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Insecticidal Action of PF2 Lectin from *Olneya tesota* (Palo Fierro) against *Zabrotes subfasciatus* Larvae and Midgut Glycoconjugate Binding

IRLANDA LAGARDA-DIAZ,[†] ANA M. GUZMAN-PARTIDA,[†] GABRIEL URBANO-HERNANDEZ,[†] M. MAGDALENA ORTEGA-NIEBLAS,[‡] M. REFUGIO ROBLES-BURGUEÑO,[†] JOY WINZERLING,[§] AND LUZ VAZQUEZ-MORENO*,[†]

Centro de Investigacion en Alimentacion y Desarrollo, A.C. Apartado Postal 1735, Hermosillo, Mexico, Departamento de Investigaciones Cientificas y Tecnologicas de la Universidad de Sonora, Apartado Postal 1819, Hermosillo, Mexico, and Department of Nutritional Sciences, University of Arizona, Tucson, Arizona 85721

Zabrotes subfasciatus (Boheman) is the main pest of common beans (*Phaselous vulgaris*). Some wild legume seeds may contain lectins with insecticidal activities against this insect. The larval developments of *Z. subfasciatus* on seeds of *Olneya tesota* (a desert wild legume) and on artificial seeds containing purified PF2 lectin were evaluated. PF2 susceptibility to proteolysis was assessed by incubation with midgut extract at different times. PF2 binding to midgut glycoconjugates was assessed by histochemistry. A reduced level of oviposition and a lack of emergence of adult beetles were observed in *O. tesota* seeds (compared to common beans), and in artificial seeds containing PF2 at 0.5 and 1%. PF2 was resistant to larval midgut proteolysis for 24 h, while PHA-E (lectin control) was fully digested after 4 h. Histochemistry analysis of midguts incubated with PF2 showed recognition for microvillae and possibly with peritrophic gel. On the other hand, PHA-E exhibited no interaction with larval midgut glycoproteins. Proteolysis resistance and glycan recognition could in part explain why PF2 is toxic to *Z. subfasciatus* while PHA is not.

KEYWORDS: Insecticidal lectins; wild legume; *Zabrotes subfasciatus*; insect midgut

1. INTRODUCTION

Common bean (*Phaseolus vulgaris*) is produced and consumed in more than three-quarters of developing countries where it constitutes a major source of dietary protein (*1, 2*). However, a substantial amount of the stored bean is lost due to insect damage. In Mexico, the bruchid *Zabrotes subfasciatus* Boheman (Mexican bean weevil) is the main postharvest pest of the common bean (*3, 4*). When left untreated, *Z. subfasciatus* grows exponentially and can completely destroy the stored beans (*5*). Weevil populations are significantly reduced in the presence of chemical pesticides (*6*); however, consumers are concerned with the residual chemical toxicity. The use of natural insecticides may be an alternative to avoid this residual toxicity (*7, 8*). Plant-derived products offer several potential benefits, safety to producers and consumers and the fact they are environmentally friendly. Some defense plant proteins are highly selective for pests which reduce the impact on nontarget species, including mammals. Utilization of a pest-resistant plant could be an effective way to assist farmers in reducing losses due to pest (*8*).

Lectins are ubiquitous proteins or glycoproteins that reversibly bind to specific mono- or oligosaccharides (*9*). Lectins have been extensively studied in animals and plants. Among the various roles attributed to plant lectins is defense against pathogens and phytophagous insects (*10*). Lectins from *Triticum vulgaris* agglutinin (WGA) and *Galanthus nivalis* agglutinin (GNA) are toxic to Lepidopteran, Homopteran, and Coleopteran insects, while *Canavalia ensiformis* lectin (Con A) shows an insecticidal effect against *Lacanobia oleracea* in tomato and *Nilaparvata lugens* in rice (*10*−*12*). *Arachis hypogeae* agglutinin (PNA) shows an inhibitory effect on development of *Callosobruchus maculatus* larvae (*13, 14*). The insecticidal activity of plant lectins is associated with their sugar binding properties; the gut epithelial cells of insects are lined with glycoproteins that are important in maintaining the normal function of the gut and also in providing a number of choices for lectin binding (*15*−*17*). However, knowledge about the mechanism of action of plant lectins is still limited. The activity has been associated with the lectin midgut epithelium binding which can cause damage to epithelial cells, disruption of nutrient

* To whom correspondence should be addressed. Telephone or fax: +52-662-2800381. E-mail: lvazquez@ciad.mx.
[†] Centro de Investigacion en Alimentacion y Desarrollo.
[‡] Departamento de Investigaciones Cientificas y Tecnologicas de la Universidad de Sonora.
[§] University of Arizona.

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Table 1. Number of Eggs and Emergence of *Z. subfasciatus* in Natural and Artificial Seeds Using the No-Choice Conditions Bioassay[a]

seeds	no. of eggs[b]	oviposition (%)	emergence (%)
natural			
O. tesota	4.0 ± 1	6	0
P. vulgaris	63.0 ± 0	100	81
artificial			
0.5% PF2 lectin	6.5 ± 0.5	34	0
1.0% PF2 lectin	4.0 ± 0	21	0
without PF2 lectin	19.0 ± 0	100	95

[a] All experiments were conducted in quadruplicate. [b] The standard deviation is given.

assimilation (*18*−*20*), and absorption of potentially harmful substances (*21*). Lectins have the potential of being used as naturally occurring insecticide agents against pests that restrain crop yield (*22*).

