Antibiosis of Maize Inbred Lines to the Carmine Spider Mite, *Tetranychus cinnabarinus*

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The antibiosis of ten *Zea mays* L. inbred lines to the carmine spider mite, *Tetranychus cinnabarinus* (Boisduval) (Acari: Tetranychidae), was evaluated. Two maize inbred lines previously reported as resistant to this spider mite and a susceptible inbred line were compared with B96 (formerly called 41:2504B), reported as being resistant to the two-spotted spider mite *T. urticae* and to the European corn borer (*Ostrinia nubilalis* Hübner). Other lines were derived from B96. All lines originated in the U.S.A. and were tested in Israel at two different growth stages. Four days after inoculation of detached leaf squares with adult mites, significant differences in susceptibility were observed among lines. At the 3-leaf stage lines B68, B96, B79, A619, B65, B49 and B64 reduced the average mite daily fecundity by 43%, 64%, 66%, 67%, 77%, 81% and 87%, respectively, as compared with the most susceptible line, B52. At the flowering stage, the average reduction in mite daily fecundity was much lower: inbred lines B64, A619 and B96 reduced the average mite daily fecundity by 48%, 51% and 86%, respectively, whereas the seven other genotypes had an intermediate or a susceptible reaction to the carmine spider mite, with A661 being the most susceptible. Our results show that B96 could be used as a source of resistance in developing improved resistance of inbred lines of maize to carmine spider mites.

KEY WORDS: *Tetranychus cinnabarinus*; carmine spider mite; antibiosis; resistance; maize.

INTRODUCTION

There is growing public concern over the impact of chemicals on the environment and the greater number of cases of pest resurgence, secondary pest outbreaks and increasing frequency of resistance of arthropods to pesticides (5,18,25). This has led to a change in the direction of research in crop pest control towards integrated pest management including the development and the utilization of resistant crop varieties. This study describes a comparison of antibiosis to the carmine spider mite of ten maize inbred lines including B96, which had been reported previously as being highly resistant to the two-spotted spider mite.

The two-spotted spider mite, *Tetranychus urticae* (L.), and the carmine spider mite, *T. cinnabarinus* (Boisduval), are serious pests commonly infesting maize (*Zea mays* L.) growing in arid and semi-arid regions (4,19,20). By piercing and sucking the foliar tissue,

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spider mites (Acari: Tetranychidae) cause gradual drying of the leaves, leading to yield reduction or total loss of grain yield (1,2,4,22).

Although genetic resistance to spider mites has been reported in many field and vegetable crops (3,5-7,12,14-17,24), very limited work has been reported in maize (10,11,13,14,20). Resistance of *Z. mays* lines to *T. cinnabarinus* was evaluated previously under laboratory conditions (13). We found significant differences among 36 lines tested 4 days after the inoculation of detached leaf squares with adult female mites when plants were tested at the 3-leaf stage. Seven of the lines harbored significantly fewer mites than the other non-susceptible lines. The most resistant inbred lines reduced the average daily mite fecundity by 88% to 83%, compared with the susceptible inbred H84 (13). Many authors have shown that the basis of the host plant resistance to spider mites is related to the biochemical composition of the leaves (3,9). In another study we compared antibiosis of several selected maize lines differing in their susceptibility to carmine spider mites (14). Significant differences were observed among inbred lines, 4 days after inoculation of detached leaf squares at the 3-leaf stage and at the flowering stage. No significant association was found in spider mite resistance of the lines when tested on young and mature maize plants, indicating that flowering greatly affects pest resistance. Thus, although it is simpler, cheaper and faster to compare antibiosis on young plants, it is imperative to verify resistance at both growth stages. Host plants may differ greatly in basic chemical composition and secondary plant metabolites through different growth stages (9,23). Inbred lines that display high levels of resistance to mites at both the 3-leaf and flowering stages are of interest for breeding and commercial purposes.

The inbred line B96 (41:2504B), originating from the Argentinean line maize 'Amargo', is highly resistant to the European corn borer (ECB), *Ostrinia nubilalis* Hübner, and has been used as a non-recurrent parent in developing novel genotypes of maize. This maize inbred line was also found highly resistant to the two-spotted spider mite (*T. urticae*) in both laboratory and field tests in the U.S.A. (10). We report here a comparison of the antibiosis of resistant (A619 and H100) and susceptible maize lines (A661) at two different growth stages, with the inbred line B96 and six other genotypes.

