $p = 0.136$), and nor did the crop biomass (Fig. 9.6f, $F1.893 = 2.031$, $p = 0.123$). In contrast, the natural enemy biomass did have significant effect on the pest biomass (Fig. 9.6d, $F6.505 = 9.478$, $p < 0.001$).

The relationship between crop richness and predator-prey interactions as they relate to pest-natural enemy systems is a very important topic in ecology, and greatly affects biological control services. The effects of crop arrangement on predator-prey interactions have received much attention as the basis for pest population management. Our results show that the biomass of pests and their natural enemies increase with increasing crop biomass, and decrease with decreasing crop biomass; however, the effects of plant biomass on the pest and natural enemy biomass were not significant. The relationship between adjacent trophic levels was significant (such as pests and their natural enemies, or crops and pests), whereas non-adjacent trophic levels (crops and natural enemies) did not significantly interact with each other. The ratio of natural enemy/pest biomass was the highest in the areas of four crop species that had the best biological control service. Having either low or high crop species richness did not enhance the pest population management service, and lead to loss of biological control. Although the resource concentration hypothesis was not well supported by our results, high crop species richness could suppress the pest population, indicating that crop species richness could enhance biological control services. These results could be applied in habitat management aimed at biological control, and provide the theoretical basis for agricultural landscape design, suggesting new methods for integrated pest management (Zhao et al. 2013).

9.4.2 Landscape-Level Drivers of Biocontrol at County Scale

9.4.2.1 Regional Landscape System Composed of Wheat and Shelterbelts

An regional landscape system composed of wheat farmland and shelterbelts was designed and implemented by Ouyang Fang, Dong Zhaoke and Ge Feng for 2 years during 2012–2013, and was located in Yucheng county, Dezhou City, Shandong Province, China, nearby Yucheng Experimental Station of the Chinese Academy of Sciences (36.57 °N, 116.36 °E) (Fig. 9.7).

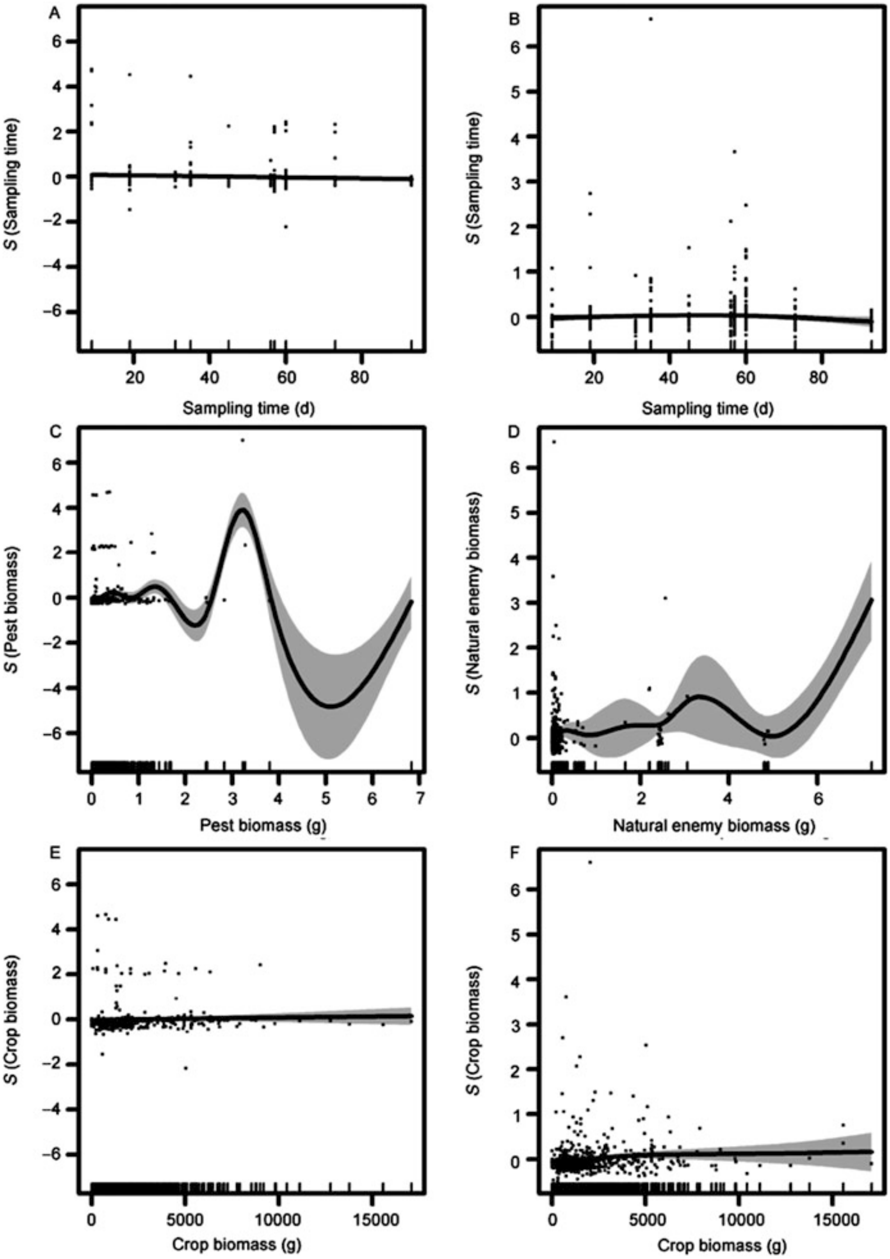

Fig. 9.6 Relationships of sampling time and biomass in a generalized additive model (GAM). **a**, **c**, and **e** indicate the effects of sampling time, pest biomass, and crop biomass on natural enemies; **b**, **d**, and F indicate the effects of sampling time, natural enemy biomass, and crop biomass on pests

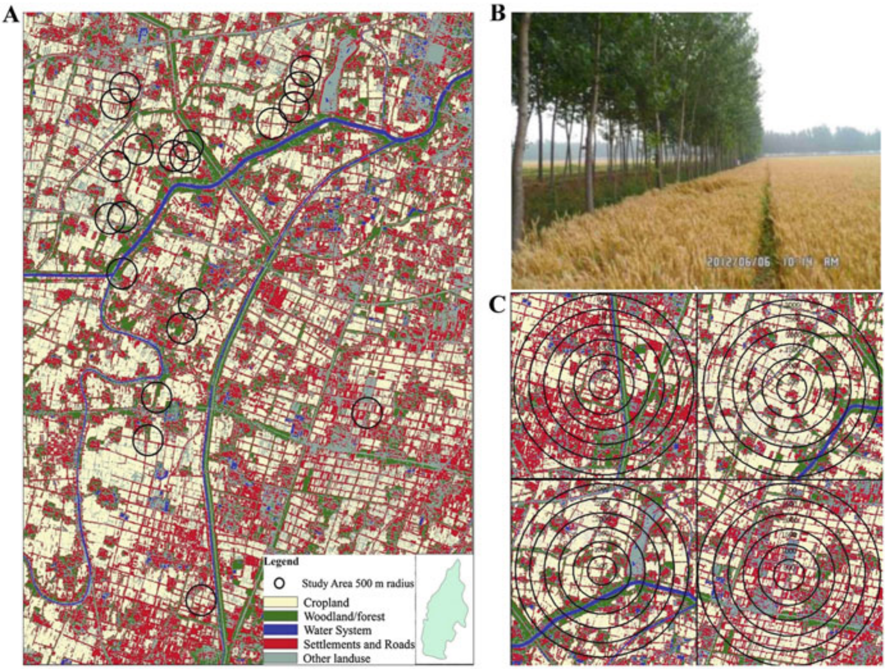

Fig. 9.7 Study region and study sites. (**a**) Twenty-eight study sites in Yucheng county, Dezhou city, Shandong province, China. (**b**) Agricultural landscape system composed of wheat farmland and shelterbelts. (Photo by Ouyang Fang in 2012 at in Yucheng county, Dezhou City, Shandong Province, China. (**c**) Four study sites with the six nested spatial scales used in the analysis)

Wheat was grown in rotation with maize. Networks of forest belts on farmland have been established to reduce the effects of wind. This type of intercropping of poplar (*Populus alba* L.) and agricultural crops began several decades ago. We selected 28 study sites, which covered a gradient from extremely simple landscapes to relatively complex landscapes. The study sites were all located within approximately 600 km^2. Each site was separated by 0.2–4 km from other sites. Field geospatial data were collected using a handheld GPS receiver.

Natural enemies of herbivorous pests in cropping systems may relocate to adjacent habitats in response to declining habitat quality in heterogeneous landscapes. In this study, we measured the cross-edge spillover of ladybeetles from wheat fields to shelterbelts, and tested how landscape variables at various spatial scales influence ladybeetle populations. We conducted a large-scale sampling study of agricultural landscapes differing in structural complexity during 2012 and 2013. The effects of landscape variables (i.e., landscape diversity and the percentage of woody habitats) on the ladybeetle abundance were investigated. *Propylea japonica* and *Harmonia axyridis* (Pallas) were the dominant ladybeetle species. The abundances of ladybeetles in spillover were positively correlated with the percentage of woody area, and negatively correlated with landscape diversity and edge density of crop habitats. It indicates that a low diversity landscape with a large area of shelterbelts supports larger ladybeetle abundance in spillover compared with a high diversity landscape with a limited area of shelterbelts. By contrast, greater numbers of within-field ladybeetles were associated with landscape diversity increase. Landscape features at the spatial scale of 2.5–3 km could best predict the abundance of ladybeetles in spillover, whereas the best predictive model for ladybeetle abundance within the field was at the 1.5 km scale. These results suggest that the landscape variables influence ladybeetle abundance differently in spillover, and within the fields. The introduction of shelterbelts in the agricultural landscape could enhance the conservation of ladybeetle populations (Dong et al. 2015).

9.4.3 Landscape-Level Drivers of Biocontrol at Province Scale

9.4.3.1 Regional Landscape System Composed of Wheat and Surrounding Vegetation

A regional landscape system composed of wheat farmland and surrounding vegetation was designed and implemented by Ouyang Fang, Men Xinyuan and Ge Feng in 2010, and was located in Shandong Province, China (Fig. 9.8). Wheat farmland

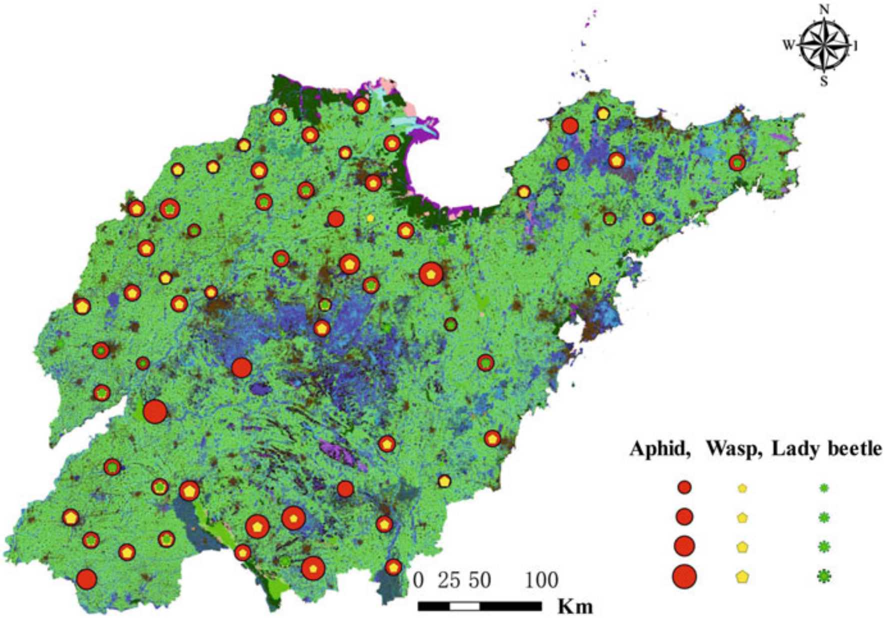

Fig. 9.8 Land coverage type distribution and the survey sites of wheat aphids and their two natural enemy insects (parasitic wasp and predatory lady beetle) in Shandong province

formed the main body, with surrounding vegetation as background, such as forest, grassland, shrub, wetland, building area etc.

Knowing the ecological effects of agricultural landscape pattern on population dynamics of insect pests and their natural enemies is fundamental for ecological regulation and management of insect pests. Previous research has studied the ecological effects of simple landscape factors on insects on the micro scale; however, only few of them focused on the ecological effects of multiple landscape factors on the macro scale. In this study, we took the planting region of wheat in Shandong province, China, as a typical example. We analyzed the ecological effects of agricultural landscape patterns (component type, component proportions and shape structure) on population dynamics of an insect pest (wheat aphids) and their two natural enemy insects (parasitic wasp and predatory lady beetle) on the basis of remote sensing data, land coverage type distribution, and survey data of insect population dynamics in the field. Landscape indices describing characters of agricultural landscape pattern in Shandong province are given in Table 9.3.

The results show that the higher number of patch types in the agricultural landscape helped to increase the population numbers of wheat aphids and predatory lady beetles per unit area. High patch density and edge density enhanced the population numbers of the two natural enemy insects (parasitic wasp and predatory lady beetle) per unit area. The relative contributions of three landscape factors (component type, component proportions and shape structure) in explaining the variation of insect population numbers (wheat aphids, parasitic wasp and predatory lady beetle) were quantified. For the wheat aphids, the total variation explained by the above three landscape factors was only 9.81%, while the three landscape factors accounted for 25.87% of the total variation for the parasitic wasp, and 47.86% for the predatory lady beetle (Fig. 9.9). The results suggest that the optimization of agricultural landscape such as crop patterns and non-crop habitats can help to directly regulate and increase the relative abundance and richness of natural enemy insects, and to indirectly suppress and reduce the population numbers of wheat aphids, enhancing regional level biological control for pests on wheat.

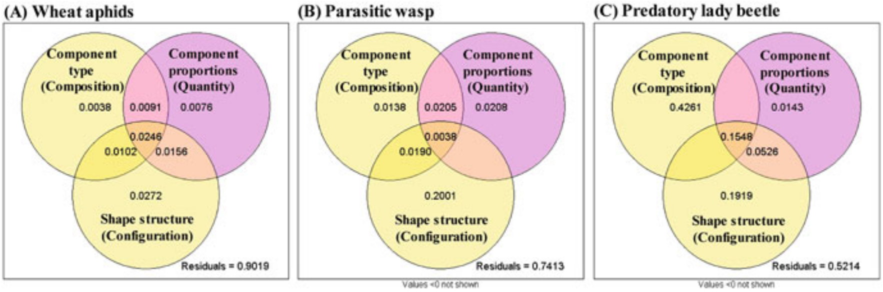

Fig. 9.9 Variation partitioning of ecological effects of component type, component proportions and shape structure on wheat aphids (**a**), parasitic wasp (**b**) and predatory lady beetle (**c**)

Table 9.3 Landscape indices describing characters of agricultural landscape pattern in Shandong province

Landscape index	Landscape	Class							
	Composition								
Patch type (PT)	Province scale	Forest	Grassland	Wetland	Water	Cropland	Orchard	Urban	Other
Patch richness (PR)	8								
Patch richness density (PRD)	0.0001								
	Quantity								
Patch density (PD)	1.7833	0.3310	0.2203	0.0058	0.1421	0.3562	0.0053	0.7026	0.0200
Proportion of land (PLAND)	100.00	11.8543	4.0458	0.6052	5.4752	60.7451	0.6053	15.9053	0.7637
Mean patch area (MPA)	56.0752	35.8148	18.3648	104.7396	38.5215	170.5388	113.8315	22.639	38.1061
Shannon diversity index (SHDI)	1.2359								
Simpson diversity index (SIDI)	0.5869								
	Configuration								
Edge density (ED)	42.9760	16.0811	7.4663	0.2668	4.8831	34.2787	0.4500	22.0885	0.4373
Landscape shape index (LSI)	428.4147								
Mean shape index (MSI)		462.0171	367.0517	34.2696	212.4886	435.6638	57.2701	548.5535	57.6263
Fractal dimension (FRACT)	1.3094	1.2166	1.2168	1.144	1.2325	1.3675	1.1952	1.2247	1.1585

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Chapter 10
IPM and Pollinator Protection in Canola Production in the USA

Anamika Sharma and Gadi V. P. Reddy

10.1 Canola Production in the USA and Pollination

Among oilseed crops, brassicas are the second largest group of crops grown globally after soybean (*Glycine max* (L.) Merr.). Oil-producing brassicas are generally derived from two species, *Brassica napus* L. and *Brassica campestris* L. (Gupta 2016). The Canola Council of Canada defines canola as "Seeds of the genus *Brassica* (*B. napus*, *B. rapa* (=*B. campestris*) or *B. juncea*) from which the oil shall contain less than 2% erucic acid in its fatty acid profile and the solid component shall contain less than 30 micromoles of any one or any mixture of 3-butenyl glucosinolate, 4-pentenyl glucosinolate, 2-hydroxy-3 butenyl glucosinolate, and 2-hydroxy- 4-pentenyl glucosinolate per gram of air-dry, oil-free solid" (Canolacouncil.org 2018). These all traits of oil are considered to be healthy for human consumption (Canolacouncil.org 2018). In Canada, a low erucic acid rapeseed was developed and released as the cultivar "Oro" in 1968. Several other cultivars with low erucic acid levels were also released later, and the first canola cultivar "Tower" was released in 1974 (Gupta 2016). Besides Canada, canola is also produced in Europe, Asia, Australia, New Zealand, and the United States. After 1985, concerted efforts to grow canola on a large scale began in the USA (Raymer 2002). It has been mainly grown in North Dakota, followed by Oklahoma, Kansas, Texas, Minnesota,

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A. Sharma (✉)
IPM Innovation Lab, Center for International Research, Education, and Development (CIRED), Virginia Polytechnic Institute and State University, 526 Prices Fork Road, Blacksburg, VA, USA
e-mail: anamika@vt.edu

G. V. P. Reddy (✉)
Insect Management Research Unit, USDA-ARS-Southern, 141 Experiment Station Rd., Stoneville, MS, USA
e-mail: gadi.reddy@usda.gov

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Montana, Idaho, and Oregon. Currently about 1.7 million acres of canola are grown in the USA (uscanola.com 2018).

