Journal of Pest Science
https://doi.org/10.1007/s10340-023-01716-3

ORIGINAL PAPER

Laboratory and field evaluation of maize resistance to the two-spotted spider mite, *Tetranychus urticae*

Ning Di[1,2] · Zi Shi[1,3] · James D. Harwood[1] · Yafeng Chen[1,2,5] · Jiarong Wang[1,3] · Zhengyang Zhu[1,2] · Su Wang[1,2] · Nicolas Desneux[4] · Jiuran Zhao[1,3] · Wen Ren[1,3]

Received: 31 January 2023 / Revised: 29 September 2023 / Accepted: 6 November 2023

Abstract

Spider mites are some of the most important agricultural pests worldwide, jeopardizing maize production across many continents. However, mechanisms of resistance to spider mite in maize remain unclear. Here, we report data from resistant and susceptible maize varieties, Jingke968 and Xianyu335 along with their parental lines, and investigate their responses to *Tetranychus urticae* (Acari: Tetranychidae). A significantly lower female adult mite population and total mite numbers were recorded in the resistant variety, Jingke968, compared to Xianyu335. Similarly, mite fecundity on Jingke968 was substantially lower compared to Xianyu335, which could be associated with its highly resistant parental line Jing92. Stomatal dimensions also showed a close correlation with the fitness of spider mites on the two maize varieties and four parental lines. *Tetranychus urticae* population numbers under field conditions showed lower mite densities (numbers per plant) in Jingke968 and Jing92 (75% and 89% lower, respectively) than the most sensitive line PH6WC, hinting toward a high level of resistance to spider mites in these maize lines. Our study provides new insights for mechanisms of physical defense to spider mites in maize and contributed to the basis for pest control and breeding of maize varieties.

Keywords Pest resistance · Maize · Spider mites · Stomatal dimensions

Introduction

Modern breeding efforts have resulted in a significant increase in maize yields (*Zea mays* L.), making it one of the most important crops in the world (Calderon-Vazquez et al. 2008; Duvick 2005; Jiao et al. 2012). However, maize is threatened by the infestation of insects and other herbivores worldwide (Karjagi et al. 2017). Spider mites (Acari: Tetranychidae) such as *Tetranychus urticae* Koch, *T. cinnabarinus* (Boisduval) and *T. truncatus* Ehara are important pests of key crops (Bui et al. 2018; Nieberding et al. 2022), such as maize in dry regions of northern China (Guo et al. 1998, 2013). In addition, these phytophagous mites can also transmit plant viruses to crops (de Lillo et al. 2021).

The most commonly utilized method of management for spider mites is pesticide (acaricide) application but their effectiveness is reduced because of increasing resistance (Dermauw et al. 2013; Xu et al. 2018, 2023), and pesticides are also known to have multiple side effects on non-target arthropods (Desneux et al. 2007). In addition to chemical insecticides, transgenic crops expressing *Bacillus thuringiensis* (*Bt*) toxins have been tested and

---

Communicated by Cesar Rodriguez-Saona.

Ning Di and Zi Shi have contributed equally to the work.

✉ Jiuran Zhao
maizezhao@126.com

✉ Wen Ren
renwen@maizedna.org

[1] Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, China

[2] Key Lab of Natural Enemies Insects, Ministry of Agriculture and Rural Affairs, Beijing, China

[3] Beijing Key Laboratory of Maize DNA Fingerprinting and Molecular Breeding, Beijing, China

[4] Université Côte d'Azur, INRAE, UMR ISA, 06000 Nice, France

[5] Beijing Xicheng Vocational School, Beijing 100037, China

are reported to be effectively against many lepidopteran and coleopteran pests (Betz et al. 2000; Wu et al. 2008; Zhu et al. 2022), but they are not effective against non-target pests such as spider mites and aphids (Dutton et al. 2004; Ramirez-Romero et al. 2008; Li and Romeis 2010). The release of natural enemies could be an effective alternative to chemical pesticides but the high costs of such biological control agents, as well as their environmental requirements, currently prevent their wide scale adoption in cropping systems (Parolin et al. 2013; Jactel et al. 2019; Loomans 2021; Verheggen et al. 2022). The development of pest resistant maize varieties is therefore a particularly safe, economical and effective approach to overall pest management (Karjagi et al. 2017).

Plants have established a variety of defense mechanisms to resist pests during the process of their coevolution with herbivores (Ramawat and Goyal 2020). The resistance mechanisms in plants against herbivores can be categorized into three types: antixenosis, antibiosis and tolerance (Sulistyo and Inayati 2016). Antixenosis influences the behavior of pests and directs them away from the host, whereas antibiosis affects their biology and results in detrimental effects on herbivores, for example, increased mortality and reduced reproduction (Peterson et al. 2017). Tolerance does not alter host–pest relationships, but refers to the ability to withstand or recover from pest damage through physiological regulations and adjustments of plants (Koch et al. 2016).

Physical traits play an important role in herbivore resistance (Carmona et al. 2011) with leaf thickness, trichome density and stomatal density all influencing pest feeding behavior and damage (Agrawal 1998; Carmona et al. 2011; Stavrinides and Skirvin 2003). For example, trichome density can affect the movement of mites; however, they can also offer attachments to prevent mites shedding from the leaf surface (Nihoul 1993) and provide shelter to avoid predators (Yin et al. 2022). Stomata are exploited as one of the feeding routes by spider mites, so higher stomatal density typically results in higher mite fitness (Skorupska 1998).

Jingke968 (JK968) and Xianyu335 (XY335) are two of the most commonly planted maize varieties in China, and their parental lines have completely different genetic backgrounds (Chen et al. 2022a). Previous studies have documented that JK968 exhibits stronger mite resistance than XY335 in the field (Chen et al. 2022b) although the specific mechanism of mite resistance in JK968 remains unclear. The objectives of the present study were to: (1) determine the differences of *T. urticae* population and fecundity on JK968/XY335 and their parental lines; (2) elaborate on the effects of physical traits of maize leaves on the fitness of *T. urticae*; and (3) describe the relationship between spider mite resistance among hybrids and their parental lines.

