JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Efficiency of Mannose-Binding Plant Lectins in Controlling a Homopteran Insect, the Red Cotton Bug

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Yield losses of different crops due to the attack of various classes of insects are a worldwide problem. Sucking type homopteran pests causing damage to many crop species are not controlled by commonly known insecticidal proteins, namely, *Bacillus thuringiensis* δ-endotoxin (Bt). This study describes the purification of mannose-binding lectins from three different monocotyledonous plants (*Allium sativum*, *Colocasia esculenta*, and *Diffenbachia sequina*) and their effects on a homopteran insect, the red cotton bug. All of them had a detrimental effect on the growth and development of the insect, where *A. sativum* bulb lectin showed the highest mortality of all, in particular. The same bulb lectin not only affected the growth and fecundity of the insect but also imparted drastic changes in the color, weight, and size, even on the second generation of the insects which have been reared on artificial diet supplemented with a sublethal dose of the lectin. Thus, this finding opens up a possibility of using this lectin as an important component in crop management.

KEYWORDS: Mannose-binding plant lectins; homopterans; *Bacillus thuringiensis*; LC_{50}

INTRODUCTION

Cotton, the prime cash crop of India, has the greatest insect predation problem. In India, to protect the crop from insect attack, huge amounts of pesticides (54% of the total pesticide consumed in India) are applied to the cotton fields. In recent years, as a consequence of the extensive use of synthetic pesticides in controlling the most damaging lepidopteran pest, the cotton boll worm (*1*), other pests, namely, aphids, whiteflies, mealybugs, and mites, have emerged in a major way. Likewise, the red cotton bug (*Dysdercus koenigii/cingulatus*) has become an important pest. Apart from cotton, this homopteran group of insects also feed on okra, maize, pearl millet, etc. The adult bugs are 0.6−1 cm in length, slender, and colored crimson red with white bands across the abdomen. The membranous parts of forewings, antennae, and scutellum are black. The adults and nymphs both feed gregariously on the leaves and the green bolls. The attacked bolls do not open properly; as a result, the quality of the lint is reduced, the oil content of the seed decreases, and the germination of the seeds is affected. During ginning, the lint becomes stained with the crushed nymphs and deposited excreta, which affects the market value of the crop. Lint also is stained by the crushed bacterium *Nematospora gosypii*, transmitted into the boll by this bug. Unfortunately, the insect is not affected by an established biocontrol agent like Bt (*Bacillus thuringiensis* endotoxin). Due to the typical feeding habit of sucking away the free sugars and amino acids from the plant sap (*2*), it does not depend on the protease or amylase type of digestive enzymes. Therefore, controlling these insects by inhibitors of any of the above-mentioned enzymes is of no use.

Alternatively, several plant lectins have been reported previously to have detrimental effects on several pests (*3*−*11*). With these in mind, mannose-binding lectins, namely, ASAI and -II, CEA, and DEA, have been isolated and purified from bulbs of *Allium sativum*, tubers of *Colocasia esculenta*, and leaves of *Dieffenbachia sequina*, respectively. This study describes the purification of these lectins, monitoring of their insecticidal activity on the red cotton bug, and determination of the LC_{50} values of the toxins via an insect bioassay with different concentrations of the three above-mentioned lectin types. Analyses of the effects of these lectins on mortality rate at nymphal stages have been carried out. These lectins have a detrimental effect on the fecundity pattern and the rate of growth and development. Additionally, drastic phenotypic changes in the next generation of insects, hatched from the eggs laid by the adult insects, which survived on diet supplemented with low concentrations of lectin have been observed.

MATERIALS AND METHODS

Plant Material. Bulbs of *A. sativum* (garlic), leaves of *D. sequina*, and tubers of *C. esculenta* were collected from local fields. Red cotton bugs were collected from local gardens.

