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Immunological Assessment of an Insecticide Resistance-Associated Esterase in the Western Corn Rootworm

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In previous investigations, we have determined that organophosphate resistance in the western corn rootworm, *Diabrotica virgifera virgifera*, is at least partially attributed to a group of non-specific carboxylesterases referred to as group II. Antiserum raised against a purified 66-kDa group II esterase is specific for the denatured enzyme. This antiserum reacts similarly with both beetle homogenates from resistant and susceptible populations, although there is much higher signal intensity in immunoblots of resistant relative to susceptible beetles. These results suggest that overproduction of group II esterases is the underlying basis of esterase-mediated resistance in *D. v. virgifera* by demonstrating that (1) group II esterases are immunologically indistinguishable between the resistant and susceptible populations, and (2) the intensity differences are due to increased group II esterase proteins in the resistant population. The diagnostic potential of immunological-based assays was tested with a traditional diagnostic concentration bioassay and a biochemical-based native PAGE assay. Significant correlations were observed among all three diagnostic assays (regression coefficients ranging from 0.95 to 0.96). These results demonstrate the importance of the 66-kDa protein as a resistance-associated biochemical marker, thus emphasizing the potential for 66-kDa protein-targeted immunoassays in resistance monitoring programs. Arch. Insect Biochem. Physiol. 58:157–165, 2005. © 2005 Wiley-Liss, Inc.

KEYWORDS: immunoassay; esterase; insecticide resistance monitoring; *Diabrotica virgifera virgifera*

INTRODUCTION

The western corn rootworm, *Diabrotica virgifera virgifera* LeConte, is the most economically and environmentally important insect pest of maize in North America (Chang, 1973; Levine and Oloumi-Sadeghi, 1991). Synthetic insecticides and crop rotation have been the traditional rootworm management strategies for many years. However, the remarkable capacity of this insect to evolve resistance has resulted in the failure of both broad spectrum insecticides (Levine and Oloumi-Sadeghi, 1991; Meinke et al., 1998) and crop rotation (Levine et al., 2002) as

rootworm management options. Widespread resistance of *D. v. virgifera* to organochlorine insecticides in the 1950s resulted in the adoption of adult control in certain areas of Nebraska (Meinke et al., 1998). Adult control, which involves the application of foliar insecticides to suppress adult populations and reduce egg laying to decrease the population density in the following season (Pruess et al., 1974), has been highly effective over several decades. Microencapsulated methyl-parathion (PennCap-M®, Elf Atochem North America, Inc., Philadelphia, PA) has been the most commonly used organophosphate insecticide to control adult

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D. v. virgifera in Nebraska because of its low cost and relatively long persistence. However, the repetitive application of methyl-parathion in continuous corn fields led to detectable levels of organophosphate resistance by the mid 1990s (Meinke et al., 1997).

Studies of organophosphate resistance mechanisms have revealed high levels of general esterase activity in resistant *D. v. virgifera* populations from across South-Central Nebraska (Zhou et al., 2002, 2003, 2004). Other evidence, such as in vivo metabolism experiments with ¹⁴C-labeled ethyl-parathion, and synergism bioassays with the esterase inhibitor DEF (*S,S,S*-tributyl phosphorotrithioate) indicated the involvement of esterase-mediated resistance in both larvae and adult *D. v. virgifera* (Miota et al., 1998; Wright et al., 2000).

Three distinct groups of esterase isozymes (I, II, and III) are identifiable by native polyacrylamide gel electrophoresis (PAGE). Of the three esterase groups, group II esterases are overproduced in resistant individuals (Zhou et al., 2002). Elevated activity of group II esterase isozymes is highly correlated with resistance and these enzymes provide a reliable biochemical marker for the detection of methyl-parathion resistance in individual rootworms (Zhou et al., 2002, 2003). Recent investigations of partially purified group II esterases and the resulting physical, kinetic, and insecticide inhibition properties all suggested the overproduction of resistance-associated esterase as the molecular basis of organophosphate resistance in *D. v. virgifera* (Zhou et al., 2004). In this study, we report on the development of immunoassays for detection of the group II esterase from both resistant and susceptible *D. v. virgifera* populations. These results provide insight into the molecular basis of resistance and diagnostic potential of group II esterase antiserum for detection of resistance among field populations.

