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DETECTION OF PROLONGED DIAPAUSE OF NORTHERN CORN
ROOTWORM IN MICHIGAN (COLEOPTERA: CHRYSOMELIDAE)D. A. Landis¹, E. Levine², M. J. Haas¹ and V. Meints³

ABSTRACT

Prolonged diapause of northern corn rootworm, while known from other Midwestern states, has not previously been reported in Michigan. Populations of northern corn rootworm, (*Diabrotica barberi*) from two first-year corn fields in Genesee County, Michigan were examined for prolonged egg diapause. Prolonged diapause was suspected in these populations due to an unusually high proportion of northern versus western corn rootworms in these fields. Eggs obtained from females collected at these sites were reared in the laboratory for two years. The presence of the prolonged diapause trait was confirmed in one population by eggs which hatched following two simulated winters (7.3%). None of the eggs in the second population hatched following the second chill period, however, some eggs in this population remained in apparent diapause at the end of two years. The potential for using observed population shifts in favor of *D. barberi* as an early warning of the expansion of prolonged diapause in a population is discussed.

Northern corn rootworm (NCR), *Diabrotica barberi* Smith and Lawrence, and western corn rootworm (WCR), *Diabrotica virgifera virgifera* LeConte, are the most serious insect pests of corn in the Midwest, accounting for approximately 1 billion dollars annually through lost yield and costs of control (Metcalfe 1986). Rootworm eggs are laid in the soil of corn fields in late summer, overwinter in diapause and typically hatch the following spring (Branson and Krysan 1981). Larval feeding on corn root systems is usually confined to fields which have been in corn for two or more years. Management of corn rootworms includes the use of adult monitoring and thresholds to predict the need to control rootworms where corn follows corn as well as, crop rotation, tillage, host plant resistance, cultural, biological and insecticidal controls (Levine and Oloumi-Sadeghi 1991). In Michigan, rootworm management is usually accomplished by crop rotation, i.e. planting corn following a non-host crop (soybeans, dry beans, alfalfa, sugar beets or small grains), or by use of insecticides where fields are not rotated (Landis and Giebink 1992a). Scouting for adults to predict the potential for damage in the following year is becoming more prevalent as a decision tool.

Rootworm management is complicated by the occurrence of prolonged (extended) diapause in some NCR populations (Levine and Oloumi-Sadeghi

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1991). Prolonged diapause occurs when eggs pass through more than one winter (chill period) before hatching. This allows for the possibility of damage in first year corn (e.g. corn, soybean, corn rotation) and makes prediction of appropriate management tactics more difficult. Chiang (1965) reported that a small percentage (0.3%) of NCR eggs collected from a Minn. corn field hatched after passing through two winters. More recently, prolonged diapause of NCR has been confirmed from Minn. (Krysan et al. 1986), S. Dak. (Krysan et al. 1984, 1986), N. Dak. and Ill. (Levine et al. 1992). The maximum reported percentage of eggs hatching following more than one chill period (simulated winter) is 51% from east central Ill. (Levine et al. 1992). Damage to first year corn, suggesting the occurrence of the prolonged diapause trait, has occurred in Iowa (Tollefson 1988). Prolonged diapause of NCR has not previously been reported from Michigan.

In many cases, the occurrence of prolonged diapause is detected only after reports of damage (lodged plants) in first-year corn fields. For first-year damage to occur, two criteria must be met. The prolonged diapause trait must be present and there must be sufficiently high numbers of NCR in a field so that the population that diapause for two winters is adequate to cause lodging. In Michigan, NCR are at present relatively uncommon, with 95% or greater of the rootworm population made up of WCR. This situation would tend to lead to minimal damage to first-year corn even if the prolonged diapause trait were present in NCR and may complicate or delay the detection of the phenomenon.

Beginning in 1987, unusually high proportions of NCR beetles were observed in a localized area of Richfield township in Genesee County, Michigan. In August, 1989, an exceptionally high proportion of NCR (> 85%) was observed in two first-year corn fields (following winter wheat) in this area. Prolonged diapause of NCR was one possible explanation for these observations and the following studies were conducted to determine if the trait was present in these populations.