Purification of Lectin from Garlic Bulbs. Garlic bulbs (10 g) were homogenized with a blender in 1 M ammonium sulfate. Five to ten milliliters of an ammonium sulfate solution was used per gram of fresh tissue. The extract was filtered through cheesecloth and centrifuged (5000*g* for 10 min). The resulting supernatant was then frozen overnight at −20 °C. After being thawed, the suspension was centrifuged and

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10.1021/jf025660x CCC: $22.00
Published on Web 10/02/2002

the clear supernatant was loaded on a mannose−agarose column (4% cross-linked, Sigma) equilibrated with 1 M ammonium sulfate. Unbound proteins were washed with the same solution, and the ASA lectin along with alliinase desorbed at alkaline pH using unbuffered 20 mM 1,3-diaminopropane. The individual garlic bulb lectins, ASAI and ASAII, had been further purified according to the modified methods of Van Damme et al. (*12*, *13*). The pH of the affinity-purified sample was brought back to 7.5 by dialyzing against 20 mM Tris-HCl (pH 7.5). The dialyzed sample was loaded onto a DEAE-Sephacel column, washed with the same buffer at pH 6.0, and further eluted with 20 mM sodium acetate (pH 5.0). The eluted fraction, free from alliinase, was extensively dialyzed against 0.2 M NaCl (pH 6.5). The dialyzed sample was applied again onto a mannose−agarose column equilibrated with 0.2 M NaCl and allowed to bind to the column. The flow-through was collected. Thorough washing was carried out with 0.2 M NaCl. The bound protein fraction was desorbed with 50 mM sodium tetraborate (pH 9.0).

Extraction and Isolation of the Lectins from *Colocasia* and *Dieffenbachia* sps. The lectins were isolated from extracts of tubers of *Colocasia* and leaves of *Dieffenbachia* by affinity chromatography following the method of Van Damme et al. (*14*) with some modification. The tissues were homogenized in 0.2 M NaCl containing 1 g/L ascorbic acid (10 mL/g fresh weight) at pH 7.0 in a waring blender. The homogenates were filtered through cheesecloth and centrifuged at 3000*g* for 10 min. $CaCl_2$ (20 mM) was added to the filtrate. The pH of the supernatant was adjusted to 9.0, and the supernatant was kept overnight in the cold and centrifuged at 3000*g* for 10 min. The pH of the supernatant was brought back to 4.0 and again the supernatant centrifuged at 3000*g* for 10 min. The clear supernatant was allowed to stand overnight in ice after the pH had been adjusted to 7.5; the precipitate was removed by centrifugation at 9000*g* for 20 min. The final supernatant was decanted, filtered through filter paper (Whatman 3 mm), and loaded onto a mannose−agarose column equilibrated with 20 mM Tris-HCl. After repeated loadings, the column was washed with 20 mM Tris-HCl until the OD (A_{280}) was <0.01 and the bound protein desorbed with 20 mM 1,3-diaminopropane.

SDS−PAGE Analysis. All lectin preparations after purification were analyzed by 15% SDS−PAGE according to the method of Laemmli (*15*).

Agglutination Assays. Rabbit erythrocytes were collected, washed extensively in a 0.9% saline solution, and finally made up to 20% (v/v) with 0.9% saline. Twenty microliters of an erythrocyte suspension was dispensed in each well of a microtiter plate. Eighty microliters of serially diluted lectin solutions (starting from 500 ng to 1 μg per well) were added to each well. The agglutination reaction was monitored visually, after incubation for 1 h at room temperature.

Raising a Polyclonal Antibody in Rabbit against Garlic Lectin. The purified garlic lectin fraction that eluted through a DEAE-Sephacel column was run on 15% SDS−PAGE, and the 12 kDa protein band was excised. The rabbit was injected four times with the excised band every 15 days where the gel matrix acted as the adjuvant. After the final push, the anti-ASA polyclonal serum collected from the rabbit was mixed with an equal volume of binding buffer (IgG purification kit, Pierce) and passed through a protein A−agarose column pre-equilibrated with binding buffer. After being washed with the same buffer, the antibody was finally eluted with elution buffer (supplied with the kit).