Olneya tesota PF2 lectin has been purified by affinity chromatography, using an agarose−fetuin column, from palo fierro seeds, a desert legume tree. PF2 has four subunits of 33 kDa that recognize only complex carbohydrates such as those present in fetuin and is not inhibited by simple sugars (D-galactose, D-mannose, L-fucose, D-arabinose, *N*-acetylglucosamine, *N*-acetylgalactosamine, sucrose, maltose, and lactose). Comparison of PF2 and PHA-L amino-terminal sequences showed a high degree of homology (*23*). Preliminary experiments showed that *O. tesota* seeds and seed flour were toxic to *Z. subfasciatus*. In this study, the larval development of *Z. subsfaciatus* on seeds of *O. tesota* and artificial seeds, containing purified PF2 lectin, was evaluated. In addition, lectin susceptibility to proteolysis by insect midgut enzymes and PF2 binding to glycoconjugates were assessed, showing that this lectin can potentially be used as an environmentally insecticidal agent for the postharvest preservation of common bean.

2. MATERIALS AND METHODS

2.1. Materials. Agarose activated with divinyl sulfone (Mini-Leak) was from Kem-En-Tec (Hellerup, Denmark). Broad range SDS−PAGE molecular mass standards were from Bio-Rad (Hercules, CA). Biotinylated anti-Ig was from BioGenex (San Ramon, CA). Polyclonal anti-PF2 antiserum was obtained by immunization of a New Zealand white rabbit in our laboratory according to the method of Jurd and Bog-Hansen (*24*). The rabbit received a course of two subcutaneous injections of 100 and 200 μg subsequently. Bovine fetuin, PHA-E, streptavidin−peroxidase complex, and all reagents were purchased from Sigma Chemical Co. (St. Louis, MO).

2.2. Insect Culture. Colonies of *Z. subfasciatus* were reared for several generations on *P. vulgaris* cv. Peruano and kindly donated by the Entomology Laboratory of Universidad of Sonora. Insects were handled under controlled conditions (27 °C, 65−75% relative humidity, and light for 12 h daily) according to the method of Rodriguez-Quiroz et al. (*25*).

2.3. Plant Material. Seeds of *O. tesota* were collected from mature trees located in the Sonora Desert close to Hermosillo, Mexico. Mature pods containing two to six dry seeds were collected and transported to the laboratory. Seeds were removed from pods and stored at 4 °C in paper bags.

2.4. PF2 Lectin Purification from *O. tesota* Seeds. PF2 lectin was purified according to the method of Vazquez-Moreno et al. (*23*). *O. tesota* seeds were grounded and meals defatted by hexane extraction. Hexane was removed by aeration under a chemical hood. The flour was suspended in a 0.9% NaCl solution (1:10, p/v) containing 0.02% sodium azide and 0.2 mM phenylmethanesulfonyl fluoride, stirred for 2 h at 4 °C, and then centrifuged at 800*g* for 15 min. The extract was clarified by glass fiber filtration and kept at 4 °C until use. Fetuin was coupled to activated agarose (Mini-Leak) following the procedure developed by Mini-Leak Kem-En-Tec and as reported by Vazquez-Moreno et al. (*25*). Crude extract (15 mL) was injected into an agarose−fetuin chromatography column (10 mm × 100 mm), previously equilibrated with PBS [0.02 M KH_2PO_4/K_2HPO_4, 0.9% NaCl, and 0.02% sodium azide (pH 7.2)]. Unbound protein was washed off with 10 column volumes of equilibrium buffer, whereas PF2 lectin eluted with 2 column volumes of 0.05 M glycine-HCl buffer (pH 2.5). Lectin-containing fractions were pooled, dialyzed against water at 4 °C, freeze-dried, and stored at −20 °C until use.

2.5. Preparation of Artificial Seeds. Artificial seeds (500 mg each) were made from finely ground Peruano bean flour, previously treated at 120 °C for 15 min, and mixed with PF2 at concentrations of 0.5 and 1% (w/w). A mold 8 mm in diameter was used, and newly made seeds were air-dried and coated with a 1% grenetine solution. Negative control seeds had no PF2 added.

2.6. Insect Bioassay. Ten pairs (male and female) of *Z. subfasciatus* newly emerged adults were used to infest 15 natural or artificial seeds under no-choice conditions (*3*). Females were allowed to lay eggs for 24 h, and then all insects were removed. The % oviposition [(number of eggs in seeds containing PF2 lectin)/(number of eggs in control) × 100] was counted after 14 days and emergence after 35 days. Experiments were designed with four replications.

2.7. Stability of PF2 Lectin to *Z. subfasciatus* Midgut Proteolysis. Larvae gut homogenates were prepared as described by Macedo et al. (*7*). Thirty midguts of 25-day-old larvae, selected using the method of Rodríguez-Quiroz et al. (*26*), were cold-immobilized and dissected in a cold 0.25 M NaCl solution. Larval midguts were surgically separated using tweezers. Gut portions taken were posterior to proventriculus and anterior to the Malpighian tubule segments. Only actively feeding larvae with food filling the gut tract were chosen. Dissected midguts were extracted in 1 mL of 0.1 M acetate buffer (pH 5.6) with 1 mM cysteine and 3 mM EDTA and subsequently homogenized in this solution. PF2 lectin (1 mg/mL) was incubated with midgut extracts (1:1 lectin:midgut protein ratio) for 0, 2, 4, 6, 12, and 24 h at 37 °C. Proteolysis stopped when the reaction mixture tubes were immersed in boiling water for 5 min. PHA-E lectin was used as comparative control. Proteolysis controls included incubation of midgut extracts, without lectin, for the experiment time.

2.8. Polyacrylamide Gel Electrophoresis and Immunoblotting Assay. Midgut extracts containing PF2 and PHA-E lectins were analyzed by sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE), using an 8 to 22% linear gradient gel (*28*). Samples containing 0.9 μg of protein were loaded per slot. Gels were silver stained (*29*).

