Laboratory Screening Techniques for Evaluation of Soybean Germplasm for Resistance to Twospotted Spider Mite (Acari: Tetranychidae)[1]

T. C. Elden

Soybean and Alfalfa Research Laboratory, USDA—ARS, Bldg. 470A, BARC-East, Beltsville, MD 20705 USA

J. Entomol. Sci. 34(1): 132-143 (January 1999)

Abstract Thirty-one soybean, *Glycine max* (L.) Merrill, accessions from maturity groups II through VIII were evaluated in excised and intact (whole plant) leaf bioassays to determine the ability of these bioassays to detect differences in susceptibility to the twospotted spider mite, *Tetranychus urticae* Koch. Although there were few significant differences between bioassays for variables measured within maturity groupings, the excised leaf bioassay which was easier to set up and monitor and took three-fourths less growth chamber space also had less variation among replications and repeated tests and detected a greater number of differences among accessions. Although there were significant differences among soybean accessions within a maturity group for specific variables, results suggest that high levels of resistance to the twospotted spider mite are not present in the germplasm screened. Several accessions screened, with known resistance to foliar feeding insects, were significantly less preferred for spider mite oviposition and development. However, it was apparent that the gene(s) controlling insect resistance do not impart the same level of resistance to the twospotted spider mite. Results of this study, based on differences in susceptibility among soybean accessions, demonstrate that the excised leaf bioassay should prove to be an efficient and uniform laboratory bioassay to screen soybean germplasm for resistance to the twospotted spider mite.

Key Words *Tetranychus urticae,* mites, soybean, plant resistance, screening.

The twospotted spider mite, *Tetranychus urticae* Koch, is a potentially serious pest of soybean, *Glycine max* (L.) Merrill, associated with periods of prolonged hot weather and dry conditions (Baker and Connell 1961, Gray et al. 1989, Higley et al. 1989). Air temperature, rainfall, humidity, and plant moisture are major environmental factors in spider mite population dynamics (Simpson and Connell 1973, Mellors et al. 1984, Oloumi-Sadeghi et al. 1988). Klubertanz et al. (1990) suggest that other factors such as increased developmental rates, inactivity of predators and pathogens, and rate of emigration from deteriorating food sources also may be important in the development of spider mite populations.

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 1987). Soybean cultivars resistant to spider mites have not been developed, and there are no known research programs currently

[1]Received 28 January 1998; accepted for publication 17 June 1998.

underway which involve screening and selecting for spider mite resistance in soybean.

In 1966, Carlson et al. (1979) evaluated 3500 soybean lines in maturity groups II and III, in the field, for resistance to twospotted spider mite feeding damage. At the end of the season, they reported 12 lines with low damage ratings. Mohamed and Hafez (1981) reported significant differences in counts of twospotted spider mites on 16 soybean cultivars evaluated in the field. Several laboratory and greenhouse studies, each using 12 or fewer selected soybean lines, demonstrated significant differences in twospotted spider mite feeding damage or fecundity (Bailey and Furr 1975, Carlson et al. 1979, Mohammad and Rodriguez 1985, Wheatley and Boethel 1987). Studies on spider mite-soybean interactions have been reported by Rodriguez et al. (1983), Hildebrand et al. (1986), and Brown et al. (1991).

Efforts to identify and develop soybean germplasm with resistance to the twospotted spider mite have been limited and unsuccessful due to two main factors. First, large-scale field screening is complicated by nonuniform infestations within a field and is unreliable because spider mite populations are strongly regulated by environmental factors, plant phenology, natural enemies, and cultural practices. Secondly, there does not exist an adequate laboratory technique for screening and determining specific mechanisms of spider mite resistance in soybean. The objectives of this study were (1) to develop a reliable and uniform laboratory bioassay to screen large numbers of soybean germplasm for resistance to the twospotted spider mite and (2) to use this bioassay in an attempt to identify twospotted spider mite resistance mechanisms in selected lines previously reported to have some resistance to spider mites and other lines with known resistance to foliar feeding insects.