METHODS AND MATERIALS

Two first-year corn fields in Genesee Co., MI (DeWitt and Fisher) with similar cropping histories (Table 1) and high proportions of NCR adults were selected. The soil type of the DeWitt field was a Celina loam (fine, mixed, mesic Aquic Haplaudalf), and in Fisher a Conover loam (fine-loamy, mixed, mesic Udollic Ochraqualf). Numbers of adult corn rootworm beetles per plant and proportion of NCR versus WCR were determined by counting beetles on sixty plants in each of three areas of the two fields in early August 1989 (Landis and Giebink 1992b). Corn root systems from these fields were sampled on 24 August 1989, to determine if rootworm larvae had fed on the current year's plants. Five representative areas of the field were selected and four plants chosen at random from each area. Plants were excavated and root systems returned to the lab for washing and rating of corn rootworm larval damage. Root damage was rated on a 1-6 scale (Hills and Peters 1971). Adult beetle counts on the Fisher field and four additional sites in the same township were conducted on 1-2 Aug. 1991 to assess corn rootworm density and proportions of NCR in the populations. The DeWitt field was not in corn in 1991.

Adult NCR beetles were collected from the DeWitt and Fisher fields on 28 Aug. 1989 by tapping infested silks over a funnel placed in a 3.8 l plastic jug. Beetles were also collected from the flowers of *Daucus carota*, *Solidago* spp. and *Setaria* spp. in the immediate field borders. Approximately 100 females

Table 1.—Cropping histories, mean number of corn rootworm beetles (*D. virgifera virgifera* and *D. barberi*) per plant and percentage *D. barberi* of total number of beetles observed for the study fields (Fisher and DeWitt) and other locations in Genesee Co., MI.

Field	Year	Crop	Planting date	Insecticide kg ai/ha	Mean No. adults per plant ± SEM	% <i>D. barberi</i> (N total)
Fisher ^a	1991	Corn	6/3/91	terbufos, 1.5	2.8 ± 1.8	60.0 (258)
	1990	Corn	5/5/90	phorate, 1.5	-	-
	1989	Corn	4/30/89	none	0.2	> 85.0 ^b
	1988	Wheat	10/87	none	-	-
DeWitt ^a	1991	Wheat	10/90	none	-	-
	1990	Corn	4/30/90	phorate, 1.5	-	-
	1989	Corn	4/27/89	none	0.7 ± 0.3	88.7 (138)
	1988	Wheat	10/87	none	-	-
Bird	1991	Corn (1st yr)	-	-	0.3 ± 0.1	2.6 (38)
Soper	1991	Corn (3rd yr)	-	-	0.9 ± 0.1	16.0 (79)
Zelco	1991	Corn (> 3 yr)	-	-	2.1 ± 1.0	38.0 (184)
Cullen	1991	Corn (> 3 yr)	-	-	2.9 ± 0.7	52.0 (258)

^aPlanted to corn in 1986 and 1987.

^bEstimated by crop scout.

were collected from DeWitt and 50 from the Fisher site along with equivalent numbers of males. Beetles from each population were placed in separate 30 cm³ screen cages (21 ± 2°C) and supplied with fresh corn silks and 3 cm sections of milk-stage corn ear with the kernels lacerated. Oviposition dishes (Krysan et al. 1984) were presented to the beetles from 29 Aug. through 2 Oct. and changed weekly. Soil which passed through a 6.25 mm but not through a 3.13 mm mesh screen was used as the oviposition media. Two loam soils (Colwood-Brookston and Capac) were placed in separate oviposition dishes in each cage and kept moist but not wet with distilled water.

On 2 Oct. all dishes were sent to Illinois for egg extraction and determination of diapause length using methods from Levine et al. (1992). Upon arrival, ca. one half of the dishes were examined to confirm that successful oviposition had occurred. Eggs were removed from the soil by washing through a 0.25 mm mesh sieve with 20°C tap water, counted, placed on and covered by moistened silty clay loam soil (sieved through a 0.18 mm screen) in 60 mm petri dishes (≤ 50 eggs per dish). Eggs in the remaining dishes were not removed from the oviposition soil before storage. All dishes were then placed in a dark environmental chamber (5 Oct.) set to simulate average monthly soil temperatures (1981–1984) at the 10 cm depth in Urbana, IL. (Table 2). Temperatures were adjusted monthly to mimic field conditions and distilled water was added as necessary to keep soil moist. In May 1990, all eggs were separated from the soil by washing through a 0.25 mm mesh sieve and placed on moistened filter paper in 60 mm petri dishes and returned to the chamber. Six drops of 1000 ppm benomyl was added to each filter paper at the outset to retard fungal growth (Oloumi-Sadeghi and Levine 1989). Egg hatch was monitored and recorded daily and distilled water added as needed to keep the filter paper moist. Hatched eggs, larvae, collapsed eggs and those heavily infested with fungi were removed with a camel hair brush (sterilized in alcohol and rinsed with distilled water between each use). In Sept. 1990, unhatched eggs were

Table 2. — Mean monthly soil temperature (1981-1984) at the 10 cm depth, Urbana, IL, used to mimic corn rootworm egg diapause and hatch conditions. Simulated monthly temperature equals average monthly mean (N= 4 years) and is actual temperature (± 0.5 °C) of chamber used for the experiment. Minimum and maximum monthly soil temperatures show the warmest and coldest monthly averages in data set.

