

INFLUENCE AND PERFORMANCE OF SOYBEAN LINES ISOGENIC  
FOR PUBESCENCE TYPE ON OVIPOSITION PREFERENCE  
AND EGG DISTRIBUTION BY CORN EARWORM  
(LEPIDOPTERA: NOCTUIDAE)

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(Accepted for publication 23 January 1989)

ABSTRACT

Soybean, *Glycine max* (L.) Merr., lines isogenic for dense (DE), normal (NL), and no (NO) pubescence developed from the cultivar 'Tracy-M' were used in free choice tests to determine their influence on corn earworm (CEW) *Heliothis zea* (Boddie) oviposition preference and distribution of eggs within plant canopy. Post oviposition performance of the isolines was determined. Of the total eggs deposited by CEW on excised leaves in a greenhouse oviposition arena, 64.5, 29.0, and 6.5% were on the DE, NL, and NO isolines, respectively. In a 614.2 m<sup>2</sup> field cage study, 57.0, 30.9, and 12.1% of eggs deposited were on the DE, NL, and NO isolines, respectively. Within plant egg distribution was proportionally the same for the DE and NL isolines with 83.7, 13.5, and 3.8% of eggs deposited on leaves, stems, and fruiting structures, respectively. However, the within plant egg distribution on the NO isolate was 90.0, 0.9, and 9.3% deposited on leaves, stems, and fruiting structures, respectively. Isolines with the greater number of eggs senesced later, produced fewer pods and fewer seeds per pod than isolines with fewer eggs. However, isolines with the greater number of eggs produced larger seeds, which resulted in ca. the same total seed weight per plant as isolines with fewer eggs. There were no differences among isolines grown without oviposition for pod number, seed number, or seed weight. The strong ovipositional preference among the isolines, if properly developed and used, has potential for use in insect management programs.

Key Words: *Glycine max*, *Heliothis zea*, host plant resistance, isolines, insect management.

J. Entomol. Sci. 24(3): 309-316 (July 1989)

INTRODUCTION

Type and amount of plant pubescence have been shown to influence many insect species in terms of preference, or nonpreference, for oviposition and food. Cotton, *Gossypium hirsutum* L., lines with sparse or no pubescence, when compared to lines with pubescence, are nonpreferred as an oviposition substrate by corn earworm (CEW), *Heliothis zea* (Boddie), (Lukefahr et al. 1971). There is great genetic variability within soybean, *Glycine max* (L.) Merr., germplasm for amount and type of pubescence (Bernard and Singh 1969). In soybean, certain types of pubescence appear to confer resistance to the potato leafhopper (PLH), *Empoasca fabae* (Harris) (Johnson and Hollowell 1935). However, reports conflict on amount

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and type of damage caused by PLH to pubescent types (Wolfenbarger and Slesman 1963). Panda (1969) observed a reduction in eggs deposited by CEW on soybean plants without pubescence. Studies conducted with *H. virescens* (F.) have shown that under laboratory conditions this species deposits the most eggs on excised soybean terminals from isolines with greater amounts of pubescence (Ramaswamy et al. 1987). However, no definitive study has been conducted with soybean and CEW using whole plants and field conditions to determine to what degree type and amount of pubescence influences ovipositional preference. Because variability in soybean pubescence is, in most instances, simply inherited and phenotypes are easily recognizable (Bernard and Singh 1969), the development of this factor to affect CEW populations should be relatively easy.

The purpose of this study was to determine if different amounts of soybean plant pubescence influences the ovipositional preference and within plant egg distribution of CEW to a degree which warrants full-scale evaluations and possible development for use in CEW management programs.

## MATERIALS AND METHODS

Soybean isolines classified as dense (DE), normal (NL), and no (NO) pubescence were developed from the cultivar 'Tracy-M'. Tracy-M is moderately resistant to several phytophagous insect species (Lambert and Kilen 1984). The breeding lines D62-7820 (dense pubescence) and D62-7812 (no pubescence), isolines of the cultivar 'Lee', were the donor parents. The isolines DE, NL and NO were developed by seven backcrosses to the recurrent parent (T.C.K., unpublished) and are isogenic for trichome density. Therefore, any differences among the isolines for CEW oviposition preference, within plant egg distribution and performance after exposure to ovipositing CEW moths are due to influences by pubescence.