Immunoblotting of the Lectins. Garlic lectin fraction ASA (mixture of ASAI and -II), the individual isolectins ASAI and -II, eluted through the second mannose column, purified CEA, and DEA fractions were run on 15% SDS−PAGE. Thereafter, electroblotting of proteins onto a nitrocellulose membrane (*16*) was carried out. Western blot analysis (*17*) was performed by using an anti-ASA polyclonal primary antibody and anti-rabbit IgG−horseradish peroxidase conjugates as the secondary antibody.

Insect Bioassay in Artificial Diet. Keeping in mind the fact that the sucking pests feed exclusively on the phloem saps of plants, we had set up the bioassay on an artificial diet, the composition of which was close to that of natural phloem sap. The liquid diet as described by Dadd and Mittler (*18*) has been formulated with some modification by diluting it up to 50% with water. Forty-five insects of second instar were incubated for each set in a plastic pot sealed with Parafilm, stretched over the rim. Two hundred microliters of liquid diet, supplemented with the lectins ASA, CEA, and DEA (5, 10, 15, and 20 μg/mL), was put on the Parafilm layer, and a second Parafilm membrane was stretched onto it under pressure, to make a diet pouch mimicking the normal texture and tensile strength of the ventral epidermal layer of a dorsoventral leaf. The insects feed by probing through the Parafilm membrane in a manner with which they normally probe the plant tissues and suck away phloem sap. Data on the survival of the insects were collected every 24 h for a total period of 48 h.

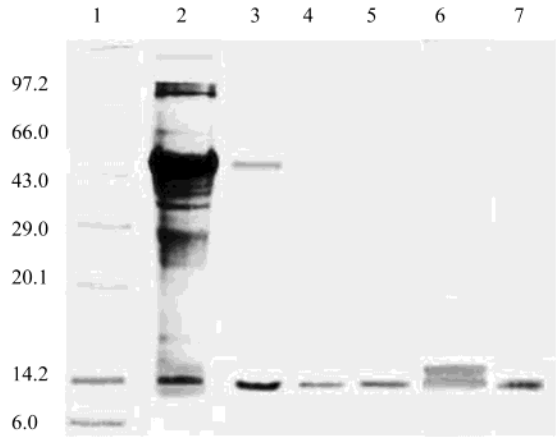

Figure 1. SDS–PAGE (15%) profile showing ASA, CEA, and DEA purification: lane 1, molecular mass markers; lane 2, crude extract from garlic bulbs; lane 3, affinity-purified sample showing ~54 kDa alliinase and ~12 kDa ASA bands; lane 4, purified fraction of ASA I; lane 5, purified fraction of ASAII; lane 6, ~12 and ~14 kDa bands of DEA; and lane 7, CEA of the ~12 kDa band of CEA.

Statistical Analysis. Corrected mortality was calculated using Abbot's formula (*19*). Statistical analysis was carried out following the standard Probit analysis methods (*20*) and factorial analysis.

Characterization of the Phenotypic Changes of the Affected Nymphs. While the bioassay was being carried out, a proportion of insects was found to survive with a diet supplemented with 5−10 μg/mL ASA; the survivors were then put back to the normal diet. The phenotypic changes of the nymphs, hatched from the eggs of insects, which survived over a period of 10 days with the lectin diet, were monitored. The sizes and weights of the nymphs were recorded every 24 h. The changes in body color were also noted.

