

University of Nebraska - Lincoln

DigitalCommons@University of Nebraska - Lincoln

Faculty Publications: Department of
Entomology

Entomology, Department of

2000

Hydrolase and Oxido-Reductase Activities in *Diuraphis noxia* and *Rhopalosiphum padi* (Hemiptera: Aphididae)

Xinzhi Ni

University of Nebraska-Lincoln

Sharron S. Quisenberry

Montana State University - Bozeman

Saowaluck Pornkulwat

University of Nebraska-Lincoln

James Lester Figarola

University of Nebraska-Lincoln

Steven R. Skoda

University of Nebraska-Lincoln, sskoda1@unl.edu

See next page for additional authors

Follow this and additional works at: <https://digitalcommons.unl.edu/entomologyfacpub>

 Part of the [Entomology Commons](#)

Ni, Xinzhi; Quisenberry, Sharron S.; Pornkulwat, Saowaluck; Figarola, James Lester; Skoda, Steven R.; and Foster, John E., "Hydrolase and Oxido-Reductase Activities in *Diuraphis noxia* and *Rhopalosiphum padi* (Hemiptera: Aphididae)" (2000). *Faculty Publications: Department of Entomology*. 286.

<https://digitalcommons.unl.edu/entomologyfacpub/286>

This Article is brought to you for free and open access by the Entomology, Department of at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Faculty Publications: Department of Entomology by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

Authors

Xinzhi Ni, Sharron S. Quisenberry, Saowaluck Pornkulwat, James Lester Figarola, Steven R. Skoda, and John E. Foster

Hydrolase and Oxido-Reductase Activities in *Diuraphis noxia* and *Rhopalosiphum padi* (Hemiptera: Aphididae)

XINZHI NI,¹ SHARRON S. QUISENBERRY,² SAOWALUCK PORNKULWAT,
JAMES LESTER FIGAROLA, STEVEN R. SKODA,³ AND JOHN E. FOSTER

Department of Entomology, University of Nebraska, Lincoln, NE 68583-0816

Ann. Entomol. Soc. Am. 93(3): 595-601 (2000)

ABSTRACT Four hydrolases and five oxido-reductases were examined using native stacking polyacrylamide gel electrophoresis. Homogenate of Russian wheat aphid, *Diuraphis noxia* (Mordvilko), bird cherry-oat aphid, *Rhopalosiphum padi* (L.), 'Arapahoe' (aphid-susceptible) and 'Halt' (aphid-resistant) wheat, *Triticum aestivum* L., and powdery mildew-infected *Erysiphe graminis* DC. ex Merat f. sp. *tritici* Em. Marchal, Arapahoe wheat leaves were assayed for enzyme activities. Pectinesterase, polygalacturonase (or pectinase), cellulase, and amylase activities were examined in the hydrolase group. Catalase, peroxidase, catechol oxidase, superoxide dismutase, and ascorbate oxidase activities were examined in the group of oxido-reductases. The two aphid species had the same hydrolases but different oxido-reductases. Although pectinesterase and cellulase enzymes were present in *D. noxia* and *R. padi*, the banding patterns were different. Polygalacturonase and δ -amylase were not detected from either aphid species. In the oxido-reductase group, catalase was detected from *D. noxia*, whereas peroxidase was detected from *R. padi*. Superoxide dismutase and ascorbate oxidase activities also were detected from both aphids. Enzyme assays using aphid head tissue that included salivary glands but excluded aphid foregut supported the enzyme assays using whole aphids. Peroxidase activity was detected from the salivary tissue of *R. padi*, but not *D. noxia*, and catalase activity was detected from *D. noxia* salivary tissue, but not *R. padi*. We suggest that the salivary enzyme difference between the 2 aphid species (i.e., catalase and peroxidase) is important in the type of damage symptom formation on susceptible wheat plants.

KEY WORDS *Diuraphis noxia*, *Rhopalosiphum padi*, *Triticum aestivum*, enzymes

ALTHOUGH APHID-PLANT INTERACTIONS have been studied extensively, considerable work has been focused on virus-aphid vector-host plant interactions rather than the direct feeding damage of aphids on plants. Mechanisms underlying how nonvector aphids and other piercing-sucking insects damage their host plants are not completely understood. It has been hypothesized that damage symptoms from feeding by piercing-sucking insects are caused by the injection of salivary phytotoxins into plants (Miles 1987, 1990; Burd et al. 1998).

The damage symptoms (i.e., necrotic spots) of greenbug, *Schizaphis graminum* (Rondani), on wheat, *Triticum aestivum* L., are thought to be the result of a hypersensitive reaction of wheat leaves to aphid feeding. Pectinases and cellulases are considered the important salivary enzymes contributing to *S. graminum* damage symptom formation on wheat (Campbell 1986, Miles 1990). Feeding damage symptoms of the

spotted alfalfa aphid, *Therioaphis trifolli maculata* (Buckton), were shown to be caused by the interruption of plant redox balance (Miles and Oertli 1993). Aphid feeding induced the accumulation of oxidases and phenolic substrates and loss of reducing activity and protein in alfalfa that caused vein clearing and localized browning of cells surrounding aphid feeding sites (Jiang and Miles 1993). Additionally, catechol oxidase and peroxidase from saliva of the rose aphid, *Macrosiphum rosae* (L.), oxidized phenolics and other allelochemicals in its food plants (Peng and Miles 1991). Thus, previous work suggests that the mechanism of direct aphid damage to plants may involve either hydrolases or oxido-reductases from aphid saliva. In the saliva and salivary glands of hemipteran insects, 4 hydrolases and 5 oxido-reductases have been detected (Madhusudhan et al. 1994). The hydrolases degrade polysaccharides in the cell wall, whereas the oxido-reductases interrupt redox balance of plants by affecting the generation and removal of hydrogen peroxide (Table 1).

