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DIAGNOSTIC ALLELES FROM ELECTROPHORESIS DISTINGUISH TWO NOCTUID PEST SPECIES, *HYDRAECIA IMMANIS* AND *H. MICACEA* (LEPIDOPTERA: NOCTUIDAE).

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ABSTRACT

Native hop vine borer (*Hydraecia immanis*) and introduced potato stem borer (*H. micacea*) populations in Midwest corn have reached noticeable levels near the Great Lakes plant community ecotone between boreal forests and temperate deciduous forests. The hop vine borer is more specialized in its diet and occurs in corn generally south of the plant community ecotone, whereas the potato stem borer is polyphagous and occurs in corn mostly north of the Great Lakes plant transition zone. We analyzed the genetic composition of each species using cellulose acetate electrophoresis and resolved 19 loci of which 6 exhibited fixed or nearly fixed allelic differences. We expect that this will be useful in determining the degree of hybridization where the two species become sympatric due to expected continued range expansions in Michigan, Wisconsin, and New York State.

Two new corn insect pest species have been detected at economically significant levels and appear to be spreading in geographical distribution in the Great Lakes region during the last 15 years. These two moth species are closely related, morphologically similar members of the genus *Hydraecia* (Lepidoptera: Noctuidae) (Forbes 1954, Godfrey 1981). The damage caused to corn plants across several midwestern states by the hop vine borer, *H. immanis* (Guenée) is also very similar to that caused by the potato stem borer, *Hydraecia micacea* Esper (Giebink et al. 1984, Deedat et al. 1983).

The hop vine borer and potato stem borer arose rather suddenly as Midwest corn pests in the late 1970's and early 1980's. The potato stem borer, *H. micacea*, was introduced from Europe in the early 1900's to North America (New Brunswick, Nova Scotia and Quebec) (Gibson 1908). In Europe, it was a widespread pest of a variety of crops including hops, corn, rhubarb, and potatoes (Deedat and Ellis 1983). The geographic distribution of the potato stem borer appears to have been spreading from localized populations in Wisconsin, Michigan, New York and Canada (Muka 1976, Rings and Metzler 1982, Giebink et al. 1984). Foodplant records (Hawley 1918, Jobin 1963) and recent feeding studies (Deedat et al. 1983, Giebink et al. 1992,) suggest that the potato stem borer is both naturally and potentially more polyphagous than the hop vine borer. The hop vine borer is a native North American insect that has been known primarily from the mid-1800's as a pest of hops in New York and Wisconsin (see review by Hawley 1918). It has been of economic significance in more than 50 counties in Minnesota, Iowa, Illinois and Wisconsin

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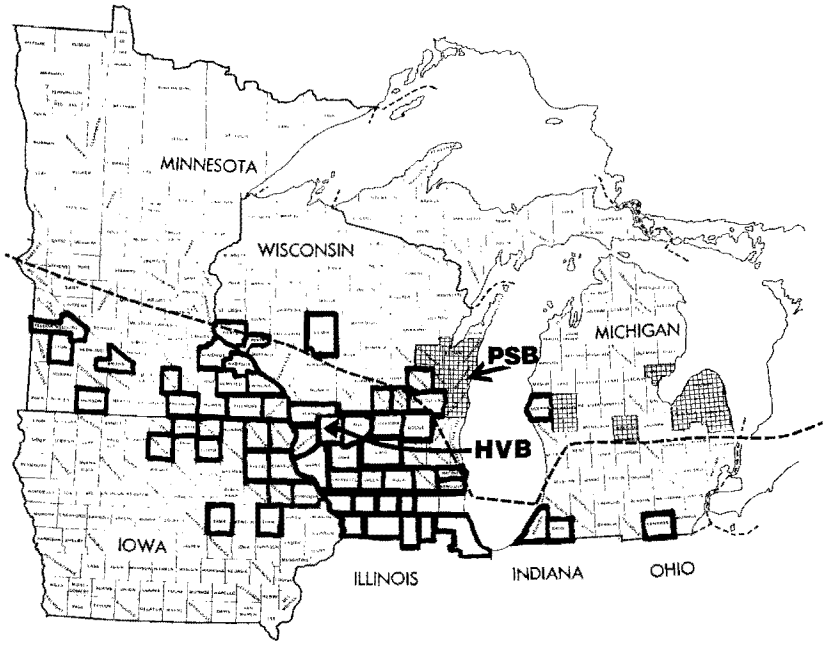