An immunoblot assay was performed as described by Towbin et al. (*30*). Proteins separated by SDS−PAGE were transferred onto a nitrocellulose membrane at a rate of 2.5 mA/cm^2 for 40 min, using a semidry blotter (Labconco). Membranes were blocked for 1.5 h with PBS containing 2% bovine serum albumin (BSA). Undigested PF2 and PHA-E lectins were independently detected with polyclonal anti-PF2 antiserum and anti-PHA antiserum, respectively (1:4000, incubation for 2 h), followed by treatment with biotinylated anti-rabbit Ig (BioGenex) for 2 h and streptavidin−peroxidase complex for 1.5 h. The color reaction was developed at room temperature by addition of peroxidase substrate, 0.075% 3,3′-diaminobenzidine·4HCl (DAB).

2.9. Lectin Histochemistry. Larval midguts of the insect were prepared as described by Macedo et al. (*7*). Instar (16, 20, and 25 days old) larvae midguts were collected in 70% ethanol and embedded in paraffin wax (*31*). Tissue sections of 2 μm were used to assay PF2 recognition. PHA-E was used as a control. Both lectins were biotinylated using the protocol proposed by Hofmann et al. (*32*).

Tissues dehydrated overnight at 37 °C were dewaxed in xylene and hydrated through a graded sequence of alcohol solutions (from 100 to 50%) and finally in bidistilled water. The treatment of heat-induced epitope retrieval (HIER) was applied to rehydrated tissues in 10 mM sodium citrate (pH 7.2) using a microwave for 5 min (*33*). Endogenous peroxidase activity was blocked with 3% H_2O_2 for 5 min. To block unspecific binding sites, tissues were covered with 1% BSA in 0.01 M PBS for 1 h. Slides were then incubated at room temperature with biotinylated PF2 or PHA-E (20 μg/mL) for 45 min, and binding was revealed by treatment with streptavidin−peroxidase complex over 30 min; then samples were immersed in DAB (0.2 mg/mL) with H_2O_2

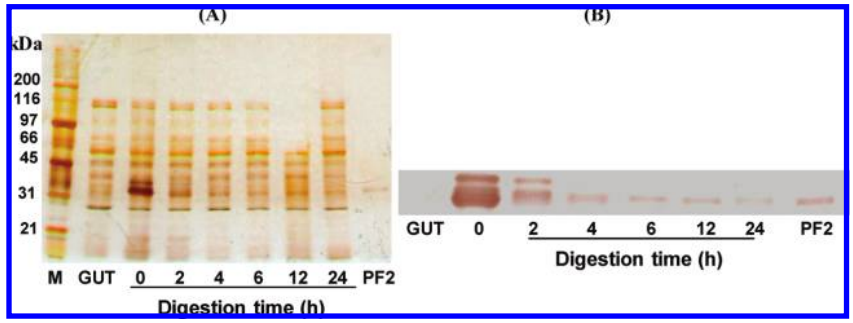

Figure 1. SDS−PAGE patterns (A) and Western blot analysis (B) of PF2 lectin digested by a midgut extract of *Z. subfasciatus*. (A) PF2 lectin was incubated with larvae midgut extract (25 days old) for 0, 2, 4, 6, 12, and 24 h at 37 °C. Purified PF2 lectin was used as a control. Proteolysis was stopped by immersing the reaction mixture tube in boiling water for 5 min. Gels were loaded with 0.9 μg of protein per slot and silver stained. (B) SDS−PAGE-separated proteins were transferred onto a nitrocellulose membrane and detected with a polyclonal anti-PF2 antiserum followed by treatment with biotinylated anti-rabbit Ig, streptavidin−peroxidase complex, and DAB. Lane M contained molecular mass markers, and lane GUT contained midgut extract.

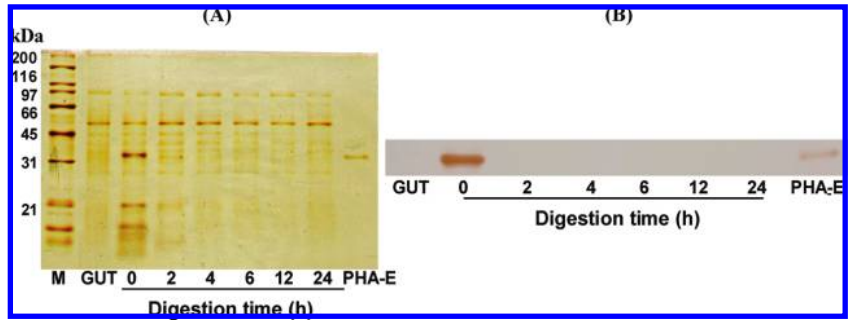

Figure 2. SDS−PAGE patterns (A) and Western blot analysis (B) of PHA-E lectin digested by a midgut extract of *Z. subfasciatus*. (A) PHA-E lectin was incubated with midgut extract from 25-day-old larvae for 0, 2, 4, 6, 12, and 24 h at 37 °C. Purified PF2 lectin was used as a control. Proteolysis was stopped by immersing the reaction mixture tube in boiling water, and 0.9 μg of protein was loaded per slot in the gel and silver stained. (B) SDS−PAGE-separated proteins were transferred onto a nitrocellulose membrane and detected with polyclonal anti-PHA antiserum followed by treatment with biotinylated anti-rabbit Ig, streptavidin−peroxidase complex, and DAB. Lane M contained molecular mass markers, and lane GUT contained midgut extract.