Materials and methods

Plants and insects

Maize materials

All maize seeds were obtained from Beijing Key Laboratory of Maize DNA Fingerprinting and Molecular Breeding at the Beijing Academy of Agriculture and Forestry Sciences (BAAFS, Beijing, China). Seeds were sterilized by soaking in 75% ethanol for 20 s (Beijing Tong Guang Fine Chemicals Company, China), rinsed twice in distilled water, soaked in distilled water for 24 h and germinated on a wet filter paper in a growth incubator (MH351, Sanyo, Japan) set to 27 ± 1 °C, 40 ± 5% RH, 16:8 L:D photoperiod, 3000 lux fluorescent light. Maize lines and their relationship are shown in Table 1. Germinated seeds were transferred into plastic pots (15 cm diameter, 15 cm height) with matrix soil (Pindstrup Mosebrug A/S, Denmark) and vermiculite (volume ratio = 3:1) in an incubator. The plants were used for assays at the V3 stage, a stage with three visible collared leaves.

Mite rearing

Two hundred females and 200 males of *T. urticae* were collected from the colony at the Laboratory of Applied Entomology, Institute of Plant and Environment Protection (IPEP) and BAAFS. The mites were reared on common bean (*Phaseolus vulgaris* L.) plants to establish a new colony in insect cages (60 cm × 60 cm × 60 cm) made of 120 mesh net (bore diameter 0.125 mm) in a growth chamber as described above. Sufficient fresh host plants were supplied to maintain the colony by adding new bean plants every 10 days. To obtain female adults at the same age, the following treatments were undertaken. A sponge, 6 cm diameter and 2 mm thickness, was placed in a 9 cm plastic Petri dish. Afterward, a whole fresh kidney bean leaf (approx. 30 cm^2) was placed on the sponge and surrounded by absorbent cotton along the edges of the leaves. Distilled water was applied to the Petri dish using a plastic dropper to maintain moisture on the cotton and prevent the mite from escaping. Ten female adults from the colony were transferred to the leaves, and the Petri dishes were transferred to the incubator. (The condition was the same as described above.) Female adults were allowed to oviposit for 24 h and were subsequently removed. Then,

Table 1 The genetic relationship between different maize lines

Maize varieties	Female parent	Male parent
Jingke968 (JK968) (resistant)	Jing724 (J724)	Jing92 (J92)
Xianyu335 (XY335) (sensitive)	PH6WC	PH4CV

the eggs were allowed to develop for about 5 days in the same Petri dish until they hatch. The newly hatched nymphs were carefully transferred to new Petri dishes separately and reared until emergence for about 4 days, and newly emerged females and males aged within 2 days were selected. The selected females and males were then reared for another day. After which 10 females and 10 males in good viability were paired in a Petri dish and allowed to mate for 24 h, then mated females were randomly chosen for use.

Laboratory evaluation of *Tetranychus urticae* total and female adult population numbers on JK968/XY335 and their parental lines

Six plants of each maize variety at similar height (V3 stage) were selected for the experiments. Forty mated female mites 4–5 days were gently transferred to each plant with a brush. To prevent mites from escaping and migrating among the different plants, each plant was covered by a cage made of a transparent cylindrical plastic screen (14 cm diameter, 60.0 cm height). The top of the cylindrical cage was covered with 120 mesh net, and the bottom was plugged into the soil (approximate depth of 1 cm). The numbers of mites on each plant were counted and recorded everyday for 25 days using a hand magnifier. The assay was conducted in growth incubators as described above. The observations were categorized into 5 stages, after 5 days each based on the mite population growth tendency.

The method for evaluating female mite population numbers was the same as above with only numbers of female adults recorded. The number of female adults were counted and recorded everyday for 30 days, and the replication number for each line was 6. The observations were categorized into 6 stages based on the female mite population growth tendency (every 5 days each).

Fecundity of *T. urticae* on various maize lines

The assay was undertaken using leaf discs (1 cm^2) placed on a sponge (4 cm diameter, 2 mm thick) in Petri dishes (5.5 cm diameter). Leaf discs were obtained from the third leaf of 3-leaf-stage plants using a hole puncher and one Petri dish contained one leaf disc; one female adult mite aged 4–5 days was placed on each leaf disc. To keep the leaves fresh and prevent mites escaping, distilled water was added to the sponge to maintain moisture. Petri dishes were maintained in the incubator (conditions as described above) and the number of eggs recorded at 24 h and 48 h after infestation with the mites, after which female adults were removed to prevent further oviposition. After 5 days, the number of hatched eggs were checked and recorded. The number of replicates for each maize variety was 12–18.

Hatching rate and fecundity were calculated as follows:

$$\text{Hatching rate} = \text{Number of hatched larvae/total egg number} \times 100\%$$

$$\text{Fecundity} = \text{Number of eggs} \times \text{hatching rate}$$

Leaf tissue structures of different maize lines

To measure leaf thickness, stomatal density and stomatal size, the middle part of the second leaf of the V3 stage was used. Leaf thickness was determined under optical microscope using the paraffin section technique (Atkinson and Wells 2017). Stomatal density and size on the adaxial and abaxial section of the leaf were photographed by scanning electron microscope (SU8100, HITACHI, Japan), and 10 fields were measured for each line. The stomatal number was counted in a 1-mm^2 field to determine stomatal density with 6 replicate plants for each line with 6 replicates performed for each line.

Field population numbers of *T. urticae*

Analyses of the field numbers of *T. urticae* were conducted at the experimental site of Seven Star Apple Cooperative in Changping district, Beijing (E116° 23′ 09″, N40° 10′ 42″). Six maize inbred lines (same lines as in the laboratory evaluation) were planted in May 2020. Four square blocks of 56.25 m^2 were established, each divided into 9 small square plots of 6.25 m^2. Test blocks were covered with a 40-mesh insect-proof netting (7.5 m × 3.5 m) to avoid mite escaping and environmental impacts, such as strong wind and heavy rain. Each maize line was planted randomly in the small square plots except for the middle one, and the remaining 3 plots were left empty as a blank control. A total of 40 plants were planted in one plot with plant spacing of 0.3 m and row spacing of 0.6 m. No insecticides were applied throughout the experiment. Fifty female adults aged younger than 10 days of *T. urticae* were transferred onto each maize plant as previously described (Kamali et al. 1989) at 14 leaf collar maize phenological stage (V14). The numbers of *T. urticae* on all leaves of 5 maize plants were examined every 5 days following a 5-point sampling method (the disparate chiasmata was first determined as the central sampling point, and then two points on each diagonal line with the same distance from the central sample point were selected as the remaining sample points) in each plot. To avoid marginal effects, the outermost plants in each plot were not included. The field experiment ceased when maize grain entered the dehydration phase because the damage of *T. urticae* would not cause yield loss after that time (Archer et al. 1997).