MATERIALS AND METHODS

D. v. virgifera Populations

Three populations were collected in Nebraska during the summer of 1996 and 1997. The Saun-

ders County population was collected from areas where adult management is not practiced and has previously been shown to be highly susceptible to methyl-parathion (Meinke et al. 1998). In contrast, the resistant Phelps County and York County populations originated from areas where continuous corn production and adult control practice have been used for more than two decades and where field control failures had become common. Previous bioassays with methyl-parathion indicated 12.5- and 16.4-fold higher LD₅₀ values in the York and Phelps populations, respectively.

Field populations of adult rootworms used in diagnostic bioassays were collected in Nebraska and Colorado during the summer of 1999 before seasonal insecticide applications had begun. The field populations were maintained in the laboratory at ambient temperature with a 14:10 h (L: D) photoperiod and were fed a standard diet of head lettuce and sweet corn before being frozen at -80°C and stored for subsequent experiments.

Chemicals

DEAE Sepharose, hydroxyapatite, and all other reagents and solvents were purchased from Sigma Chemical Co. (St. Louis, MO) unless stated otherwise. Electrophoresis reagents were purchased from Bio-Rad Laboratories (Hercules, CA). Polyvinylidene difluoride (PVDF) membrane was from Millipore (Boston, MA). The protein assay kit was from Pierce (Rockford, IL). The methyl-parathion (99%) used in diagnostic concentration bioassays was purchased from Chem Service (West Chester, PA).

Diagnostic Concentration Bioassay

Bioassays were conducted in 20-ml glass scintillation vials containing a diagnostic concentration of 0.5 µg methyl-parathion per vial (Zhou et al., 2002). Insecticide dilutions were prepared in acetone using technical grade methyl-parathion of >99% purity. Vials were treated with 0.5 ml of insecticide dilution and rolled until dry in a fume hood. For each population, 10 beetles of mixed sex were placed in 10 replicate vials (plus 2 ac-

etone control vials). Vials containing beetles were held at 22°C in darkness for 4 h, before mortality was scored based on a lack of coordinated movement by the beetles.

Protein Purification and Polyclonal Antiserum Synthesis

Resistance-associated group II esterases were partially purified from rootworm abdominal tissues as previously described by Zhou et al. (2004). Soluble proteins from abdominal homogenates were prepared by differential centrifugation (the homogenates were centrifuged at 10,000g for 20 min, and the resulting supernatants were filtered through glass wool, and then centrifuged at 100,000g for 1 h). Group II esterases were partially purified by sequential column chromatography (ion-exchange chromatography followed by hydroxyapatite chromatography). Following hydroxyapatite chromatography, fractions containing resistant and susceptible group II esterases were pooled, concentrated, and viewed on Coomassie-stained SDS-PAGE gels (Laemmli, 1970). Three predominant protein bands (20-, 29-, and 66-kDa) were present in greater quantities in the resistant preparation; two of them (29- and 66-kDa proteins) were chosen for development of antisera based on the size range of previously reported resistance-associated esterases (Zhou et al., 2004). Both the 29- and 66-kDa protein bands were purified to homogeneity through preparative electrophoresis involving zinc staining (Bio-Rad, Hercules, CA), excision, destaining and electro-elution (Bio-Rad 422 Electro-Eluter) as described by Zhou et al. (2004). After dialyzing against 5 mM Tris buffer for 24 h at 4°C (to remove SDS), pure proteins were concentrated to approximately 50–100 µl and submitted to the University of Nebraska antibody core facility.

Polyclonal antisera against the purified 29- and 66-kDa proteins were raised in New Zealand white rabbits and BALB/c mice, respectively (two animals each), by injecting the purified proteins three times bi-weekly. After the second injection of antigen for both rabbits and mice, microliter quantities (≤ 20 µl) of serum were obtained and preliminary West-

ern blots were performed to compare with pre-immune sera. This comparison enabled us to evaluate (1) if an immunological response was occurring and (2) if the antisera specifically recognized antigenic proteins. At 1 week after the last injection, the rabbit and mouse with the best immunological responses were sacrificed and their blood serum used as polyclonal antibody sources.

