MONITORING *DIABROTICA VIRGIFERA VIRGIFERA* (LECONTE) IN MICHIGAN SOYBEAN FIELDS AND SUBSEQUENT ADULT EMERGENCE IN ROTATED AND CONTINUOUS CORNFIELDS.

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ABSTRACT

Injury to rotated corn by *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) has become a common occurrence across east-central Illinois and north-west Indiana. We report results of a three-year survey (1999-2001) of soybean fields for *D. v. virgifera* in the lower peninsula of Michigan. We monitored *D. v. virgifera* with unbaited Pherocon AM yellow sticky traps. Only 3 out of 131 fields were above the action threshold of more than 3 beetles/trap/day that indicates injury to rotated corn may occur. In general the greatest number of beetles were collected in southwest Michigan, near the border with Illinois and Indiana. However, a single field in central Michigan (Clinton County) had populations above the action threshold. To confirm that *D. v. virgifera* oviposition occurred in these soybean fields, we monitored adult emergence when these fields were planted to corn the following year. As a comparison, we also monitored emergence in adjacent continuous cornfields. The highest number of *D. v. virgifera* collected occurred in southwest Michigan (Berrien County) where nearly twice as many *D. v. virgifera* emerged from rotated than continuous cornfields during 2002. In central Michigan we observed the opposite relationship, more beetles emerged from continuous than rotated cornfields. We suggest that large numbers of beetles collected in soybean fields of Clinton County most likely emigrated from silage corn that had been harvested and were not a rotation-resistant population. The pattern of adult *D. v. virgifera* emergence within rotated cornfields in central Michigan may represent the innate capacity of this species to use soybeans as an oviposition site rather than evidence of rotation-resistant beetles.

Crop rotation has failed to protect corn from larval western corn rootworm (*Diabrotica virgifera virgifera* LeConte) injury resulting in significant damage to first-year corn in parts of the Midwestern US (Levine et al. 2002). The current explanation (Levine et al. 2002) is that adult female *D. v. virgifera* emigrate from cornfields in which they emerge and immigrate to soybean fields where they oviposit. Where growers practice a corn-soybean rotation, larvae emerging the year following a soybean crop encounter corn whose roots they damage (O'Neal et al. 2001). We refer to *D. v. virgifera* capable of injuring rotated corn as rotation-resistant, in that it is similar to more traditional forms of resistance, such as the failure of an insecticide to control an insect pest. The occurrence of rotation-resistance was first recorded in Ford County in east-central Illinois (Levine and Oloumi-Sadeghi 1996). The strict use of a corn-soybean rotation in this region is considered responsible for the development of rotation-resistance (Onstad et al. 2001).

O'Neal et al. (1999) tracked the immigration of *D. v. virgifera* into soybean fields with unbaited Pherocon AM yellow sticky traps in east-central Illinois. They observed large numbers (nearly 30 beetles/trap/day) of adult females throughout soybean fields during the month of August. O'Neal et al. (2001) successfully predicted injury to rotated corn by monitoring populations of *D. v. virgifera* the prior year in soybean fields with Pherocon AM yellow sticky traps.

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O'Neal et al. (2001) found that when soybean fields with a mean of 3 beetles/trap/day were rotated the following year to corn, there was risk for *D. v. virgifera* larval injury.

Using sweep-net data from soybeans, Onstad et al. (1999) modeled the apparent spread of this phenomenon from a point of origin in east-central Illinois. They suggested that movement of beetles on prevailing easterly weather systems accounted for the large numbers of beetles found in soybean fields of Indiana and western Ohio. Their model further predicted an increasing spread of rotation-resistant *D. v. virgifera* into the lower peninsula of Michigan.

Initial survey efforts in Michigan found large numbers of *D. v. virgifera* in soybean fields of southern Michigan, and documented rootworm injury to corn following soybeans, as well as alfalfa and wheat (DiFonzo 1998). Although populations of northern corn rootworms (*D. barberi* Smith and Lawrence) with a prolonged egg diapause have been reported in Michigan (Landis et al. 1992), these recent reports of injury to rotated corn were attributed to *D. v. virgifera*.