Statistical analysis

All analyses were undertaken using R 4.0.2 software (R Development Core Team 2020). For counting mite numbers, the observations were categorized into 5 stages, after 5 days each and the daily average amount of mites at each stage were calculated. We performed generalized linear models (GLMs: 'glm' function ('stats' package)) to clarify the linear or nonlinear relationship between the response variables, and the model was fitted with a Poisson distribution. We first ran the full model, which included all fixed effects and subsequently removed all non-significant effects by stepwise model selection (Akaike information criterion: AIC).

The data of daily average amount of total mites and data of the field population numbers were transformed by $\lg(x+1)$ to fit of the model. One-way ANOVA was used to compare the difference of mite density and field population numbers among these six maize lines fitted with 'aov' function, and the post hoc analysis Tukey's HSD was performed with 'Tukey HSD,' with both functions being available in the 'stats' package. The 24 h egg number, 48 h egg number, hatching rate and 48 h fecundity differences on different maize varieties were analyzed using one-way ANOVA with the package 'stats' and Duncan's multiple range test with the package 'agricolae'. Leaf thickness, stomatal density and stomatal size across different maize lines were also analyzed using one-way ANOVA (Duncan's test).

To analyze the correlation between physical characteristics of maize leaves and mite resistance, we divided all data into mite resistance indices (including daily average number of mites, daily average number of female adult mites, 24 h egg number, 48 h egg number, hatching rate and 48 h fecundity) and leaf tissue structures indices (including adaxial stomatal length, adaxial stomatal width, adaxial stomatal density and leaf thickness). PCA of mite resistance indices and leaf tissue structure indices was conducted using the 'pca' function. Further, the Spearman correlation between each of the two variables was conducted using 'corr.test' function in the 'psych' package.

Results

Tetranychus urticae population development on JK968/XY335 and their parents

Fifteen days after feeding, substantial lesions were observed on the leaves of XY335 and its parental lines (PH6WC and PH4CV) (Fig. S1), whereas JK968 and its parental lines (J724 and J92) exhibited fewer lesions. The interaction between stage and maize lines was significant on the total number of *T. urticae* ($Z = -7.769$, $P < 0.001$). The number of mites on the parental lines of XY335, PH6WC and PH4CV was substantially elevated with increase in incubation time, whereas it remained low across 25 days of incubation in other tested lines. The female adult number of *T. urticae* per plant peaked after 11–15 days (Fig. 1B) with the exception of JK968 and its paternal line, J92, in which the peak occurred after 15–20 days with relatively low absolute numbers and decreased thereafter. The effect of maize lines on number of female adult mites was significantly different ($P < 0.05$ for all stages), being highest on PH6WC followed by PH4CV, J724, XY335, JK968 and J92, respectively. The limited and delayed development of female adults in JK968 and J92 further indicates a more robust resistance to spider mites in these two lines.

Furthermore, the daily average number of mites showed similar patterns between genotype, with the lowest recorded on J92 and highest on pH6WC (Fig. 1C, D). One-way ANOVA indicated significant differences among total mite numbers ($F = 100.7$; $df = 5, 30$; $P < 0.001$) and female adult numbers ($F = 8.527$; $df = 5, 30$; $P < 0.001$) in different maize lines. There was no significant difference in the number of female adult mites between JK968 and XY335 ($P = 0.956$), but the total number of mites on XY335 was significantly higher than JK968 ($P < 0.001$).

Fecundity of *Tetranychus urticae* on different maize lines

There were significant differences in 24 h egg number ($F = 15.460$, $df = 5$, $P < 0.001$), 48 h egg number ($F = 28.710$, $df = 5$, $P < 0.001$), hatching rate ($F = 6.1230$, $df = 5$, $P < 0.001$) and the 48 h fecundity ($F = 32.620$, $df = 5$, $P < 0.001$) between different maize lines (Fig. 1E–H). These parameters were all greatest on PH6WC and significantly lower on JK968 and J92, indicating that mite fecundity was substantially compromised on the latter two lines.

Leaf structures and their relationship with mite resistance

To investigate the relationship between certain leaf physical characteristics and mite resistance, stomatal size, stomatal density and leaf thickness were measured. These all revealed significant differences between maize lines (Fig. 2). J92 exhibited the smallest stomatal length on both the abaxial and adaxial side of the leaves, whereas J724 was the smallest in stomatal width and density on both leaf sides. Contrary to these results, stomatal length, width and density of XY335 were generally higher than those of JK968 except for the density on the abaxial side, and such differences may be inherited from their parental lines, since J92 and J724 exhibited the lowest stomatal length and the least stomatal width and density, respectively. Additionally, leaf thickness of XY335 was significantly lower than JK968.

To further explore the effect of leaf physical features on mite resistance, a principal component analysis (PCA) was performed. It revealed that stomatal length was positively correlated with all resistance indicators of mites, whereas