Electrophoresis and Western Blot Analysis

Native-PAGE was conducted on 7.5% resolving gels with 4% stacking gels and a discontinuous tris-glycine running buffer system. Volumes of supernatant containing 10 µg protein were mixed with 1.5 µl of loading buffer (tris-glycine running buffer containing 20% sucrose and 0.05% xylene cyanol) prior to loading onto gels. Electrophoresis was conducted at 4°C and 120 V for 1.5 h. Esterase bands were then visualized by incubating the gels in 100 ml 0.02 M sodium phosphate buffer (pH 7.0), with 2% (v/v) 30 mM α -naphthyl acetate dissolved in acetone at 30°C for 15 min before adding 0.04% fast blue BN salt (tetrazotized *O*-dianisidine) (w/v).

Western blot analyses with denatured proteins were carried out following separation of 20 µg denatured rootworm supernatant on 12% SDS-PAGE gels (Laemmli, 1970). Proteins were transferred from gels to nitrocellulose membranes for 1 h at 100 V and 4°C using a wet-cell transfer apparatus (Bio-Rad, Hercules, CA). Transfer occurred in Tris-glycine buffer containing 20% v/v methanol, and transfer was confirmed by reversible staining with Ponceau-S (Sigma, St. Louis, MO). Membranes were briefly washed in phosphate-buffered saline (PBS) and blocked overnight at 4°C in 20 ml of PBS + 0.05% v/v Tween-20 (PBST) containing 15 mg/ml non-fat dried milk (Bio-Rad). Membranes were then incubated for 1 h in PBST and primary antiserum prepared at either 1:500 or 1:1,000 dilutions, washed 3 times for 5 min in PBST, incubated 1 h in PBST and 1:1,000 goat anti-mouse (for the 66-kDa antiserum detection) or goat anti-rabbit IgG (for the 29-kDa antiserum detection) alkaline phosphatase conjugate (Boehringer-Mannheim; Indianapolis, IN), washed 5 times for 5 min

in PBST, and developed for 3–5 min in BCIP-NBT substrate (Sigma) to visualize antibody-conjugate. Immunoblots with native proteins were performed in a similar manner to that reported for denatured proteins, although the initial separation of proteins was done under non-denaturing conditions (Zhou et al., 2003).

Diagnostic Assays

Individual rootworm adults from 10 different populations that exhibited varying levels of susceptibility at a diagnostic methyl-parathion concentration (Zhou et al., 2003) were examined by both native-PAGE assays and Western blots using the 66-kDa protein antiserum. In order to quantify esterase activities on the native-PAGE and immunological responses on the immunoblot, the relative intensity of staining was measured by densitometric scanning using a Gel Doc 2000 gel documentation system interfaced with Quantity One software (Bio-Rad). The diagnostic potential of the 66-kDa protein antiserum was evaluated by the two-way regression analyses of diagnostic concentration bioassays, native PAGE assays, and immunoassays developed in this study. The appropriate summary statistics were analyzed by SAS PROC CORR (SAS Institute, 2001).

RESULTS

Immunological Responses of the 29- and 66-kDa Protein Antiserum

Examination of post-hydroxyapatite fractions by SDS PAGE revealed three predominant protein bands of approximately 66-, 29-, and 20-kDa that exhibited increased staining intensity in the resistant relative to the susceptible population (Fig. 1A). Western blot analyses of 66-kDa protein antiserum under denaturing conditions revealed a single band corresponding in size to the purified 66-kDa protein (Fig. 1B) in both resistant and susceptible populations. The immunological responses (staining intensity) of the 66-kDa protein are clearly elevated in resistant York and Phelps County populations

compared to the susceptible Saunders County population, and no differences between sexes were detected. This is consistent with the native-PAGE results shown in Figure 1C. The antiserum to the 66-kDa protein apparently did not react with native proteins as Western blots of native gels did not reveal any bands (data not shown).

The 29-kDa protein antiserum cross-reacted with multiple proteins (20-, 29-, and 66-kDa) under denaturing conditions. Although increased signals were observed in resistant populations for the lower molecular weight proteins (20- and 29-kDa), such intensity differences were observed only in adult males (Fig. 1D). In contrast, the 66-kDa protein was present in both males and females, and exhibited increased intensity in the resistant strain. In Western analysis of native gels with individual adult males and females (20 males and 20 females per population), the 29-kDa antiserum readily discriminated between resistant and susceptible males, but not females (Fig. 2).