Our objective was to survey soybean fields for *D. v. virgifera* in the lower peninsula of Michigan, following the protocol of O'Neal et al. (2001). We surveyed soybean fields for three years beginning in the summer of 1999. To confirm if *D. v. virgifera* had oviposited in select soybean fields, we monitored adult *D. v. virgifera* emergence in the following year when that field was planted to corn. Wild type *D. v. virgifera* are not considered to use soybean as an oviposition site (Shaw et al. 1978), as their fidelity to corn is considered high (Levine and Oloumi-Sadeghi 1991). More beetles should emerge from a rotated field where rotation-resistance occurs than where populations are comprised of only wild type *D. v. virgifera*. As a comparison, we followed the emergence of rootworms from adjacent rotated and continuous cornfields as this may further reveal the presence of rotation-resistant *D. v. virgifera*.

MATERIALS AND METHODS

Survey of Michigan Soybean Fields. In the summer of 1999, we supplied volunteer growers, scouts and extension agents with unbaited Pherocon AM yellow sticky traps (Great Lakes IPM, Vestaburg MI) to monitor 71 soybean fields for adult *D. v. virgifera*. Volunteers placed one trap along each of four field edges, 10 m into the soybean field. Traps were supported on metal posts and adjusted weekly to a height just above the soybean canopy. Traps were changed each week beginning in July through the end of August, and returned to Michigan State University for counting beetles. Volunteers followed the same protocol in 2000 and 2001, to monitor 33 and 27 fields respectively.

Adult Emergence.

Emergence Cage. We monitored adult rootworm emergence with a wooden cage that fits around the base of a corn plant (Rondon 2002). Cages are placed around the stalk when the corn is approximately 0.5 m tall and the plant is able to continue growing unimpeded by the cage. Emerging beetles are collected when they enter a 5.5 cm diameter glass jar through an inverted funnel attached to the top of the cage.

Due to the large number of soybean fields surveyed, we were unable to monitor adult emergence in all fields when they were rotated to corn the following year. Therefore, we selected rotated cornfields in which large numbers ($\geq$ 3.0 beetles/trap/day) of *D. v. virgifera* had been captured the year before. Where possible, we monitored adult emergence from an adjacent or nearby (within 1 km) cornfield that had been planted to corn the year before.

Wild type *D. v. virgifera* is considered not to use soybean as an oviposition site (Shaw et al. 1978), whereas rotation-resistant beetles oviposit a portion of their eggs outside of cornfields. In east-central Illinois where injury to rotated

corn is common, sampling soil for *D. v. virgifera* eggs in rotated corn and soybean suggest that both are used as oviposition sites (C. Pierce, pers. comm.). We hypothesized that equal, or greater emergence in a rotated versus an adjacent continuous cornfield would indicate the presence of rotation-resistant *D. v. virgifera*. Emergence data were used to test this hypothesis, by comparing the ratio of emergence in an adjacent pair of rotated and continuous cornfields to a 1:1 ratio with a Chi-square goodness of fit test ($X2$, Sokal and Rohlf 1995).

Adult Emergence: 2000. In the 1999 survey, trap catches in a single soybean field in Clinton County (in central MI) were greater than 3 beetles/trap/day, therefore, rootworm emergence in this field was monitored in 2000 when the field was rotated to corn. An adjacent continuous cornfield (planted to corn for 5 years) was monitored as a comparison. Both fields were managed using conventional agronomic practices by the grower cooperator and treated at planting with chlorpyrifos (Lorsban 15 G, Dow AgroSciences, 3.6 kg per acre). Although the application of a soil insecticide at planting can protect corn roots from larval rootworm feeding, it is not considered to significantly affect adult emergence (Gray et al. 1992). On 12 July, twenty emergence cages were distributed across four rows in each field. The rows were approximately 12 m apart; cages within a row were separated by 66 m from each other and the edge of the field. Cages were checked once a week at which time all beetles were removed.