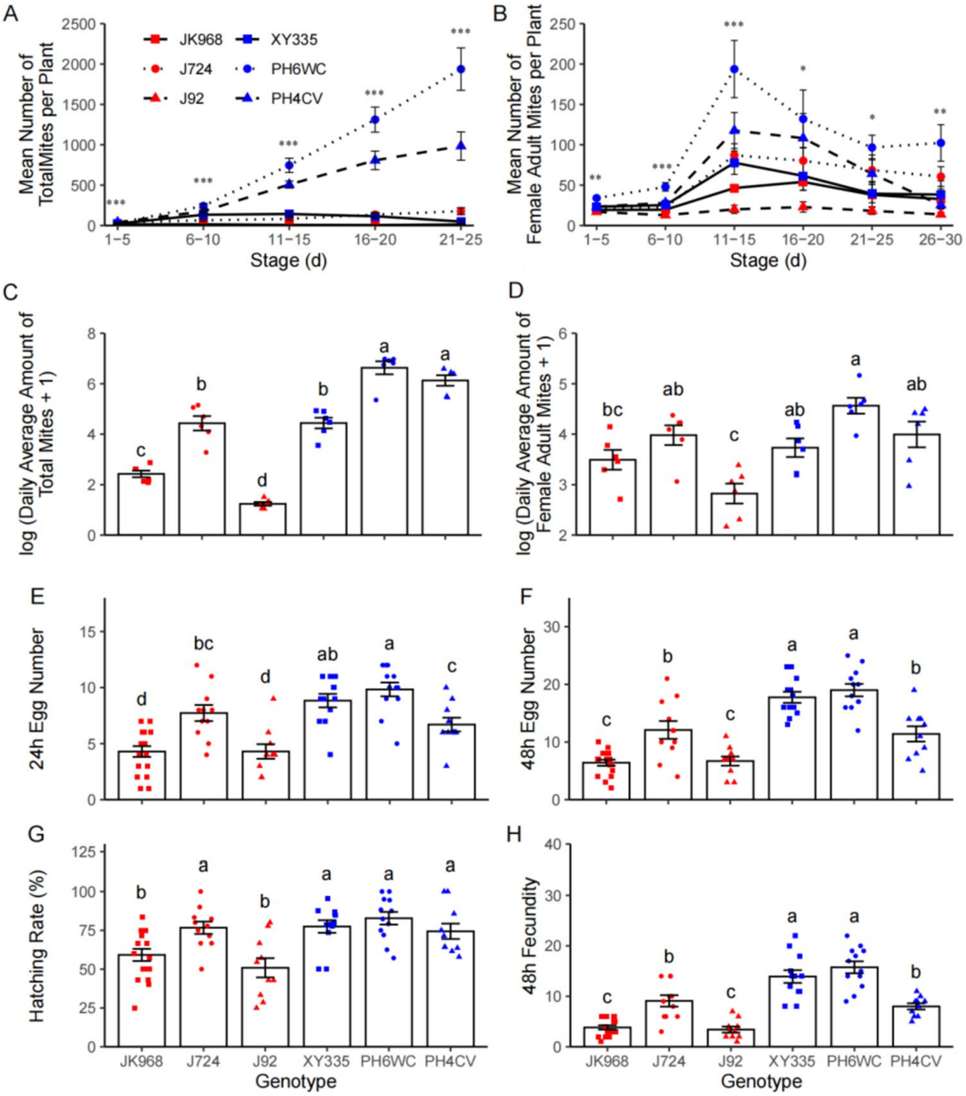

Fig. 1 Comparison of mite resistance of different maize lines. **A** Mean number of total mites per plant on different maize lines; **B** mean number of female adult mites per plants on different maize lines; **C** daily average number of total mites on different maize lines; **D** daily average number of female adult mites on different maize lines; **E** 24 h egg number; **F** 48 h egg number; **G** hatching rate; **H** 48 h fecundity of *T. urticae* on different maize lines. The stars above data points mean significant differences at $P < 0.05$*, $P < 0.01$** and $P < 0.001$*** between different maize lines; the letters above bars represent statistically significant at $P < 0.05$ between different maize lines

leaf thickness was negatively correlated with those indicators (Fig. 3). Stomatal width and stomatal density were positively correlated with total number of mites and female adult mite. Additionally, all mite resistance indicators were positively correlated with each other. These results were consistent with those obtained by Spearman correlation analysis (Fig. S3). These data suggest that the higher resistance to spider mite in JK968 and J92 may be partially due to its preferable leaf physical characteristics.

Field numbers of *Tetranychus urticae*

The number of *T. urticae* remained at a relatively low level until the middle of July in all maize genotypes (Table 2), after which it gradually increased to its peak in early August. Genotype showed a significant influence on colony numbers (F = 87.09; df = 5, 18; $P < 0.001$), with the highest and lowest number of mites observed in PH6WC and J92, respectively. Similar to data under laboratory conditions, JK968 and its parental line, J92, exhibited more robust spider mite resistance in the field compared to other maize lines, with fewer numbers of mites per plant.

Discussion

Plant herbivore resistance is the result of a coevolutionary process between herbivores and plants, thereby providing protection for plants against pests (Ramawat and Goyal 2020). Breeding of maize varieties with resistance to spider mites is the most economical and effective method to restrict damage and yield losses caused by this herbivore. Presently, few studies have fully addressed spider mite resistance in maize (Bui et al. 2021); in this study, we evaluated *T. urticae* resistance of JK968/XY335 and their parental lines. Our research reveals that *T. urticae* resistance is different

