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The African yam bean seed lectin affects the development of the cowpea weevil but does not affect the development of larvae of the legume pod borer

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Abstract

Artificial feeding assays were used to study the effect of purified galactose-specific lectins from African yam beans (*Sphenostylis stenocarpa*) on development of larvae of the cowpea weevil, *Callosobruchus maculatus* (Coleoptera: Bruchidae) and the legume pod-borer, *Maruca vitrata* (Lepidoptera: Pyralidae). Inhibition of development of *C. maculatus* was observed when larvae were fed on artificial cowpea seeds containing 0.2%, 2.0% and 5.0% (wt/wt) of dietary lectin. Larval mortality was between 30% and 88%, while delays in total developmental time ranged between 7 and 13 days. The lectin had no effect on development of larvae of *M. vitrata*, when tested through topical artificial diet incorporation assays, except at the extremely high dose of 35% dietary level.

Keywords: African yam beans; *Callosobruchus maculatus*; Cowpea weevil; Insect bioassays; Legume pod borer; *Maruca vitrata*; *Sphenostylis stenocarpa*

1. Introduction

Insect pests are the major constraint restricting increased grain legume production in the tropics and subtropics (Singh & Jackai, 1985; Singh, Jackai, Dos Santos & Adalla, 1990). In Sub-Saharan Africa, the main pulse is cowpea (*Vigna unguiculata*), whose success can largely be attributed to its adaptability for growth in stressful environments, especially in marginal, arid and semi-arid areas (Hall, Singh & Ehlers, 1997). In this crop, the legume pod-borer, *Maruca vitrata* (Fab., syn. *M. testulalis* Geyer), [Lepidoptera: Pyralidae] alone causes 20–80% of total yield losses, which often rise to 100% in seasons of severe infestation (Jackai, Singh, Raheja & Wiedijk, 1985; Sharma, 1998). Apart from the pod borer, coleopteran insects from the family *Bruchidae* cause serious grain losses in storage. Key among these pests is the cowpea weevil (*Callosobruchus maculatus* Fab.) which is especially damaging in the storage environment of low-resource, smallholder farmers in Africa who largely store their seeds in baskets, granaries and on open floors (Kitch, Bottenberg & Wolfson, 1997). Under severe periods of infestation, post-harvest seed losses due *C. maculatus* can reach 100% within a period of 6 months (Hall et al., 1997). To address the cowpea pest problem, efforts by scientists at the International Institute of Tropical Agriculture (IITA) and elsewhere, have been concentrated mainly on increasing host plant resistance levels through conventional breeding and other pest management practices (Singh, Jackai, Thottappilly, Cardwell & Meyers, 1992; Jackai & Adalla, 1997). So far, moderate levels of resistance to *C. maculatus* have been

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attained (Adjadi, Singh & Singh, 1985; Singh et al., 1992), but none to the pod borer.

IITA scientists have also identified wild cowpeas (*Vigna* species) and other non-*Vigna* legumes that have good sources of resistance to pod borer, *C. maculatus* and other pre- and post-flowering pests of cowpea (Jackai, Padulosi & Ng, 1996). Among these species, the African yam bean (*Sphenostylis stenocarpa*) has been singled out for isolation of proteins which may have a role in protecting cowpea against a pest complex that includes the pod borer, *C. maculatus* and pod-sucking bugs (*Clavigralla tomentosicollis*). The African yam bean (AYB) is an underutilized, semi-cultivated crop that has failed to gain popularity comparable to that of other edible African legumes such as cowpea and pigeon pea. The reasons for this are not clear, but may be attributed to toxicity present in the edible parts, namely seeds and tubers (Asuzu & Undie, 1986). Among plant defense factors, there is increasing evidence that lectins provide defense against insects and other herbivores (Chrispeels & Raikhel, 1991; Peumans & Van Damme, 1995). These are a large and heterogenous group of proteins that have the ability to bind reversibly to carbohydrates (free or conjugated) (Van Damme, Peumans, Barre & Rougé, 1998). Their potential role in insect control has been assessed mainly through insect feeding experiments utilizing artificial diets or seeds containing lectin preparations which are either incorporated into the diet and seeds, or applied topically (Czapla, 1998).