(0.005%) for 10 s at room temperature. Finally, tissues were rinsed in water, counterstained with Mayer's hematoxylin, dehydrated, cleared, and mounted (*31*). The intensity of lectin binding (anti-PF2) was subjectively scaled into four categories [(−) negative, (+) weak, (++) moderate, and (+++) strong], as reported by Aoki et al. (*34*).

2.10. Protein Determination. Protein concentrations were estimated as described by Lowry et al. (*35*) using BSA as a standard.

3. RESULTS AND DISCUSSION

3.1. *Z. subfasciatus* Bioassay in *O. tesota* Seeds and Artificial Seeds. *Z. subfasciatus* (Boheman) is a devastating storage bean pest, where damage is revealed as circular holes or "windows" left after insect emergence (*27*). In the search for molecules that confer plant resistance against insects, some interesting candidates have been found in the family of lectins and lectin-like proteins (*36*).

Natural seeds of *O. tesota* and *P. vulgaris* contain ~1% PF2 and PHA-E lectin, respectively (*23*). Both seeds were used to test for oviposition and emergence of *Z. subfasciatus* (**Table 1**). *O. tesota* seeds presented a reduced number of eggs (4 ± 1), while *P. vulgaris* showed a total of 63 ± 0. Interestingly, none of the eggs deposited on *O. tesota* emerged as an adult insect, whereas control bean seeds showed 81% emergence of adult insects. Other experiments using seeds without testa (data not shown) yielded consistent results, thus disputing the fact that the effect of a reduced level of oviposition and a lack of emergence was due to testa hardness and the presence of secondary metabolites like phenols. Furthermore, the α-amylase inhibitor for *P. vulgaris* has been implicated in the inhibition of *Z. subfasciatus* (*16*, *37*). However, the α-amylase inhibitor of *O. tesota* seeds (αAI-PF) has a poor inhibitory activity against the bruchid (*38*).

To determine the influence of PF2 lectin in *O. tesota* resistance to *Z. subfasciatus*, PF2 was purified and added at concentrations of 0.5 and 1% to artificial seeds made with bean flour. Again, seeds containing PF2 lectin (**Table 1**) showed a reduced percentage of oviposition (34%) and no emergence of adults in relation to control insects reared on seeds containing only *P. vulgaris* flour (95% emergence). The addition of PF2 lectin effectively prevented emergence of adult beetles. This strongly suggests that the *O. tesota* resistance to *Z. subfasciatus* in part is due to the presence of PF2 lectin. Notably, the levels of lectins (1 and 0.5%) are equal to and half of those naturally found in legume seeds, respectively. *Talisia esculenta* lectin (mannose/glucose-binding) produced 90% mortality to *Z. subfasciatus* when incorporated into an artificial diet at a level of 2% (w/w) and 50% mortality when incorporated at a level of 1% (*7*). Results with PF2 lectin incorporated at a level 0.5% into the diet of *Z. subfasciatus* were comparable to the concentrations at which *T. esculenta* and *Bauhinia monandra* lectins caused 50% mortality (*7*, *39*). The most insecticidal lectins recognize only simple sugars (*7*, *17*, *40*, *41*). This is advantageous since studies had proven that if a lectin interacts with simple sugars and those sugars are present in the plant, they can compete for the carbohydrate-binding site and reduce toxicity (*38*). Vazquez-Moreno et al. (*23*) and Urbano-Hernández (*39*) showed that PF2 lectin recognizes complex carbohy-