Diagnostic Potential of the 66-kDa Protein Antiserum

Ten populations exhibiting mortality ranging from 13.3 to 97.0% at a diagnostic methyl-parathion concentration (Table 1) were evaluated by both biochemical- and immunological-based diagnostic assays. Among the 10 populations, the staining intensity of group II esterase isoforms on native-PAGE (Fig. 3A) and the signal intensity of the 66-kDa protein bands on immunoblots (Fig. 3C) increase incrementally in association with their resistance status. Regression analyses for comparisons of methyl-parathion percent diagnostic mortality (Table 1), relative intensity of group II esterases on native gels (Fig. 3B), and relative intensity of 66-kDa protein band in immunoblots (Fig. 3D) are shown in Table 2. Regression analyses indicate a strong relationship (r^2 values ranging from 0.95 to 0.96) among all three diagnostic assays. These results clearly document the importance of the 66-kDa protein as a resistance-associated biochemical marker and the diagnostic potential of this 66-kDa protein antiserum.

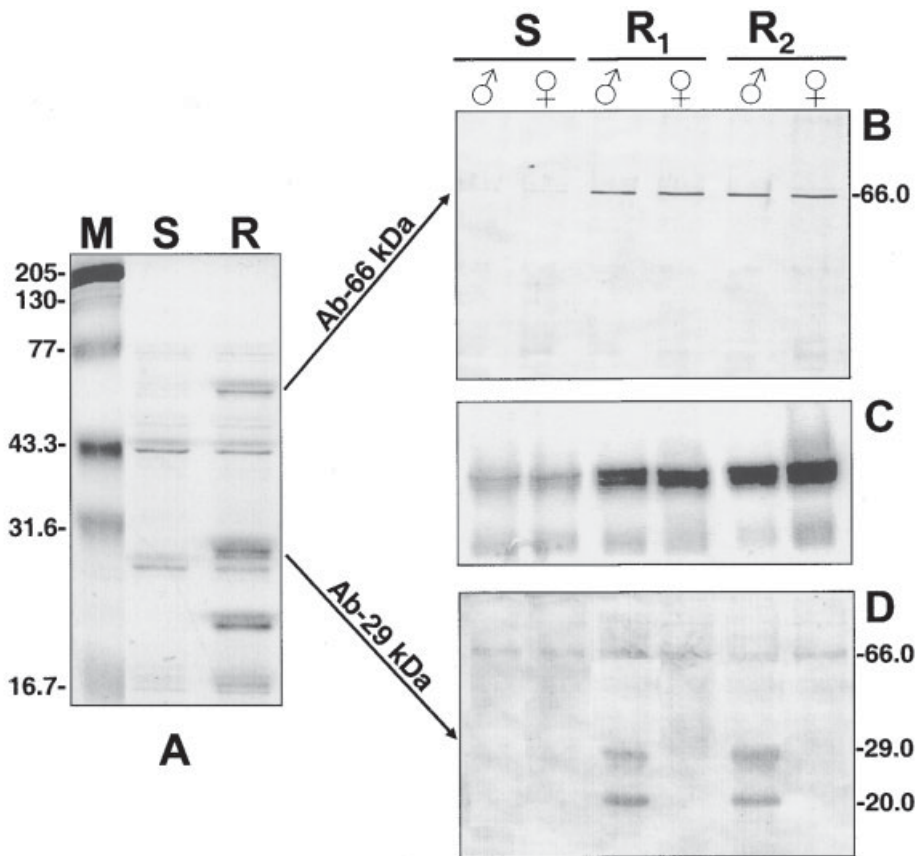


Fig. 1. Immunological reactions of the 29- and 66-kDa antisera with denatured proteins from resistant and susceptible *D. v. virgifera*. A: SDS PAGE stained with Coomassie blue to show partially purified group II esterases (post-ion exchange fractions). B: Western blot probed with 66-kDa antiserum C: Native PAGE stained to show esterase activity in crude homogenates. D: Western blot probed with 29-kDa antiserum. Abdominal tissues of 10 same-sex beetles (σ or ρ) from resistant York County (R_1), Phelps County (R_2), and susceptible Saunders County (S) populations were used as enzyme sources for this study.