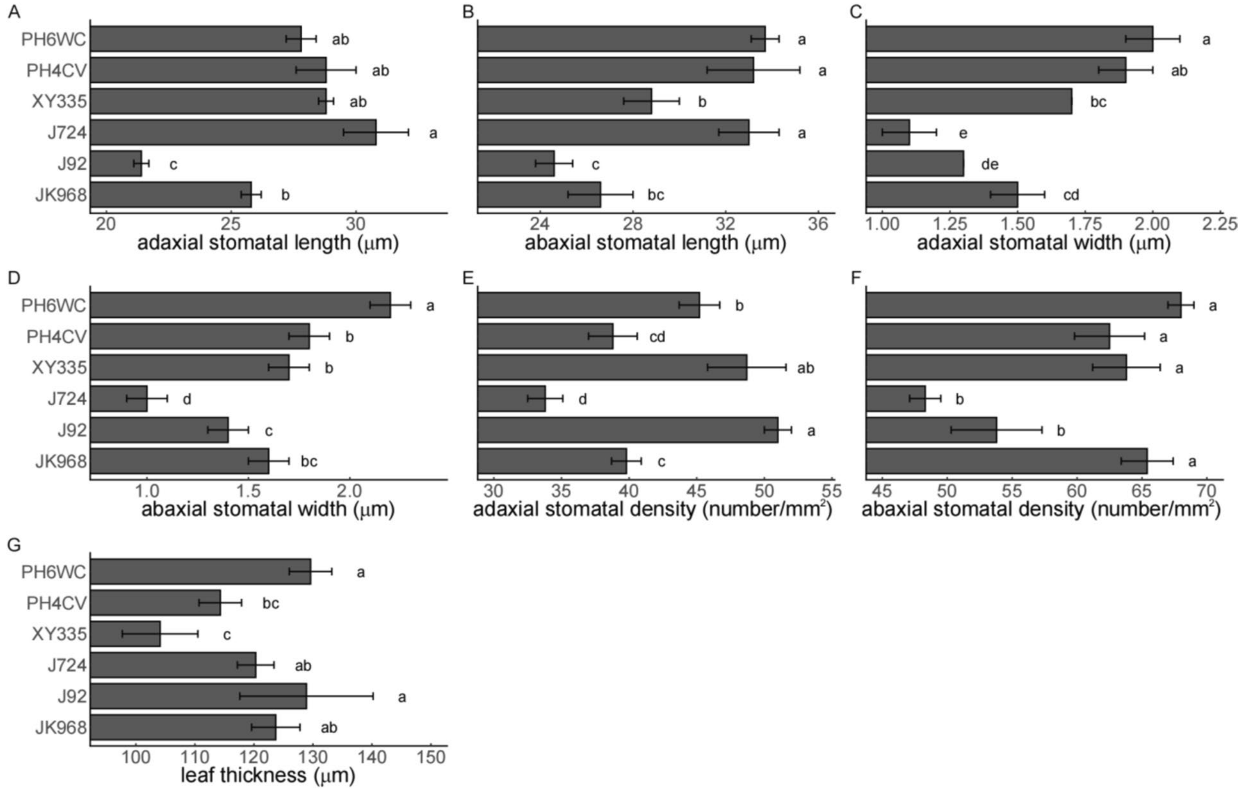

Fig. 2 Leaf structures of different maize lines. **A** Adaxial stomatal length (μm); **B** abaxial stomatal length (μm); **C** adaxial stomatal width (μm); **D** abaxial stomatal width (μm); **E** adaxial stomatal density (number/mm^2); **F** abaxial stomatal density (number/mm^2); **G** leaf thickness (μm). The lowercase letters above the bars indicate significant difference at $P < 0.05$

between two widely cultivated maize varieties in China, JK968 and XY335, and confirmed that JK968 is a variety with higher resistance to *T. urticae* infection. Our data also indicated that leaf physical characteristics play an important role in spider mite resistance in maize. These results provided a new avenue for the breeding of new maize varieties with high levels of spider mite resistance.

In this study, we documented that both JK968 and J92 showed significantly higher resistance to *T. urticae* than XY335 and its parental lines. Although no significant difference was observed in the population of female adults on JK968 and XY335, *T. urticae* fecundity on JK968 was substantially lower. Therefore, the density of *T. urticae* on XY335 was significantly higher than JK968 during the observation stages. These phenomena suggest that JK968 inhibits the population growth by reducing fecundity, which may be inherited from its parental line, J92. This is consistent with previous studies (Liu et al. 2019a, 2019b), in which the robust resistance to *Ostrinia furnacalis* (Guenee) of JK968 was believed to be derived from J92.

Additionally, previous research has demonstrated that resistance to *O. furnacalis*, *Mythimna separata* (Walker) and *Rhopalosiphum padi* (L.) of JK968 was higher than XY335 (Liu et al. 2019c). Similarly, in our assays, the number of eggs of *T. urticae* on JK968, J92 and J724 was significantly lower on day 2 compared to the first day, leading to a delay of peak female adult population size, indicating that viability of *T. urticae* on these maize lines is lower. Furthermore, the regression analysis indicated that JK968 and J92 were not suitable for population maintenance of *T. urticae* (Fig. 2).

Plants cannot move to avoid pest infestation, but they have established a number of defense mechanisms to resist pests during the process of coevolution (Züst and Agrawal 2016; Beran and Petschenka 2022). For example, levels of benzoxazinoids, a major group of defense-related secondary metabolites, are significantly higher in JK968 than in XY335 (Liu et al. 2019c). Long-term domestication of certain traits could have led to a decrease in genetic diversity of crops (Wang et al. 1999) and the loss of important genes (Gao et al. 2019). At present, herbivore resistance of hybrids is often derived unintentionally, resulting in uncertainty in pest resistance (Chen et al. 2015), and a previous study has shown that some American maize varieties have lost certain mechanisms of herbivore resistance, such as the release of volatiles to attract predators, which was present in some Chinese commercial maize (Gao et al. 2019).

Aside from secondary metabolites, physical resistance traits also play a great role in plant herbivore resistance

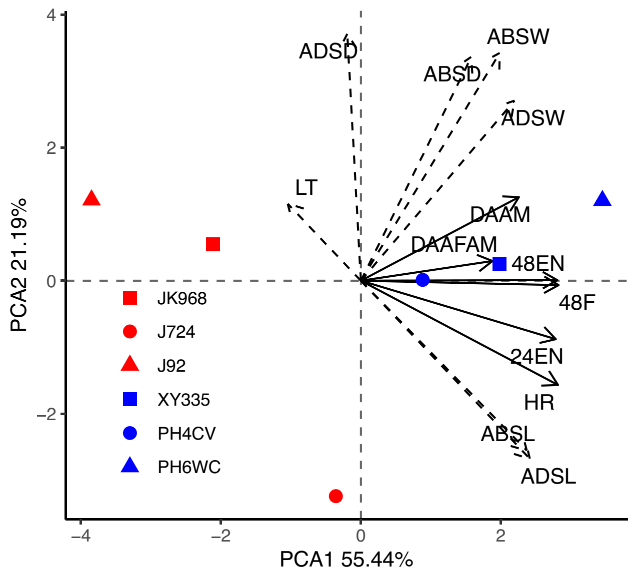

Fig. 3 Principal component analysis of mite resistance indexes (solid line) and leaf tissue structures indexes (dotted line) on different maize lines. *ADSL* adaxial stomatal length (μm), *ABSL* abaxial stomatal length (μm), *ADSW* adaxial stomatal width (μm), *ABSW* abaxial stomatal width (μm), *ADSD* adaxial stomatal density (number/mm^2), *ABSD* abaxial stomatal density (number/mm^2), *LT* leaf thickness (μm), *24EN* 24 h egg number, *48EN* 48 h egg number, *HR* hatching rate, *48F* 48 h fertility, *DAAFAM* daily average amount of female adult mite, *DAAM* daily average amount of mite

(Carmona et al. 2011). Since physical structures of leaves can all influence the feeding behavior of, and damage caused by, pests in many different ways, the leaf physical characteristics should also be considered as targets for breeding and/or engineering of different varieties with levels of pest resistance (Agrawal 1998; Glas et al. 2012). Stomata are one of the feeding channels of spider mites and higher stomatal density positively influences spider mite fitness (Skorupska 1998). In our study, stomatal size showed impact on *T. urticae* fitness, probably because the narrow stomata may prevent the mouthparts of *T. urticae* entering the leaf, but this needs to be further studied.

