Behavioral changes of the European corn borer reared continuously on a meridic diet

by

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INTRODUCTION

Research on the biology, biological control, chemical control, genetics, and plant resistance of the European corn borer, Ostrinia nubilalis (Hübner), has been facilitated in recent years by rearing large numbers of insects on a meridic diet. At present, several species of plant feeding Lepidoptera are reared on meridic diets, i.e. the corn earworm, Heliothis zea (Boddie); the fall armyworm, Spodoptera frugiperda (J. E. Smith) (Burton and Perkins 1972); the southwestern cornborer, Diatrea grandiosella (Dyar) (Davis et al. 1972); sugar cane borer Diatraea saccharalis (Fabricius) (Ortego et al. in press). Very little progress could be made in many research programs without larval rearing techniques.

Several changes have been observed in the behavior of laboratory-adapted insects when compared with the standard (wild) type. Ruprah and Treece (1968) reported that laboratory reared face flies were attracted to feces diets containing alfalfa or grass rather than feces diets containing corn silage. Significant differences in pupal production were recorded when face flies were exposed to feces from cows on different diets (Treece 1966). As measured by larval weight, adult body size, adult longevity and reproductive capacity, a population of laboratory adapted Cochliomyia hominivorax (Coquerel) differed from a population of the wild type; larvae of wild type flies

weighed 90.5 mg while larvae of laboratory-adapted populations weighed 73 mg (Spates and Hightower 1970). Spates and Hightower (1967) noted that screw worm flies reared in the laboratory for 5 and 15 generations showed reduced sexual aggressiveness in comparison with wild type males.

Nongenetic behavioral changes can be either laboratory conditioned or alterations in the morphological or physiological processes. Inadequate or unsuitable nutrition, disease, effects of treatment and handling, lack of exposure to appropriate sensory stimuli and entrainment to factory photoperiod regimens can result in altered and probably deficient performance of necessary traits (Chambers 1977). Bush (1974) suggested that genetic and conditioned effects may be mutually reinforcing through the process of reproductive isolation. This would seem most relevant to insects like the European corn borer that have had a long-term laboratory colonization.

Data collected during the past several years show that the European corn borer reared continuously on a meridic diet cannot be used in evaluating corn genotypes for resistance because leaf feeding damage is too low for measuring differential resistance (Guthrie et al. 1971). Cultures of corn borers reared 7 to 14 generations on a meridic diet caused extensive damage to leaf tissue on a susceptible inbred line of dent corn (WF9), corn borer cultures reared for 17 to 22 generations on a meridic diet lost some virulence to survive on WF9, and corn

borer cultures reared for 46 to 108 generations on a meridic diet caused little damage to leaf tissue of WF9 (Guthrie et al. 1974). Rathore et al. (1976) found this loss of virulence to be heritable and the gene action to be additive.

Cultures of corn borers reared 1 generation on corn plants and 8 generations on a meridic diet each year may maintain their ability to survive on corn plants (W. D. Guthrie, unpublished data).

At present 8 cultures of the European corn borer are maintained in the laboratory at the Corn Insects Research Unit, Ankeny, Iowa. Origins of the cultures are as follows: M133 was reared on a meridic diet containing wheat germ for 133 generations, M160 was reared on a meridic diet containing dried-ground corn leaves (WF9 x M14) instead of wheat germ for 160 generations.

Corn borer cultures CI31AF, Oh43F, and WF9F were reared for 13 generations on corn and 104 generations on a meridic diet containing wheat germ (1 generation each year on corn and 8 generations each year on a meridic diet from 1965 through 1978). Corn borer cultures CI31AL, Oh43L, and WF9L were reared 11 generations on corn and 108 generations on a meridic diet containing wheat germ (1 generation each year on corn and 8 generations each year on a meridic diet from 1965 through June 1976 then 11 generations each year on a meridic diet from July 1976 through 1978). Egg masses from the CI31AF and CI31AL

cultures were placed on 90 plants of an inbred line of dent corn (CI31A) each year during the midwhorl stage of plant growth. Infestations were made in 8 applications of 2 masses each spaced 1 day apart for a total of ca. 400 eggs per plant; 300-400 larvae were recovered ca. 16 days after egg hatch and were placed in 3-dram vials containing a plug of diet. These 2 cultures were reared the remainder of the year on a meridic diet. The same technique was used for rearing corn borer cultures Oh43F and Oh43L on inbred line Oh43 and for rearing corn borer cultures WF9F and WF9L on inbred line WF9.

The purpose of this study was to determine the behavioral changes in 8 cultures of the European corn borer reared for long periods of time on a meridic diet. A culture of borers reared for 5 generations on a meridic diet (M5) was used as a check. These studies were conducted over a period of 7 months. Each culture, therefore, was advanced 3 generations from the start to the end of the experiments. Several experiments were conducted and a tremendous amount of data was collected.

LARVAL SURVIVAL AND DEVELOPMENT OF EUROPEAN CORN BORERS REARED CONTINUOUSLY ON A MERIDIC DIET IN 3-DRAM VIALS

Experiment I

Studies of the life history of laboratory-adapted colonies of the Caribbean fruit fly revealed increased fecundity and decreased developmental time (Leppla et al. 1976). Life history values might be considered the most basic of generalized changes to indicate adaptive changes during mass production of insects (Chambers 1977).

Methods and materials

This experiment was conducted to determine if European corn borers reared for many generations on a meridic diet have changed in percentage survival to pupation, percentage survival to adults, days to pupation, days to adult emergence, and weight of female and male pupae.

First instar larvae from 9 corn borer cultures (M5, M160, M133, CI31AF, CI31AL, Oh43F, Oh43L, WF9F, and WF9L) were reared individually on plugs of diet placed in 3-dram vials. M160 was reared for 160 generations on a meridic diet containing dried-ground corn leaves instead of wheat germ. Leaves of a corn borer susceptible single cross hybrid (WF9 x M14) were cut during the whorl stage of plant development; these leaves were dried, ground into a fine powder, and stored in plastic bags

under -23°C temperature for 13 years (1966-1978). All other cultures were reared on a meridic diet containing wheat germ. The M5 culture (reared for 5 generations on a meridic diet) was used as a check.