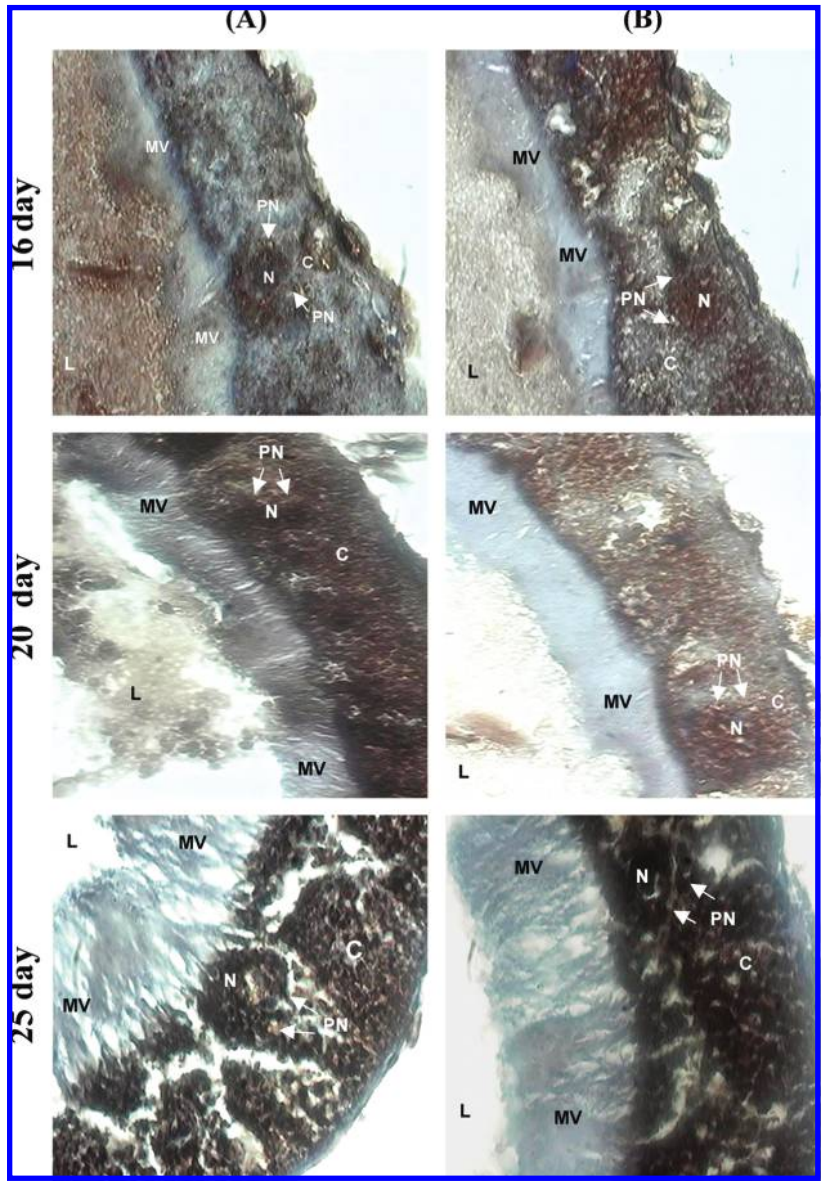

Figure 3. Light microscopy studies of *Z. subfasciatus* midgut from different larval stages treated with (A) PF2 and (B) PHA-E. Midgut tissues were embedded in parafilm, treated with sodium citrate buffer, and incubated with biotinylated (A) PF2 and (B) PHA-E. Interactions were revealed with streptavidin−peroxidase complex and DAB, after the samples had been counterstained with Mayer's hematoxylin: L, lumen; MV, microvillae; C, cytoplasm; N, nucleus; PN, perinuclear region. The magnification was 1000×.

drates (a tetrasialylated triantennary oligosaccharide) of fetuin. These studies were conducted using affinity chromatography and liquid chromatography−mass spectrometry.

3.2. Stability of PF2 Lectin to *Z. subfasciatus* Midgut Proteolysis. Despite the substantial structural information about plant lectins, little is known about their mode of insecticidal action. Nevertheless, a prerequisite for toxicity is that lectins should be able to survive the hostile proteolytic environment of the insect midgut. Thus, in vitro susceptibility of PF2 lectin was evaluated and PHA-E was used as a control. Results showed that PF2 lectin is resistant to protease digestion for up to 24 h as shown by a band at 32 kDa (**Figure 1A**). In contrast, PHA-E was fully digested after 4 h (**Figure 2A**). These data were confirmed by Western blot using antiserum raised against either lectin (**Figures 1B** and **2B**). Proteolysis of lectins appears to be insect gut-dependent; other lectins with insecticidal activity, such as *Griffonia simplicifolia II*, *Ulex europeus*, *T. esculenta*, and *B. monandra*, are also resistant to proteolysis by different insect digestive enzymes (*20, 7, 39*). The degree of resistance to proteolysis of plant lectins may be variable (*10, 43, 44*).

The production of resistant lectins to proteolysis represents an effective strategy developed by some plants against their depredators (*44*). The stability to proteolysis of most lectins could result from a stable tertiary structure over a wide pH and temperature range, or one able to withstand heat (*20*). In addition, some insects could not possess gut proteases capable of digesting plant lectins (*9, 20*). Alternatively, the lectin binding to glycoconjugates in the gut may somehow protect them from proteolytic activity, therefore stabilizing them. *G. simplicifolia II* mutant lectins lacking carbohydrate binding showed an absence of insecticidal activity and proteolysis sensitivity to digestive enzymes (*45*).

3.3. Carbohydrate Recognition by PF2 Lectin on *Z. subfasciatus* Larval Sections. Previous studies have substantiated that lectins and others sugar-binding proteins (chitinases, arcelins, and vicilins) that interact with insect midgut structures are a predominant factor for their insecticidal action (*15−18, 20, 40−42, 45−49*). Lectins are thought to exert a toxic effect via binding to the peritrophic membrane and brush-border microvilli of epithelial cells (*50, 51*). Histochemistry studies of

Table 2. Evaluation of Lectins Binding to *Z. subfasciatus* Midgut Glycoconjugates[a]

	16-day-old larvae		20-day-old larvae		25-day-old larvae	
localization	PF2	PHA-E	PF2	PHA-E	PF2	PHA-E
microvilli	−	−	+++	−	+	−
cytoplasm	+	+++	+++	++	+++	+++
perinuclear region	++	+++	+++	++	+++	+++

[a] The intensity of lectin binding (anti-PF2) was subjectively scaled into four categories: (−) negative, (+) weak, (++) moderate, and (+++) strong, as reported by Aoki et al. (*34*).

bruchid larvae midgut sections and glycoconjugate recognition by PF2 lectin were undertaken in three different instar larvae (corresponding to 16, 20, and 25 days old). PHA-E lectin was used as a control.

Insects such as *Z. subfasciatus* do not present a well-defined peritrophic membrane (*52*, *53*); instead, they have a peritrophic gel along the whole midgut that separates the midgut cells from the food (*54*). PF2 lectin recognized structures at the boundaries of the microvillae in the 16-day-old larvae (**Figure 3**), due to the presence of glycoconjugates in the peritrophic gel, as reported for other bruchid beetles using different sugar binding proteins (*47*, *52*−*54*). PHA-E exhibited a similar but less intense interaction. On the other hand, larvae that were 20 and 25 days old exhibited interaction of PF2 lectin with the microvillae, whereas PHA-E did not bind (**Table 2** and **Figure 3**). Both lectins recognized glycans of the epithelial cells (in the perinuclear region and cytoplasm) in all larval stages studied. The distinct intensity of interaction for both lectins at different stages of *Z. subfasciatus* larval development may indicate changes in the expression of carbohydrates related to larval development. In insects, like in other species, the composition of carbohydrates along the luminal surface may vary between different gut sections and age groups (*55*, *56*).

