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Short-term responses of spider mites inform mechanisms of maize resistance to a generalist herbivore

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Plants are attacked by diverse herbivorous pests with different host specializations. While host plant resistance influences pest pressure, how resistance impacts the behaviors of generalist and specialist herbivores, and the relationship to resistance, is less well known. Here, we investigated the short-term (<1 h) behavioral changes of a generalist herbivore, the two-spotted spider mite (TSM), and a specialist herbivore, the Banks grass mite (BGM), after introduction to no-choice Tanglefoot leaf-arenas (2 × 2 cm) of three maize inbred lines (B73, B75, and B96). The widely-used inbred line B73 is susceptible to spider mites, while B75 and B96 are known to be mite resistant, especially to TSM. Video tracking was used to record TSM and BGM walking, probing, feeding, resting, web-building and travel distance on arenas of each line. Mite oviposition was also recorded after 72 h. B75, a resistant line, decreased the feeding behavior (i.e., time) of both mite species compared to B73 (susceptible control) and B96. Moreover, TSM appeared to be sensitive to both resistant lines (B75 and B96) with reduced oviposition, and increased resting and web-building times compared to susceptible B73. In contrast, the specialist BGM showed no difference in oviposition, resting and web-building time across all maize inbred lines. Our findings of quite broad and short-term responses of TSM to B75 and B96 are consistent with a role for constitutive or rapidly induced plant defenses in maize in conferring TSM resistance. Other mechanisms of plant resistance may be needed, however, for defense against specialists like BGM.

Keywords *Tetranychus urticae*, *Oligonychus pratensis*, Host plant resistance, Oviposition, Web-building

Host plant resistance through genetic modification or traditional plant breeding is an ecologically-based pest management tactic, alleviating concerns associated with reliance on pesticide use and pesticide resistance^{1,2}. The development of a pest resistant plant is a multifaceted evaluation that includes phenotyping multiple plant lines by high-throughput screening¹. Once candidate resistant lines are identified, pest behaviors and development can be analyzed on plant tissues through a variety of methods to understand the resistance mechanisms³. Some of the studies that evaluated resistant plants in cropping systems such as maize, rice, cowpea and soybean showed that plant resistance traits reduced pest behaviors such as feeding^{4–9} and oviposition^{10–12}, and increased walking as well as resting behaviors^{5,13–17}. However, plants encounter diverse herbivorous pests with different levels of host specialization (i.e., generalists, that feed on many plant species and specialists that feed on a single plant family or species), and this specialization may alter the interaction with developed resistance traits¹⁸.

Historically, evaluation has often focused on either generalist or specialist herbivores, most of which have been insects, and comparatively few studies have assessed plant resistance to phylogenetically related herbivores that vary in host plant specialization¹⁸. Nevertheless, among mite herbivores, a recent study using maize (*Zea mays*) revealed that benzoxazinoids (e.g., DIMBOA), a class of plant defense compounds widely distributed in the grass family (Poaceae)¹⁹, reduced the population growth of the generalist two-spotted spider mite (*Tetranychus urticae*, TSM), while the specialist Banks grass mite (*Oligonychus pratensis*, BGM) was unaffected²⁰. However, the population growth of spider mites depends on host plant acceptance (e.g., the proportion of females settling on the host) and host plant suitability (e.g., oviposition on the host plant)²¹. Host plant acceptance also relates to non-preference or antixenosis (avoidance, a behavioral response of arthropods), while host plant suitability can

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be determined by antibiosis (adverse effects of a host plant on arthropod growth, development and fecundity). Resistance mechanisms can be quantified by behavioral parameters such as movement (walking and resting), feeding on a host plant, and oviposition, which have all previously been reported to be impacted by host plant resistance^{4–17,22}. As opposed to major insect herbivores of maize, spider mites also engage in web-building or spinning that facilitates oviposition, mate finding, locomotion, dispersal, and colonization, and can protect against natural enemies and acaricides^{23–25}. The extent of variation in web-building traits among spider mite herbivores, and hence the relationship to resistance and host specialization, remains incompletely understood.