Ingredients in the diet and preparation of the diet were similar to that used by Guthrie et al. (1971). The vials of diet were placed in trays containing 9 rows of 17 vials/row. Each row contained a corn borer culture. A randomized block design was used with corn borer cultures randomized within each tray, and each tray was 1 replication. The experiment was replicated 12 times for a total of 204 larvae/culture (1836 larvae for the 9 cultures). The incubation room was maintained at a temperature of 27-28°C, 75% RH, and constant light.

Results and discussion

Percent survival to pupation was high and did not differ significantly for 8 (a range of 95.1 to 98.0%) of the 9 cultures of corn borers; corn borer culture WF9F had 81.4% survival to pupation. Percent survival to adult emergence was also high and did not differ significantly (a range of 90.2 to 96.1%) for 8 of the 9 cultures; WF9F had 80.9% survival to adult emergence (Table 1).

The range in number of days to pupation for the 9 cultures was 15.5 for CI31AF to 19.3 for M160; corn borer culture M160 took 2.0 to 3.8 days longer to pupate than did the other 8

Table 1. Larval survival and development of European corn borer cultures reared on a meridic diet

Cultures	Percent survival to		Number of days to		Pupal weight (mg)	
	Pupation[a]	Adult[a]	Pupation[a]	Adult[a]	Female[a]	Male[a]
M5	98.0a	96.1a	17.3b	24.3b	102.6c	76.9ab
M160	96.6a	96.1a	19.3a	26.4a	101.7c	78.5a
M133	95.1a	93.1a	16.3c	23.2c	100.9c	73.0b
C131AF	97.6a	94.1a	15.5d	22.5c	109.6ab	78.4a
C131AL	95.1a	90.2a	16.1cd	22.7c	113.3a	78.7a
Oh43F	97.1a	92.7a	16.2c	22.8c	105.9bc	73.7b
Oh43L	97.6a	94.6a	16.1cd	23.1c	102.6c	76.9ab
WF9F	81.4b	80.9b	16.1cd	22.8c	105.4bc	73.6b
WF9L	96.1a	95.6a	15.8cd	22.6c	110.1ab	79.2a

[a]Means followed by the same letter do not differ at the 5% level of probability (Duncan's Multiple Range Test).

cultures. M160 also took 2.1 to 3.9 days longer to emerge as adults than did the other 8 cultures (Table 1).

Differences in female and male pupal weights for the 9 cultures were significant. The range in pupal weights of female corn borers was 100.9 mg for M133 to 113.3 mg for CI31AL. The range in male pupal weights was 73.0 mg for M133 to 81.9 mg for M5 (Table 1). Female pupae of cultures reared continuously on a meridic diet (M133 and M160) weighed ca. the same as the check culture (M5) but weighed less than most of the other cultures (reared 1 generation on corn and 8 generations each year on a meridic diet). Male pupae of all corn borer cultures weighed ca. the same as the check culture (M5).

Experiment II

This experiment was conducted to determine if corn borer culture M160, which had been reared on a meridic diet containing dried-ground corn leaves instead of wheat germ for 160 generations, can be reared on a diet containing wheat germ, and to determine if corn borer cultures (M133, CI31AF, CI31AL, Oh43F, Oh43L, WF9F, and WF9L), which had been reared on a meridic diet containing wheat germ for many generations, can be reared on a diet containing dried-ground corn leaves.

Methods and materials

The corn leaves were from the same source as reported in Experiment I (WF9 x M14 leaves stored at -23°C for 13 years). A culture reared on a diet containing wheat germ for 5 generations (M5) was used as a check.

Larvae were reared individually on plugs of diet placed in 3-dram vials; the vials were placed in trays containing 9 rows of 17 vials/row. A split-plot design was used with the 9 corn borer cultures randomized on the whole plot area and the 2 diets randomized on the split-plot area. Twelve replications (2 trays/replication) were used for a total of 204 larvae/corn borer culture on each diet (3,672 larvae were used for the experiment). The incubation room was maintained at a temperature of 27-28°C, 75% R.H., and constant light.

The criteria used for evaluating the effect of diet on larval survival and development were: (1) percentage survival to pupation, (2) percentage survival to adults, (3) days to pupation, (4) days to adults, and (5) weight of female and male pupae.

Results and discussion

The analysis of variance for percent survival to pupation showed significant difference between the main effect of cultures. The main effect of diets and the interaction of cultures x diets were not significant. Percent survival to

pupation was high for all 9 cultures reared on a diet containing wheat germ with a range of 93.4 for the check (M5) to 99.0 for Oh43F. Percent survival to pupation was high for 8 of the 9 cultures reared on a diet containing dried-ground corn leaves; M160 had 81.4% survival to pupation; survival to pupation for the other 8 cultures ranged from 91.7% to 100% (Table 2).

The analysis of variance for percent survival to adult emergence showed significant differences between the main effect of cultures. The main effect of diets and the interaction of cultures x diets were not significant. Percent survival to adult emergence for larvae reared on a diet containing wheat germ ranged from 90.2 for M5, M133, and Oh43L, to 95.6 for WF9F. Percent survival to adult emergence for larvae reared on a diet containing dried-ground corn leaves ranged from 79.9 for M160 to 98.5 for CI31AL.

Larval survival to pupation and to adult emergence was higher for corn borer culture M160 that was reared on a diet containing wheat germ than on a diet containing dried-ground corn leaves; larval survival of the other 8 cultures was nearly equal on the 2 diets (Table 2).