The damage symptoms (i.e., leaf folding, rolling, and chlorotic streaks) caused by Russian wheat aphid, *Diuraphis noxia* (Mordvilko), feeding is still not well understood (Burd et al. 1998), although the effect of aphid feeding on plant photosynthesis has been examined (Fouche et al. 1984, Kruger and Hewitt 1984,

This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or a recommendation by the USDA for its use.

¹ To whom correspondence should be addressed.

² College of Agriculture, Office of the Dean and Director, P.O. Box 172860, Montana State University, Bozeman, MT 59717-2860.

³ MLIRU, USDA-ARS, Department of Entomology, University of Nebraska, Lincoln, NE 68583-0938.

Table 1. Principles of enzymatic activity assays

Enzyme	Standard		Type of protein	Principle
	EC No.	Subunit		
Hydrolases				
Pectinesterase	3.1.1.11	—	—	Pectin + nH ₂ O <u>pectinesterase</u> → n methanol + pectate
Polygalacturonase	3.2.1.15	—	—	Random hydrolysis of 1,4- α -D-galactosiduronic linkages in pectate and other galacturonans
α -amylase	3.2.1.1	—	—	Endohydrolysis of 1,4- α -glucosidic linkages in oligo- and polysaccharides
Cellulase	3.2.1.4	—	—	Endohydrolysis of 1,4- β -D-glucosidic linkages in cellulose
Oxido-reductases				
Catalase	1.11.1.6	Tetramer	Fe protein	2H ₂ O ₂ <u>catalase</u> → 2H ₂ O + O ₂
Peroxidase	1.11.1.7	Uncertain	Fe protein	Donor + 2H ₂ O ₂ <u>peroxidase</u> → oxidized doner + 2H ₂ O
Catechol oxidase	1.14.18.1	—	Cu protein	2 Catechol + O ₂ <u>catechol oxidase</u> → 2 1,2-benzoquinone + 2H ₂ O
Superoxide	1.15.1.1	—	Cu & Zn, or Fe or Mn protein	O ₂ ⁻ + O ₂ ⁻ + 2H ⁺ <u>superoxide dismutase</u> → O ₂ + 2H ₂ O ₂
Dismutase	—	—	—	—
Ascorbate oxidase	1.10.3.3	—	Cu protein	2 L-ascorbate + O ₂ <u>ascorbate oxidase</u> → 2 dehydroascorbate + 2H ₂ O

After International Union of Biochemistry 1978, Vallejos 1983, and Murphy et al. 1996.

Burd and Elliott 1996). *D. noxia* extract influenced photosynthesis of isolated wheat chloroplasts (Kruger and Hewitt 1984). Chloroplast membrane and photosynthetic pigments were the important sites responding to *D. noxia* feeding (Fouche et al. 1984). *D. noxia* feeding also caused significant changes of chlorophyll *a* fluorescence induction kinetics in susceptible wheat leaves (Burd and Elliott 1996).

The objective of this study was to compare the hydrolase and oxido-reductase activities from whole aphids and salivary glands of symptom-eliciting *D. noxia* and nonsymptom-eliciting *R. padi* using native polyacrylamide gel electrophoresis (PAGE). Four hydrolases (pectinesterase, polygalacturonase, δ -amylase, and cellulase) and 5 oxido-reductases (catalase, peroxidase, catechol oxidase, superoxide dismutase, and ascorbate oxidase) commonly found in the saliva of homopteran and heteropteran insects (Madhusudhan et al. 1994) were examined in the 2 cereal aphids and their host plants. Because whole-body catalase and peroxidase activities were different between the 2 aphid species, salivary gland catalase and peroxidase activities also were examined. We present the results of enzyme activity assays for 4 hydrolases and 5 oxido-reductases from the 2 aphid species and wheat plants.

Materials and Methods

Plants and Aphids. The plants were grown in pots (10 cm diameter) of a soil mixture of silty clay loam soil, peat moss, and sand (2:1:1 ratio) in a growth chamber at 21 \pm 1°C, a photoperiod of 16:8 (L:D) h, and 40–50% RH. The colony of *D. noxia* was originally established in 1994 from field-collected aphids near Scottsbluff, NE. The *R. padi* colony was established in the fall of 1996 from field-collected aphids near Lincoln, NE. Both aphid colonies were maintained on 'Stephens' (susceptible) wheat at 21 \pm 1°C, a photoperiod of 16:8 (L:D) h, and 40–50% RH. The aphid colonies were maintained on young wheat seedlings at the 3–5 leaf stages (stages 13–15 (Zadoks et al. 1974).