Materials and Methods

Mite colonies. Twospotted spider mite colonies used in this study originated from mites collected on soybeans in the field and were reared on foliage of 'Henderson' bush lima beans, *Phaseolus lunatus* L., 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 diam) were drilled in the Petri dish tops to reduce condensation on the lids. Twenty dishes containing 5, 1- to 3-d-old, female spider mite adults were set up weekly. Individual females were transferred, using a size 000 insect pin attached to a small wooden handle, to young, 1- to 3-wk-old, excised lima bean leaves (≈ 60 × 40 mm) centered on the filter paper setting on the sponge within the dish. 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 selections. Thirty-one soybean accessions from maturity groups II through VIII were evaluated in this study. Most accessions were selected based on reported susceptibility or resistance to the twospotted spider mite in previous studies (Carlson 1969, Bailey and Furr 1975, Carlson et al. 1979, Mohamed and Hafez 1981). Two studies, Mohammad and Rodriguez (1985) and Wheatley and Boethel (1987), included in their evaluations several lines with known resistance to foliar feeding insects. Commercial cultivars from each of the maturity groups and several additional insect resistance lines, not previously evaluated for spider mite resistance, were included in this study.

Selected soybean accessions were planted and tested by maturity group(s) to allow comparisons within the same growth stage. Accessions in maturity group IV were tested as a separate group and maturity groups II and III, V and VI, VII and VIII were combined and tested as separate groups. Henderson bush lima bean, on which spider mite colonies were reared, was included in all tests to provide a standard across tests and maturity groups.

Soybean seeds were germinated in 35-cm diam 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 approximately 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 every two months with a water-soluble 5-11-26 fertilizer.

Excised leaf bioassay. Seven accessions in maturity group IV were screened in three tests, five accessions in maturity grouping VII and VIII were screened in four tests, and ten accessions in maturity grouping II and III and nine accessions in maturity grouping V and VI were screened in five tests. There were four replications in all tests. The 3 to 5 tests in each of the maturity groupings represented two different sets of plants planted and tested over a 2-y period.

Soybean leaflets (≈ 60 × 40 mm) from the second trifoliolate leaf from plants in the V3 to V4 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 d 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, similar to that used by Gray et al. (1989), 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).

Intact leaf bioassay. The 31 soybean accessions screened in the excised leaf bioassay also were screened separately by maturity groupings in two intact leaf (whole plant) tests, with four replications in each test. The two tests in each maturity grouping were from the same set of plants which also represented one of the two sets of plants used in the excised leaf bioassay, which were screened over a 2-yr period.

The middle leaflet from the second trifoliolate leaf from plants in the V3 to V6 stage of development was infested with 5, 1- to 3-d-old, female spider mite adults. Plants were supported by metal stakes, which projected from the soil within the pot. The petioles of infested leaflets were coated with petroleum jelly to discourage mites from leaving the leaflets. Plants were placed in pans, for bottom watering, on carts in a walk-in growth chamber maintained at 24 to 26°C, 50 to 90% RH, and a photoperiod of 15:9 (L:D) h. Three d after infestation, living and dead adults and adults that were missing or became trapped on the petroleum jelly were recorded and removed from the leaflets. After 10 d, the total number of progeny and feeding damage were recorded similar to the methods used in the excised leaf bioassay.

Mite development in both the excised and intact leaf bioassays 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 in the excised leaf bioassay or were missing or trapped in the intact leaf bioassay after 3 d are reported as the percentage of adults that left the leaf.

Statistical analysis. Soybean accessions were evaluated in a randomized block design with four replications (infested leaflets) in each test. The excised leaf bioassay was repeated from 3 to 5 times (tests) and represented two different sets of plants planted and tested over time by maturity groupings. The intact leaf bioassay was repeated twice and represented the same set of plants. For each bioassay, the general linear model (GLM) procedure of SAS (SAS Institute 1988) was used to analyze the data for each maturity grouping separately, combined over tests, with a model containing the effects of tests, entry, rep(test), and test × entry. The GLM also was used to compare bioassays by analyzing data for each maturity grouping separately, combined over all tests in the excised and intact leaf bioassays, with a model containing the effects of bioassay, entry, rep(bioassay), and bioassay × entry. Mean comparisons were conducted using the Ryan-Einot-Gabriel-Welsch (REQWQ) multiple range test. Partial correlations between dependent variables, adjusted for model effects, are reported. Significance is reported at the 5% level.