The analysis of variance for number of days to pupation and number of days to adult emergence showed significant differences between the main effect of cultures, the main effect of diets, and the interaction of cultures x diets. The range

Table 2. Larval development and survival of European corn borer cultures reared continuously on two diets

Cultures	Percent survival to				No. of days to				Pupal weight (mg)			
	Pupation		Adult		Pupation		Adult		Female		Male	
	WG[a]	Lf(0)[b]	WG[a]	Lf(0)[b]	WG[a]	Lf(0)[b]	WG[a]	Lf(0)[b]	WG[a]	Lf(0)[b]	WG[a]	Lf(0)[b]
M5	93.4	91.7	90.2	91.2	17.2	18.1	24.3	24.7	98.0	83.3	71.7	72.3
M160	95.1	81.4	92.7	79.9	17.2	19.0	24.2	25.4	101.5	99.9	77.5	78.0
M133	95.6	95.1	90.2	88.7	16.6	17.6	23.4	24.2	100.7	102.0	76.0	75.6
CI31AF	94.6	93.6	93.1	90.7	16.4	17.3	23.6	24.2	106.7	103.6	77.2	75.4
CI31AL	95.1	99.5	93.6	98.5	16.4	16.0	23.3	22.6	109.9	109.1	76.3	80.4
Oh43F	99.0	99.5	95.1	97.6	16.4	16.4	23.4	23.3	110.4	106.4	78.3	80.7
Oh43L	93.6	93.2	90.2	91.7	16.3	16.1	23.0	22.8	104.7	101.1	75.1	75.7
WF9F	97.1	100.0	95.6	93.6	16.3	16.8	23.2	23.9	105.5	102.6	74.8	77.9
WF9L	96.1	96.6	95.1	95.1	16.4	17.2	23.3	24.1	108.4	108.7	78.9	79.8
LSD.05												
Any 2 means between cultures for the same diet	2.4		2.4		0.7		0.8		1.9		1.1	
Any 2 means between diets for the same culture	6.4		6.4		0.7		0.8		4.8		2.7	

[a]Meridic diet containing wheat germ as an ingredient.

[b]Meridic diet containing dried-ground corn leaves (stored at -23°C for 13 years) as an ingredient.

in number of days to pupation for the 9 cultures reared on a diet containing wheat germ was 16.3 for Oh43L and WF9F to 17.2 for M5 (check) and M160 (Table 2), whereas the range for the 9 cultures reared on a diet containing dried-ground corn leaves was 16.0 for CI31AL to 19.0 for M160. The range in number of days to adult emergence for 9 cultures of corn borers reared on a diet containing wheat germ was 23.0 for Oh43L to 24.3 for M5. The range in number of days to adult emergence for 9 cultures reared on a diet containing dried-ground corn leaves was 22.6 for CI31AL to 25.4 for M160. Larval development of M160 (reared on either a diet containing wheat germ or dried-ground corn leaves) was slower than larval development of most of the other 8 cultures. In general, larval development of the other 8 cultures was nearly equal on the 2 diets (Table 2).

The analysis of variance for weights of female and male pupae showed significant differences between the main effects of cultures and the main effects of diets. The interaction of cultures x diets was not significant. The range in female pupal weights for larvae reared on a diet containing wheat germ was 98.0 mg for M5 to 110.4 mg for Oh43F (Table 2). The range in female pupal weights for larvae reared on a diet containing dried-ground corn leaves was 83.3 mg for M5 to 109.1 mg for CI31AL. M133 and M160 pupae (reared on either diet) weighed more than did pupae of the check culture (M5) but less than several of the other cultures. Female pupae of M5 weighed

less on the diet containing dried-ground corn leaves than on the diet containing wheat germ. Pupal weights of the other 8 cultures were nearly equal on the 2 diets. The range in male pupal weights for cultures reared on a diet containing wheat germ was 71.7 mg for M5 to 78.9 mg for WF9L and for cultures reared on a diet containing dried-ground corn leaves was 72.3 mg for M5 to 80.7 mg for Oh43F; the M133 and M160 pupae weighed more than did the check pupae (M5) but less than pupae of some other cultures. Pupal weights of most of the cultures were equal on the 2 diets.

Experiment III

This experiment was conducted to determine if dried-ground corn leaves (WF9 x M14) stored at a temperature of -23°C for 13 years have deteriorated as an ingredient in a diet for rearing the European corn borer and to determine performance of corn borer cultures reared on 3 diets.

Methods and materials

The following 3 diets were used for rearing the 9 cultures of corn borers (M5, M160, M133, CI31AF, CI31AL, Oh43F, Oh43L, WF9F, WF9L): (1) meridic diet containing wheat germ, (2) meridic diet containing dried-ground leaves of WF9 x M14 (stored at -23°C for 13 years), and (3) meridic diet containing dried-ground leaves of B73 x M017 (stored at -23°C for 1 month); B73 x M017 is susceptible to the European corn borer.

Larvae were reared individually on plugs of diet placed in 3-dram vials; the vials were placed in trays containing 9 rows of 17 vials/row. A split-plot design was used with the 9 corn borer cultures randomized on the whole plot area and the 3 diets randomized on the split-plot area. Six replications (3 trays/replication) were used for a total of 102 larvae/corn borer culture on each diet (2,754 larvae for the experiment). The incubation room was maintained at a temperature of 27-28°C, 75% RH, and constant light.

The criteria used for evaluating the effect of diet on larval survival and development were: (1) percentage survival to pupation, (2) percentage survival to adults, (3) days to pupation, (4) days to adult emergence, and (5) weights of female and male pupae.

Results and discussion

The analysis of variance for percent survival to pupation and percent survival to adult emergence showed no significant differences between the main effect of cultures, the main effect of diets, and the interaction of cultures x diets. The main effect of cultures and diets are of little interest and the means are not recorded. The data for the interaction of cultures x diets are recorded in Table 3.