TSM and BGM are well-documented maize herbivores that can cause severe crop damage and economic losses^{26,27}. Evaluation of plant resistance to spider mites is of utmost importance for their sustainable management, and of special urgency as both TSM and BGM have become resistant to acaricides in many chemical classes^{28,29}. Recently, in a greenhouse study we screened a large number of inbred maize lines for resistance (antibiosis) to TSM and BGM³⁰. While we found that most maize lines were susceptible to BGM and TSM, a small number of lines were resistant (lines B49 and B75) or highly resistant (B96) to TSM. For B96, our findings confirmed several earlier reports that this line is highly resistant to generalist spider mites, including TSM and the closely related generalist carmine spider mite, *Tetranychus cinnabarinus*^{11,12}, and in subsequent work we showed that B75 and B96 are resistant to TSM in the field as well³¹. Although B75 and B96 are highly resistant to TSM, they were not observed to be markedly resistant to BGM^{30,31}. Line B73, on the other hand, is highly susceptible to TSM, as it is to BGM^{20,31}. B73 is an agriculturally important maize inbred line that has contributed disproportionately to the development of many elite maize varieties³². Interestingly, both B75 and B96 maize were previously reported to have higher DIMBOA levels than B73^{33,34}, a finding that may also explain why B75 and B96 are highly resistant to the European corn borer (*Ostrinia nubilalis*)^{11,35}. While population growth of TSM and BGM has been evaluated on the B73, B75 and B96 maize inbred lines, little is known about how B75 and B96 resistance to major arthropod pests impacts the behaviors of the generalist TSM and the specialist BGM.

Economically damaging TSM and BGM outbreaks on maize in field settings have typically been associated with drought stress, and recently the resistance of B75 and B96 to TSM was demonstrated to be persistent even under drought stress conditions³¹, highlighting the potential importance of the arthropod resistance mechanisms of B75 and B96 for durable pest suppression. Our previous work evaluated longer-term interactions (one to many days) between these two mite species and each of the three maize lines investigated in this study^{30,31,36}. Strikingly, in response to *T. urticae*, in earlier work we found dramatic induced expression of many maize defense genes in the resistant lines B75 and B96 at 24 h post-infestation, a potential mechanism of defense³⁶. To further understand the resistance mechanisms of B75 and B96, in the current study we investigated behavioral changes that have been associated with arthropod resistance for the generalist TSM and specialist BGM on detached leaf arenas of B73, B75 and B96 using video tracking within the first hour post-infestation (a time point at which only constitutive plant defenses, or rapidly induced ones, would be expected to impact variation in plant resistance). Our study allowed us to evaluate a variety of mite behaviors including probing, walking, and web-building for each mite species exposed to dramatic differences in levels of maize resistance to arthropod herbivores.

Material and methods

Maize lines and plant maintenance

Plants were grown at Utah State University's Research Greenhouse and Laboratory, Logan, UT. Three maize inbred lines were selected for the study (B73, B75, and B96). B73 is susceptible to both BGM and TSM^{20,31}, while B75 and B96 have moderate- and high-level resistance to TSM, respectively^{30,31}. The maize inbred lines used in this study were publicly available to the study participants via the U.S. National Plant Germplasm System with no restrictions on their use for the work performed in this study; their use in this study complies with relevant institutional, national, and international guidelines and legislation.

Two seeds per pot for each maize inbred line (B73, B75, and B96) were sown in 3.5 L pots filled with soil (Sunshine Mix #3, Sun Gro Horticulture, MA), 8 pots per line, distributed in a complete randomized design. Maize plants were grown under greenhouse-controlled conditions (25 ± 2 °C, 60 ± 5% RH, 16:8 h. (L:D) photoperiod) and fertigated at a rate of 4.8 kg/100L of 21N-5P-20 K Peters Excel Water Soluble Fertilizer mixture (ICL Specialty Fertilizers, SC, USA) by using drip tape (DIG Corporation, CA, USA; 12.7 mm and 6.35 mm diameter tubing with 3.8 L/hr compensating emitters). At 8 weeks of age, plants were used to evaluate spider mite behavior.

Video tracking spider mite behavior on maize lines

We conducted a 3 × 2 factorial design experiment using three levels of maize resistance (susceptible B73, and resistant lines B75 and B96) and two levels of mites (TSM and BGM).

A rectangular plastic box (20 × 15 cm, Webstaurant Store, PA, USA) was used as an experimental unit and each treatment (3 maize inbred lines × 2 mite species) was replicated six times. A 3 × 3 cm leaf-cutting from the middle section of the 8th leaf from each plant was collected, excluding the leaf midrib. We placed leaf-cuttings abaxial side up for each respective inbred line on a wet cotton pad on a plexiglass sheet fitted within the rectangular plastic box to prevent the leaf arena from desiccating. To ensure the cotton remained moist, boxes were filled halfway so the ends of the cotton pads were in contact with the water (the ends draped over the plexiglass sheet into the water below to allow wicking). A 2 × 2 cm no-choice arena was created by placing Tanglefoot (The Scotts Miracle-Gro Company, OH, USA) non-phytotoxic wax barriers on the edges of each leaf-cutting to keep mites on the feeding site and prevent escape. BGM and TSM colonies used in the study were maintained in lab conditions [28 ± 2 °C, 50 ± 5% RH, 16:8 h (L:D) photoperiod] on B73 maize. These colonies have been described previously^{30,31}, while the colonies were maintained long-term on B73 maize, their reproductive rate on B73 (susceptible) was found to be similar to that of nearly 35 other inbred maize lines excepting B75, B96, and B49 (which

are resistant to *T. urticae*)³⁰. One newly emerged adult female mite, mated and starved overnight, was introduced into the arena of each respective maize inbred line by using a fine paintbrush immediately after the leaf was cut.

