STUDY TITLE

Efficacy of Cry1F Events TC1360 and TC1507

DATA REQUIREMENTS

Not Published

AUTHORS

[redacted]

STUDY COMPLETED ON

November 20, 1999

PERFORMING LABORATORY

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LABORATORY STUDY ID

PHI99-024

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No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA Section 10 (d)(1)(A)(B), or (C).

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Date: 11/29/99

STATEMENT OF COMPLIANCE

WITH GOOD LABORATORY PRACTICE STANDARDS

This study was not conducted in compliance with the requirements of 40 CFR Part 160.

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SIGNATURES OF APPROVAL

Study Number: PHI99-024

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Study Initiation Date: April 15, 1999

Records Retention: All study-specific raw data, methods, final reports and facility records will be retained at Pioneer Hi-Bred International, Johnston, Iowa.

Specimen Storage: Not applicable

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Efficacy of Cry1F Events TC1360 and TC1507

1.0 ABSTRACT

The purpose of this study was to evaluate Cry1F maize events TC1360 and TC1507 for efficacy against the most important Lepidopteran pests of maize in the United States. The insects tested were:

- European corn borer, *Ostrinia nubilalis* (Hübner);
- Southwestern corn borer, *Diatraea grandiosella* (Dyer);
- black cutworm, *Agrotis ipsolon* (Hugnagel);
- corn earworm, *Helicoverpa zea* (Boddie);
- fall armyworm, *Spodoptera frugiperda* (J.E. Smith).

TC1360 and TC1507 were evaluated in multiple hybrid backgrounds to test the efficacy across maize genotypes. Non-Bt isolines were included as controls, and current commercial events were included for comparison purposes.

Tables 1-3 describe the scoring schemes for European corn borer, corn earworm and fall armyworm. Results of the efficacy evaluations are shown in Tables 4-8.

TC1360 is no longer in product development and will not be part of the Cry1F registration package.

Table 1. Pioneer European Corn Borer Leaf Feeding Scoring System[1]

RATING	DESCRIPTION
9	No visible leaf injury or a small amount of pin or fine shot-hole type injury on a few leaves.
8	Small amount of shot-hole type lesions on a few leaves.
7	Shot-hole injury common on several leaves.
6	Several leaves with shot-hole and elongated lesions.
5	Several leaves with elongated lesions (ca. ½").
4	Several leaves with elongated lesions (ca. 1").
3	Long lesions common on about one-half of leaves.
2	Long lesions common on about two-thirds of leaves.
1	Most of leaves with long lesions

[1] From Guthrie, *et al.* (1960) with 1-9 in reverse order.

Table 2. Pioneer Corn Earworm Ear Damage Scoring System

RATING	DESCRIPTION
9	No damage to eartips or kernels, slight damage to silks or husks
8	Slight damage to silks, husks, or eartips but no kernel damage
7	Small damage to silks, husks, or eartips and slight damage to kernels (1-2 kernels damaged or lost)
6	Small damage to silks, husks, or eartips, and 0.1 - 1.0 cm (>2) kernels damaged or lost
5	Moderate damage to silks, husks or eartips, with 1.1 - 2.0 cm kernels lost
4	Moderate damage to silks, husks or eartips, with 2.1 - 3.0 cm kernels lost
3	Heavy damage to silks, husks or eartips, with 3.1 - 4.0 cm kernels lost
2	Heavy damage to silks, husks or eartips, with 4.1 - 5.0 cm kernels lost
1	Heavy damage to silks, husks or eartips, with 5.1(+) cm kernels lost

Table 3. Pioneer Fall Armyworm Leaf Feeding Scoring System[1]

RATING	DESCRIPTION
9	No visible leaf damage or only pinhole lesions present on whorl leaves.
8	Pinhole and small circular lesions present on whorl leaves.
7	Small circular lesions and a few small elongated (rectangular-shaped) lesions of up to 1.3 cm (1/2 ") in length present on whorl and furl leaves.
6	Several small to mid-size 1.3 to 2.5 (1/2" to 1") in length elongated lesions present on a few whorl and furl leaves.
5	Several large elongated lesions greater than 2.5 cm (1") in length present on a few whorl and furl leaves and/or a few small- to mid-sized uniform to irregular-shaped holes (basement membrane consumed) eaten from the whorl and/or furl leaves.
4	Several large elongated lesions present on several whorl and furl leaves and/or several large uniform to irregular-shaped holes eaten from furl and whorl leaves.
3	Many elongated lesions of all sizes present on several whorl and furl leaves plus several large uniform to irregular-shaped holes eaten from furl and whorl leaves.
2	Many elongated lesions of all sizes present on most whorl and furl leaves plus many mid- to large-sized uniform to irregular shaped holes eaten from the whorl and furl leaves.
1	Whorl and furl leaves almost totally destroyed.

[1] From Davis *et al.* (1992) 14-day rating scale with 1-9 in reverse order.

Table 4. European Corn Borer Efficacy Data (Johnston, Iowa, 1999)

Hybrid Group	Event	ECB1 Leaf Damage Rating[1,2]	Ear Shank Tunneling[1] (inches/plant)	Stalk Tunneling[1] (inches/plant)
Mycogen Hybrid M	TC1360	9.0a	0.0c	0.0c
Mycogen Hybrid M	TC1507	9.0a	0.0c	0.0c
Mycogen Hybrid M	Non-Bt	6.3b	0.2a	1.4b
Pioneer Hybrid N	TC1360	9.0a	0.0c	0.1c
Pioneer Hybrid N	TC1507	9.0a	0.0c	0.0c
Pioneer Hybrid N	Non-Bt	4.7b	0.1b	1.2b
Pioneer Hybrid D	TC1360	9.0a	0.0c	0.1c
Pioneer Hybrid D	Mon810	9.0a	0.0c	0.0c
Pioneer Hybrid D	Non-Bt	4.3b	0.0c	1.7a
Pioneer Hybrid D	Non-Bt	4.3b	0.1b	0.9b
Pioneer Hybrid P	TC1507	9.0a	0.0c	0.1c
Pioneer Hybrid P	Mon810	9.0a	0.0c	0.0c
Pioneer Hybrid O	TC1507	9.0a	0.0c	0.0c
Pioneer Hybrid O	Mon810	9.0b	0.0c	0.1c
Pioneer Hybrid Q	TC1507	9.0a	0.0c	0.0c

[1] Values are means of three replications at one site, planted in a randomized complete block design. Each replication consisted of the average score of up to 10 plants per entry. Means followed by the same letter are not statistically different (P = 0.05).

[2] Based upon Guthrie *et al.* (1960) with 1-9 in reverse order. See Table 1.

Table 5. Southwestern Corn Borer Efficacy Data (Plainview, Texas and Union City, Tennessee, 1999)

Hybrid Group	Event	Southwestern Corn Borer Tunneling[1] (inches/plant)
Mycogen Hybrid M	TC1360	0.0c
Mycogen Hybrid M	TC1507	0.0c
Mycogen Hybrid M	Non-Bt	3.5b
Pioneer Hybrid N	TC1507	0.1c
Pioneer Hybrid N	Non-Bt	3.9ab
Pioneer Hybrid D	TC1360	0.0c
Pioneer Hybrid D	TC1507	0.1c
Pioneer Hybrid D	Mon810	0.0c
Pioneer Hybrid D	Non-Bt	4.5a
Pioneer Hybrid D	Non-Bt	3.9ab
Pioneer Hybrid P	TC1507	0.0c
Pioneer Hybrid P	Mon810	0.0c
Pioneer Hybrid O	TC1507	0.0c
Pioneer Hybrid O	Mon810	0.0c
Pioneer Hybrid R	TC1507	0.0c

[1] Values are means of three replications at each of two sites, planted in a randomized complete block design. Each replication consisted of the average score of up to 10 plants per entry. Means followed by the same letter are not statistically different (P = 0.05).

Table 6. Corn Earworm Efficacy Data (Kauai, Hawaii, 1999)

Hybrid Group	Event	Ear Damage Rating[1,2]
Mycogen Hybrid M	TC1360	5.7b
Mycogen Hybrid M	TC1507	5.4bc
Mycogen Hybrid M	Non-Bt	3.8d
Pioneer Hybrid N	TC1360	5.3c
Pioneer Hybrid N	TC1507	5.0c
Pioneer Hybrid N	Non-Bt	3.6d
Pioneer Hybrid D	TC1360	6.6a
Pioneer Hybrid D	Mon810	6.5a
Pioneer Hybrid D	Non-Bt	3.4d
Pioneer Hybrid D	Non-Bt	3.8d
Pioneer Hybrid P	TC1507	5.6bc
Pioneer Hybrid P	Mon810	5.4bc
Pioneer Hybrid O	TC1507	5.6bc
Pioneer Hybrid O	Mon810	6.1ab
Pioneer Hybrid Q	TC1507	5.8abc

[1] Values are means of three replications at each of two sites, planted in a randomized complete block design. The two sites were planted during different growing cycles. Each replication consisted of the average score of up to 10 plants per entry. Means followed by the same letter are not statistically different (P = 0.05).

[2] See Table 2 for scoring system.

Table 7. Black Cutworm Efficacy Data (Huxley, Iowa and Johnston, Iowa, 1999)

Hybrid Group	Event	Mean Plant Survival (%)[1]
Mycogen Hybrid M	TC1360	100a
Mycogen Hybrid M	TC1507	98a
Mycogen Hybrid M	Non-Bt	50c
Pioneer Hybrid N	TC1360	100a
Pioneer Hybrid N	TC1507	88ab
Pioneer Hybrid N	Non-Bt	27d
AgrEvo	Cry 9CStarlink	81b

[1] Values are means of three replications at each of two sites, planted in a randomized complete block design. Each replication consisted of eight plants per entry. Means followed by the same letter are not statistically different (P = 0.05).

Table 8. Fall Armyworm Efficacy Data (Salinas, Puerto Rico, 1999)

Hybrid Group	Event	Fall Armyworm Leaf Damage Rating[1,2]
Mycogen Hybrid M	TC1360	9.0a
Mycogen Hybrid M	TC1507	9.0a
Mycogen Hybrid M	Non-Bt	1.0b
Pioneer Hybrid N	TC1360	8.5a
Pioneer Hybrid N	TC1507	8.0a
Pioneer Hybrid N	Non-Bt	3.3b
Pioneer Hybrid D	TC1360	8.7a
Pioneer Hybrid D	Mon810	7.0a
Pioneer Hybrid D	Non-Bt	1.0b
Pioneer Hybrid D	Non-Bt	1.0b
Pioneer Hybrid P	TC1507	8.5a
Pioneer Hybrid P	Mon810	2.7b
Pioneer Hybrid O	TC1507	8.3a
Pioneer Hybrid O	Mon810	2.7b
Pioneer Hybrid Q	TC1507	9.0a

[1] Values are means of three replications at one site, planted in a randomized complete block design. Means followed by the same letter are not statistically different (P = 0.05).

[2] Based upon Davis *et al.* (1992) with 1-9 in reverse order. See Table 3.

Conclusions:

Results from the 1999 field evaluations show that TC1360 and TC1507 provide excellent control of European corn borer, Southwestern corn borer, black cutworm, and fall armyworm and suppression of corn earworm. In all cases, both events were statistically more efficacious than the non-Bt isoline. TC1360 and TC1507 provide a broader spectrum of Lepidopteran control than the commercial transgenic events tested.

2.0 INTRODUCTION

The Cry1F maize events TC1360 and TC1507 were evaluated for efficacy against the most important Lepidopteran pests of maize in the United States. The insects tested were:

- European corn borer, *Ostrinia nubilalis* (Hübner);
- Southwestern corn borer, *Diatraea grandiosella* (Dyer);
- black cutworm, *Agrotis ipsolon* (Hugnagel);
- corn earworm, *Helicoverpa zea* (Boddie);
- fall armyworm, *Spodoptera frugiperda* (J.E. Smith).

Hybrids derived from TC1360 and TC1507 were compared to non-Bt isolines, and current commercial events were included for comparison purposes. TC1360 is no longer in product development and will not be part of the Cry1F registration package.

3.0 METHODS

At all locations, the hybrids were grown using the standard agronomic practices of the area, except foliar insecticides were not used when the insect population being tested was present. In addition, the Cry1F entries were segregating for the presence of Cry1F, so negative segregants were removed from the plot either by glufosinate application or by detection of the Cry1F protein through ELISA determination. Unless otherwise noted, each experimental unit consisted of a 15-ft. single row plot bordered on each end by 2.5-ft. alleyways. All plots were planted with a 30 inch row spacing

3.1 European Corn Borer

The European corn borer trial was planted at Johnston, IA in a randomized block design with three replications. At the V6 plant stage, entries that were segregating for the presence of Cry1F were leaf painted with Liberty herbicide mixed at a rate of 1.25 ml Liberty / 100 ml water. The mixture was applied to the upper surface of the V6 leaf with a cotton swab. Seven days later,

plants were evaluated for Liberty resistance and all Liberty-susceptible plants within the Cry1F plots were removed.

To evaluate for first generation European corn borer resistance, neonate larvae were infested beginning at the V6 plant stage. At V6 and every 2-3 days after, 50 larvae were applied to the whorl of each plant until 4 successful infestations had occurred. An infestation is deemed successful if there is no rainfall within 12 hours of application. Approximately 21 days after the last infestation, the plots are rated for ECB leaf feeding damage using a 1-9 rating scale (Table 2).

To evaluate for second generation European corn borer resistance, neonate larvae were infested beginning when the majority of the plants within the experiment were at 50% pollen shed. On that date and every 2-3 days after, plants were infested with 50 neonate larvae per date until 4 successful infestations had occurred. The larvae were applied to the leaf axil area from 3 leaves above the primary ear to 1 leaf below the primary ear and on the tip of the primary ear. Approximately 60 days after the last infestation, the plots were evaluated for shank and stalk tunneling damage. The primary ear shank and stalk were split longitudinally and the amount of tunneling was measured. Stalks were split from four nodes above the primary ear to the ground. Shanks were split from the ear to the stalk. Stalk tunnels are measured to the nearest inch, with the exception that any tunnel less than 1 inch is length is considered to be 1 inch. Because tunnels in the ear shank are generally much shorter, tunnels are measured to the tenth of an inch. Up to 10 plants per plot were evaluated, and the first and last plant within each plot were not evaluated to eliminate edge effects near the alleyways. The values within a plot were then combined to yield an average amount of stalk and shank tunneling per plant. Plot values were analyzed as a randomized block design using the SAS JMP software package (SAS Institute; 1995). Mean separations were conducted by calculating 2 X Mean Standard Error confidence intervals.

3.2 Southwestern Corn Borer

The plots were planted at Union City, TN and Plainview, TX. At both locations, the plots were planted after the normal corn planting period had concluded to ensure a natural infestation of Southwestern corn borer. The test was conducted in a two location, randomized design with three replications at each location. Entries that were segregating for the presence of Cry1F were sprayed with Liberty herbicide at a rate of 1.25 ml Liberty / 100 ml water to remove all non-transgenic plants from the rows.

When it was estimated that Southwestern corn borer damage had reached its peak, the plots were evaluated for feeding damage by splitting up to 10 plants per plot. The first and last plant within each plot was not evaluated to eliminate edge effects near the alleyways. The bottom 6 nodes of each stalk were split in half longitudinally using a sharp knife. The amount of stalk tunneling in each plant that was caused by Southwestern corn borer feeding was measured in inches. Stalk tunnels are measured to the nearest inch, with the exception that any tunnel less than 1 inch is length was recorded as 1 inch. The measurements within a plot were then combined to yield an average tunnel length per plant for the plot.

Plot values were analyzed as a two location randomized block design using the SAS JMP software package (SAS Institute; 1995). Mean separations were conducted by calculating 2 X Mean Standard Error confidence intervals.

3.3 Corn Earworm

Hybrids from Cry1F events TC1360 and TC1507 were tested against Corn earworm, *Helicoverpa zea* (Boddie) on the island of Kauai in Hawaii. The experiment was planted during two separate growing cycles. The first was planted in April of 1999 at Waimea, HW. The second planting was July 1999 at Koloa, HW. At both locations, the plots were planted after the majority of the corn in the surrounding area had been planted to ensure a heavy natural infestation of corn earworm.

The trials were conducted in a randomized block design with three replications at each planting date. Entries that were segregating for the presence of Cry1F were sprayed with Liberty herbicide at a rate of 1.25 ml Liberty / 100 ml water to remove all non-transgenic plants from the rows.

When plants reached the dough stage, the plots were evaluated for corn earworm damage using a 1-9 damage rating scale (Table 1). Husks were pulled back on the primary ear of up to 10 plants in each plot. The first and last plant within each plot were not evaluated to eliminate edge effects near the alleyways. Ears within each plot were rated individually, and then those ratings were averaged to yield the overall plot score. The number and instar of live corn earworm larvae in each plot was also recorded to determine if the Cry1F entries caused a reduction in corn earworm populations, or if they delayed corn earworm development. Plot values were analyzed as a two location randomized block using SAS JMP software (SAS Institute; 1995). Mean separations were conducted by calculating 2 X Mean Standard Error confidence intervals.