Figure 1. Distribution of noticeable levels of damage to corn (reported from 1978–1989) caused by the hop vine borer (*H. immanis*—indicated on map by heavy outlining) and the potato stem borer (*H. micacea*—indicated by cross-hatching) in the Great Lakes region of North America. Locations of our population samples are indicated by arrows for both species. In Michigan, all potato stem borer reports are since 1987.

especially since the increase in reduced tillage/conservation tillage corn (Fig. 1).

The subtle differences between these congeners in their biology (Deedat et al. 1983; Giebink et al. 1984), diapause physiology (Giebink et al. 1985, Levine 1988), and morphology (Godfrey 1981) have been described recently. The recent shift of both species to corn at damaging levels is described in relation to the Great Lakes plant ecotone and insect hybrid zone (Scriber and Hainze 1987). To our knowledge, the genetics of neither species has been reported. We felt it important to investigate the population genetics of these new pest species especially in view of possible genetic introgression of polyphagous genes into the stenophagous hop vine borer populations as the geographic distributions of the two species come into contact and overlap (Fig. 1). Giebink (1988 and unpublished) has observed interspecific matings when the two species are caged together in the laboratory.

Our initial objective was to assess the genetic make-up, identify fixed allele (diagnostic) differences and determine allele frequencies of each *Hydraecia* species. We obtained specimens from the center of the hop vine borer infestation in corn (possibly the result of a major host shift) and from the center of the newly discovered populations of potato stem borer (likely to be the founding populations for the State of Wisconsin). Comparisons of allele

frequencies at polymorphic loci would be particularly useful for monitoring any genetic divergence in the periphery of geographically spreading populations of each species in subsequent years. This would also give us an indication about the degree of hybridization and genetic introgression between the two species where they become sympatric in the Great Lakes region.

METHODS AND MATERIALS

Adult specimens were reared from larvae collected in corn plants from Richland County Wisconsin (hop vine borer) and Manitowoc County, Wisconsin (potato stem borer) and were analyzed using cellulose acetate electrophoresis according to the methods of Hagen and Scriber (1989 and 1991). After eclosion as adults, males and females of both species were frozen at -80°C to preserve tissues. All samples were processed at 5°C in a cold room. Tissue extracts were prepared by grinding 1/4-1/2 of the abdomen of each moth in 250 μl of an extraction buffer (0.1 M tris, pH 7.0, with 40mg EDTA, 10mg NADP, 20mg NAD, and 250μl B-mercaptoethanol per 100ml). The thorax and remaining abdomen were returned to the freezer and saved for later use, if needed. Extracts were centrifuged for 8 min at 14,000 x g and the supernatant was applied to thin layer cellulose acetate plates ("Titan III", Helena Laboratories, Beaumont, Texas) for electrophoresis. All plates were run at 4°C and specific conditions for each enzyme are listed in Table 1. Running buffers are adapted from those of Richardson et al. (1986): A–10 mM phosphate, 2.5 mM citric acid, pH 6.4; B–20 mM phosphate, pH 7.0; C–50 mM tris, 20 mM maleic acid, pH 7.8; D–15 mM tris, 5 mM EDTA, 10 mM $MgCl_2$, 5.5 mM boric acid, pH 7.8; I–25 mM tris, 192 mM glycine, pH 8.5.

Enzyme stains followed standard recipes (Harris and Hopkinson 1978, Richardson et al. 1986), scaled to approximately 10 ml total volume, and applied to plates as overlays mixed 1:5 with 1.5% agar. HBDH and LDH stains required extra NAD; activity for these enzymes was only detected in abdomen preparations. Bands for highly active enzymes (Apk, G3pdh, Gpi, Pepla, and Tpi) were most clearly resolved when the sample extract was diluted 1:3 with buffer prior to loading plates.