Larval survival to pupation and to adult emergence for 9 cultures of corn borers were relatively high on the 3 diets; statistically, larval survival of the 8 cultures was as high

Table 3. Larval survival and development of European corn borer cultures reared continuously on three meridic diets

Cultures	Percent survival						No. of days		
	To pupation			To adult			To pupation		
	WG[a]	LF(0)[b]	LF(N)[c]	WG[a]	LF(0)[b]	LF(N)[c]	WG[a]	LF(0)[b]	LF(N)[c]
M5	87.3	89.2	86.3	86.3	87.3	80.4	16.8	16.3	16.4
M160	91.2	94.1	98.0	89.2	94.1	93.1	17.4	17.4	17.7
M133	92.2	94.1	98.0	90.2	90.2	97.1	16.8	17.2	17.6
CI31AF	98.0	86.3	90.2	92.2	79.4	83.3	16.4	16.7	16.6
CI31AL	95.1	96.1	94.1	93.1	95.1	92.2	16.2	15.6	16.0
Oh43F	92.2	97.1	98.0	86.3	95.1	95.1	15.9	15.7	16.0
Oh43L	98.0	92.2	93.1	95.1	90.2	93.1	16.2	17.0	16.2
WF9F	93.1	96.1	95.1	90.2	89.2	94.1	16.6	16.8	16.9
WF9L	91.2	95.1	95.1	91.2	89.2	89.2	16.3	16.9	16.7
LSD.05									
Any 2 means between cultures for the same diet								0.5	
Any 2 means between diets for the same culture								0.3	

[a]Meridic diet containing wheat germ as an ingredient.

[b]Meridic diet containing dried-ground corn leaves (WF9 x M14) stored at -23°C for 13 years as an ingredient.

[c]Meridic diet containing dried-ground corn leaves (B73 x MO17) stored at -23°C for 1 month as an ingredient.

Pupal weight (mg)								
To adult			Female			Male		
WG[a]	LF(0)[b]	LF(N)[c]	WG[a]	LF(0)[b]	LF(N)[c]	WG[a]	LF(0)[b]	LF(N)[c]
23.5	23.1	23.1	99.8	102.9	107.5	72.0	76.9	78.8
24.3	24.8	25.1	99.5	101.7	98.8	74.7	75.4	77.2
23.8	25.0	25.7	102.2	103.9	98.8	79.5	79.2	78.5
23.4	23.9	23.9	107.8	109.7	106.2	78.7	84.4	78.5
22.9	22.0	22.6	106.8	110.2	108.6	78.6	83.2	79.4
22.7	22.2	22.4	109.6	110.1	109.9	78.8	82.4	82.2
23.0	22.4	22.6	109.0	107.4	111.4	80.1	85.6	83.3
23.3	23.8	24.4	105.5	110.2	106.4	79.6	83.4	82.1
23.2	24.3	24.1	114.0	109.6	108.6	84.1	86.0	84.8
	0.7			3.8			2.9	
	0.2			2.0			1.6	

as the check culture (M5). In general, larval survival to pupation and to adult emergence were equal or nearly equal for each culture on the 3 diets (i.e. diets containing wheat germ, dried-ground corn leaves stored at -23°C for 13 years, and dried-ground corn leaves stored at -23°C for 1 month).

The analysis of variance for number of days to pupation showed no significant difference between the main effect of cultures and diets. The interaction of cultures x diets was significant. The analysis of variance for number of days to adult emergence showed significant differences for the main effect of cultures, the main effect of diets, and the interaction of cultures x diets. In general, corn borer cultures M133 and M160 developed slower than the check culture (M5) and slower than most of the other cultures (Table 3). Also, the rate of larval development was equal or nearly equal for each culture on the 3 diets.

The analysis of variance for female pupal weights showed significant differences between the main effect of cultures. Differences between the main effect of diets and the cultures x diets interaction were not significant. Differences between the main effect of cultures and diets were significant for male pupal weights; the interaction of cultures x diets was not significant. In general, weights of female and male pupae of cultures M133 and M160 were equal to or almost equal to pupal

weights of the check culture (M5) but were less than pupal weights of the other 6 cultures (reared 1 generation each year on corn, 8 generations each year on a meridic diet). Pupal weights for most cultures were nearly equal on the 3 different diets.

Conclusions

The data for the 3 experiments show that European corn borer cultures reared for many generations on a meridic diet containing dried-ground corn leaves (M160) can be reared on a meridic diet containing wheat germ and that corn borer cultures reared for many generations on a meridic diet containing wheat germ (M133, CI31AF, CI31AL, Oh43F, Oh43L, WF9F, WF9L) can be reared on a diet containing dried-ground corn leaves. Larval survival and development were equal or nearly equal on diets containing dried-ground corn leaves stored at a temperature of -23°C for 13 years compared with dried-ground corn leaves stored at a temperature of -23°C for 1 month.

In general, larvae reared for 133-160 generations on a meridic diet survived as well and the pupae weighed as much as a check culture (M5) but larval development (number of days to pupation and to adult emergence) was slower than the check culture (M5). Larval survival of corn borer cultures reared for 1 generation each year on corn in the field and 8 generations each year on a meridic diet in the laboratory (CI31AF,

CI31AL, Oh43F, Oh43L, WF9F, WF9L) was high; in general, the rate of larval development of these cultures was faster and pupal weights were higher than the check culture (M5).

LARVAL SURVIVAL AND DEVELOPMENT OF THE EUROPEAN CORN BORER REARED ON A MERIDIC DIET IN DISHES

Millions of European corn borer egg masses are produced in the laboratory each season for artificial infestation of maize plants (Guthrie et al. 1971). For large scale production of egg masses, larvae are reared in plastic dishes (25.4 cm diam. and 8.9 cm deep) containing 930 g of diet.

The 8 corn borer cultures (M160, M133, CI31AF, CI31AL, Oh43F, Oh43L, WF9F, and WF9L) used in this study were reared for many generations on plugs of diet in 3-dram vials (1 larva/vial). The M5 (check) culture was reared for 2 generations on plugs of diet in 3-dram vials and for 3 generations in dishes.

Experiment IV

Methods and materials

This experiment was conducted to determine if corn borer larvae reared for many generations on plugs of diet in 3-dram vials survive and develop rapidly under crowded conditions in dishes and to determine if the larvae will crawl out of the diet into strips of corrugated paper to pupate.