Adult emergence: 2001. Since survey results of soybean fields in 2000 did not reveal any rotated cornfields at risk for *D. v. virgifera* injury (Table 1), we sought assistance from extension agents in selecting additional field sites in 2001. We selected three pairs of fields, comprised of a rotated and a continuous cornfield no less than 50 m apart. One pair of fields was located in St. Joseph County, Indiana (approx. 6 km from the Michigan border) where injury to rotated corn was reported. Two additional sites in Kalamazoo County, Michigan, did not have a history of injury to rotated corn. We used 20 emergence cages per cornfield. Cages were arranged in the same manner as in 2000, sealed on 6 July and checked every three to four days. None of the fields were treated with soil insecticide.

Adult emergence: 2002. From the 2001 survey, sticky trap catches in two soybean fields in Berrien County were over threshold. In 2002, we selected these two fields to track emergence, as well as a field in Clinton County. Although not above threshold, this Clinton County field was included as it had surpassed the threshold in 1999 suggesting it as possible source of rotation-resistant *D. v. virgifera*. The rotated fields in Berrien County were planted without a soil insecticide. Only a single continuous cornfield was found as a comparison for both rotated cornfields, at a distance of ~1400 m from both fields. An additional continuous cornfield could not be located near these Berrien County rotated cornfields in 2002. The rotated cornfield in Clinton County was treated with imidacloprid, 1.34 mg a.i. (Gauchoä, Bayer) / kernal seed treatment, but no soil insecticide. The continuous cornfield was within 100 m of the continuous cornfield monitored in 2001 and treated with chlorpyrifos (3.6 kg a.i. per acre). In both Clinton and Berrien County, we used 20 emergence cages per cornfield. All cages were sealed on 8 July.

RESULTS

Survey of Michigan soybean fields. During the three years of our survey, we tracked *D. v. virgifera* in 131 fields in 26 counties (Table 1). Only three fields in two of the 26 counties surveyed had a mean higher than 3 beetles/trap/day. The majority of Michigan soybean fields surveyed had very low numbers (1 to 5 per trap) of adults and often only during one or two weeks of the eight-week sampling period. Only three fields, one in Clinton County in 1999 (Fig. 1a) and two in Berrien County in 2001 (Fig. 1b), had trap catches that indicated injury to rotated corn may occur (O'Neal et al. 2001). With the exception of Clinton

Table 1. Mean (beetles/trap/day/field) numbers of *D. v. virgifera* in Michigan soybean fields.

County	County mean[a] (number of fields sampled)					
	1999		*2000*		*2001*	
Allegan	0.46	(1)	NA		NA	
Arenac	NA		0.0	(4)	NA	
Barry	0.24	(4)	NA		NA	
Bay	0.0	(2)	NA		NA	
Berrien	1.1	(5)	0.22	(4)	1.95	(4*)
Branch	0.59	(4)	NA		NA	
Calhoun	0.42	(3)	0.05	(1)	0.17	(3)
Cass	1.03	(6)	0.30	(7)	1.18	(3)
Clinton	1.82	(3*)	0.21	(1)	1.04	(1)
Eaton	0.23	(3)	NA		NA	
Hillsdale	0.57	(3)	NA		NA	
Huron	0.22	(5)	NA		NA	
Ingham	0.22	(1)	NA		NA	
Ionia	0.64	(1)	0	(1)	NA	
Isabella	0.62	(1)	0.17	(1)	0.09	(1)
Kalamazoo	0.60	(3)	0.05	(2)	0.46	(3)
Lapeer	NA		0.01	(2)	0.09	(2)
Lenawee	0.36	(3)	0.07	(2)	0.05	(3)
Livingston	0.17	(3)	NA		NA	
Monroe	0.07	(3)	0.12	(4)	0.01	(5)
Saginaw	0.0	(3)	NA		0.02	(1)
St. Joseph	0.21	(7)	0.13	(4)	0.24	(1)
Sanilac	0.65	(4)	NA		NA	
Shiawassee	0.07	(1)	NA		NA	
Tuscola	0.29	(1)	NA		NA	
Washtenaw	1.35	(1)	NA		NA	
Total fields monitored	**71**		**33**		**27**	

[a] Mean calculated from the mean of each field. Field means calculated from a four week period starting on the last week of July to the third week of August.
* Indicates county contained an individual field that had a mean beetle/trap/day above the action threshold in that year.