Interpretation of bands and allozyme nomenclature follows conventional systems (Richardson et al. 1986, Hagen and Scriber 1989). Isoenzyme loci are numbered from cathode to anode (Ac-1, Ac-2). For purposes of this study, alleles at a locus are assigned letters from least to most cathodal according to mobility. Voucher specimens have been retained in the J.M. Scriber research collection at Michigan State University.

RESULTS

Distinct, readily scorable bands were obtained for 19 enzyme-encoding loci (Table 1). Two enzymes were not resolvable on cellulose acetate: Ldh (lactate dehydrogenase) and Sod (superoxide dismutase). Six enzymes showed apparently fixed differences between the species (Table 2): Aat (Aspartate amino transferase), Ac-1 (aconitase-1), Ak (Adenylate kinase), Hbdh (Hydrozybutyrate dehydrogenase), Mdh (Malate dehydrogenase) and P3gdh (3phosphoglycerate dehydrogenase), and Mpi is nearly fixed. These fixed differences were consistent for both males and females in both species.

Table 1. Allozymic loci resolved for *Hydraecia immanis* and *H. micacea*. Electrophoretic conditions found to give optimal resolution on cellulose acetate plates are indicated for each enzyme. Origin positions (an = anode, ce = center, ca = cathode) are selected to keep migrating enzymes centered on the plate. Running buffers are described in the text.

LOCUS	ENZYME NAME (E.C. NUMBER)	ELECTROPHORESIS CONDITIONS			
		Buffer	Origin	Time	Voltage
Aat-1	Aspartate aminotransferase (2.6.1.1)	I	an	35 min.	275V
Ac-1	Aconitase (4.2.1.3)	A	an	35 min.	*
Ac-2					
Acp	Acid phosphatase (3.1.3.2)	C	ce	45 min.	275V
Ak	Adenylate kinase (2.7.1.20)	C	an	35 min.	275V
Ald	Aldolase (4.1.2.13)	I	an	35 min.	275V
Apk	Arginine phosphokinase (2.7.3.3)	C	an	35 min.	275V
Gapdh	Glyceraldehyde phosphate dehydrogenase (1.2.1.12)	A	ce	35 min.	250V
Gpi	Glucose phosphate isomerase (5.3.1.9)	I	an	30 min.	275V
G3p	Glycerol-3-phosphate dehydrogenase (1.1.1.8)	C	an	30 min.	275V
Hbdh	Hydroxybutyrate dehydrogenase (1.1.1.30)	D	an	35 min.	300V
Idh-2	Isocitrate dehydrogenase (1.1.1.42)	A	an	35 min.	*
Mdh-1	Malate dehydrogenase (1.1.1.37)	C	ce	30 min.	275V
Mpi	Mannose-6-phosphate isomerase (5.3.1.8)	I	an	30 min.	275V
Pgd	Phosphogluconate dehydrogenase (1.1.1.44)	D	an	35 min.	*
Pgm	Phosphoglucomutase (2.7.5.1)	I	an	35 min.	275V
P3gdh	3-phosphoglycerate dehydrogenase (1.1.1.95)	C	an	35 min.	275V
Sordh	L-iditol dehydrogenase (1.1.1.14)	I	an	35 min.	275V
Tpi	Triose phosphate isomerase (5.3.1.1)	I	an	35 min.	275V

*Voltage is adjusted to maintain current between 12–15 mA per plate.
#Consistent resolution achieved to date only on starch gel medium using citrate buffer, pH 6.4, from Clayton and Tretiak (1972), Hagen and Scriber (1989).

DISCUSSION

While the native hop vine borer is polymorphic at 63% of the loci (12 of 19), the introduced potato stem borer is polymorphic for only 5% of the loci. This may reflect a possible genetic bottleneck or founder effect (Barton 1989). With six allozyme loci exhibiting fixed differences of the 19 enzymes resolved, we now have excellent biochemical diagnostic capabilities for distinguishing the two species. If these apparently fixed differences hold up for different geographic populations, we will have identified an excellent indicator for any natural hybridization that may occur in areas such as central Michigan, central Wisconsin or New York where the populations of the two species are becoming sympatric. Morphology and color types intermediate between *H. immanis* and *H. micacea* have been described by Forbes (1954). We could presumably now identify and classify such intermediates with these biochemical characters as well

The first potato stem borer populations in Wisconsin were discovered in corn fields of Manitowoc and Kewaunee counties in 1982 (Fig. 1; Giebink et al. 1984). From these northeastern locations, subsequent populations with economically damaging levels were reported moving to the southwest in Brown, Outagamie, Calumet and Sheboygan counties by 1987 (Bruce Giebink, pers.