3.4 Black Cutworm

The black cutworm trials were conducted in a three location, randomized block design with three replications at each location. The trials were planted at Huxley, IA, Johnston, IA and Fowler, IN. The black cutworm infestation did not cause damage at the Fowler, IN location so that location was dropped from the data analysis. Each experimental unit consisted of 10 ft single row plots that were enclosed with metal sheeting to eliminate plot to plot movement of black cutworm larvae. When plants were at the VE leaf stage, entries that were segregating for the presence of Cry1F were sampled and tested using an ELISA designed to test for the presence of Cry1F protein. Plants that were found to be non-transgenic were removed from the plot. All plots were then thinned to leave 8 plants per enclosed row. At V1, each seedling was infested with 3-4 third instar black cutworm larvae.

After infestation, the number of plants that were destroyed by black cutworm feeding was recorded daily to monitor progression of black cutworm feeding. On day 13, the final number of plants surviving was recorded. The number of surviving plants per plot was analyzed as a two location randomized block using SAS JMP software (SAS Institute; 1995). Mean separations were conducted by Least Significant Difference.

3.5 Fall Armyworm

Hybrids from Cry1F events TC1360 and TC1507 were tested against fall armyworm at Salinas, Puerto Rico. The experiment was planted during two separate growing cycles. The first was planted in April of 1999 and the second planting was July 1999. For both planting dates, the plots were planted after the majority of the corn in the surrounding area had been planted to ensure a heavy natural infestation of fall armyworm.

The trials were conducted in a randomized block design with three replications at each planting date. Entries that were segregating for the presence of Cry1F were sprayed Liberty herbicide at a rate of 1.25 ml Liberty / 100 ml water to remove all non-transgenic plants from the rows. When the majority of the plots reached pollen shed, the plots were evaluated for fall armyworm leaf feeding damage. A 1-9 rating scale based on the 14-day leaf feeding scale described by Davis et al. (1992) was used to evaluate the plots (see Table 3). Each plot was given a single 1-9 value.

The July 1999 planting date was severely impacted by the presence of leaf disease. Those plots had to be destroyed before the plots could be evaluated, so that data was not included in the final analysis. The data from the April planting data were analyzed as a randomized block design using the SAS JMP software package (SAS Institute; 1995). Mean separations were conducted by calculating 2 X Mean Standard Error confidence intervals.

4.0 RESULTS

4.1 European Corn Borer (Table 4)

The artificial infestation level in this trial was not exceptional, but it was adequate to show the efficacy of the events against both first and second generation European corn borer. All of the TC1360 and TC1507 scored a 9 for ECB1 leaf damage and had 0 inches of shank tunneling. In the stalk, there were only a few short tunnels. These tunnels were most likely where late instar larvae had moved from the control rows onto the Cry1F material. In all cases, the tunnels were of less than one inch and no surviving larvae were found on the Cry1F hybrids.

4.2 Southwestern Corn Borer (Table 5)

Insect pressure was excellent at both locations as shown by the non-Bt isolines averaging 3.5 to 4.5 inches of stalk tunneling. All of the TC1360 and TC1507 hybrids averaged 0.0 to 0.1 inches of tunneling per plant. Any tunnels that were found on those entries were only small entrance holes where a late instar larva had moved onto the plant and attempted to tunnel into the stalk. No live larvae were found on any of the TC1507 or TC1360 hybrids. From these results it is obvious that both events show excellent control of Southwestern corn borer even under heavy natural infestations.

4.3 Corn Earworm (Table 6)

Corn earworm pressure was excellent during both planting dates as shown by the non-Bt isolines averaging 3.4 to 3.8 on the 1-9 rating scale. In all hybrid backgrounds, Cry1F events TC1360 and TC1507 scored significantly ($P<0.05$) higher than their non-Bt isolines and equal to the Mon810 entries. Mean scores did vary across hybrids within events probably due to differences in husk cover and silk channel length in the different hybrids. More open husks and shorter silk channels allow corn earworm larvae to establish on the ear more easily than closed husks and long silk channels (Wiseman *et al.* 1977). The results show that the Cry1F events TC1360 and TC1507 offer some protection against corn earworm feeding damage on the developing ear. Based upon the number and instar of corn earworm larvae on TC1360 and TC1507, it appears that expression of Cry1F reduces corn earworm survival and delays larval development, but some larvae do survive to pupation. This level of control, although significant, is not complete control as seen with the other Lepidopteran insects tested.

4.4 Black cutworm (Table 7)

There were statistically more surviving plants in the two Cry1F events compared to their non-transformed isolines. Both hybrids from Cry1F event TC1360 had 100% plant survival across all reps and locations, and hybrids from Cry1F event TC1507 had at least 88% plant survival. Both events were statistically more protective than the Cry9c Starlink event in at least one hybrid. In this study, MON810 hybrids were not included as controls because they do not offer BCW activity.

4.5 Fall Armyworm (Table 8)

Results from the Puerto Rico trial show clearly the complete control that Cry1F events TC1360 and TC1507 provide against fall armyworm. All hybrids in these two events scored 8 or better on the 1-9 rating system. Fall armyworm larvae are very mobile. Therefore, in most cases the level of damage seen on the Cry1F hybrids was caused by fall armyworm larvae moving from plot to plot. The non-Bt rows were fed upon to the extent that larval competition caused larvae to find alternative feeding sites. These late instar larvae do not survive exposure to TC1360 and TC1507; however, they can cause enough damage to drop the plot score from 9 to an 8.

5.0 CONCLUSIONS

Results from the 1999 field evaluations show that TC1360 and TC1507 provide excellent control of European corn borer, Southwestern corn borer, black cutworm, and fall armyworm and suppression of corn earworm. In all cases, both events were statistically more efficacious than the non-Bt isoline. TC1360 and TC1507 provide a broader spectrum of Lepidopteran control than the commercial transgenic events tested. TC1360 is no longer in product development and will not be part of the Cry1F registration package.

2.0 BIBLIOGRAPHY

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Table 1. Pioneer European Corn Borer Leaf Feeding Scoring System[1]

RATING	DESCRIPTION
9	No visible leaf injury or a small amount of pin or fine shot-hole type injury on a few leaves.
8	Small amount of shot-hole type lesions on a few leaves.
7	Shot-hole injury common on several leaves.
6	Several leaves with shot-hole and elongated lesions.
5	Several leaves with elongated lesions (ca. ½").
4	Several leaves with elongated lesions (ca. 1").
3	Long lesions common on about one-half of leaves.
2	Long lesions common on about two-thirds of leaves.
1	Most of leaves with long lesions

[1] From Guthrie, *et al.* (1960) with 1-9 in reverse order.

Table 2. Pioneer Corn Earworm Ear Damage Scoring System

RATING	DESCRIPTION
9	No damage to eartips or kernels, slight damage to silks or husks
8	Slight damage to silks, husks, or eartips but no kernel damage
7	Small damage to silks, husks, or eartips and slight damage to kernels (1-2 kernels damaged or lost)
6	Small damage to silks, husks, or eartips, and 0.1 - 1.0 cm (>2) kernels damaged or lost
5	Moderate damage to silks, husks or eartips, with 1.1 - 2.0 cm kernels lost
4	Moderate damage to silks, husks or eartips, with 2.1 - 3.0 cm kernels lost
3	Heavy damage to silks, husks or eartips, with 3.1 - 4.0 cm kernels lost
2	Heavy damage to silks, husks or eartips, with 4.1 - 5.0 cm kernels lost
1	Heavy damage to silks, husks or eartips, with 5.1(+) cm kernels lost

Table 3. Pioneer Fall Armyworm Leaf Feeding Scoring System[1]

RATING	DESCRIPTION
9	No visible leaf damage or only pinhole lesions present on whorl leaves.
8	Pinhole and small circular lesions present on whorl leaves.
7	Small circular lesions and a few small elongated (rectangular-shaped) lesions of up to 1.3 cm (1/2 ") in length present on whorl and furl leaves.
6	Several small to mid-size 1.3 to 2.5 (1/2" to 1") in length elongated lesions present on a few whorl and furl leaves.
5	Several large elongated lesions greater than 2.5 cm (1") in length present on a few whorl and furl leaves and/or a few small- to mid-sized uniform to irregular-shaped holes (basement membrane consumed) eaten from the whorl and/or furl leaves.
4	Several large elongated lesions present on several whorl and furl leaves and/or several large uniform to irregular-shaped holes eaten from furl and whorl leaves.
3	Many elongated lesions of all sizes present on several whorl and furl leaves plus several large uniform to irregular-shaped holes eaten from furl and whorl leaves.
2	Many elongated lesions of all sizes present on most whorl and furl leaves plus many mid- to large-sized uniform to irregular shaped holes eaten from the whorl and furl leaves.
1	Whorl and furl leaves almost totally destroyed.

[1] From Davis *et al.* (1992) 14-day rating scale with 1-9 in reverse order.

Table 4. European Corn Borer Efficacy Data (Johnston, Iowa, 1999)

Hybrid Group (doses of recurrent parent)	Event	ECB1 Leaf Damage Rating[1,2]	Ear Shank Tunneling[1] (inches/plant)	Stalk Tunneling[1] (inches/plant)
Mycogen Hybrid M	TC1360	9.0a	0.0c	0.0c
Mycogen Hybrid M	TC1507	9.0a	0.0c	0.0c
Mycogen Hybrid M	Non-Bt	6.3b	0.2a	1.4b
Pioneer Hybrid N (3)	TC1360	9.0a	0.0c	0.1c
Pioneer Hybrid N (2)	TC1507	9.0a	0.0c	0.0c
Pioneer Hybrid N	Non-Bt	4.7b	0.1b	1.2b
Pioneer Hybrid D (3)	TC1360	9.0a	0.0c	0.1c
Pioneer Hybrid D	Mon810	9.0a	0.0c	0.0c
Pioneer Hybrid D	Non-Bt	4.3b	0.0c	1.7a
Pioneer Hybrid D	Non-Bt	4.3b	0.1b	0.9b
Pioneer Hybrid P (2)	TC1507	9.0a	0.0c	0.1c
Pioneer Hybrid P	Mon810	9.0a	0.0c	0.0c
Pioneer Hybrid O (2)	TC1507	9.0a	0.0c	0.0c
Pioneer Hybrid O	Mon810	9.0b	0.0c	0.1c
Pioneer Hybrid Q (2)	TC1507	9.0a	0.0c	0.0c

[1] Values are means of three replications at one site, planted in a randomized complete block design. Each replication consisted of the average score of up to 10 plants per entry. Means followed by the same letter are not statistically different (P = 0.05).

[2] Based upon Guthrie *et al.* (1960) with 1-9 in reverse order. See Table 1.

Table 5. Southwestern Corn Borer Efficacy Data (Plainview, Texas and Union City, Tennessee, 1999)

Hybrid Group	Event	Southwestern Corn Borer Tunneling[1] (inches/plant)
Mycogen Hybrid M	TC1360	0.0c
Mycogen Hybrid M	TC1507	0.0c
Mycogen Hybrid M	Non-Bt	3.5b
Pioneer Hybrid N	TC1507	0.1c
Pioneer Hybrid N	Non-Bt	3.9ab
Pioneer Hybrid D	TC1360	0.0c
Pioneer Hybrid D	TC1507	0.1c
Pioneer Hybrid D	Mon810	0.0c
Pioneer Hybrid D	Non-Bt	4.5a
Pioneer Hybrid D	Non-Bt	3.9ab
Pioneer Hybrid P	TC1507	0.0c
Pioneer Hybrid P	Mon810	0.0c
Pioneer Hybrid O	TC1507	0.0c
Pioneer Hybrid O	Mon810	0.0c
Pioneer Hybrid R	TC1507	0.0c

[1] Values are means of three replications at each of two sites, planted in a randomized complete block design. Each replication consisted of the average score of up to 10 plants per entry. Means followed by the same letter are not statistically different (P = 0.05).

Table 6. Corn Earworm Efficacy Data (Kauai, Hawaii, 1999)

Hybrid Group	Event	Ear Damage Rating[1,2]
Mycogen Hybrid M	TC1360	5.7b
Mycogen Hybrid M	TC1507	5.4bc
Mycogen Hybrid M	Non-Bt	3.8d
Pioneer Hybrid N	TC1360	5.3c
Pioneer Hybrid N	TC1507	5.0c
Pioneer Hybrid N	Non-Bt	3.6d
Pioneer Hybrid D	TC1360	6.6a
Pioneer Hybrid D	Mon810	6.5a
Pioneer Hybrid D	Non-Bt	3.4d
Pioneer Hybrid D	Non-Bt	3.8d
Pioneer Hybrid P	TC1507	5.6bc
Pioneer Hybrid P	Mon810	5.4bc
Pioneer Hybrid O	TC1507	5.6bc
Pioneer Hybrid O	Mon810	6.1ab
Pioneer Hybrid Q	TC1507	5.8abc

[1] Values are means of three replications at each of two sites, planted in a randomized complete block design. The two sites were planted during different growing cycles. Each replication consisted of the average score of up to 10 plants per entry. Means followed by the same letter are not statistically different (P = 0.05).

[2] See Table 2 for scoring system.

Table 7. Black Cutworm Efficacy Data (Huxley, Iowa and Johnston, Iowa, 1999)

Hybrid Group	Event	Mean Plant Survival (%)[1]
Mycogen Hybrid M	TC1360	100a
Mycogen Hybrid M	TC1507	98a
Mycogen Hybrid M	Non-Bt	50c
Pioneer Hybrid N	TC1360	100a
Pioneer Hybrid N	TC1507	88ab
Pioneer Hybrid N	Non-Bt	27d
AgrEvo	Cry 9CStarlink	81b

[1] Values are means of three replications at each of two sites, planted in a randomized complete block design. Each replication consisted of eight plants per entry. Means followed by the same letter are not statistically different (P = 0.05).

Table 8. Fall Armyworm Efficacy Data (Salinas, Puerto Rico, 1999)

Hybrid Group	Event	Fall Armyworm Leaf Damage Rating[1,2]
Mycogen Hybrid M	TC1360	9.0a
Mycogen Hybrid M	TC1507	9.0a
Mycogen Hybrid M	Non-Bt	1.0b
Pioneer Hybrid N	TC1360	8.5a
Pioneer Hybrid N	TC1507	8.0a
Pioneer Hybrid N	Non-Bt	3.3b
Pioneer Hybrid D	TC1360	8.7a
Pioneer Hybrid D	Mon810	7.0a
Pioneer Hybrid D	Non-Bt	1.0b
Pioneer Hybrid D	Non-Bt	1.0b
Pioneer Hybrid P	TC1507	8.5a
Pioneer Hybrid P	Mon810	2.7b
Pioneer Hybrid O	TC1507	8.3a
Pioneer Hybrid O	Mon810	2.7b
Pioneer Hybrid Q	TC1507	9.0a

[1] Values are means of three replications at one site, planted in a randomized complete block design. Means followed by the same letter are not statistically different (P = 0.05).

[2] Based upon Davis *et al.* (1992) with 1-9 in reverse order. See Table 3.

APPENDIX 1

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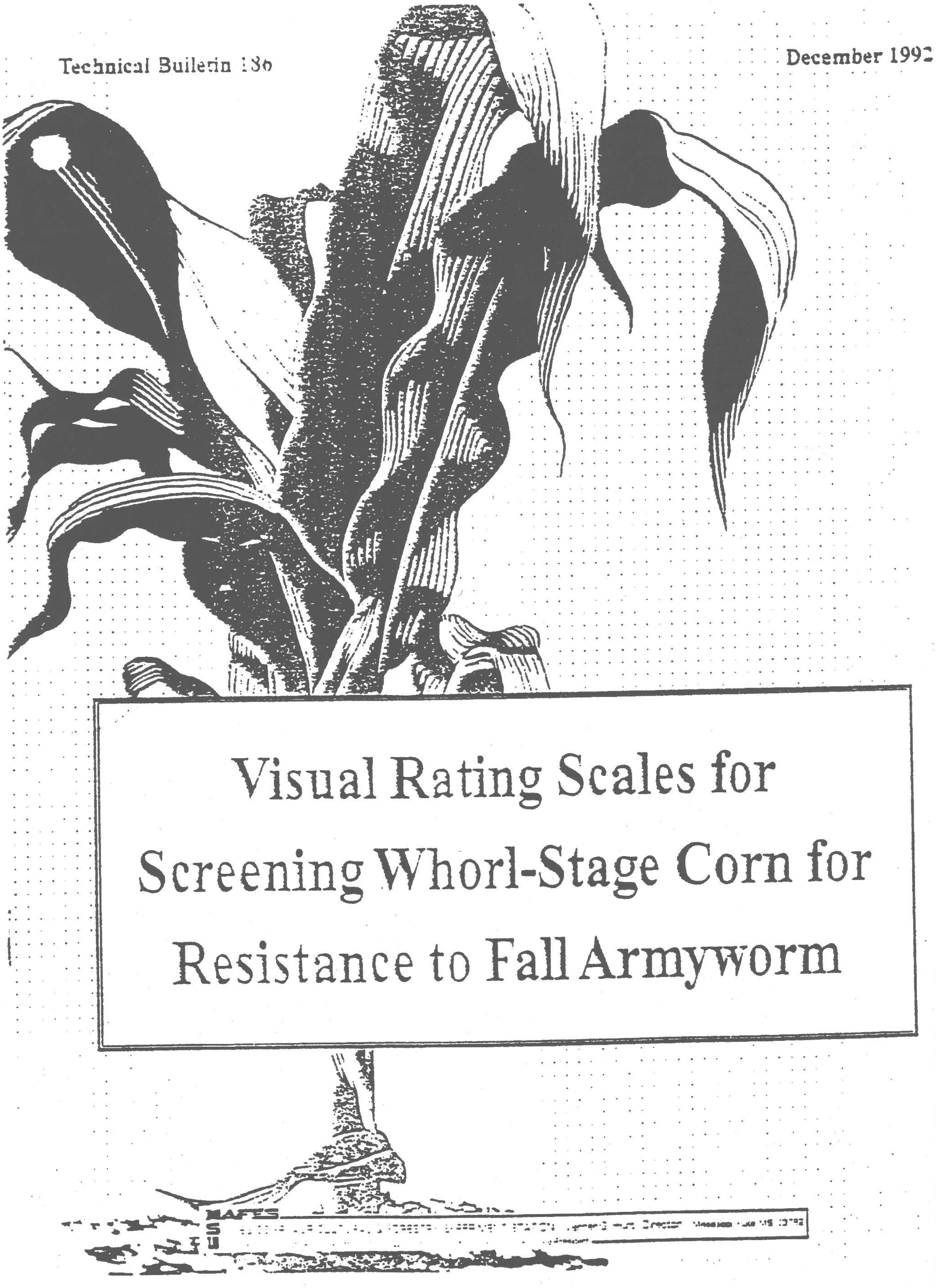

Visual Rating Scales for Screening Whorl-Stage Corn for Resistance to Fall Armyworm

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Visual Rating Scales for Screening Whorl-Stage Corn for Resistance to Fall Armyworm

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Published by the Department of Information Services, Division of Agriculture, Forestry, and Veterinary Medicine, Mississippi State University. Edited by Keith H. Remy, Publications Coordinator. Cover designed by Gae Mott, Student Artist.