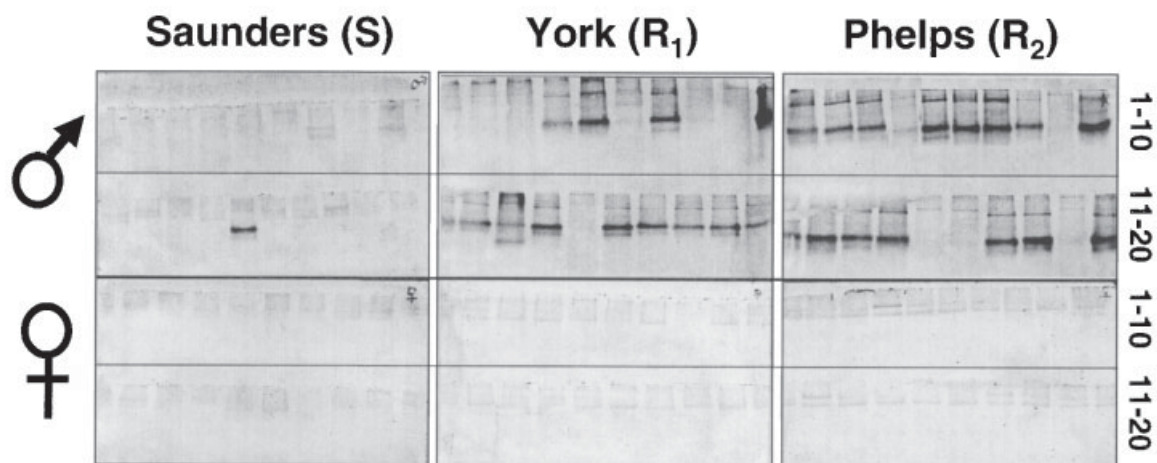


Fig. 2. Western blots of individual beetle protein extracts probed with 29-kDa antiserum (10 per gel, 20 per sex per population), following separation by native PAGE. The individual homogenates were prepared from the resistant York County (R_1), Phelps County (R_2), and susceptible Saunders County (S) populations.

TABLE 1. Descriptions and Methyl-Parathion Susceptibility Levels of Populations Compared in Native PAGE and Western Blotting Studies

Population no.	Collection site	County (state)	% Mortality (SE) ^a
1	Mead	Saunders (NE)	97.0 (1.5)
2	Collette	Yuma (CO)	92.0 (3.6)
3	Johnson	Saunders (NE)	80.3 (4.9)
4	Trumbull II	Clay (NE)	70.0 (7.3)
5	Lochland	Adams (NE)	69.5 (9.7)
6	Nelson	Phelps (NE)	45.0 (6.7)
7	Colo-1	Yuma (CO)	44.0 (9.5)
8	Hampton	Hamilton (NE)	32.0 (7.7)
9	Karrs	Adams (NE)	22.8 (7.2)
10	Keene	Kearney (NE)	13.3 (4.6)

^aPercentage mortality in bioassays with a diagnostic concentration of methyl-parathion (see text for methods).

DISCUSSION

Following the partial purification of group II esterases, three putative esterase proteins (20, 29, and 66-kDa) were enriched in resistant relative to susceptible populations. Two of these resistance-associated proteins (29- and 66-kDa) were purified to homogeneity and used as antigens to develop polyclonal antisera. The 29-kDa antibody recognized two lower molecular weight resistance-associated proteins (20- and 29-kDa) but only in male beetles. The existence of a male-linked esterase (*Est-m*) in *D. v. virgifera* (Ruud et al., 1988; McDonald et al., 1990) with a molecular weight of approximately 78-kDa and its activity localized in the abdomens of virgin males has been previously documented. The two male-linked proteins (20- and 29-kDa) identified in the present study are much smaller than *Est-m*, although Rudd et al. (1988) suggested the existence of other sex-linked loci in both *D. v. virgifera* and *D. barberi*. The role of these male-linked proteins is unclear, especially in relation to resistance, since there are no apparent differences in susceptibility to methyl-parathion between male and female beetles (Zhou, unpublished data), and no indication of sex-linked inheritance of esterase activity as determined by native PAGE analysis of individual rootworms obtained from reciprocal crosses (Parimi et al., 2003).