MATERIALS AND METHODS

Plant material

Three inbred lines that had previously displayed resistance to the carmine spider mite (13,14) were selected for this study: A619 and H100 as resistant lines, and A661 as the susceptible one (14). The other seven inbred lines: B52, previously reported as being resistant to ECB, B96 and five genotypes which had B96 in their pedigree (Table 1), were obtained from the Corn Insect Research Unit (USDA-ARS-MWA, Ankeny, IA, USA).

Maintenance of the mite stock culture

The strain of *T. cinnabarinus* used in this study originated from infested leaves of cotton collected at the Newe Ya'ar Research Center in 1991. Since then, new spider mites from different sources have been added three times a year to the original population to avoid inbreeding depression. Rearing was done on trifoliate leaves of 2–3-week-old kidney bean (*Phaseolus vulgaris* L.) plants grown in 25×32×8-cm-high pots. The mites were reared in a controlled climate room at 25–27°C, 60±5% r.h. and 16 h of light supplied by a series of fluorescent lamps, providing a light intensity of *ca* 2000 lux. The individual mites used for the bioassays were collected and transferred to the plants using a fine-hair brush. Mites were routinely transferred from aging plants to younger ones by placing old leaves infested with mites near 7- to 10-day-old seedlings (14).

Preparation of maize plants and screening for resistance

In all experiments, maize seeds were germinated in 7×7×17-cm-high pots using a peat:vermiculite (1:1) growth medium, watered with a standard nutrient solution. The environmental conditions in the rearing room were 26±1°C, 55–65% r.h., and 16 h of light from Gro-lux lamps providing a light intensity of *ca* 2000 lux.

Three-leaf stage. Five seedlings from each of the ten maize lines were grown and used for testing. To prevent outside infestation, pots with plants were placed in tin trays containing a thin layer of water (14). When seedlings reached the 3-leaf stage, four 20 × 20 mm squares were punched from the leaves of each plant and placed, upside down, on filter paper in petri dishes. Five adult mites, 3 to 5 days old, were transferred to each square from the original breeding colony on bean.

Each filter paper was placed on a sheet of foamed plastic, floating on water in a 90-mm-diameter petri dish. Dishes with mites were kept in the controlled climate room as described above. Records on live, dead and repelled mites, as well as oviposition data, were taken 4 days following incubation. Mites that were found on the foam were scored as repelled. Previous experiments showed that mites under these experimental conditions had low rates of oviposition beyond the stage of 4 days after infestation (17).

Flowering plants. The same five seedlings of each of the ten lines that were evaluated at the 3-leaf stage were transplanted into 20-*l* pots and placed in a greenhouse. When the plants started to flower, four 20 × 20-mm leaf-tissue squares were punched out of the third leaf from the stem apex.

Experimental design

Recent studies indicated the efficiency of the analysis of leaf squares for antibiosis, as compared with the whole plant (3,10). Each inbred line was represented by five plants. Four leaf-squares were cut out from each plant. Thus, all together 100 mites were tested for each line at each stage of development. The experiments had a completely randomized design. In subsequent analyses of variance of the data, the five plants were taken as five blocks and all of each plant's squares for a particular line were considered to constitute a plot in the analyses. Differences between lines were evaluated by using Duncan's multiple range test at 5% level of probability. The analyses for percentages were carried out after angular transformation.

RESULTS AND DISCUSSION

The antibiosis of maize inbred lines to spider mites was evaluated at the 3-leaf stage. No significant differences were observed among plants (blocks) within maize inbred lines for the evaluated criteria at both growth stages. Results are presented in Table 1. Unlike its performance in our previous study (14), the inbred line A661 did not show significant difference in susceptibility in comparison with the most susceptible inbred line B52. All other lines reduced the number of eggs/female/day (E/F/D) significantly, as compared with the B52 line. In relation to other measures, all lines that were considered resistant in our previous study (14) caused a significant reduction in numbers of live mites and significant repellence of mites, compared with the susceptible B52 line. Since selection for resistance to the spider mite and other pests is restricted mainly to evaluating plant effect on oviposition (3,13,21), the numbers based on this measurement showed significant differences ($P<0.05$) among genotypes, even though data were collected only 4 days after inoculation (Table 1).