Results and Discussion

Maturity groups II and III. Significant differences among soybean accessions for all variables in the excised leaf bioassay were demonstrated in this maturity grouping (Table 1). Adult mortality in the intact leaf bioassay had a significant F value (P = 0.034) for entries in the model. However, the Ryan-Einot-Gabriel-Welsch multiple range test which is one of the more powerful conservative tests, in that it strongly controls the experimentwise error rate, failed to distinguish significant differences among entries for adult mortality in this bioassay (Table 1). The breeding line L62-561 and PIs 86452 and 88492 had the highest percentages of adult mortality in the excised leaf bioassay which was a reflection of the highest percentages of adults leaving the leaf and becoming trapped on the filter paper. L62-561 also had the lowest number of progeny in both the excised and intact leaf bioassays. Carlson (1969) and Carlson et al. (1979) reported reduced mite development in these three accessions. L62-561 and PI 86452 are glabrous soybean lines which lack simple plant hairs. Elden (1997) reported that glabrous soybean isolines were less preferred by the twospotted spider mite than normal or dense isolines which was expressed by more adults on the glabrous isolines leaving the leaf possibly in search of a more preferred oviposition site. He also demonstrated that even though more adults left the glabrous isolines in this no-choice test, there were no significant differences in progeny development between pubescent isolines within genotypes.

Feeding damage differences among accessions were significant in both the excised and intact leaf bioassays. However, there were no significant correlations of adult mortality or number of progeny with feeding damage in this or any of the other maturity groupings tested. The cultivar 'Williams' which has demonstrated tolerance to spider mite damage in the field (Carlson et al. 1979, Rodriguez et al. 1983) was tolerant to feeding damage but did not exhibit antibiosis or nonpreference resistance in the present study or in a study by Wheatley and Boethel (1987).

Means for all variables in the excised leaf bioassay were significantly higher (P <

Table 1. Twospotted spider mite adult mortality, adults that left the leaf, progeny, and feeding damage on selected soybean accessions in maturity groups II and III in excised and intact leaf laboratory bioassays

Accession	Maturity group	% adult mortality		% adults left leaf		Progeny/♀/d		Feeding damage*	
		Excised	Intact	Excised	Intact	Excised	Intact	Excised	Intact
Wells	II	18.0d	17.5a	11.0cd	5.0a	8.4bc	7.9a	2.6b	1.4c
L62-561	II	43.0a	17.5a	38.0a	7.5a	7.3c	6.3a	2.0cd	1.9bc
L67-3388	II	26.0bcd	2.5a	18.0bcd	0.0a	8.6bc	7.7a	3.1a	2.9b
Guelph	III	19.0d	5.0a	14.0cd	0.0a	8.5bc	7.6a	1.1e	1.4c
Wayne	III	21.0cd	15.0a	13.0cd	2.5a	9.6ab	8.2a	1.9d	1.0c
Williams	III	23.0cd	17.5a	15.0cd	2.5a	9.5ab	9.1a	1.3e	1.3c
PI 70212	III	19.0d	10.0a	7.0d	0.0a	9.0ab	7.8a	2.4bc	1.8c
PI 85473	III	18.0d	2.5a	8.0d	0.0a	10.3a	8.1a	2.5b	1.9bc
PI 86452	III	36.0abc	15.0a	25.0abc	10.0a	8.4bc	8.4a	3.5a	3.9a
PI 88492	III	40.0ab	7.5a	31.0ab	7.5a	8.6bc	6.7a	2.5b	1.3c
Control**		14.0d	0.0a	8.0d	0.0a	9.8ab	9.6a	1.1e	1.0c
Mean		25.2	10.0	17.1	3.2	8.9	7.9	2.2	1.8
F		6.78	2.14	9.45	1.71	5.08	1.38	53.78	11.79
P		<0.001	0.034	<0.001	0.099	<0.001	0.213	<0.001	<0.001
CV		67.6	138.3	86.3	253.1	18.7	28.1	22.8	40.8

Means with the same letter within a column are not significantly different ($P > 0.05$) according to the Ryan-Einot-Gabriel-Welsch multiple range test (SAS Institute 1988). Excised leaf bioassay means of 5 tests and 4 replications per test ($n = 20$). Intact leaf bioassay means of 2 tests and 4 replications per test ($n = 8$).