In contrast to the 29-kDa antiserum and based on multiple lines of evidence, the 66-kDa antiserum is considered to be diagnostic for a group II esterase that is at least partially responsible for re-

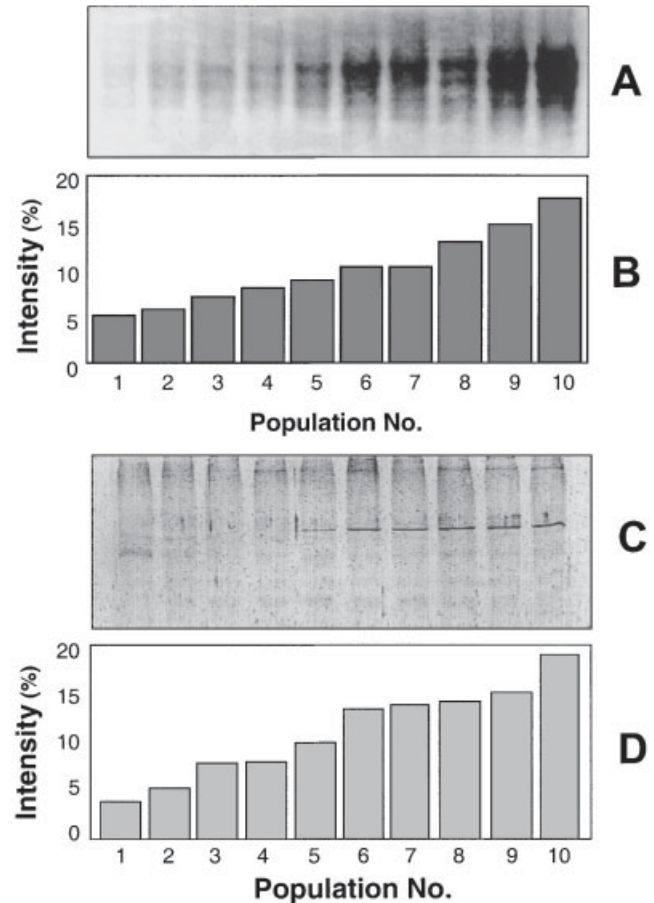


Fig. 3. Comparison of an immunological-based assay with a biochemical-based diagnostic native PAGE assay. A: Native PAGE assay result. B: Densitometric data corresponding to staining intensity shown in A (α -naphthyl acetate as substrate). C: Immunoassay result. D: Densitometric data corresponding to staining intensity shown in C (blot developed with BCIP-NBT substrate). A total of 10 *D. v. virgifera* populations with various susceptibilities to the diagnostic concentration of methyl-parathion (Table 1) are used to evaluate the diagnostic potential of the 66-kDa specific immunoassay developed in this study.

TABLE 2. Regression Analyses Comparing % Mortality in Methyl-Parathion Diagnostic Concentration Bioassays (Table 1), Relative Intensity of Group II Esterase Activity (Visualized by Native PAGE Gels; Fig. 3B), and Relative Intensity of Immunological Response With 66-kDa Antiserum (Visualized by Western Blots; Fig. 3C)

Dependent variable	Independent variable	Slope	Y-intercept	r ^{2a}
% Mortality	PAGE relative intensity	-0.13	17.55	0.95
% Mortality	Western relative intensity	-0.17	19.92	0.96
PAGE relative intensity	Western relative intensity	1.28	-2.96	0.95

^aCoefficient of determination indicating the goodness-of-fit of regression lines.

sistance. Resistance-associated esterases may confer resistance either by qualitative modification of enzyme structure that increases catalytic efficiency or by quantitative modification that involves overproduction of esterases that act to sequester insecticidal esters (Hemingway, 2000). Based on the physical and biochemical properties of the partially purified group II esterase (66-kDa protein) (Zhou et al., 2004), we propose the overexpression of group II esterases as the underlying basis of esterase-mediated resistance in *D. v. virgifera*. The immunoassay results shown here support this conclusion by demonstrating that increased titers of group II esterase proteins are associated with resistance.

We have previously shown that the *Myzus persicae* antiserum (Zhou et al. 2004) to the E4 esterase that confers organophosphate resistance in this aphid is cross-reactive with a 66-kDa protein from both resistant and susceptible *D. v. virgifera* populations but that signal intensity was much stronger in preparations from resistant populations. It is highly likely that the resistance-associated E4 antiserum and 66-kDa antiserum recognizes the same group II esterase (approximately 66-kDa in size) from *D. v. virgifera*. Based on their immunological responses (signal intensity), group II esterases are immunologically indistinguishable between resistant and susceptible *D. v. virgifera* populations, and therefore different expression levels exhibited on immunoblots are a direct result of the overproduction of group II esterases in resistant populations. There are two types of genetic changes that may lead to this overproduction. The first is a stable, heritable change in the copy number of the gene (gene amplification) that would lead to the multiple gene copy numbers and increased levels of mRNA and protein. The second change involves modification of regulatory DNA sequences outside the protein coding region, which results in increased protein synthesis. Gene amplification has been conclusively documented in the aphids, *Myzus persicae* (esterases E4 and FE4) (Field et al., 1988; Field and Devonshire, 1998) and *Schizaphis graminum* (Type I esterases) (Siegfried et al., 1997; Ono et al., 1999), mosquitoes, *Culex quinquefasciatus*, *C. pipens*, *C. tarsalis*, and *C. tritaeniorhynchus* (esterases A and B) (Mouches et al., 1986;