Canola plants are self-fertile and mostly self-pollinated. Wind pollination is common (Buntin et al. 2017), but studies have reported a modest dependency on pollinators (Klein et al. 2007; Westcott and Nelson 2001). Although insects are often considered as only supplemental pollinators, canola is highly attractive to pollinating insects because it is an early and a rich source of nectar (Buntin et al. 2017). Canola provides a nutritional balance of amino acids, protein and fats to the bees (Somerville 2002). Common insect pollinators of canola include honey bees, bumble bees, large carpenter bees, and some native solitary bees (e.g., Andrenidae, Colletidae, Halictidae, Megachillidae, and Xylocopidae) (Badenes-Pérez et al. 2017; Buntin et al. 2017). Although honey bees are considered to be responsible for 90% of insect pollination on canola, other foraging insect families such as Diptera (Syrphidae, Bombyliidae, and Calliphoridae) also play an important role in cross pollination, while species of Lepidoptera, Coleoptera, Hemiptera and Neuroptera also frequently visit canola crops (Westcott and Nelson 2001; Badenes-Pérez et al. 2017). Several reports have found higher seed yields in the presence of honey bees. Research in Australia in 1997 found an increase in yield of 18% on the variety Karoo (Manning and Boland 2000), while research in Canada found an improvement in seed yield of 46% in the presence of honey bees (Sabbahi et al. 2005). Increase in the number of fertile pods due to pollination causes a greater yield (Manning and Wallis 2005). The presence of pollinators [(*Apis dorsata* Fabricius, *A. florea* Fabricius (both Apidae), and *Halictus* sp. Latreille (Halictidae)] on canola has been reported to increase both the number and weight of seeds per pod (Ali et al. 2011; Shakeel and Inayatullah 2013). Bees may also cause earlier seed set, resulting in shorter, more compact plants with an even seed maturity, making such canola crops easier to harvest and less prone to the pod shattering (Somerville 2002; Gavloski 2017). According to the canola council of Canada, pollinators are vital for hybrid seed production as they are necessary for the pollen delivery from the male parent lines to female parent lines (Clay 2009; Durán et al. 2010). Both *Apis mellifera* L. (Hymenoptera: Apidae) and alfalfa leafcutting bees, Megachile rotundata Fab. (Hymenoptera: Megachilidae) play an important role in pollination, with an opportunity to provide diversified honey bee products (Hoover and Ovinge 2018). Seed germination rate also has been reported to increase in the insect pollinated canola crops (Kevan and Eisikowitch 1990). Moreover, bees promote higher yields through better ripening of seeds. More uniform flowering and earlier pod-setting (Abrol 2007), greater number of pods per plant and seeds per pod, an overall increase in seed weight, and a reduction in initiation of blooming time (Sabbahi et al. 2005; Gavloski 2017) are the other benefits from the presence of insect pollinators. It is also reduces the flowering period. Although it has never been directly tested, the fungal disease stem rot, *Sclerotinia sclerotiorum* (Helotiales: Sclerotiniaceae) could be reduced by a shorter flowering period (Gavloski 2017). Similarly, another beneficial aspect of insect pollination in canola is the dispersal of entomopathogenic fungi; one study done in Canada found higher mortality of *Lygus* sp. (Hemiptera: Miridae) when honey bees were used to spread *Beauveria bassiana* (Al Mazra'awi et al. 2006; Gavloski 2017).

In the USA, honey bees are an important part of agriculture as managed pollinators. In 2000, an estimated 2.9 million bee colonies were recorded in the United States (as reported by beekeepers with five or more colonies) (Morse and Calderone 2000). Lately, US agriculture has shown increased dependence on pollinators (from 1992 to 2009) in several crops (Calderone 2012). In canola, both managed and wild bees play an important role (Morandin and Winston 2005). Wild species of pollinators can provide pollination services in the absence of managed pollinators. For instance, the stingless bees *Plebeia emerina* Friese and *Tetragonisca fiebrigi* Schwarz (both Hymenoptera: Apidae) showed similar pollination efficiency as *A. mellifera* in terms of fruit setting in canola (Witter et al. 2015).

10.2 Integrated Pest Management (IPM) for Canola

The major insect pests of canola in USA are the cabbage seedpod weevil, *Ceutorhynchus assimilis* (Paykull) (syn. *Ceutorhynchus obstrictus* [Marsham]); bertha armyworm, *Mamestra configurata* Walker; *Phyllotreta cruciferae* Goeze; crucifer and striped flea beetle, *Phyllotreta striolata* (Fabricius) (Coleoptera: Chrysomelidae); tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois) (Hemiptera: Miridae) and diamondback moth, *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae). A number of minor pests also attack canola in the USA, including cabbage and turnip aphid, *Brevicoryne brassicae* (Linnaeus) (Hemiptera: Aphididae), *Hyadaphis erysimi* (Kaltenbach) Hemiptera: Aphididae) and various grasshoppers (Weiss et al. 2018; Reddy 2017). Several bacterial, fungal, viral, and phytoplasmal pathogens also reduce yield in canola (Kharbanda et al. 2018). In general, all groups of pesticides including herbicides, insecticides, fungicides and seed treatments are used in the USA on canola crops to increase yield (Raymer 2002; Johnson et al. 2010).

The main role of IPM techniques is to promote the integration of all available pest control techniques to reduce pest populations. Such techniques should be economically justifiable and minimize risks to human health and the environment. Two important components of canola IPM are cultural controls (planting dates, increased seeding rates) and insecticide treatments. Determining an accurate action threshold and the development of pest prediction models for pest monitoring could help to reduce the insecticide application without losing yield due to insect infestation (Sekulic and Rempel 2016). IPM strategies for the majority of the important insect pests of canola include monitoring and forecasting of insect populations to achieve their proper management. To accurately estimate damage, proper trapping methods and correct estimation of thresholds are extremely important for the effective management of insect pests (Gavloski 2017).

Flea beetles (P. cruciferae and P. striolata) are the most economically damaging insect pests of canola in the northern USA and Canada, and foliar damage from flea beetles can causes yield reduction of 10–50% during medium to high level of population outbreaks (Sekulic and Rempel 2016). Cultural strategies for controlling flea beetles include higher seeding rates, planting bigger sized seeds, early seeding, and reduced tillage (Elliott et al. 2008; Cárcamo et al. 2008). The use of resistant varieties with greater trichome density can also be helpful in reducing flea beetle damage (Gavloski 2017). However, at present no resistant varieties of canola are known for flea beetle (Gavloski 2017). Entomopathogenic fungi (*Beauveria bassiana* (Bals.-Criv.) Vuill. (Hypocreales: Cordycipitaceae) and *Metarhizium brunneum* Petch (Hypocreales: Clavicipitaceae)), and the nematode *Steinernema carpocapsae* (Rhabditida: Steinernematidae) are potential biocontrol agents useful against flea beetles (Reddy et al. 2014), but these agents have not been studied on a wide scale. Neonicotinoid insecticides used as seed treatments provide early season protection for a short period, and season long control may require an additional foliar application (Reddy 2017). Various pyrethroid, carbamate, and organophosphate insecticides are foliar insecticides used for control of flea beetles in canola (Gavloski 2017), as well as for suppression of other major insects (*P. xylostella, M. configurata* and *L. lineolaris*). Moreover, the larval parasitoids *Diadegma insulare* (Hymenoptera: Ichneumonidae) and *Microplitis plutellae* (Hymenoptera: Braconidae), and the pupal parasitoid *Diadromus subtilicornis* (Hymenoptera: Ichneumonidae), also serve as biological control agents for control of diamondback moth *P. xylostella.* For *M. configurata,* fall tillage as a cultural control has been found to reduce outbreaks of this pest, while the native endoparasitoid, *Banchus flavescens* (Hymenoptera: Ichneumonidae) can cause up to 40% parasitism (Wylie and Bucher 1977), and a nuclear polyhedrosis virus also causes some level of mortality for insect pests (Gavloski 2017; Reddy 2017).

10.3 Impact of IPM Methods on Pollinators

Along with managed bees, populations of wild pollinators (*Bombus* spp.) are strongly influenced by various pesticides in specific weather conditions (Turnock et al. 2006), and it has been estimated that 20% of all honeybee colonies are adversely affected while 5% of bee colonies in the USA die during the winter due to severe pesticide exposure. This mortality of the bees is responsible for crop production loss of 13.3 million dollars each year (Pimentel and Burgess 2014; Meikle et al. 2017). In North America, the main crops, including canola, wheat, maize, soybean and cotton, represent approximately 115 million hectares of annual production. Neonicotinoid insecticides are routinely applied to seeds to protect these crops from early season insect pests (Krupke and Long 2015). The use of neonicotinoid insecticides as seed treatments in canola to control flea beetle populations (*P. cruciferae*, *P. striolata, Psylliodes punctulata* Melsh) began in the mid-1990s (Cutler et al. 2014). Seed treatments (imidacloprid, clothianidin, and thiamethoxam) are used to prevent damage at the most vulnerable initial stage of the canola crop. Use of seed treatments reduces the number of foliar insecticide applications needed, and seed treatments are thought to be less harmful than foliage treatments to pollinators, as well as having a low pesticide residual effect for human health (Sekulic and Rempel 2016). Insecticides applied as seed coatings move from seeds into the young growing roots and leaves, and provide post emergence protection to young plants. These pesticides are then transported systemically within the developing plants to the leaves and flowers where, although they occur only in small quantities, they can pose a threat to pollinators, especially to bees (Sur and Stork 2003). In general, exposure of bees to neonicotinoids applied as seed treatments through pollen and nectar of treated crops has been found to be negligible (Maus et al. 2003), and seed treatments have less of the active ingredient per unit area compared to foliar application, which reduces the damage to non-target pollinators, minimizing the likelihood of exposure (Sekulic and Rempel 2016).

Canola has bright, visible flowers and produce copious amounts of nectar and pollen that attract pollinators (Thom et al. 2016). In canola the seed treatment has been found to have an almost negligible effect on honey production by the managed honey bee colonies, exposed to the treated canola crop (Cutler and Scott-Dupree 2007; Cutler et al. 2014). However, wild bee populations showed a decline after being exposed to conventional seed treatment insecticides in canola crops (Scott-Dupree et al. 2009). The direct contact toxicity of imidacloprid, clothianidin, deltamethrin, spinosad and novaluron as seed treatment and foliar application was tested on populations of common eastern bumble bees [*Bombus impatiens* (Cresson) (Hymenoptera: Apidae)], alfalfa leafcutting bees [*Megachile rotundata* (F.) (Hymenoptera: Megachilidae)], and blue orchard bee [*Osmia lignaria* Cresson (Hymenoptera: Megachilidae)] (Scott-Dupree et al. 2009). Among five insecticides used, only novaluron was nontoxic to the tested pollinators in laboratory. The other four chemicals (imidacloprid, clothianidin, deltamethrin, spinosad) showed high but varying degrees of toxicity between the three-pollinator species (Scott-Dupree et al. 2009) (Table 10.1). This research also indicates the need to test on wild bee species as well as honey bees, as wild bees are more representative of the specific agricultural system (Scott-Dupree et al. 2009), and also raise the point that determining the impact of seed treatment on bees cannot allow us to predict the effect of these treatments on all pollinators. Furthermore, new laboratory approaches are needed to infer real-world consequences of exposure to realistic field levels of neonicotinoids, since only field based studies have to date predicted the negative effects of such exposure (Lundin et al. 2015).

Major groups of insecticides, including neonicotinoid, pyrethroid, organophosphate, and carbamate insecticides have been found to be the most toxic for bees in the USA (Frazier et al. 2015; Lundin et al. 2015; Hladik et al. 2016). Oxadiazines (indoxacarb), thiourea derivatives (diafenthiuron), avermectins (emamectin benzoate), spinosyns (spinosad), diamides (chlorantraniliprole), benzoylureas (flufenoxuron, lufenuron), pyridine azomethine derivatives (pymetrozine), phenylpyrazoles (fipronil), neonicotinoids (thiamethoxam, clothianidin, imidacloprid) and organophosphate (profenofos) are the insecticides commonly applied to control insect pests worldwide (for an example, *P. xylostella*), and all of these groups have been found to be harmful to pollinators to some extent (Badenes-Pérez et al. 2017; Abrol and Thakur 2016).

Laboratory studies have shown a variety of harmful effects on bees from systemic neonicotinoid pesticides, such as impaired learning and memory loss (Ciarlo et al. 2012; Rortais et al. 2005). In France, low levels of imidacloprid were found in

Table 10.1 Impact of pesticides on pollinators reported on canola

Pollinator	Location	Pesticide used	Impact on pollinator	Sources
Bombus impatiens, Megachile rotundata, Osmia lignaria	Canada	Imidacloprid, clothianidin, deltamethrin, spinosad, and novaluron	From highly to moderately toxic	Scott-Dupree et al. (2009)
Apis mellifera	Canada	Clothianidin	No long term impact	Cutler and Scott-Dupree (2007)
Apis mellifera	Canada	Clothianidin	Low risk	Cutler et al. (2014)
Apis mellifera	Canada	Carbaryl	Presence in pollens	Kevan et al. (1984)
Apis mellifera	France	Imidacloprid	Presence in pollens	Chauzat et al. (2006)
Apis mellifera	Germany	Clothianidin	Mortality	Heimbach (2015)
Apis mellifera	India	Oxadiazines thiourea derivatives avermectins spinosyns diamides benzoylureas pyridine azomethine derivatives phenylpyrazoles neonicotinoids organophosphate	Toxic	Abrol and Thakur (2016)

a high percentage of pollen samples of canola, along with maize and sunflower (Chauzat et al. 2006). It has also been found that fungicides and adjuvants can disrupt nest recognition in solitary bee species (Artz and Pitts-Singer 2015). In the USA, insecticides, fungicides and herbicides were detected in tissues of native bees (Hladik et al. 2016). In some cases, combination of insecticides, fungicides, herbicides and adjuvants cause more severe effects than any one item alone (Hooven et al. 2013; Mullin et al. 2015). Seed treatments contaminate soil, water, and plant products such as pollen and nectar, and these contaminated materials can have a negative impacts on bee health (Krupke and Long 2015). In the USA, foraging of *A. mellifera* is reported to be reduced on crops like cotton, blueberries, alfalfa, corn, and pumpkins in the presence of different pesticides (Frazier et al. 2015). Pesticide drift and the collection of nectar and nesting material from pesticide-contaminated plants can also cause poisoning of bees. The classic indications of bee poisoning due to pesticides are unusual numbers of dead and dying honey bees in front of the hives, increased defensiveness, abnormal behavior of being extremely lethargic or aggressive and confused, disorientation, dead brood, and poor queen development (Hooven et al. 2013). It has been found that neonicotinoid insecticides can harm populations of both managed and wild bees (Mullin et al. 2010; Goulson 2013; van der Sluijs et al. 2013).

The use of pesticides, herbicides, and the introduction of genetically modified (GM) crops have influenced natural population of pollinators. For instance, a study was conducted in northern Alberta, Canada, in organic, conventional, and herbicide-resistant, genetically modified (GM) canola fields (*Brassica napus* and *B. rapa*). When wild bee populations were assessed in those fields, the greatest pollination deficit was recorded in GM fields, followed by conventionally managed fields, while in organic fields no pollination deficit was recorded (Morandin and Winston 2005). A study in Finland (Hokkanen et al. 2017) found drastic decline in yield trends of canola associated with neonicotinoid use and simplified landscapes. Genetically modified herbicide resistant crops have increased the use of nonselective herbicides and hence causes change in habitat diversity, which in turn causes nutritional stress for pollinators (Sharma et al. 2018). Genetically modified canola such as Roundup ready and Cibus are available in the USA, and these cultivars provide opportunities to the use of non-selective herbicides on canola. Although not much is known about the impact of herbicide resistant canola on pollinators, however, in general an increase in herbicide use due to availability of GM crops impacts pollinators. The main reason of poisoning is the addition of adjuvants, which are added to increase the efficiency of herbicides (Mullin et al. 2015; Shrestha et al. 2017; Sharma et al. 2018).

Pollinator populations can potentially be reduced due to exposure to different kinds of pesticides, exotic pathogens, agricultural intensification, habitat alteration and fragmentation, nutritional stress, and the loss of genetic variation (Calderone 2012). Two possible causes of the decline of populations and genetic variability of invertebrate pollinators, particularly native bees in North America, were suggested by Cane and Tepedino (2001): (1) monoculture grain crops do not provide sustainable food to pollinators, and (2) widespread habitat destruction due to removal of other flowering plants. Therefore, the restoration of plant biodiversity improves habitats for domestic and wild bees and other beneficial insects. Floral resource availability is known to be the primary direct factor influencing bee population abundance, while invasive parasites, pathogens, foraging range, and diet breadth are known to limit bee populations (Roulston and Goodell 2011). Tolerating certain weed species within crop fields can provide food resources and habitat to pollinators, as will the appropriate management of hedgerows, field margins and non-cropped areas (Nicholls and Altieri 2013). Variation in response to insecticides is due to variation in direct (food resources, nesting resources and incidental risks) and indirect factors (grazing, invasive species, habitat complexity and land management). Although food availability is a major regulating factor affecting pollinator populations, manipulative experiments to explore different factors and relationships between indirect factors and floral resources based on environmental circumstances are still needed (Roulston and Goodell 2011). Canola itself provides an excellent source of pollen and sugars to bees (Westcott and Nelson 2001). Hence, canola as a crop certainly provides diversification to the landscape otherwise dominated by grain crops, and increases the economic value of the landscape by providing a good food source to pollinators (Eberle et al. 2015).

Hooven et al. (2013) examined the impact of pesticides on bee behavior and proposed a number of ways to reduce bee poisoning. The selection of low toxicity pesticides with less residual toxicity, reducing pesticide drift and water contamination, and avoiding tank mixing can reduce bee poisoning. Moreover, it is important that pesticide applicators should be informed about the effects of both active and inert components of the products which they apply. The inclusion of pollinator management in IPM is necessary in the present circumstances (Biddinger and Rajotte 2015), which includes the accommodation of pollinator protection by adjusting the pesticide regime, and selecting of new and less harmful pesticides. This also includes concentrating on alternative pollinators and improving the insect pollinator community (Wheelock et al. 2016).

10.4 Conclusion

Canola production in the USA has a promising future for growers as well as for the overall economy, despite the presence of some important insect pests, pathogens and weeds. Although conventional pesticides seem to be most reliable tool at present to deal with canola insect pests, pathogens and weeds, caution should be taken in choosing the pesticide, timing and method of application. Even though canola can be self-fertile, pollinators find the copious amount of nectar from the blooming flowers of canola quite attractive, and this attraction of pollinators strengthens the economic value of canola by increasing landscape diversity and improving the health of pollinators. Improving the public understanding on the importance of biodiversity of insect pollinator community, both around canola crops and in general, is urgently needed. Bees are the most abundant pollinators in canola. Therefore, caution regarding pesticide application and blooming time of canola should be taken by growers. Both growers and beekeepers should be appropriately informed about how to reduce the bee poisoning. Various biological control methods have shown great potential to perform the same service as various pesticides without the harm to the pollinator community, and these control methods should be promoted among growers. Since canola is a natural attractant for pollinators, better communication between growers and beekeepers is required to reduce possible pesticide drift and the collection of contaminated nectar by bees (Fig. 10.1).

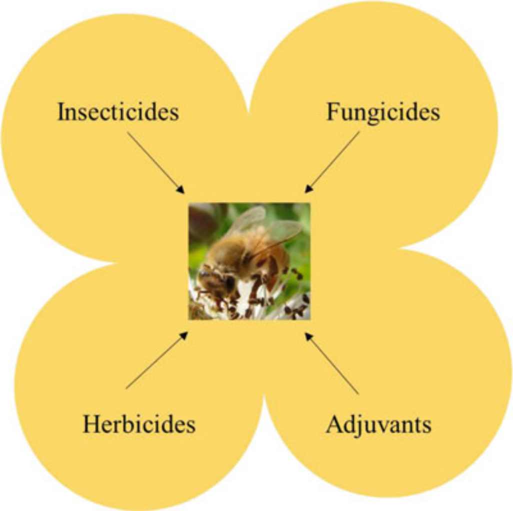

Fig. 10.1 A cumulative and synergistic effect of pesticides on pollinators could be greater than their individual impact. (Photo credit: Dr. Ramesh Sagili)

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Part III
Specific Techniques to Enhance Ecostacking

Chapter 11
A Study and Application of Biological Control Technique Using the Parasitic Natural Enemy *Aphidius gifuensis* (Hymenoptera: Braconidae) to Control *Myzus persicae* (Hemiptera: Aphidiidae) in China

Yanbi Yu, Hailin Yang, Zhonglong Lin, Limeng Zhang, Xinghui Gu, Chunming Li, Xinzhong Wang, Bin Chen, and Li Chengyun

11.1 Introduction

As one of the most important tobacco growing regions in the world, tobacco-planting area in the Yunnan Province has maintained at about 469 thousand hectares, which is 35% of Chinese tobacco growing area, and 20% of world's tobacco area. Yunnan province is also one of the famous planting areas of high-quality tobacco in the world, and a globally important production base. Tobacco is a pillar industry of the Yunnan province, and more than eight hundred thousand households have benefited from tobacco planting and have lifted themselves out of poverty. The Yunnan tobacco planting regions are generally located in remote mountainous areas, which have a fine scenery but a fragile ecological environment. Yunnan tobacco planting has the following five characters: Wide distribution of tobacco planting, diverse climate and environment, smallholder farmers dominating the

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Y. Yu · Z. Lin
Yunnan Tobacco Company of China National Tobacco Corporation (CNTC), Beijing, China

H. Yang · L. Zhang · X. Gu
Yuxi Branch of Yunnan Tobacco Company of CNTC, Beijing, China

C. Li
Honghe Branch of Yunnan Tobacco Company of CNTC, Beijing, China

X. Wang
Dali Branch of Yunnan Tobacco Company of CNTC, Beijing, China

B. Chen · L. Chengyun (✉)
State Key Laboratory for Conservation and Utilization of Bio-Resources in Yunnan, College of Plant Protection, Yunnan Agricultural University, Kunming, China

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tobacco production landscape, different backgrounds for growers, and many factors interacting for tobacco leaf yield and quality.