* Feeding damage rated on a 1 (normal green, no apparent damage) to 4 (extensive yellowing, moderate necrosos) scale.

** Control; leaf of Henderson bush lima bean.

0.002) than those in the intact leaf bioassay. The coefficients of variation for all variables in the intact leaf bioassay were approximately twice those in the excised leaf bioassay which helps explain why means among accessions for adult mortality, adults that left the leaf, and progeny were not significantly different in the intact leaf bioassay.

Maturity group IV. There were no significant differences among soybean accessions for any of the variables except feeding damage in either the excised or intact leaf bioassay (Table 2). There were significant differences between the lima bean control and one or more soybean accessions for all variables in the excised leaf bioassay. PI 80837, the source of moderate Mexican bean beetle, *Epilachna varivestis* Mulsant, resistance in the soybean cultivar 'Shore' (Smith et al. 1975), had the lowest number of mite progeny in both types of bioassays but there was no indication of antibiosis. PI 157409, with appressed pubescence, was reported by Carlson (1969) to support moderate spider mite populations in greenhouse cage tests. In the present study, PI 157409 had the highest percentages of adult mortality and adults that left the leaf. However, these variables were not significantly different from other soybean accessions and had no effect on the number of progeny.

The number of progeny was significantly greater and feeding damage significantly less ($P < 0.001$) in the intact leaf bioassay than in the excised leaf bioassay. Differences in coefficients of variation were moderate between types of bioassays with higher values for adult mortality and number of progeny and lower values for adults leaving the leaf and feeding damage in the excised leaf bioassay.

Maturity groups V and VI. The main effects of accessions for all variables in both the excised and intact leaf bioassays were significant (Table 3). However, with the exception of feeding damage, the Ryan-Einot-Gabriel-Welsch multiple range test identified few significant differences among soybean accessions for the other variables in the excised leaf bioassay and no significant differences in the intact leaf bioassay. The soybean cultivar 'Tracy-M', a herbicide-tolerant cultivar with moderate resistance to several foliar feeding insects (Lambert and Kilen 1984), had the highest percentages of adult mortality and adults that left the leaf, lowest number of progeny, and second lowest feeding damage across excised and intact leaf bioassays combined. However, excluding feeding damage, Tracy-M was only significantly different from one other soybean accession in the excised leaf bioassay for adults that left the leaf and number of progeny. Wheatley and Boethel (1987) reported that the twospotted spider mite laid significantly fewer eggs on Tracy-M than 8 other soybean genotypes in a growth chamber leaf disk test.

The mean number of progeny in the intact leaf bioassay was significantly greater ($P = 0.004$) than in the excised leaf bioassay. Adults that left the leaf and feeding damage means in the excised leaf bioassay were significantly greater ($P < 0.006$) than in the intact leaf bioassay. Differences in coefficients of variation between types of bioassays were moderate with lower values for all variables in the excised leaf bioassay.

Maturity groups VII and VIII. None of the differences among soybean accessions for any of the variables in the intact leaf bioassay were significant (Table 4). In the excised leaf bioassay, there were significant differences among soybean accessions for number of progeny and feeding damage. All of the soybean accessions in this group, except the commercial cultivar 'Bragg', are resistant to foliar-feeding insects (Van Duyn et al. 1971, Lambert and Kilen 1984). Three of these lines, PI 171451, PI 229358, and D75-10169, had significantly fewer progeny in the excised leaf bioassay

Table 2. Twospotted spider mite adult mortality, adults that left the leaf, progeny, and feeding damage on selected soybean accessions in maturity group IV in excised and intact leaf laboratory bioassays