Whole-Aphid and Plant Sample Preparations. *Diuraphis noxia* and *R. padi* were collected by harvesting

the aphid-infested wheat seedlings at the soil surface and freezing aphids and plants at -20°C. Immediately after quick freezing, aphids were removed from the wheat leaves, weighed, and stored at -80°C. Because the aphids used in this study were removed from wheat plants, it was possible that the gut of aphids contained plant enzymes ingested from wheat leaves. We therefore used aphid-susceptible 'Arapahoe' (Hein 1992) and 'Stephens' (Quisenberry and Schotzko 1994) wheat and resistant 'Halt' wheat (Quick et al. 1996) as controls to show enzyme activities from the aphids and their food plants. Powdery mildew-infected, *Erysiphe graminis* DC. ex Merat f. sp. *tritici* Em. Marchal, Arapahoe wheat was also assayed to demonstrate possible enzyme activity similarities between fungus and aphid damage. Plant samples were collected by harvesting the 2nd or the 3rd leaves of 4-leaf stage plants (Zadoks et al. 1974). Aphid or plant material (0.3 g material per treatment) was homogenized in 100 μ l of cold sample buffer (0.1 M potassium phosphate buffer, pH 7.5, 0.05% tracking dye—bromothymol blue, and 12.5% glycerol). Samples were centrifuged at 14,000 \times g for 15 min and the supernatant used for electrophoresis. Each enzyme activity assay was repeated 3 times.

Aphid Salivary Gland Sample Preparation. Because peroxidase and catalase activities were found in aphid whole-body assays, aphid salivary glands were assayed for peroxidase and catalase using excised aphid heads. Excised heads were used because dissecting and collecting uninjured salivary glands from the minute aphids (<2 mm) was extremely difficult. Thus, the excised aphid heads included salivary glands but excluded the foregut. We used 600 excised aphid heads as a sample for the enzyme assays. Aphid heads were immediately stored in a microcentrifuge tubes with 200 μ l cold (4°C) extracting buffer (0.1 M potassium phosphate buffer pH 7.5 and 10% glycerol). A sample was homogenized using a chilled Teflon microtube pestle. The homogenate was centrifuged at 4°C with 14,000 \times g for 15 min, the supernatant removed, and diluted 2:1 (supernatant: gel loading buffer). The diluted sample (30 μ l) was loaded to a sample well on

a gel. The gel loading buffer consisted of 0.05 M Tris-Cl (pH 6.8), 0.1% bromophenol blue, and 10% glycerol. Two samples (each containing 600 aphid heads) were examined for each aphid species, and the assays were repeated 5 or 6 times per sample.

Commercial Enzyme Standards. Commercial standards were used as positive control for the experiment. The commercial standards were: pectinesterase (EC 3.1.1.11), polygalacturonase (EC 3.2.1.15), amylase (EC 3.2.1.1), cellulase (EC 3.2.1.4), catalase (EC 1.11.1.6), peroxidase (EC 1.11.1.7), catechol oxidase (EC 1.14.18.1), superoxide dismutase (EC 1.15.1.1), and ascorbate oxidase (EC 1.10.3.3) (Sigma Chemicals, St. Louis, MO).

Electrophoresis Conditions. A Bio-Rad Protean II vertical mini-gel apparatus was used for electrophoresis. Native discontinuous (or stacking) polyacrylamide gels were used in this study. The stacking gel used was 5%, and the separating gel was 10% (except 12% for catalase). A continuous buffer system (Tris-glycine buffer, pH 8.3) was used. Electrophoresis was conducted at 4°C, with 24 mA or 110 v.

Gel Incubation and Staining. After electrophoresis was completed, gels were incubated in 10–100 ml of substrate or staining solution. The volume of the incubation and staining solutions varied among the enzymes assayed. Unless the procedures were modified and so stated, all 9 enzymes assayed in this report were determined by following the protocols described by Vallejos (1983), Madhusudhan et al. (1994), and Murphy et al. (1996). All gels were scored for presence or absence of the enzymes and photographed immediately after incubation, staining, and destaining of the enzyme activities. Gels were fixed in a gel fixation solution (acetic acid/methanol: water in a 1:2:7 ratio), and photographed in a gel storage solution (50% glycerol). The incubation and staining solutions for the enzymes assayed are summarized as follows:

Pectinesterase and Polygalacturonase. The gels with both standards were incubated first in 0.4% pectin dissolved in 100 ml of 0.1 M malic acid (pH 3) at 35°C on a shaker for 4 h. The gels were then transferred to stain in 100 ml ruthenium red (0.02%) for 1 h and destained in water overnight. Pectinesterase activity was shown by dark red-stained zones (bands) over the pink background color of the gel, and the polygalacturonase action was shown by a colorless or pale zone on the gel. The procedure used in pectic enzyme assays was a combination of the reports by Cruickshank and Wade (1980) on plants, and Laurema et al. (1985) and Shen et al. (1996) on insects.

α -Amylase. The gels with standard amylase were incubated in 0.5% of soluble starch in 0.002 M CaCl₂ and 0.1 M Tris-Cl (pH 7.6) for 2 h. The incubated gels were stained with a mixture of 3% KI and 1.3% I₂ for 10 min. Amylase bands were achromatic against a dark blue background (Lacks and Springhorn 1980).

Cellulase. Postelectrophoresed gels were placed in a 2% agar overlay containing 0.1% carboxymethyl cellulose in 0.05 M disodium phosphate and 0.0125 M citric acid (pH 6.3) and incubated in 40°C for 3 h. Both the overlay and the gels were stained with 0.1% congo

red and destained in 1 M NaCl for 20 min. Cellulase activity was shown as orange-red bands. The procedure used to detect cellulase activity was modified after Béguin (1983).

