Influence of Soybean Lines Isogenic for Pubescence Type on Twospotted Spider Mite (Acarina: Tetranychidae) Development and Feeding Damage[1]

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ABSTRACT Soybean lines isogenic for glabrous, normal, and dense pubescence developed from the genotypes 'Davis', 'Tracy-M', and D75-10169 were used in studies of the impact of pubescence on twospotted spider mite, *Tetranychus urticae* Koch, development and feeding damage under controlled environmental conditions and a known infestation rate. Significant differences in progeny and feeding damage of the twospotted spider mite between genotypes were observed. There were no significant differences between isolines within genotypes for number of progeny. Glabrous isolines had significantly more adult mites leave the excised plant leaf and significantly less feeding damage than the normal or dense isolines. Results suggest that leaf pubescence, or in this case lack of, could be a potential factor in the control of the twospotted spider mite on soybean. This study also demonstrates a reliable and uniform laboratory procedure, under controlled conditions, for rearing the twospotted spider mite, screening soybean germplasm, and determining resistance mechanisms.

Key Words *Tetranychus urticae,* mites, pubescence, plant resistance, soybean

The twospotted spider mite, *Tetranychus urticae* Koch, is among the arthropod pests of soybean. Serious infestations have been associated with periods of prolonged hot weather and dry conditions (Baker and Connell 1961, Oloumi-Sadeghi et al. 1988, Gray et al. 1989).

Breeding for spider mite resistance has been limited to a relatively few crops including cassava, cotton, strawberry, cucumber, and peanuts (De Ponti 1977 and 1985, Brandenburg and Kennedy 1985). Various soybean germplasms have been examined for their relative ability to withstand mite injury (Carlson 1969, Carlson et al. 1979, Bailey and Furr 1975, Mohamed and Hafez 1981, Mohammad and Rodriguez 1985, Wheatley and Boethel 1987). However, soybean germplasms resistant to spider mites have not been identified, and there is no information of current programs trying to screen and select for spider mite resistance in soybean.

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Plant pubescence, or density of trichomes (hairs), can have a significant effect on the insect-plant relationship of herbivores colonizing a specific plant species. Lambert and Kilen (1989) and Lambert et al. (1992) demonstrated that soybean lines isogenic for dense pubescence, when compared with lines isogenic for normal or glabrous pubescence, were resistant to the larval stage of several lepidopterous defoliators but more susceptible to adult oviposition. Elden and Lambert (1992) demonstrated that normal and dense pubescent soybean isolines caused a significant reduction in potato leafhopper, *Empoasca fabae* (Harris), feeding damage, and oviposition when compared to glabrous isolines. The objectives of the present study were to develop a reliable and uniform laboratory procedure for rearing the twospotted spider mite and screening soybean germplasm for resistance, and to investigate the influence of soybean lines isogenic for pubescence type on twospotted spider mite development and feeding damage under controlled environmental conditions, and at known infestation rates.

Materials and Methods

Twospotted spider mite colonies used in this study were reared on 'Henderson' bush lima beans in a walk-in growth chamber maintained at 24 to 27°C, 50 to 90% RH, and a photoperiod of 15:9 (L:D) h. Spider mites were reared in plastic Petri dishes (100 × 20 mm) containing a circular sponge (80 × 5 mm) saturated with distilled water over which was placed a piece of No. 1 qualitative filter paper (90 mm). Five holes (1 mm) were drilled in the Petri dish tops to prevent condensation from forming on the lids. Twenty dishes containing 5, 1 to 3-d-old, female spider mite adults were set up weekly. Individual females were transferred to young, 1 to 3-wk-old, excised bean leaves (≈60 by 40 mm) centered on the filter paper setting on the sponge within the dish using a size 000 insect pin attached to a small wooden handle. After 3 d, females were removed from the leaves so that developing populations would be of a relatively uniform age for subsequent colonization and testing.

Soybean isolines with glabrous, normal, and dense pubescence from the cultivars 'Davis' and 'Tracy-M' and the breeding line D75-10169 were used in this study (Kilen and Lambert 1993). Davis is a productive cultivar of Maturity Group VI, highly susceptible to foliar-feeding insects (Caviness and Walters 1966). Tracy-M is a herbicide-tolerant selection from 'Tracy' of Maturity Group VI, moderately resistant to several foliar feeding insects (Hartwig et al. 1980, Lambert and Kilen 1984, Lambert and Hamer 1988). D75-10168 is a registered germplasm line of Maturity Group VII, highly resistant to foliar feeding insects (Hartwig et al. 1984, Lambert and Kilen 1984, Lambert and Hamer 1988).