### *Oviposition Preference, Greenhouse Study*

Oviposition preference among the isolines was tested in two environments. In the first environment, plants were grown in a greenhouse during November. To prevent fruiting, the light period was extended 4 h with artificial lighting. Plants were grown 4 per 4 liter pot. At the V-5 development stage (Fehr and Caviness 1977), a fully expanded leaf was excised from plants at the third node from the plant apex. The excision was done under water and the submerged leaf petiole placed in a tube (floral pik) containing water. A saran screen cage (1.22 m wide  $\times$  1.22 m high  $\times$  6.1 m long), the floor of which was covered with a 2.54 cm thick styrofoam sheet, was utilized as an oviposition arena. The tubes with leaves were pressed into the styrofoam sheet in a grid pattern with 20.3 cm between leaves in a randomized complete block design with 40 replications. Thus, leaves were in four row with 30 leaves per row. Fifteen pairs of CEW adults from a laboratory culture were released at 4:00 p.m. CDT within the cage four days after eclosion and allowed to remain overnight. The number of eggs deposited on each leaf was then determined. Data were analyzed by ANOVA procedure (SAS Institute 1985) and means separated by Waller-Duncan K-ratio t-tests at the  $P \leq 0.05$  level (Waller and Duncan 1969).

### *Oviposition Preference, Field Cage Study*

In the second environment, the isolines were planted during the last week of May inside a 19.2  $\times$  32.0 m cage frame (Lambert 1984). Plots were 5.3 m long  $\times$  4

rows on 0.7 m centers in a randomized complete block design with 12 replications. Plantings were six plots wide by six plots long within the cage. After emergence, plants were thinned to 20 per meter of row. Standard agronomic production techniques were used. At the R-1 stage of development (Fehr and Caviness 1977), a screen covering was placed over the cage frame and remained in place until plant senescence. On the day the screen cover was put in place 2000 pairs of four day old CEW imagos were released inside at 4:00 p.m. CDT. The insects were from a laboratory culture which had been reared for more than 10 generations. However, insects from the local wild population are systematically introduced into this culture annually. After 72 hours, 10 plants were randomly removed from each of the two center rows of each plot. These plants were carefully placed into plastic bags and transported to the laboratory in containers cooled with ice. Each plant was examined under 3× magnification and the number of eggs on leaves, stems, and fruiting racemes was determined. All parts of a plant excluding leaf surfaces and fruiting raceme were classified as stem. This included main stem, branches, and leaf petioles. Each bag was carefully examined for eggs dislodged from plants. The ANOVA procedure (SAS Institute 1985) was used to analyze data and the Waller-Duncan K-ratio t-test at the  $P \leq 0.05$  level (Waller and Duncan 1969) used to determine differences among isolines for egg deposition on leaves, stems, fruiting racemes, and whole plants. Chi<sup>2</sup> comparisons were used to determine within plant differences in oviposition preference among leaves, stems, and fruiting racemes.

#### *Leaf Trichome Density and Length*

Ten fully expanded leaves were collected from the top third of plants in the two center rows of each plot in the field cage study. Leaves were collected randomly in an effort to establish the average pubescence type and density to which insects were exposed. Total area of each leaf was measured with an area meter. By use of a dissecting microscope, the number of trichomes on a 20 mm<sup>2</sup> area of leaf laminae located between the midrib and first lateral vein was determined at each of two locations on the adaxial and abaxial surfaces. The total complement of trichomes is present at leaf initiation making trichome density on a fully expanded leaf a function of trichome number and leaf expansion. The amount of leaf expansion varies depending on the environment in which plants are grown. Therefore, leaf laminae area and trichome number per cm<sup>2</sup> are reported.