On the other hand, binding of a particular lectin to insect gut does not necessarily mean that it is toxic (*40*). PHA-E, like PF2, binds midgut glycan structures (in the cytoplasm and perinuclear region) of *Z. subfasciatus*; however, PHA-E is not toxic to this insect and, in fact, is the most common pest of *P. vulgaris* (*4*). The resistance to proteolysis of PF2 in the midgut could be a very important factor inr its toxicity. Zhu-Salman et al. (*20*) evaluated the binding of the recombinant GSII lectin to the gut of *C. maculatus* larvae and its digestion by catepsines from insects and suggested that the binding of GSII lectin to midgut carbohydrates and proteolytic resistance are independent. However, both properties facilitated the efficacy of GSII lectin-like as a plant defense molecule.

3.4. Conclusions. Proteolysis resistance of PF2 lectin to midgut enzymes and glycan recognition microvilli and the possibility of glycan of a peritrophic gel at early larval stages could explain PF2 toxicity to *Z. subfasciatus* and *P. vulgaris* susceptibility. PF2 is the first reported insecticidal lectin not inhibited by simple sugars and specific only to complex carbohydrates.

ABBREVIATIONS

GNA, *G. nivalis* agglitinin; Con A, *Ca. ensiformis*; PNA, *A. hypogeae* agglutinin; GSII, *G. simplicifolia II* lectin; PHA-E,*P. vulgaris*; DAB, 3,3′-diaminobenzidine•4HCl; αAI-PF, inhibitor of *O. tesota* seed.

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LITERATURE CITED

(1) Schmale, I.; Wäckers, F. L.; Cardona, C.; Dorn, S. Biological control of the bean weevil, *Acanthoscelides obtectus* (Say) (Col.: Bruchidae), by the native parasitoid *Dinarmus basalis* (Rondani) (Hym: Pteromalidae) on small-scale farms in Colombia. *J. Stored Prod. Res.* **2006**, *42*, 31–46.

(2) Cardona, C. Common Beans: Latin America. Crop Post-Harvest. *Sci. Technol.* **2007**, *2*, 145–150.

(3) Schoonhoven, A. V.; Cardona, C.; Valor, J. Resistance to the bean weevil and the Mexican bean weevil (Coleoptera: Bruchidae) in non-cultivated common bean accessions. *J. Econ. Entomol.* **1983**, *76*, 1255–1259.

(4) Lopez-Perez, E.; Rodriguez-Hernandez, C.; Ortega-Arenas, L. D.; Garza-Garcia, R. Biological Activity of *Senecio salignus* root against *Zabrotes subfasciatus* in stored bean. *Agrociencia* **2007**, *41*, 95–102.

(5) Hall, A. E.; Singh, B. B.; Ehlers, J. D. Cowpea breeding. *Plant Breed Rev.* **1997**, *15*, 217–274.

(6) Daoust, R. A.; Roberts, D. A.; Neves, B. P. Distribution, biology and control of cowpea pest in Latin America. In *Cowpea Research, Production and Utilization*; Singh, S. R., Rachie, K. O., Eds.; Wiley: London, 1985; pp 249−264.

(7) Macedo, M. L. R.; Freire, M. G. M.; Novello, J. C.; Marangoni, S. *Talisia esculenta* lectin and larval development of *Callosobruchus maculatus* and *Zabrotes subfasciatus* (Coleoptera: Bruchidae). *Biochim. Biophys. Acta* **2002**, *1571*, 83–88.

(8) Singh, B. B.; Singh, S. R.; Adjadi, O. Bruchid resistance in cowpea. *Crop Sci.* **1985**, *25*, 736–739.

(9) Peumans, W. J.; Van Damme, E. J. M. Lectins as Plants Defense Proteins. *Plant Physiol.* **1995**, *109*, 347–352.

(10) Carlini, C. R.; Grossi-de-Sa, M. F. Plant toxic proteins with insecticidal properties. A review on their potentialities as bioinsecticides. *Toxicon* **2002**, *40*, 1515–1539.

(11) Fitches, E.; Gatehouse, A. M. R.; Gatehouse, J. A. Effects of snowdrop lectin (GNA) delivered via artificial diet and transgenic plants on the development of the tomato moth (*Lacanobia oleracea*) larvae in laboratory and glasshouse trials. *J. Insect Physiol.* **1997**, *43*, 727–739.

(12) Powell, K. S. Antimetabolic effects of plant lectins towards nymphal stages of the planthoppers *Tarophagous proserpina* and *Nilaparvata lugens*. *Entomol. Exp. Appl.* **2001**, *99*, 1–77.

(13) Murdock, L. L.; Huesing, J. E.; Nielsen, S. S.; Pratt, R. C.; Shade, R. E. Biological effects of plant lectins on the cowpea weevil. *Phytochemistry* **1990**, *29*, 85–89.

(14) Gatehouse, A. M. R.; Howe, D. S.; Flemming, J. E.; Holder, V. A.; Gatehouse, J. A. Biochemical basis of insect resistance in winged bean (*Psophocarpus tetragonolobus*) seeds. *J. Sci. Food Agric.* **1991**, *55*, 63–74.

(15) Eisemann, C. H.; Donaldson, R. A.; Pearson, R. D.; Cadogan, L. C.; Vuocolo, T.; Tellam, R. L. Larvicidal activity of lectins on *Lucilia cuprina*: Mechanism of action. *Entomol. Exp. Appl.* **1994**, *72*, 1–10.

(16) Chrispeels, M. J.; Raikhel, N. V. Lectins, lectin genes, and their role in plant defense. *Plant Cell* **1991**, *3*, 1–9.