Catalase. Gels were soaked for 45 min in a solution of 10 ml 0.05 M potassium phosphate buffer (pH 7.0), 1.25 ml of 0.4% 3,3'-diaminobenzidine, and 0.1% of horseradish peroxidase. Gels were then rinsed with deionized water and immersed in 0.02 M hydrogen peroxide until achromatic bands appeared on a dark brown background (Gregory and Fridovich 1974).

Peroxidase. Gels were incubated at room temperature on a shaker with 100 ml of acetate buffer (pH 5.0), 50 μ l of 30% hydrogen peroxide, and redox dye (3-amino-9-ethylcarbazole). The solution was made by first dissolving 0.05 g of the redox dye in 3 ml of N, N-dimethylformamide. The dissolved redox dye was then mixed with 100 ml of acetate buffer and the hydrogen peroxide was added just before incubation. Thin dark red bands appeared in 5 min and reached maximum intensity in 30 min.

Catechol Oxidase. Gels were incubated at room temperature on a shaker in 0.01 M DL-3,4-dihydroxyphenylalanine (DOPA) in 0.1 M potassium phosphate buffer (pH 7.4) for 30–60 min. Catechol oxidase activity was shown by a dark band in a clear background.

Superoxide dismutase. The electrophoresed gels were incubated for 30 min in 0.00245 M nitro-blue tetrazolium at dark at room temperature on a shaker. Incubated gels were then transferred to a solution containing 0.036 M potassium phosphate, 0.028 M TEMED, and 25 (M ascorbic acid for 15 min in the dark. Gels were then placed under a fluorescent lamp for 20 min. Superoxide dismutase activity was shown by clear bands on a blue background (Beauchamp and Fridovich 1971).

Ascorbate Oxidase. Gels were first incubated in 0.1 M of hydrogen peroxide for 20 min, and then incubated in nitro-blue tetrazolium. After that, the procedure for detection of superoxide dismutase also was used to detect ascorbate oxidase. Ascorbate oxidase activity was shown by clear bands on a blue background (Maccarrone et al. 1990).

Results and Discussion

Hydrolase Activities from Whole Aphids. Pectinesterase activity was detected from *D. noxia*, *R. padi*, and the 3 wheat samples. Polygalacturonase activity was not detected from either aphid or plant samples (Fig. 1). No (α)amylase activity was detected from *D. noxia* or *R. padi*, but was detected from the wheat samples (Table 2). Cellulase activity was detected from both aphids and wheat samples (Table 2), although the banding patterns of the enzymes varied among the aphids and plants. In the group of hydrolases, both *D. noxia* and *R. padi* showed pectinesterase and cellulase enzyme activities, whereas neither aphid species exhibited polygalacturonase and α -amylase activities. Among the wheat samples examined, no differences were observed in hydrolase enzymes. Pectinesterase,

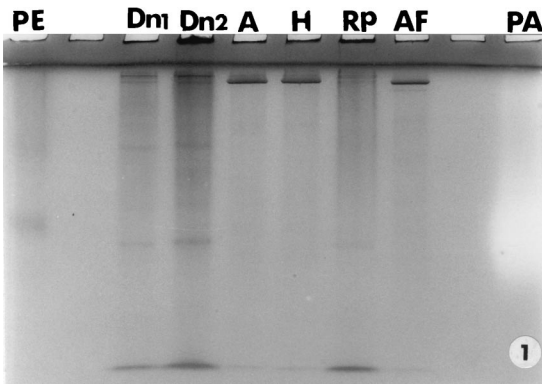


Fig. 1. Stained native polyacrylamide gel (10%) showing enzyme activities of pectinesterase and polygalacturonase. Pectinesterase activity shown by dark bands, whereas polygalacturonase (or pectinase) was not detected in aphid and plant samples, except polygalacturonase standard (PA). Abbreviations for sample wells: PE, pectinesterase standard (5 μ l of 2.18 μ g protein per μ l); Dn1, *D. noxia* aphid with 10 μ l sample loaded; Dn2, *D. noxia* sample with 15 μ l sample loaded; A, Arapahoe wheat (15 μ l). H, Halt wheat (15 μ l); Rp, *R. padi* aphid (15 μ l); AF, fungus (i.e., *E. graminis tritici*) infected Arapahoe wheat (15 μ l); and PA, polygalacturonase standard (5 μ l of 0.016 μ g protein per μ l) showing negative-stained bands.

amylase, and cellulase were detected from the wheat plants, but not polygalacturonase (Table 2).

However, in another symptomatic cereal aphid, *S. graminum*, hydrolases, in particular pectic enzymes, were considered important in the necrotic damage symptom development (Ma et al. 1990, Miles 1990). Polysaccharase and carbohydrase enzymes from *S. graminum* elicit plant wound response by degrading cell walls or producing cell wall fragments (Campbell and Dryer 1985, 1990). Furthermore, salivary polysaccharases (e.g., pectinases and cellulases) have been hypothesized to enable intercellular stylet penetration of aphid and other piercing-sucking insects (Miles 1990).