Number of trichomes were analyzed with plots as samples, leaves as subsamples and location on leaves as sub-subsamples. Data were analyzed by the ANOVA procedure (SAS Institute 1985) and mean separation determined by Waller-Duncan K-ratio t-test at the  $P \leq 0.05$  level (Waller and Duncan 1969).

#### *Post Oviposition Performance of Isolines*

Neonate through third stage larvae chew through the walls of flower buds and small pods and move inside these structures to feed. The task of daily dissecting and microscopically examining large numbers of these structures to determine the size and dynamics of the larval population was beyond our resources. Also, there is no method for accurately determining, on a daily basis, the absolute larval population on fruiting soybeans in small plots. Therefore, we opted to determine the performance of the isolines with and without CEW oviposition.

In addition to growing the isolines inside the field cage, they were grown adjacent to the cage in the same experimental design used inside the cage.

Senescence date, pods per plant, seeds per pod, weight of single seed and weight of total seeds per plant were determined for ten plants selected randomly from each replication under oviposition and nonoviposition conditions. Data were analyzed by the ANOVA procedure (SAS Institute 1985) and mean separations determined by Waller-Duncan K-ratio t-tests at the  $P \leq 0.05$  level (Waller and Duncan 1969).

## RESULTS AND DISCUSSION

### *Oviposition Preference, Greenhouse Studies*

In the greenhouse oviposition arena, 64.5, 29.0, and 6.5% of the egg were deposited on leaves from the DE, NL, and NO isolines, respectively (Table 1). These data show a significant ovipositional preference among isolines.

Table 1. Eggs deposited by corn earworm on excised leaves of Tracy-M soybean isolines in a greenhouse.

Isoline Pubescence Type	No. eggs/leaf*
Dense (DE)	26.0 a
Normal (NL)	11.7 b
None (NO)	2.6 c

\* Means not followed by same letter are significantly different ( $P \leq 0.05$ ).

### *Oviposition Preference, Field Cage Studies*

In the field cage, 57.0, 30.9, and 12.1% of eggs were deposited on the DE, NL, and NO isolines, respectively (Table 2). Again, there was a strong ovipositional preference among the isolines with only 54.1% as many eggs deposited on the NL isolate as on the DE isolate. Only 39.3% as many eggs were deposited on the NO isolate as on the NL isolate. Less than 0.01% of eggs from any of the isolines were dislodged within the plastic bags. Therefore, dislodged eggs did not influence the outcome of the study.

Table 2. Eggs deposited by corn earworm on leaves, stems, and flowering racemes and on whole plants of Tracy-M soybean isolines.\*

Plant part	Isoline Pubescence Type		
	Dense (DE)	Normal (NL)	None (NO)
Leaves	89.2 a,x	48.5 a,y	20.9 a,z
Stems	14.9 b,x	7.8 b,y	0.2 c,z
Flowering racemes	4.0 c,x	2.2 c,y	1.9 b,y
Whole plant	108.1 x	58.5 y	23.0 z

\* Means within columns not followed by same letter, a, b, or c, or within rows x, y, or z, are significantly ( $P \leq 0.05$ ) different.

Within plant egg distribution (Table 2) was proportionally the same for the DE and NL isolines. Approximately 84, 13, and 4% of eggs were deposited on leaves, stems, and fruiting structures, of the DE and NL isoline respectively. Terry et al. (1987) found in plant samples from fields of 'Ransom' and 'Bragg' soybeans with multiple planting dates that leaves, stems, and fruiting racemes made up 89.6, 10.1, and 0.3%, respectively, of the surface area. It is probable that this egg distribution primarily reflects the relative surface area of plants for egg deposition made up by these structures. Although, based on relative surface areas, there appears to be a slight preference for fruiting structures. Egg distribution on the NO isoline was 90.9, 0.9, and 9.3% of eggs deposited on leaves, stems, and fruiting racemes, respectively. Thus, when compared to the DE and NL isolines, stems were much less preferred and fruiting structures more preferred as oviposition sites on the NO isoline. We observed that moths seemed to have difficulty maintaining position while in the act of ovipositioning on the NO isoline. This was especially true on stems of this isoline, which probably accounts for some of the within plant egg distribution on the NO isoline. However, it does not appear to account for all of the differences among isolines for egg distribution because there were no easily observable differences in the ability of an ovipositioning moth to hold position on the DE and NL isolines. Thus, the differences in preference among isolines may reflect other differences such as a tactile response to amount of pubescence.