Accession	Maturity group	% adult mortality		% adults left leaf		Progeny/♀/d		Feeding damage*	
		Excised	Intact	Excised	Intact	Excised	Intact	Excised	Intact
Clark 63	IV	20.0ab	12.5a	13.3abc	10.4a	6.9b	10.5a	2.6e	1.5cd
Cutler 71	IV	10.0ab	20.8a	8.3abc	8.3a	6.2b	10.6a	3.4bcd	1.9bcd
Douglas	IV	13.3ab	20.8a	11.7abc	20.8a	6.5b	10.0a	3.1cde	1.3d
PI 80837	IV	15.0ab	14.6a	10.0abc	6.3a	5.9b	9.2a	2.8de	2.1abc
PI 157409	IV	30.0a	10.4a	26.7a	6.3a	6.5b	9.3a	3.7abc	2.8a
PI 506654	IV	16.7ab	8.3a	11.7abc	6.3a	6.7b	9.6a	4.3a	2.3ab
PI 507073	IV	25.0ab	10.4a	23.3ab	4.2a	7.5b	10.4a	4.1ab	2.6a
Control**		1.7b	6.3a	1.7c	4.2a	10.0a	10.2a	1.3f	1.4d
Mean		15.2	13.2	12.4	8.1	6.8	10.0	3.2	2.0
F		2.92	0.92	3.10	1.39	10.87	0.89	26.78	9.44
P		0.007	0.508	0.005	0.224	<0.001	0.531	<0.001	<0.001
CV		121.0	114.0	127.5	152.4	20.3	16.1	18.6	24.9

Means with the same letter within a column are not significantly different ($P > 0.05$) according to the Ryan-Einot-Gabriel-Welsch multiple range test (SAS Institute 1988). Excised leaf bioassay means of 3 test and 4 replications per test ($n = 12$). Intact leaf bioassay means of 2 tests and 4 replications per test ($n = 8$).

* Feeding damage rated on a 1 (normal green, no apparent damage) to 4 (extensive yellowing, moderate necrosos) scale.

** Control; leaf of Henderson bush lima bean.

Table 3. Twospotted spider mite adult mortality, adults that left the leaf, progeny, and feeding damage on selected soybean accessions in maturity groups V and IV in excised and intact leaf laboratory bioassays

Accession	Maturity group	% adult mortality		% adults left leaf		Progeny/♀/d		Feeding damage*	
		Excised	Intact	Excised	Intact	Excised	Intact	Excised	Intact
Essex	V	15.0a	12.5a	9.0abc	10.0a	7.3bc	8.8ab	2.4b	1.9bc
Forrest	V	24.0a	17.5a	22.0ab	2.5a	5.6d	7.7ab	2.8b	3.0a
Hill	V	28.0a	22.5a	23.0ab	17.5a	7.2bc	7.9ab	2.6b	2.3abc
S-100	V	18.0a	10.0a	14.0abc	0.0a	6.9bcd	9.2ab	2.8b	2.1abc
Shore	V	19.0a	10.0a	13.0abc	2.5a	7.2bc	7.1b	2.4b	1.5c
York	V	18.0a	27.5a	12.0abc	7.5a	6.4bcd	8.9ab	3.3a	2.1abc
MBB 84-20	V	19.0a	22.5a	15.0abc	12.5a	6.7bcd	8.5ab	1.8c	1.3c
Davis	VI	13.0a	12.5a	7.0bc	5.0a	7.6b	9.8ab	2.8b	2.6ab
Tracy-M	VI	30.0a	27.5a	24.0a	20.0a	5.8cd	6.9b	1.8c	1.6bc
Control**		15.0a	5.0a	5.0c	0.0a	9.7a	11.0a	1.0d	2.1abc
Mean		19.9	16.8	14.4	7.8	7.0	8.6	2.3	2.1
F		2.19	2.04	3.23	2.44	10.45	2.94	24.73	4.94
P		0.026	0.053	0.001	0.021	<0.001	0.007	<0.001	<0.001
CV		86.0	93.6	115.9	166.1	22.3	24.1	25.1	32.4

Means with the same letter within a column are not significantly different ($P > 0.05$) according to the Ryan-Einot-Gabriel-Welsch multiple range test (SAS Institute 1988). Excised leaf bioassay means of 5 tests and 4 replications per test ($n = 20$). Intact leaf bioassay means of 2 tests and 4 replications per test ($n = 8$).

* Feeding damage rated on a 1 (normal green, no apparent damage) to 4 (extensive yellowing, moderate necrosos) scale.