(17) Du, J.; Foissac, X.; Carss, A.; Gatehouse, A. M. R.; Gatehouse, J. A. Ferritin acts as the most abundant binding protein for snowdrop lectin in the midgut of rice brown planthoppers (*Nilaparvata lugens*). *Insect Biochem. Mol. Biol.* **2000**, *30*, 297–305.

(18) Czapla, T. H.; Lang, B. A. Effect of plant lectins on the larval development of European corn borer (Lepidoptera: Pyralidae) and southern corn rootworm (Coleoptera: Chrysomelidae). *J. Econ. Entomol.* **1990**, *83*, 2480–2485.

(19) Sharon, N.; Lis, H. History of lectins: From hemagglutinin to biological recognition molecules. *Glycobiology* **2004**, *14*, 53R–62R.

(20) Zhu-Salzman, K.; Shade, R. E.; Koiwa, H.; Salzman, R. A.; Narasimhan, M.; Bressan, R. A.; Hasegawa, P. M.; Murdock, L. L. Carbohydrate binding and resistance to proteolysis control insecticidal activity of *Griffonia simplicifolia* lectin II. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 5123–5128.

(21) Pusztai, A.; Clarke, E. M.; King, T. P. The nutritional toxicity of *Phaseolus vulgaris* lectins. *Proc. Nutr. Soc.* **1979**, *38*, 115–120.