County, all counties that averaged more than one beetle/rap/day were located within the first two tiers of counties bordering Illinois and Indiana (Fig. 2).

Adult emergence: 2000. All beetles collected in the rotated and continuous cornfields at the Clinton County site were *D. v. virgifera*, and many still teneral when removed from the emergence cages, as evidenced by soft, white elytra that had not yet sclerotized completely. We collected a total of 35 adult *D. v. virgifera* in the rotated cornfield and 178 were collected in the adjacent continuous cornfield. The ratio of emergence (rotated: continuous) was significantly different from a 1:1 ratio (Table 2).

Adult emergence: 2001. At both Michigan sites and the Indiana site, we collected more adult *D. v. virgifera* in the continuous than rotated cornfield. We collected the greatest number of adults (63) from a rotated cornfield at our Indiana site in 2001 (Table 2). However, the greatest total (rotated and continuous combined) number of *D. v. virgifera* collected was from our Climax field site in Kalamazoo County, Michigan.

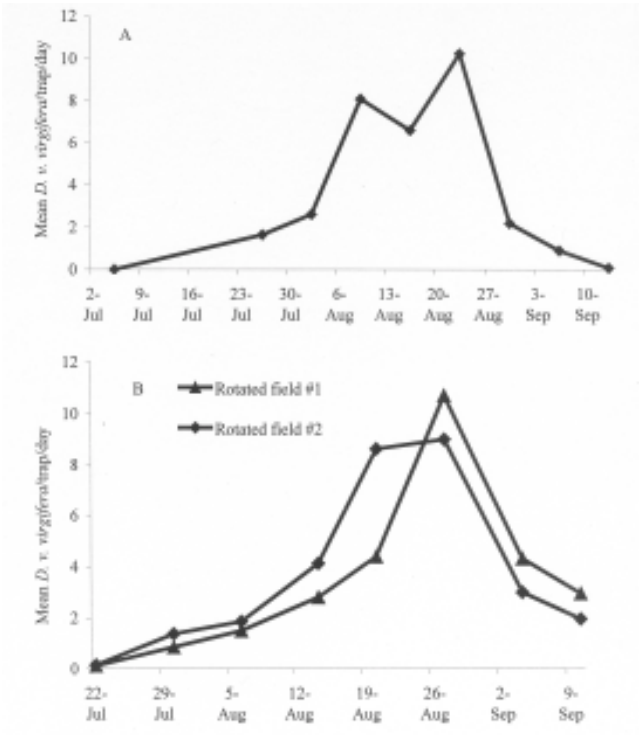

Figure 1. Mean number of beetles per trap per day collected in a soybean field of (A) Clinton County, Michigan during the 1999 growing season and two soybean fields in (B) Berrien County during the 2001 growing season.

For the site in Indiana, the numbers of beetles emerging in the rotated and continuous cornfields was statistically indistinguishable from a 1:1 ratio (P = 0.16; Table 2). In both Michigan sites the ratio of emergence between the rotated and continuous cornfields was significantly different from an expected ratio of 1:1 (Table 2). The rotated fields in Michigan never contributed more than 16% of the total beetles collected for the two fields combined.

Adult emergence: 2002. In 2002, the greatest number of *D. v. virgifera* collected during the three-year study occurred in southwest Michigan (Berrien County, Table 2) where nearly twice as many *D. v. virgifera* emerged from rotated than continuous cornfields. The number of *D. v. virgifera* at Clinton County during 2002 was similar to that of 2000, with fewer beetles emerging from the rotated than the continuous cornfield.