In this study, we found that parent with naturally high resistance to spider mites could be associated with the mite resistance performance of related hybrids in both laboratory and field conditions. Additionally, sensitive foreign parents led to compromised mite resistance of the hybrid XY335 (Fig. S1; Fig. 2), indicating that domestication could cause resistance loss to spider mites. Our work not only establishes the basis for studies on the mechanism of mite resistance, but also provides new insights into the control of spider mites and future crop breeding practices. Furthermore, domestication of crops and early spread have been shown to mediate the emergency and to influence the genetic variation of pest populations (Bernal et al. 2019), and the susceptibility of crop strains to pests from different geographical populations varies substantially (Gill et al. 2022; Mansour et al. 1993; Tadmor et al. 1999). Thus, whether mite resistance of less domesticated germplasms is generally better can be evaluated using more representative inbred lines from both local and exotic regions. Similarly, future studies on the resistance to spider mites from different geographical populations on JK968 and resistance inherited from native parents during the breeding process may also provide insights into future control efforts.

Author contributions

NDi, ZS, JZ, ND and RW designed the research. YFC, JRW and ZYZ conducted the experiments. NDi, JH, ZS, SW and RW contributed new analytical methods. YFC analyzed data. YFC, NDi, JH, ZS and WR wrote the manuscript. JZ provided materials and supervised the project. All authors read, revised and approved the manuscript.

Table 2 Field numbers of *Tetranychus urticae* on six maize lines

Time maize lines	JK968	J92	J724	XY335	PH4CV	PH6WC
1	34.7 ± 8.8b	6.5 ± 0.8c	118.5 ± 33.5ab	78.7 ± 23.5ab	116.5 ± 15.9a	138.1 ± 20.9a
2	21.5 ± 2.2c	8.9 ± 1.1d	116.5 ± 17.7a	76.5 ± 6.4ab	52.5 ± 5.3b	78.2 ± 3.2ab
3	14.9 ± 2.5b	7.2 ± 1.2b	89.0 ± 18.7a	74.0 ± 6.5a	43.5 ± 7.8a	57.9 ± 5.3a
4	2.0 ± 0.6a	1.3 ± 0.3a	2.7 ± 0.3a	2.7 ± 0.3a	2.0 ± 0.0a	3.0 ± 0.6a
5	10.5 ± 1.3b	2.8 ± 0.7c	75.5 ± 2.2a	76.3 ± 4.7a	66.4 ± 7.9a	88.8 ± 15.4a
6	129.3 ± 6.4c	13.2 ± 2.0d	233.8 ± 11.0b	347.0 ± 6.6b	130.8 ± 26.3c	586.0 ± 14.3a
7	319.3 ± 62.5c	36.3 ± 2.7d	883.3 ± 140.5b	1312.0 ± 155.8ab	1530.0 ± 137.5ab	3646.7 ± 1146.5a
8	1916.0 ± 280.8c	1060 ± 46.2d	3006.0 ± 87.8bc	4542.0 ± 577.4ab	3372.0 ± 324b	6596.0 ± 887.5a
9	3459.3 ± 149.5b	1554.0 ± 224.2c	9740.7 ± 650.2a	9386.9 ± 961.6a	9646.5 ± 386.2a	13,770.7 ± 641.94a
10	3160.3 ± 461.7ab	1516.3 ± 228.5b	5513.3 ± 826.3a	4610.8 ± 842.0a	4655.5 ± 1137.8a	5701.4 ± 1013.0a

Data were means ± SE. Same letters in a line means no significant difference between different maize lines

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10340-023-01716-3.

Acknowledgements We would like to thank Dr. Ningxing Huang for his help in providing spider mites to start the colony.

Funding The present study was funded by Sci-Tech Innovative Ability Project from Beijing Academy of Agriculture and Forestry Sciences (KJCX20200420; KJCX20230417), Beijing Innovation Team of the Modern Agricultural Research System (BAIC08-2023-YJ02), Beijing Nova Program (20220484220) and the High-level Returned Talents grant from Ministry of Human Resources and Social Security of the People's Republic of China.

Availability of data and materials All data are available in the manuscript, and materials are available upon requests.

Declarations

Conflict of interest The authors declare that they have no conflict of interest. ND serves as Editor-in-Chief of Journal of Pest Science and was not involved in the review process and decisions related to this manuscript.

Ethical approval Not applicable.

References

Agrawal AA (1998) Induced responses to herbivory and increased plant performance. Science 279(5354):1201–1202. https://doi.org/10.1126/science.279.5354.1201

Archer T, Peairs F, Mihm J (1997) Mechanisms and bases of resistance in maize to mites. In: Insect resistant maize: recent advances and utilization. Proceedings of an international symposium held at the International Maize and Wheat Improvement Center

Atkinson JA, Wells DM (2017) An updated protocol for high throughput plant tissue sectioning. Front Plant Sci 8:1721. https://doi.org/10.3389/fpls.2017.01721

Beran F, Petschenka G (2022) Sequestration of plant defense compounds by insects: from mechanisms to insect–plant coevolution. Annu Rev Entomol 67:163–180. https://doi.org/10.1146/annurev-ento-062821-062319

Bernal JS, Dávila-Flores AM, Medina RF, Chen YH, Harrison KE, Berrier KA (2019) Did maize domestication and early spread mediate the population genetics of corn leafhopper? Insect Sci 26(3):569–586. https://doi.org/10.1111/1744-7917.12555

Betz FS, Hammond BG, Fuchs RL (2000) Safety and advantages of *Bacillus thuringiensis*-protected plants to control insect pests. Regul Toxicol Pharmacol 32(2):156–173. https://doi.org/10.1006/rtph.2000.1426

Bui H, Greenhalgh R, Ruckert A, Gill GS, Lee S, Ramirez RA, Clark RM (2018) Generalist and specialist mite herbivores induce similar defense responses in maize and barley but differ in susceptibility to benzoxazinoids. Front Plant Sci 9:1222. https://doi.org/10.3389/fpls.2018.01222

Bui H, Greenhalgh R, Gill GS, Ji M, Kurlovs AH, Ronnow C, Lee S, Ramirez RA, Clark RM (2021) Maize inbred line B96 is the source of large-effect loci for resistance to generalist but not specialist spider mites. Front Plant Sci 12:693088. https://doi.org/10.3389/fpls.2021.693088

Calderon-Vazquez C, Ibarra-Laclette E, Caballero-Perez J, Herrera-Estrella L (2008) Transcript profiling of *Zea mays* roots reveals gene responses to phosphate deficiency at the plant-and species-specific levels. J Exp Bot 59(9):2479–2497. https://doi.org/10.1093/jxb/ern115