Aphids are one of the most destructive pests on earth, and one of the main pests in agricultural production systems. *Myzus persicae* Sulzer is an aphid species with a wide host range, whose feeding and deposition of honeydew can directly injure the host plant causing yield reduction (Kulash 1949; Stary 1970). In addition, the most harm from *M. persicae* results from its ability to transmit over one hundred viral diseases, among over four hundred host plant species (Mackauer and Way 1976) leading to a decrease of tobacco quality. The control of this pest is still largely based on insecticides in Yunnan (Zhao et al. 1980), which leads to problems of resistance, difficulty of control, killing of natural enemies, decrease of biodiversity, excessive pesticide residues, safety of product quality by repeated applications, inappropriate application methods, and incorrect application rates. It is important to conduct research and to apply biological control technology to replace chemical control by natural methods, ecological principle and systemic methodology.

Aphidius gifuensis Ashmaed (Hymenoptera: Braconidae) is an important natural enemy and has been found attacking aphids in various habitats. This natural parasitic enemy is widely distributed all over the world and occurs, for example, in China, India, Canada, USA and so on, which provides a good ecological basis for wider application. Many studies have been conducted on the biology and ecology of this insect (Bi et al. 1993; Lu et al. 1993). The biological characters of *A. gifuensis* are shown in Fig. 11.1: Female *A. gifuensis* mating with a male, finding the host aphid, laying eggs in aphid individuals. These eggs grow by absorbing the nutrients from the aphid, and the process of *A. gifuensis* growth is also the process of death of the aphid.

There are two bottlenecks for large-scale application of biological control for the aphids by using *A. gifuensis*. The first is large-scale production and release of *A. gifuensis*. The second is how to spread this technology to technicians and farms, and make smallholders benefit from the technology. Thus, after research, we have established two kits of effective, economic, and convenient high-density breed-

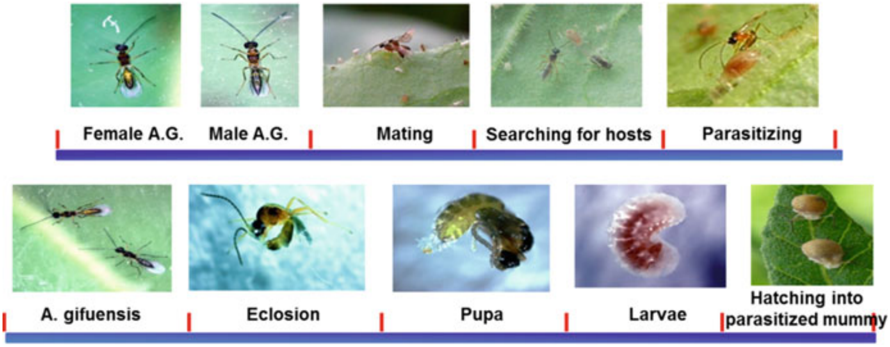


Fig. 11.1 Biological characters of *Aphidius gifuensis*

ing technology for different application areas; adult-plant breeding and seedling breeding, published industry-level standards, constructed “one plus two” model of technology extension, achieving large-scale application of the technology of aphid control by *A. gifuensis*.

11.2 Technology System

Large scale, high-density mass rearing of *A. gifuensis* is mainly divided into three parts: (1) cultivating host plants for large population of *M. persicae*, (2) breeding large population of *M. persicae*, (3) high-density breeding of *A. gifuensis* on *M. persicae.* Optimum conditions and procedures for the technology are described below.

11.2.1 Breeding on Mature Plants

11.2.1.1 Cultivating Host Plants

A proper host plant with high resistance to TMV is selected, such as tobacco (variety Yunyan 203), radish (white radish), and Chinese cabbage (variety Chinese 82). When starting the mass-rearing, seed host plants and transplant them after 70–80 days. After cultivating for 25–30 days, inoculate with aphids and breed them for 15–20 days in the greenhouse.

11.2.1.2 Breeding Large Populations of *Myzus persicae*

When the tobacco plants have 6–8 effective leaves, each plant is inoculated with 20 healthy aphids. Breeding the aphids for 15–20 days is at the temperature of 17 °C to 27 °C, humidity of 50% to 80%, in a greenhouse (50 m × 12 m × 4.6 m) (Deng et al. 2006; Wu et al. 2000; Yang et al. 2009).

11.2.1.3 High-Density Breeding of *A. gifuensis*

The breeding process for *A. gifuensis* is shown in Fig. 11.2. When the population of aphids reaches 2000 ahpids per plant, release *A. gifuensis* at the ratio of *A. gifuensis* to aphids between 1 per 50 to 1 per 100. When *A. gifuensis* lay their eggs, a number of parasitized aphids are obtained. Parasitized aphids will turn into mummified aphids, from which new generation of *A. gifuensis* will emerge. The parasitism rate is higher than 90% after 10 to 15 days. (Wei et al. 2003; Yang et al. 2009).

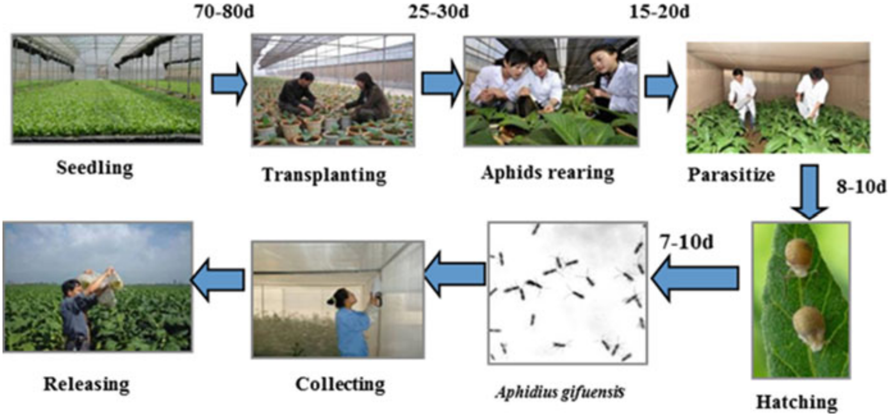


Fig. 11.2 Breeding process for *A. gifuensis*

Every tobacco plant can produce 6000–10,000 *A. gifuensis*. If each small greenhouse (3 m × 3 m × 2 m) can support 28 plants, then 16.8 thousand of *A. gifuensis* can be produced in one greenhouse and can be used for 6 hectares of tobacco plants.

11.2.2 Breeding on Seedling Plants

11.2.2.1 Cultivating Host Plants

Tobacco variety Yunyan 203 with high resistance to TMV is selected and bred according to GB/25241 (tobacco intensive cultivation technology discipline). Tobacco seedlings with 5 leaves and 1 heart are used to rear aphids by the method of breeding aphids and *A. gifuensis* separately. Tobacco seedlings in the cat-ear period are used to grow aphids by the method of breeding aphids and *A. gifuensis* at the same time.

11.2.2.2 Breeding Large Population of *Myzus persicae*

Breeding aphid and *A. gifuensis* separately: on tobacco with 5 leaves and 1 heart, inoculate aphids to leaves according the standard of 10 aphids per plant. The conditions involve air temperature from 20 to 30 °C and humidity from 60% to 80%. After 10 to 12 days, average population of aphids reaches 200 per plant for breeding natural enemies.

Breeding aphids and *A. gifuensis* at the same time: on tobacco at the cat-ear period, inoculate aphids to leaves according the standard of 2.5 aphids per plant (aphids with parasitism rate from 40% to 60%, or parasitoid/aphid ratio controlled from 5% to 10% are used). The condition is with the temperature from 20 to 30 °C, and humidity from 60% to 80%.

11.2.2.3 High-Density Breeding of *A. gifuensis* on the Aphids

Breeding aphids and *A. gifuensis* separately: According to the population of aphids on a single plant, *A. gifuensis* or parasitic aphids are inoculated in the greenhouse. After 17 days, the population of parasitized aphids would reach one hundred thousand per square meter.

Breeding aphids and *A. gifuensis* at the same time: *A. gifuensis* is inoculated at the same time with the aphids. *A. gifuensis* will emerge from the parasitized aphids and parasitize other aphids. If the parasitism ratio is too high, aphids need to be inoculated, while if the parasitism ratio is too low, *A. gifuensis* need to be inoculated. After 23 days, the population of parasitized aphids will reach 49 thousand per square meter.

11.2.3 Collection and Storage of A. gifuensis

There are two methods for collection of *A. gifuensis.* Automatic collection: collection bag is placed in the breeding tents; *A. gifuensis* would fly into the bag because of its phototaxis. Artificial collection: self- invented simple collection devise, or automatic collection devises are used to collect *A. gifuensis* in the tents, and *A. gifuensis* is stored in container.

11.2.4 Release of A. gifuensis

The release process of *A. gifuensis* are shown in Fig. 11.3. We use different methods to collect parasitized aphids and *A. gifuensis* from the large-scale feeding rooms. The methods of releasing *A. gifuensis* are the release of parasitized aphids, release of *A. gifuensis*, and dispersion in field. When releasing parasitized aphids, leaves or seedlings with parasitized aphids are hung onto plants. When releasing *A. gifuensis*, *A. gifuensis* is taken to the field in a collection bag or a collection bottle, to be released before 12 o'clock noon without rain. Transportation time needs to be less than 3 hours. When dispersed directly in the field, *A. gifuensis* is breed in a breeding tent in the field. Open the tent when the parasitism ratio is up to 90%, and *A.gifuensis* will fly out to find aphids. Occurrence of aphids is used to ensure the release time and population density. When population of aphids on one plant reaches 1–5, *A. gifuensis* is released on the scale of 200 to 500 per mu (3–8 per m^2). The second and third releases are according to aphid occurrence. When population of aphids on one plant reached 6 to 20, *A. gifuensis* is released on the scale of 500 to 1000 per mu

Collection of A. gifuensis Storage of A. gifuensis Product of A. gifuensis (Boxes of parasitized aphids) Product of A. gifuensis (Cards of parasitized aphids)

Fig. 11.3 Release process of *A. gifuensis*

(7–15 per m^2). When population of aphids on one plant is more than 20, *A. gifuensis* is released on the scale of 1000–1200 per mu (15–18 per m^2).

11.2.5 Conservation of Aphids and A. gifuensis in Winter

11.2.5.1 Conservation of Aphids in Winter

The main method of conservation of aphids in winter is conservation by host plants in greenhouses. Aphids are collected from the wild and inoculated onto healthy tobacco seedling plants, radish, and other host plants. The breeding condition is at temperature from 17 °C to 27 °C, and humidity from 50% to 80%. Status of aphids and hosts are monitored. Old, weak, ill aphids and hosts with virus are removed three times. Purified, rejuvenated and virus-free aphids are obtained.

11.2.5.2 Conservation of *A. gifuensis* in Winter

The methods of conservation of *A. gifuensis* are conservation on host plants in greenhouses, and cold storage of parasitized aphids. *A. gifuensis* are collected from the wild and used to parasitize aphids for three times. The breeding condition is at temperature from 17 °C to 27 °C and humidity from 50% to 80%. Purified and rejuvenated *A. gifuensis* are obtained. When using cold storage, parasitized aphids are collected by brush, or collection devise, from hosts and put into tubes and kept at 4 °C to 5 °C. Seedlings or other plants with parasitized aphids could also be directly put into 4 to 5 °C. Emergence rate maintains at 90% after storage for 20 days.

11.3 Application Systems

11.3.1 Technical Standard

As shown in the Fig. 11.4, an industrial standard named "Technical schedule of control of aphids by *A. gifuensis*" was published, including conservation, rejuvenation, large-scale breeding, collection and release. Construction standard for conservation breeding facility was published, technical process was clarified, and technical manual was unified. 506 breeding bases were built in 16 provinces (region, city) in China, with a total area of 420 thousand square meters, and breeding capacity of 24 billion per breeding period.

11.3.2 Training

The training system has four levels, including industrial level, provincial level, municipal level and county level, built from organization of research and application in tobacco industry, and organization of agricultural application in government (Figs. 11.5 and 11.6). We built the training platform, carried out theoretical training and practical training for technical backbone, technician, extension workers and farmers about key points and difficulties in technology and operation. We have trained more than 3000 backbones, more than 20,000 technicians, and provided more than 1200 thousand pieces of technical material. More than 120 thousand farms have benefited from this.

11.3.3 Goal Setting

According to the occurrence of aphids and release times of *A. gifuensis* in different tobacco-planting areas, *A. gifuensis* was released following five standards, including

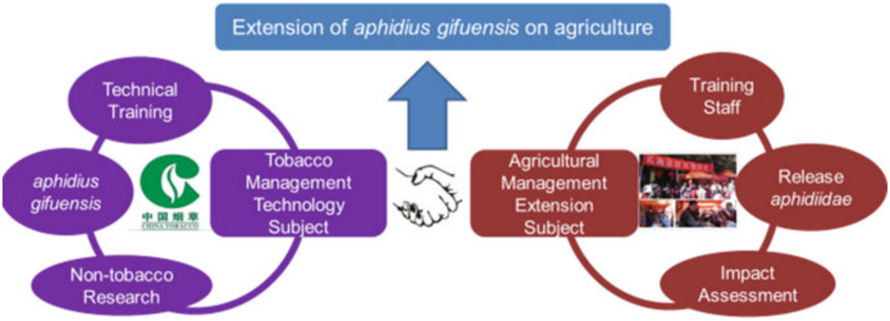


Fig. 11.4 Extension system

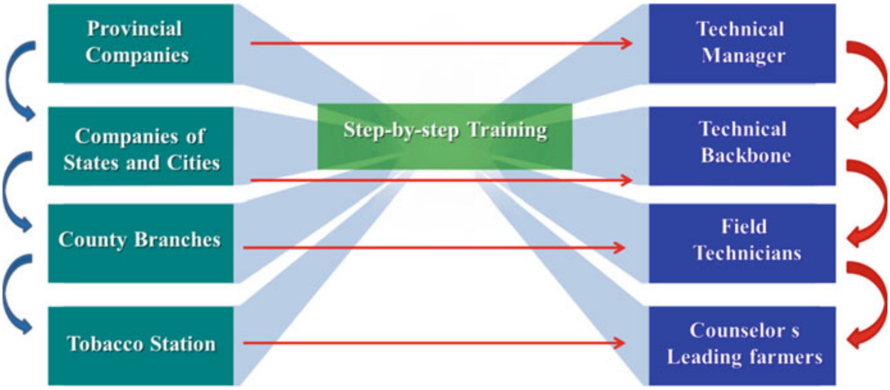


Fig. 11.5 Training system

Fig. 11.6 Four-level training

7500 per hectare, 12,000 per hectare, 15,000 per hectare, 18,000 per hectare and 22,500 per hectare. Also, according to the features of different areas, application areas were set to 4 levels including 30%, 50%, 80% and 95% of tobacco-planting area.

11.3.4 Match Funding

This technical application was divided into technical projects. Each province matched funding by 75–150 yuan per hectare, according to the set application areas.

11.3.5 Evaluation

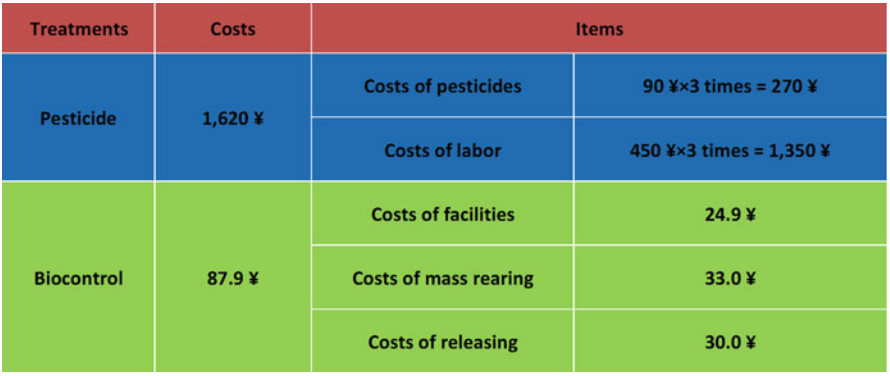
Evaluation was carried out to check implementation, supply, application scale, and control efficiency of this technology on different levels. Rewards and punishments were made so that evaluation results are related to salary of technicians, and funding in the next year.

11.4 Effects

Compared to chemical control of aphids, the cost of aphid control using *A. gifuensis* is much lower. The cost of biological control is about 87.9 yuan per hectare, compared to 1620 yuan per hectare by chemical control (Fig. 11.7).

Aphids were controlled well because of long-term releases of *A. gifuensis*. Meanwhile, population of useful natural enemies such as *Coccinella septempunctata*, *Harmonia axyridis, Episyrphus balteatus, Chrysopa sinica, Lycosa pseudoamulata* increased markedly, protecting biodiversity in the field, and proving the long-term ecological effect of biological control.

This technology has been applied in tobacco-planting area of Yuxi from 2000 onwards. By 2010 this technology covered Yuxi tobacco-planting area 100%, and began to be applied in Yunnan province. In 2013, it covered 90% of tobacco planting fields in Yunnan province. Since then, this technology has been applied in many provinces of China from 2014. Step by step, this technology has covered 100% of Yunnan tobacco-planting area and 90% of Chinese tobacco-planting area. Efficiency of aphid control in the field reached 80%, better than with using pesticides. The total application area in China reached 45263.2 thousand mu from 2010 to 2015, with a total decrease in applied pesticides reaching 1966.05 tons, and total decrease of control cost reaching 1.523 billion. 8.778 billion of financial loss was saved,



Treatments	Costs	Items	
Pesticide	1,620 ¥	Costs of pesticides	90 ¥×3 times = 270 ¥
		Costs of labor	450 ¥×3 times = 1,350 ¥
Biocontrol	87.9 ¥	Costs of facilities	24.9 ¥
		Costs of mass rearing	33.0 ¥
		Costs of releasing	30.0 ¥

Fig. 11.7 Comparison of the cost of biological and chemical control against aphids

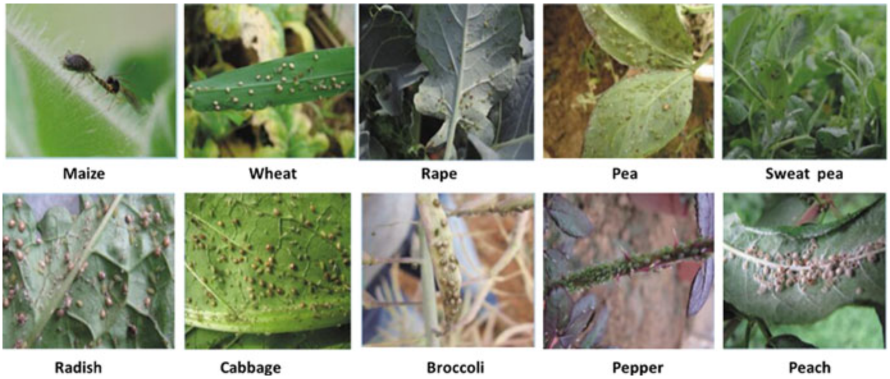


Fig. 11.8 Aphid control on other crops by *A. gifuensis*

and one million farm households benefited from this technology. This technology became the biological control technology with the largest application area in China. Control methods for aphids have transformed from mainly by pesticides to mainly by biological control, promoting the control strategy of pests changing from passive to active, with significant social benefits.