TABLE 1. Antibiosis of maize inbred lines at the 3-leaf growth stage to the carmine spider mite *Tetranychus cinnabarinus.* Measurements were taken at the flowering growth stage, 4 days following inoculation of detached leaf squares (five females/square).

Line^z	% Live^y		% Dead		% Repelled		E/F/D^x		% Diff.^{w}
B52	25a	(4.5)	10a	(3.5)	65a	(3.5)	1.4a	(0.18)	
A661	10ab	(7.6)	1ab	(1.0)	89b	(7.5)	1.2a	(0.30)	-2
H100	15ab	(6.3)	8ab	(4.1)	77ab	(9.1)	1.1b	(0.30)	-7
B68	2b	(1.2)	3ab	(2.0)	95b	(3.2)	0.8bc	(0.21)	-13
B96	8ab	(4.8)	1ab	(0)	91b	(4.8)	0.5bc	(0.21)	-26
B79	1b	(1.0)	4ab	(1.9)	95b	(2.7)	0.5bc	(0.20)	-26
A619	3b	(2.0)	1ab	(1.0)	96b	(2.4)	0.5bc	(0.14)	-29
B65	3b	(1.2)	2ab	(1.2)	95b	(1.6)	0.3bc	(0.06)	-48
B49	6b	(6.0)	3ab	(2.0)	91b	(5.6)	0.3bc	(0.06)	-51
B64	2b	(2.0)	1ab	(1.0)	97b	(2.0)	0.2c	(0.05)	-86

^{z}Listed in descending order from the most susceptible to the most resistant line.
^{y}Within columns, mean separation by Duncan's multiple range test (30 d.f. for error). Figures followed by a common letter do not differ significantly at the 5% level. S.E.M. of five plants is given in parentheses.
^{x}Eggs per female per day.
^{w}Percentage of difference as compared with the most susceptible line (100 × (E/F/D$_{B52}$ − E/F/D$_{of\ line}$) / E/F/D$_{B52}$).

Evaluations of antibiosis to spider mites of the same plants at the flowering stage are presented in Table 2. At this growth stage, the E/F/D was much higher, ranging from 1.1 to 7.9. Only line B96 caused a significant reduction in the average number of E/F/D, and daily mite fecundity was reduced by 86% compared with A661, the most susceptible line. All other lines, which showed a moderate to high degree of resistance at the 3-leaf stage, lost – in different ratios – their relative resistance to the spider mites at the flowering stage. Lines A619 and B64 still displayed moderate resistance and significantly reduced the average mite daily fecundity by $\sim$50% as compared with the most susceptible line, A661. Similar behavior, but to a lesser extent, was seen with genotypes B49, H100 and B68, which reduced significantly the average mite daily fecundity by 26% to 29%, as compared with A661, the most susceptible line.

Interestingly, genotypes B65 and B79, which showed significant resistance at the 3-leaf stage, lost their resistance to the spider mite at the flowering stage. With regard to other measurements, there were no significant differences in the percentage of dead mites associated with any line except of B96. Only lines A619 and B96 caused a significant reduction in the number of live mites and a significant increase in the level of repelled mites. The mites had been reared on beans and were immediately transferred to maize

TABLE 2. Antibiosis of maize inbred lines at the flowering growth stage to the carmine spider mite, *Tetranychus cinnabarinus.* Measurements were taken at the flowering growth stage, 4 days following inoculation of detached leaf squares (five females/square).