Carmona D, Lajeunesse MJ, Johnson MT (2011) Plant traits that predict resistance to herbivores. Funct Ecol 25(2):358–367. https://doi.org/10.1111/j.1365-2435.2010.01794.x

Chen YH, Gols R, Benrey B (2015) Crop domestication and its impact on naturally selected trophic interactions. Annu Rev Entomol 60:35–58. https://doi.org/10.1146/annurev-ento-010814-020601

Chen C, Liu X, Li S, Liu C, Zhang Y, Luo L, Miao L, Yang W, Xiao Z, Zhong Y, Li J, Chen R, Chen S (2022a) Co-expression of transcription factors ZmC1 and ZmR2 establishes an efficient and accurate haploid embryo identification system in maize. Plant J 111(5):1296–1307. https://doi.org/10.1111/tpj.15888

Chen Y, Wang S, Zhao J, Ren W, Shi Z, Di N, Jin D (2022b) Effects of leaf physical characters of maize hybrid Jingke 968 on the resistance to *Tetranychus urticae* Koch (Acari: Tetranychidae). J Environ Entomol 44(01):229–235. https://doi.org/10.3969/j.issn.1674-0858.2022.01.26

de Lillo E, Freitas-Astúa J, Kitajima E, Ramos-González P, Simoni S, Tassi A, Valenzano D (2021) Phytophagous mites transmitting plant viruses: update and perspectives. Entomol Gen 41:439–462. https://doi.org/10.1127/entomologia/2021/1283

Dermauw W, Wybouw N, Rombauts S, Menten B, Vontas J, Grbic M, Clark RM, Feyereisen R, Van Leeuwen T (2013) A link between host plant adaptation and pesticide resistance in the polyphagous spider mite *Tetranychus urticae*. Proc Natl Acad Sci USA 110(2):E113-122. https://doi.org/10.1073/pnas.1213214110

Desneux N, Decourtye A, Delpuech JM (2007) The sublethal effects of pesticides on beneficial arthropods. Annu Rev Entomol 52:81–106. https://doi.org/10.1146/annurev.ento.52.110405.091440

Dutton A, Obrist L, D'Alessandro M, Diener L, Muller M, Romeis J, Bigler F (2004) Tracking Bt-toxin in transgenic maize to assess the risks on non-target arthropods. IOBC WPRS Bull 27(3):57–63. https://eurekamag.com/research/004/370/004370290.php

Duvick DN (2005) The contribution of breeding to yield advances in maize (*Zea mays* L.). Adv Agron 86:83–145. https://doi.org/10.1016/S0065-2113(05)86002-X

Gao L, Gonda I, Sun H, Ma Q, Bao K, Tieman DM, Burzynski-Chang EA, Fish TL, Stromberg KA, Sacks GL, Thannhauser TW, Foolad MR, Diez MJ, Blanca J, Canizares J, Xu Y, van der Knaap E, Huang S, Klee HJ, Giovannoni JJ, Fei Z (2019) The tomato pan-genome uncovers new genes and a rare allele regulating fruit flavor. Nat Genet 51(6):1044–1051. https://doi.org/10.1038/s41588-019-0410-2

Gill GS, Bui H, Clark RM, Ramirez RA (2022) Spider mite resistant maize lines, B75 and B96, maintain resistance under water-stress. J Pest Sci 96(3):1117–1132. https://doi.org/10.1007/s10340-022-01584-3

Glas JJ, Schimmel BC, Alba JM, Escobar-Bravo R, Schuurink RC, Kant MR (2012) Plant glandular trichomes as targets for breeding or engineering of resistance to herbivores. Int J Mol Sci 13(12):17077–17103. https://doi.org/10.3390/ijms131217077

Guo F, Zhang Z-Q, Zhao Z (1998) Pesticide resistance of *Tetranychus cinnabarinus* (Acari: Tetranychidae) in China: a review. Syst Appl Acarol 3(1):3–7. https://doi.org/10.11158/saa.3.1.1

Guo Y-L, Jiao X-D, Xu J-J, Yang S, Duan X-K, Zhang J-P (2013) Growth and reproduction of *Tetranychus turkestani* and *Tetranychus truncatus* (Acari: Tetranychidae) on cotton and corn. Syst Appl Acarol 18(1):89–98. https://doi.org/10.11158/saa.18.1.10

Jactel H, Verheggen F, Thiéry D, Escobar-Gutiérrez AJ, Gachet E, Desneux N (2019) Alternatives to neonicotinoids. Environ Int 129:423–429. https://doi.org/10.1016/j.envint.2019.04.045

Jiao Y, Zhao H, Ren L, Song W, Zeng B, Guo J, Wang B, Liu Z, Chen J, Li W, Zhang M, Xie S, Lai J (2012) Genome-wide genetic changes during modern breeding of maize. Nat Genet 44(7):812–815. https://doi.org/10.1038/ng.2312

Kamali K, Dicke FF, Guthrie WD (1989) Resistance-Susceptibility of maize genotypes to artificial infestations by two spotted spider mites. Crop Sci 29:936. https://doi.org/10.2135/cropsci1989.0011183X002900040020x

Karjagi CG, Sekhar J, Lakshmi SP, Suby S, Kaur J, Mallikarjuna M, Kumar P (2017) Breeding for resistance to insect pests in maize. In: Arora R, Sandhu S (eds) Breeding insect resistant crops for sustainable agriculture. Springer, Berlin, pp 201–229. https://doi.org/10.1007/978-981-10-6056-4

Koch KG, Chapman K, Louis J, Heng-Moss T, Sarath G (2016) Plant tolerance: a unique approach to control hemipteran pests. Front Plant Sci 7:1363. https://doi.org/10.3389/fpls.2016.01363

Li Y, Romeis J (2010) Bt maize expressing Cry3Bb1 does not harm the spider mite, *Tetranychus urticae,* or its ladybird beetle predator, *Stethorus punctillum*. Biol Control 53(3):337–344. https://doi.org/10.1016/j.biocontrol.2009.12.003

Liu S, Guo J, He K, Wang Q, Wang Z (2019a) Evaluation of the resistance of corn hybrid "Jingke968" and its parents to the Asian corn borer by using life table technique. Plant Prot 45(01):93–97. https://doi.org/10.16688/j.zwbh.2018086