Moreover, *A. gifuensis* has been applied in the biological control on *Myzus persicae* on other crops besides tobacco, reaching good control efficacy (Fig. 11.8).

11.5 Ecological Effects on Biodiversity After the Augmentative Release of *Aphidius* in the System

11.5.1 Effect of the Aphdius gifuensis Releasing in the Tobacco Field on the Arthropod Community Structure and Diversity

A systematic investigation on the arthropod community in the tobacco field and in the edge of the tobacco fields was conducted in Lincang, Chuxiong, Midu county of Dali, Longyang in Baoshan from 2016–2017.

In Lincang county in 2016, the number of arthropod communities in *A. gifuensis* release tobacco fields was significantly lower than that in fields where no *A. gifuensis* was released ($F = 25.39$, $P < 0.01$), but the Shannon index and the richness index was higher (Shannon index: $F = 18.36$, $P < 0.01$; richness index: $F = 21.43$, $P < 0.01$). There were 15 species of arthropods belonging to 3 classes from 11 families of 8 orders in *A. gifuensis* release tobacco fields, but 3 orders from 8 families and 14 species in fields without releases. There were 11 species that were found in the *A. gifuensis* release and in non *A. gifuensis* release tobacco fields. They were *Myzus persicae*, *Epistrophe balteata*, *Pirata subpiraticus*, *Pirata subpiraticus*, *Sympiezomias velatus*, *Trialeurodes vaporariorum*, *Liriomyza sativae*, *Spodoptera litura*, *Heliothis assulta*, *Cyrtopeltis tenuis*, *Bradybaena ravida* and *Bradybaena similaris*.

Myzus persicae and *Trialeurodes vaporariorum* were the predominat species in the *A. gifuensis* release and no *A. gifuensis* release tobacco field, the relative abundances of *M. persicae* and *T. vaporariorum* being 33.92% and 61.44% in *A.gifuensis* release tobacco fields, and 61.53% and 35.49% in no *A. gifuensis* release tobacco fields.

The community diversity in the *A. gifuensis* release tobacco fields was similar to that in the no *A. gifuensis* release tobacco fields. The similarity of the arthropod community in *A. gifuensis* release and no *A. gifuensis* release tobacco fields was 0.61, so that the community diversity in *A. gifuensis* release tobacco field was half-similar with that in no *A. gifuensis* release tobacco field.

11.5.2 Arthropod Community Diversity in the Ridge of Tobacco Fields

There were 45 species of arthropods in the ridge of *Aphdius gifuensis* release tobacco fields, which belonged to 30 families from 11 orders and 3 classes, but there were 35 species of arthropods in the ridge of non *Aphdius gifuensis* release tobacco fields, belonging to 21 families from 10 orders and 3 classes.

The occurrence of arthropods in the ridge of *A. gifuensis* release, and no *A. gifuensis* release tobacco fields, had the maximum arthropod community diversity index and richness index in the late July in the ridges of *A. gifuensis* release tobacco fields, but was in early August in no *A. gifuensis* release tobacco fields in Midu county in 2016. The result indicates that the arthropod community in the ridge of *A. gifuensis* release tobacco fields was significantly different from that in no *A. gifuensis* release tobacco fields.

There were 45 species of arthropods, which belonged to 30 families from 11 orders and 3 classes, in the *Aphdius gifuensis* release fields, but there were 35 species of arthropods, which belonged to 21 families from 10 orders and 3 classes in the non *Aphdius gifuensis* release fields in Eryuan county tobacco planting areas in 2016.

11.5.3 The Willingness of Farmers Was Improved

Farmers' willingness increased in those *Aphidius* release areas, where farmers grow crops like rice, corn, vegetables and flowers or fruits. They like to buy *Aphidius* and release them in their own crop, field, fruit orchard, or vegetable greenhouse.

A survey reveals that many farmers know that *Aphidius* is very useful for the control of ahpids, reaching more than 80% of vegetable and tobacco planting areas in Baoshan, Dali, and Dehong county of Yunnan province. They know that *Aphidius* is a very important natural enemy of aphids, and that it is useful for the control on aphids on tobacco and in many other crops. Moreover, oilseed rape is an important crop in Baoshan city, where many farmers grow oilseed rape. They all like to control rape aphids by releasing *Aphidius* in the rapeseed fields, such as in Tengchong city of Baoshan city in the province.

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Chapter 12
Current Knowledge on the Migratory Moth *Autographa gamma* as Basis for Future Chemo-ecological Research

Paul G. Becher and Santosh V. Revadi

12.1 Introduction

Migration is one of the many mechanisms organisms use to maximize their fitness under annual seasonal changes. Insects from several taxonomic orders initiate migration to resource-rich habitats because of unfavorable conditions in their current environment (Chapman et al. 2015). Migrating individuals show higher reproductive success and longevity compared to the ones that prevail (Spitzer et al. 1984; Chapman et al. 2012). However, the changing global climate affects the seasonal climate and consequently plant phenology and insect physiology (Hoffmann and Sgrò 2011; Kraaijenbrink et al. 2017). For example, studies investigating the impact of climate change on insect pests and their host plants show accelerated development of the insect pest as a result of an earlier onset of the growing season (Altermatt 2010; Van Asch et al. 2013; Baker et al. 2015), thereby increasing the impact and pest risk on agricultural production (Porter et al. 1991). More research is needed to understand how climate affects insect physiology such as the migratory behavior of agricultural pests.

One such agricultural pest exposed to changing climate and plant phenology is the long distance migratory silver Y moth, *Autographa gamma*, (Lepidoptera: Noctuidae), a polyphagous pest on a wide range of cultivated crops that include sugar beet, cabbage, maize, potato, and legumes (Carter 1984; Chapman et al. 2012), spread across Asia, Africa and Europe. The species migrates in high numbers from the Mediterranean basin to central and Northern Europe to temporarily avoid hot climate. However, with transient resources and decreasing temperature later

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P. G. Becher · S. V. Revadi (✉)
Chemical Ecology Unit, Department of Plant Protection Biology, Swedish University of Agricultural Sciences, Alnarp, Sweden
e-mail: paul.becher@slu.se; revadi.santosh@slu.se

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in the season, the descendants of migrants re-migrate southward after two to four generations (Chapman et al. 2012).

Most of our knowledge on the silver Y moth is derived from the studies on seasonal migration (Pedgley and Yathom 1993; Chapman et al. 2012), few physiological studies on digestion and dietary quality of host plants (Naseri et al. 2016), pheromone communication (Dunkelblum and Gothilf 1983), nectar foraging (Plepys et al. 2002a) and behavioral responses in the context of migration and reproduction triggered by photoperiod and temperature (Hill and Gatehouse 1993; Alois et al. 2002). The silver Y moth stands out as one of the important pest species that undertake inter-continental migration, while surprisingly little is known about the host plant localization and mate finding. In this brief review, we outline the life cycle and seasonal migration of *A. gamma* and propose future lines of research on chemoreception as a basis for the development of sustainable pest control.

12.2 The Life Cycle of the Silver Y Moth

Adult females of *A. gamma* show a high fecundity. On a host plant, a female lays eggs individually on the underside of the leaves. The number of eggs laid during life time ranges from few hundred to more than 1000 eggs per female (Harakly 1975; Spitzer et al. 1984). However, high fecundity does not guarantee high offspring survival because of density-dependent mortality and other factors. High temperature has a significant negative effect on *A. gamma*. The female moths lay a maximum number of eggs at 16 °C and fewer eggs at temperatures above 20 °C; female longevity reduces significantly by half at 25 °C (Hill and Gatehouse 1992). Larval development takes about 13–17 days (six larval instars) depending upon host plants and temperature (Harakly 1975; Golikhajeh et al. 2016). The optimum temperature for larval development is around 13–19 °C. Temperature above 20 °C increases larval development time and reduces pupal weight (Hill and Gatehouse 1992; Alois et al. 2002). The larvae are smooth, pale green with white stripes on the dorsal side or sometimes turn dark green when crowded on the host plants (Harakly 1975; Golikhajeh et al. 2016). The larvae crawl like an inchworm, and can easily reach 2.5–3 cm in length during late instar. Eventually, larvae pupate at the underside of the leaf covered in a silken cocoon.

12.3 Migratory Behavior

The migration of silver Y moth to new breeding grounds, involving the finding of host plants and mates is a remarkable behavior. Migration and reproduction are physiologically regulated and alternate in the migratory individuals (Johnson 1969; Wilson 1969; Gatehouse 1997). Triggered due to unfavorable breeding conditions, reproductively immature moths migrate into new breeding sites. The condition is termed as oogenesis-flight syndrome or pre-reproductive phase (PRP) (Johnson 1969; Hill and Gatehouse 1993). Hill and Gatehouse 1993, describe photoperiod and temperature as factors affecting development and PRP in *A. gamma*.

After migration, the PRP in adults is terminated by a long photoperiod and temperature, and physiology changes for reproduction (Hill and Gatehouse 1993). In other migratory lepidopteran species, photoperiod, temperature and endocrine gland secretions (juvenile hormone activity) are shown to play a pivotal role in terminating reproductive diapause in both sexes (Gadenne et al. 1993; Anton et al. 2007). Unlike in wild populations, silver Y moth females under laboratory conditions directly become sexually mature post-emergence from the pupal stage. The calling behavior and pheromone production occur during the whole scotophase and females remain sexually active as long as 10 days (Mazor and Dunkelblum 2005; Groot 2014). It can be hypothesized that a long duration of calling and pheromone production in females increases the probability to attract males in space and time, as a critical parameter for successful reproduction of insects that migrate into new habitats.

12.4 Pheromone Communication

The individual components and their ratio in the pheromone blend make sexual communication in lepidopteran moths species-specific (Baker 2008). In the sexually mature silver Y moth two female sex pheromone components have been identified (Dunkelblum and Gothilf 1983). The main component is (*Z*)-7-dodecenyl acetate (Z7-12:OAc) and the minor component is (*Z*)-7-dodecenyl alcohol (Z7-12:OH) (Dunkelblum and Gothilf 1983; Tóth et al. 1983). Males are attracted to Z7-12:OAc by itself and the minor component augments attraction (5–10% of Z7-12:OH). However, an increase in the proportion of Z7-12:OH (50–100%) drastically reduces attraction and eventually courtship attempts in males (Dunkelblum and Gothilf 1983; Mazor and Dunkelblum 1992). Other minor components in the gland extracts have not been identified yet, and their functions are not known (Mazor and Dunkelblum 1992). The silver Y moth belongs to the subfamily Plusiinae and shares pheromone blend components with the sibling species (Linn et al. 1984; Mazor and Dunkelblum 1992). For example, *Cornutiplusia circumflexa* uses the same pheromone components but with an inverse ratio of Z7-12:OAc and Z7-12:OH compared to the silver Y moth. Also, in the cabbage looper, *Trichoplusia ni* whose main pheromone component is Z7-12:OAc, a small percentage of males show attraction to the silver Y moth virgin females (Berger 1966; Mazor and Dunkelblum 1992). Cross attraction between sympatric species is broadly attributed to similar seasonal phenology and dial activity pattern (Groot 2014). Synthetic pheromone lures are used for monitoring of *A. gamma*. The data presented in Fig. 12.1 summarizes the trapping of *A. gamma* in pea crop in the Scanian region of Sweden from 1998 to 2015.

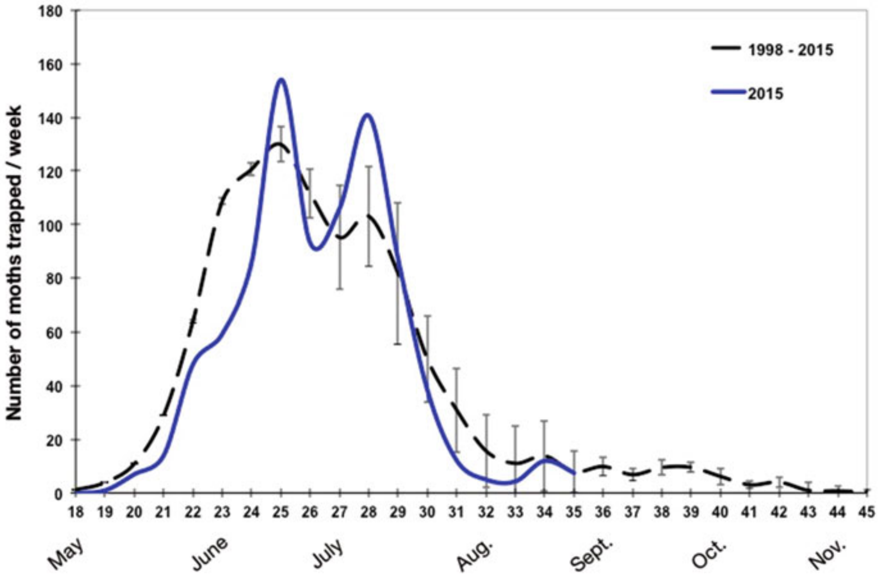

Fig. 12.1 Mean weekly catches of silver Y moths from 1998 to 2015, in pea fields in southern Sweden using pheromone traps. The black line indicates average catches over 18 years and the blue line represents catches during the year 2015. The error bars shows standard error

12.5 Natural Enemies of the Larvae

Migration into a new habitat often guarantees an ‘enemy-free space’ (Chapman et al. 2015). However, after migration, *A. gamma* encounters a number of parasitoids like the polyembryonic wasp, *Copidosoma floridanum,* the braconid wasp, *Cotesia plutellae* and several *Trichogramma* spp. that can successfully parasitize the larvae (Guerrieri and Noyes 2005; Tremblay and Masutti 2005; Klug and Meyhöfer 2009; Polaszek 2010). We isolated the egg-larval parasitoid *C. floridanum* (Chalcidoidae: Encyrtidae) from the first seasonal generation of *A. gamma* larva collected from a field in Sweden, which means, eggs get parasitized immediately after the arrival of the moths (Fig. 12.2). The wasp, *C. floridanum* is specialized on species of the Plusiinae subfamily in Noctuidae. A single egg produces up to ca. 2000 progenies from the parasitized larva (Ode and Strand 1995). Development of *C. floridanum* is synchronized with the development of the host *A. gamma* (Strand 1989). Another parasitoid, *C. plutellae* is a branocid wasp that occasionally parasitizes caterpillars of the silver Y moth (Kaneko 1993). We also isolated *Cotesia* sp. from *A. gamma* larvae collected from the field in Sweden (Fig. 12.3). The parasitic larvae emerge from late-instar larvae, spin a cocoon and eventually develop into adults. Similarly, several species in the genus *Trichogramma* successfully parasitize silver Y moth larvae. Interestingly, some species like *T. brassicae* overwinter on silver Y moth

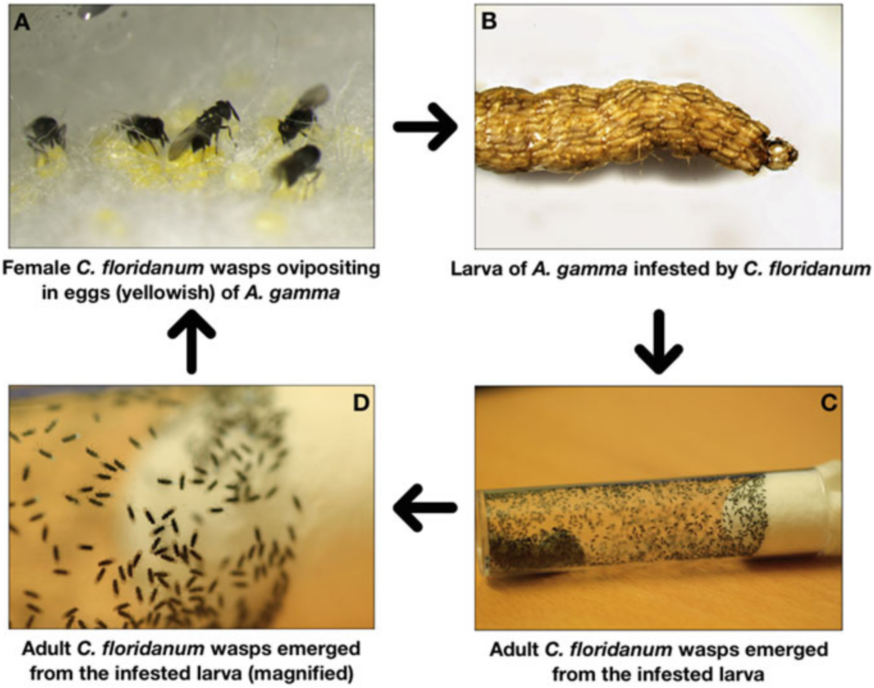

Fig. 12.2 Life cycle of the polyembryonic wasp *C. floridanum* infesting *A. gamma*. (**a**) The adult wasps ovipositing in the eggs of *A. gamma*. (**b**) Developing pupae of the parasitic wasp inside the *A. gamma* larva. (**c** and **d**) Adult wasps emerged from the larva

Fig. 12.3 *Cotesia* sp. larvae (black arrow) emerging from a caterpillar of *A. gamma*, before spinning individual cocoons

eggs in northern Switzerland (Babendreier et al. 2003) which illustrates *A. gamma* encounter natural enemies in their migrated habitat.

12.6 Olfaction

Lepidopteran moths are attracted to flowers for nectar feeding before mating (Raguso and Willis 2002; Saveer et al. 2012). The adult silver Y moth are attracted to a wide range of plants for nectar feeding (Plepys et al. 2002a) and provide pollination services (Plepys et al. 2002b). We presume that migrated adults of the silver Y moth refuel their energy sources before scouting for a potential mate and a suitable host plant as in other migratory and non-migratory lepidopterans (Brower et al. 2006; Saveer et al. 2012).

In another migratory lepidopteran, the monarch butterfly, *Danaus plexippus,* genome sequencing has elucidated many interesting facts about migration-associated behavior and physiological adaptations (Zhan et al. 2011). The silk moth, *Bombyx mori*, a non-migratory lepidopteran was used as a ‘template species’ to understand olfaction, particularly the evolution of protein families involved in chemoreception in the monarch butterfly. Strikingly, in the phylogenetic analysis, monarch butterfly antennae possess a species-specific odorant receptor (ORs) clade, more ionotropic receptors (IRs) and less gustatory receptors (GRs) compared to the silk moth. Furthermore, in addition to migratory related adaptation, it is speculated that the exceptional differences in the protein families are related to overwintering site selection, olfaction-based foraging and oviposition site selection in the monarch butterfly (Zhan et al. 2011). This assessment, nevertheless, needs to be functionally examined. However, the differences in the chemosensory receptor repertoire in the phylogenetic analysis between the nocturnal *moth B. mori* and the diurnal *migratory* butterfly *D. plexippus* opens an interesting opportunity to understand the evolution of chemoreceptors in *migratory moths*, using *A. gamma*. Recent transcriptomic and phylogenetic analyses on the migratory moth, *Mythimna separata* show that many sensory genes are conserved (Liu et al. 2017), but the evolution of migration-related chemoreceptors are not studied for moths.

12.7 Future Perspectives

Functional analyses at a genomic and transcriptomic level are needed to understand the gene expression governing insect chemoreception and behavior. These analyses will provide fundamental insights into insect ecology and evolution. In the species that are ecologically and/evolutionarily important but lack the genome sequenced, RNA-Seq provides an opportunity to understand gene expression and phylogenetic relations with other species (Haas et al. 2013). RNA sequencing on the migratory insect antenna further facilitates characterization of genes involved in chemoand mechanosensation that are crucial for migration (Zhan et al. 2011). There is evidence of evolutionary adaptations in relation to migration in the chemosensory genes in other species (Zhan et al. 2011). These findings suggest that the migratory silver Y moth likely has similar adaptations, but our understanding of these adaptations is still unclear. By surveying the literature, we suggest that future work should be directed towards understanding the genetic basis of chemoreception in this species.