RESULTS

Analysis of *A. sativum* Lectins (ASAI and ASAII) *Colocasia* Agglutinin (CEA) and *Dieffenbachia* Agglutinin (DEA) via SDS−PAGE. The first affinity column-purified fractions of garlic bulb lectin, ASA, were checked via 15% SDS−PAGE, which demonstrated that in all fractions, there have been 54 kDa bands of contaminating alliinase (*21*) as shown in **Figure 1**. These pooled fractions, after passing through a DEAE-Sephacel column, have been eluted as ASA, free from alliinase. The ASA fraction was reloaded onto a mannose−agarose column pre-equilibrated with 0.2 M NaCl. The first fraction containing ASAI was obtained in the flow-through after a thorough washing with 0.2 M NaCl, and the second fraction, ASAII, was obtained by eluting from the column through 50 mM sodium tetraborate. On native PAGE, ASAI, ASAII, DEA, and CEA all generated bands in the 25 kDa range (data not shown) which resolved on 15% SDS−PAGE as 11.5 and 12.5 kDa in the case of ASAI (**Figure 1**, lane 4), a single band of 12 kDa in the case of ASAII (**Figure 1**, lane 5), ~12 and ~14 kDa bands in the case of DEA (**Figure 1**, lane 6), and a single band in the ~12 kDa range (**Figure 1**, lane 7) in the case of CEA. This result revealed that ASAI is a dimer of two

Figure 2. Western blot analysis of three lectins: lane 1, ASA; lane 2, CEA; and lane 3, DEA.

heteromeric subunits, whereas ASAII is a homodimer of 12 kDa subunits (*12*). DEA is a complex of two subunits of ~12 and 14 kDa, and CEA is a homodimer of ~12 kDa subunits, as also demonstrated in earlier reports (*12*, *14*).

Agglutination Assays. The agglutination assays performed with two garlic lectins, ASAI and ASAII, *Colocasia* agglutinin (CEA), and *Dieffenbachia* agglutinin (DEA) on rabbit erythrocytes demonstrated that the erythrocytes agglutinate readily; however, mannose was able to inhibit the agglutination reaction at 10 mM in the case of ASAI and ASAII, but in case of DEA and CEA, inhibition took place at a relatively higher concentration of 80−90 mM.

Immunoblotting of ASAI, ASAII, CEA, and DEA. The immunoblotting experiment carried out with all aforementioned lectins using the ASA antibody demonstrated that ASAI, ASAII, CEA, and DEA all gave positive signals which indicated that all four lectins are cross-reactive to the anti-ASA antibody (**Figure 2**).

Insect Bioassay with an Artificial Diet Supplemented with Different Concentrations of ASA, CEA, and DEA. The rate of mortality was recorded every 24 h when the bioassay was conducted with an artificial diet supplemented with different doses of ASA, CEA, and DEA (each lectin at 5, 10, 15, and 20 μg/mL). The rate of mortality increased with time and dose. The highest mortality for the red cotton bug with ASA was found to be 77.8% followed by those of CEA (51%) and DEA (43%) with each lectin at 20 μg/mL as shown in **Figure 3**.

These data demonstrated that the expected mortality rate apparently increased with the dose in the case of all three lectins; however, the efficiency of the toxin is judged by its appreciable killing ability at comparatively lower doses. Hence, two factors, dose and control agent (type of lectin), have been taken into consideration together.

Effect of Control Agent and Dose Together. When the results of the bioassay carried out with different lectins at different doses were analyzed, ASA exhibited the highest mortality of 77.8% at a dose of 20 μg/mL. This value is followed by those of CEA and DEA (~51 and ~43%, respectively) at a dose of 20 μg/mL (**Figure 4**). At doses of 15, 10, and 5 μg/mL, ASA exhibited 66.7, 53.3, and 24.4% mortality, respectively, which were still higher in comparison to those for different doses of DEA and CEA as evidenced from **Figure 3**. This indicated that ASA lectin is the most efficient anti-insect component among the three lectins that were tested, at both