Line[z]	% Live[y]		% Dead		% Repelled		E/F/D[x]		% Diff.[w]
A661	75a	(1.6)	2a	(1.2)	23a	(1.2)	7.9a	(0.42)	
B52	76a	(5.3)	1a	(1.0)	23a	(6.0)	7.8ab	(0.29)	-2
B65	75a	(3.6)	2a	(1.1)	23a	(5.4)	7.4ab	(0.32)	-7
B79	73a	(6.8)	2a	(1.2)	25a	(9.6)	6.9b	(0.63)	-13
B49	70a	(6.6)	1a	(1.0)	29a	(6.6)	5.9c	(0.30)	-26
H100	69a	(4.0)	4a	(1.9)	27a	(3.4)	5.9c	(0.12)	-26
B68	66a	(3.2)	3a	(1.9)	31a	(4.8)	5.6c	(0.46)	-29
B64	66a	(9.4)	3a	(1.6)	31a	(4.4)	4.1d	(0.37)	-48
A619	49b	(7.5)	2a	(2.0)	49b	(6.8)	3.9d	(0.36)	-51
B96	36c	(4.0)	13b	(5.1)	51b	(5.6)	1.1e	(0.12)	-86

[z] Listed in descending order from the most susceptible to the most resistant line.

[y] Within columns, mean separation by Duncan's multiple range test (30 d.f. for error). Figures followed by a common letter do not differ significantly at the 5% level. S.E.M. of five plants is given in parentheses.

[x] Eggs per female per day.

[w] Percentage of difference as compared with the most susceptible line (100 × (E/F/D$_{A661}$ − E/F/D$_{of\ line}$) / E/F/D$_{A66}$).

plants. Thus, they could have been stressed while adapting to a new host. However, this effect alone can not account for the large differences observed among the maize inbred lines.

The observed reduction in oviposition and in the E/F/D is corroborated by the data on live mites and on repelled mites. The lower the average daily fecundity, the higher the percentage of live mites repelled. For example, lines with 1.1 and 3.92 E/F/D had an average of 36% and 49% live mites and 51% and 49% repelled mites, respectively, whereas lines with 4.13 to 7.79 E/F/D had approximately 66–76% live mites and 23–31% repelled mites. This explains in part the lower rate of reproduction on the more resistant genotypes as compared with the rate of reproduction on the susceptible ones.

Resistance to the carmine spider mite was apparent in a limited number of maize genotypes. Both antixenosis (the effect of plant resistance on insect behavior) and antibiosis (the effect of plant resistance on insect biology) are possible mechanisms of resistance manifested by the resistant lines (21). Both the developmental and physiological state of the hosts, as well as environmental factors, may have profound effects on susceptibility (9,14). Many authors have shown that the biochemical constituents of plants are dependent on the physiological condition of the plant (9,23). This affects the availability of nutrients and existence of anti-insect factors in the plant leaves, and may partially explain the apparent breakdown of resistance at the flowering stage in the inbred lines investigated (9).

A previous report (10) indicated a cross tolerance to ECB and to the two-spotted spider mite. Maize lines B52, B68 and B86, which are resistant to the ECB (8), are also partially resistant to *T. urticae* (10). In our study B52 was susceptible, whereas B68 was moderately resistant, to *T. cinnabarinus.* The inbred lines B49, B64, B65 and B68 are descendants of B96, the mite resistant line, in their pedigree. Interestingly, all these lines showed high levels of antibiosis to *T. cinnabarinus.* The moderate resistance found in B64 and B68 could be attributed mainly to the B96 germplasm in their pedigree, suggesting that mite resistance is transferred genetically. Further investigation is necessary to determine whether the genes controlling resistance to the two-spotted mite (10) are the same as those controlling the resistance to the carmine spider mite reported here. However, resistance is probably governed by more than one gene since the performance of these lines was inferior to their ancestor (Table 2). It might be advisable and ultimately lead to greater stability to develop inbred lines with multiple resistances to a number of insect pests, even though the level of resistance might be compromised. It would also be of interest to investigate the relationship between mite resistance and ECB resistance.

Our data, based on antibiosis and repellence evaluations (3,21), suggest that additional sources of resistance to mites can be identified in many genotypes, including in corn belt material, and might be utilized in breeding programs. The high degree of resistance shown by B96 and its progeny can be related to its tropical origins, which emphasizes the importance of the tropical genetic reservoir for the identification of genes controlling pest resistance. Further progress in introgression of resistance to mites into elite germplasm could be achieved by understanding the inheritance of resistance, and the components contributing to this resistance.

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