Although both *D. noxia* and *S. graminum* cause leaf chlorosis on wheat plants, the symptoms are different. *D. noxia* feeding prevents wheat leaf unfolding and causes rolling and systemic leaf chlorotic streaks on young leaves (Burd et al. 1993), whereas *S. graminum* causes localized necrotic spots (Ryan et al. 1990). Polygalacturonase and pectinesterase were detected from *S. graminum* feeding sites on agar-pectin plates, but not from *R. padi* feeding sites (Ma et al. 1990). Our examination of *D. noxia* feeding sites on agar-pectin plates did not show polygalacturonase activity (X.N. and S.S.Q., unpublished data). The comparisons suggest that the mechanism of *D. noxia* damage symptom formation is different from that of *S. graminum*. Additionally, we detected pectinesterase activity from *R. padi* that was not detected using the method of Ma et al. (1990). The difference between the results reported by Ma et al. (1990) and our results is probably related to the high sensitivity of the modified pectic enzyme activity assay we used.

Erysiphe graminis tritici infection caused limited damage symptoms on wheat plants surrounding its infection sites, which is different from the systemic damage observed after *D. noxia* feeding (Burd and Elliott 1996). We found that *E. graminis tritici*-infected wheat had pectinesterase activity, but not polygalacturonase activity (Fig. 1). Because both aphid stylets and fungal hyphae penetrate wheat leaf surfaces, it is possible that aphid and fungus penetration through plant tissues are related to pectic enzymes (e.g., pectinesterase). The pectinesterase bands observed in the assays of *D. noxia*, *R. padi*, and *E. graminis tritici*-infected wheat leaves were different and also may contribute to the variations observed in damage symptom formation among the species (Fig. 1). Further experiments are needed to assay pectinesterase activities in aphid salivary glands excluding pectic substrate. Because ruthenium red also is a protein stain, it may elicit false positive results for pectinesterase activity.

Furthermore, pectinesterase activity has been detected at the feeding site of the cassava mealybug,

Table 2. Enzymatic activity assays of hydrolases and oxido-reductases using stacking native PAGE electrophoresis

Enzyme	Standard		<i>D. noxia</i>	<i>R. padi</i>	wheat-s ^a	wheat-r ^b	wheat-s + fungus ^c
	EC No.	Activity					
Hydrolases							
Pectinesterase	3.1.1.11	3	3	3	3	3	3
Polygalacturonase	3.2.1.15	3	1	1	1	1	1
Amylase	3.2.1.1	3	1	1	3	3	2
Cellulase	3.2.1.4	3	3	2	3	3	3
Oxido-reductases							
Catalase	1.11.1.6	3	3	1	3	3	3
Peroxidase	1.11.1.7	3	1	2	3	3	3
Catechol oxidase	1.14.18.1	3	2	2	2	1	2
Superoxide dismutase	1.15.1.1	3	3	3	3	3	3
Ascorbate oxidase	1.10.3.3	3	3	3	3	3	3

1, no enzyme bands detected; 2, faint enzyme bands detected; 3, distinct enzyme bands detected.

^a Wheat-s, susceptible Arapahoe wheat.

^b Wheat-r, resistant Halt wheat.

^c Wheat-s fungus, *E. graminis tritici*-infected Arapahoe wheat.

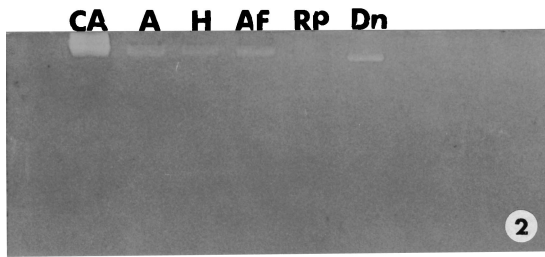


Fig. 2. Negatively stained gel (12%) showing catalase activity. Catalase activity shown by the clear (or achromatic) bands against the dark background. CA, catalase standard (5 μ l of 1.18 μ g protein per μ l); A, Arapahoe wheat (5 μ l); H, Halt wheat (5 μ l); AF, fungus (i.e., *E. graminis tritici*) infected Arapahoe wheat (15 μ l); Rp, *R. padi* aphid (5 μ l); and Dn, *D. noxia* aphid (5 μ l).

Phenacoccus manihoti Matile-Ferreto (Calatayud et al. 1996). Because *P. manihoti* and *D. noxia* have similar habitat colonization strategies (i.e., prefer to colonize and live under covered areas or rolled leaves), the presence of pectinesterase enzymes and absence of polygalacturonase enzymes could be one of the evolutionary strategies of the homopterans occupying cryptic habitats.

Oxido-Reductase Activities from Whole Aphids.

Among the 5 enzymes examined in the group of oxido-reductases, *D. noxia* showed catalase activity (Fig. 2; Table 2) and *R. padi* showed peroxidase activity (Fig. 3; Table 2). All 5 oxido-reductase enzyme activities were detected from the wheat plants, except that catechol oxidase activity was absent from the aphid-resistant Halt wheat leaves.