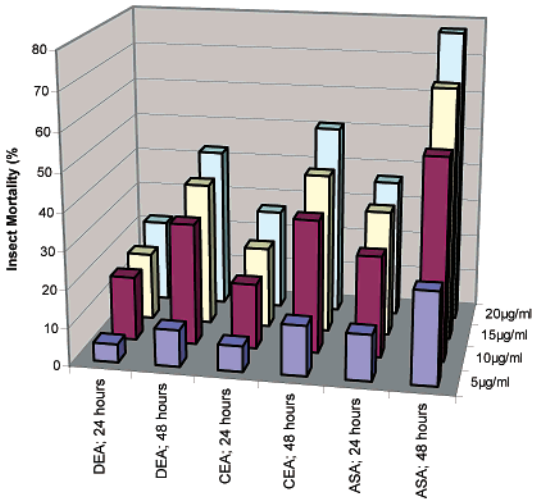

Figure 3. Percentage of insect mortality at 24 and 48 h when the bioassay was conducted with an artificial diet supplemented with the three tested lectins at 5, 10, 15, and 20 μg/mL.

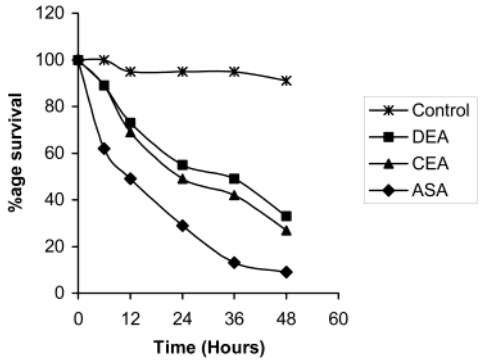

Figure 4. Insect mortality with the three different lectins at 20 μg/mL, supplemented with artificial diet after 48 h.

high and low doses and the LC_{50} of which is expected to be close to 15 μg/mL. However, for obtaining correctly estimated LC_{50} values for the lectins on the red cotton bug, a detailed statistical analysis has been performed.

Determination of LC_{50} Values of Different Lectins. While the results of the bioassay are being analyzed, the proportion of test subjects, which die even without any toxin (natural mortality, C), has also to be considered. The total mortality (P') is worked out from the formula considering a proportion (P) which would have survived if no toxin had been applied. Therefore, $P' = C + P(1 - C)$ (Abbot's formula).

Two types of mortality operate independently (natural death and death due to the toxin effect). From the above equation, it can be deduced that when the total proportion dead is P', the proportion killed by the toxin alone is P [$=(P' - C)/(1 - C)$.

With the equation for calculating the dose-dependent LC_{50} value in mind, first, the percentages of mortality were calculated which were further corrected to probit. The log dose of ASA corresponding to a probit value of 5.00 was converted, and an LC_{50} value of 11.3 μg/mL was derived (**Figure 5**). With the standard deviation in mind, the exact LC_{50} value for ASA against the red cotton bug was calculated to be 11.2 $\pm$ 1.06 μg/mL. Likewise, the same values for DEA and CEA were determined to be 22.3 $\pm$ 1.22 and 19.9 $\pm$ 0.98 μg/mL, respectively.

Characterization of the Phenotypic Changes of the Lectin-Treated Insect Progeny. The nymphs, hatched from the eggs laid by the adults, that survived with the lectin-containing diet (5 and 10 μg/mL) for a period of 10 days looked quite different

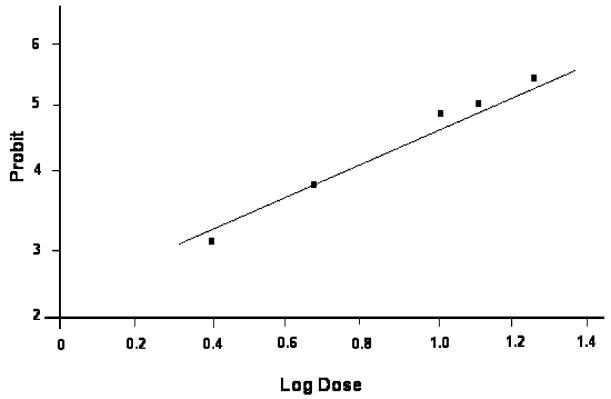

Figure 5. Probit analysis of mortality against the log dose of ASA for the determination of LC_{50}.