Table 2. Allele frequencies for hop vine borer and potato stem borer adults at 19 enzyme loci.

		SPECIES	
ENZYME	ALLELE	*H. immanis* (n)	*H. micacea* (n)
AAT-1	A	1.0	0
	B	0	1.0
		(21)	(30)
AC-1	A	0	1.0
	B	.39	0
	C	.61	0
		(21)	(5)
AC-2	A	.24	0
	B	.45	.90
	C	.31	.10
		(21)	(5)
ACP	A	1.0	1.0
		(25)	(27)
AK	A	0	1.0
	B	1.0	0
		(29)	(5)
ALD	A	1.0	1.0
		(29)	(5)
APK	A	1.0	1.0
		(29)	(5)
G3P	A	1.0	1.0
		(25)	(26)
GAPDH	A	1.0	1.0
		(40)	(3)
GPI	A	.02	0
	B	.95	1.0
	C	.02	0
		(21)	(5)
HBDH	A	.15	0
	B	0	1.0
	C	.85	0
		(24)	(28)
IDH-2	A	1.0	1.0
		(26)	(26)
MDH	A	0	1.0
	B	1.0	0
		(21)	(5)
MPI	A	.01	1.0
	B	.82	0
	C	.17	0
		(36)	(36)
P3GDH	A	0	1.0
	B	1.0	0
		(40)	(3)
PGD	A	1.0	1.0
		(23)	(24)
PGM	A	.10	0
	B	.79	1.0
	C	.11	0
		(35)	(36)
SORDH	A	.06	0
	B	.94	1.0
		(40)	(5)
TPI	A	1.0	1.0
		(40)	(5)

comm.). In southwestern Wisconsin, the hop vine borer occupies at least 20 counties at economically significant levels for corn growers (Fig. 1). A similar geographic pattern of distribution of the two species appears to have occurred in Michigan. The first potato stem borer populations were detected in 1987 in the northeast "thumb" region of Michigan (Sanilac Co.) and Arenac Co. (slightly to the north). Subsequent populations (in Newago, Tuscola and Huron counties) have been reported damaging corn in Michigan (D. Landis, pers. comm.). As in Wisconsin, the hop vine borer records of distribution are confined to the south and southwestern part of the State (Berrien, Cass, Oceana, and Lenawee counties; Fig. 1).

The rather narrow Great Lakes plant community ecotone (Curtis 1957) separates the two species of *Hydraecia* in both Michigan and Wisconsin. Hybridization (or any genetic introgression) in the hybrid zone may contribute to wider larval host plant use abilities, altered voltinism patterns, reduced developmental temperature thresholds of the larva and possibly changes in oviposition preferences (Scriber 1988, Scriber and Lederhouse 1992). Such introgression has been shown to affect all of these ecologically important traits for the eastern tiger swallowtail butterfly at the same ecotone locations in Michigan and Wisconsin where the northern species (*P. canadensis*) meets the southern species (*P. glaucus*) (Ritland and Scriber 1985, Rockey et al. 1987, Hagen et al. 1991, Scriber 1990, Scriber et al. 1989, 1991). This plant transition zone is in fact closely aligned with other geographic sutures for insect species distributions (Scriber and Hainze 1987, Scriber and Lederhouse 1992).

For both the southern hop vine borer and the northern potato stem borer, we are currently monitoring the geographic spread of damaging populations and hope to subsequently assess the degree of genetic introgression in sympatric areas directly by use of the six diagnostic allozyme alleles that distinguish the two species at their allopatric locations. If genetic introgression occurs between these species, the economic impact on numerous crop systems in these areas will require careful analysis and perhaps different management programs.

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