Soybean seeds were germinated in 35-cm plastic pots, containing a 3:1:1 (v/v/v) peat moss/vermiculite/perlite potting mixture, in the greenhouse at temperatures ranging from 24 to 30°C and a photoperiod of 15:9 (L:D) h provided by supplemental high-pressure sodium lighting. At ≈ 8 d of age, soybean seedlings were removed from the pots, inoculated with *Bradyrhizobium japonicum* to insure nodulation, and transferred singly into 10-cm diam clay pots containing a 1:1 mixture of soil and potting mixture. Plants were fertilized bi-monthly with a water-soluble 5-11-26 fertilizer.

The nine soybean isolines were screened in eight laboratory tests, with four replications in each test, for resistance to twospotted spider mite development and feeding damage. The eight tests represented four different sets of plants with two tests per planting tested over a 12-mo period. Soybean leaflets (≈ 60 × 40 mm) from the second trifoliolate leaf from plants in the V4 to V5 stage of development (Fehr and Caviness 1977) were excised from the plant and placed on filter paper, with the abaxial (bottom) surface facing up, in the Petri dish cages as described above. Each leaflet was infested with 5, 1 to 3-d-old, female spider mite adults and cages placed in the walk-in growth chamber described above. Three days after infestation, living and dead adults and adults that left the leaf and became trapped on the moistened filter paper were recorded and removed from the leaflets. Additional water was applied to the sponge and filter paper after 7 d. After 10 d, the total number of living progeny (nymphs and neonate adults) and feeding damage based on foliar discoloration were recorded. A scale of 1 to 4 was used for scoring feeding damage (1 = normal green, no apparent damage; 2 = pale green, some yellowing; 3 = moderate yellowing, some necrosis; 4 = extensive yellowing, moderate necrosis).

Mite development was converted to and is reported as the number of progeny per female per day surviving 10 d after initial adult infestation. Adult mortality is reported as the percentage of infestation adults dead after 3 d. Infestation adults that left the leaf and became trapped on the filter paper after 3 d are reported as the percentage of adults that left the leaf.

The treatment structure for all tests was 3 genotypes with 3 isolines of varying pubescence nested within genotypes. These tests were repeated eight times, twice each in June, August, and October of 1993 and twice in May of 1994. The experimental design for all tests was a randomized complete block with four replicates in each test. For each test by treatment combination (8 × 9), the mean was calculated and these 72 means were used as observations for a combined analysis using all available data. The general linear model (GLM) procedure of SAS (SAS Institute 1988) was used to analyze the data, for all dependent variables, with a model containing the effects of test, genotype, and isoline nested within genotype. Mean comparisons were conducted using Fisher's protected least significant difference (FPLSD) as indicated by the analysis of variance. Partial correlations between dependent variables, adjusted for the effects of test, genotype, isoline and genotype*isoline are reported. Significance is reported at the 5% level.

Results

The density of simple hairs (trichomes) on the abaxial (bottom) surface was 358 trichomes/cm^2 for the Davis dense isoline and 201 trichomes/cm^2 for the normal isoline (Elden and Lambert 1992). Pubescence on the abaxial leaf surface of Tracy-M dense and normal isolines averaged 369 and 190 trichomes/cm^2, respectively. Pubescence on the D75-10169 dense and normal isolines averaged 287 and 98 trichomes/cm^2, respectively. No trichomes were observed on leaves of Davis, Tracy-M, or D75-10169 glabrous isolines.

The ≈ number of surviving twospotted spider mite progeny per female per day after 10 d in the laboratory colonies was 7. Completion of the mites life cycle, egg to adult, in laboratory colonies and tests averaged 9 d under the conditions imposed. The effect of switching host plants, rearing spider mites on lima bean and testing progeny on soybean, had no effect on twospotted spider mite growth, development, or fecundity in this study nor in numerous other tests conducted by this author to select for spider mite resistance in selected soybean germplasm (Elden, unpubl. data).

Differences among tests for all variables were significant with progeny differences being the greatest, and adult mortality being the least. Data for progeny development, adult mortality, adults that left the leaf and became trapped on filter paper, and feeding damage on the soybean genotypes and isolines tested are presented in Table 1.

Progeny differences between isolines differing in pubescence within genotypes were not significant. The main effect of genotype was significant ($P < 0.01$) with D75-10169 having significantly fewer progeny than Davis ($P < 0.01$) or Tracy-M ($P < 0.001$). The number of progeny was negatively correlated with infestation adults that left the leaf ($r = -0.26$, $P = 0.04$).

Adult mortality was significantly greater for the glabrous isoline of Tracy-M than for the normal ($P = 0.02$) or dense ($P = 0.04$) isolines of this genotype. Adult mortality also was significantly greater for the glabrous isoline of D75-10169 than for the normal ($P = 0.03$) or dense ($P < 0.001$) isolines of this genotype. Differences between genotypes were not significant. Adult mortality was positively correlated with feeding damage ($r = 0.26$, $P = 0.04$).