Visual Rating Scales for Screening Whorl-Stage Corn for Resistance to Fall Armyworm

Introduction

Screening for leaf feeding resistance in corn, *Zea mays* L., to the fall armyworm, *Spodoptera frugiperda* (J.E. Smith), a major pest of this crop in the southeastern United States, consists of two components. The first component (infestation) brings the insect and host plant together. After some specified time, the second component (evaluation) characterizes the results of their interaction.

Corn has been screened for leaf feeding resistance since the mid-1970's at the Crop Science Research Laboratory (USDA-ARS) located at Mississippi State University. Over the years, the procedures for infesting corn with fall armyworms and evaluating the response of the plants to their attack have evolved with efforts to improve our ability to separate resistant from susceptible corn. In the early years, we relied on large, naturally occurring field populations of the fall armyworm to infest our late-planted corn for resistance screening.

By 1980, we were able to successfully rear the fall armyworm on a meridic diet in the laboratory (Davis, 1989). This allowed us to infest plants at the desired growth stage with a pre-selected number of fall armyworm neonate larvae. Now, each plant is infested in the mid-whorl stage of growth with 30 larvae. The larvae are mixed in corn cob grits and dispersed directly into the whorls of the plants using a hand-held plastic device (Davis et al., 1989; Mihm, 1989). This procedure has provided excellent uniformity of infestation, with plant escapes being rare.

An evaluation technique for characterizing the responses of plants to an insect's attack should be accurate and capable of differentiating among small differences in plant damage. Also, the technique should be fast and easy to execute because thousands of plants normally must be evaluated during the process of identifying and developing resistant germplasm.

To meet these criteria, we developed rating scales to visually evaluate differences in the degree of leaf feeding damage among corn genotypes. The scales are based on types and numbers of feeding lesions present on the leaves 7 and 14 days after infestation. The scales range from 0 to 9, where 0 indicates no visible damage and 9 indicates heavy damage. The 7-day scale was developed in 1987 to minimize the effect of migratory mid-instar larvae on rating scores, and to provide us an opportunity to control with insecticides the larvae feeding in the plant whorls after plants are rated. Control is not possible 14 days after infestation because most of the larvae have left the whorls to pupate in the soil.

Also in 1987, we revised the 14-day scale that we had previously developed for rating artificially infested mid-whorl stage corn for degree of leaf damage (Williams et al., 1983). The primary reasons for revising the scale were to parallel the 7-day scale in plant anatomy and insect feeding lesion terminology and to adjust scores for damage undoubtedly caused by migratory larvae on resistant plants.

These two rating scales have been used in our screening program since 1988 (Williams et al., 1989; Callahan et al., 1992). Also, we shared the scales with other plant resistance researchers. Some have reported adopting the scales for their use in screening for fall armyworm leaf feeding resistance in corn and sorghum (Diawara et al., 1990; Diawara et al., 1991; Wiseman and Isenhour, 1992). Only brief descriptions of the scales were presented in these publications. We report here the: (1) methodology used to devise the scales, (2) efficiency of the scales in separating resistant from susceptible corn genotypes, and (3) correlations between scales.

Methodology

Additional knowledge of the feeding behavior of the fall armyworm was needed to devise a damage scale based on 7 days of feeding and to revise the 14-day scale. Observations were made every other day on the progression of fall armyworm larval feeding on susceptible and resistant corn plants infested with 30 neonate larvae at the mid-whorl stage of growth. Types and sizes of lesions were recorded at each observation. Photographic slides of the damage were taken periodically to allow further study of lesion types. Information on lesion type and size was used in devising the 7-day scale and revising the 14-day scale.

Experiments were conducted in 1987 and 1988 to determine the efficiency of these scales in separating resistant from susceptible corn. In 1987, 15 inbred lines with known levels of susceptibility to leaf feeding by the fall armyworm were grown in a randomized complete block design in single-row plots, 5.1 m (16 ft) long with 1.0 m (38 in) between rows, with two replications. Each row was planted with 35 seeds and later thinned to 20 plants. Agronomic practices common to our area were used to grow the plants. When the plants were at the mid-whorl stage, each plant was infested with 30 neonate larvae. Seven days after infestation, each plant within a row was visually rated using the 7-day scale. The same plants were scored a week later using the revised 14-day scale. The following year, a similar experiment was conducted using 20 inbred lines. The inbreds were planted in three replications.

Data (plot means) were subjected to the ANOVA (Steel and Torrie 1980). Means were separated for significance using Student-Newman-Keuls' test. Correlations between rating scales were calculated for each experiment.

Because plant host and temperature are major factors governing larval growth rate, the number of heat units accumulated during the 7-day and 14-day feeding periods was calculated from temperature records provided by the Mississippi Agricultural and Forestry Experiment Station. The heat units for a given period were accumulated by summing daily heat units, which were estimated by subtracting the base temperature of 50°F from the mean daily temperature. As temperature influences insect growth rate and resulting damage to the plant by the larvae, this factor should be considered when making a decision on the number of days after infestation to evaluate the responses of the plants to the insects' attack.

Larval Feeding Habits

When mid-whorl stage corn is infested with fall armyworms, the larvae feed on leaf tissue within the whorl for their entire developmental period. Different types of feeding lesions are made as the larvae progress through their various instars (Fig. 1). Pinholes, small circular lesions, and small elongated (rectangular shaped) lesions of 1.3 cm (1/2 in) or less in length and about 0.2 cm (1/16 in) in width are made by 1- to 3-day-old larvae. These lesions occur normally between leaf veins.

When the larvae are about 5 days old, they begin to make mid-sized (up to 2.5 cm [1 in] in length) to large (greater than 2.5 cm in length) elongated lesions. These lesions usually range from 0.3 to 0.6 cm

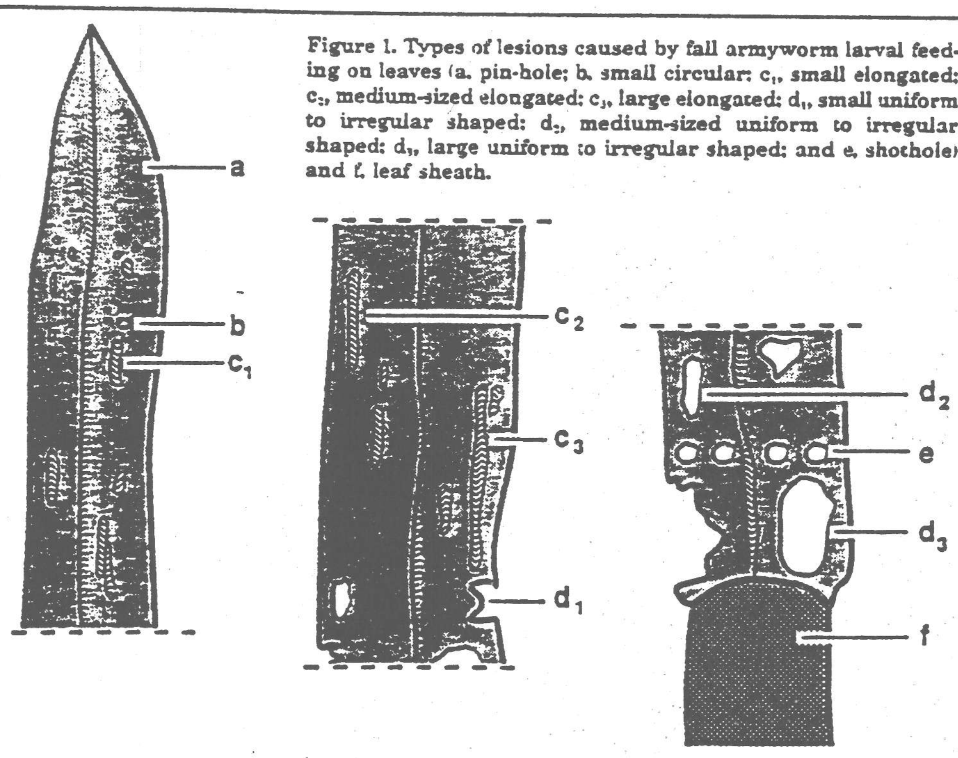

Figure 1. Types of lesions caused by fall armyworm larval feeding on leaves (a, pin-hole; b, small circular; c_1, small elongated; c_2, medium-sized elongated; c_3, large elongated; d_1, small uniform to irregular shaped; d_2, medium-sized uniform to irregular shaped; d_3, large uniform to irregular shaped; and e, shothole) and f, leaf sheath.

(1/8 to 1/4 in) in width. Larvae less than 5 days old feed as leaf surface scrapers and the basement membrane of the leaf remains intact.

When the larvae reach 6 to 7 days old, they begin to eat completely through the leaf, consuming the basement membrane. This type of lesion may be uniform or irregular in shape and range from small to medium in size. Leaf tissue may be completely consumed along the margins and inner portions of the leaf.

As the larvae continue to grow, larger portions of the leaves are consumed. Such lesions have been observed to be as large as 1.9 cm wide by 7.6 cm in length (3/4 in by 3 in). Additionally, when late instar larvae feed on the upper whorl and furl leaves, they will often eat directly through these tightly furled leaves. When the leaves unfurl, a series of uniform to irregular shaped lesions can be observed across the leaf. Often, these lesions resemble a line of shotholes. If the larvae feed through the tip of the furled leaves, the distal end of these leaves is truncated.

Information on fall armyworm larval feeding habits was used to devise a scale based on less than 14 days of larval infestation on the corn plants and to determine when an early leaf-damage rating should be taken. Seven days after infestation was chosen because (1) damage was easily visible, (2) differences between resistant and susceptible genotypes were evident, and (3) it was a transition period between larvae being leaf scrapers and becoming total leaf tissue consumers.

Table 1. A 7-day rating scale for visually estimating damage on leaves of mid-whorl stage corn caused by fall armyworm larval feeding.

Score	Description
0	No visible damage.
1	Only pinhole lesions present on whorl leaves.
2	Pinholes and small circular lesions present on whorl leaves.
3	Pinholes, small circular lesions and a few small elongated (rectangular shaped) lesions of up to 1.3 cm (1/2") in length present on whorl and furl leaves.
4	Small elongated lesions present on whorl leaves and a few mid-sized elongated lesions of 1.3 to 2.5 cm (1/2" to 1") in length present on whorl and/or furl leaves.
5	Small elongated lesions and several mid-sized elongated lesions present on whorl and furl leaves.
6	Small and mid-sized elongated lesions plus a few large elongated lesions of greater than 2.5 cm (1") in length present on whorl and/or furl leaves.
7	Many small and mid-sized elongated lesions present on whorl leaves plus several large elongated lesions present on the furl leaves.
8	Many small and mid-sized elongated lesions present on whorl leaves plus many large elongated lesions on the furl leaves.
9	Many elongated lesions of all sizes on whorl and furl leaves plus a few uniform to irregular shaped holes (basement membrane consumed) eaten from the base of the whorl and/or furl leaves.

Visual Rating Scale

The 7-day and 14-day scales for evaluating the degree of leaf damage caused by fall armyworms feeding on mid-whorl-stage corn are shown in Tables 1 and 2. Both scales utilize a 0 to 9 range of scores based on lesion types and number that occur on the whorl ..

Table 2. A 14-day rating scale for visually estimating damage on leaves of mid-whorl stage corn caused by fall armyworm larval feeding.

Score	Description
0	No visible damage.
1	Only pinhole lesions present on whorl leaves.
2	Pinhole and small circular lesions present on whorl leaves.
3	Small circular lesions and a few small elongated (rectangular-shaped) lesions of up to 1.3 cm (1/2") in length present on whorl and furl leaves.
4	Several small to mid-sized 1.3 to 2.5 cm (1/2" to 1") in length elongated lesions present on a few whorl and furl leaves.
5[1]	Several large elongated lesions greater than 2.5 cm (1") in length present on a few whorl and furl leaves and/or a few small- to mid-sized uniform to irregular shaped holes (basement membrane consumed) eaten from the whorl and/or furl leaves.
6[1]	Several large elongated lesions present on several whorl and furl leaves and/or several large uniform to irregular shaped holes eaten from furl and whorl leaves.
7	Many elongated lesions of all sizes present on several whorl and furl leaves plus several large uniform to irregular shaped holes eaten from the whorl and furl leaves.
8	Many elongated lesions of all sizes present on most whorl and furl leaves plus many mid- to large-sized uniform to irregular shaped holes eaten from the whorl and furl leaves.
9	Whorl and furl leaves almost totally destroyed.

[1]Scores 5 and 6 adjust for feeding damage caused by migratory mid-instar larvae. Plants that have feeding by migratory larvae exhibit a break in the sequence of types of larval feeding lesions. For example, a plant suffering from migratory larval feeding may exhibit pinholes, small circular lesions, and then small to large eaten out holes in the leaves. Missing in the feeding sequence are the mid-sized and large elongated lesions.

Table 3. Mean leaf feeding ratings for 15 corn inbred lines infested at the mid-whorl stage with 30 neonate fall armyworm larvae per plant (1987).[1]

Inbred	Susceptibility Classification[2]	Rating	
		7-Day	14-Day
NC252	S	7.7 a[3]	9.0 a
NC256	S	7.1 a	9.0 a
Tx601	S	7.1 a	8.9 a
Ab24E	S	7.0 a	8.9 a
NC262	S	6.8 ab	8.7 a
NC254	S	6.6 ab	9.0 a
NC258	S	6.4 ab	8.5 a
Mp496	R	5.8 abc	6.3 b
Mp703	R	5.2 bc	5.9 bc
Mp702	R	4.3 c	5.2 bc
Mp704	R	4.3 c	5.4 bc
Mp708	R	4.2 c	6.0 bc
Mp707	R	4.1 c	5.7 bc
Mp701	R	4.1 c	4.9 c
Mp705	R	4.0 c	6.4 bc

[1]The number of heat units (degrees above 50 °F) accumulated from day 1 through day 7 was 175.0 and 372.4 for the 14-day period.
[2]S = leaf feeding susceptible; R = leaf feeding resistant.
[3]Means within a column followed by the same letter do not differ significantly at the 0.05 level of probability (Student-Newman-Keuls' test).

Table 4. Mean leaf feeding ratings for 20 corn inbred lines infested at the mid-whorl stage with 30 neonate fall armyworm larvae per plant (1988).[1]

Inbred	Susceptibility Classification[2]	Rating	
		7-Day	14-Day
N236	S	8.1 a[3]	8.8 a
L873	S	7.5 ab	8.6 a
SC343	S	7.3 ab	8.7 a
NC252	S	7.0 bc	8.5 a
SC229	S	6.8 bc	8.3 a
B86	S	6.3 cd	8.2 a
Mp72:299	S	6.2 cd	8.1 a
Tx601	S	6.1 cd	8.2 a
Ab24E	S	6.0 cd	8.6 a
Va35	S	5.4 d	7.3 a
Mp496	R	4.5 e	6.1 b
Mp706	R	3.9 ef	5.9 bc
Mp702	R	3.7 fg	4.9 ed
Mp701	R	3.2 fgh	5.3 cd
Mp705	R	3.2 fgh	5.0 cd
Mp703	R	3.0 fgh	4.3 e
Mp707	R	3.0 fgh	4.3 e
Mp704	R	2.7 gh	4.9 ed
Mp78:518(MS)	R	2.6 gh	4.6 ed
Mp78:518(CIMMYT)	R	2.5 h	4.5 ed

[1]The number of heat units (degrees above 50 °F) accumulated from day 1 through day 7 was 131.5 and 392.0 for the 14-day period.
[2]S = leaf feeding susceptible; R = leaf feeding resistant.
[3]Means within a column followed by the same letter do not differ significantly at the 0.05 level of probability (Student-Newman Keuls' test).

and furl leaves. Lesion types range in severity from pinhole, small circular, small elongated, mid-sized elongated, large elongated to small, mid-sized and large uniform/irregular shaped holes eaten from the leaves. This severity range parallels the feeding sequence of the larvae as they grow to maturity.