Using a Canon Eos 5D Mark III camera and 65 mm MP-E lens, each female mite was recorded by video for 50 min following mite introduction to each respective arena. Each video was examined for six behaviors that included the total time that each mite spent (1) walking, (2) probing, (3) feeding, (4) resting, (5) web-building, and (6) traveling (total distance traveled in cm) for each mite in an arena using a behavior tracking software (OpenCV mite tracer, <https://github.com/HMKRL/OpenCV-mitetracer>). Briefly, videos were uploaded to the software, a tracer was placed on each spider mite, and the software tracked movement in the video and generated a path plot (see Appendix A: Fig. S1). The distance traveled by spider mites in arenas, and the times the mites moved and stopped were recorded. Manual visual inspection of each video was used to further assign times for specific behaviors (i.e., probing, feeding, resting, and web-building). Resting time was assessed as the time that mites were not moving, probing or feeding. Probing was apparent when a mite stopped its movement, short feeding events occurred in place, and forelegs showed a variety of small tactile movements. Web-building was assessed when mites swaying their forelegs in a side-to-side motion and observation of threads of silk fibers left on the leaf surface²⁵. Note that there is variation in the types of web structures produced by spider mites from silk threads and balls to more extensive silk production similar to a nest with eggs and spider mites³⁷. Here, the midrib of the leaf, where spider mites are primarily found with extensive web structures on maize plants, was not a part of the arena owing to requirements for imaging (a flat leaf surface) and therefore limited the ability to evaluate extensive web-building.

Finally, oviposition (number of eggs deposited) was recorded for each replicate 72 h post mite introduction using a stereomicroscope (Leica S6 D Greenough, NJ, USA).

Statistical analysis

Data from mite oviposition on maize inbred lines were analyzed using a generalized linear model (Proc Glimmix; SAS 9.4 M4 University edition) within two-way ANOVA that included maize resistance (B73, B75, and B96) and mites (TSM and BGM) as fixed factors. Oviposition data were log transformed to conform to the assumptions of normality and heteroscedasticity. Video recordings (50 min each) were analyzed in 10 min intervals (0 to 10 min, 10 to 20 min, etc.). Proportions of time that each mite spent walking, probing, feeding, and resting within the 10 min intervals were analyzed using a generalized linear model (Proc Glimmix; SAS 9.4 M4 University edition) within two-way ANOVA and repeated measures (5 time intervals) with a beta distribution³⁸. Data for web-building and travel distance were square-root transformed and analyzed using two-way ANOVA (maize inbred lines) with repeated measures (5 time intervals) using Proc Glimmix (SAS 9.4 M4 University edition). Where ANOVAs were significant, post hoc tests were performed using Tukey's HSD (Honestly Significant Difference) to separate significant differences among main effects of maize inbred lines or time. For further analysis of significant three-way interactions, we used the LSMESTIMATE statement (Proc Glimmix) with Tukey–Kramer adjustment. For instance, when mite walking time revealed a three-way interaction (line × mite × time), each mite species was independently analyzed at each time period comparing maize inbred lines.

Results

Oviposition by TSM and BGM on susceptible and resistant inbred maize lines

Mite oviposition on detached leaf enclosures 72 h post mite introduction was significantly affected by a maize resistance × mite interaction ($F_{2,27} = 4.81$, $P = 0.01$, Fig. 1, Table 1). To further assess the interaction, we analyzed oviposition for each mite species independently by comparing maize inbred lines. The interaction appeared to be driven by a reduction in oviposition by TSM on B75 ($P < 0.01$, LSMESTIMATE Tukey adjustment) and B96 ($P < 0.01$, LSMESTIMATE Tukey adjustment) representing 0.8 ± 0.58 eggs/female (83% decrease) and 1.2 ± 0.58 eggs/female (74% decrease), respectively, compared to 4.66 ± 0.52 eggs/female on the control (previously reported TSM-susceptible) B73 line (Fig. 1). In contrast, BGM oviposition was not significantly different among susceptible and resistant maize lines (Fig. 1).