The majority of artificial diet bioassays utilizing plant lectins have focused mainly on *C. maculatus* (Janzen, Juster & Liener, 1976; Gatehouse, Dewey, Dove & Fenton, 1984; Murdock, Huesing, Nielson, Pratt & Shade, 1990; Gatehouse, Howe, Flemming, Hilder & Gatehouse, 1991; Huesing, Murdock & Shade, 1991a). However, little work has been done that pertains to antiinsect activities of plant and non-plant proteins against the pod borer. The recent study by Omitogun, Jackai & Thottappilly (1999) is, therefore, among the first of its kind on this insect. These workers found that incorporation of lectin-enriched extracts from AYB in artificial diet (1.0%) or artificial seeds (5.0%) significantly affected survival of *C. maculatus*, *M. vitrata* and *C. tomentosicollis* larvae. We have recently purified the AYB lectin by affinity chromatography based on its specificity for galactose (Machuka, Okeola, Van Damme, Chrispeels, Van Leuven & Peumans, 1999). In the present study, our purpose was to investigate the effect of the purified lectin on development of larvae of *C. maculatus* and *M. vitrata*. Broadly, the ultimate goal of this research is to identify insecticidal proteins from AYB, and the genes encoding them, for deployment in a transgenic approach to control cowpea insect pests.

2. Results and discussion

We used lectins purified from AYB seeds to test their efficacy against the pod borer and the cowpea weevil, in artificial diet and seed bioassays, respectively. This study was a follow up of previous bioassays that utilized crude AYB seed lectin-enriched fractions separated by ammonium sulphate precipitation (Omitogun et al., 1999). Findings from this study implicated lectins as a possible source of the observed insecticidal activities against these two pests as well as pod sucking bugs. The effect of lectins from two AYB collections (Enugu 95-3 and Umueze 98-3-2) on survival of *C. maculatus* larvae are shown in Fig. 1. Larval mortalities were identical in both AYB collections, being 30.0% and 36.7%, at 0.2% and 2.0% dietary lectin levels, respectively. At 5.0% level, mortalities were 63.3% and 88.8% with artificial cowpea seeds (ACS) containing lectins from Enugu 95-3 and Umueze 98-3-2, respectively. Survival on intact Ife Brown seeds (susceptible control) was 95.0%, whereas no insects survived on TVnu 72 (resistant control) seeds. These data show that the yam bean lectin is insecticidal to *C. maculatus* larvae, although the potency of this lectin is lower than what is reported for garlic, rice, snowdrop, pea and wheat lectins (Czapla, 1998). For example, wheat germ agglutinin (WGA) caused about 64.0% mortality of *C. maculatus* larvae when incorporated in ACS at about 1.8% level (wt/wt) (Murdock et al., 1990).

The Enugu 95-3 lectin caused an average delay in developmental time of 7.5, 7.8 and 11.3 days at 0.2%, 2.0% and 5.0% dietary levels, respectively, compared to susceptible control seeds (Table 1). Average delays of 7.7, 6.4 and 13.0 days were caused by the lectin from UM98-3-2 at 0.2%, 2.0% and 5.0%, respectively. At the three dietary concentrations tested, the delays in total developmental time (TDT) caused by lectins from the two AYB collections were statistically significant when compared to susceptible controls. The TDT at 0.2% and 2.0% levels was not significantly different between these two treatments. However, increasing the lectin concentration to 5.0% resulted in, approximately, two-fold increase in TDT. Similar effects of plant lectins on development of the cowpea weevil are well documented, as well as cases in which the lectins were not potent (Czapla, 1998). For example, Murdock et al. (1990) showed that WGA, which has *N*-acetylglucosamine carbohydrate binding specificity, delays *C. maculatus* development by 6.5 and 22.8 days at 0.2% and 1.0%, respectively. The same authors also reported that peanut and osage ogange lectins (*N*-acetylgalactosamine/galactose-specific) delay development by 10.0 and 8.5 days at 1.0% levels, respectively. However, at least 10 other plant lectins tested were not as effective (Murdock et al., 1990). The results of this