Peroxidase and Catalase Activities from Aphid Salivary Glands. The results of salivary gland enzyme assays supported the results of whole-aphid assays in that peroxidase activity (Fig. 4) was detected in the homogenates of *R. padi*, but not those of *D. noxia*. Additionally, catalase activity was detected from *D.*

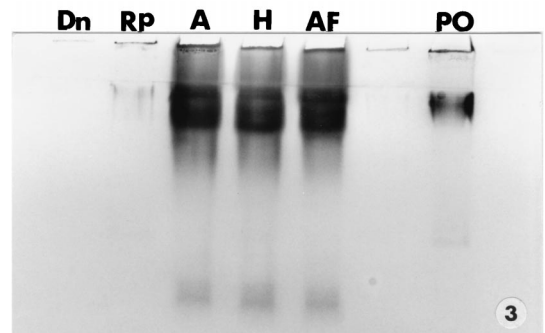


Fig. 3. Positively stained polyacrylamide gel (10%) showing the presence of peroxidase. Peroxidase activity shown by the dark bands. PO, peroxidase standard (5 μ l of 1.05 μ g protein per μ l); Dn, *D. noxia* aphid (15 μ l); Rp, *R. padi* aphid (15 μ l); A, Arapahoe wheat (15 μ l); H, Halt wheat (15 μ l); and AF, fungus (i.e., *E. graminis tritici*) infected Arapahoe wheat (15 μ l).

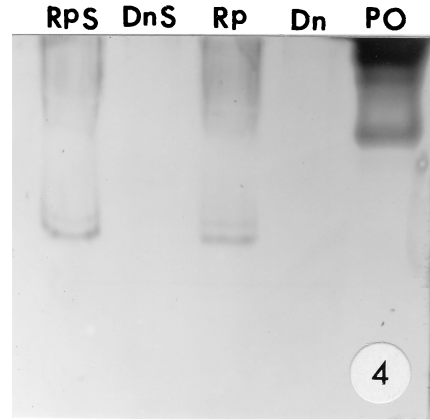


Fig. 4. Positively stained native polyacrylamide gel (10%) showing the presence of peroxidase in aphid head and whole aphid homogenates. Peroxidase activity shown by the dark bands. RPS, *R. padi* aphid head sample including salivary glands (30 μ l); DnS, *D. noxia* aphid head sample including salivary glands (30 μ l); Rp, whole aphid sample of *R. padi* (30 μ l); Dn, whole aphid sample of *D. noxia* (30 μ l); PO, peroxidase standard (10 μ l of 1.05 μ g protein per μ l).

noxia (shown by an arrow in Fig. 5), but not from *R. padi*.

Because we found similar hydrolases and different oxido-reductases from the enzyme activity assays, we postulate that the difference in the salivary enzyme activities of *D. noxia* and *R. padi* cause variation in damage elicitation in susceptible wheat plants. Although the biological function of peroxidase (found in *R. padi*) and catalase (found in *D. noxia*) is the same, that is, to remove hydrogen peroxide in plants, peroxidase needs a donor to reduce hydrogen peroxide, but catalase does not (Table 1). *D. noxia* feeding damage (i.e., leaf rolling and systemic chlorotic streaks) on wheat could be caused by the interruption of normal wheat plant redox balance when aphid salivary en-

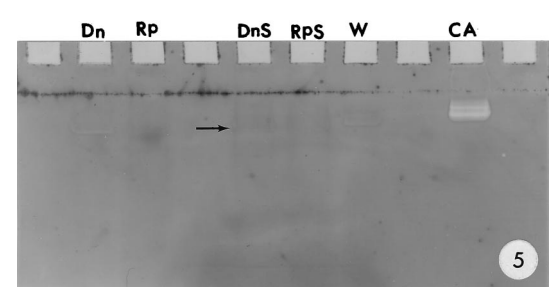


Fig. 5. Negatively stained polyacrylamide gel (12%) showing the presence of catalase activity. Catalase activity shown by a clear band on a dark background. Dn, whole aphid sample of *D. noxia* (30 μ l); Rp, whole aphid sample of *R. padi* (30 μ l); DnS, *D. noxia* aphid head sample including salivary glands (30 μ l); RPS, *R. padi* aphid head sample including salivary glands (30 μ l); W, aphid fodder plants 'Stephens' wheat plants (30 μ l); CA, catalase standard (5 μ l of 1.18 μ g protein per μ l).

zymes are injected into leaves during feeding. In particular, the presence of catalase and absence of peroxidase in *D. noxia* salivary glands could be an important factor contributing to the severe *D. noxia* damage elicitation on cereals.

Catalase is a heme-protein (Table 1) with a chelate or tetrapyrrole structure. Plant cytochromes and chlorophyll *a* and *b* also have a similar chelate structure (Raven et al. 1986, Mengel and Kirby 1987). Catalase and cytochromes have a similar structure of an iron-containing chelate ring attached to protein, whereas chlorophylls *a* and *b* attach the chelate rings on a long, insoluble carbon-hydrogen chain (Raven et al. 1986). The chlorophylls have a magnesium within the chelate ring rather than an iron (Mengel and Kirby 1987). Also, catalase removes hydrogen peroxide in plant tissue and converts it to water and oxygen. Catalase and superoxide dismutase play a key role in photorepiration and glycolytic pathways of plants (Marchner 1986). In plant chloroplasts, the chlorophylls and protein complex can convert light energy to chemical energy during photosynthesis. Cytochromes also are important in electron transfer in plant photosynthesis and respiration processes (Raven et al. 1986). Therefore, the catalase from aphid salivary glands could affect normal plant metabolism by affecting the availability of chelate for chlorophyll synthesis and interruption of normal redox balance in plants by affecting electron transfer during plant photosynthesis and respiration.