(22) Macedo, M. L. R.; de Castro, M. M.; Freire, M. G. Mechanisms of the insecticidal action of TEL (*Talisia esculenta* lectin) against *Callosobruchus maculatus* (Coleoptera: Bruchidae). *Arch. Insect Biochem. Physiol*. **2004**, *56*, 84–96.
(23) Vazquez-Moreno, L.; Ortega-Nieblas, M.; Robles-Burgueño, M. R.; Ramos-Clamont, G. Purification of complex carbohydrate specific lectins from *Olneya tesota* seeds using tandem affinity chromatography. *IJBC* **2000**, *5*, 83–90.
(24) Jurd, R. D.; Bog-Hansen, T. C. In *Production of polyvalent antibodies for immunoelectroforesis. Gel electrophoresis of proteins. The practical approach series*; Rickwood, D., Harris, B. D., Eds.; IRL Press: Cary, NC, 1990; pp 366−376.
(25) Vazquez-Moreno, L.; Calderon de la Barca, A. M.; Robles-Burgeño, M. R. Comparasion of divinylsulphone-activated agarose with different matrix materials to purify lectins by affinity chromatography. In *Lectins: Biology, Biochemistry, Clinical Biochemistry*; Van Driessche, E., Franz, H., Beeckmans, S., Pfiiller, U., Allikorm, A., Bog-Hansen, T. C., Eds.; Textop: Hellerup, Denmark, 1993; pp 110−114.
(26) Rodriguez-Quiroz, M.; Valdez-Carrasco, J.; Vera-Graziano, J.; Castillo-Morales, A. Identificación de ínstares larvales de *Zabrotes subfasciatus* (Boh.)(coleoptera: bruchidae) mediante las dimensiones de sus cápsulas cefálicas. *Agrociencia* **2000**, *31*, 349–352.
(27) Schoonhoven, A. V.; Cardona, C.; Valor, J. Resistance to the bean weevil and the Mexican bean weevil (Coleoptera: Bruchidae) in non-cultivated common bean accessions. *J. Econ. Entomol*. **1983**, *76*, 1255–1259.
(28) Laemmli, U. K. Cleavage of structural proteins during the assembly of head of bacteriophage T4. *Nature* **1970**, *227*, 680–685.
(29) Wray, D.; Graykowski, E. A.; Notkins, A. L. Role of mucosal injury in initiating recurrent aphthous stomatitis. *Br. Med. J*. **1981**, *283*, 1569–1570.
(30) Towbin, H.; Staehelin, T.; Gordon, J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. *Proc. Natl. Acad. Sci. U.S.A*. **1979**, *76*, 4350–4354.
(31) Whiteland, J. L.; Nicholls, S. M.; Shimeld, D.; Easty, D. L.; Williams, N. A.; Hill, T. J. Immunohistochemical Detection of T-cell Subsets and Other Leukocytes in Paraffin-embedded Rat and Mouse Tissues with Monoclonal Antibodies. *J. Histochem. Cytochem*. **1995**, *43*, 313–320.
(32) Hofmann, K.; Titus, G.; Montibeller, J. A.; Finn, F. M. Avidin binding of carboxyl-substituted biotin and analogues. *Biochemistry* **1982**, *21*, 978–984.
(33) Schorge, J. O.; Lea, J. S.; Garner, E. O.; Duska, L. R.; Miller, D. S.; Coleman, R. L. Cervical adenocarcinoma survival among Hispanic and white women: A multicenter cohort study. *Am. J. Obstet. Gynecol*. **2003**, *188*, 640–664.
(34) Aoki, D. M. D.; Shiro, N. M. D.; Rihachi, I. M. D.; Hayato, K.; Hiroshi, H. M. D. Differences in lectin binding patterns of normal endomerium and endometrium adenocarcinoma, woth special reference to staining with *Ulex eupopeaus* Agglutinin 1 and peanut Agglutinin. *Gynecol. Oncol*. **1990**, *37*, 338–345.
(35) Lowry, O. H.; Rosebrough, N. J.; Farr, A.; Randall, R. J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem*. **1951**, *193*, 265–275.
(36) Sharma, H. C.; Sharma, K. K.; Crouch, J. H. Genetic transformation of drops for insect resistance: Potential and limitations. *Crit. Rev. Sci*. **2004**, *23*, 47–72.
(37) Payan, F. Structural basis for the inhibiton of mammalian and insect α-amylases by proteins inhibitors. *Biochim. Biophys. Acta* **2004**, *1696*, 171–180.
(38) Guzman-Partida, A. M.; Jatomea-Fino, O.; Robles-Burgueño, M. R.; Ortega-Nieblas, M.; Vazquez-Moreno, L. Characterization of α-amylase inhibitor from Palo Fierro seeds. *Plant Physiol. Biochem*. **2007**, *45*, 711–715.
(39) Urbano-Hernádez, G. Identificación de estructuras glicosídicas reconocidas por la lectina PF2 en fetuína y tejidos linfoides. Ph.D. Thesis, Centro de Investigación en Alimentación y Desarrollo A.C., Hermosillo, Sonora, Mexico, 2007.
(40) Macedo, M. L. R.; Freire, M. G.; Barbosa, M.; Breitenbach, L. C. Insecticidal action of *Bauhinia monandra* leaf lectin (BmoLL) against *Anagasta kuehniella* (Lepidoptera: Pyralidae), *Zabrotes subfasciatus and Callosobruchus maculates* (Coleoptera: Bruchidae). *Biochem. Physiol. A: Mol. Integr. Physiol*. **2007**, *146*, 486–498.
(41) Bandyopadhyay, S.; Roy, A.; Das, S. Binding of Garlic (*Allium sativum*) Leaf Lectin to the Gut Receptors of Homopteran Pests is Correlated to Its Insecticidal Activity. *Plant Sci*. **2001**, *161*, 1025–1033.
(42) Macedo, M. L. R.; de Castro, M. M.; Freire, M. G. Mechanisms of the insecticidal action of TEL (*Talisia esculenta* lectin) against *Callosobruchus maculatus* (Coleoptera: Bruchidae). *Arch. Insect Biochem. Physiol*. **2004**, *56*, 84–96.
(43) Murdock, L. L.; Shade, R. E. Lectins and protease inhibitors as plant defenses against insects. *J. Agric. Food Chem*. **2002**, *50*, 6605–6611.
(44) Brunelle, F.; Cloutier, C.; Michaud, M. Colorado potato beetles compensate for tomato cathepsin D inhibitor expressed in transgenic potato. *Arch. Insect Biochem. Physiol*. **2004**, *55*, 103–113.
(45) Zhu-Salzman, K.; Salzman, R. A. Functional mechanics of the plant defensive *Griffonia simplicifolia* lectin II: Resistance to proteolysis is independent of glycoconjugate binding in the insect gut. *J. Econ. Entomol*. **2001**, *94*, 1280–1284.
(46) Gatehouse, A. M. R.; Dewey, F. M.; Dove, J.; Fenton, K. A.; Pusztai, A. Effect of seed lectins from *Phaseolus vulgaris* on the development of the larvae of *Callosobruchus maculatus*: Mechanism of toxicity. *J. Agric. Food Chem*. **1984**, *35*, 373–380.
(47) Sales, M. P.; Sales, P. P.; Paes, N. S.; Grossi-de-Sá, M. F.; Xavier-Filho, J. Vicilins (7S storage globulins) of cowpea (*Vigna unguiculata*) seeds bind to chitinious structures of the midgut of *Callosobruchus maculatus* (Coleoptera: Bruchidae) larvae. *Braz. Med. Biol. Res*. **2001**, *34*, 27–34.
(48) Wang, P.; Granados, R. R. Molecular structure of the peritrophic membrane (PM): Identification of potential PM target sites for insect control. *Arch. Insect Biochem. Physiol*. **2001**, *47*, 110–118.
(49) Paes, N. S.; Gerhardt, I. R.; Coutinho, M. V.; Yokoyama, M.; Santana, E.; Harris, N.; Chrispeels, M. J.; Grossi-de-Sa′, M. F. The effect of arcelin-1 on the estructure of the midgut of the bruchid larvae and immunolocalization of the arcelin protein. *J. Insect Physiol*. **2000**, *46*, 393–402.
(50) Powell, K. S.; Spence, J.; Bharathi, M.; Gatehouse, J. A.; Gatehouse, A. M. R. Immunohistochemical and developmental studies to elucidate the mechanism of action of the snowdrop lectin on the rice brown planthopper, *Nilaparvata lugens*. *Entomol. Exp. Appl*. **1998**, *44*, 529–539.
(51) Habibi, J.; Backus, E. A.; Huesing, J. E. Effects of phytohemagglutinin (PHA) on the structure of midgut epithelial cells and localization of its binding sites in western tarnished plant bug *Lygus hesperus* Knight. *J. Insect Physiol*. **2000**, *46*, 611–619.
(52) Vats, L. K. Alimentary canal in bruchid larvae (bruchidae: Coleoptera). *Bull. Punjab Univ*. **1976**, *27*, 103–106.
(53) Terra, W. R. The Origin and Functions of the Insect Peritrophic Membrane and Peritrophic Gel. *Arch. Insect Biochem. Physiol*. **2001**, *47*, 47–61.
(54) Terra, W. R. Evolution of digestive system of insects. *Annu. Rev. Entomol*. **1990**, *35*, 181–200.
(55) Alcoy, J.; Goyal, V.; Lukacs, N. W.; Taylor, R. L.; Strout, R. G.; Ward, H. D.; Pereira, M. E. A. Glycoconjugates of the intestinal epithelium of domestic fowl (*Gallus domesticus*): A lectins histochemistry study. *Histochem. J*. **1989**, *21*, 187–193.
(56) Pohlmeyer, I.; Jorns, J.; Schumacher, U.; Van Damme, E. J. M.; Peumans, W. J.; Pfüller, U. Lectin histochemical investigations of the distal gut of chicks with special emphasis on the follicle-associated epithelium. *J. Vet. Med., Ser. A* **2005**, *52*, 138–146.

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