DISCUSSION

The initial spread of *D. v. virgifera* into Michigan during the early 1970's (Ruppel 1975) also began in the southwest corner of the lower peninsula. The first confirmed reports of *D. v. virgifera* in Michigan were from Southwest Michigan (Cass, Van Buren and Allegan Counties) in 1971. By 1974, *D. v. virgifera* had spread across the lower peninsula of Michigan. However, the reports of putative rotation-resistant populations *D. v. virgifera* has not been as rapid, nor has it consistently increased from year to year (Table 1). Model based predictions suggested that rotation-resistant *D. v. virgifera* would spread throughout lower Michigan by 2005, at a rate of 10-30 km/yr (Onstad et al. 1999). More recent models that incorporated sampling data presented here, indicate that these earlier predictions overestimated the rate of spread of rotation-resistance (Onstad et al. in press).

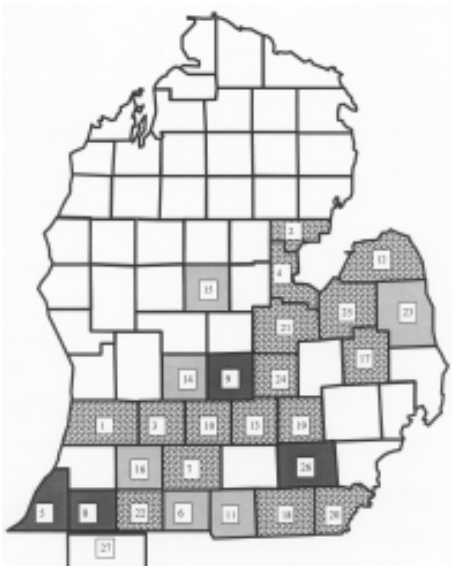

Figure 2. Counties in which *D. v. virgifera* were collected in soybean fields using Pherocon AM yellow sticky traps. Shading represent mean beetles/trap/day for a four week period of oviposition spanning the last week of July and the first 3 weeks of August; dark gray > 1.0; 0.5 < light gray < 1.0; dotted < 0.5; white = no traps deployed. Michigan Counties included: 1. Allegan, 2. Arenac, 3. Barry, 4. Bay, 5. Berrien, 6. Branch, 7. Calhoun, 8. Cass, 9. Clinton, 10. Eaton, 11. Hillsdale, 12, Huron, 13, Ingham, 14 Ionia, 15. Isabella, 16. Kalamazoo, 17. Lapeer, 18. Lenawee, 19. Livingston, 20. Monroe, 21. Saginaw, 22. St. Joseph, 23. Sanilac, 24. Shiawassee, 25. Tuscola, 26. Washtenaw. In Indiana, two fields were monitored for adult *D. v. virgifera* emergencein St. Joseph county (27).

Table 2. Total number of *D. v. virgifera* collected in emergence cages in adjacent continuous and rotated cornfields.

Site	Year	Field type[a]	Sum[b]	Percent[c]	X^2	P[d]
Clinton Co. Michigan	2000	Continuous	178	84		
		Rotated	35	16	96	*P < 0.01*
South Bend, Indiana	2001	Continuous	84	57		
		Rotated	63	43	3	*P* = 0.16
Kalamazoo Co. Michigan	2001	Continuous	9	100		
(Mendon)		Rotated	0	0	9	*P* < 0.01
Kalamazoo Co. Michigan	2001	Continuous	247	92		
(Climax)		Rotated	22	8	188	*P* < 0.01
Clinton Co. Michigan	2002	Continuous	165	82		
		Rotated	35	18	84.5	*P* < 0.01
Berrien Co. Michigan	2002	Continuous	264	40	(28)[e]	
		(Rotated field #1)	391	60	114	*P* < 0.01
		(Rotated field #2)	664	72	306	*P* < 0.01