Photosynthetic rates of isolated wheat chloroplasts were altered when exposed to *D. noxia* extract (Kruger and Hewitt 1984); however, no mechanism was suggested. Burd and Elliott 1996 reported that chlorophylls *a* and *b* and total chlorophyll content was significantly reduced after *D. noxia* fed on susceptible 'Pavon' and 'TAMW-10' wheat. However, chlorophyll content was not significantly reduced in resistant PI 366616 and PI 372129 wheat. Aphid infestation significantly altered the chlorophyll fluorescence induction kinetics and reduced the photochemical efficiency of photosystem II (Burd and Elliott 1996). Thus, the systemic chlorosis of the wheat leaves caused by *D. noxia* feeding could result from interruption of plant redox balance that influences electron transfer. This would result in damage to the chloroplast membrane cause either reduction in chlorophyll synthesis or increase chlorophyll degradation.

Comparison of enzyme activities from symptom-eliciting and nonsymptom-eliciting aphids suggest that the presence of catalase in *D. noxia* salivary secretion is an important contributing factor to leaf chlorosis in susceptible wheat plants. The difference observed in pectinesterase activity between the 2 aphids also could contribute to differences observed in damage symptom formation on wheat. Conversely, previous researchers (Campbell and Dryer 1985, 1990; Ma et al. 1990) reported pectinases and cellulases were the enzymes responsible for *S. graminum* damage symptom formation on wheat.

Acknowledgments

We thank L. L. Silberman for providing the stock colony of *R. padi*, A. E. Seltzer for the assistance on plant and aphid colony maintenance, R. D. Peterson and D. B. Taylor for their advice and helpful discussions on electrophoresis (Department of Entomology, University of Nebraska), and K. W. Lee (Department of Biology, University of Nebraska) for his assistance with photographic work. We thank L. G. Higley and Z. B. Mayo (Department of Entomology, University of Nebraska), 2 anonymous reviewers, and editor for their constructive comments and suggestions on early drafts of the manuscript. This work was supported by Grant No. NEB-17-066 of the Agricultural Research Division, Institute of Agriculture and Natural Resources, University of Nebraska—Lincoln. This is Journal Series No. 12,037 of Agricultural Research Division, Institute of Agriculture and Natural Resources, University of Nebraska—Lincoln.

References Cited

- Beauchamp, C., and I. Fridovich. 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Anal. Biochem.* 44: 276–287.
- Béguin, P. 1983. Detection of cellulase activity in polyacrylamide gels using Congo red-stained agar replicas. *Anal. Biochem.* 131: 333–336.
- Burd, J. D., and N. C. Elliott. 1996. Changes in chlorophyll a fluorescence induction kinetics in cereals infested with Russian wheat aphid (Homoptera: Aphididae). *J. Econ. Entomol.* 89: 1332–1337.
- Burd, J. D., R. L. Burton, and J. A. Webster. 1993. Evaluation of Russian wheat aphid (Homoptera: Aphididae) damage on resistant and susceptible hosts with comparisons of damage ratings to quantitative plant measurements. *J. Econ. Entomol.* 86: 974–980.
- Burd, J. D., R. A. Butts, N. C. Elliott, and K. A. Shufran. 1998. Seasonal development, overwintering biology, and host plant interactions of Russian wheat aphid (Homoptera: Aphididae) in North America, pp. 65–99. *In* S. S. Quisenberry and F. B. Peairs [eds.], *A response model for an introduced pest—the Russian wheat aphid*. Thomas Say Publications. Entomological Society of America, Lanham, MD.
- Calatayud, P. A., B. Boher, M. Nicole, and J. P. Geiger. 1996. Interactions between cassava mealybug and cassava: cytochemical aspects of plant cell wall modifications. *Entomol. Exp. Appl.* 80: 242–245.
- Campbell, B. C. 1986. Host-plant oligosaccharins in the honeydew of *Schizaphis graminum* (Rondani) (Insecta: Aphididae). *Experientia* 42: 451–452.
- Campbell, B. C., and D. L. Dreyer. 1985. Host plant resistance of sorghum: differential hydrolysis of sorghum pectic substances by polysaccharases of greenbug biotypes, *Schizaphis graminum* (Homoptera: Aphididae). *Arch. Biochem. Physiol. Insects* 2: 203–215.
- Campbell, B. C., and D. L. Dreyer. 1990. The role of plant matrix polysaccharides in aphid-plant interactions, pp. 149–170. *In* R. K. Campbell and R. D. Eikenbary [eds.], *Aphid-plant genotype interactions*. Elsevier, Amsterdam.
- Cruickshank, R. H., and G. C. Wade. 1980. Detection of pectic enzymes in pectin-acrylamide gels. *Anal. Biochem.* 107: 177–181.
- Fouche, A., R. L. Verhoeven, P. H. Hewitt, M. C. Walters, C. F. Kriel, and J. DeJager. 1984. Russian wheat aphid, *Diuraphis noxia*, feeding damage on wheat, related cereals and *Bromus* grass species, pp. 22–23. *In* M. C. Walters [ed.], *Progress in Russian wheat aphid (Diuraphis noxia)*