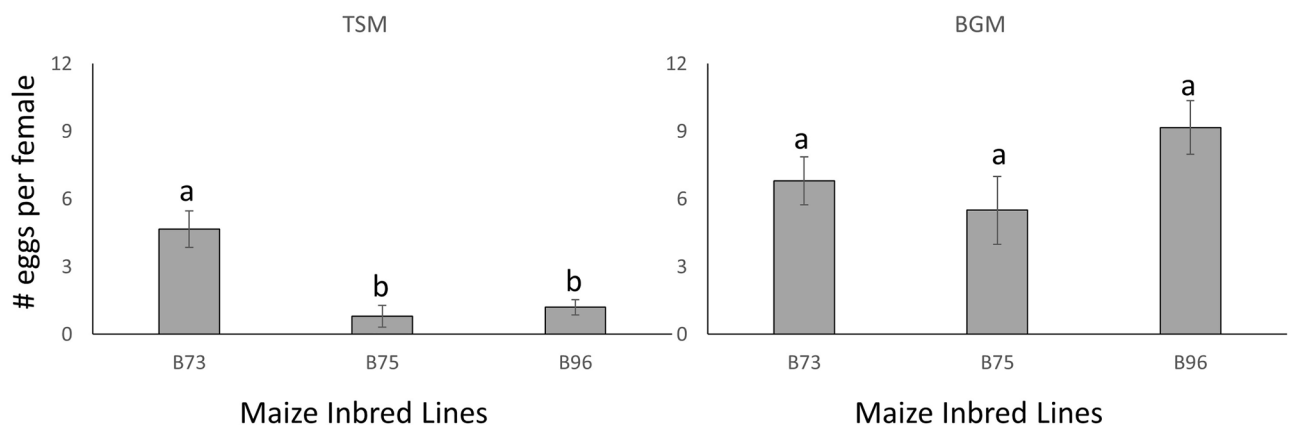


Fig. 1. Mean (\pm SE) oviposition (number of eggs per female) by TSM and BGM on susceptible B73 and resistant B75 and B96 maize inbred lines at 72 h post-mite introduction.

Response	Effect	Num DF	Den DF	F Value	P
Oviposition	Resistance	2	27	4.16	0.026
	Mite	1	27	41.05	<0.01
	Resistance*Mite	2	27	4.81	0.016
Probing time	Resistance	2	26	0.33	0.721
	Mite	1	26	7.88	0.009
	Mite*Resistance	2	26	2.38	0.112
	Time	4	104	2.65	0.037
	Time*Resistance	8	104	1.01	0.435
	Time*Mite	4	104	0.29	0.882
	Time*Mite*Resistance	8	104	0.46	0.884
Feeding time	Resistance	2	26	8.73	<0.01
	Mite	1	26	2.44	0.130
	Mite*Resistance	2	26	2.50	0.101
	Time	4	104	1.69	0.159
	Time*Resistance	8	104	1.33	0.235
	Time*Mite	4	104	1.52	0.201
	Time*Mite*Resistance	8	104	0.91	0.508
Walking time	Resistance	2	26	11.60	<0.01
	Mite	1	26	0.68	0.415
	Mite*Resistance	2	26	3.41	0.048
	Time	4	102	0.89	0.472
	Time*Resistance	8	102	0.49	0.858
	Time*Mite	4	102	1.46	0.221
	Time*Mite*Resistance	8	102	2.19	0.033
Resting time	Resistance	2	26	5.22	0.012
	Mite	1	26	0.44	0.511
	Mite*Resistance	2	26	4.91	0.015
	Time	4	104	1.82	0.129
	Time*Resistance	8	104	1.22	0.296
	Time*Mite	4	104	2.59	0.041
	Time*Mite*Resistance	8	104	0.38	0.932
Web-building time	Resistance	2	26	0.33	0.721
	Mite	1	26	7.88	0.009
	Mite*Resistance	2	26	2.38	0.112
	Time	4	104	2.65	0.037
	Time*Resistance	8	104	1.01	0.435
	Time*Mite	4	104	0.29	0.882
	Time*Mite*Resistance	8	104	0.46	0.884
Travel distance	Resistance	2	26	7.36	0.002
	Mite	1	26	0.02	0.901
	Mite*Resistance	2	26	1.14	0.335
	Time	4	104	2.46	0.050
	Time*Resistance	8	104	1.65	0.119
	Time*Mite	4	104	0.37	0.828
	Time*Mite*Resistance	8	104	1.16	0.332

Table 1. ANOVA results of spider mite behavior (oviposition, probing, feeding, walking, resting, web-building, and travel distance) on susceptible and resistant inbred maize lines. DF: degrees of freedom; Num: numerator; Den: denominator; F: *F* value; P: *p*-value.

Mite probing and feeding time on susceptible and resistant maize lines

Overall, TSM spent more time (6.78 ± 0.87 mins) probing leaves than BGM (3.53 ± 0.59 mins) on all maize inbred lines (mite: $F_{1,26} = 7.88$, $P < 0.01$, Fig. 2, Table 1). For both TSM and BGM, mites probed leaves more in the first 10 min (1.76 ± 0.35 min), then probing was reduced and stayed constant (from 0.84 ± 0.19 min to 0.85 ± 0.22 min) for the remaining time (time: $F_{4,104} = 2.65$, $P = 0.03$, Fig. 2, Table 1).