Plants that show migratory larval feeding will have a break in the sequence of feeding lesions. Those plants that have only pinholes, small circular, and small elongated lesions on leaves but regular/irregular shaped lesions eaten from the furl leaves are examples of the effects of migratory larval feeding. The mid-sized and large elongated lesions are missing. Scores of 5 and 6 in the 14-day scale adjust for damage caused by migrating mid-instar larvae onto resistant plants. Also, the scales reflect the degree of larval antibiosis and/or nonpreference possessed by the plant.

As the adverse effects on larval growth and/or survival increase, leaf damage decreases. The rating scale described by Guthrie et al. (1960) for screening whorl-stage corn for resistance to the European corn borer, *Ostrinia nubilalis* (Hübner), was used as a model for formulating the scales.

When rating plants, we identify the types of lesions present first and then consider the number of lesions. The most severe lesion type(s) immediately gives the rater the approximate score. A final score is arrived at quickly by then observing lesion number. The association between rating score and leaf feeding damage caused by fall armyworm larvae is illustrated in Figures 2-5. At 7 days after infestation, the plant in Figure 2 rates a score of 4, whereas, the plant in Figure 3 rates a score of 9 using the 7-day scale. At 14 days after infestation, the plant in Figure 4 rates a score of 5 and the plant in Figure 5 rates a score of 9 using the 14-day scale.

Screening Efficiency

Both the 7-day and the 14-day scales are efficient in separating resistant from susceptible corn genotypes as shown in Tables 3 and 4. Additionally, the scales are highly correlated (r values for the two experiments equal 0.93 and 1987 and 0.97 in 1988).

Summary and Conclusions

Two rating scales have been devised and are in use for visually evaluating mid-whorl-stage corn plants for differences in degree of leaf feeding damage caused by the fall armyworm. They are based on the types of feeding lesions and numbers of these lesions present on the whorl and furl leaves 7 and 14 days after infesting each plant with 30 neonate larvae.

Under our normal summer temperatures, the larvae will have caused long elongated lesions and will have just begun to eat out uniform to irregular shaped holes from the leaves of highly susceptible genotypes by 7 days after infestation. By the 14th day after infestation, feeding on susceptible genotypes will have ceased, and the larvae will have entered the soil to pupate. In other locations, where temperatures are significantly cooler or warmer than ours, the day selected for rating may need to be adjusted to fit the larval feeding characteristics under their normal temperatures.

The 7-day and 14-day scales are highly correlated in their ability to separate resistant from susceptible genotypes. Either or both scale(s) can be used in screening for resistance and for experiments where leaf feeding ratings are needed. Thus, having available both 7-day and 14-day rating scales provides flexibility. The advantages of the 7-day scale are that it minimizes the effect of leaf damage caused by migrating mid-instar fall armyworm larvae by the rating occurring prior to most of the migration, and that it provides the researcher with the opportunity to use an insecticide to kill the larvae after rating while they are in the whorls. Management of these larvae is important where this pest poses a problem to other experiments and surrounding plant hosts. The 14-day scale gives the researcher a view of the total feeding damage caused by the larvae. It also provides an opportunity to determine if the plants show any late resistance response to the insect that would not be apparent at 7 days after infestation. An example of a delayed resistance response was observed by Diawara et al. (1990) in some converted sorghums being screened for resistance to the fall armyworm.

In conclusion, these scales have proven to be: (1) accurate in their abilities to separate resistant from susceptible genotypes, (2) capable of differentiating among small differences in damage, (3) fast (requiring only 1 to 2 minutes per row of 20 plants, and (4) easy to execute after some practice.

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Figure 2. Mid-whorl-stage corn plant with leaf feeding damage caused by fall armyworm larvae 7 DAI has a visual rating score of 4.

Figure 9. Mid-whorl-stage corn plant with leaf feeding damage caused by fall armyworm larvae 7 DAI has a visual rating score of 9.

Figure 4. Mid-whorl-stage corn plant with leaf feeding damage caused by fall armyworm larvae 14 DAI has a visual rating score of 5.

Figure 5. Mid-whorl-stage corn plant with leaf feeding damage caused by fall armyworm larvae 14 DAI has a visual rating score of 9.

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Leaf and sheath feeding resistance to the EUROPEAN CORN BORER in eight inbred lines of DENT CORN

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CONTENTS

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LEAF AND SHEATH FEEDING RESISTANCE TO THE EUROPEAN CORN BORER IN EIGHT INBRED LINES OF DENT CORN

W. D. GUTHRIE[1], F. F. DICKE[2], and C. R. NEISWANDER[3]

INTRODUCTION

The development of hybrids resistant to the European corn borer, *Pyrausta nubilalis* (Hbn.), has been in progress for over 30 years and has become an integral part of the corn breeding programs in several states and some seed companies. These investigations began when practically all corn grown was open-pollinated. For several years the work on varietal resistance consisted of testing open-pollinated varieties, inbred lines, and hybrids to locate resistant germ plasm.

At the present time the practice of direct extraction of lines from special crosses, commonly referred to as second cycle breeding, and from special synthetic varieties is being used extensively. Agronomically desirable lines with a good level of resistance, even though one of the parents used was susceptible, have been produced with this breeding method. In some instances the procedure of breeding commonly referred to as transference through backcrossing in combination with various methods of intensification or recurrent selection has been used successfully.

Most of the varietal resistance factors studied thus far are most effective against the newly-hatched larvae of the first-brood infestation on corn in the "whorl" stage of growth. Such resistance is usually referred to as resistance to larval establishment and survival. For the European corn borer it is actually resistance to leaf blade feeding. However, some growth inhibiting effects and abnormal mortality have been observed in the third and fourth instar larvae which feed mainly on

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the midrib and the leaf sheath. The study reported herein was designed to extend our information on both leaf blade and sheath feeding resistance of the first-brood infestation.

REVIEW OF LITERATURE

Three of the earliest reports of differences in borer survival in strains of corn were those of European investigators. Roubaud (1928) artificially infested five French varieties of corn with newly-hatched larvae and found almost complete mortality of larvae on Dent de Cheval. The variety Hâtif d' Auxonne was partially resistant. Roubaud suggested that since the varieties of corn grown in the United States and Canada were more favorable for the development of the corn borer than were the European and particularly the French varieties, the American strains should be replaced by the more resistant European strains. Hase (1929) also found the variety Pferdezahn (Dent de Cheval) to be practically immune to corn borer attack; of 4,368 larvae deposited on 728 plants, less than 1 percent were recovered at harvest. The two German varieties, Baden and Pommern, favored the development of borers.

Ellinger and Chorine (1930, 1931) reported that a South African corn (Natal), which was believed to be of the same origin as Dent de Cheval, was resistant to the corn borer.

In the early research, the differences in borer survival between open-pollinated varieties, inbred lines, or hybrids were thought to be due to the time of planting, to the seasonal habit or size of growth, and the stage of maturity of the corn plant; the early maturing strains of corn were less resistant to attack by the borer than late strains (Felt 1921, Caffrey 1924, Cutright and Huber 1928, Huber *et al.* 1928, Salter *et al.* 1928, Neiswander and Huber 1929, Patch 1929, Kelsheimer and Polivka 1931, Ficht 1936, Meyers *et al.* 1937, Huber 1939).

Meyers *et al.* (1937) reported that Jablonowski, who studied the corn borer for more than 30 years in Hungary, noted, as early as 1898, that early varieties suffered more from corn borer attack than the later varieties; in 1918 early varieties suffered 50 percent greater loss than later varieties. These authors also reported that a Russian, Krassilstchik, noted differences in infestation between varieties in 1914-1915.

One of the early reports on breeding for corn borer resistance was made by Marston (1930a, 1930b, 1931, 1933). The variety Maize Amargo, which is a flint variety developed in Argentina from material believed to have been introduced from Hungary, was observed to be resistant to corn borer attack. This variety was crossed with several native dent varieties. The F_1 generation of these crosses was heavily infested (1930a), leading Marston to believe that susceptibility was dominant and resistance recessive. In the F_2 generation there was a lighter infestation on the plots of Maize Amargo crosses than in the native parent plots. Of 935 F_3 families tested, 227 were not infested. Marston interpreted this as a simple Mendelian ratio of 3 to 1 and believed that the resistance of Maize Amargo was a simple recessive character. Marston (1933) also showed that the resistance of Maize Amargo was transmissible to the progeny of its crosses.

Marston and Mahoney (1933) crossed Maize Amargo with a few varieties of sweet corn in an attempt to select resistant sweet corn types. Lines of Golden Bantam × Maize Amargo were selected which were highly resistant and equal to inbred lines of Golden Bantam in other respects.

In a later article, Marston (1936) reported that Michigan hybrid 561, which had Maize Amargo in its pedigree, was resistant to attack. Patch *et al.* (1938), however, reported this hybrid to be no more resistant in respect to the number of mature borers surviving from a given number of eggs than the susceptible single cross, A × Tr. In all resistance tests reported by Marston and co-workers, the infestation originated from natural oviposition.

Since the early work on resistance, many investigators have discovered that most strains of corn vary somewhat in their comparative resistance and tolerance. The following investigators have reported on differences in relative resistance among open-pollinated varieties, inbred lines, and hybrids: Huber and Herr 1931; Huber 1937; Patch 1937; Patch and Bottger 1937; Thompson 1938, 1939; Huber and Stringfield 1940; Thompson 1940; Patch *et al.* 1941; Pepper and Garrison 1941; Thompson 1941; Huber and Stringfield 1942; Patch *et al.* 1942; Patch and Deay 1948; Patch and Everly 1948; Patch *et al.* 1951. Some of these tests were made under infestations originating from the natural oviposition, whereas others were made under an artificial infestation simulating as nearly as possible natural infestation conditions.

Dicke and Penny (1956) list the source of resistance in new experimental field corn inbreds.

The genetic basis of corn borer resistance has been postulated by several investigators. Patch *et al.* (1942) concluded from tests on a large number of open-pollinated varieties, inbred lines, and dent corn hybrids during the period 1930 to 1939 that the different inbred lines varied in their inherent resistance to survival of the corn borer, and that this resistance was transmitted to hybrids. The authors suggested that resistance was the result of an undetermined number of multiple factors, and that lines showing the greatest degree of resistance contained the largest number of these factors. In these tests the plots were artificially infested, and the number of borers surviving in the summer was determined by dissections. The relative resistance was measured as the percentage deviation of the observed population of borers from the predicted population on the date of silking of the strain of corn.

From a study of 977 sweet corn inbreds, which were artificially infested, Schlosberg and Baker (1948) found that 44 manifested some resistance, and the results obtained from single crosses indicated incomplete dominance of either resistance or susceptibility. The intercrosses of resistant and susceptible parents generally showed results intermediate between those obtained from crosses within resistant and susceptible groups of inbreds.

The results obtained by Singh (1953), in a study of the segregation for leaf feeding differences in the F_2 and first backcross generation of a cross between a resistant and susceptible inbred line, showed a slight tendency for phenotypic dominance of susceptibility. The results also gave an excellent fit to a two-factor pair hypothesis.

Ibrahim (1954) used a large number of chromosomal interchange lines to determine which chromosomes carried genes differentiating the borer resistance of inbred line A411, which was derived from A344 × L317, from the susceptibility of inbred line A344. His data indicated that the resistance of A411 was due to at least one gene in the long arm of chromosome 3, one in the long arm of chromosome 4, and probably one in the long arm of chromosome 5. The resistance of A411 was considered dominant in all crosses studied.

Penny and Dicke (1956) reported on resistance to leaf feeding of a group of F_3 and backcross progenies of a susceptible × resistant cross, M14 × MS1. Segregation of genes for borer resistance at three or more loci with at least partial phenotypic dominance of susceptibility was indicated. In a B14 × N32 cross, one or two gene pairs for leaf feeding resistance were indicated on the basis of individual plant segregations in F_2 and first backcrosses. The F_3 and selfed backcross progeny ratings could not be explained on a single locus basis.

In a later article, Penny and Dicke (1957) reported that ratings of leaf feeding on plants in F_2 and backcross progenies from two susceptible × resistant crosses (the susceptible parents were M14 and WF9; the resistant parent, gl_7 v_{17}, used in both crosses was a stock homozygous for two very closely linked genes, glossy, gl_7, and virescent, v_{17}) indicated that resistance differences were conditioned by segregation of genes at a single locus. The resistance gene was linked with gl_7 v_{17} genes of the resistant parent with cross-over frequencies estimated at from 31 to 37 percent.

The role of chemical substances recovered from the corn plants in inhibiting the growth of larvae has been studied by Beck (1951, 1957a, 1957b), Smissman *et al.* (1957a, 1957b), and Loomis *et al.* (1957). These authors have isolated several chemical factors that inhibit the development of larvae, designated as resistance factors RFA, RFB, and RFC.

Resistance Factor A, 6-methoxybenzoxazolinone, is ether-soluble (Beck *et al.* 1957; Smissman *et al.* 1957a, 1957b; Loomis *et al.* 1957). Neither Resistance Factor B, which is water-soluble, nor Resistance Factor C, which is ether-soluble, have been isolated and characterized (Beck *et al.* 1957).

The inbred lines WF9, W204, W210D, W22, and W22RB were utilized by Beck (1957c) in a study of the role of growth-inhibiting chemical factors in the resistance to the establishment of corn borer larvae. Total resistance factor activity, as determined by bioassay, was reported to be in close agreement with field test ratings of the inbreds. Resistance Factor A was primarily responsible for resistance to leaf feeding on early plant growth stages but negligible after the development of a visible tassel. Resistance Factors B and C contributed about equally to the resistance factor activity found in internode, leaf sheath, husk, and silk tissues.

Painter (1941, 1951, 1954, 1958) and Snelling (1941a, 1941b) discuss the complex of insect resistance in crop plants. These authors list many references on insect resistance.

MATERIALS AND METHODS

Eight inbred lines of dent corn were assembled for a study of their resistance to survival of the first and second larval instars, as expressed by feeding damage to the leaf blade in the whorl stage of development, and their degree of resistance to the third and fourth larval instars, as expressed by feeding lesions on the sheath, midrib, and around the collar. The eight lines were selected on the basis of observed differences in establishment and survival of the first and second larval instars and also on survival of the third and fourth larval instars. However, no quantitative study of resistance of these lines to third and fourth instar larvae has been made. Information on the origin of the lines studied is as follows:

The experimental inbred (W24 × Ind. B2)-2-38-1-Sel. was developed by L. H. Penny and F. F. Dicke at Ankeny, Iowa. W24 is a first cycle line from the open-pollinated variety Golden Daybreak formerly grown in Minnesota. Ind. B2 was developed from Reid yellow dent at the Purdue University Agricultural Experiment Station by R. R. St. John. This line was used in the parent single cross as a source of both moderate leaf feeding and sheath feeding resistance.

W22R was recovered from (W22 × Hy). The recurrent parent (W22) was developed from a single cross (Ill.B10 × W25). Hy, used as the non-recurrent parent, was derived from the variety Illinois High Yield by A. M. Brunson at the Kansas Agricultural Experiment Station and further selected by C. R. Holbert in Illinois after the fifth generation of inbreeding. W22R was developed in Wisconsin by N. P. Neal.

A295 is a direct extraction from (A344 × L317), selection 1088. The original selection was made in an F_3 population by F. F. Dicke in a cooperative resistance breeding project between the U. S. Department of Agriculture and the Minnesota Agricultural Experiment Station from which it was released. Minn. A344 is a sub-strain of Ia. 153 which was derived from the variety Minn. 13. L317 is a derivative from the open-pollinated variety Lancaster Surecrop. Both of these inbreds were originated by M. T. Jenkins at the Iowa Agricultural Experiment Station.

Oh43 is a derivative of (Oh40B × W8), Oh40B being a direct isolate of a composite of eight Lancaster Surecrop lines. W8 is a second cycle inbred derived from (Minn. 13 × Ill. A48) and developed at the Wisconsin Agricultural Experiment Station by N. P. Neal.

Oh51A is a recovered line from (Oh51 × Oh17). Oh51 was used as the recurrent parent and is a derivative of the open-pollinated variety, Clarage. Oh17 was used as the non-recurrent parent and was developed from an ear to row breeding stock. Oh43 and Oh51A were developed by G. H. Stringfield at the Ohio Agricultural Experiment Station.

B14 was developed from a Stiff Stalk synthetic variety by G. F. Sprague at the Iowa Agricultural Experiment Station. This inbred was included because of its outstanding stalk qualities.

M14 was developed by B. E. Moews of Granville, Illinois. It was derived from a single cross (BR10 × R8) and released from the Illinois Agricultural Experiment Station. The origin of BR10 and R8 is obscure.

WF9 was derived from the open-pollinated variety, Reid yellow dent, by R. R. St. John at the Purdue University Agricultural Experiment Station. This inbred is probably the most extensively used inbred in making up commercial hybrids.