Month	Mean Monthly Soil Temperatures		
	Max. Temp. °C	Min. Temp. °C	Simulated Temp. °C
January	2.4	-2.8	0.0
February	6.8	-1.5	1.7
March	11.3	0.0	4.4
April	18.3	3.9	10.6
May	22.5	10.1	16.7
June	26.9	17.2	22.2
July	29.1	20.2	24.4
August	28.2	20.0	24.4
September	26.3	14.2	20.6
October	21.8	8.6	15.0
November	15.3	2.9	8.9
December	9.4	-1.5	3.3

again placed in soil dishes (as above) and returned to the soil temperature chamber. The procedure was repeated in 1991.

RESULTS

In the DeWitt field, 63% of the plant roots sampled in 1989 were fed upon by corn rootworm larvae, however, no root systems had damage ratings greater than 2, indicating minimal damage. The mean root rating for the field was 1.6 (N=19, SEM = 0.11). In the Fisher field, 86% of the plant roots had detectable rootworm damage. Most damaged plants (76.2%) had root ratings of 2, however, single plants with root ratings of 4 and 5 were recorded and both were lodged. The mean root rating for the Fisher field was 2.1 (N=21, SEM=0.19). Yield losses from rootworm damage depend on the biological, environmental and agronomic factors which occur prior to and following damage, however, a root rating of 2.75-3.0 (1-6 scale) is considered by many entomologists to be the minimum level at which the potential for economic damage exists (Mayo 1986).

A total of 101 viable eggs laid by NCR from the DeWitt population, were recovered in May 1990 following the first chill period. During the first observation (hatching) period (May-Sept. 1990) 8 of these eggs died and 76 hatched. At the end of the period, 17 viable (diapausing) eggs were returned to a soil dish for a second chill period. Following the second chill period, (May 1991) 6 had died, 1 was lost and 10 remained viable. During the second observation period, 4 eggs died and 6 hatched. When calculated as a percentage of the eggs which hatched in two years, 92.7% of the eggs in the DeWitt population exhibited a one year diapause and 7.3% had a two year diapause.

In the Fisher population, 41 viable eggs were recovered following the first chill period. In the first observation period, 6 of these eggs died and 18 hatched. At the end of the period, 17 viable eggs remained. In May 1991, 4 eggs were recovered: 12 had died and 1 was lost. Two eggs died in the subsequent observation period and none hatched. The two remaining viable eggs

were placed back into soil for a third chill period. All of the eggs which hatched from the Fisher population exhibited a one year diapause. None showed a two year diapause, however, two eggs remained in diapause at the end of the study.

DISCUSSION

The unusually large percentage of NCR adults in first-year corn fields combined with the presence of root damage was strongly indicative of prolonged diapause in these populations. However, it could not be ruled out that wheat residue or weeds had stimulated egg laying by NCR following wheat harvest in 1988. The subsequent egg diapause studies confirmed that the prolonged diapause trait is present in at least one and perhaps both of these populations.

In the Fisher population, all of the eggs which hatched within two years did so in the first summer, however, two eggs remained in diapause at the end of the study. In other studies (Levine et al. 1992), between 25 and 100% of the eggs which remained in diapause after two years hatched in year three (Mean=49.3%, SEM=9.2%, N=7) with a much smaller percentage hatching in year four. Thus, it is possible that the remaining eggs in this study are exhibiting a three year or longer diapause. If both of the remaining eggs hatched in a subsequent year they would represent a prolonged diapause in 10% of the Fisher population.