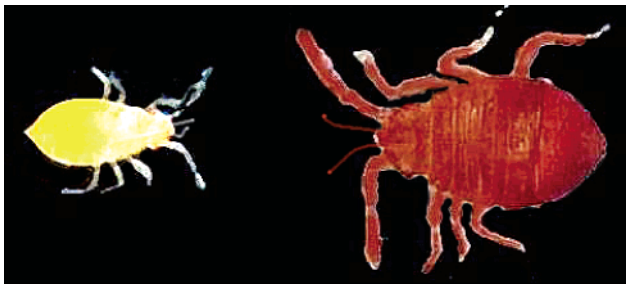

Figure 6. Morphological changes of the nymphs from the eggs of red cotton bugs reared on an ASA-containing diet. Nymphs of affected insects (yellow) and unaffected insects (red) are shown. Both insects are of the same instar and age.

from the nymphs of normal insects. A significant reduction in the size in treated nymphs was evident in contrast to the nymphs of adult insects reared on a normal artificial diet. A similar phenomenon was observed when the average weights of the normal and treated insects of the same age were analyzed. The average weight of normal insects was approximately 25 ± 0.02 mg, while that of the treated ones was found to be 15 ± 1.0 mg. **Figure 6** depicts a significant change in color of the insects of the same age from red to yellowish and a size reduction of ~50% in contrast to the nymphs of adult insects reared on a normal diet.

DISCUSSION

In the past few years, several lectins have been used by early workers to monitor the effect on cowpea weevil (*22*), aphids (*3*, *4*), and potato leafhopper (*23*). In the study presented here, three lectins, ASA (admixture of isolectins I and II), CEA, and DEA, were bioassayed on the red cotton bug to judge their efficacy on the growth and developmental pattern, survival rate, and fecundity. As ASAI and ASAII are a mixture of isolectins (*12*), the ASA lectin (mixture of I and II) was used for the bioassay experiments. When the results were compared, the mortality with ASA scored highest at doses of 20 and 15 μg/mL followed by CEA and DEA. The data obtained from these three lectins with the red cotton bug assay clearly demonstrated that two factors, namely, the source of the lectin and the lectin dose, contributed significantly to judging the effectiveness of the lectins in controlling the insects. When the data shown in **Figure 3** were analyzed, the most interesting point that has become evident is 10 μg/mL DEA and CEA caused 32.2 and 35.2% insect mortality, respectively, after 48 h of treatment which is close to the percentage mortality (33%) with 15 μg/mL ASA after incubation for 24 h. Therefore, the third factor, the "duration" of the treatment, should also be considered when judging the efficiency of the control agent. Because the sucking pests are able to build up a huge population within a short span of time, one has to be careful to note the minimum time required to arrest the growth of the insects, or until mortality is evident. From the result shown in **Figure 3**, it was clearly understood that ASA had a marked effect on the growth and survival rate of the insects at a comparatively lower dose and with a shorter period of incubation, which led us to investigate the site of action of ASA and another garlic lectin (ASAL, the garlic leaf lectin) on insect gut (*11*) and the effect of ASA on phenotypic characters in the insect. Probit analysis was performed to statistically represent the actual LC_{50} value of the effective lectin (ASA). It is absolutely essential to calculate the LC_{50} of the toxin specifically for the target insect, well in advance, for attaining that level of expression of the toxin in a transgenic situation for protecting the crop from the aforementioned insect. In the case of the red cotton bug, the LC_{50} value of ASA was calculated to be 11.3 ± 1.06 μg/mL. In all experimental sets, allowances were made for natural mortality as recorded in the control sets. The red cotton bug, although reported to be a moderately damaging pest, sometimes can cause massive deterioration of the full blooming of the plant. Rearing of these insects in our laboratory for studying the mechanism of action of lectin on insect midgut has been successfully described in earlier studies, which demonstrated the binding of lectin to the insect midgut epithelium (*11*). This binding might be causing interference with nutrient uptake, leading to the death of the insect. Studies have been undertaken to probe this aspect further.