Each dish was infested with 40 black-head egg masses (ca. 1000 eggs). Two dishes were used for each of the 9 cultures over a period of 10 days for a total of 20 dishes/

corn borer culture (1000 egg masses/dish = ca. 20,000 eggs/culture); each dish constituted 1 replication in a randomized block design. Strips of corrugated paper (2.5 cm wide, treated in hot wax) were placed in the dishes for pupation as described by Guthrie et al. (1971). The incubation room was maintained at a temperature of 27-28°C, 75% RH, and constant light.

Twenty-one days after egg hatch, the corrugated strips containing pupae were placed in oviposition cages inside a large room (27-28°C and 75-80% RH) for moth emergence. After all moths had emerged from the strips, pupal cases were counted.

Some larvae remained in the dishes to pupate; the number of these larvae was recorded.

Results and discussion

The analysis of variance showed significant differences between corn borer cultures in percentage of larvae crawling out of the diet into strips of corrugated paper to pupate. Fewer larvae of corn borer culture M160 (reared on a meridic diet containing dried-corn leaves for 160 generations) crawled out of the diet into corrugated strips to pupate than did the larvae of check culture (M5) and the other 8 cultures (Table 4). The same percentage of larvae of M133 (reared on a meridic diet containing wheat germ for 133 generations) and larvae of the check culture (M5) crawled out of the diet into corrugated

strips to pupate. A high percentage of larvae of corn borer cultures reared for 1 generation each year on corn in the field and 8 generations each year on a meridic diet in the laboratory (CI31AF, CI31AL, Oh43F, Oh43L, WF9F, WF9L) crawled out of the diet into corrugated strips to pupate. Therefore, certain behavioral patterns of corn borer cultures reared in the laboratory do change over time.

Table 4. Larval survival and development of European corn borer larvae reared on a meridic diet in dishes and percentage of larvae that crawled into strips of corrugated paper to pupate vs percentage of larvae that remained in the dish to pupate

Cultures	Percentage of larvae in		Total individuals counted
	Dish[a]	Strip	
M5	27.6	72.4	15,069
M160	35.7	64.3	12,211
M133	28.0	72.0	12,780
CI31AF	17.9	82.1	18,974
CI31AL	20.6	79.4	17,223
Oh43F	16.6	83.4	17,823
Oh43L	17.1	82.9	18,057
WF9F	16.0	84.0	17,997
WF9L	17.7	82.3	16,690

[a]LSD.05 any 2 means between the percentage of cultures in the dish = 3.1.

MATING BEHAVIOR OF EUROPEAN CORN BORERS REARED CONTINUOUSLY ON A MERIDIC DIET

Fye and LaBrecque (1966) noted that 3 races of male houseflies, Musca domestica (L) reared for many generations in the laboratory were less acceptable to females than were the males of natural populations. Laboratory caged apple maggots, Rhagoletis pomonella (Walsh) were unable to mate or oviposit until 7-8 days after emergence indicating that there are mating behavioral changes in laboratory insects (Prokopy et al. 1972).

Experiment V

Methods and materials

Virgin female and male moths of 8 cultures of corn borers (M160, M133, CI31AF, CI31AL, Oh43F, Oh43L, WF9F, and WF9L) and a culture used as a check (M5) were placed in oviposition cages (100 females, 115 males/cage). The oviposition room was operated at a temperature of 27°C during 18 hrs each day and 16-20°C during 6 hrs each day. The cycling temperature was required to insure adequate mating as reported by Guthrie et al. (1971). A randomized block design with 6 replications was used for a total of 600 female and 690 male moths/culture or 11,610 moths for the 9 cultures. The moths were allowed to mate in the cages for 6 days. Female moths were then killed and were preserved in 75% alcohol; female moths were dissected and the

number of spermatophores in each female of each corn borer culture was counted; spermatophores are an index of the number of times female moths mate (Showers et al. 1974).

Results and discussion

The analysis of variance showed significant differences between corn borer cultures in mating behavior based on spermatophore counts. Corn borer cultures M5, M160, M133, Oh43F, and WF9L mated a maximum of 4 times; CI31AL and Oh43L mated a maximum of 5 times; and CI31AF and WF9F mated a maximum of 6 times (Table 5). Mean spermatophore counts for the 9 cultures ranged from 1.2 to 2.1 spermatophores/female moth. Corn borer cultures M133 and M160 had about the same number of spermatophores as did the check culture (M5). Corn borer cultures CI31AF, CI31AL, Oh43L, and WF9F had the greatest number of spermatophores (Table 5). In general, corn borer cultures reared continuously on a meridic diet in the laboratory (M133, M160) mated as many times as did a check culture (M5), whereas most of the corn borer cultures reared 1 generation each year on corn in the field and 8 generations each year on a meridic diet in the laboratory (CI31AF, CI31AL, Oh43L, WF9F) mated more times than did the check culture (M5).

Table 5. Mating frequencies of European corn borer cultures reared continuously on a meridic diet

Cultures	Percentage of females with 0 to 6 spermatophores							
	0	1	2	3	4	5	6	Mean[a]
M5	20.7	48.0	22.0	8.7	0.6	0.0	0.0	1.2
M160	16.0	33.3	36.7	13.3	0.7	0.0	0.0	1.5
M133	23.3	46.0	20.7	8.0	2.0	0.0	0.0	1.2
CI31AF	2.7	29.3	36.0	24.7	5.3	1.3	0.7	2.1
CI31AL	2.7	25.7	38.0	26.3	4.0	3.3	0.0	2.0
Oh43F	16.0	40.7	33.3	8.7	1.3	0.0	0.0	1.4
Oh43L	6.7	24.4	39.3	26.3	2.7	0.6	0.0	1.9
WF9F	5.3	27.3	40.7	22.7	2.0	0.7	1.3	1.9
WF9L	24.0	42.0	24.0	9.3	0.7	0.0	0.0	1.2

[a]LSD.05 any 2 means between cultures = 0.45.