Karunaratne et al., 1998; Hemingway et al., 1998), and brown planthoppers, *Nilaparvata lugens* (NI-EST1) (Small and Hemingway, 2000). The combined results from biochemical, kinetic, and inhibition characterizations (Zhou et al., 2004), as well as immunological analyses (this study) imply an over-expression of the resistance-associated esterase in *D. v. virgifera*. However, it is not possible to determine whether such over-expression is due to gene amplification or a change in gene regulation that results in increased protein synthesis.

Immunological and biochemical-based techniques that diagnose both the qualitative and quantitative differences in detoxification enzymes offer tremendous opportunities to improve the speed, accuracy, and sensitivity of resistance monitoring (Denholm et al., 1990). Immunoassays for detecting resistant individuals have been developed for a number of insect pests and have been adapted to microplate format (such as Enzyme-linked immunosorbent assay, ELISA) for screening of large numbers of individuals in a relatively short period of time (Devonshire et al., 1986; Siegfried et al., 1997). In this report, immunological-based assays provide comparable results to both conventional diagnostic concentration bioassays as well as biochemical-based native PAGE assays. The low background exhibited by this technique (without contamination from other esterases) is a major advantage over biochemical-based native PAGE or model substrate assays. However, given that the 66-kDa antiserum only recognizes denatured protein, development of immunological-based microplate assays (ELISA) for rapid detection of resistance would likely require further development. Once in place for routine execution, immunoassays in conjunction with diagnostic insecticide bioassays would provide a means to effectively monitor the onset of resistance development and to precisely document the distribution of resistant populations, with the end result being a better understanding of the effects of insecticide selection on resistance evolution.