These studies were initiated in 1955 and concluded in 1956. With minor exceptions, the experimental methods were similar for the two-year period. The inbred lines were planted in randomized blocks consisting of 26-foot single-row plots with sixfold replication. In order to escape an infestation from the natural moth population, the plots were planted two to three weeks later than normal, depending on seasonal weather conditions.

The experimental plots followed clover in the rotation, and 300 pounds per acre of a 5-10-10 analysis fertilizer were applied in the row before planting. A 33 1/3 percent ammonium nitrate fertilizer was applied as a side-dress application at the rate of 100 pounds per acre when the plants were 12 to 15 inches in extended leaf height.

In 1955 each plant in the experimental plots was artificially infested with six egg masses (approximately 120 eggs), whereas in 1956 the plants were infested with five egg masses (approximately 100 eggs) per plant. The egg masses were incubated to near the hatching stage before being placed in the whorl of the plants.

In 1956 the application of egg masses was made when the plants were in the mid-whorl stage of growth. The height of the inbred lines, as measured from ground level to the tip of the longest leaf, ranged from 27.0 to 32.1 inches. In 1955 the application of egg masses was made when the plants were in a slightly later stage of development. The inbred lines ranged from 27.4 to 36.9 inches in extended leaf height. These tests simulated, as nearly as possible, the natural first-brood infestation.

The pattern of larval survival on the inbred lines was determined by dissecting samples at intervals of 5, 10, 20, and 30 days after egg hatch. In 1955 a four-plant sample was dissected in each plot 5, 10, 20, and 30 days after egg hatch. In 1956 a four-plant sample in each plot was dissected five days after egg hatch, whereas a six-plant sample was dissected 10, 20, and 30 days after egg hatch. The samples on each of the 5-, 10-, 20-, and 30-day intervals were taken at random from all plots in a split-plot arrangement.

Leaf feeding ratings and lesion and burrow counts were made 20 and 30 days after egg hatch.

A nine-class rating scale was used for evaluating borer leaf feeding in the whorl stage of plant development. Only injury caused by larvae feeding in the whorl was used in the leaf rating determinations, i.e., lesions on the sheath collar, sheath, and midrib were not considered in these determinations. In the relative resistance scale, lines which rated 1 to 3 are considered resistant, lines which rated 4 to 6 are considered intermediate in resistance, and lines which rated 7 to 9 are considered highly susceptible. Classification into a resistant, intermediate, or susceptible class is dependent upon the size and shape of leaf injuries, and rating within each class is determined by the number of holes or amount of feeding. A general description of the visual leaf feeding rating classes for evaluating the amount of plant injury for different levels of larval establishment and survival is given in the following summary:

Class 1. No visible leaf injury or a small amount of pin or fine shot-hole type of injury on a few leaves.

Class 2. Small amount of shot-hole type lesions on a few leaves.

Class 3. Shot-hole injury common on several leaves.

Class 4. Several leaves with shot-hole and elongated lesions.

Class 5. Several leaves with elongated lesions.

Class 6. Several leaves with elongated lesions (about 1 inch).

Class 7. Long lesions common on about one-half of the leaves.

Class 8. Long lesions common on about two-thirds of the leaves.

Class 9. Most of the leaves with long lesions.

Examples of classes 1, 5, and 9 are illustrated in Figures 1-3. Dicke (1954) has discussed the biology of the first brood of the corn borer in the corn plant and its relation to the leaf feeding rating system which is important in resistance investigations.

Lesions on the sheath, midrib, and around the collar are caused primarily by the feeding of the third and fourth larval instars. The lesion counts were made on the basis of the number and size of the lesion, i.e., a midrib or sheath lesion 1 to 1½ inches long was counted as one lesion, but a midrib or sheath lesion 6 inches in length was counted as four lesions. Likewise, a lesion which girdled one-third of the collar was counted as one lesion, a lesion which girdled two-thirds of the collar was counted as two lesions, and a lesion which completely girdled the collar, as in Figure 4, was counted as three lesions. This method gave a better index of injury to the midrib, sheath, and sheath collar than is

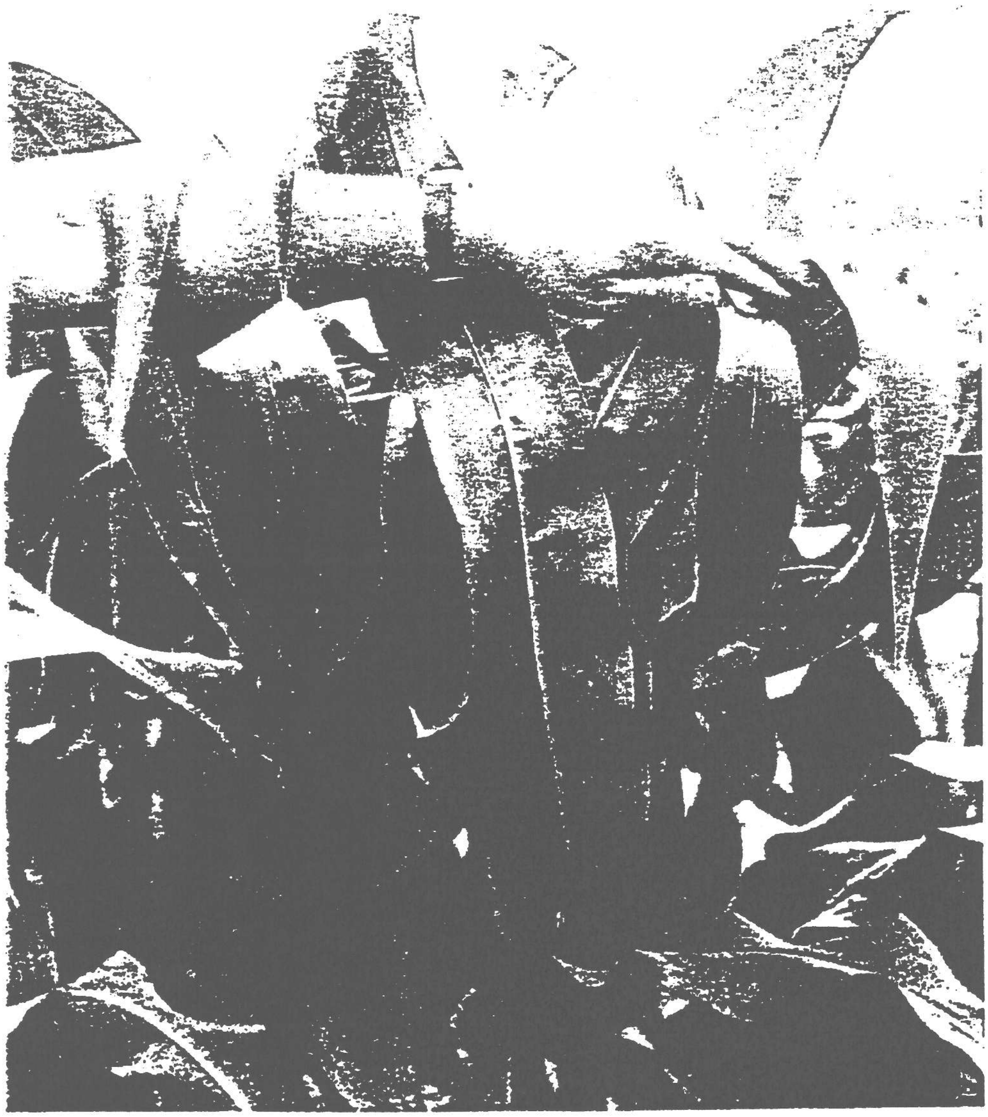

Fig. 1.—A class 1 visual leaf rating showing small and few lesions caused by young larvae feeding in the whorl. Typical of a resistant reaction to the first-brood corn borers.

possible by disregarding the size of the lesion. Figures 5 and 6 show typical feeding of third and fourth instar larvae on the sheath and midrib.

Fig. 2.—A class 5 visual leaf rating showing several leaves with elongated lesions. Typical of an intermediate reaction to the first-brood corn borer.

Fig. 3.—A class 9 visual leaf rating showing numerous elongated lesions caused primarily by first and second instar larvae feeding in the whorl. Typical of a highly susceptible reaction to the first-brood corn borer.

Fig. 4.—Lesion caused primarily by third and fourth instar larvae feeding in the sheath collar.

The methods used in first-brood resistance studies have evolved slowly. During the early work, the criterion used as an index for open-pollinated, hybrid, or inbred line performance was the number of larvae that survived which was usually determined by dissection in late July or

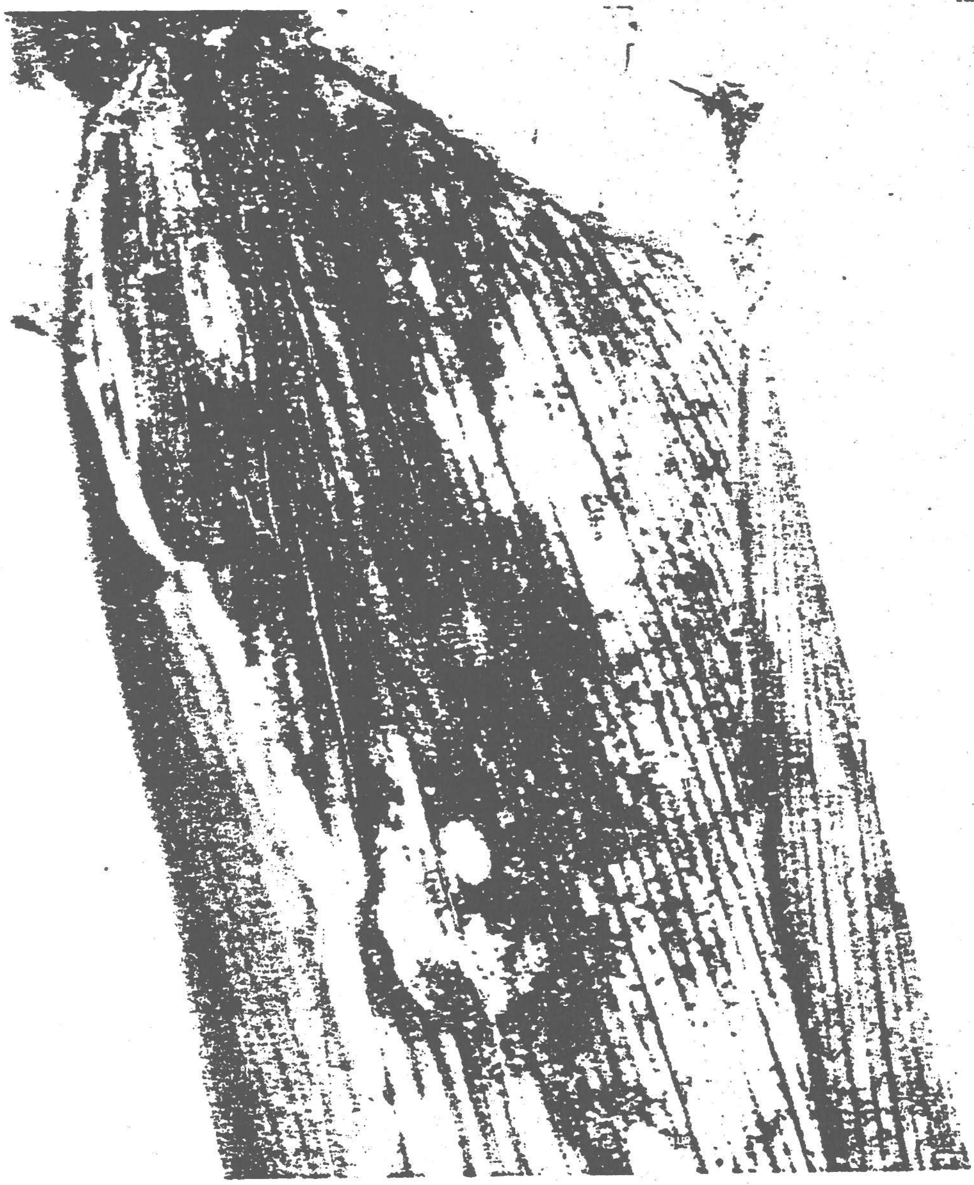

Fig. 5.—Lesions caused primarily by third and fourth instar larvae feeding in the sheath.

early August. This method involved a great deal of work for evaluating only a few hundred entries. Huber (1937) used leaf feeding puncture counts as an index of resistance to first instar larvae. Patch and Everly (1945) used mature larvae in the fall and also leaf feeding ratings (class 0 = least, to 10 = highest infestation level) for evaluating

Fig. 6.—Lesions caused primarily by third and fourth instar larvae feeding in the midrib of the leaf.

a group of inbred lines and hybrids. The leaf feeding rating method and the sheath and midrib lesion count method and combinations thereof were perfected and used on a large scale for evaluating inbred lines and hybrids by F. F. Dicke[4]. A nine-class scale was used for evaluating inbred lines and a five-class scale for evaluating hybrid material (class 1 = least, to 5 or 9 = highest infestation level). He considered the whorl type feeding and also the midrib, sheath, and sheath collar type feeding in the class scale. In tests on hybrids and late generation inbreds, samples of ten plants were usually artificially infested with three or four egg masses (60 or 80 eggs) per plant. The inbreds were rated on a plot basis before pollination. This system preserves the resistant cultures for pollination and progeny testing which is particularly valuable in individual plant selections in segregating populations to study inheritance of resistant factors. This is an excellent method for screening a large amount of material. The highly susceptible material can easily be discovered and discarded. To establish an accurate evaluation of resistance, several tests are essential because activities of several predators may destroy the egg masses or young larvae on certain entries. By using this visual method for evaluation of inbred lines or hybrids, one investigator can test many fold the amount of material as would be possible by dissection. However, for detailed information on the nature of resistance a combination of methods is most desirable.

Since the plots were planted in randomized blocks and the plant dissections were made in a split-plot arrangement, the data on surviving larvae, leaf feeding ratings, and lesion and burrow counts were analyzed according to split-plot procedure (Cochran and Cox 1950). The inbred lines were on the whole plot area, and the dissection intervals of 5, 10, 20, and 30 days after egg hatch were on the split-plot area.

In order to determine the degree of association between surviving larvae, leaf feeding ratings, lesion counts, and burrows, mean values for the lines were used to obtain simple correlation coefficients. Regression coefficients were also computed. By using the means of the six replications in the correlation and regression coefficient determinations, the errors involved in estimating the means were ignored. Larval establishment and survival, resulting from the artificial infestations, were on a much higher level in 1956 than in 1955. Therefore, correlation and regression coefficients were determined for only the 1956 data.

All data were analyzed on a probability per plant basis. The analysis of variance for the data is presented in Appendix Tables 6-8.

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[4]Unpublished data.

EXPERIMENTAL RESULTS

LARVAL ESTABLISHMENT AND SURVIVAL

The data on larval survival, leaf feeding ratings, and lesion and burrow counts obtained in these studies are summarized in Tables 1-5. The conclusions arrived at are based on the two-years' results. However, since a much higher level of larval establishment was obtained in 1956 than in 1955, the data for 1956 give a better picture of what is expected when high populations prevail. In a study of this kind it is imperative to have a high level of larval establishment and survival in order to obtain expression of the resistance factors that exist. The level of larval survival in 1955 was too low to show differences, at the desired magnitude, between inbred lines.

The level of larval survival in the inbred lines in 1956 was satisfactory for evaluating varying levels of resistance by the four criteria: (1) surviving larvae, (2) leaf feeding ratings, (3) lesion counts, and (4) burrows.

TESTS OF DENT CORN INBRED LINES, 1955 AND 1956

The levels of significant differences for the eight inbred lines in 1955 and 1956 are indicated by the usual characters in Table 1. The analysis was based on mean number of larvae, leaf feeding ratings, lesions, and burrows per plant. The analysis of data for 1955 shows significant differences among inbreds, dissection intervals, and the interaction of inbreds × dissection intervals for larvae, significant differences among inbreds for leaf feeding ratings, and significant differences among inbreds and dissection intervals for burrows. There were no significant differences among inbreds, dissection intervals, or the interaction of inbreds × dissection intervals for lesions.

The analysis of data for 1956 shows significant differences among inbreds, dissection intervals, and the interaction of inbreds × dissection intervals for larvae, leaf feeding ratings, lesions, and burrows.

Since the performance of the inbred lines for each dissection interval of 5, 10, 20, and 30 days after egg hatch is of primary interest, the data on the main effect of dissection intervals recorded in Table 2 are of little interest. The over-all effect of inbred lines on larval survival is also of little interest and can be determined by computing means for the dissection intervals of each inbred line, as presented in Tables 3 and 4.

The interaction of inbreds × dissection intervals, which measures the rate of larval mortality, is of greatest interest. These data are reported in Tables 3 and 4 for 1955 and 1956, respectively. Although the analysis of variance for the 1955 data (Table 1) shows significant differences among inbred lines, dissection intervals, and the interaction of inbreds × dissection intervals for larvae, an examination of the data (Table 3) reveals that these differences were due primarily to the differential in larval survival among lines five days after egg hatch. The differences among lines for larvae 10, 20, and 30 days after egg hatch and for leaf feeding ratings, lesions, and burrows 20 and 30 days after egg hatch were small and of minor importance.