It has been shown that moths modulate their sensory and behavioral responses to chemical cues, depending on feeding-state, mating-state and age (Anton et al. 2007; Saveer et al. 2012; Kromann et al. 2014). Migration might be an additional factor modulating chemosensory-mediated behavior. Migratory insects show distinct behavior during the migratory or reproductive phase. We know that migratory insects including the silver Y moth, respond to flowering plants for feeding cues (Plepys et al. 2002a; Brower et al. 2006), but it is not known if moths modulate their olfactory preferences towards pheromone and host plant cues. We therefore suggest silver Y moth as a model to study migration-induced olfactory modulation.

Performance in herbivorous insects largely depends on female host plant choice. The mother's experience influences host plant preference (Thöming et al. 2013; Carrasco et al. 2015). For a migratory species, host plant selection gets more complex as the adults typically lack prior experience of the new landscape. The seasonal migrant silver Y moth is a polyphagous pest but in the north of Europe, the pest is predominantly reported on sugar beet and peas (Golikhajeh et al. 2016; Lemic et al. 2016; Hauer et al. 2017). Host plant selection and specifically the underlying olfactory plant signals are not known. And, to which extent migration to a new and previously not experienced environment influences host plant selection still needs to be studied.

Host plant odors have the potential to complement pheromone-based control for sustainable pest management strategies (Rodriguez-Saona and Stelinski 2009; Witzgall et al. 2010). Generally, host plant choice and rejection are influenced by kairomones and herbivore-induced plant volatiles, respectively (Zakir et al. 2013). Non-host volatiles and herbivore-induced volatiles possibly disrupt oviposition site selection in the silver Y moth and merit further investigation. Future research elucidating the behavioral manipulation using semiochemicals could help in optimizing potential pest management methods. A better understanding of chemical communication, sensory physiology and ecology underlying the migratory behavior, mate-finding and host plant choice will contribute to the improvement of silver Y moth control methods.

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Chapter 13
How Microbiome Approaches Can Assist Industrial Development of Biological Control Products

Manuela Rändler-Kleine, Adrian Wolfgang, Kristin Dietel, Helmut Junge, Tomislav Cernava, and Gabriele Berg

13.1 Introduction

Biologicals, which were initially niche products for sustainable agriculture, are increasingly becoming important and their market share is steadily increasing. Promising candidates for novel biocontrol products can be found in various natural ecosystems and especially as part of the indigenous plant microbiota. Identifying and isolating such microorganisms is crucial for the development of advanced pathogen management strategies in sustainable agriculture. The first step of a microbiome-driven screening is often based on a general assessment of the plant microbiome and the identification of positive interactions of specific microorganisms with the host. Each plant species and crop cultivar harbours a certain proportion of unique and specific microorganisms (Berg 2009). Here, plants in undisturbed, natural ecosystems were identified as treasure chests for the identification of highly efficient BCAs. An enormous microbial diversity was shown for mosses, which are the oldest terrestrial plants on earth (Bragina et al. 2015; Bragina et al. 2012), lichen as long-life symbiotic systems (Cernava et al. 2015), but also for medicinal plants (Köberl et al. 2013). In recent studies, plant endophytes were

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M. Rändler-Kleine · G. Berg
Institute of Environmental Biotechnology, Graz University of Technology, Graz, Austria

A. Wolfgang
ACIB, Austrian Centre of Industrial Biotechnology, Graz, Austria

K. Dietel · H. Junge
ABiTEP GmbH, Berlin, Germany

T. Cernava (✉)
Institute of Environmental Biotechnology, Graz University of Technology, Graz, Austria

College of Tobacco Science, Guizhou University, Guiyang, Guizhou, China
e-mail: tomislav.cernava@tugraz.at

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often in the focus of studies targeted at the identification of novel strains that are applicable for biological control. It was shown that endophytes and in particular seed endophytes offer a great potential for new biocontrol agents (Bergna et al. 2018). One of the advantages of many endophytes is that they are able to colonize specific niches inside the plant, similar to plant pathogens, but without negative effects on the host's health (Hardoim et al. 2015). Therefore, they offer enormous potential as a biocontrol agents (Shahzad et al. 2017). Microbiome studies were also successfully implemented to identify so far unknown modes of action. In this context it was shown that many biocontrol products are able to induce shifts in the plant microbiome that have positive effects on pathogen susceptibility of the host plant (Erlacher et al. 2014; Schmid et al. 2011; Schmidt et al. 2012; Schreiter et al. 2014). The exact mechanisms of these shifts have not yet been finally elucidated; however, it is assumed that they interact directly with both the pathogen and the plant and indirectly stimulate the immune system of the plant (Berg 2009; Lugtenberg and Kamilova 2009). In order to be able to use these effects in upcoming approaches, it would be necessary to develop BCAs that can be employed for targeted microbiome management.

During the development of novel biologicals, especially the interplay of plant genotype, environment, microbiome and management challenges constitute an important barrier for successful integration of beneficial microorganisms into plant breeding and sustainable agriculture. Extensive microbiome and -omics datasets provide new strategies to integrate crucial information into efficient engineering of microbial interactions (Foo et al. 2017; Klitgord and Segre 2011). With the help of increasingly cost-effective analysis by Next-Generation Sequencing (NGS) technologies, it is possible to optimize various parameters (Fig. 13.1) at the molecular level that promote resilience or certain types of interactions of novel biocontrol agents (Bergna et al. 2018; Hugerth and Andersson 2017). These optimizations play an increasing role in the industrial development of novel products.

13.2 Deciphering the Interactions of BCAs in Microbial Communities

For the successful implementation of novel BCAs, the understanding of their interaction with the host plant, pathogens, and the indigenous microbiota are essential. Microbiome studies that were conducted in the last years have substantially improved our knowledge related to the complexity and structure of microbial communities. We are gradually increasing our understanding how these complex communities are organized and interact with each other (Sergaki et al. 2018). Experimental approaches with reproducible conditions and variable biotic as well as abiotic factors were used to decipher relationships between the microbiota and plant phenotypes. Biotic factors that can be addressed include the presence, absence, and abundance of special microbes, genetic modification of selected microorganisms

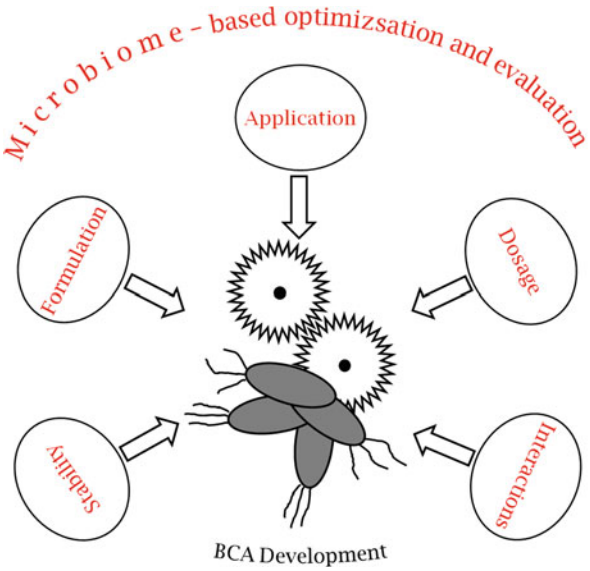

Fig. 13.1 Illustration of different strategies for the implementation of microbiome studies to improve biological control agents. Microbiome studies can complement different steps during the development of BCAs and provide complementary data for their implementation. The improvement of BCAs relies on the integration of multiphase approaches and microbiome studies can used to design, optimize and evaluate advanced products

and detailed assessment of their growth conditions (Busby et al. 2017; Mueller and Sachs 2015; Vandenkoornhuyse et al. 2015). Abiotic factors that are important for agricultural applications relate to environmental conditions and include soil type, temperature, humidity, as well as light intensity and quality (Sams 1999). Studies that are conducted under controlled conditions allow a simplified interpretation of the results, as interference factors are minimized. However, multispecies model systems are needed to determine microbial interactions as well as the structure and function of the community *in planta*. Such artificial systems, adapted to the host plant, also allow to assess the impact of BCAs on the microbiome (Niu et al. 2017; Sergaki et al. 2018). Such modelling contributes to a deeper understanding of microbiome-related functions and can help to develop next-generation applications of biologicals in sustainable agriculture. Moreover, a reconstruction of the microbial community can significantly help to decipher the interactions between microorganisms that have a direct or indirect impact on plant growth (Hartman et al. 2017). Complementary assessments of the response of single microorganism to the plant microbiome based on transcriptomics or proteomics provide useful information of genes which are involved in biological control or plant growth promoting processes (PGP) and also in compiling of beneficial microorganism (Fernandez et al. 2013; Mauchline et al. 2006). Deeper insights into the mechanisms of plant-microbial interactions can be provided by expression studies targeting specific genes. These studies can also decipher how the community adapts to changing environmental conditions. They can complement findings obtained with genomics and metagenomics approaches, which only identify the presence or absence of genes, but not their expression levels (Molina et al. 2012; Sheibani-Tezerji et al. 2015). For example, Camilios-Neto et al. have observed an upregulation of nutrient uptake and cell cycle genes of *Azospirillum brasilense* populated wheat roots by using dual RNAseq transcription profiling (Camilios-Neto et al. 2014). Comparative transcriptional analyzes can also elucidate mechanisms how endophytes contribute to induce resistance to plant diseases or promoting plant growth (Dinkins et al. 2010). An additional method termed as 'proteomics' is the investigation of proteins which are expressed in an organism based on mass spectrometry, which is the dominant technological platform for such analyses (Wilkins et al. 1996). It is suitable for a comprehensive characterization of the entire protein collection which is expressed by a microbial community in a natural sample (Maron et al. 2007). Due to various available extraction methods, the total protein content can also be directly extracted from the plant endosphere. Depending on the analyzed host and/or environmental conditions, a respective protein fingerprint can be generated, which represents the effects on the metabolite production spectrum (Kaul et al. 2016; Maron et al. 2007). In order to decipher microbial functioning, protein fingerprints of selected microorganisms under different stress states can be compared. This can indicate specific roles of microorganisms in the plant system under these stress conditions.

13.3 Support of Novel Application Strategies

BCAs as well as plant growth promoting microorganisms (PGPM) serve the host plant either by stimulating plant growth, suppressing plant diseases or pests, or both and can simultaneously interact with the plant or soil microbiome. The best-studied BCA and PGPM model organisms belong to the genera *Azospirillum, Bacillus, Pseudomonas, Rhizobium, Serratia, Stenotrophomonas* and *Streptomyces* as well as fungal genera such as *Ampelomyces*, *Coniothyrium* and *Trichoderma* (Berg 2009; de Vrije et al. 2001; Franken 2012) and many of them are available as microorganisms-based formulations for agricultural applications (Berg 2009). However, their efficiency depends on the concentration and persistence of the introduced microorganisms (Finkel et al. 2017). For successful establishment of a BCA, adequate formulation and application is essential (Walker et al. 2004). The ability of a BCA to establish itself in root-associated microbial communities is termed 'rhizosphere competence'. Rhizosphere competence is essential for interactions between PGPMs and plants that depend on close proximity between the involved organisms. Applied microorganisms do not necessarily need to dominate root-associated microbial communities but have to be abundant enough to affect plant performance. Rhizosphere competence can be visualized and evaluated with fluorescence in-situ hybridization (FISH) and confocal laser scanning microscopy (CLSM), which is a highly useful tool to complement NGS approaches. FISH-CLSM provides three-dimensional visualization of spatial distribution, number, size and shape of bacterial colonies. However, targeted CLSM analyses require hybridization of microorganisms with the fluorescent FISH probes and these probes can be more or less discriminative towards a certain taxonomic group of microorganisms. For strain-specific visualization of plant colonization by BCAs, the use of genetically modified cells expressing fluorescent protein markers like “green fluorescent protein” (gfp) or DsRed is a more precise method (Fig. 13.2). For example, the commercially available BCA *Bacillus velezensis* FZB42 which is currently one of the best-researched BCAs, could be identified on roots of several host plants, including *Arabidopsis thaliana* (Dietel et al. 2013) tomato (Fig. 13.2c),

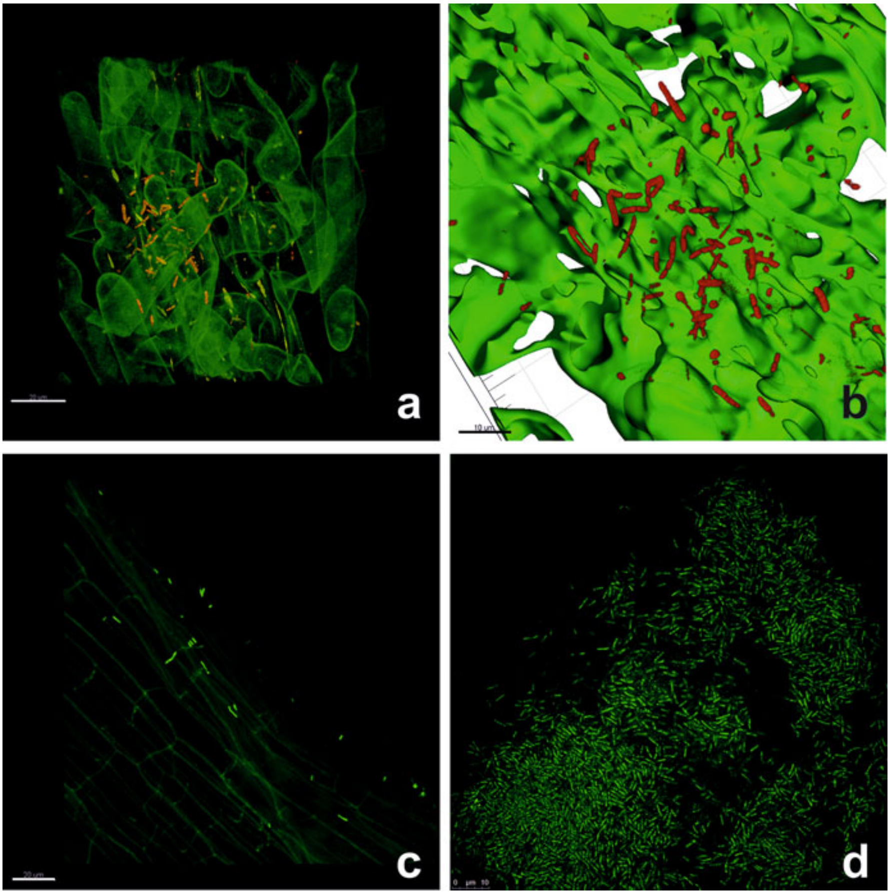

Fig. 13.2 (**a**) FISH-CLSM of the root hair zone of 14 days old oilseed rape (*Brassica napus*), seed-primed with *Bacillus velezensis* FZB42. FISH probes EUBII-mix (Bacteria, red), LGC (*Firmicutes*, yellow) and calcofluor white (plant cell walls, green); (**b**) FISH-CLSM of the root hair zone of 14 days old oilseed rape (*Brassica napus*), seed-primed with *Bacillus velezensis* FZB42. The micrographs were processed with IMARIS (Bitplane AG, Zürich, Switzerland); FISH probes: LGC (*Firmicutes*, red) and calcofluor white (plant cell walls, green); (**c**) CLSM of a lateral root of a 14-day-old tomato seedling, (*Solanum lycopersicum* cv ‘Moneymaker’), seed-primed with gfp-labelled *Bacillus velezensis* FZB42 (light green); (**d**) (d) Bacterial suspension of gfp-labelled Bacillus velezensis FZB42 showing autofluorescence

maize (Fan et al. 2012), lettuce (Chowdhury et al. 2015), sorghum (Zachow et al., unpublished data) and oilseed rape (Fig. 13.2a,b) amongst others. The combination of visualization and microbiome data creates a substantially more precise picture of prevalent interactions within rhizosphere communities.

In addition, microbiome studies can be helpful to provide information that influences application strategies for microbial inoculants (Busby et al. 2017). By integrating microbiome studies it was shown that the application of the commercially available inoculant *Bacillus velezensis* FZB42 on lettuce had only a minor effect on the indigenous bacterial community of the rhizosphere (Erlacher et al. 2014). However, the bacterial community showed a significant shift over a period of 2–5 weeks after planting. It was also observed that the saprophytic pathogen *Rhizoctonia solani* AG1-IB can affect the microbial community after inoculation (Chowdhury et al. 2013). Plant pathogens like *Rhizoctonia solani* often cause "microbiome diseases"; because their presence results in a loss of microbial diversity and dysbiosis in soil and consequently in the rhizosphere and endosphere of plants (van Elsas et al. 2012).

A new application approach for microbial inocula optimized for specific cropping systems includes the production of plant/soil-optimized microbes and plant/soil-optimized microbiomes. It is known that the soil microbiota adapt to the present crop over the course of time to improve the plant-microbe interactions (Berendsen et al. 2012). This is the reason why microbiome studies to investigate this adaption respectively shaping of the microbiome are very suitable to develop application strategies of new products to reach optimal effects under natural conditions. Identification of the optimal microbiota, which are specific for a certain crop is one of the major aims of microbiome studies. A potential application that could result from such studies is the development of seed coatings with microbes which are specialized for a distinct type of soil and environment. This would simplify the application procedure in terms of the handling and time requirement for the farmers (Dawar et al. 2014; O'Callaghan 2016). Another promising approach could be not only to implement microbes in or to the plant, but also to focus on the plant breeding. It is a common procedure to select plants with an improved yield and resistances, but microbiota mostly remain ignored. In that respect it is important to address the microbiome, more precisely the beneficial constituents of the microbiome, which can play an important role for maintaining yield and plant growth (Vandenkoornhuyse et al. 2015) under unfavorable conditions. Many studies support the idea to integrate the plant microbiome in crop breeding (Berg and Raaijmakers 2018; Mendes et al. 2018). This could be an important tool in the future to develop novel plant phenotypes (Wei and Jousset 2017). For example, pathogen tolerance is a highly desired trait in plant breeding. Mendes et al. (2018) investigated the bacterial communities in bean cultivars with different grades of resistance towards Fusarium oxysporum and found the amount rhizobacteria to be correlated with pathogen tolerance. In addition, the development of plant cultivars that serve as a kind of probiotics to improve plant and animal health is an ongoing approach based on microbiome studies (Mueller and Sachs 2015).

13.4 Development of Applications Based on Synthetic Communities

The knowledge that the composition of the microbiome has a major impact on how plants increase productivity and tolerance against biotic and abiotic stress (Lebeis 2015; Schlaeppi and Bulgarelli 2015) provides the basis to design functional microbiota for future applications in agriculture. A promising strategy for the improvement of BCA applications is the development of synthetic communities with complementary functioning of the included strains. In most natural habitats, microorganisms co-occur with different species in a microbial community. These communities take part in complex metabolic interactions and nutrient exchanges, which stabilize microbial co-existence. There are different approaches that show how synthetic communities can be generated. For example, Busby et al. (2017) describe how to develop a plant microbiome model as the first step towards an implementation of synthetic communities. Such models provide information about system level interactions between two or more organisms. Resources that are required for such approaches include annotated genomes, organized collections of mutants, standardized protocols, central databases and large-scale field experiments. They can cover the entire experiment spectrum from the Petri dish to the greenhouse and finally the field. In order to transfer such processes to cultures that are evolutionarily distant from each other, a sufficient number of model plant species is needed (Chang et al. 2016). So far, a good progress was achieved in case of legumes such as *Medicago* (Stanton-Geddes et al. 2013), rice (Edwards et al. 2015; Knief et al. 2012; Sessitsch et al. 2011), Sorghum (Ramond et al. 2013) and tomato (Tian et al. 2015).