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LITERATURE CITED

- Chang HC. 1973. Bionomics of the northern and western corn rootworms. *Annu Rev Entomol* 18:47–72.
- Denholm I, Gorman KJ, Moores GD, Devonshire AL. 1990. Biochemical assays for insecticide resistance: strengths and limitations. *Brighton Crop Prot Conf Pests Dis* 3:1175–1180.
- Devonshire AL, Moores GD, French-Constant RH. 1986. Detection of insecticide resistance by immunological estimation of carboxylesterase activity in *Myzus persicae* (Sulzer) and cross reaction of the antiserum with *Phorodon humuli* (Schrank) (Hemiptera: Aphididae). *Bull Ent Res* 76:97–107.
- Field LM, Devonshire AL, Forde BG. 1988. Molecular evidence that insecticide resistance in peach-potato aphids (*Myzus persicae* Sulz.) results from amplification of an esterase gene. *Biochem J* 251:309–312.
- Field LM, Devonshire AL. 1998. Evidence that the E4 and FE4 esterase genes responsible for insecticide resistance in the aphid *Myzus persicae* (Sulzer) are part of a gene family. *Biochem J* 330:169–173.
- Hemingway J. 2000. The molecular basis of two contrasting metabolic mechanisms of insecticide resistance. *Insect Biochem Mol Biol* 30: 1009–1015.
- Hemingway J, Hawkes N, Prapanthadara L, Jayawardenal KG, Ranson H. 1998. The role of gene splicing, gene amplification and regulation in mosquito insecticide resistance. *Philos Trans R Soc Lond B Biol Sci* 353:1695–1699.
- Karunaratne SHPP, Vaughan A Paton MG, Hemingway J. 1998. Amplification of a serine esterase gene is involved in insecticide resistance in Sri Lankan *Culex tritaeniorhynchus*. *Insect Mol Biol* 7:307–315.
- Laemmli UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227:680–685.
- Levine E, Oloumi-Sadeghi H. 1991. Management of *Diabrotica* rootworms in corn. *Annu Rev Entomol* 36:229–255.
- Levine E, Spencer JL, Isard SA, Onstad DW, Gray ME. 2002. Adaptation of the western corn rootworm to crop rotation: evolution of a new strain in response to a management practice. *Am Entomol* 48:64–107.
- McDonald IC, Krysan JL, Johnson OA. 1990. Studies of electrophoretic variation in *Diabrotica* as influenced by the age, sex, or diet of adult beetle (Coleoptera: Chrysomelidae). *Ann Entomol Soc Am* 83:1192–1202.
- Meinke LJ, Siegfried BD, Wright RJ, Chandler LD. 1997. Western corn rootworm resistance to insecticides: current situation in Nebraska. *Proc Illinois Agric Pestic Conf* 88–92.
- Meinke LJ, Siegfried BD, Wright RJ, Chandler LD. 1998. Adult susceptibility of Nebraska western corn rootworm (Coleoptera: Chrysomelidae) populations to selected insecticides. *J Econ Entomol* 91:594–600.
- Miota F, Scharf ME, Ono M, Marcon P, Meinke LJ, Wright RJ, Chandler LD, Siegfried BD. 1998. Mechanisms of methyl and ethyl-parathion resistance in the western corn rootworm (Coleoptera: Chrysomelidae). *Pestic Biochem Physiol* 61:39–52.
- Mouches C, Pasteur N, Berge JB, Hyrien O, Raymond M, De Saint Vincent BR, De Silvestri M, Georghiou GP. 1986. Amplification of an esterase gene is responsible for insecticide resistance in a California *Culex* mosquito. *Science* 233:778–780.
- Ono M, Swanson JJ, Field LM, Devonshire AL, Siegfried BD. 1999. Amplification and methylation of an esterase gene associated with insecticide-resistance in greenbugs, *Schizaphis graminum* (Rondani) (Homoptera: Aphididae). *Insect Biochem Mol Biol* 29:1065–1073.
- Parimi S, Scharf ME, Meinke LJ, Chandler LD, Siegfried BD. 2003. Inheritance of methyl-parathion resistance in Nebraska western corn rootworm populations (Coleoptera: Chrysomelidae). *J Econ Entomol* 96:131–136.
- Pruess KP, Witkowski JF, Raun ES. 1974. Population suppression of western corn rootworm by adult control with ULV malathion. *J Econ Entomol* 67:651–655.

- Ruud SK, McDonald IC, Gabrielson DA, Johnson OA. 1988. Species distribution and characterization studies of sex-limited esterases within the *virgifera* group of *Diabrotica* (Coleoptera: Chrysomelidae). *Ann Entomol Soc Am* 81:954–963.
- SAS Institute. 2001. SAS user's guide: statistics. Cary, NC: SAS Institute.
- Siegfried BD, Swanson JJ, Devonshire AL. 1997. Immunological detection of greenbug (*Schizaphis graminum*) esterases associated with resistance to organophosphate insecticides. *Pestic Biochem Physiol* 57:165–170.
- Small GJ, Hemingway J. 2000. Molecular characterization of the amplified carboxylesterase gene associated with organophosphorus insecticide resistance in the brown planthopper, *Nilaparvata lugens*. *Insect Mol Biol* 9:647–653.
- Wright RJ, Scharf ME, Meinke LJ, Zhou X, Siegfried BD, Chandler LD. 2000. Larval susceptibility of an insecticide-resistant western corn rootworm (Coleoptera: Chrysomelidae) population to soil insecticides: laboratory bioassays, assays of detoxification enzymes, and field performance. *J Econ Entomol* 93:7–13.
- Zhou X, Scharf ME, Parimi S, Meinke LJ, Wright RJ, Chandler LD, Siegfried BD. 2002. Diagnostic assays based on esterase-mediated resistance mechanisms in western corn rootworms (Coleoptera: Chrysomelidae). *J Econ Entomol* 95:1261–1266.
- Zhou X, Scharf ME, Meinke LJ, Chandler LD, Siegfried BD. 2003. Characterization of general esterases from methyl-parathion resistant and susceptible populations of western corn rootworm (Coleoptera: Chrysomelidae). *J Econ Entomol* 96:1855–1863.
- Zhou X, Scharf ME, Sarath G, Meinke LJ, Chandler LD, Siegfried BD. 2004. Partial purification and characterization of a methyl-parathion resistance-associated general esterase in *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae). *Pestic Biochem Physiol* 78:114–125.