#### *Leaf Trichome Density and Length*

Trichome numbers on the isoline classified as DE were 38.2 and 61.4% more numerous on the abaxial and adaxial surfaces, respectively, than on the isoline classified as NL (Table 3). No trichomes were found on the isoline classified as NO. Trichomes were of varied lengths, ranging from 0.3 to 1.0 mm long. All trichomes were of the simple, unicellular type (Metcalf and Chalk 1979).

Table 3. Leaf laminae area and number of trichomes for Tracy-M soybean isolines.\*

Isoline Pubescence Type	Leaf Laminae area cm <sup>2</sup>	Trichomes/cm <sup>2</sup> on Abaxial Laminae	Trichomes/cm <sup>2</sup> on Adaxial Laminae
Dense (DE)	50.1 a	459.2 a	949.3 a
Normal (NL)	44.9 a	332.2 b	588.1 b
None (NO)	47.3 a	†	†

\* Means within columns not followed by the same letter are significantly different ( $P \leq 0.05$ ).

† No trichomes were found on the NO isoline.

#### *Post Oviposition Performance of Isolines*

There were no significant differences among isolines grown outside the cage for date of plant senescence, pods per plant, seeds per pod or seed weight per plant (Table 4). However, except for seed size and senescence date, every parameter measured was larger for the isolines grown outside of the cage than for isolines grown inside the cage (Table 4). Senescence date was earlier and seed size smaller for isolines grown outside the cage. This indicates that insects had an effect on all isolines grown inside the cage. However, since the screen cover was left in place

Table 4. Performance of Tracy-M soybean isolines with and without exposure to corn earworm (CEW) oviposition during first (R-1) fruiting.\*

Isoline pubescence type	Senescence Date		No. Pods Plant		No. Seeds/ Pod		Single Seed Wt (mg)		Total Seed Wt/plant (g)	
	w/out†	w/‡	w/out	w/	w/out	w/	w/out	w/	w/out	w/
	ovip	ovip	ovip	ovip	ovip	ovip	ovip	ovip	ovip	ovip
Dense (DE)	Oct 12	Oct 24	42.1 a	31.0 a	2.6 a	2.2 a	115 a	153 a	11.6 a	10.4 ab
Normal (NL)	Oct 12	Oct 21	42.9 a	32.4 a	2.4 a	2.2 a	116 a	131 b	11.9 a	9.3 a
None (NO)	Oct 12	Oct 16	42.7 a	39.4 b	2.4 a	2.3 b	118 a	120 c	12.1 a	10.9 b

\* Means within columns not followed by the same letter are significantly different ( $P \leq 0.05$ ).

† Without oviposition.

‡ With oviposition.

from the time of insect release until plant senescence, it is possible it may have had a slight, uniform effect on plant development.

When exposed to ovipositioning CEW moths, there were more pods per plant and more seed per pod on the NO isoline than on the NL and DE isolines. Examination of pods showed an equal number of seed sites per pod for all three isolines, but many missing seeds due to consumption by CEW. The DE and NL isolines had more seed and pods per plant consumed than did the NO isoline. Seed size was significantly ( $P \leq 0.05$ ) different among all isolines when exposed to oviposition. The DE isoline with the greatest egg population had the largest seed. The NO isoline with the smallest egg population had the smallest seed. Although there were fewer seeds per pod on the DE isoline than on the NO isoline, yields were not significantly different due to compensation by producing large seed. Although total seed weight was significantly less on the NL isoline than on the NO isoline, the difference was small.