Another approach is to identify the ‘core’ microbiome of a distinct plant system. The core microbiome contains all microbial taxa that are present in most samples of a particular group of plants (Bulgarelli et al. 2013; Lundberg et al. 2012). By focusing on the core microbiome, researchers can filter out transient associations and focus on stable taxa that have a more likely impact on the host’s phenotype. Such culture-independent studies (often based on sequencing of ITS and 16S rRNA amplicons) are often compared to microbiomes of the same plant species from different environments. The complemetary identification of the functional core on a metagenomic and metatranscriptomic basis, reveals information related to common functions that are relevant for the respective plant groups (Vandenkoornhuyse et al. 2015).

The major cultivatable phyla for the reconstruction of synthetic communities are *Actinobacteria, Proteobacteria, Bacteroidetes,* and *Firmicutes.* Many studies with extensive characterizations of these microorganisms were done so far and included genome sequencing and phenotypic characterisation (Bai et al. 2015; Mauchline et al. 2015). Currently, common biological agents on the agricultural market are designed as single microorganism products (bacteria or fungi), which can improve the crop yield, plant growth and fitness. However, recent research showed that the application at the microbiome level is more promising (Woo and Pepe 2018). The most important barrier in this context is to isolate the relevant microbial species and prepare the applicable consortia, because of the complexity of the microbe-plant interactions. As already shown by Swenson et al., artificial selection steps can also modulate the characteristics of complete ecosystems (Day et al. 2011; De Roy et al. 2014; Swenson et al. 2000). Thus, experiments with synthetic communities provide a possibility to develop a molecular basis in order to transfer findings, which were observed under natural conditions to a defined test system. The results obtained from these experiments can be subsequently further tested and verified in field and greenhouse experiments before large-scale application can be ensured.

By employing synthetic communities and a top-down strategy, distinct bacterial families were identified which likely contribute the suppression of the pathogenic fungus *Rhizoctonia solani* and expand the earlier findings and knowledge on “suppressive soils” (Mendes et al. 2011; van der Voort et al. 2016). The bottom-up strategy is often limited by the requirement of culture collections with specific microorganisms for the reconstitution of synthetic communities under gnotobiotic conditions, which then imitate the natural environment (Vorholt et al. 2017).

13.5 Biotechnological Production of BCAs

The pure culture represents a milestone for modern biotechnology and is still essential for the mass production of, for example, amino acids, organic acids, antibiotics and enzymes (Sabra et al. 2010). However, in view of the limitations in the production of pure cultures including high energy requirements during fermentation and high raw material costs, only about 1–10% of all microorganisms can be cultivated in pures culture (Streit et al. 2004). Therefore, strategies making use of microbial co-fermentations should be reconsidered for future applications, which also include biotechnological production of BCAs. Among various process steps that are important for the development of biologicals, the production process itself has to be carefully designed. For the manufacturer it is essential that the production process for a microbial product is economical, which means the application of cost-effective raw materials and a low energy consumption at a maximum yield. Upcoming approaches to advance BCA production could profit from a direct implementation of synthetic communities in bioreactors.

In their natural habitats, production processes of microorganisms are often more efficient, because the respective process is coordinated by the community as a whole (Straight and Kolter 2009). Communities can catalyze many processes more efficiently, because the organisms combine metabolic pathways and enzymatic systems in cooperation with each other (Rosche et al. 2009). Similarly, the distribution of metabolic activities among the relevant members of the community leads to an optimization of functionality and reduction of metabolic stress on the individual member (Biliouris et al. 2012; Brenner et al. 2008). In successful implementations, co-cultures with different (often two) defined species are applied, in which the different properties of microbial growth and metabolism are used for fermentation.

In addition, microbial communities can be used to produce a specific product spectrum from mixed substrates, resulting in substrate reduction and product purification. So far, mixed cultures are increasingly used in environmental remediation (activated sludge in wastewater treatment) and energy production (biogas production). The production of multi-species communities in a bioreactor requires adequate monitoring. With the available tools, the analysis of microbiome structures including their variation at different levels of analysis can be performed reliably. The challenge, despite technological developments, is still to link the microbiome structure with its performance. Koch et al. contrasted a molecular and a cell-based method of microbiome diagnostics for monitoring the production process (Koch et al. 2014). Cell-based investigations that are performed during the generation time allow monitoring of the microbiome structure in process-relevant time course. However, to be able to monitor all cells, the use of a general marker is needed. The monitoring by means of classical microbiome studies does not allow the detection of functionally relevant changes in the microbiome, for example strain-specific metabolic pathways (Koch et al. 2014). Moreover, predicting the performance of microbial communities is very complex. Functional capacity including its metabolic pathways, replication, interaction and interplay with its local environment must be considered and integrated into the production process (Koch et al. 2014). The general requirements in practice are the successful product formation in a defined time period and maintenance of process stability.

The success story of products based on *Bacillus* and *Peanibacillus* in the field of agriculture are mostly due to biotechnological and sustainability reasons. The advantage of these bacterial genera is that, in contrast to Gram-negative bacteria, they are able to form spores. This property offers a huge benefit in the formulation of the products as well as in terms of durability (Pérez-García et al. 2011; Sorokulova et al. 2008). The extreme robustness of the spores thus permits the possibility to adapt the formulation of the customer requirement or the field of application (Brar et al. 2006; Lim and Kim 2010). In future applications, genera with similar properties could be co-formulated and the process optimized by implementation of microbiome-based monitoring of the fermentation. With the knowledge and experience concerning the production and application of BCAs from recent years and the new technological possibilities, it will soon be possible to develop new biologicals that are more efficient and cost-efficient.

13.6 Risk Assessment and Registration of New Products

In addition to other applications, microbiome studies can also be implemented to facilitate the registration of new products. Here the question often arises how the introduced microorganisms will interact with the natural biodiversity and if they will spread in the environment. For modern agriculture, effective and innovative crop protection is essential. Therefore, novel bioprotection technologies are an important tool for the EU to facilitate the implementation of the Directive on the Sustainable Use of Pesticides (Directive 2009/128/EC) successfully. However, novel biologicals currently require a costly and time-consuming registration that often hinders their implementation on the market. Unfortunately, the EU cannot fully exploit the potential of this fast-growing industry, which is dominated by SMEs (small and medium-sized enterprises). Hence, the IBMA (International Biocontrol Manufacturers Association), for instance, requires regulatory authorities, legislation, procedures and data acquisition and, especially, high-level scientific experts that conduct risk-based assessments in close cooperation with producers / applicants. Strasser (2010) describes, that the following components are necessary to evaluate the environmental risk of the application of biologicals: persistence of the active ingredient, dispersal potential, range of non-target organisms that are affected, and direct and indirect effects on the ecosystem. Microbiome studies and other community-spanning analyses can potentially support the evaluation of the environmental risk in more detail than conventional studies (Strasser 2010). In addition, NGS techniques are highly suitable for risk assessment, as they can identify potential pathogenicity factors and antibiotic resistance on genome level. They can be applied to investigate the production potential of bioactive metabolites at genomic and transcriptomic levels and also track the establishment of implemented strains through amplicon libraries or metagenomics (Berg et al. 2013).

Many biocontrol products show low persistence on plants and soil (Scherwinski et al. 2008). They are only detectable in the new habitat for a certain period of time. The demonstration of these properties by means of microbiome studies allows an evaluation of the influence of the biocontrol product on the habitat to be treated and thus can simplify the registration of the product. Little information exist about the impact on the native microbial community following the application of microbial inoculants (Trabelsi and Mhamdi 2013), and also about its survival and persistence after application in the field. Nevertheless, this information is important because it helps to understand the biological environmental dependency of the inoculation approaches and the environmental impact assessment of selected microorganisms. Here, targeted analyses will likely be conducted in the future on the basis of NGS approaches. In one instance, microbiome studies have identified the genus *Stenotrophomonas* among other Gram-negative bacteria as highly efficient antagonists of phytopathogenic fungi that also has positive effects on plant growth (Cernava et al. 2015). It is known that isolates of the genus *Stenotrophomonas* produce stress-reducing substances such as spermidine that can mitigate negative effects of drought in the host plant. However, this genus also harbours opportunistic human pathogens that are closely related to environmental strains (Berg and Martinez 2015). Only with a comprehensive evaluation of all strain-specific characteristics, the risk connected to the application of a potential BCA can holistically assessed. In case of the strain *Stenotrophomonas rhizophila* DSM14405T, which is applied as a Stress Protection Agent (SPA), genome sequencing and transcriptomic studies have been used to optimize the production process and to identify mechanisms of action and risk factors. Due to the ability to promote plant growth and to protect the roots from biotic and abiotic stress, this strain stands out to use it as a biocontrol product (Egamberdieva et al. 2010). It was also found that this strain produces antibiotics, lytic enzymes, and osmoprotective agents (Egamberdieva et al. 2010; Ryan et al. 2009). Alavi et al. could show that with the help of genomics as well as transcriptomics and physiological approaches it is possible to distinguish between beneficial and pathogenic bacteria (Alavi et al. 2014). The obtained results are highly valuable for the registration as a stress protection agent, because they confirmed that no pathogenicity factors are involved in this plant-bacteria interaction.

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Chapter 14
Beneficial Fungi for Promoting Plant Health in Cassava: Ecostacking Prospects for the Management of Invasive Pests

A. Nasruddin, Ingeborg Menzler-Hokkanen, and Heikki M. T. Hokkanen

14.1 Introduction

Innovative approaches to redesigning agricultural systems are urgently needed. A crucial way of "ecologically intensifying" agricultural production relies on designing cropping systems that mimic the diversity of natural ecosystems through lengthening and diversifying crop rotations and reducing tillage intensity (e.g. conservation agriculture). Minimal soil disturbance (reduced or no tillage) and permanent soil cover (mulch) combined with rotations facilitate to conserve, improve and make more efficient use of natural resources. These practices not only reduce soil degradation but also contribute to sustained agricultural production including biological control of pests and diseases.

Plant pathologists have for a long time studied the concept of "suppressive soils", trying to understand the mechanisms involved in plant disease suppression. We propose to expand the concept to "insect pest suppressive soils", to apply this new concept, and to provide data on the occurrence and importance of soil-borne insect pathogens in pest population suppression. Agricultural fields usually harbor only low numbers of beneficial insect antagonists such as entomopathogenic nematodes (EPN) and fungi (EPF), so that their role in pest population dynamics currently is negligible (Hokkanen and Menzler-Hokkanen 2018). Yet simple improvements in field and crop management can quickly increase the numbers of EPN and EPF to levels that will impact the peak pest populations.

A. Nasruddin (✉)
Department of Plant Protection, Faculty of Agriculture, Universitas Hasanuddin, Makassar, Indonesia

I. Menzler-Hokkanen · H. M. T. Hokkanen
Department of Environmental and Biological Sciences, University of Eastern Finland, Kuopio, Finland

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Published data show that most, if not all, entomopathogenic fungi are able to colonize tissues of at least some plant species as symptomless endophytes. Most data so far refer to *B. bassiana* as an endophyte, but also *M. anisopliae* and *Lecanicillium lecanii* have been shown to colonize plant tissues. Depending on the plant species and the isolate of the EPF, these interactions could be beneficial to both the plant and the fungus, neutral or even antagonistic (Vidal and Jaber 2015). Recently Greenfield et al. (2016) showed that several strains of *Beauveria bassiana* and *Metarhizium anisopliae* can endophytically colonize cassava roots following soil drench inoculation. Colonization rates, distribution in different plant parts, and impacts on plant growth varied depending on the inoculant strain. No information concerning possible impacts on pest insects in this system are available.

Data published in this respect are highly variable, and not consistent with regard to the underlying mechanisms that might explain the effects. Although several studies report adverse effects of endophytic EPF on herbivorous insects, most have either not tested, or not observed, direct mycosis on the herbivores feeding on the plant tissues (Vidal and Jaber 2015). Therefore, the mode of action of endophytic EPF in most of the studies remains obscure, but likely involves the activation of various plant defense mechanisms.

Possible impact of other soil-borne beneficial microbes on herbivorous insects feeding on crop plants has only seldom been studied. Recently it has been shown that *Trichoderma harzianum* endophytic colonization of tomato plants enhances its indirect defense against aphids (Coppola et al. 2017). Earlier, Gange et al. (2005) reported that different combinations of three species of the mycorrhizal fungus *Glomus* affected host acceptance by the leaf-mining insect *Chromatomyia syngenesiae*, and two seed-feeding insects (*Tephritis neesii* and *Ozirhincus leucanthemi*).

In this chapter we review and report on the spread of invasive mealybugs in important cassava growing regions in the province of South Sulawesi, Indonesia, and on our studies to implement ecostacking (Hokkanen 2017) strategies in the case of controlling the cassava mealybug *Phenacoccus manihoti*. The possibility to utilize beneficial fungi for activating plant defences and for direct biocontrol play a central role in this strategy.

14.2 Basic Principles of Ecostacking in the Context of Cassava Pest Management

Functional biodiversity has been shown to influence at all its levels the level and quality of biological control services to crops. Examples of demonstrated beneficial impacts to crop protection include increasing the genetic diversity within the target crop plants (e.g. Grettenberger and Tooker 2017), the composition of beneficial microbial assemblages in the soil (e.g. Pineda et al. 2017; Prieto et al. 2017), the botanical diversity within the target field (e.g. Balzan 2017; Adhikari and Reddy 2017; Schröder et al. 2017), and diversity of the landscape surrounding the field (e.g. Steingröver et al. 2010). Intense research effort has addressed the functioning of each of the diversity levels listed above, and their potential for exploitation in crop protection has been demonstrated. The possibility of combining such beneficial biocontrol effects provided by the different levels of biodiversity in an additive or even synergistic manner, has not been explored systematically. Hokkanen (2017) proposed the concept of "ecostacking," where maximum use of biological control services in an agroecosystem can be achieved by conserving and stacking functional biodiversity.

Stacking of beneficial ecological traits in an ecosystem is analogous to the concept of stacking functional genetic traits to produce improved, transgenic crops (c.f. Hokkanen and Menzler-Hokkanen 2017). In the context of biological pest control, it implies combining available biocontrol services based on functional biodiversity from all diversity levels and types. In this process, the different types of biological control must be fully and functionally integrated with all other components of the cropping system, including biotic and abiotic conditions, and management of the agroecosystem.

In the case of cassava growing, the impacts on the population levels and dynamics of key pests and their natural enemies needs to be considered in the context of the following factors: landscape around the cassava field, crop margin composition and possible use of trap and nursery crops, botanical diversity within the cassava field, genetic diversity of the cassava plant in the plantation, possible soil organic amendments, and beneficial microbes in the soil of the field. Detailed studies on how the listed variables affect key cassava pests and their natural enemies are lacking for the most parts. Therefore, our approach on ecostacking in cassava cultivation has to rely on common generalities amended with specific information whenever possible.

At least one study has addressed the possibility of using varietal mixtures of cassava for possible benefits to pest management. Gold et al. (2011) studied the effects of cassava varietal mixtures on the population dynamics of the whiteflies *Aleurotrachelus socialis* and *Trialeurodes variabilis* under outbreak conditions in Colombia. A regional variety of cassava was grown in pure stand, and mixed with a variety believed to have partial resistance to *T. variabilis*. The dominant pest species in the system, *A. socialis*, showed no varietal preference, and population levels on the regional cultivar were similar between treatments. One of the two cultivars was less preferred by *T. variabilis*, and its presence in mixed variety plots lowered populations on the regional cassava. Yield losses, primarily due to *A. socialis*, were equal for regional cassava in mixed and in pure stands. Nevertheless, the authors concluded that the use of varietal mixtures provides a potential strategy for lowering herbivore load and increasing yields in areas with heavy *T. variabilis* attack.

Increasing within-field botanical diversity requires knowledge of the ecology of the pest species. Uninformed increase in diversity can even aggravate pest problems (reviewed by Landis et al. 2000). Trials on intercropping cassava with maize and groundnuts actually increased the severity of the root mealybug *Stictococcus vayssierei* infestation (Ngeve 2003). Increased pest pressure was a consequence of using other suitable host plants of the mealybug as the intercropping species. Therefore, knowledge of the alternative host plants of the pest is critical, so that additional food resources to a pest that is targeted for control, are not added to the system.

An interesting aspect of ecostacking is the provision of suitable carbohydrate sources to support the action of various groups of natural enemies. Cassava provides such carbohydrate sources by exuding droplets rich in sugar at the base of its petioles, and sometimes at the midrib. This extrafloral nectar is composed of reducing sugars, fructofuranosides, and amino acids (Bakker and Klein 1992). Production sites are mainly located on younger leaves. Organisms feeding on cassava extrafloral nectar include ants, parasitoids, lacebugs and predatory mites (Bakker and Klein 1992). Laboratory studies have shown that natural sugar solutions such as honey, honeydew, and extrafloral nectar, have a positive effect on the survival of predatory mites, and sometimes on reproduction (Bakker and Klein 1992).

14.3 Cassava Cultivation, Pests and Pest Management in South-Sulawesi, Indonesia

Cassava is the third major staple food crop after rice and corn in Indonesia (Balitbang Pertanian 2008). Indonesia is the third world’s largest cassava producer after Nigeria and Thailand (Worldatlas.com 2018) and the fifth largest cassava exporter (Suherman 2014). National cassava production is more than 23 million tons per year. Cassava harvest area is about 950,000 ha per year, with an average yield of 23.37 tons per ha (BPS 2016). This is far below the genetic potential of the currently available cultivars, ranging from 20 to 102 tons/ha (Balitkabi 2017). The province of South Sulawesi is one the major cassava-producing areas in the country with cultivation area and total production of 26.785 ha and 565.958 tons, respectively (BPS 2016). As more food and bioethanol industries are being developed throughout the country, the demand for cassava is increasing.

Wahono (2006) reports that corn is the most efficient source of bioethanol, followed by sugar cane and cassava. However, corn production in Indonesia does not meet the demand, and the cane is mostly used for sugar production. Both commodities are currently still imported to satisfy the national needs. Therefore, cassava has the highest potential to be grown for bioethanol production based on technological, environmental, and economic considerations (Ginting et al. 2006).

Cassava has suffered until now so little from pest and disease damage that they have been considered insignificant in reducing plant yields. Even the cassava cultivation guide, published by the Indonesian government as recently as in 2008, pest and disease management is not included in the guide book except for the use of herbicides for weed control (Balitbang Pertanian 2008). The low productivity with respect to yield potential is due to poor plant management practices. For example, most smallholder farmers do not apply fertilizer, or any kind of pest controls. However, in recent survey we found significant plant damage due to mealybug and mite infestations. Other insects were also found infesting the plants, such as the spiraling whitefly (*Aleurodicus dispersus*), sweet potato whitefly (*Bemisia tabaci*), and thrips (unpublished data). Insect pests of cassava are among the least studied crop pests in South Sulawesi.

Importantly, a new invasive cassava pest, the cassava pink mealybug, *Phenacoccus manihoti* Matile-Ferrero, (Hemiptera: Pseudococcidae) appeared on cassava plants in SE Asia in 2008 (Thailand), and has since then spread to other countries, including Indonesia, Vietnam and Cambodia. Climatic suitability analysis using CLIMAX software indicates that Indonesia is one of the potential distribution areas of the pest in SE Asia (Parsa et al. 2012). In 2014, the pest was reported causing damage on cassava plants in Java (Rauf 2014), and by 2017 the pest has already spread throughout Java Island (Abduchalek et al. 2017). The presence of the cassava mealybug on cassava in South Sulawesi was first recorded in 2016 in all main cassava-producing regions of the province (Fig. 14.1). The pest has quickly spread to the neighboring West Sulawesi province, and now the pest has been found causing damage to cassava crops throughout both provinces (Mewar 2018).