- Mordv.) research in the Republic of South Africa. Technical Communication 191, Department of Agriculture, Republic of South Africa.
- Gregory, E. M., and I. Fridovich. 1974. Visualization of catalase on acrylamide gels. *Anal. Biochem.* 58: 57-62.
- Hein, G. L. 1992. Influence of plant growth stage on Russian wheat aphid, *Diuraphis noxia* (Homoptera: Aphididae), reproduction and damage symptom expression. *J. Kans. Entomol. Soc.* 65: 369-376.
- International Union of Biochemistry. 1978. Enzyme nomenclature 1978: recommendations of the nomenclature. Academic, New York.
- Jiang, Y., and P. W. Miles. 1993. Responses of a compatible lucerne variety to attack by spotted alfalfa aphid: changes in the redox balance in affected tissues. *Entomol. Exp. Appl.* 67: 263-274.
- Kruger, G.H.J., and P. H. Hewitt. 1984. The effect of Russian wheat aphid (*Diuraphis noxia*) extract on photosynthesis of isolated chloroplasts: preliminary studies, pp. 34-37. In M. C. Walters [ed.], *Progress in Russian wheat aphid (Diuraphis noxia Mordv.) research in the Republic of South Africa*. Technical Communication 191. Department of Agriculture, Johannesburg, Republic of South Africa.
- Lacks, S. A., and S. S. Springhorn. 1980. Renaturation of enzymes after polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. *J. Biol. Chem.* 255: 7467-7473.
- Laurema, S., A.-L. Varis, and H. Miettinen. 1985. Studies on enzymes in the salivary glands of *Lygus rugulipennis* (Hemiptera: Miridae). *Insect Biochem.* 15: 211-224.
- Ma, R., J. C. Reese, W. C. Black IV, and P. Bramel-Cox. 1990. Detection of pectinesterase and polygalacturonase from salivary secretions of living greenbugs, *Schizaphis graminum* (Homoptera: Aphididae). *J. Insect Physiol.* 36: 507-512.
- Maccarrone, M., A. Rossi, and G. A'andrea. 1990. Electrophoretic detection of ascorbate oxidase activity by photoreduction of nitroblue tetrazolium. *Anal. Biochem.* 188: 101-104.
- Madhusudhan, V. V., G. S. Taylor, and P. W. Miles. 1994. The detection of salivary enzymes of phytophagous Hemiptera: a compilation of methods. *Ann. Appl. Biol.* 124: 405-412.
- Marschner, H. 1986. Functions of mineral nutrients: micronutrients, pp. 269-340. In H. Marschner [ed.], *Mineral nutrition of higher plants*. Academic, London.
- Mengel, K., and E. A. Kirby. 1987. Plant nutrients, pp. 11-24. In K. Mengel and E. A. Kirby [eds.], *Principles of plant nutrition*. International Potash Institute, Switzerland.
- Miles, P. W. 1987. Feeding processes of Aphidoidea in relation to effects on their food plants, pp. 321-339. In A. K. Minks and P. Harrewijn [eds.], *Aphids, their biology, natural enemies and control*, vol. A. Elsevier, Amsterdam.
- Miles, P. W. 1990. Aphid salivary secretion and their involvement in plant toxicoses, pp. 131-147. In R. K. Campbell and R. D. Eikenbary [eds.], *Aphid-plant genotype interactions*. Elsevier, Amsterdam.
- Miles, P. W., and J. J. Oertli. 1993. The significance of antioxidants in the aphid plant interaction: the redox hypothesis. *Entomol. Exp. Appl.* 67: 275-283.
- Murphy, R. W., J. W. Sites, jr., D. G. Buth, and C. H. Haufler. 1996. Proteins: isozyme electrophoresis, pp. 51-120. In D. M. Hillis, C. Moritz, and B. K. Mable [eds.], *Molecular systematics*, 2nd ed. Sinauer, Sunderland, MA.
- Peng, Z., and P. W. Miles. 1991. Oxidases in the gut of an aphid, *Macrosiphum rosae* (L.) and their relation to dietary phenolics. *J. Insect Physiol.* 37: 779-787.
- Quick, J. S., G. E. Ellis, R. M. Normann, J. A. Stromberger, J. F. Shanahan, F. B. Peairs, J. B. Rudolph, and F. Lorenz. 1996. Registration of 'Halt' wheat. *Crop Sci.* 36: 210.
- Quisenberry, S. S., and D. J. Schotzko. 1994. Russian wheat aphid (Homoptera: Aphididae) population development and plant damage on resistant and susceptible wheat. *J. Econ. Entomol.* 87: 1761-1768.
- Raven, P. H., R. F. Evert, and S. E. Eichhorn. 1986. *Biology of plants*, 4th ed. Worth, New York.
- Ryan, J. D., A. T. Morgham, P. E. Richardson, R. C. Johnson, A. J. Mort, and R. D. Eikenbary. 1990. Greenbug and wheat: a model system for the study of phytotoxic Homoptera, pp. 171-186. In R. K. Campbell and R. D. Eikenbary [eds.], *Aphid-plant genotype interactions*, Elsevier, Amsterdam.
- Shen, Z., J. C. Reese, and G. R. Reeck. 1996. Purification and characterization of polygalacturonase from the rice weevil, *Sitophilus oryzae* (Coleoptera: Curculionidae). *Insect Biochem. Mol. Biol.* 26: 427-433.
- Vallejos, C. E. 1983. Enzyme activity staining, pp. 469-516. In S. D. Tanksley and T. J. Orton [eds.], *Isozymes in plant genetics and breeding*, part A. Elsevier, Amsterdam.
- Zadoks, J. C., T. T. Chang, and C. F. Konzak. 1974. A decimal code for the growth stages of cereals. *Weed Res.* 14: 415-421.

Received for publication 22 June 1999; accepted 8 August 1999.