Mite feeding time was dependent on the maize inbred line (Resistance: $F_{2,26} = 8.73$, $P < 0.01$, Table 1). Specifically, mite feeding time across species over 50 min was reduced on B75 (16 ± 2.22 min) as compared to

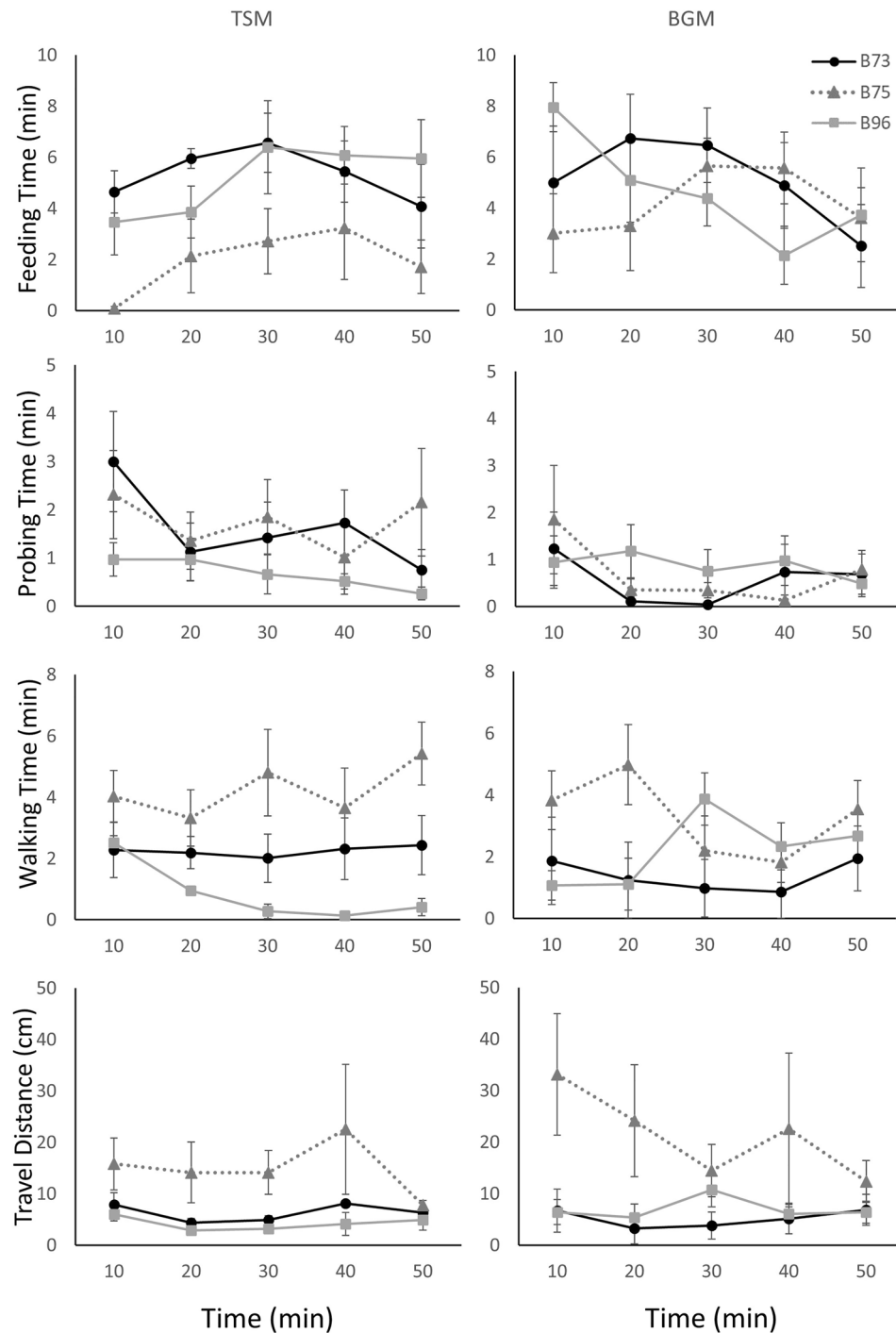


Fig. 2. Mean (\pm SE) time that TSM and BGM spent probing, feeding, and walking and their mean (\pm SE) travel distance in each 10-min interval on susceptible B73 (—●—) and resistant B75 (··▲··) and B96 (—■—) maize inbred lines.

26.18 \pm 2.12 min on B73 ($P < 0.01$, Tukey) and 24.52 \pm 2.34 min on B96 ($P < 0.01$, Tukey), respectively (Fig. 2). Surprisingly, no differences were found in mite feeding time on the control B73 and resistant B96 lines ($P = 0.76$, Tukey).