Although the level of larval establishment and survival in 1955 was very low, a trend in inbred performance is obvious. B14 (12.3 larvae per plant) followed by WF9 (6.9 larvae per plant) harbored the greatest population five days after egg hatch. W22R, A295, and Oh43 harbored a population of 1.4, 1.9, and 2.3 larvae per plant, respectively, five days after egg hatch, whereas (W24 × B2)-2-38-1, Oh51A, and M14 harbored similar populations of 3.5, 4.0, and 4.0 larvae per plant, respectively. The larval population in all lines decreased appreciably between five and ten days after egg hatch, but did not decrease appreciably from 10 days to 20 and 30 days after egg hatch. By the end of 10, 20, and 30 days after egg hatch there was not much differentiation in larval survival between any of the lines. Although B14 had the highest population five days after egg hatch, the larval population in this inbred decreased rapidly, and at the end of 30 days after egg hatch the population was at a low level.

The low leaf feeding ratings, lesion counts, and burrows for all lines in 1955 resulted from the high larval mortality that occurred within five days after egg hatch; practically all of the larvae had perished by the end of ten days after egg hatch. The larval population was too low in most of the inbreds five days after egg hatch to cause extensive damage to leaf tissue.

A much higher level of larval establishment and survival resulted from the artificial infestation in 1956. Therefore, the resistance effect on the larvae over the 30-day period was measured with more reliability. All four dissection intervals will, therefore, be used as a basis for determining inbred performance. The leaf feeding ratings 20 days after egg hatch and the lesion and burrow counts 30 days after egg hatch will be used as a basis for determining inbred performance. The reason for using only the 20-day leaf feeding ratings and 30-day lesion counts is discussed in a later section entitled "Best Time for Making Leaf Feeding Ratings and Lesion Counts in Resistance Investigations." The following discussion is based on the data recorded in Table 4. The standard error of the means and the differences between inbreds required for significance at the 5 percent probability level are indicated.

The majority of the larvae were in the first and second instar stage of development during the first ten days after egg hatch. Therefore, larval mortality during this period was used as an index to the degree of resistance of the inbred lines to the whorl type of feeding by the first and second instar larvae. Larval mortality between the 10- and 20-day dissection intervals was used as an index to the degree of resistance of the lines to the third and fourth larval instars. However, mortality of the fourth larval instar also occurred beyond 20 days after egg hatch. The majority of the larvae were in the fifth instar stage of development on the 30-day dissection interval.

Based on the four criteria, mean number of larvae, leaf feeding ratings, lesions, and burrows per plant, the experimental inbred (W24 × B2)-2-38-1 was highly resistant to the first and second larval instars. This line may also be resistant to the third and fourth larval instars. However, since the rate of larval mortality was so rapid (only 4.5 larvae per plant survived five days after egg hatch thus resulting in a larval mortality of 95.5 percent), it is difficult to measure the resistance of this line to the feeding of the third and fourth instar larvae. In order to determine conclusively if resistance factors of inbred lines, which are highly resistant to leaf feeding of the early larval instars, are effective against the third and fourth instar larvae, the infestation would have to originate from third instar larvae. There was appreciable larval mortality on this line beyond five days after egg hatch; only 2.7, 1.6, and 1.0 larvae surviving 10, 20, and 30 days after egg hatch, respectively. The fast rate of larval mortality was also reflected in the low leaf feeding rating (1.6 per plant), lesion count (2.4 per plant), and burrows (0.8 per plant) of this line.

W22R was highly resistant to the first and second larval instars. The rate of larval mortality was rapid in this inbred, 4.6 larvae per plant surviving five days after egg hatch; 2.3, 3.0, and 2.4 larvae per plant survived 10, 20, and 30 days after egg hatch, respectively. This line also had a low leaf feeding rating (1.7 per plant) and burrow count (2.5 per plant). However, the lesion count was somewhat higher (4.5 per plant) than would be expected from the fast mortality rate.

A295 was highly resistant to the first and second larval instars. The rate of larval mortality, however, was somewhat slower in A295 than it was in (W24 × B2)-2-38-1 and W22R; 7.7, 3.9, 2.2, and 1.9

larvae per plant surviving 5, 10, 20, and 30 days after egg hatch, respectively. The leaf feeding rating (2.4 per plant) and burrow count (2.8 per plant) were also low for A295. The lesion count (4.2 per plant) was similar to the lesion count for W22R.

Oh43 was resistant to the first and second larval instars but somewhat susceptible to the third and fourth instars. The rate of larval mortality was rather fast but slower than in the three inbreds discussed above; 9.7 larvae per plant surviving five days after egg hatch. As is reflected in the low leaf feeding rating (1.8 per plant), the larval population of 9.7 per plant five days after egg hatch and then a decrease in population to 3.5 larvae per plant ten days after egg hatch was not high enough to cause extensive damage to whorl leaf tissue. However, the larval population of 3.9 and 2.4 per plant 20 and 30 days after egg hatch appears to be high enough to cause considerable damage to the midrib, sheath, and collar as is reflected by the lesion count of 5.6 per plant. Oh43 had 2.8 burrows per plant 30 days after egg hatch.

Oh51A appears to be somewhat susceptible to the first and second larval instars but resistant to the third and fourth instars. The rate of larval mortality was considerably slower than in the four previously discussed inbreds. The larval population of 14.1 and 5.3 per plant five and ten days after egg hatch, respectively, is reflected in an intermediate leaf feeding rating (3.8 per plant). The population died out rapidly beyond ten days after egg hatch, 3.7 and 2.0 larvae per plant remaining 20 and 30 days after egg hatch, respectively, which is reflected in a low lesion count of 2.9 per plant. Oh51A also had a low number of burrows (3.3 per plant).

B14 was highly susceptible to the first and second larval instars. However, it was indicated that this line may be resistant to the third and fourth larval instars. The rate of larval mortality was slow, 21.7 larvae per plant surviving five days after egg hatch. The high number of larvae surviving five days after egg hatch plus the 8.8 larvae per plant, which survived ten days after egg hatch, is reflected in the high leaf feeding rating of 7.0 per plant. On the basis of the high number of larvae which B14 harbored five and ten days after egg hatch, the lesion count of 5.5 per plant is rather low. It appears that this phenomenon is due to the fact that larval mortality in B14 proceeded at a fast rate (5.8 and 3.1 larvae per plant surviving 20 and 30 days after egg hatch, respectively) beyond ten days after egg hatch. Therefore, B14 had almost as low a population 30 days after egg hatch as some of the more resistant lines. B14 also had a relatively low number of burrows (3.0 per plant) which indicates that this inbred is resistant to stalk invasion.

M14 was susceptible to all types of larval feeding. Although the larval population five days after egg hatch (13.9 per plant) was lower in this inbred than in B14 and Oh51A, the population remained at a relatively high level and there was practically no mortality beyond ten days after egg hatch (5.9, 6.3, and 5.2 larvae per plant surviving 10, 20, and 30 days after egg hatch, respectively). The leaf feeding rating, lesion count, and number of burrows were 5.5, 8.3, and 6.9 per plant, respectively.

WF9 was highly susceptible to all types of feeding. This inbred had the highest susceptibility of any line in the test. The rate of mortality was slow (32.5 larvae per plant surviving on the five-day dissection interval). The larval population, consisting of 12.9, 9.8, and 8.9 per plant 10, 20, and 30 days after egg hatch, respectively, remained at a high level and did not decrease appreciably beyond ten days after egg hatch. The high larval population is also reflected in a high leaf feeding rating (7.5 per plant), lesion count (10.4 per plant), and burrow count (8.6 per plant).

Larval mortality in inbred lines, 1955 and 1956.—As is shown in Table 5, most of the larval mortality in the eight inbred lines in 1955 and 1956 occurred during the first few days after egg hatch. This phenomenon has been noted by many investigators and reported by Painter and Ficht (1924), Caesar (1925, 1926), Springer (1930), Huber (1936), and Patch (1943). In 1955 there was appreciable larval mortality in all lines beyond five days after egg hatch but practically no mortality in nearly all of the lines beyond ten days after egg hatch. In 1956 there was appreciable mortality in all lines beyond five days after egg hatch and appreciable mortality in a few lines beyond 10 and 20 days after egg hatch.

Correlation of four criteria for determining corn borer damage.—The data in Table 4 indicate that the eight inbred lines possess different types of resistance and various factors for resistance. The simple correlation coefficients recorded in Figures 7-9 show that on the whole, the four criteria of surviving larvae, leaf feeding ratings, lesion counts, and burrows, for determining corn borer damage or for evaluating inbred lines for corn borer resistance, were equally effective and are highly correlated. Very high correlation coefficients were obtained for larvae 30 days after egg hatch vs. lesion counts 30 days after egg hatch (.95,

Figure 7) and larvae 30 days after egg hatch vs. burrows 30 days after egg hatch (.96, Figure 8). A correlation coefficient of .77 was obtained for larvae 30 days after egg hatch vs. leaf ratings 20 days after egg hatch (Figure 9). Dicke (1954) reported simple correlations of .882 for

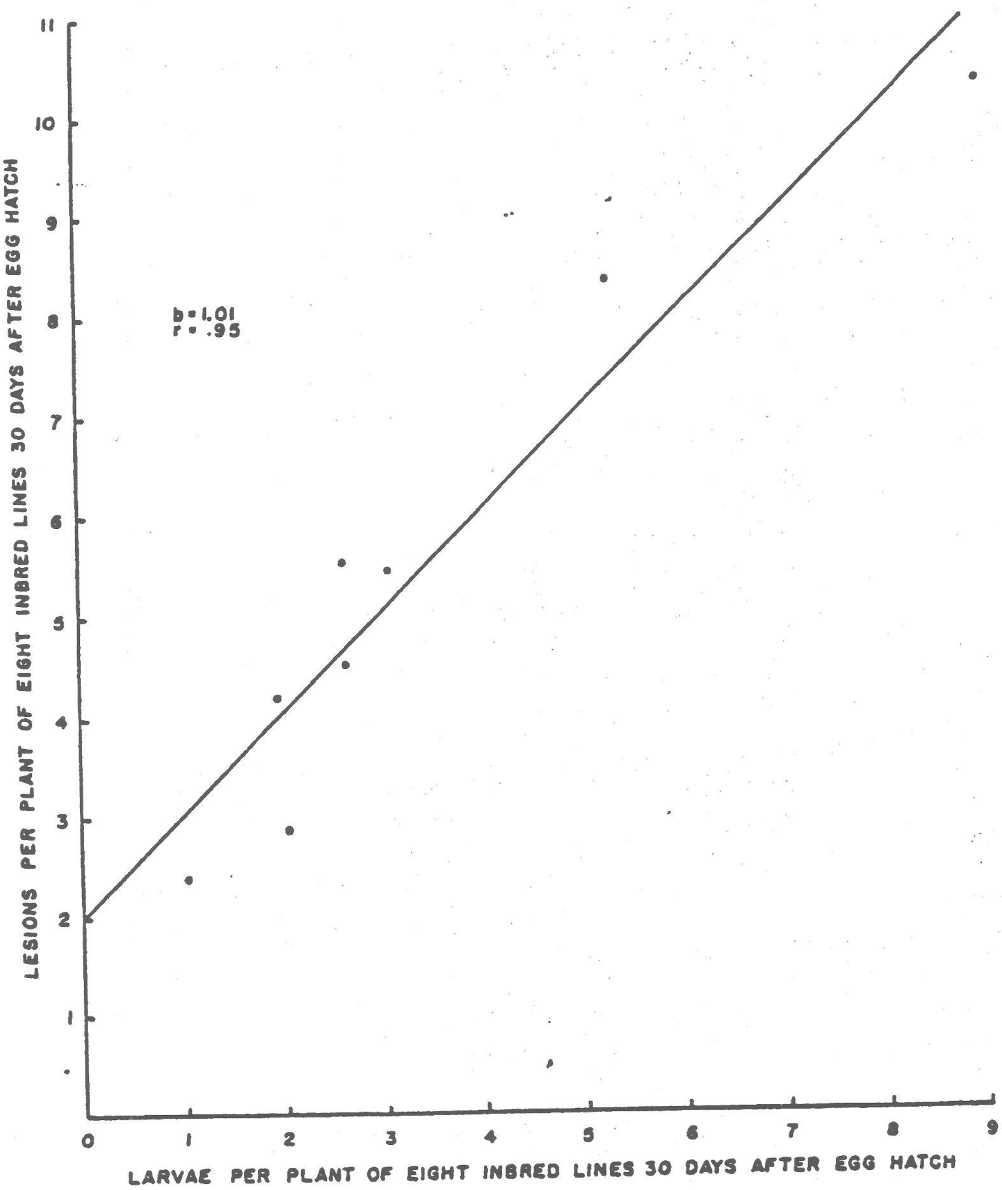

Fig. 7.—Regression and correlation between surviving larvae per plant 30 days after egg hatch and the lesion count per plant 30 days after egg hatch for 8 inbred lines of dent corn, 1956.

larvae per plant vs. leaf rating per plant and .869 for larvae per plant vs. lesions per plant; this study was made with 24 inbred lines. Correlation coefficients which were computed for the 1956 data but not recorded graphically were: lesion counts per plant 30 days after egg hatch vs. burrows per plant 30 days after egg hatch, .93; leaf ratings per plant 20 days after egg hatch vs. burrows per plant 30 days after egg hatch, .75; and leaf ratings per plant 20 days after egg hatch vs. lesion counts per plant 30 days after egg hatch, .72.

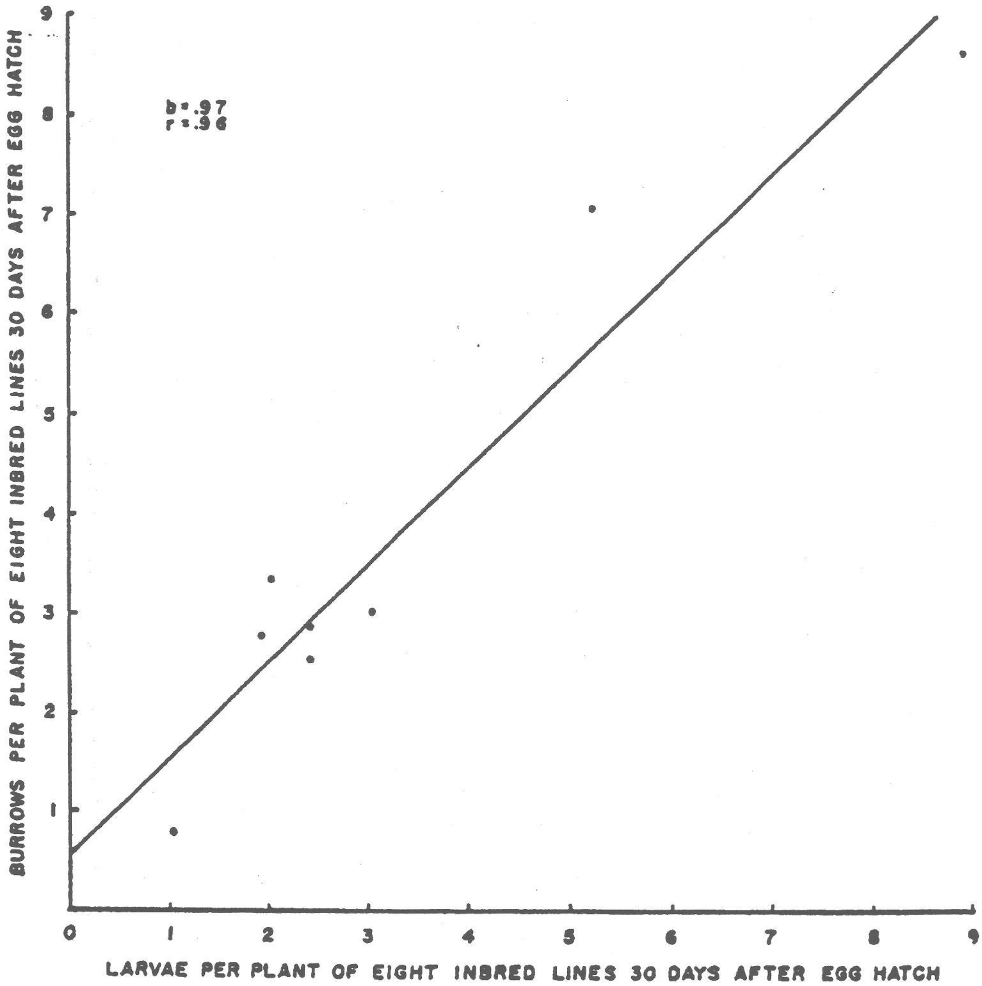

Fig. 8.—Regression and correlation between surviving larvae per plant 30 days after egg hatch and the burrows per plant 30 days after egg hatch for 8 inbred lines of dent corn, 1956.

On the basis of all eight inbred lines, the regression coefficient (b) in Figures 7-9 shows that there was an increase of 1.01 lesions per plant for every increase of 1.00 larva per plant (Figure 7); there was an increase of .97 burrows per plant for every increase of 1.00 larva per plant (Figure 8); and there was an increase of .74 class in leaf feeding rating per plant for every increase of 1.00 larva per plant (Figure 9).