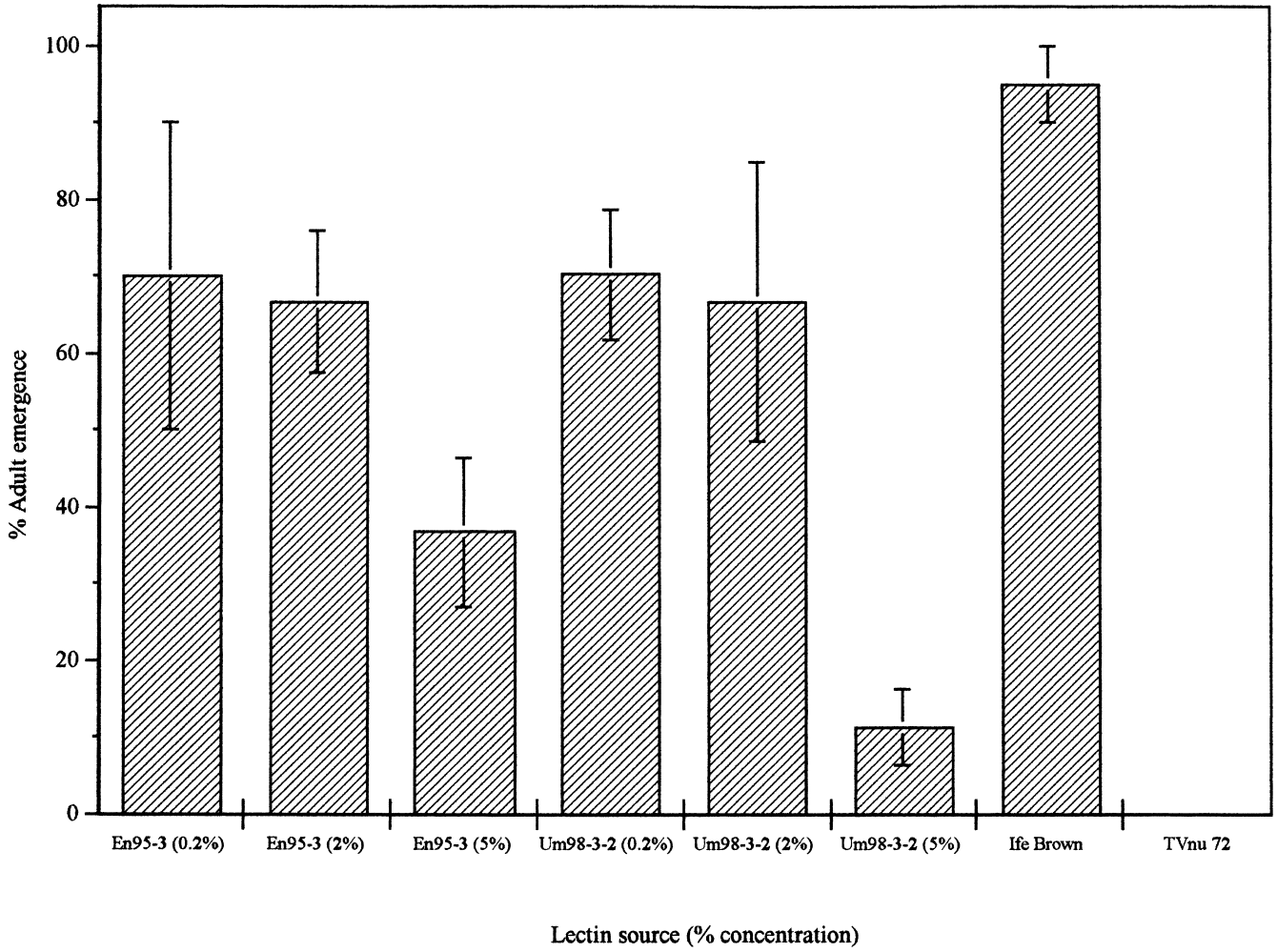

Fig. 1. Percent adult emergence of *Callosobruchus maculatus* fed on artificial seeds containing *Sphenostylis* lectins.

study pertaining to TDT are in line with the report by Omitogun et al. (1999), that suggested a correlation between hemagglutinating activity and developmental time in *C. maculatus* but not *M. vitrata*.