To control the cassava mealybug in Africa, a parasitoid wasp, *Anagyrus lopezi* (De Santis) (Hymenoptera: Encyrtidae) was introduced from Uruguay and released to the field for the first time in Nigeria in 1981 (Herren and Lema 1982). Since the introduction, the parasitoid has spread to 25 African countries and effectively suppresses the mealybug populations. In 2011, *A. Lopezi* was introduced into

Fig. 14.1 *Phenacoccus manihoti* infestation and its symptoms on cassava in South Sulawesi, Indonesia. (**a**). *P. manihoti* population at the base of leaf petiole. (**b**). Heavy *P. manihoti* infestation shortening stem internodes. (**c**). Terminal shoot deformation due to *P. manihoti* infestation

Thailand from Benin, and it has significantly reduced plant damage due to *P. manihoti* (FAO 2011). Inspired by the previous successes, Indonesian government introduced *A. lopezi* from Thailand to Java in 2014 (Rauf 2014).

Introduced *A. lopezi* showed good potential for use in a biological control program for cassava mealybug. All mealybug-infested cassava control plants contained in cages died after 2 months. However, when the infested plants were caged with three pairs of *A. lopezi*, the plant mortality rate was 20%, and the mealybugs parasitism rate was recorded at 25%. Field experiment showed that the parasitoid was capable of surviving, reproducing, and establishing in the agroclimatic conditions of Bogor, West Java, Indonesia (Abduchalek et al. 2017).

14.4 Survey of Pest Incidence and Notes on Natural Enemies Encountered in the Field

In South Sulawesi Province, proportion of cassava crop infested by mealybugs tended to increase during the survey in all locations: 9.0, 16.5, and 39.4% in 2016, 2017, 2018, respectively. Farmers interviewed in 2016 were not aware of mealybug presence, and did not apply any control measures for any pest on their crops, including insecticides. However, in 2018, about 50% of the interviewed farmers realized the damage caused by mealybugs, especially those who cultivate larger areas on the west coast. Because of economic considerations they are reluctant to apply any control measures against the pest. Thus, this insect has become a serious threat to the sustainability of cassava cultivation in the province. In general, proportion of mealybug infestation in the west coast was higher than in the east coast. This was most likely due to cassava planting area being larger in the west coast than in the east coast. Approximately 75% of all cassava plantations in South Sulawesi is cultivated in three regencies in the west coast area: Gowa, Maros, and Takalar (BPS 2016). Mealybug infestation affects the plant height with varying degrees, depending on the time of the first infestation. Plants infested at ages of 1, 2, and 3 moths, suffered in our studies a height reduction of about 45%, 34%, and 20%, respectively.

During the surveys, several natural enemies were found feeding on the mealybugs, such as coccinellids and praying mantids. Several ant species were associated with the presence of mealybugs on a plant, including *Solenopsis* sp. and *Crematogaster* sp., which are known predatory ants on several important plant pests such as the corn stem borer (*Ostrinia furnacalis*) (La Daha et al. 2016), and the sugarcane borer (*Diatraea saccharalis*) (De Oliviera et al. 2012). However, ants are also reported to have mutualistic associations with mealybugs. Mealybugs provide sugar for the ants, and ants protect the mealybugs from their natural enemies (Cheng et al. 2015). Such associations have been reported between the cassava mealybug and several ant species, *Camponotus, Crematogaster* and *Pheidole* (Hymenoptera: Formicidae) (Cudjoe et al. 1993).

14.5 Greenhouse Experiment on Bottom-Up Effects of Beneficial Fungi

An experiment was conducted with cassava in the greenhouse at the University of Helsinki, Finland, to explore possible bottom-up effects of soil inoculants on plant growth, health, and on pests. Cassava cv. HB60 (obtained from Kasetsart University, Thailand) was grown from small cuttings in the greenhouse at +25 °C, 70–90% RH. Initially the cuttings were grown in small 1 dl pots using standard commercial potting soil, inoculated with beneficial microbes at the rate of 10^5 cfu/g of soil. After the plants had several leaflets, they were transferred to 1-litre pots containing standard potting soil. The plants were fertilized and watered as needed. Treatments with the beneficial fungi included *Trichoderma harzianum* (T22, product Trianum™), *Glomus* spp. (product Symbio™), *Beauveria bassiana* (GHA, obtained from Stefan Jaronski, USDA), and *Metarhizium robertsii* (obtained from Stefan Jaronski, USDA). In addition, a control treatment with mealybugs only, and a control treatment without beneficial fungi and without mealybugs were included. When plants were 30 cm tall, they were manually infested with 20 female cassava mealybugs *Phenacoccus manihoti* (originating from Universitas Hasanuddin, Makassar, South Sulawesi, Indonesia). Spider mites *Tetranychus urticae* naturally infested all cassava plants in the greenhouse. Plant growth and population development of these pests were followed for a period of 4 weeks.

The results (Table 14.1) show that plants grown in soil treated with *Metarhizium robertsii* grew best (relative to untreated control without mealybugs), had fewer spider mites and mealybugs, and produced the highest number of extrafloral nectaries. Second best treatment was with the mycorhizal fungus *Glomus* spp. (Symbio™). All treatments with beneficial fungi improved plant growth relative to untreated control with mealybugs, but only *Metarhizium* and *Glomus* stimulated the production of extrafloral nectar. *Metarhizium* treatment appeared effective in preventing population growth of the cassava mealybug, and supported only low numbers of spider mites relative to all other treatments.

14.6 Potential of Beneficial Fungi as Biopesticides Against Mealybugs

Not many fungal entomopathogens have been reported to effectively infect mealybugs in the field, or to have potential to be used as a biological control agent. For example, *Neozygites fumosa* (Speare) Remaudière & Keller (Zygomycetes: Entomophthorales) naturally infected *Phenacoccus herreni* Cox & Williams in the field at rates ranging from 9–64.6% (Delalibera et al. 1997). In our 2018 survey, at the peak of the cassava mealybug populations, 30–40% of the field populations of the mealybug were naturally infected by an entomopathogenic fungus of *Fusarium* sp.

Table 14.1 Outcomes of bottom-up treatments of cassava potting soil with beneficial fungi on plant height, production of extrafloral nectaries (number of droplets per petiole), and population development of the cassava mealybug and spider mites

Treatment	Plant height (cm)	Relative to control 2	Extrafloral nectaries	Relative to control 2	Mites and mealybugs[a]
Trichoderma harzianum	88,3	79	3,3	94	Spider mites +++ MB ++
Glomus spp. (Symbio™)	89	80	4,6	131	Spider mites ++ MB +
Beauveria bassiana	86,3	78	3,5	100	Spider mites ++ MB +++
Metarhizium robertsii	97,1	87	4,9	140	Spider mites — MB +
Control 1 with mealybugs	78	70	3,5	100	Spider mites ++ MB +++
Control 2 without mealybugs	111,3	100	3,5	100	Spider mites ++ MB −−−

[a] Plants naturally infested by spider mites (*Tetranychus urticae*), manually inoculated with mealybug *Phenacoccus manihoti* (MB). +++ = high abundance, +++ = medium abundance, + = low abundance, −−− = very low numbers or absent

Table 14.2 A/verage daily mortality of the mealybug *Phenacoccus manihoti* after being sprayed with entomopathogenic *Fusarium* sp., isolated from three different insect hosts

Original host	Percent of infected mealybugs						
	Jan 9	Jan 10	Jan 11	Jan 12	Jan 13	Jan 14	Jan 5
Bactrocera dorsalis	0	0	11	16	32	52	81
Aphis gossypii	0	2	9	29	6	72	98
Mealybug	0	0	15	28	43	63	96
Control	0	0	0	1	1	1	1

In a field experiment with four locally isolated fungal entomopathogens: *Paecilomyces* sp., *Beauveria bassiana*, *Fusarium* sp., and *Trichoderma* sp., all were capable of infecting mealybugs in the field. However, *Fusarium* sp. was the most effective against *P. manihoti* with a mortality rate of 62.3%.

When the mealybug population was treated by spraying with *Fusarium* spores of at a concentration of 10^6 conidia/ml of sterile water, 81–98% of the individuals were killed in the greenhouse (Table 14.2, Fig. 14.2). This appears promising as a biocontrol agent against *P. manihoti* (Fig. 14.3).

Fig. 14.2 98% mortality of *Phenacoccus manihoti* 1 week after being sprayed with *Fusarium* conidia at the concentration of 10^6 conidia/ml of sterile water

14.7 Potential of Using Resistant Cultivars Against Mealybugs

We conducted a field study with 6-weeks old plants of six different cultivars. The plants were artificially infested with 10 adults of *P. manihoti* per plant. Six weeks after the infestation, the total number of mealybugs and bunchy-tops per plant were determined. In addition, the plant height was measured, and compared to the plant height of the uninfested plants of the respective cultivar. The results show that the plant response varied between the tested cultivars. The most resistant cultivar was Malang-4, with the average total number of 8 mealybugs per plant, no bunchy-top, and only about 4% plant height reduction. The most susceptible cultivar, in turn, was Adira-1 with 1343 mealybugs per plant, 4 bunchy-tops per plant, and 74% plant height reduction (Table 14.3). These results indicate a great potential to employ resistant cultivars in cassava production, in particular when combined with biological and other control methods.

Fig. 14.3 Reaction of six cassava cultivars to infestation with the cassava mealybug *Phenacoccus manihoti* (left to right): Adira-4, Malang-4, Litbang UK-1, Litbang UK-2, Malang-1, and Adira-1

Table 14.3 Average number of mealybugs per plant, number of bunchy-tops per plant, and percent reduction of plant height for six cassava cultivars

Variety	Number of mealybugs/plant	Number of bunchy-tops/plant	% height reduction
Adira 1	1343	4	74
Malang 1	589	3	63
Litbang UK-2	103	2	25
Litbang UK-1	784	3	16
Malang-4	8	0	4
Adira	824	3	30

14.8 Future Prospects of Ecostacking in Promoting Plant Health in Cassava

The cassava mealybug *Phenacoccus manihoti* has quickly infested the cassava growing regions of the province of South Sulawesi, Indonesia. Like at other invaded locations, it has become a serious threat to cassava growing within a few years after invasion.

Biological control of the cassava mealybug and other cassava pests can gain high importance when synergy obtainable from the various ecostacking techniques is exploited. This can lead to full and successful control of cassava mealybugs not only in South Sulawesi, but overall in South-East Asia.

Several techniques may be employed, such as parasitoid releases, soil inoculation (or stake treatment) with selected beneficial microbes, and direct treatment by spraying mealybug pathogenic fungi on the crop. Enhancement of the impact of natural enemies such as parasitoids and predators via stimulation of the production of extrafloral nectaries by the cassava plant remains to be studied, but appears as a promising addition to the ecostacking techniques in this case.

In addition, the large differences in varietal resistance between cassava cultivars can be utilized, while supporting natural biological control functions via informed management of the soil, vegetation, habitat, and landscape, can further enhance biological control efficacy and stabilize the production system against severe pest outbreaks.

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Chapter 15
Bees and Medicinal Plants – Prospective for Entomovectoring

Ekaterina Kozuharova, Ina Aneva, and Dave Goulson

15.1 Introduction

We constantly search for new bioactive compounds and natural products (e.g., Watson et al. 2001; Nash 2007; Atanasov et al. 2015; Waltenberger et al. 2016; Liu et al. 2017b). We put considerable effort into understanding how they work in the human body (e.g., Saxena et al. 1993; Asano et al. 2000; Nash et al. 2011; Butterweck et al. 2011; Wang et al. 2014, 2016, 2017a, b, c; Liu et al. 2017a, b; Tewari et al. 2018). We restlessly try to optimize the yield of plant bioactive compounds (e.g., Bimakr et al. 2012; Azmira et al. 2013; Atanasov et al. 2015). Medicinal plants are living creatures and we rely on their successful reproduction to supply these important plant substances. Many of the medicinal plants depend on bees for their seed set. What then is the impact of pollinator decline on the resources of medicinal plants?

Both wild and managed pollinators have globally significant roles in crop pollination, IPBES (2016). Pollinators are in trouble, globally, and with them agricultural products worth more than $200 billion annually (FAO 2017). But if pollinator decline is a hazard for agricultural productivity, it is not less dangerous for wild plants. Perring and Farrell (1977) found that a large proportion of endangered plant species depend on bees for their survival. Of the 321 species included, 27% are from families considered to be pollinated by bees. Therefore insect pollinator decline reflects in increasing the numbers of endangered plants in the UK. Zych

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E. Kozuharova (✉)
Faculty of Parmacy, Deapartment of Pharmacognosy, Medical University of Sofia, Sofia, Bulgaria

I. Aneva
Institute of Biodiversity and Ecosystem Research, BAS, Sofia, Bulgaria

D. Goulson
School of Life Sciences, University of Sussex, Brighton, UK

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and Jakubiec (2008) present similar data on the pollination of Polish red list plants. Thus, pollinators are fundamental to maintaining both agricultural productivity, and biodiversity.

Habitat destruction, loss of flower resources, and increased use of pesticides (neonicotinoids and others) are causing declines in pollinator abundance and diversity (Potts et al. 2010, 2015; Goulson et al. 2015). Decline of pollinators in North-West Europe is well documented (Biesmeijer et al. 2006; Carvalheiro et al. 2013). Ollerton and co-authors (2014) reveal that 23 species of bees and flower-visiting wasps have gone extinct in the UK. They also found that the rate of extinction was highly variable, and raised the question whether these extinctions have stopped, or whether they will continue in the future. The study demonstrates the importance of maintaining the year-on-year effort of recording natural history data (Ollerton et al. 2014). The relationship between the use of pesticides and the decline of pollinators is an important and controversial topic that is unlikely to go away any time soon. Neonicotinoids are often applied as systemic seed treatments to crops, and have negative impact on pollinators when they appear in floral nectar and pollen (Wright et al. 2015; Stanley et al. 2015a, b). There is strong evidence that this scales up to causing loss of bee biodiversity (Goulson et al. 2015; Woodcock et al. 2016).

The relative importance of pollinating honey bees, Apis mellifera, versus other species in providing pollination has been debated for more than 20 years (Aebi et al. 2012; Ollerton et al. 2012). The role of wild bees in the pollination process should not be neglected. Despite the fact that beekeepers now lose about 40% of their colonies each year due to problems such as *Varroa* mites, the spread of inhospitable industrial farmland, and exposure to pesticides, the total number of managed honey bees worldwide has risen by 45% over the last half century, driven by economic factors (Aizen and Harder 2009). Honey bees are in no danger of extinction. There are data that commercially managed bumblebees and honey bees may be contributing to wild pollinator decline (Hatfield et al. 2012). Commercial beekeeping often involves maintaining bees at high densities, making it easy for diseases to pass from bee to bee. Companies routinely feed bumblebees with pollen gathered by honey bees, helping diseases to spread between species. It has been demonstrated that commercially produced bumblebee colonies often carry multiple, infectious parasites that pose a significant risk to native and managed pollinators (Goka et al. 2001; Graystock et al. 2013, 2015). Managed bees may transmit new diseases to wild bees, or they may allow existing diseases to multiply and “spill back” into wild populations (Goulson and Hughes 2015). Additionally commercial bumblebees such as Bombus terrestris may turn into a dangerous, invasive pest (Dafni and Shmida 1996; Dafni 1998; Dafni et al. 2010; Schmid-Hempel et al. 2014).

It is difficult to find data ranking the economical importance of medicinal plants. One possible approach is tracing the herbal medicinal product registrations (Kozuharova and Getov 2012). This reveals that *Hypericum perforatum* L., *Valeriana officinalis* L., *Crataegus monogyna* Jacq. (Lindm.), *Matricaria recutita* L., *Melissa officinalis* L., *Silybum marianum* (L.) Gaertner, *Equisetum arvense* L., *Thymus sp. div., Urtica dioica* L., *Urtica urens* L., *Plantago lanceolata* L. along with some other species are amongst the most popular medicinal plants. These are plants included in Pharmacopeias (e.g. Europaean Pharmacopeia). Important Pharmacopeian plants include also *Gentiana lutea* L., *Arctostaphylos uva-ursi* (L.) Spreng., *Vaccinium myrtillus* L., *Primula veris* L., *P. elatior* (L.) Hill, *Melilotus officinalis* (L.) Lam., *Leonurus cardiaca* L., *Potenilla erecta* (L.) Raeusch., etc.). Many of these, as well other species such as *Ononis spinosa* L., *Rosa canina* L., *Oreganum vulgare* L. are collected in the wild, and used commercially in large quantities for herbal teas by different producers in Bulgaria (Kathe et al. 2003). Home use of medicinal plants is also registered (Kathe et al. 2003; Kozuharova et al. 2013; Dragoeva et al. 2015; Mincheva et al. 2016). *Gentiana lutea* L. *Valeriana officinalis* and *Arctostaphylos uva-ursi* are protected by the Biodiversity act, and forbidden for commercial collection from wild populations (Evstatieva and Hardalova 2004). Some species such as Valeriana officinalis*, Glaucium flavum, Althaea officinalis* and *Silybum marianum* are cultivated, but cultivation of medicinal and aromatic plants on about 3000 ha provides only less than 25% of the total annual harvest in Bulgaria *(*Kathe et al. 2003; Evstatieva 2006).

There are fungal diseases that infect the medicinal plants via the flower (Kenneth and Palti 1984; Dedej et al. 2004; Antonovics 2005; Ngugi and Scherm 2006; Munda 2011). Additionally medicinal plants suffer from pests associated with their flowers. Insects whose larvae damage the seed/fruit set of *Gentiana lutea, G. punctata, G. asclepiadea, G. pneumonanthe* and *G. cruciata,* belonged mainly to Coleoptera (Curculionidae) and Diptera (Tephritidae and Cecidomiidae). The larvae of lycaenid butterflies, *Maculinea* spp. (Lepidoptera), were recorded only in seeds of *G. asclepiadea, G. pneumonanthe* and *G. cruciata* (Kozuharova et al. 2018). Also bruchid beetles damage seed set of many medicinal plants members of family Fabaceae (Smith et al. 2001; Delobel and Delobel 2006; Tyler and Tyler 2016).

The aim of this study is to evaluate the dependency on insect pollinators for the reproduction of medicinal plants, with a view to identifying which of them may potentially be at risk due to wild bee declines. Furthermore, the potential to use entomovectoring for the protection of medicinal plants via targeted precision biocontrol (Hokkanen et al. 2015) is discussed.

15.2 Material and Methods

In our assessment, we used Bulgarian medicinal plants as a model. We classified 712 plants recognized by the Medicinal Plants Act (2000) as spore and gymnosperm plants, and angiosperm (flowering) plants. Then we divided the flowering plants according to their biological type after Jordanov (1964–1995) and Kozuharov (1992). The biological type gives information about the breeding system (Richards 1990). The flowering plants were also analyzed according to their functional flower morphology and pollination syndromes, described by Faegri and van der Pijl (1971) as anemophilous (wind pollinated) and entomophilous (insect pollinated) plants.