Mite walking time and travel distance on susceptible and resistant inbred maize lines

Mite walking time was significantly affected by a maize resistance \times mite \times time interaction, which appeared to be driven by no change in BGM walking time but increased TSM walking time due to maize resistance over the 50 min observation period ($F_{8,104} = 2.40$, $P = 0.02$, Fig. 2, Table 1). This was confirmed by evaluating each mite

species (TSM and BGM) independently, comparing maize inbred lines across time. At 30- and 50-min intervals ($P=0.02$ and $P=0.03$, respectively, LSMESTIMATE Tukey adjustment), TSM walking time was greater on the resistant line B75 (4.8 ± 1.85 min and 5.43 ± 2.15 min) compared to the resistant B96 line (0.27 ± 0.66 min and 0.41 ± 0.26 min) (Fig. 2). In contrast, BGM walking time appeared to have no significant change on maize inbred lines throughout the experiment ($P>0.05$, LSMESTIMATE Tukey adjustment) (Fig. 2).

Travel distance for each mite was significantly affected by maize resistance ($F_{2,26}=7.36$, $P<0.01$, Table 1) and time ($F_{4,104}=20.08$, $P<0.01$, Table 1). Specifically, the travel distance for each mite was greater on the resistant B75 line (92.01 ± 12.66 cm) compared to 28.9 ± 3.35 cm for the control B73 ($P<0.01$, Tukey) and 27.95 ± 3.26 cm for the resistant B96 lines ($P=0.01$, Tukey), respectively (Fig. 2). Further, travel distance by each mite was greater for the first 20 min, then was reduced and stayed constant for the remainder of the study (Fig. 2).

Mite resting time on susceptible and resistant inbred maize lines

Resting time (i.e., time associated with arrested mite movement when not probing or feeding) was significantly affected by resistance \times mite ($F_{2,26}=4.91$, $P<0.01$) and mite \times time ($F_{4,104}=2.59$, $P=0.04$) interactions (Fig. 3, Table 1). To further assess the resistance \times mite interaction, we analyzed mite resting time for each mite species independently by comparing maize inbred lines. The resistance \times mite interaction revealed that the resting time increased for TSM on leaves of resistant lines B96 (16.56 ± 2.71 mins) ($P<0.01$, LSMESTIMATE Tukey adjustment) and B75 (10.15 ± 2 mins) ($P<0.01$, LSMESTIMATE Tukey adjustment), compared to the control B73 (4.04 ± 1.02 mins). As recognized with other behavior traits, no such effect was apparent for BGM (Fig. 3, Table 1). The mite \times time interaction was also further analyzed for each mite species by comparing each time period. BGM's resting time increased from 1.09 ± 0.56 mins at the initial 10 min interval to 3.25 ± 0.67 min in the final 50 min interval ($P=0.02$, LSMESTIMATE Tukey adjustment). Alternatively, the resting time for TSM stayed constant at an average of 1.97 ± 0.27 mins throughout the experiment ($P=0.99$, LSMESTIMATE Tukey adjustment).

Mite web-building time on susceptible and resistant inbred maize lines

Web-building time was significantly affected by a resistance \times mite interaction ($F_{2,26}=5.95$, $P<0.01$, Table 1). To further assess the interaction, we analyzed mite web-building time for each mite species independently by comparing maize inbred lines. Here, the time spent web-building for TSM was higher on resistant lines B75