Surprisingly, peculiar phenotypic changes have been observed in the nymphs developed from the eggs laid by the females that survived on a sublethal dose of ASA. Drastic changes in color and reductions in the size and weight of the nymphs have been evident. No such phenotypic changes in the progeny of insects treated with any other type of lectin have been reported in previous work. In the experimental setup presented here, as ASA has been found to be the most effective toward the red cotton bug among the three tested lectins, attempts have been made to study the fate of the insects which had survived the treatment with ASA only and not the other two lectin types.

Nevertheless, from the results presented above, ASA may be considered to be a potent control component of IPM (integrated pest management) due to not only its ability to control the survival rate and fecundity of the insects but also its efficacy in influencing the normal metabolism of the next generation of the affected insects. The second phenomenon poses an additional advantage in controlling the population development of attacking insects.

ABBREVIATIONS USED

Bt, *B. thuringiensis*; ASA, *A. sativum* agglutinin; CEA, *C. esculenta* agglutinin; DEA, *D. sequina* agglutinin; LD_{50}, minimum dose (of the toxin) resulting in 50% mortality of the test subjects; DEAE, diethylaminoethyl; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; IgG, immunoglobulin G.

ACKNOWLEDGMENT

We are thankful to the Bose Institute for providing the facilities. We acknowledge the Council of Scientific and Industrial Research, Department of Atomic Energy, Government of India.

LITERATURE CITED

(1) Ajiri, D. S.; Mali, A. R.; Shelake, S. S.; Patil, C. S.; Subhedar, A. J. A status paperon problem of whitefly, *Bemisia tabaci* (Gennadius), in cotton and other crops in Western Maharastra. In *Status of whitefly in cotton*; College of Agriculture: Pune, India, 1986; p 7.

(2) Sogowa, K. Studies on feeding habits of the brown plant hopper I. Effects of nitrogen deficiency of host plant insect feeding. *Nippon Oyo Dobutsu Konchu Gakkaishi* **1970**, *14*, 101−106.

(3) Hilder, V. A.; Gatehouse, A. M. R.; Powell, K. S.; Boulter, D. Proteins with insecticidal properties against homopteran insects and their use in plant protection. PCT Int. Appl. WO 93/04177, 1993.

(4) Hilder, V. A.; Powell, K. S.; Gatehouse, A. M. R.; Gatehouse, J. A.; Gatehouse, L. N.; Shi, Y.; Hamilton, W. D. O.; Merryweather, A.; Newell, C. A.; Timans, J. C. Expression of snowdrop lectin in transgenic tobacco plants resulted in added protection against aphids. *Transgenic Res.* **1995**, *4*, 18−25.

(5) Powell, K. S.; Gatehouse, A. M. R.; Hilder, V. A.; Van Damme, E. J. M.; Peumans, W. J.; Boonjawat, J.; Horsham, K.; Gatehouse, J. A. Different antimetabolic effects of related lectins towards nymphal stages of *Nilaparvata lugens*. *Entomol. Exp. Appl*. **1995**, *75*, 61−65.

(6) Gatehouse, A. M. R.; Powell, K. S.; Van Damme, E. J. M.; Peumans, W. J.; Gatehouse, J. A. Insecticidal properties of plant lectins: their potential in plant protection. In *Lectins. Biomedical Perspectives*; Pustzai, A. J., Ed.; Taylor and Francis: Basingstoke, U.K., 1995.

(7) Gatehouse, A. M. R.; Down, R. E.; Powell, K. S.; Sauvion, N.; Rahbe, Y.; Newell, C. A.; Merryweather, A.; Hamilton, W. D. O.; Gatehouse, J. A. Transgenic potato plants with resistance to the peach potato aphid *Myzus persicae*. *Entomol. Expl. Appl*. **1996**, *79*, 295−307.