The medicinal plants that require insect vectors for their pollen transport were analyzed further: entomophilous pollination syndromes were assessed according to the functional morphology and access to nectar and pollen of their “blossoms” (flower or compact inflorescence). The medicinal plants were divided into the following functional morphology classes (Fig. 15.1):

- dish/bowl type with free access to the nectar and pollen, radial symmetry of the “blossom”;
- dish/bowl+funnel tube with radial symmetry of the “blossom” (capitulum), individual flowers are tubes with free access to the pollen but proboscis is required to reach the nectar;

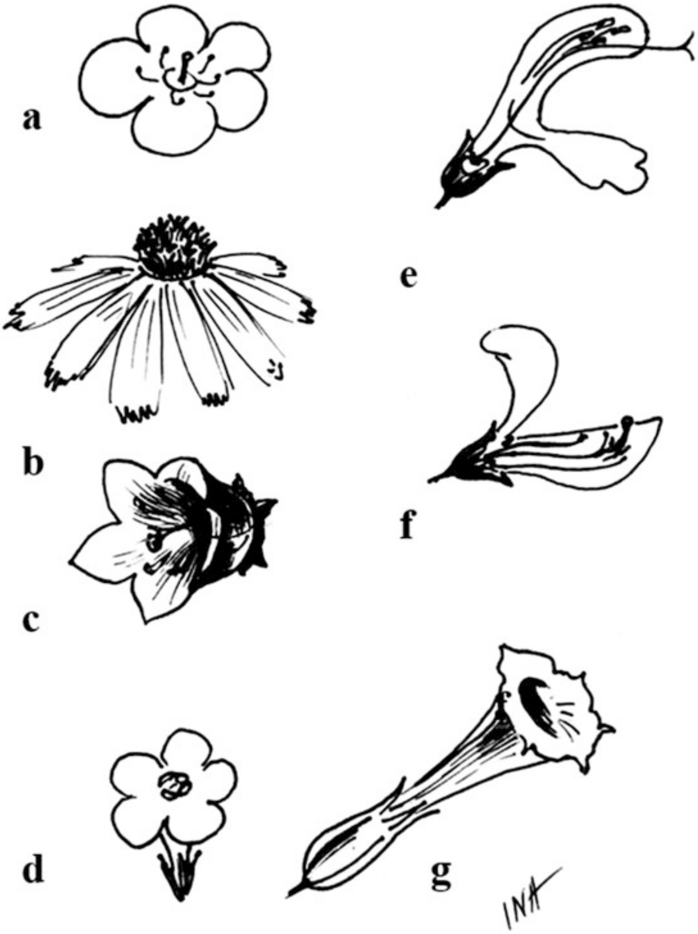

Fig. 15.1 Functional morphology classes in medicinal plants: (**a**) – dish/bowl, (**b**) – dish/bowl+funnel tube, (**c**) – bell, (**d**) – funnel shallow, (**e**) – flag, (**f**) – gullet, (**g**) – funnel deep

- bell – more or less hidden nectar, wide corolla tube, radial symmetry to slight zygomorphy;
- funnel shallow with hidden nectar, narrow but short corolla tube, radial symmetry to slight zygomorphy;
- funnel deep – hidden nectar, narrow and deep corolla tube, radial symmetry to slight zygomorphy;
- flag – sexual organs are found in the lower part, zygomorphy pollen is deposited on the abdominal side of the insect, stemotribic pollination;
- gullet – sexual organs are restricted to the functionally upper side, zygomorphy pollen is deposited on the dorsal side of the insect and upper part of the head, more or less hidden nectar, nototribic pollination.

Descriptive statistics were used to analyze the data obtained. Adaptations like spontaneous self-pollination ability, vegetative propagation and apomixis are also discussed.

15.3 Results and Discussion

Spore and gymnosperm comprise only a few of the Bulgarian medicinal plants (4.2%). The angiosperm/flowering medicinal plants (682 species) are predominantly perennials (Fig. 15.2). They are also known as polycarpic plants that bloom several times in their life cycle. Monocarpic plants that blooms only once in their life cycle (annual and biennials) comprise about one quarter (Fig. 15.2). Monocarpic plants often have the ability to spontaneous self pollination (Richards 1990) even though

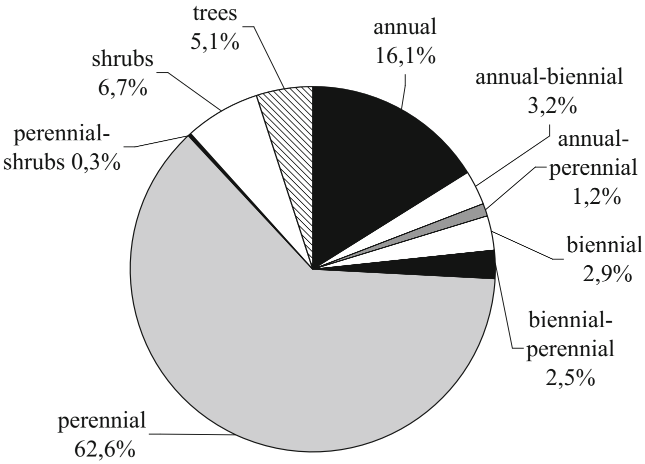

Fig. 15.2 Biological types of medicinal plants recognized by Bulgarian Medicinal plant Act (2000)

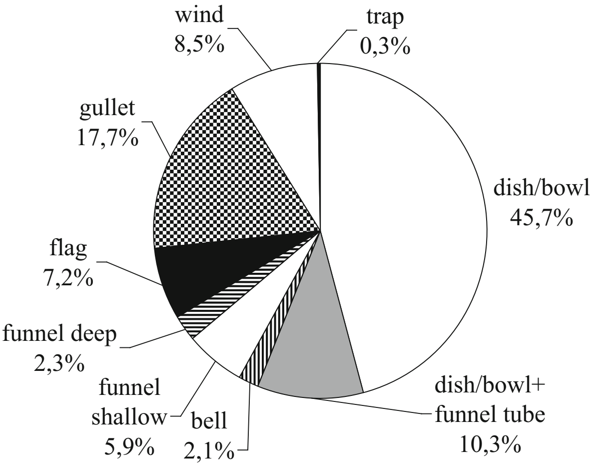

Fig. 15.3 Medicinal plants recognized by the Bulgarian Medicinal plant Act (2000) classified according to the functional morphology of their "blossoms" (flower or compact inflorescence)

they would cross-pollinate in the presence of pollinators. For example, the medicinal plant *Centaurium erythrea* Rafn. is cross-pollinated primarily by hoverflies, but has a surprising and elegant way of realizing delayed selfing, in which the anthers curl at the end of a flowers' life in order to shed pollen and guarantee self-pollination (Brys et al. 2011). Overall, more than three quarters of the medicinal plants potentially need pollen vectors for their seed production.

The analysis of the functional flower morphology of angiosperm medicinal plants reveals that those with blossoms adapted to wind pollination are few (8.5%, Fig. 15.3). This implies that about 91.5% medicinal plants potentially need insect pollinators for their seed production, as these are the major animal pollen vectors in the temperate zone, where Bulgaria is located.

The dish/bowl blossoms dominate among medicinal plants (Figs. 15.1 and 15.3).

More than a half of the plants with dish/bowl pollination syndrome are perennials (Table 15.1). Thus, potentially they need pollen vectors and are unlikely to self-pollinate. Pollinators may be nectar and/or pollen collecting bees, but also short-tongued insects like flies, wasps, and even anthophilous beetles. Some may have alternative mechanisms such as vegetative propagation, and yet be actively pollinated by bees, like for instance *Geranium macrorrhizum* L. (Fig. 15.4, Kozuharova 2008). Others, like *Alchemilla* sp. div. and *Hypericum perforatum* L. may be visited by bees (Lazarova and Yurukova 2007; Arnold et al. 2009; Girard et al. 2012; Fig. 15.4), but are known as well to have alternative reproductive mechanisms such as apomixis (Sepp et al. 2000; Köster 2010; Matzk et al. 2001, 2003).

Next most abundant plants have dish/bowl+funnel tube blossoms. These also have radial symmetry of the blossom (capitulum). Individual flowers in the capitulum are tubes or funnels with nectar and pollen more or less accessible (Figs. 15.1 and 15.3). The tube flowers require proboscis to reach the nectar, and therefore, wild bees and honey bees are very active pollinators (Fig. 15.4). Nearly half of the plants in this blossom class are monocarpic (Table 15.1). Some of these plants are

Table 15.1 Functional flower morphology of entomophilous angiosperm medicinal plants and their biological types

Functional blossom morphology and biological types	Number of plant species	Percent
Dish/bowl		
Annual	42	13,5%
Annual-biennial	13	4,2%
Annual-perennial	6	1,9%
Biennial	7	2,2%
Biennial-perennial	11	3,5%
Perennial	181	58,0%
Perennial-shrubs		
Shrubs	29	9,3%
Trees	23	7,4%
Total	312	
Dish/bowl + funnel tube		
Annual	20	28,6%
Annual-biennial	2	2,8%
Annual-perennial		
Biennial	7	10,0%
Biennial-perennial	3	4,3%
Perennial	38	54,3%
Perennial-shrubs		
Shrubs		
Trees		
Total	70	
Bell		
Annual		
Annual-biennial	3	21,4%
Annual-perennial		
Biennial		
Biennial-perennial		
Perennial	11	78,6%
Perennial-shrubs		
Shrubs		
Trees		
Total	14	
Funnel shallow		
Annual	5	12,4%
Annual-biennial	1	2,4%
Annual-perennial		
Biennial	2	5,0%
Biennial-perennial	1	2,4%
Perennial	25	62,5%
Perennial-shrubs		
Shrubs	6	15,3%
Trees		
Total	40	

(continued)

Table 15.1 (continued)

Functional blossom morphology and biological types	Number of plant species	Percent
Funnel deep		
Annual	1	6,2%
Annual-biennial	1	6,2%
Annual-perennial		
Biennial		
Biennial-perennial		
Perennial	14	87,6%
Perennial-shrubs		
Shrubs		
Trees		
Total	16	
Flag		
Annual	12	24,5%
Annual-biennial	1	2,0%
Annual-perennial		
Biennial		
Biennial-perennial		
Perennial	25	51,0%
Perennial-shrubs		
Shrubs	9	18,4%
Trees	2	4,1%
Total	49	
Gullet		
Annual	15	12,4%
Annual-biennial	1	0,8%
Annual-perennial	1	0,8%
Biennial	4	3,3%
Biennial-perennial	2	1,7%
Perennial	94	77,7%
Perennial-shrubs	2	1,7%
Shrubs	2	1,7%
Trees		
Total	121	
Trap		
Annual		
Annual-biennial		
Annual-perennial		
Biennial		
Biennial-perennial		
Perennial	2	100%
Perennial-shrubs		
Shrubs		
Trees		
Total	2	

Fig. 15.4 Some examples of plants and their pollinators: *Bombus lapidarius* on *Lamium maculatum; B. lapidarius* on *Centaurea jacea; B. hortorum* on *Digitalis graniflora; Apis mellifera* on *Geranium macrorrhizum; A. mellifera* on *Taraxacum officinalis; B. argillaceus* on *Digitalis lanata*

likely to self-pollinate, as is well known for the important medicinal plants *Silybum marianum* (L.) Gaerth. (Alemardan et al. 2013). Nevertheless, the monocarpic *Onopordum acanthium* L., *O. tauricum* Willd., and *Carduus acanthoides* L. are valuable honey plants (Lazarova and Yurukova 2007), and food sources for wild bees (Tomozei 2002; Celary and Wiśniowski 2007; Özbek 2014). Some perennials like *Taraxacum officinale* Web. and *Achillea millefolium* gr. are well known for their apomictic reproduction (Richards 1990; Terziiski et al. 1995; Guo et al. 2008.) and yet they are actively visited by pollinators (Fig. 15.4). The perennials *Tussilago farfara* L., *Petasites hybridus* (L.) Gaertn. (P. *officinalis Moench.*) and P. *albus* (L.) Gaertn. possess strong ability for vegetative propagation (Myerscough and Whitehead 1966; Bostock 1980; Richards 1990), but as they bloom early in the spring they are very attractive food plants for bees (Debrunner and Meier 1998; Warakomska and Kolasa 2003; Haratym and Weryszko-Chmielewska 2012).

Funnel blossoms have more or less hidden nectar. The depth of the corolla tube restricts the pollinators. Among medicinal plants, "funnel shallow" blossoms are well presented and much more numerous than "funnel deep" blossoms (Figs. 15.1 and 15.3). "Funnel shallow" blossom is characterised by hidden nectar, narrow but short corolla tube, radial symmetry to slight zygomorphy. Nectar is accessible to short tongued insects – wild and honey bees and bee flies. Medicinal plants with this pollination syndrome are predominantly polycarpic (Table 15.1) and therefore potentially dependent on their pollinators. Here we can list *Buglossoides* *purpurocaerulea* (L.) Lohnst., *Pulmonaria officinalis* L., *Symphytum officinale* L. – their pollinators are hoverflies, butterflies, and bumblebees, and even buzz pollination may be present as it is in the last species (Corbet et al. 1988; Woodward and Laverty 1992; Goulson et al. 1998; Brys et al. 2008; Jacquemyn et al. 2013; Nocentini et al. 2013; De Luca and Vallejo-Marín 2013). *Vaccinium* species are also known as buzz-pollinated plants, but their flowers are also rich in nectar (Knudsen and Olesen 1993; Dupont and Olesen 2009). Research on *Vaccinium myrtillus, V. vitis-idaea,* and *V. uliginosum,* which are quite popular for their medicinal properties, reveals that even though they are partially self-compatible and to a different extent capable of spontaneous self-pollination, they have poor capacity to self in the absence of pollinators (Jacquemart and Thompson 1996). Bumble bees and honey bees can be used as vectors for antagonistic fungi and bacteria to control *Monilinia* pathogens on *Vaccinium* (Munda 2011; Menzler-Hokkanen and Hokkanen 2017).

"Funnel deep" blossom is characterised by hidden nectar, with narrow and deep corolla tube, radial symmetry to slight zygomorphy. Nectar is accessible to insects with long proboscis – butterflies and moths, long tongued bumblebees or sweat bees; for example *Calystegia sepium* (L.) Rr. is pollinated by bumblebees, small bees, and syrphid flies (Ushimaru and Kikuzawa 1999). In this group the most interesting from medicinal point of view is *Saponaria officinalis* L. It is pollinated by nocturnal moths (Jürgens et al. 1996, 2003; Wolff et al. 2006), but it also has the capability for reproducing clonally (Davis and Turner-Jones 2008).

Specialization in pollination syndromes is expressed in flag and gullet syndromes. They usually require large bees as pollen vectors, for example *Megachile* sp. div., *Andrena* sp. div., *Osmia* sp. div., bumblebees and honey bees, which are usually generalists and polylectic (Proctor et al. 1996; Waser et al. 1996; Goulson 2006). Medicinal plants with gullet blossoms are about twice as numerous as those with flag blossoms (Figs. 15.1 and 15.3) but they are both predominantly polycarpic (Table 15.1) and consequently dependent on their bee pollinators. For example bumblebees are the pollen vectors of *Lamium maculatum* L. (Fig. 15.4), *Digitalis lanata Ehrh.* (Fig. 15.4) and *Digitalis grandiflora Mill.* (Fig. 15.4). Bees can be used as vectors for entomopathogenic fungi or bacteria to control Bruchidae pests on medicinal plants from the family Fabaceae with flag blossoms (Tyler and Tyler 2016; Menzler-Hokkanen and Hokkanen 2017).

Bell blossom is characterized by more or less hidden nectar, wide corolla tube, radial symmetry to slight zygomorphy. Few medicinal plants posses this syndrome (Fig. 15.3) and they also are mainly polycarpic (Table 15.1). In this group are vulnerable and popular medicinal plants such as *Atropa belladonna* L., *Gentiana lutea* L., and *G. punctata* L. Bees can be used as vectors for entomopathogenic fungi or bacteria to control the Diptera or Coleoptera pests on the seed set of gentians (Kozuharova et al. 2018; Menzler-Hokkanen and Hokkanen 2017).

Trap pollination syndrome among medicinal plants is presented only by two species of *Arum* (Fig. 15.3).

This analysis of the Bulgarian medicinal plants is highly approximate as we attempt to infer likely pollinators and breeding system based on life history and

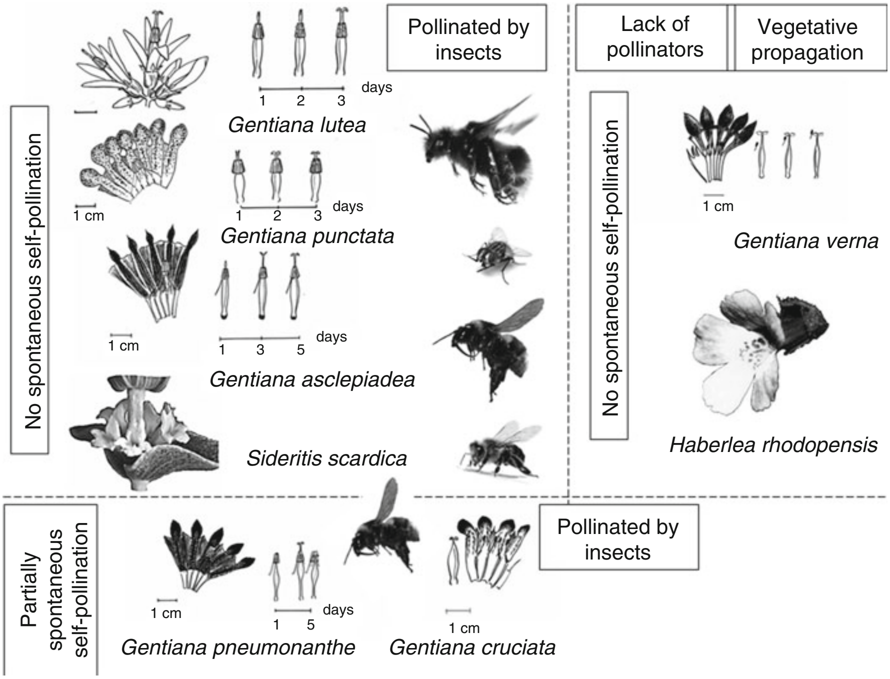

Fig. 15.5 Ability for spontaneous self-pollination, and overview of reproductive strategies in medicinal plants

flower morphology. Detailed research is required on each particular species as was shown by Ollerton et al. (2009), but this is a huge task and unlikely to be completed in the near future. Research on pollination and breeding systems of medicinal plants (Fig. 15.5) is only at its beginning.

Our experiments show that Gentiana lutea, G. punctata and *G. asclepiadea* are not spontaneously self-pollinated, but their wild populations are actively pollinated predominantly by bumblebees and flies (*Thricops* sp. div). *G. pneumonanthe* and G. cruciata have limited capacity for spontaneous self-pollination, but their populations are mainly outcrossing because they are actively pollinated by bumblebees. G. verna does not set seed when flowers are isolated from pollinators, although they are self-compatible. Pollinators with long proboscis are morphologically adapted, but they are extremely scarce. The reduced fruit set due to pollinator limitation is compensated by a strong ability of vegetative propagation (Kozuharova 1994a, b; Kozuharova and Hadzieva 2006).

Our experiments show that Haberlea rhodopensis is self-compatible, but not spontaneously self-pollinated and autogamous. Phenology of the populations, herkogamy and weak protrandry are mechanisms which favour the outcrossing in natural populations. Pollinators, however, in situ are practically absent. The plant compensates the pollinator limitation by intensive vegetative propagation (Bogacheva-Milkoteva et al. 2013a, b).

Our preliminary observations reveal that *Sideritis scardica* is visited by bumblebees in situ and ex situ in small patches. When it is cultivated on a larger scale (about 10–20 ares), honeybees provide the pollination service. It is not capable of spontaneous self-pollination as the experimentally excluded from pollinators inflorescences (n = 5) did not produce seed (Kozuharova et al., unpublished data).

15.4 Conclusion

More than three quarters of medicinal plants are likely to need insects, predominantly bees, as pollen vectors for their seed production. Despite the fact that some of them have additionally alternative strategies to ensure reproduction, such as spontaneous self-pollination, vegetative propagation, or apomixis, the importance of bees and other insect pollinators is clear. Pollinator decline (Potts et al. 2010, 2015; BeeInformed 2014–2015; Goulson et al. 2015) is therefore an important hazard for the existence and production of medicinal plants. This predominant dependence of medicinal plants on insects for pollination provides also an excellent potential for using these pollen vectors in the context of biological control of insect pests and diseases via entomovectoring (Hokkanen and Menzler-Hokkanen 2009; Smagghe et al. 2012; Hokkanen et al. 2015; Menzler-Hokkanen and Hokkanen 2017).

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