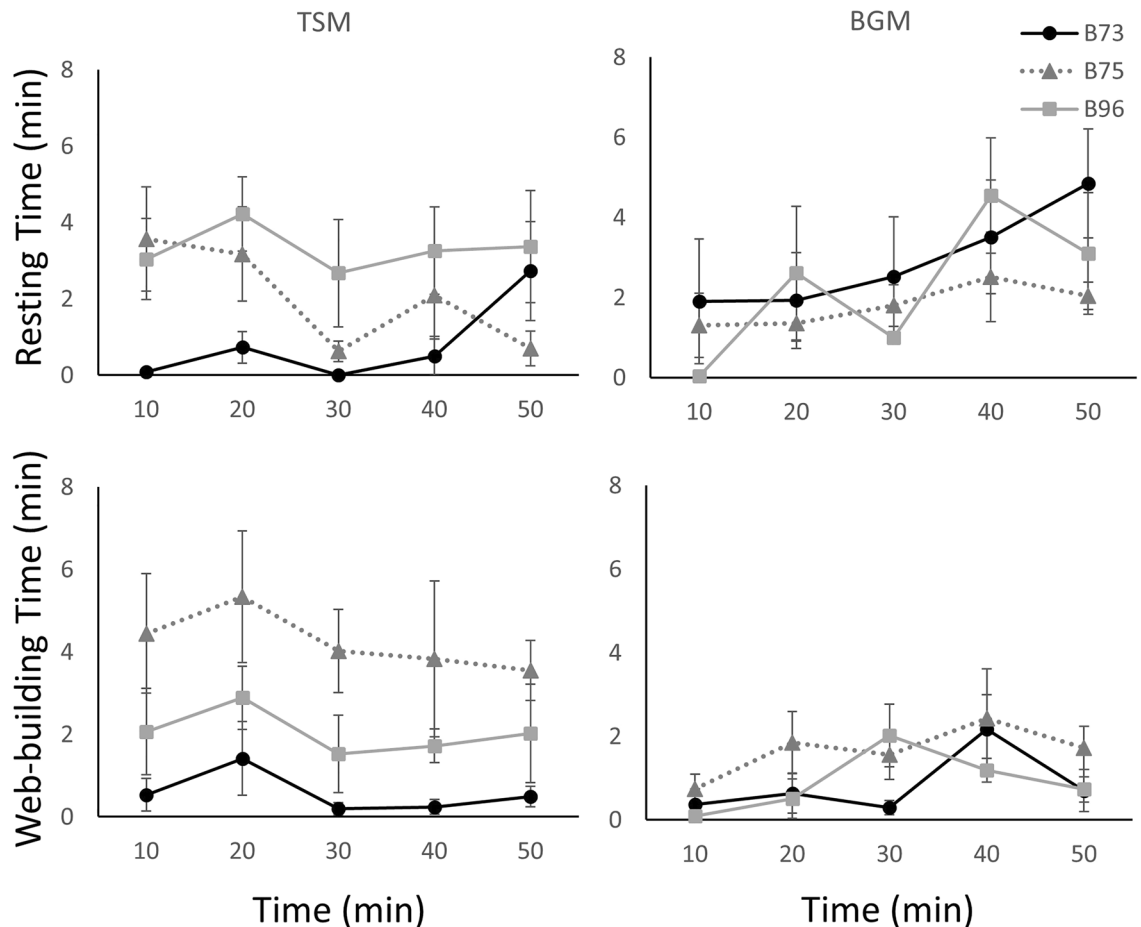


Fig. 3. Mean (\pm SE) time that TSM and BGM spent resting and web-building in each 10-min interval on susceptible B73 (\bullet) and resistant B75 (\blacktriangle) and B96 (\blacksquare) maize inbred lines.

(21.19 ± 4.23 min) ($P < 0.01$, LSMESTIMATE Tukey adjustment) and B96 (10.22 ± 2.04 min) ($P < 0.01$, LSMESTIMATE Tukey adjustment), compared to the control B73 (2.86 ± 0.52 mins). Despite the observed differences for TSM, no significant differences were observed for BGM's time spent web-building across all maize inbred lines (Fig. 3).

Discussion

In previous studies using *in planta* leaf enclosures in greenhouse and field settings, we demonstrated that B75 and B96 are highly resistant to TSM as compared to B73; in contrast, no significant differences for BGM were observed^{30,31}. Critically, while our current study required the use of leaf cuttings—i.e., detached leaf arenas immobilized for imaging—we found that resistance among (1) maize lines and (2) between mite species was identical to what was observed *in planta*. Therefore, we were able to assess phenotypes in an assay amenable to behavioral study where variation in maize resistance nonetheless recapitulated that observed on whole plants in agricultural settings.

We found that multiple behaviors related to mite feeding varied by maize line and mite species. Although exposure to resistant B75 resulted in behavioral changes of increased travel distance and reduced feeding common to both mite species, on B75 and B96 TSM-specific behavioral changes including increased resting and web-building time were observed as compared to these same behaviors on susceptible B73 (Table 1). Consistent with the lack of reduced egg-laying by BGM on TSM-resistant maize lines (B75 and B96) observed in this and earlier studies^{30,31}, our findings suggest that the specialist BGM is well adapted to maize resistance strategies as fewer behaviors were significantly affected by maize genotype (further, the mean numbers of eggs deposited by BGM on B73, B75 and B96 were greater than for TSM on any maize genotype; Fig. 2).