The DeWitt population, had a significant proportion of the individuals with a two year diapause (7.3%). This supports the hypothesis that root damage observed in the DeWitt field in 1989 was due to eggs laid in 1987 when the field was last in corn. Failure to detect a two year diapause from the Fisher population suggests that damage in 1989 was either due to eggs laid in 1986 (three year diapause) or that oviposition occurred in the wheat stubble or weeds in 1988. However, it is possible that the smaller initial egg sample size (N=41 for Fisher, N=101 for DeWitt) may have inhibited our ability to detect a low level of two year diapause in the Fisher population. While oviposition by rootworm adults in crops preceding corn has been proposed as an explanation for damage to first-year corn (Branson and Krysan 1981, Hill and Mayo 1980), studies examining oviposition in soybeans (Shaw et al. 1978) and small grain stubble (Gustin 1984) indicate that non-corn oviposition is minimal.

Prolonged diapause is believed to be an adaptation of the NCR to the cultural practice of crop rotation (Krysan et al. 1986). There is a greater incidence of two year diapause in areas where corn is commonly grown in a two year rotations (e.g. corn, soybeans, corn) than in areas of continuous corn production (Krysan et al. 1986, Ostlie 1987). Levine et al. (1992) provide evidence that NCR may also adapt to longer rotations. A population from a South Dakota site where various two to four year rotations prevail, had 20.6, 20.9 and 9.6% of the eggs hatching after two, three and four winters respectively. Among Michigan cash grain producers, corn is typically grown in a two year (31% of acres) or three year rotation (23% of acres) with small grains, beans or sugar beets. This includes some rotations which have corn following corn (e.g. corn, corn, soybeans). A smaller amount (10%) is planted as continuous corn with four year or longer and "other" rotations making up the remainder of the acreage (Chase et al. 1990).

In our study area of Genesee County, numerous rotational patterns exist, including continuous corn as well as three and four year rotations with corn, wheat and soybeans. Although two year crop rotations are practiced on some farms, they not particularly common in this area and are not used on the

farms where we conducted our studies (DeWitt was corn in four out of six years and Fisher five out of six). While it may not be immediately clear from these rotational patterns why prolonged diapause is occurring, it should be noted that development of the trait must be viewed at the population rather than individual field level. As long as a substantial number of fields in an area have rotations which include corn on a two to four year basis, then selection for prolonged diapause can occur particularly since interfield movement of adult beetles is well known (Lance et al. 1989, Naranjo 1991). Eggs laid in corn in the DeWitt or Fisher fields in 1987 were subjected to a wheat, corn sequence in the next two years. Any individuals previously selected for prolonged diapause would have increased fitness under these conditions.

A unique feature of this study is that the presence of prolonged diapause was detected primarily from the presence of a high proportion of NCR versus WCR beetles in these fields, not from observation of damage in first-year corn. Several farms in this area have been scouted regularly since 1985 by employees of a crop consulting firm. Experience indicated that in this area of the state, WCR made up > 95% of the corn rootworm population, with NCR typically < 5%. A high proportion of NCR was first recorded in 1987 from one field in this immediate area. In 1989, observations of several fields with very high proportions of NCR (>85% Fisher, and 89% DeWitt) indicated a very unusual situation and prompted these investigations. The 1991 adult survey data (Table 1) indicate that four out of the five fields sampled in this area continue to have a greater than expected proportion of NCR versus WCR beetles. The Fisher field had 60% NCR with a density of 2.8 beetles per plant (Table 1). The Soper, Zelco and Cullen fields also had elevated proportions of NCR ranging from 16 to 52% of the total population. All of these are unusually high in contrast to the normal level of < 5% NCR in most areas of Michigan. The Bird field had a more typical situation with 0.3 beetles per plant and 2.6% NCR.

Although individual plants in the Fisher and DeWitt fields exceeded the minimum root rating for economic damage (3 on a scale of 1-6), the field means (root ratings = 2.1 and 1.6) were well below economic levels. In Michigan, and other states where WCR predominates, a sudden unexplained shift in the species composition of a corn rootworm population towards the NCR may be an early warning signal of the expansion of the prolonged diapause trait in the population. Since this warning may be detected prior to economic damage in first-year corn (as in this study), appropriate management practices can be initiated earlier, perhaps by several years, than if lodging of first-year corn was the only symptom. Observation of a population shift towards NCR could be caused by a number of other factors and should only be used as a signal that prolonged diapause may be present. In particular, since WCR eggs are less cold hardy than NCR eggs (Palmer et al. 1977, Gustin 1983), widespread population shifts towards NCR following extremely cold winters may not be diagnostic. Increased abundance of NCR without a concomitant increase in proportion of NCR versus WCR may also simply reflect better overall survival and may not be important in signaling prolonged diapause. Rearing experiments, although tedious, are at present the only reliable method for positive confirmation of prolonged diapause and should be conducted if the trait is suspected.

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