EFFECT OF TEMPERATURE AND HUMIDITY ON THE HATCHABILITY OF EUROPEAN CORN BORER CULTURES REARED CONTINUOUSLY ON A MERIDIC DIET

Eight corn borer cultures (M160, M133, CI31AF, CI31AL, Oh43F, Oh43L, WF9F, WF9L) have been maintained in the laboratory for many generations. In general, 9 to 11 generations were obtained each year. Large egg masses were selected for advancing each corn borer culture to the next generation.

Corn borer cultures M160 and M133 were reared each generation on plugs of diet in 3-dram vials (1 larva/vial; 500 larvae/generation). Cultures CI31AF, CI31AL, Oh43F, Oh43L, WF9F, and WF9L were reared from 1965-1976 on plugs of diet in 3-dram vials (2 larvae/vial, 500 larvae/generation), and from 1977-1978 on diet in dishes (930 g of diet/dish, infested with ca. 1000 eggs). Culture M5 (check) was reared for 2 generations on plugs of diet in 3-dram vials and for 3 generations on diet in dishes.

European corn borers reared continuously on a meridic diet cannot be used in screening corn genotypes for resistence or susceptibility because leaf feeding damage is too low for measuring differential resistance (Guthrie et al. 1971). In host plant resistance research disks of waxed paper (1.3 cm diam.), containing 1 or 2 egg masses/disk, are pinned onto celotex boards (Guthrie et al. 1965); these boards are wrapped in moist paper, placed in plastic bags, and incubated at a

temperature of 27-28°C for ca. 4 days. During most years corn plants are not of sufficient height to infest with egg masses when the 1st masses are produced; 300-500 thousand egg masses, therefore, are incubated for 10-12 days at a temperature of 16°C and high humidity.

Several experiments were conducted to determine: (1) if egg masses produced by corn borer cultures reared for many generations on a meridic diet have increased or decreased in size, (2) if hatchability of egg masses from these cultures incubated at a temperature of 16°C and 27°C and high humidity has deteriorated, and (3) if a relatively low humidity affects hatchability of corn borer cultures that had been reared for many generations under high humidity conditions. A decrease in hatchability would partially explain the low level of larval survival on corn plants under field conditions.

Experiment VI

Methods and materials

Virgin moths of 9 corn borer cultures were placed into oviposition cages. A randomized block design with 6 replications (100 females and 115 males in 1 cage for each culture = 1 replication) were used. The method of egg production was reported by Guthrie et al. (1971).

Female moths oviposited on sheets of waxed paper placed on top of each cage (2 sheets of 15 cm x 60 cm/cage). The

waxed paper containing egg masses was replaced with new paper daily for 9-10 days. A total of 5,400 female moths was used in the experiment; these moths would be expected to produce ca. 54,000 egg masses (ca. 1,350,000 eggs); thus a 10% sample of egg masses on each sheet of waxed paper (taken at random) was used to estimate (1) the size of egg mass for each culture, (2) percentage of eggs that hatched, (3) percentage of eggs that embryonated without hatching, and (4) percentage of sterile eggs.

Disks of waxed paper containing 1 egg mass/disk were cut out each day and pinned onto celotex boards; these boards were wrapped in moist paper, placed in plastic bags, and incubated at 27-28°C for 4 days. At the black-head stage of embryonic development, the boards with egg masses were spread out to allow the hatching larvae to crawl away. The egg masses (after hatching) were observed under a compound light microscope. A total of 2,781 egg masses (56,050 eggs) were examined.

Results and discussion

Table 6 shows that corn borer culture Oh43L had the smallest number of eggs/mass (13.3), whereas corn borer culture CI31AF had the largest number of eggs/mass (19.4). Egg masses of corn borer cultures M133 and M160 contained the same number of eggs as the check culture (M5) and did not differ significantly from most of the other cultures.

Table 6. Size of egg masses and hatchability of European corn borer egg masses incubated at a temperature of 27-28°C and high humidity

Cultures	No. of eggs counted	Egg mass size[a]	Range	Percent hatched[a]	Percent embryonated without hatching	Percent sterile[a]
M5	5,852	16.3b	3-59	74.2a	13.0ab	12.8cd
M160	6,228	16.1b	2-78	64.5b	11.3abcd	24.2a
M133	5,500	16.3b	2-69	66.9b	13.8a	19.3ab
CI31AF	4,428	19.4a	3-56	76.9a	12.4abc	10.6d
CI31AL	5,197	18.3ab	1-72	67.3b	10.6bcd	22.1ab
Oh43F	4,685	17.6ab	1-53	78.0a	9.9cde	12.1cd
Oh43L	4,015	13.3c	2-53	74.5a	7.5e	18.0bc
WF9F	5,106	17.7ab	3-71	73.9a	8.5de	17.6bc
WF9L	5,039	17.6ab	1-66	75.3a	11.9abc	12.9cd

[a]Means within a column followed by the same letter do not differ significantly at 5% level of probability (Duncan's Multiple Range Test).

Hatchability of eggs for corn borer cultures M133 (reared for 133 generations on a meridic diet containing wheat germ) and M160 (reared for 160 generations on a diet containing dried-ground corn leaves) was significantly lower than was hatchability of eggs from the check culture (M5) and was significantly lower than was most of the cultures reared 1 generation each year on corn in the field and 8 generations each year on a meridic diet in the laboratory (CI31AF, CI31AL, Oh43F, Oh43L, WF9F, WF9L).

Experiment VII

Methods and materials

Egg masses from female moths used in Experiment VI were utilized in this experiment. A randomized block design with 4 replications was used. Each replication contained 50 egg masses of each of the 9 corn borer cultures for a total of 200 egg masses/culture. Disks of waxed paper containing 1 egg mass/disk were cut out and pinned onto celotex boards (50 masses/board). The boards were wrapped in moist paper, placed in plastic bags, and incubated at a temperature of 27-28°C for 4 days. At the black-head stage of embryonic development, the boards were spread out to allow the hatching larvae to crawl away. The egg masses (after hatching) were observed under a compound light microscope; 1,800 masses (38,813 eggs) were examined.