** Control; leaf of Henderson bush lima bean.

Table 4. Twospotted spider mite adult mortality, adults that left the leaf, progeny, and feeding damage on selected soybean accessions in maturity groups VII and VIII in excised and intact leaf laboratory bioassays

Accession	Maturity group	% adult mortality		% adults left leaf		Progeny/♀/d		Feeding damage*	
		Excised	Intact	Excised	Intact	Excised	Intact	Excised	Intact
PI 171451	VII	47.5a	37.5a	36.3a	17.5a	3.7b	4.9a	1.8b	2.6ab
PI 227687	VIII	33.8ab	27.5a	26.3ab	15.0a	5.6a	3.7a	1.8b	2.9a
PI 229358	VII	52.5a	30.0a	37.5a	10.0a	3.5b	4.4a	2.9a	2.1ab
D75-10169	VIII	48.8a	32.5a	43.8a	15.0a	3.4b	3.9a	2.3ab	2.0ab
Bragg	VII	40.0ab	30.0a	35.0a	10.0a	6.3a	5.9a	2.4ab	3.1a
Control**		18.8b	32.5a	7.5b	20.0a	7.3a	5.7a	1.0c	1.6b
Mean		40.2	31.7	31.0	14.6	5.0	4.8	2.0	2.4
F		4.60	0.26	4.74	0.51	12.29	0.89	13.10	4.34
P		0.001	0.932	0.001	0.768	<0.001	0.501	<0.001	0.004
CV		57.9	60.0	75.9	109.0	38.0	58.3	36.2	32.4

Means with the same letter within a column are not significantly ($P > 0.05$) according to the Ryan-Einot-Gabriel-Welsch multiple range test (SAS Institute 1988). Excised leaf bioassay means of 4 tests and 4 replications per test ($n = 16$). Intact leaf bioassay means of 2 tests and 4 replications per test ($n = 8$).

* Feeding damage rated on a 1 (normal green, no apparent damage) to 4 (extensive yellowing, moderate necrosos) scale.

** Control; leaf of Henderson bush lima bean.

than Bragg or PI 227687 which also had the lowest adult mortality of all lines. Wheatley and Boethel (1987) reported that significantly more twospotted spider mite eggs were laid on PI 227687 than on PI 171451 or PI 229358.

Adult mortality and number of progeny were not significantly different between the excised and intact leaf bioassays. The number of adults which left the leaf was significantly greater ($P < 0.001$) and feeding damage significantly lower ($P = 0.019$) in the excised leaf bioassay. Coefficients of variation were lower in the excised leaf bioassay for adult mortality, adults that left the leaf, and number of progeny.

Even though high levels of resistance to the twospotted spider mite were not identified in the soybean accessions screened in this study, results do demonstrate that the laboratory bioassays are capable of detecting differences in low levels of resistance or in susceptibility to the mite. Analysis of data between the excised and intact leaf bioassays for the different maturity groupings demonstrated few significant differences and, with two exceptions, the bioassay × entry interactions were nonsignificant. These data imply that the two bioassays are equally effective. However, in an overall comparison of the excised and intact leaf bioassays used in this study, the excised leaf bioassay demonstrated less variation among replications and tests for all variables measured and detected greater differences among accessions. The ability to infest, monitor, and record spider mite growth and development in the laboratory under a microscope and the fact that one replication of an intact leaf test utilizes the growth chamber space of four replications of a excised leaf test also favors the excised leaf bioassay as a more efficient, but not necessarily more effective bioassay.

In this study on 31 selected soybean accessions mean separations based on the conservative REQWQ test are presented to avoid falsly identifying resistant sources. The more liberal LSD mean separation test was included in all analyses (not presented) and did detect a greater number of significant differences among soybean accessions. For mass screening a large number of soybean lines an initial screening using the excised leaf bioassay and a liberal means comparison to avoid falsly rejecting potential sources of resistance would be more appropriate. Selected lines from the initial screening could then be rescreened using the intact leaf bioassay to confirm and help determine the mechanism of resistance.

Although there were significant differences among soybean accessions for specific variables, when you compare differences for all variables among accessions within a maturity grouping, results suggest that high levels of resistance to the twospotted spider mite are not present in any of the soybean lines screened in this study. These data also suggest that the gene(s) responsible for resistance to foliar feeding insects do not impart the same level of resistance to the twospotted spider mite. The USDA Agricultural Research Service maintains a collection of soybean germplasm comprised of over 14,000 strains. Most of this germplasm has been evaluated for resistance to the major disease and insect pests of soybean in the U.S. Fewer than 3500 soybean strains have been evaluated for resistance to spider mites and almost all of these were evaluated in the field. The present study demonstrates a laboratory bioassay to screen for spider mite resistance within the wide genetic diversity of a very large germplam collection.