Probing and feeding by herbivores are two important behaviors that associate with host plant resistance to herbivores. Specifically, increased frequency of probing and decreased feeding by cowpea aphid (*Aphis craccivora* Koch), soybean aphid (*Aphis glycines* Matsumura), and a leafhopper (*Cicadulina storeyi* Naudé) were reportedly due to resistance properties of cowpea⁵, soybean⁹ and maize¹⁵, respectively. While probing by both BGM and TSM was not impacted by maize resistance, feeding time was reduced on resistant B75 as compared to susceptible B73, especially for TSM over the 50 min observation window. Surprisingly, feeding time for neither mite species was reduced on resistant B96 as compared to susceptible B73. For insect herbivores, increased duration of resting has also been shown to be proportional to host plant resistance^{5,15,16}. For instance, on maize the leafhopper *Cicadulina storeyi* exhibited greater resting activities on resistant as compared to susceptible varieties¹⁵. In our study, relative to B73 increased resting on B75 and B96 was TSM specific. Collectively, our findings suggest that while interactions between spider mite herbivores and maize lines are complex, impacts on behavior are likely a component to the resistance (antibiosis) observed for B75 and B96 for TSM. Indeed, these interactions were occurring at a short time scale in this study, but longer-term studies³¹ in the field with the same species and maize lines suggest that these behaviors may collectively persist to show sustained differences on each of the maize lines. The underlying complexity also suggests that B75 and B96 may possess partially distinct resistance mechanisms, a conclusion supported as well by quantitative trait locus mapping of TSM resistance in B75 and B96³⁰, and by a recent transcriptomic study with these lines that revealed dramatic induced upregulation of both shared as well as distinct sets of known anti-herbivore maize defense genes in response to TSM at 24 h post infestation³⁸. The shorter time frame of our study, however, raises the possibility that constitutive differences in plant defenses (or ones that are rapidly induced) play a role in the variation in resistance to *T. urticae* observed among B73, B75, and B96 in this and earlier studies^{30,31,36}. However, future studies are required to assess this conjecture, as some patterns were complex, and (albeit to a lesser extent) differences were also observed for BGM that performs well on all maize lines included in the study. The behavioral differences observed in our study also suggest that B75 and B96 should be evaluated for resistance by antixenosis, which also involves behavior (note that our study did not employ an experimental design with host choice as needed to directly test for antixenosis / host nonpreference).

Both B75 and B96 have higher DIMBOA levels compared to susceptible B73, and mutant maize plants that cannot produce benzoxazinoid compounds are more susceptible to TSM as assessed by oviposition²⁰. However, while DIMBOA can hinder the performance of insect herbivores by reducing oviposition and fecundity, it may not deter feeding^{39,40}. A possibility is that the decrease in feeding time on resistant B75 by both mite species could be due to other factors such as high fiber content and cell wall phenolics as reported for other maize herbivores³⁹. In fact, Ji et al.³⁶ recently reported changes in the expression of maize cell wall biosynthesis genes in response to TSM in B75 that were not observed (or as dramatic) in either B73 or B96.

Among major arthropod herbivores of maize, the web-spinning capability of spider mites is unique, and is known to serve many purposes such as protection from natural enemies and acaricides, mate finding, locomotion and dispersal and colonization^{23–25,37,41}. The amount of silk produced in the web-spinning behavior of mites depends on temperature, air humidity, smoothness of substrate, plant species and other unknown factors^{23–25,37}. In our study, TSM web-building was increased on B75 and B96 as compared to B73. Since temperature and humidity were the same for all treatments, leaf surface properties of B75 and B96 maize inbred lines may have played a role in TSM web-building behavioral changes. These differences were also apparent without the midrib of the leaf, a major area on maize plants where spider mites colonize. Interestingly, one factor that web-building may not depend on is feeding, as starved spider mites produce silk as well²⁴. According to Oku et al.⁴¹, producing webbing is costly, and resource allocation by TSM in web-building can result in a decrease in egg-laying. In the present study, while TSM spent more time web-building on B75 and B96, it also had reduced egg-laying on B75 and B96 as compared to B73. This raises the possibility that reduced egg-laying by TSM on resistant lines may not be solely due to direct negative effects of resistance in B75 and B96, but to indirect effects as well that impact resource allocations to different behaviors.

Concluding remarks

This study evaluated the behavioral changes of the generalist TSM and the specialist BGM on resistant B75 and B96 maize inbred lines compared to susceptible B73. Exposure of both mite species to resistant line B75 resulted in several behavioral changes, while additional ones associated with reduced fecundity were observed for the generalist TSM on both TSM-resistant B75 and B96 (i.e., increased resting and web-building time). Our findings suggest that evolution of traits impacting behaviors may have contributed to the grass-specialist BGM's ability to overcome intra-specific variation in maize defense pathways that are highly effective against the generalist spider mite TSM.

Data availability

The datasets generated during and/or analysed during the current study are available in the DRYAD repository <https://doi.org/10.5061/dryad.h18931zrr>. Custom code used for the analyses is publicly available on GitHub (<https://github.com/HMKRL/OpenCV-mitetrace>).

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Author contributions

RR, GG, HB, and RC conceived the project and analyses, and RR supervised the study. GG performed mite experiments, data collection, analyses and prepared figures. HL performed mite experiments and data collection. GG and RR wrote the manuscript, with input from all other authors, who read and approved the final version for publication.

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Competing interests

The authors declare no competing interests.

Additional information

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