Adults that left the leaf were significantly greater for the glabrous isolines of Tracy-M ($P = 0.03$) and D75-10169 ($P = 0.04$) than for the normal isolines of these genotypes. The glabrous isoline of D75-10169 also had significantly more ($P < 0.001$) adults leave the leaf than its dense isoline. Differences between genotypes were not significant. The number of adults that left the leaf was positively correlated with adult mortality ($r = 0.86$, $P < 0.001$). Seventy-one percent of the adult mortality (22% for all tests) was accounted for by adults leaving the leaf and becoming trapped on filter paper. Mites in this study and in our laboratory colonies would approach the edge of the leaflets, probe the filter paper, and back away from the edge. Two conditions, crowding and severe feeding damage, were observed in our laboratory colonies to cause mites to leave the leaf and become trapped on the filter paper. Because infestation adults were only on the leaflets for 3 d in these tests neither crowding nor severe damage occurred within this time frame or even after 10 d when progeny and feeding damage were recorded.

Feeding damage was significantly less for the glabrous isolines of all genotypes than for the normal or dense isolines with one exception. The glabrous isoline of Tracy-M was not significantly different from its normal isoline. The P value for the main effect of isoline within genotype was < 0.001. Feeding damage on the genotype Tracy-M was significantly less ($P = 0.02$) than on Davis. Feeding damage was positively correlated with adults that left the leaf ($r = 0.33$, $P = 0.01$).

Table 1. Twospotted spider mite progeny, adult mortality, adults that left leaf, and feeding damage on soybean isolines with dense, normal, and glabrous pubescence.

Isoline	n	Genotype: Davis	Tracy-M	D75-10169
			Progeny*	
Glabrous	8	7.3	7.7	5.0
Normal	8	7.3	7.2	5.2
Dense	8	5.6	6.9	5.2
Genotype mean	24	6.7 x	7.3 x	5.1 y
			Adult mortality, %	
Glabrous	8	22.0 a	30.9 a	34.5 a
Normal	8	18.9 a	19.0 b	23.9 b
Dense	8	17.9 a	20.4 b	15.4 b
Genotype mean	24	19.6	23.4	24.6
			Adults left leaf, %†	
Glabrous	8	16.4 a	24.0 a	26.5 a
Normal	8	13.7 a	13.9 b	16.9 b
Dense	8	9.4 a	15.0 ab	8.1 b
Genotype mean	24	13.2	17.6	17.2
			Feeding damage‡	
Glabrous	8	1.6 b	1.6 b	1.7 b
Normal	8	2.9 a	2.2 ab	2.7 a
Dense	8	3.2 a	2.4 a	3.0 a
Genotype mean	24	2.6 x	2.1 y	2.5 xy

*Individual leaflets infested with 5 female adults for 3 d and progeny recorded after 10 d.

**Within a column (isoline a, b, c) or row (genotype x, y, z) least square means followed by the same letter are not significantly different ($P > 0.05$); Fisher's LSD test. Mean of 8 tests.

†Percent infestation adults which left leaflet and became trapped on filter paper after 3 d.

‡Feeding damage based on filiar discoloration after 10 d: 1 = normal green, no apparent damage; 2 = pale green, some yellowing; 3 = moderate yellowing, some necrosis; 4 = extensive yellowing; moderate necrosis.

Discussion

This study demonstrates a reliable and uniform laboratory procedure, under controlled conditions, for rearing the twospotted spider mite, screening soybean germplasm for resistance, and determining resistance mechanisms.

Data from this study demonstrate that glabrous soybean isolines are less preferred by the twospotted spider mite than normal or dense pubescence isolines. This nonpreference is expressed by adults leaving the leaflet, possibly in search of a preferred oviposition site. Even though more adults left the glabrous isolines, there were little differences in progeny development between isolines within genotypes. There were small but significant differences between genotypes for number of progeny and feeding damage. Tracy-M which had the lowest feeding damage also had the highest number of progeny, suggesting that differences in tolerance to feeding damage may exist among soybean genotypes. There was no evidence of antibiosis on any of the isolines or genotypes tested in this study.

Twospotted spider mite adults in this no-choice laboratory study appeared to prefer pubescent soybean lines over glabrous lines. Leaf pubescence, or in this case lack of, could be a potential factor in the control of the twospotted spider mite on soybean. Infestation adults which left the leaflets in this study became trapped on the filter paper and died. Adult mites on a glabrous soybean plant in the field may tend to roam more in search of a more suitable host than adults on a pubescent plant, but this study demonstrated that overall oviposition and progeny development was not affected in a no-choice situation. Progeny development on the soybean isolines tested in this study differed little from that of our laboratory colonies which also suggest that these isolines do not possess antibiosis resistance to the twospotted spider mite.

Keeping in mind that glabrous soybean isolines are more susceptible than pubescent isolines to foliar feeding lepidopterous pests (Lambert and Kilen 1989, Lambert et al. 1992) and the potato leafhopper (Elden and Lambert 1992), their use as a source of resistance to the twospotted spider mite would be practical only in a situation where these other arthropod pests are not a problem.

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