[a] Continuous cornfields were fields that were planted to corn for a minimum of two years, rotated cornfields were planted to soybeans in the previous year.
[b] The sum is calculated from the total number of beetles collected in the cages during the sampling period. Twenty emergence cages per field were used in 2001 and 40 cages per field were used in 2000.
[c] The percentage is calculated based on the contribution of each field to the total number of beetles collected in the two adjacent fields.
[d] *P* value is for X^2 comparison of the percent contribution of each field to a 1:1 ratio.
[e] Berrien Rotated field #2 was used to a calculate the percentage and subsequent X^2 value

Surprisingly, the only Michigan soybean field considered to be at risk for injury during our survey was in Clinton County, far north of the Illinois border. In 1999 and again in 2001 when this field was planted to soybean, we observed high numbers of *D. v. virgifera* but did not observe significant root injury, or large numbers of adults emerging from this field when it was rotated to corn the following years. Recent modeling of the spread of rotation-resistant *D. v. virgifera* indicates that Clinton County is outside of the predicted range (Onstad et al. in press). We suggest that the practice of chopping corn for silage and not rotation-resistance may explain the large numbers of *D. v. virgifera* found in this Clinton County soybean field.

In Clinton County, 30% of the farms raise cattle and of these nearly one-third are dairy farms (Anon. 1997). Corn grown for silage represents ca. 20% of the total corn production in this county (Anon. 1997). Silage corn is typically harvested in late August, forcing *D. v. virgifera* to leave this habitat. The particular soybean fields we studied in Clinton County in 1999 were in close proximity (2 km) to early-harvested silage cornfields. This apparently forced *D. v. virgifera* into soybeans where some subsequent oviposition occurred.

The effect of harvesting corn for silage on *D. v. virgifera* emigration has not been studied. However, the effect of corn maturation and phenology has been shown to affect *D. v. virgifera* immigration and emigration (Hill and Mayo 1974, Darnell et al. 2000). As corn ages the availability and quality of adult feeding sites decreases, producing an increase in emigration. We suggest that within Clinton County, harvesting silage early in the season when adult *D. v. virgifera* were still present would mimic the effect that corn in an advanced phenological state has on adult dispersal. This effect would be accentuated in areas of relatively concentrated silage corn production, like Clinton County.

In contrast, in Berrien County, where large numbers of beetles have also been trapped, only 9% of the farms raise cattle with only 25 dairy farms (less than 2% of the total number of farms in the county) and only ca. 2% of the total corn production is silage (Anon. 1997). Silage corn bordered none of the soybean fields monitored in Berrien County. In this county, absence of corn cut for silage and its proximity to known problem areas in Illinois and Indiana suggest that behaviors associated with rotation-resistance may be occurring.

Without a morphological or genetic marker, it is not possible to separate wild type from rotation-resistant populations. However, comparison of adult emergence from adjacent rotated and continuous cornfields suggests that *D. v. virgifera* in Clinton County is unlikely to be rotation-resistant. Of the pairs of rotated and continuous cornfields monitored for *D. v. virgifera* emergence (Table 2), only those in South Bend, Indiana (2001) and Berrien County, Michigan had a ratio of emergence that was 1:1, or exceeded a 1:1 ratio in favor of rotated corn. However, the ratio in Clinton County exceeded a 1:1 ratio in favor of continuous corn.

As we have observed in Clinton County, large numbers of beetles found in soybeans may not result in significant egg-laying. Shaw et al. (1978) also found large numbers of *D. v. virgifera* in Illinois soybean fields and after extensive soil sampling, did not find evidence of oviposition. Ideally, survey work of *D. v. virgifera* adults in soybean fields should be coupled with some follow-up to determine if egg-laying actually occurred. Soil-sampling for eggs is difficult and requires a large sample size for statistically powerful results. Emergence cages offer a less-labor intensive compromise to egg-sampling and can substantiate inferences from trapping data regarding rootworm oviposition in soybeans.

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