Although the data show high correlations between the four criteria used for determining relative plant damage, certain facets of information in Table 4 should be pointed out. Larvae per plant 30 days or

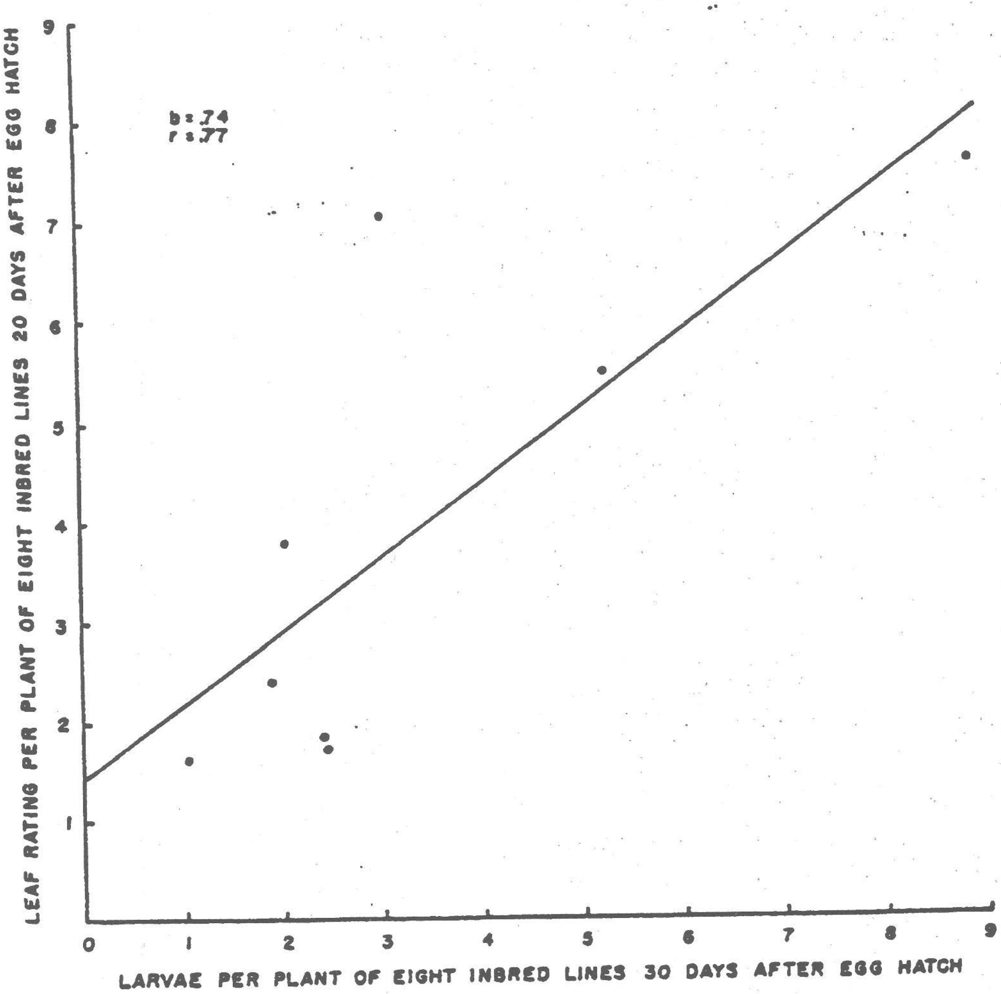

Fig. 9.—Regression and correlation between surviving larvae per plant 30 days after egg hatch and the leaf ratings per plant 20 days after egg hatch for 8 inbred lines of dent corn, 1956.

later after egg hatch may be a good index of inbred performance for some lines but not for others. Likewise, leaf feeding ratings or lesion counts may be a good index of the performance of some lines but not others. Leaf feeding ratings, as an index to the degree of damage caused by first and second instar larvae, and lesion counts, as an index to the degree of damage caused by third and fourth instar larvae, appear to be good criteria for determining the performance of most inbred lines. Larvae per plant 30 days or later after egg hatch would give an inaccurate interpretation of the performance of B14 because the larval population is rather low at this time, and one would conclude that B14 is resistant. However, B14 harbors a high population five and ten days after egg hatch. The larvae feed in the whorl of the plant at this time and if a high population exists, considerable damage is done to leaf tissue. Also the leaf feeding rating used alone as an index to the performance of B14 would be misleading as the rating of 7.0 would indicate high susceptibility. Lesion counts would also be misleading but might be the best single method. With an inbred such as B14, it appears that only a detailed study of the larval survival pattern, leaf feeding rating, lesion count, and burrows would estimate its true performance.

The leaf feeding rating of Oh51A would indicate an intermediate reaction to the early larval feeding, thus a moderately high level of infestation during the first few days after egg hatch, whereas the low lesion count of Oh51A would indicate a fast mortality rate of the third and fourth larval instars. Therefore, the combination of leaf ratings and lesion counts would be a better index to the performance of Oh51A than the larvae surviving 30 days after egg hatch.

A combination of leaf feeding ratings and lesion counts would be as good an index for the performance of W22R, A295, and Oh43 as the larvae surviving 30 days after egg hatch.

These data further indicate that any one of the four criteria of larvae, leaf feeding ratings, lesion counts, and burrows would be equally effective in measuring the performance of inbred lines falling in the extreme classes such as (W24 × B2)-2-38-1, M14, and WF9, i.e., any one of the criteria would indicate that (W24 × B2)-2-38-1 is highly resistant to all types of feeding and that M14 and WF9 are susceptible to all types of feeding.

Vouk (1930) tested certain varieties of corn in Yugoslavia, which were resistant to the European corn borer in certain parts of Europe, and found that the number of larvae surviving from an artificial infestation was practically the same for all varieties, but the damage suffered by the plants, infested with an equal number of larvae, was different for the varieties. From 2.5 to 3.5 larvae per plant were recovered for all varieties in the fall. In two varieties, White-row and Cinquantino, all the plants were broken and dried with poorly developed ears. The stronger and more robust varieties like Pferdezahn were fully matured and showed little injury. The author interpreted this as a constitutional resistance of certain varieties although the susceptibility of the plants to larval infestation was the same. Although the number of larvae recovered by Vouk in the fall was practically the same for all varieties, the rate of mortality must have been much slower in the varieties White-row and Cinquantino. The number of larvae recovered in the fall was a poor index to the comparative performance of the varieties. It appears that leaf feeding ratings and probably lesion counts would have been better criteria for measuring the comparative performance of these varieties.

Best time for making leaf feeding ratings and lesion counts in resistance investigations.—The data in Table 4 show that in these experiments the best time to make leaf feeding ratings of inbred lines is 20 days after egg hatch as the leaves of the plants are closely bunched at this time, and it is easier to visualize the relative amount of leaf damage. In most cases the leaf feeding ratings 30 days after egg hatch were considerably lower than they were 20 days after egg hatch. The best time to make lesion counts is 30 days after egg hatch. The lesion counts were considerably higher on the 30-day dissection interval. However, temperature plays a part in how rapidly the larvae and corn plants develop. In warmer areas these periods might be shortened somewhat.

The results of these investigations show that when evaluating a large number of inbred lines, where the time consumed in dissecting a certain number of plants in each plot is prohibitive, leaf feeding ratings made about three weeks after egg hatch, as an index to the mortality of the first and second larval instars, and lesion counts made from four to five weeks after egg hatch, as an index to the mortality of the third and fourth instar larvae, are good criteria for evaluating the performance of inbred lines. If the time consumed in counting the lesions of a certain number of plants in a plot is prohibitive, a rating scale similar to the one used for leaf ratings could be utilized on a plot basis. F. F. Dicke[3] is investigating the possibility of utilizing a ratio between leaf ratings and lesion counts as an index to inbred performance.

[3]Unpublished data.

TABLE 1.—Summarized analysis of mean number of larvae, leaf feeding ratings, lesions, and burrows per plant of eight inbred lines of dent corn (6 replications). Wooster, Ohio, 1955 and 1956

Source of variation	Larvae		Leaf ratings		Lesions		Burrows	
	1955	1956	1955	1956	1955	1956	1955	1956
Inbreds	**	**	**	**	ns	**	*	**
Dissection interval [1]	**	**	ns	**	ns	**	**	**
Inbred × dissection interval	**	**	ns	*	ns	**	ns	**

* Significant at the 5 percent probability level.

** Significant at the 1 percent probability level.

ns Nonsignificant.

[1] Inbred lines were dissected at intervals of 5, 10, 20, and 30 days after egg hatch.

TABLE 2.—Mean number of larvae, leaf feeding ratings, lesions, and burrows per plant of eight inbred lines of dent corn by dissection intervals (6 replications). Wooster, Ohio, 1955 and 1956

Dissection interval[1]	Larvae		Leaf ratings		Lesions		Burrows	
	1955	1956	1955	1956	1955	1956	1955	1956
5	4.5	13.6	--	--	--	--	--	--
10	1.1	5.7	--	--	--	--	--	--
20	0.9	4.5	1.4	3.9	0.6	2.9	0.4	0.9
30	0.9	3.4	1.2	3.0	0.8	5.5	1.2	3.9
LSD .05	0.77	1.16	ns	**	ns	**	**	**

[1] Days after egg hatch on which the plants were dissected.

TABLE 3.—Mean number of larvae, leaf feeding ratings, lesions, and burrows per plant by inbred line and dissection interval (6 replications). Wooster, Ohio, 1955[1]

Inbred line	Dissection interval[2]									
	Larvae				Leaf ratings[3]		Lesions[4]		Burrows	
	5	10	20	30	20	30	20	30	20	30
(W24 x B2)-2-38-1	3.5	0.2	0.3	0.4	1.0	1.0	0.3	0.5	0.1	0.4
W22R	1.4	0.7	0.7	1.0	1.1	1.0	0.7	0.6	0.4	1.6
A295	1.9	0.2	0.5	0.8	1.0	1.0	0.5	0.7	0.2	1.2
Oh43	2.3	0.9	0.8	0.4	1.0	1.0	0.4	0.8	0.4	0.7
Oh51A	4.0	1.5	1.5	0.9	1.2	1.3	0.9	1.0	1.0	1.4
B14	12.3	1.3	0.9	0.7	2.0	1.3	0.3	0.7	0.1	1.2
M14	4.0	2.4	1.2	1.4	1.1	1.5	0.9	1.2	0.6	1.6
WF9	6.9	1.9	1.4	1.3	2.5	1.7	0.5	0.9	0.4	1.8
Standard error of difference between										
Any two means between dissection intervals for the same inbred	1.09				0.32		0.37		0.41	
Any two means between inbreds for the same dissection interval	1.06				0.37		0.40		0.41	
LSD .05										
Any two means between dissection intervals for the same inbred	2.18				ns		ns		ns	
Any two means between inbreds for the same dissection interval	2.11				ns		ns		ns	

[1] Planted in single row 26-foot plots on May 20.

[2] Number of days plants were dissected after egg hatch (infested with 120 eggs per plant).

[3] The leaf ratings were made on a 9 class basis (class 1 = least, class 9 = highest infestation level).

[4] Lesions refer to feeding damage on the midrib, sheath, and around the collar

TABLE 4.—Mean number of larvae, leaf feeding ratings, lesions, and burrows per plant by inbred line and dissection interval (6 replications). Wooster, Ohio, 1956[1]

Inbred line	Dissection interval[2]									
	Larvae				Leaf ratings[3]		Lesions[4]		Burrows	
	5	10	20	30	20	30	20	30	20	30
(W24 x B2)-2-38-1	4.5	2.7	1.6	1.0	1.6	1.3	1.9	2.4	0.2	0.8
W22R	4.6	2.3	3.0	2.4	1.7	1.1	2.9	4.5	0.4	2.5
A295	7.7	3.9	2.2	1.9	2.4	1.4	1.9	4.2	0.6	2.8
Oh43	9.7	3.5	3.9	2.4	1.8	1.1	3.6	5.6	0.9	2.8
Oh51A	14.1	5.3	3.7	2.0	3.8	1.4	2.3	2.9	0.9	3.3
B14	21.7	8.8	5.8	3.1	7.0	5.7	2.8	5.5	0.6	3.0
M14	13.9	5.9	6.3	5.2	5.5	4.5	4.3	8.3	1.8	6.9
WF9	32.5	12.9	9.8	8.9	7.5	7.6	3.6	10.4	1.5	8.6
Standard error of difference between										
Any two means between dissection intervals for the same inbred	1.66				0.43		0.89		0.45	
Any two means between inbreds for the same dissection interval	1.74				0.33		1.10		0.58	
LSD .05										
Any two means between dissection intervals for the same inbred	3.29				0.86		1.80		0.92	
Any two means between inbreds for the same dissection interval	3.47				0.68		2.23		1.19	

[1] Planted in single row 26-foot plots on June 7.

[2] Number of days plants were dissected after egg hatch (infested with 100 eggs per plant).

[3] The leaf ratings were made on a 9 class basis (class 1 = least, class 9 = highest infestation level).

[4] Lesions refer to feeding damage on the midrib, sheath, and around the collar.

TABLE 5.—Summarized data showing larval mortality as expressed by percent survival on eight inbred lines of dent corn at intervals of 5, 10, 20, and 30 days after egg hatch (6 replications). Wooster, Ohio, 1955 and 1956

Inbred line	Days after egg hatch[1]							
	5		10		20		30	
	1955	1956	1955	1956	1955	1956	1955	1956
(W24 × B2)-2-38-1	3.0	4.5	0.2	2.7	0.3	1.6	0.3	1.0
W22R	1.2	4.6	0.6	2.3	0.6	3.0	0.9	2.4
A295	1.5	7.7	0.2	3.9	0.4	2.2	0.6	1.9
Oh43	2.2	9.7	0.6	3.5	0.6	3.9	0.4	2.4
Oh51A	3.5	14.1	1.2	5.3	1.3	3.7	0.7	2.0
B14	7.8	21.7	1.1	8.8	0.8	5.8	0.5	3.1
M14	3.4	13.9	1.8	5.9	1.0	6.3	1.1	5.2
WF9	5.7	32.5	1.6	12.9	1.2	9.8	1.0	8.9

[1] Each plant in 1955 was artificially infested with 120 eggs, whereas the plants in 1956 were infested with 100 eggs per plant.

SUMMARY

The European corn borer larval survival pattern of eight inbred lines of dent corn, (W24 × B2)-2-38-1, W22R, A295, Oh43, Oh51A, B14, M14, and WF9, was determined at intervals of 5, 10, 20, and 30 days after egg hatch under a uniform simulated natural first-brood infestation (an artificial infestation of 100 to 120 eggs per plant was made in the mid-whorl stage of plant development). Relative leaf feeding ratings, midrib, sheath and collar lesion counts, and stalk burrows were made 20 and 30 days after egg hatch. These investigations were initiated in 1955 and concluded in 1956.

The objective of this study was to extend our information on both leaf blade and sheath feeding resistance of the first-brood infestation.

The level of larval establishment and survival in 1955 was too low to show differences, at the desired magnitude, between inbred lines. The level of larval establishment in the inbred lines in 1956 was satisfactory for evaluating varying levels of resistance by the four criteria of surviving larvae, leaf feeding ratings, lesion counts, and number of burrows.

Based on the four criteria, the experimental inbred (W24 × B2)-2-38-1 was highly resistant to the first and second larval instars. This line may also be resistant to third and fourth instar larvae. The inbreds W22R and A295 were highly resistant to the first and second instars. Oh43 was resistant to the first and second instars but somewhat susceptible to the third and fourth instars, whereas Oh51A was somewhat susceptible to the first and second instars but resistant to the third and fourth instars. B14 was highly susceptible to the first and second instars, but appeared to be resistant to the third and fourth instars. B14 also possessed resistance to stalk invasion. WF9 and M14 were highly susceptible to all types of feeding.

The greatest differential in larval survival between lines was on the five-day dissection interval. Most of the mortality in the eight inbred lines occurred during the first few days after egg hatch.

The results of these investigations indicate that when evaluating a large number of inbred lines, where the time consumed in dissecting a certain number of plants in each plot is prohibitive, leaf feeding ratings (on a plot basis) made about three weeks after egg hatch, as an index to the mortality of the first and second larval instars, and lesion counts made about five weeks after egg hatch, as an index to the mortality of the third and fourth instar larvae, are good criteria for evaluating the performance of inbred lines. If the time consumed in counting the lesions of a certain number of plants in a plot is prohibitive, a rating scale similar to the one used for leaf feeding ratings could be utilized on a plot basis.

Although the four indices for determining corn borer damage were highly correlated, the data show that the number of larvae surviving 30 days or later after egg hatch may be a good index of inbred performance for some lines but not others. The relative leaf feeding rating used alone would also give an inaccurate interpretation of the performance of some lines. A combination of leaf feeding ratings and lesion counts is a good index to the performance of most inbred lines.