The criteria used for evaluating hatchability of eggs for the 9 corn borer cultures were: (1) percentage of eggs that hatched, (2) percentage of eggs that embryonated without hatching, and (3) percentage of sterile eggs.

Results and discussion

The data in Table 7 show that hatchability of eggs for corn borer culture M160, incubated at a temperature of 27°C and high humidity, was significantly lower than was hatchability of eggs for the check culture (M5), i.e., 58.6% vs 76.4%, and was significantly lower than the hatchability of eggs for most of the cultures reared for 1 generation each year on corn in the field and 8 generations each year on a meridic diet in the laboratory (corn borer cultures, CI31AF, CI31AL, Oh43F, Oh43L, WF9L). Hatchability of eggs for corn borer culture M133 was 8.1% lower than was hatchability of eggs for the check culture but was 9.7% higher than was hatchability of eggs for corn borer culture M160; these differences were not statistically significant.

Experiment VIII

Methods and materials

Egg masses from female moths used in Experiment VI were utilized in this experiment. Methods were identical to the methods used in Experiment VII except that the egg masses (on

Table 7. Hatchability of European corn borer egg masses incubated at a temperature of 27-28°C and high humidity

Cultures	Percent hatched	Percent embryonated without hatching	Percent sterile[a]
M5	76.4a	5.7a	17.4abc
M160	58.6b	10.5a	31.8a
M133	68.3ab	8.3a	27.3ab
CI31AF	77.2a	8.6a	15.5abc
CI31AL	78.6a	8.8a	12.1bc
Oh43F	76.2a	9.5a	5.8c
Oh43L	80.5a	8.9a	10.0bc
WF9F	74.4ab	9.8a	13.1bc
WF9L	82.8a	9.6a	5.0c

[a]Means within each column followed by the same letter do not differ significantly at 5% level of probability (Duncan's Multiple Range Test).

celotex boards) from the 9 corn borer cultures were incubated at a temperature of 16°C and ca. 95% RH. In 10-12 days (blackhead stage of embryonic development) the boards were spread out in a room operating at a temperature of 27-28°C and 75-80% RH to allow the hatching larvae to crawl away. A total of 1,800 egg masses (39,580 eggs) were examined for: (1) percentage of eggs that hatched, (2) percentage of eggs that embryonated without hatching, and (3) percentage of sterile eggs.

Results and discussion

The data in Table 8 show that hatchability of eggs for corn borer culture M160, incubated at a temperature of 16°C and high humidity, was significantly lower than was hatchability of eggs for the check culture (M5) i.e. 50.2% vs 73.4%. Hatchability of eggs for M160 was also significantly lower than was hatchability of eggs for a culture reared for 133 generations on a meridic diet containing wheat germ (M133) and for most of the cultures reared 1 generation each year on corn in the field and 8 generations each year on a meridic diet in the laboratory (CI31AF, Oh43F, Oh43L, WF9F, WF9L).

Table 8. Hatchability of European corn borer egg masses incubated at a temperature of 16°C and high humidity

Cultures	Percent hatched[a]	Percent embryonated without hatching[a]	Percent sterile[a]
M5	73.4a	8.7c	17.8b
M160	50.2b	12.5ab	37.3a
M133	67.2a	12.8a	20.0b
CI31AF	67.7a	10.7abc	21.6b
CI31AL	61.8ab	8.2c	30.0ab
Oh43F	74.4a	8.9bc	16.7b
Oh43L	70.7a	11.1abc	18.2b
WF9F	77.2a	10.2abc	12.7b
WF9L	69.5a	11.0abc	19.5b

[a]Means within each column followed by the same letter do not differ significantly at 5% level of probability (Duncan's Multiple Range Test).

Experiment IX

Methods and materials

Virgin moths of 9 corn borer cultures (including M5 as a check) were placed into oviposition cages (200 pairs of moths/cage, 1 cage/culture). Female moths oviposited on sheets of waxed paper placed on top of each cage (2 sheets of 15 cm x 60 cm/cage). Egg masses for these studies were from the 4th day of moth oviposition. Disks of waxed paper containing 1 egg mass/disk were cut out and pinned onto celotex boards (25 egg masses/board).

A randomized block design with 4 replications (25 egg masses/replication) was used for each of 3 levels of humidity. The 3 levels of humidity for incubation of egg masses were:

(1) ca. 100% RH until all eggs had hatched; the boards containing egg masses were wrapped in moist paper; placed in plastic bags, and incubated at a temperature of 27-28°C (27,224 eggs were examined)

(2) ca. 100% RH for 4 days (black-head stage of embryonic development) and 1 day at 35% RH; the boards containing egg masses were wrapped in moist paper, placed in a plastic bag and incubated at a temperature of 27-28°C for 4 days, then the egg masses were removed from the plastic bag, unwrapped, and exposed to a temperature of 27°C and 35% RH (23,279 eggs were examined)

(3) ca. 35% RH until all eggs had hatched; the boards containing egg masses were incubated at a temperature of 27-28°C and 35% RH (33,209 eggs were examined).

The egg masses (after hatching) were observed with a compound light microscope. The criteria used for evaluating hatchability of egg masses for the 9 corn borer cultures were: (1) percentage of eggs that hatched, (2) percentage of eggs that embryonated without hatching, and (3) percentage of sterile eggs.

Results and discussion

Hatchability of eggs for all corn borer cultures was high when incubated under high humidity conditions until all eggs had hatched. The range in egg hatchability was 84.9% for corn borer culture WF9F to 95.0% for corn borer culture CI31AL (Table 9). Hatchability of eggs for corn borer cultures M133 and M160 (reared on a meridic diet in the laboratory for 133 and 160 generations, respectively) was not significantly different from the check culture (M5) and was not significantly different from most of the cultures that had been reared each year for 1 generation on corn plants in the field and 8 generations each year on a meridic diet in the laboratory (CI31AF, CI31AL, Oh43F, Oh43L, WF9F, WF9L).