Liu S, Guo J, He K, Wang Q, Wang Z (2019b) Resistance evaluation of corn hybrid Jingke 968 silks to *Ostrinia furnacalis* and related resistance mechanism. J Environ Entomol 41(01):25–32. https://doi.org/10.3969/j.issn.1674-0858.2019.01.4

Liu S, Shen G, Li J, Zhang C, Saif-Ul-Mallook, Wu J, Wang L, Song W, Wang Y, Zhao J, Tang G, Qi J (2019c) Comparation of herbivore resistance among the maize lines Xianyu335, Zhengdan958, and Jingke968. J Maize Sci 27(06):52–57. https://doi.org/10.13597/j.cnki.maize.science.20190609

Loomans AJ (2021) Every generalist biological control agent requires a special risk assessment. Biocontrol 66(1):23–35. https://doi.org/10.1007/s10526-020-10022-1

Mansour F, Bar-Zur A, Abo-Moch F (1993) Resistance of maize inbred lines to the carmine spider mite, *Tetranychus cinnabarinus* (ACARI: Tetranychidae): evaluation of antibiosis of selected lines at different growth stages. Maydica 38:309–311. https://doi.org/10.2135/cropsci1989.0011183X002900040020x

Nieberding CM, Kaisin A, Visser B (2022) Inbreeding and learning affect fitness and colonization of new host plants, a behavioral innovation in the spider mite *Tetranychus urticae*. Entomol Gen 42:531–538. https://doi.org/10.1101/2021.06.29.450353

Nihoul P (1993) Controlling glasshouse climate influences the interaction between tomato glandular trichome, spider mite and predatory mite. Crop Prot 12(6):443–447. https://doi.org/10.1016/0261-2194(93)90005-4

Parolin P, Bresch C, Ruiz G, Desneux N, Poncet C (2013) Testing banker plants for biological control of mites on roses. Phytoparasitica 41:249–262. https://doi.org/10.1007/s12600-012-0285-6

Peterson RKD, Varella AC, Higley LG (2017) Tolerance: the forgotten child of plant resistance. PeerJ 5:e3934. https://doi.org/10.7717/peerj.3934

Ramawat KG, Goyal S (2020) Co-evolution of secondary metabolites during biological competition for survival and advantage: An overview. In: Mérillon J-M, Ramawat KG (eds) Co-evolution of secondary metabolites. Springer, Berlin, pp 3–17. https://doi.org/10.1007/978-3-319-96397-6_45

Ramirez-Romero R, Desneux N, Chaufaux J, Kaiser L (2008) Bt-maize effects on biological parameters of the non-target aphid *Sitobion avenae* (Homoptera: Aphididae) and Cry1Ab toxin detection. Pestic Biochem Physiol 91:110–115. https://doi.org/10.1016/j.pestbp.2008.01.010

Skorupska A (1998) Morphologico-anatomical structure of leaves and demographic parameters of the hawthorn spider mite, *Tetranychus viennensis* Zacher and the two-spotted spider mite, *Tetranychus urticae* (Koch) (Acarina, Tetranychidae) on selected scab-resistant apple varieties. J Appl Entomol 122(1–5):493–496. https://doi.org/10.1111/j.1439-0418.1998.tb01533.x

Stavrinides MC, Skirvin DJ (2003) The effect of chrysanthemum leaf trichome density and prey spatial distribution on predation of *Tetranychus urticae* (Acari: Tetranychidae) by *Phytoseiulus persimilis* (Acari: Phytoseiidae). Bull Entomol Res 93(4):343–350. https://doi.org/10.1079/BER2003243

Sulistyo A, Inayati A (2016) Mechanisms of antixenosis, antibiosis, and tolerance of fourteen soybean genotypes in response to whiteflies (*Bemisia tabaci*). Biodivers J Biol Divers 17(2):447–453. https://doi.org/10.13057/biodiv/d170207

Tadmor Y, Lewinsohn E, Abo-Moch F, Bar-Zur A, Mansour F (1999) Antibiosis of maize inbred lines to the carmine spider mite, *Tetranychus cinnabarinus*. Phytoparasitica 27:35–41. https://doi.org/10.1007/BF02980725

Verheggen F, Barrès B, Bonafos R, Desneux N, Escobar-Gutiérrez AJ, Gachet E, Laville J, Siegwart M, Thiéry D, Jactel H (2022) Producing sugar beets without neonicotinoids: an evaluation of alternatives for the management of viruses-transmitting aphids. Entomol Gen 42(4):491–498. https://doi.org/10.1127/entomologia/2022/1511

Wang RL, Stec A, Hey J, Lukens L, Doebley J (1999) The limits of selection during maize domestication. Nature 398(6724):236–239. https://doi.org/10.1038/18435

Wu K-M, Lu Y-H, Feng H-Q, Jiang Y-Y, Zhao J-Z (2008) Suppression of cotton bollworm in multiple crops in China in areas with Bt toxin–containing cotton. Science 321(5896):1676–1678. https://doi.org/10.1126/science.1160550

Xu D, He Y, Zhang Y, Xie W, Wu Q, Wang S (2018) Status of pesticide resistance and associated mutations in the two-spotted spider mite, *Tetranychus urticae*, in China. Pestic Biochem Physiol 150:89–96. https://doi.org/10.1016/j.pestbp.2018.07.008

Xu D, Liao H, Li L, Wu M, Xie W, Wu Q, Zhang Y, Zhou X, Wang S (2023) The CYP392D8 gene is not directly associated with abamectin resistance, a case study in two highly resistant *Tetranychus urticae* strains. Entomol Gen 43(3):679–687. https://doi.org/10.1127/entomologia/2022/1646

Yin WD, Hoffmann AA, Bai CM, Ma CS (2022) A conservative oviposition preference in spider mites for complex habitats as a preventive strategy for reducing predation risk. Entomol Gen 42(3):389–401. https://doi.org/10.1127/entomologia/2021/1282

Zhu L, Chen L, Min Y, Liu F, Liao X, Rao B, Qiu Y, Chen W, Wang K, Yang Z (2022) Acaricidal activity and field efficacy analysis of the potential biocontrol agent *Bacillus vallismortis* NBIF-001 against spider mites. Microorganisms 10(9):1750. https://doi.org/10.3390/microorganisms10091750

Züst T, Agrawal AA (2016) Mechanisms and evolution of plant resistance to aphids. Nat Plants 2(1):1–9. https://doi.org/10.1038/nplants.2015.206