APPENDIX

TABLE 6.—Analysis of variance of the data for larvae reported in Tables 2 and 3, 1955 and in Tables 2 and 4, 1956

Source of variation	d.f.	1955 Mean square	1955 F	1956 Mean square	1956 F
Whole plot					
Replications	5	5.9488	2.23 ns	61.32	5.25**
Inbreds	7	26.9931	10.11**	481.57	41.20**
Error (A)	35	2.6697		11.69	
Split plot					
Dissection interval	3	152.5246	42.06**	1026.55	123.98**
Inbred × D. I.	21	18.2022	5.02**	74.71	9.02**
Error (B)	120	3.6259		8.28	
Total	191				

**Significant at the 1 percent probability level.
ns Nonsignificant.

TABLE 7.—Analysis of variance of the data for leaf ratings, lesions, and burrows reported in Tables 2 and 3, 1955

Source of variation	d. f.	Leaf ratings Mean square	Leaf ratings F	Lesions Mean square	Lesions F	Burrows Mean square	Burrows F
Whole plot							
Replications	5	0.5542	1.08ns	1.0356	1.97ns	1.2631	2.65*
Inbreds	7	1.7827	3.48**	0.5316	1.01ns	1.2950	2.72*
Error (A)	35	0.5120		0.5262		0.4759	
Split plot							
Dissection interval	1	0.4174	1.39ns	1.0817	2.57ns	15.9740	31.41**
Inbred x D. I.	7	0.4905	1.63ns	0.0668	0.16ns	0.5415	1.06 ns
Error (B)	40	0.3005		0.4209		0.5086	
Total	95						

* Significant at the 5 percent probability level.
** Significant at the 1 percent probability level.
ns Nonsignificant.

TABLE 8.—Analysis of variance of the data for leaf ratings, lesions, and burrows reported in Tables 2 and 4, 1956

Source of variation	d. f.	Leaf ratings Mean square	Leaf ratings F	Lesions Mean square	Lesions F	Burrows Mean square	Burrows F
Whole plot							
Replications	5	1.1417	1.44ns	19.0178	3.91**	4.0174	2.79*
Inbreds	7	73.3159	92.45**	35.4223	7.28**	28.2924	19.68**
Error (A)	35	0.7930		4.8645		1.4374	
Split plot							
Dissection interval	1	19.8471	36.33**	54.2294	64.55**	215.5203	349.93**
Inbred x D. I.	7	1.6776	3.07*	12.8787	5.39**	13.1810	21.40**
Error (B)	40	0.5463		2.3892		0.6159	
Total	95						

* Significant at the 5 percent probability level.
** Significant at the 1 percent probability level.
ns Nonsignificant.

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The Florida Entomologist Vol. 60, No. 2, 1977 97

EAR CHARACTERISTICS AND MECHANISMS OF RESISTANCE AMONG SELECTED CORNS TO CORN EARWORM[1,2,3]

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ABSTRACT

Some of the ear characteristics of selected corns resistant and susceptible to the corn earworm, *Heliothis zea* (Boddie), are described. Some corns, previously described as tolerant, had long, fairly tight silk channels, and/or a large amount of silk that maintained a high moisture content over the period of earworm larval development. However, 'Zapalote Chico' had a long tight silk channel, but only a small amount of silk, and a low moisture content over this period. Its mechanism of resistance is other than tolerance, and corn earworm larvae did not readily establish on it.

Investigations of host plant resistance to insects tend to deal with the development and use of screening procedures for evaluation of the relative resistance among lines. Some investigators have gone beyond initial screening, however, and have identified mechanisms of resistance such as nonpreference, antibiosis, or tolerance (Painter 1968), and a few have attempted to delineate factors associated with the mechanisms of resistance. For example, Wiseman et al. reported (1972, 1974a) that certain "resistant" corn lines were as much infested with corn earworm, *Heliothis zea* (Boddie), as certain susceptible corn lines; thus, they identified the mechanism of resistance as tolerance.

This report describes several ear characteristics, measured over several sampling periods in 1973 and 1974, of selected corns classified previously in the field as resistant or susceptible to the corn earworm, and illustrates their respective ear damage.

METHODS AND MATERIALS

Selected corns were planted in plots consisting of six 20-ft rows, 3 ft apart and arranged in a randomized complete block design with 5 replications. 'Dixie 18' and 471-U6 X 81-1 were selected as resistant (tolerant), 409 X 20 as intermediate, and 'Asgrow 204B', 'Ioana', and 'Stowell's Evergreen' as susceptible. 'Zapalote Chico' was selected because of unpublished reports that it possessed "lethal silks" and because it had the tightest husks.

When all the ears were in full silk (3 days past initial silk) 1 row of each plot was infested (Wiseman et al. 1974b) with 30 corn earworm eggs/

[1]Lepidoptera: Noctuidae.

[2]In cooperation with the University of Georgia College of Agriculture Experiment Stations, Coastal Plain Station, Tifton, GA. Accepted for publication 25 Feb. 1977.

[3]Mention of a proprietary product in this paper does not constitute an endorsement by the USDA.

silk aggregate/ear obtained from the laboratory. From another randomly selected row, 5 silks from the top ears of 5 plants from each plot were harvested that same day (0-day), and the silk was separated into exposed silk and nonexposed silk (that portion in the silk channel to ear tip). This procedure was repeated on other rows at 5, 10, 15, and 20 days past full silk. All plants that were not in full silk at 0-day were discarded. Silk channels were measured in cm lengths for each sampling period. The silks, as separated, were weighed, oven-dried at 41°C for 24 h, and the percentage moisture determined. These same procedures were repeated at 5-day intervals through the 20th day past full silk. Only the data for the 0, 10, and 15-day sampling periods along with marginal means are shown. On the 20th day, corn earworm damage was measured as the depth of penetration (cm) of the earworm larvae into the infested ears.

Analyses of variance were made for each character measured, and data were combined over the years.

Results and Discussion

Analyses of variance for data on corn earworm damage revealed that significant differences existed among lines (Table 1). Zapalote Chico and 471-U6 X 81-1 were the least damaged. Dixie 18 and Asgrow 204B did not differ in damage when data were combined over years, but significant differences were apparent in 1974. Zapalote Chico had the tightest husks, but the other 2 resistant entries, Dixie 18 and 471-U6 X 81-1, also had fairly tight husks.

TABLE 1. Corn earworm damage and husk tightness ratings for selected corns grown in 1973 and 1974.

Entry	Husk tightness*	Earworm damage**
Dixie 18	2.2	3.8 c
Asgrow 204B	1.4	4.3 bc
Zapalote Chico	3.3	1.6 d
Ioana	1.0	5.6 a
Stowell's Evergreen	1.4	5.2 ab
409 × 20	1.6	3.8 c
471-U6 × 81-1	1.6	1.8 d

*Average husk tightness ratings shown from other experiments. Ratings were based on a visual scale of 0-5, where 0 = loose husks with ear visible and 5 = very tight husks that are tough and difficult to shuck.

**Means followed by the same letter are not significantly different at P = 0.05. Earworm damage rating was as follows: 0 = no injury, 1 = silk feeding, 2 = injury to 1 cm beyond ear tip, and 3, 4, . . . n = additional penetration into the ears in 1-cm increments.

The average lengths of the silk channel for each entry for each of 3 sampling periods are shown in Table 2. Analyses of variance revealed significant differences for silk channel lengths among entries at the various sampling periods. Asgrow 204B and Ioana had the shortest silk channels; Stowell's Evergreen was next, and the resistant entries Dixie 18, Zapalote

TABLE 2. AVERAGE LENGTH OF SILK CHANNEL (CM)* AT FULL SILK (0-DAY) AND AT 10 DAYS AND 15 DAYS AFTER FULL SILK OF SELECTED CORNS GROWN IN 1973 AND 1974.

	Average length (cm) at indicated Days after full silk			
Entry	0	10	15	Mean
Dixie 18	9.5 a	6.6 a	7.7 a	7.7 a
Asgrow 204B	9.3 a	4.2 b	3.9 b	5.8 d
Zapalote Chico	9.4 a	7.2 a	6.8 a	7.5 a
Ioana	6.0 b	4.0 b	4.4 b	4.3 e
Stowell's Evergreen	9.9 a	6.1 a	5.0 b	6.7 c
409 × 20	8.2 ab	7.9 a	7.0 a	7.8 a
471-U6 × 81-1	10.2 a	6.4 a	5.5 ab	7.1 b
Mean	8.9 a	6.1 b	5.8 c	

*Average silk channel lengths at any sample period or marginal means followed by the same letter are not significantly different at P = 0.05.

Chico, 409 X 20, and 471-U6 X 81-1 had the longest silk channels. Silk channel lengths stabilized after a sharp decline from 0-day through 10 days.

The average weight of exposed silks (that portion beyond the silk channel) and nonexposed silks of all entries is shown in Table 3. Analyses of variance revealed significant silk weight differences among entries within sampling dates. On any sampling date, however, the differences were usually greater for nonexposed silks. With the exception of Ioana, the entries previously classified as tolerant and as susceptible differed only slightly among themselves through 15 days. Generally, Ioana tended to have a relatively smaller amount of silk throughout the various sampling periods than other entries, but Zapalote Chico always had even smaller quantities of both exposed and nonexposed silks than Ioana at all sampling periods. The resistant (tolerant) Dixie 18 and 471-U6 X 81-1 both possessed very large quantities of silk, the one characteristic for which Dixie 18 and 471-U6 X 81-1 differed drastically from the equally resistant Zapalote Chico.

The percentage of moisture in the exposed and nonexposed silks of each entry at each sampling period is shown in Table 4. Moisture contents were generally above 90% at 0-day except for the exposed and nonexposed silks of Zapalote Chico, which had 82% and 87% moisture, respectively. Percentages for Zapalote Chico were low at all sampling periods, but this was not true for Dixie 18 and 471-U6 X 81-1, which had a much higher moisture content in their silks. Overall means showed that their 0-day silks were about 90% moisture for both exposed and nonexposed silks and then dropped steadily in moisture content through the 20th day.

Barber (1941) reported on behavioral observations of newly hatched corn earworm larvae. He observed that by the time corn earworm eggs begin to hatch and the silks to wilt, the larvae have penetrated into the still-moist silks since they prefer to feed under the protection of a moist environ-

TABLE 3. AVERAGE WEIGHTS* (G) OF EXPOSED (E) AND NONEXPOSED (N) SILKS OF SELECTED CORNS AT FULL SILK (0-DAY), 10 DAYS AND 15 DAYS AFTER FULL SILK. 1973-74.

	Avg weight (g) at indicated Days after full silk						Mean	
	0		10		15			
Entry	E	N	E	N	E	N	E	N
Dixie 18	36.4 a	41.9 a	14.4 abc	28.8 a	8.4 a	27.2 a	18.1 ab	31.0 ab
Asgrow 204B	28.2 a	43.7 a	16.1 ab	19.0 bc	11.1 a	13.0 d	16.8 b	25.3 c
Zapalote Chico	9.9 b	14.7 c	2.5 d	7.5 d	2.0 c	6.0 e	4.6 e	8.7 e
Ioana	34.7 a	29.2 b	9.6 bcd	15.1 c	5.5 bc	13.3 d	15.2 c	16.9 d
Stowell's Evergreen	32.7 a	48.3 a	11.5 abc	28.5 a	6.9 bc	19.9 c	17.1 b	30.0 b
409 × 20	25.2 a	27.6 b	7.5 cd	24.6 ab	5.1 bc	21.0 bc	10.8 d	24.2 c
471-U6 × 81-1	31.7 a	47.9 a	19.0 a	29.9 a	10.9 a	25.1 ab	18.7 a	32.8 a
Mean	28.4 a	36.2 a	11.5 b	21.9 b	7.2 c	17.9 c		

*Silk weights are an average of 5 silks, 5 replications, and 2 years for each sample day. Marginal means or averages within a given sample period followed by the same letter are not significantly different at P = 0.05. Entry mean includes data for all 5 sampling periods.

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TABLE 4. PERCENT* MOISTURE IN EXPOSED (E) AND NONEXPOSED (N) SILKS OF SELECTED CORNS, RECORDED AT FULL SILK (0-DAY) 10 DAYS AND 15 DAYS AFTER FULL SILK, 1973-74.

	Days after full silk							
	0		10		15		Mean	
Entry	E	N	E	N	E	N	E	N
Dixie 18	91 b	90 ab	75 bc	87 b	60 b	90 b	72 d	89 c
Asgrow 204B	90 b	90 ab	77 bc	90 b	67 bc	86 b	73 c	88 d
Zapalote Chico	82 a	87 a	35 a	79 a	31 a	77 a	48 f	79 e
Ioana	92 b	92 b	74 bc	93 b	60 b	92 b	71 e	91 b
Stowell's Evergreen	90 b	92 b	72 b	92 b	57 b	90 b	72 d	91 b
409 × 20	91 b	91 b	73 b	91 b	67 bc	91 b	74 b	91 b
471-U6 × 81-1	91 b	92 b	82 c	92 b	73 c	92 b	79 a	92 a
Mean	90 a	91 a	70 b	89 b	59 c	88 c		

*Column or marginal mean percentages followed by the same letter are not significantly different at P=0.05. Entry mean includes data from all 5 sampling periods.

ment. Thus, when earworm moths oviposit on silks of corn, the silks would then normally possess moisture in excess of 90%, and since eggs usually hatch in ca. 2 days, most corn would still have a silk moisture content of 90% or above. The exception would be corn of Zapalote Chico types.

A long, tight silk channel and a large quantity of silk that maintains a high moisture content, such as Dixie 18 and 471-U6 X 81-1, provide an ideal environment for successful establishment of corn earworm larvae and also an adequate diet of silk for development (Wiseman et al. 1976). However, the larvae stop and initiate feeding near the husk tip of Dixie 18 and 471-U6 X 81-1, probably because of a thigmotactic response. Susceptible corns seem to allow the larvae to move deeper into the silk channel before they begin feeding. Wiseman and McMillian (1973) also found differences in the movement and feeding of corn earworm larvae on 2 susceptible sweet corns: the susceptible corn, with the longer silk channel, had limited early ear feeding.

Wiseman et al. (1976) found that when corn earworm larvae were fed silks from certain tolerant and susceptible lines, their growth and mortality were essentially the same. They found this was not true for Zapalote Chico: larval growth was retarded and mortality increased. Also, no pupation had occurred by the end of the 20th day as compared to 88% for susceptible Stowell's Evergreen or tolerant 471-U6 X 81-1. Thus, these effects measured for Zapalote Chico were quite different from those for the tolerant entries Dixie 18 and 471-U6 X 81-1. Zapalote Chico possesses the tightest husk and a long silk channel, but it does not have a large amount of silk, nor does it maintain a high moisture content in the silks. The exposed silks dry rapidly and probably physically prevent most larval establishment due to the matting of the silks, or chemically by an antibiosis factor and/or by a combination of both.

In summary, the 3 resistant entries, Zapalote Chico, Dixie 18, and 471-U6 X 81-1, had the least amount of earworm damage and tightest husks (Table 1), and they had long silk channels over the entire sampling periods (Table 2). Thus, in these respects, no noticeable differences existed among the 3 resistant entries. They differed in amount of silk mass, however, (Table 3) and moisture content of silk (Table 4). Yet, they were fairly equal for resistance in the field. The resistant (tolerant) entries, Dixie 18 and 471-U6 X 81-1, possessed long, tight silk channels and large amounts of silks that maintained a high moisture content over the period of corn earworm larval development. The resistant (either antibiosis and/or nonpreferred) Zapalote Chico had a long, tight silk channel and possessed very small quantities of silk that sharply decreased in moisture over the period of insect development. Therefore, as seen in the field, the resistance mechanism of Zapalote Chico, and of Dixie 18 and 471-U6 X 81-1, were quite different.

Acknowledgment

Lila G. Adcock and Winfred N. Roberson of this laboratory are thanked for their continued contributions to the Host Plant Resistance Project.

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CONTROL OF STRIPED GRASS LOOPERS AND ARMY-WORMS IN PASTURE: 1976[1] – (*Note*). Florida has over 3 million acres of improved pasture of which ca. 200,000 acres are grown for hay. Such insect pests as the striped grass looper, *Mocis latipes* (Guenée), and fall armyworm, *Spodoptera frugiperda* (J. E. Smith), are capable of severely damaging improved pasture and hay fields (Kelsheimer, E. G., D. W. Jones, and E. M. Hodges, 1953, Agr. Exp. Sta. Cir. S-64). The economic literature of the striped grass looper as a major pest of pasturegrass has been reviewed by J. A. Reinert (1975; Ann. Ent. Soc. Am. 68:201-4), and E. O. Ogunwolu and D. H. Habeck (1975; Fla. Ent., 58:97-103).

Lepidopterous larvae in pasturegrass are usually controlled by aerial applications of approved insecticides. This study was initiated to evaluate the effectiveness of several new insecticides and formulations applied by air for control of lepidopterous larvae in pasture.

Three insecticides were evaluated for pasture caterpillar control in September 1976. These insecticides were permethrin (Ambush®), carbaryl (Sevin 4 Oil®), and carbaryl (Sevin 80S®). All materials were applied by air on 31 Aug. 1976 with a Cessna AG truck (188 series) aircraft equipped with a Transland spray system. The carbaryl-oil formulation was diluted 1:1 in fuel oil and applied with 30 D3 nozzles at 1.0 lb AI/acre. The carbaryl WP formulation and permethrin were applied in 3 gal of water per acre at 1.0 and 0.2 lb AI/acre, respectively, with 60 D6 nozzles. Three experimental plots were established in a 20 acre coastal bermuda, *Cynadon dactylon* (L.), pasture near Newberry, Fla. as 3 swaths, 60 ft. wide running the length of the pasture.

[1]Fla. Agricultural Experiment Station Journal Series No. 487.