Hatchability of eggs for all corn borer cultures was relatively high when incubated under high humidity conditions

Table 9. Hatchability of European corn borer egg masses incubated at a temperature of 27-28°C and 100% RH until egg hatch

Cultures	Percent hatched[a]	Percent embryonated without hatching[a]	Percent sterile[a]
M5	88.8ab	9.5abc	1.7a
M160	85.9b	14.0a	0.1a
M133	90.0ab	8.4bc	1.6a
CI31AF	85.1b	11.9ab	3.0a
CI31AL	95.0a	4.6c	0.4a
Oh43F	91.7ab	7.1bc	1.2a
Oh43L	89.2ab	9.4abc	1.4a
WF9F	84.9b	12.1ab	3.0a
WF9L	88.5ab	9.2abc	2.3a

[a]Means within each column followed by the same letter do not differ significantly at 5% level of probability (Duncan's Multiple Range Test).

until the black-head stage of embryonic development (ca. 4 days) and 35% RH until all eggs had hatched (ca. 1 day). The range in egg hatchability was 72.1% for corn borer culture M160 to 84.9% for corn borer culture CI31AL (Table 10). Hatchability of eggs for M133 and M160 was not statistically different from the check culture (M5) and was not significantly different from most of the other corn borer cultures.

Table 10. Hatchability of European corn borer egg masses incubated at a temperature of 27-28°C and 100% RH until black-head stage of embryonic development and at 35% RH until egg hatch

Cultures	Percent hatched[a]	Percent embryonated without hatching[a]	Percent sterile[a]
M5	77.5ab	18.9ab	3.6a
M160	72.1b	23.8a	4.1a
M133	77.3ab	18.6ab	4.1a
CI31AF	73.7ab	19.5ab	6.8a
CI31AL	84.9a	11.0b	4.1a
Oh43F	83.1ab	11.9b	5.0a
Oh43L	78.8ab	14.6ab	6.6a
WF9F	75.5ab	20.1ab	4.4a
WF9L	73.2b	23.0a	3.8a

[a]Means within each column followed by the same letter do not differ significantly at 5% level of probability (Duncan's Multiple Range Test).

Hatchability of eggs for all corn borer cultures was low when incubated under low (35% RH) humidity conditions until all eggs had hatched (Table 11). The range in egg hatchability was 19.7% for corn borer culture M5 to 37.8% for corn borer culture Oh43F. Hatchability of eggs for M133 and M160 was not significantly different from the check culture (M5) and was not significantly different from most of the other corn borer cultures.

Table 11. Hatchability of European corn borer egg masses incubated at a temperature of 27-28°C and 35% RH until egg hatch

Cultures	Percent hatched[a]	Percent embryonated without hatching[a]	Percent sterile[a]
M5	19.7c	73.2a	7.1b
M160	20.9c	67.6abc	11.5a
M133	28.9abc	63.3bcd	7.8ab
CI31AF	25.1bc	65.4abc	9.6ab
CI31AL	31.8ab	60.1bcd	8.1ab
Oh43F	37.8a	54.7bd	7.5ab
Oh43L	28.9abc	65.5abc	8.0ab
WF9F	25.0bc	69.7ab	5.3b
WF9L	25.1bc	65.9abc	9.0ab

[a]Means within each column followed by the same letter do not differ significantly at 5% level of probability (Duncan's Multiple Range Test).

Conclusions

In general, data from Experiments VI, VII, and VIII show that hatchability of eggs from a corn borer culture reared for 160 generations (M160) on a meridic diet containing dried-ground corn leaves has deteriorated. Data from Experiment IX, however, show that hatchability of eggs from M160 was as high or almost as high as was hatchability of eggs from the check

culture (M5). Hatchability of eggs from a culture reared for 133 generations (M133) on a meridic diet containing wheat germ was numerically higher but was not significantly different in most tests than was hatchability of eggs from culture M160; since these 2 cultures had lower hatchability in some tests (compared with the check culture) but not in other tests, we are not certain that hatchability of M133 and M160 explains the low level of larval survival of these 2 cultures on corn plants grown under field conditions.

Hatchability of eggs from corn borer cultures reared for 1 generation each year on corn in the field and 8 generations each year on a meridic diet in the laboratory (CI31AF, CI31AL, Oh43F, Oh43L, WF9F, WF9L) was as high or higher than was hatchability of eggs from the check culture in all tests.

DIFFERENCES IN MOUTH PARTS OF EUROPEAN CORN BORERS REARED CONTINUOUSLY ON A MERIDIC DIET

Corn borer culture M160 has been reared on a meridic diet containing dried-ground corn leaves for 160 generations. Corn borer culture M133 has been reared on a meridic diet containing wheat germ for 133 generations. These 2 cultures have lost their ability to survive on corn plants under field conditions (Guthrie et al. 1971).

When the wild borer is removed from the rigorous selection pressure of the wild environment and colonized in the laboratory under optimum conditions for growth and reproduction, weak individuals may be able to survive and increase in the population (Huggans and Guthrie 1970).

Knipling (1960) has postulated that it might be possible to develop deficiencies in immature stages of insects for their own destruction. Deformed mouth parts in larvae is one example of a deficiency that might interfere with development of an insect in nature, but would not seriously effect their survival in the laboratory. If deformed mouth parts should occur in M160 and M133, a much lower level of larval survival would probably result on corn from artificial infestation (Huggans and Guthrie 1970).

Experiment X

Methods and materials

Corn borer cultures M160, M133 and M5 (check) were individually reared on plugs of diet in 3-dram vials (1 larva/vial).

A randomized block design with 6 replications was used (51 larvae/replication/instar of each culture) a total of 306 2nd instar larvae, 306 3rd instar larvae, and 306 5th instar larvae of each culture was placed in KAAD for 24 hrs, preserved in 75% alcohol, and was examined for deficient mouth parts with a compound light microscope. Special attention was directed to differences in the mandibles because corn borer larvae have chewing mouth parts.

Results and discussion

All larvae of the 3 corn borer cultures had normal mouth parts. Deficient mouth parts, therefore, do not contribute to low survival of corn borer cultures M160 and M133 on corn plants under field conditions.

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