(8) Goldstein, I. J.; Poretz, R. D. Isolation, physicochemical characterization and carbohydrate-binding specificity of lectins. In *The Lectins: Properties, Functions and Applications in Biology and Medicine*; Liener, I. E., Sharon, N., Goldstein, I. J., Eds.; Academic Press: New York, 1986; pp 33−247.

(9) Czapla, T. H. Plant lectins as insect control proteins in transgenic plants. *Advances in insect control: The role of transgenic plants*; Carozzi, N., Koziel, M., Eds.; Taylor and Francis: Basingstoke, U.K., 1997; pp 123−138.

(10) Rao, K. V.; Rathore, K. S.; Hodges, T. K.; Fu, X.; Stoger, E.; Sudhakar, D.; Williams, S.; Christou, P.; Bharathi, M.; Bown, D. P.; Powell, K. S.; Spence, J.; Gatehouse, J. A. Expression of snowdrop lectin (GNA) in transgenic rice plants confers resistance to rice brown plant hopper. *Plant J.* **1998**, *15*, 469−477.

(11) 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* (5), 1025−1033.

(12) Van Damme, E. J. M.; Smeets, K.; Torrekens, S.; Leuven, F. V.; Goldstein, I. J.; Peumans, W. J. The closely related homodimeric and heterodimeric mannose-binding lectins from garlic are encoded by one-domain and two-domain lectin genes, respectively. *Eur. J. Biochem.* **1992**, *206*, 413−420.

(13) Van Damme, E. J. M.; Peumans, W. J. The mannose binding lectin from Alliaceae species. In *Lectins: Biology, Biochemistry, and Clinical Biochemistry*; Basu, J., Kundu, M., Chakrabarti, P., Eds.; Wiley Eastern: New Delhi, India, 1993; Vol. 9, pp 9−18.

(14) Van Damme, E. J. M.; Goossens, K.; Smeets, K.; Leuven, F. V.; Verheart, P.; Peumans, W. J. The Major Tuber Storage Protein of Araceae Species Is a Lectin. *Plant Physiol.* **1995**, *107*, 1147−1158.

(15) Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **1970**, *227*, 680−685.

(16) Towbin, H.; Stachelin, T.; Goedon, J. Electrophoretic transfer of protein from polyacrylamide gels to nitrocellulose sheets. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 4350−4354.

(17) Burnette, W. N. "Western blotting": Electrophoretic transfer of proteins from sodium dodecyl sulfate−polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radio-iodinated protein A. *Anal. Biochem.* **1981**, *112*, 195−203.

(18) Dadd, R. H.; Mittler, T. E. Permanent culture of an aphid on a totally synthetic diet. *Experientia* **1966**, *22*, 832.

(19) Abbott, W. S. A method of computing the effectiveness of insecticide. *J. Econ. Entomol.* **1925**, *18*, 265−267.

(20) Finney, D. J. *Probit analysis*, 3rd ed.; Cambridge University Press: Cambridge, U.K., 1971.

(21) Smeet, K.; Van Damme, E. J. M.; Verheart, P.; Barre, A.; Rouge, P.; Van Leuven, F. V.; Peumans, W. J. Isolation, characterisation and molecular cloning of the mannose binding lectins from the leaves and roots of garlic (*Allium sativum* L.). *Plant Mol. Biol.* **1997**, *33*, 223−234.

(22) Murdock, L. L.; Huesing, J. E.; Nielson, S. S.; Pratt, R. C.; Shade, R. E. Biological effects of plant lectins on the cowpea weevil. *Phytochemistry* **1990**, *29* (1), 85−89.

(23) Habibi, J.; Bacus, E. A.; Czapla, T. H. Plant lectins affect survival of potato leaf hopper (homopteran, Cicadellidae). *J. Econ. Entomol.* **1993**, *86* (3), 945−951.

Received for review May 9, 2002. Revised manuscript received August 22, 2002. Accepted August 24, 2002.

JF025660X