Acknowledgments

Thanks are extended to P. Hebron and C. Crowe (USDA-ARS, Beltsville, MD) for technical assistance.

0.002) than those in the intact leaf bioassay. The coefficients of variation for all variables in the intact leaf bioassay were approximately twice those in the excised leaf bioassay which helps explain why means among accessions for adult mortality, adults that left the leaf, and progeny were not significantly different in the intact leaf bioassay.

Maturity group IV. There were no significant differences among soybean accessions for any of the variables except feeding damage in either the excised or intact leaf bioassay (Table 2). There were significant differences between the lima bean control and one or more soybean accessions for all variables in the excised leaf bioassay. PI 80837, the source of moderate Mexican bean beetle, *Epilachna varivestis* Mulsant, resistance in the soybean cultivar 'Shore' (Smith et al. 1975), had the lowest number of mite progeny in both types of bioassays but there was no indication of antibiosis. PI 157409, with appressed pubescence, was reported by Carlson (1969) to support moderate spider mite populations in greenhouse cage tests. In the present study, PI 157409 had the highest percentages of adult mortality and adults that left the leaf. However, these variables were not significantly different from other soybean accessions and had no effect on the number of progeny.

The number of progeny was significantly greater and feeding damage significantly less ($P < 0.001$) in the intact leaf bioassay than in the excised leaf bioassay. Differences in coefficients of variation were moderate between types of bioassays with higher values for adult mortality and number of progeny and lower values for adults leaving the leaf and feeding damage in the excised leaf bioassay.

Maturity groups V and VI. The main effects of accessions for all variables in both the excised and intact leaf bioassays were significant (Table 3). However, with the exception of feeding damage, the Ryan-Einot-Gabriel-Welsch multiple range test identified few significant differences among soybean accessions for the other variables in the excised leaf bioassay and no significant differences in the intact leaf bioassay. The soybean cultivar 'Tracy-M', a herbicide-tolerant cultivar with moderate resistance to several foliar feeding insects (Lambert and Kilen 1984), had the highest percentages of adult mortality and adults that left the leaf, lowest number of progeny, and second lowest feeding damage across excised and intact leaf bioassays combined. However, excluding feeding damage, Tracy-M was only significantly different from one other soybean accession in the excised leaf bioassay for adults that left the leaf and number of progeny. Wheatley and Boethel (1987) reported that the twospotted spider mite laid significantly fewer eggs on Tracy-M than 8 other soybean genotypes in a growth chamber leaf disk test.

The mean number of progeny in the intact leaf bioassay was significantly greater ($P = 0.004$) than in the excised leaf bioassay. Adults that left the leaf and feeding damage means in the excised leaf bioassay were significantly greater ($P < 0.006$) than in the intact leaf bioassay. Differences in coefficients of variation between types of bioassays were moderate with lower values for all variables in the excised leaf bioassay.

Maturity groups VII and VIII. None of the differences among soybean accessions for any of the variables in the intact leaf bioassay were significant (Table 4). In the excised leaf bioassay, there were significant differences among soybean accessions for number of progeny and feeding damage. All of the soybean accessions in this group, except the commercial cultivar 'Bragg', are resistant to foliar-feeding insects (Van Duyn et al. 1971, Lambert and Kilen 1984). Three of these lines, PI 171451, PI 229358, and D75-10169, had significantly fewer progeny in the excised leaf bioassay

Simpson, K. W. and W. A. Connell. 1973. Mites on soybeans: Moisture and temperature relations. Environ. Entomol. 2: 319-323.

Smith, T. J., H. M. Camper, Jr. and J. A. Schillinger. 1975. Registration of Shore soybean. Crop Sci. 15: 100.

Van Duyn, J. W., S. G. Turnipseed and J. D. Maxwell. 1971. Resistance in soybeans to the Mexican bean beetle. 1. Sources of resistance. Crop Sci. 11: 572-573.

Wheatley, J. A. C. and D. J. Boethel. 1987. Fecundity and egg hatchability of twospotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) reared on nine soybean genotypes. J. Entomol. Sci. 22: 147-152.