When not exposed to ovipositioning CEW moths, all three isolines reached full senescence four days prior to the NO isoline with oviposition. With oviposition, the NL and DE isolines reached full senescence five and eight days, respectively, after the NO isoline. This difference in maturity was apparently caused by greater CEW damage to fruit forms on the DE and NL isolines. Delay in maturity and the development of larger seed, which compensates for fewer seed, is a common response to damage of soybean plants (Smith and Bass 1972; McAlister and Korber 1958). The high egg deposition in this study resulted in a larval population much higher than would normally be encountered under field conditions. Essentially, all fruit forms which began developing early on the three isolines were destroyed. It is possible that with a lower insect population the differences in yield would have been greater.

From these studies, it is clear that differences in pubescence of the isolines caused differences in oviposition preference and in within plant egg distribution by CEW and that differences in plant yield were caused by oviposition levels and subsequent larval populations. How these differences were brought about is to be an objective of future studies. It is probable that the differences in egg population size and within plant egg distribution alone, which resulted in different larval population sizes and distribution, caused the differences in isoline performance. However, differential mortality may have played a role as well. Regardless, the differences in oviposition preference and plant responses to oviposition were caused by the isogenic character of the isolines. These studies show that pubescence significantly influences oviposition preference of CEW and that more extensive investigations are justified. The possibility of using a DE type in combination with a NL or NO type in a trap crop insect management scheme is apparent. However, before full-scale development of pubescent types is undertaken, such matters as oviposition responses of CEW in no choice situations and behavioral and biological responses of other damaging and beneficial insect species to the different pubescent types should be investigated.

#### LITERATURE CITED

- Bernard, R. L., and B. B. Singh. 1969. Inheritance of pubescence type in soybeans: Glabrous, curly, dense, sparse, and puberulent. *Crop Sci.* 9: 192-97.
- Fehr, W. R., and C. E. Caviness. 1977. Stage of soybean development. *Coop. Ext. Serv., Ames, Iowa. Special Rep.* 80.

- Johnson, H. W., and E. A. Hollowell. 1935. Pubescent and glabrous characters of soybean as related to resistance to injury by the potato leafhopper. *J. Agric. Res.* 51: 371-81.
- Lambert, L. 1984. An improved screen cage design for use in plant and insect research. *Agron. J.* 76: 168-70.
- Lambert, L., and T. C. Kilen. 1984. Multiple insect resistance in several soybean genotypes. *Crop Sci.* 24: 887-90.
- Lukefahr, M. J., J. E. Houghtaling, and H. M. Graham. 1971. Suppression of *Heliothis* populations with glabrous cotton strains. *J. Econ. Entomol.* 64: 486-88.
- Metcalf, C. R., and L. Chalk. 1979. *Anatomy of the dicotyledons*, second edition (2 volumes). Clarendon Press, Oxford.
- McAlister, D. F., and O. A. Korber. 1958. Response of soybeans to leaf and pod removal. *Agron. J.* 50: 674-77.
- Panda, N. 1969. Pubescence as a major factor in oviposition and damage by the corn earworm, *Heliothis zea* (Boddie). Ph.D. dissertation, Univ. of Missouri, Columbia.
- Ramaswamy, S. B., Wai Keung Ma, and G. T. Baker. 1987. Sensory cues and receptors for oviposition by *Heliothis virescens*. *Entomol. Exp. Appl.* 43: 159-68.
- SAS Institute. 1985. *SAS user's guide: statistics version 5 ed.*, SAS Institute, Cary, NC.
- Smith, R. H., and M. Bass. 1972. Relationship of artificial pod removal to soybean yields. *J. Econ. Entomol.* 65: 606-08.
- Terry, I., J. R. Bradly, Jr., and J. W. Van Duyn. 1987. Within-plant distribution of *Heliothis zea* (Boddie) (*Lepidoptera*: Noctuidae) eggs on soybeans. *Environmental Entomol.* 16: 625-29.
- Waller, R. A., and D. B. Duncan. 1969. A bayers rule for the symmetric multiple comparison problem. *J. Amer. Stat. Assoc.* 64: 1484-99.
- Wolfenbarger, Dan A., and J. P. Slesman. 1963. Variation in susceptibility of soybean pubescent types, broad bean, and runner bean varieties and plant introductions to the potato leafhopper. *J. Econ. Entomol.* 56: 895-97.
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