The growth index (GI) measures the effects of the food substrate, such as dietary lectin, on both survival and developmental time (Howe, 1971). The principle behind this parameter is that adverse conditions such

Table 1
Effect of *Sphenostylis* lectins on development of *Callosobruchus maculatus* larvae in artificial seed bioassays: total developmental time (TDT), growth index (GI) and resistance index (RI)[a]

Lectin/seed source	%[b]	TDT	GI	RI
Enugu 95-3	0.2	28.13 ± 1.39b	0.16 ± 0.01b	72.28 ± 5.82b
Enugu 95-3	2.0	28.37 ± 1.07b	0.15 ± 0.01b	66.87 ± 2.83b
Enugu 95-3	5.0	31.88 ± 0.52a	0.12 ± 0.00c	54.28 ± 2.13c
Umueze 98-3-2	0.2	28.27 ± 0.80b	0.15 ± 0.01b	68.04 ± 3.18b
Umueze 98-3-2	2.0	27.00 ± 0.65b	0.16 ± 0.01b	74.03 ± 2.56b
Umueze 98-3-2	5.0	33.67 ± 0.33a	0.09 ± 0.00d	39.00 ± 2.16d
Ife brown (susceptible control seed)	–	20.62 ± 0.20c	0.22 ± 0.00a	100.00 ± 1.31a
TVnu 72 (resistant control seed)[c]	–	–	–	–

[a] TDT = total developmental time (mean developmental time per insect in days); GI = growth index [(in % adult emergence)/TDT]; RI = resistance index [(GI test material/GI susceptible) × 100]. Means in the same column with the same letter are not significantly different ($p < 0.05$).
[b] Percent (wt/wt) lectin incorporated into artificial seeds.
[c] No insects survived on intact TVnu 72 seeds, hence no entries are shown in the table.

as an unsuitable food substrate would prolong the developmental period while fewer individuals survive, resulting in a low GI value. Conversely, a suitable food substrate would result in a high GI value. Across AYB collections, no significant differences were found between treatments at dietary levels of 0.2% and 2.0%. However, increasing the concentration of the lectin to 5.0% resulted in a significant drop in GI in both AYB collections, with the lowest value of 0.09 recorded using Umueze 98-3-2 lectin. In comparison to the susceptible control, all three tested dietary lectin concentrations were effective in lowering GI. Another parameter employed to assess the effect of the yam bean lectin on *C. maculatus* development was the resistance index (RI). This parameter is based on the GI of the test material relative to the susceptible control, expressed as a percentage. Like the GI, the RI is an arbitrary measure that nevertheless helps to condense and rank the effects of the test protein from different sources. Based on this criterion, no significant differences were observed (between treatments) when Enugu 95-3 (mean RI = 69.6) and Umueze 98-3-2 (mean RI = 71.0) lectins were incorporated in artificial seeds at 0.2% and 2.0% levels, respectively. However, the drop in the RI became significant when a comparison was made between these two treatments, on the one hand and Ife Brown (susceptible control), on the other. This effect was enhanced further at 5.0% dietary levels, resulting in a significant drop in the RI to 54.0 and 39.0 in Enugu 95-3 and Umueze 98-3-2, respectively. Assuming that the rates of *C. maculatus* larval development and survival on ACS are comparable to those obtained with intact seeds (Shade, Murdock, Foard & Pomeroy, 1986), it can be inferred that the yam bean lectin inhibited larval development in this insect at all three lectin concentrations tested and that the Umueze 98-3-2 lectin is more potent than the lectin from Enugu 95-3.

Table 2 shows the data for larval survival at 7 and 10 days after infestation (DAI), feeding deterrence and weight (at 10 DAI) when pod borer larvae were fed on artificial diets containing lectins purified from 5 AYB collections, namely, Enugu 95-3, Enugu 98-2, Enugu 97-1, Umueze 98-3-2 and Umuahia 97-1. Enugu 95-3 was included in the assays because the ammonium sulphate fraction from this collection was previously shown to have the highest hemagglutination activities (HA) and to cause the highest mortality in pod borer bioassays (Omitogun et al., 1999). However, although the lectin was suspected to be responsible for the observed effects, there was no correlation between HA and survival. In this study, all experiments were carried out on separate days with more than 2 weeks intervals using different batches of insects. Initially, 5.0% (wt/wt) level of the lectin from Enugu 95-3 lectin was utilized in diet, but this did not produce a significant effect on larval development (Table 2). Increasing the dietary lectin concentration four-fold to 20% only resulted in a minimal, albeit, significant, decrease in larval survival, from 77.8% at the 5.0% dietary level to 69.4% at the 20.0% level, 10 DAI. Preliminary quantitative analysis indicated that the AYB lectin alone accounts for 25–31% of total protein per mature, dry seed (O.G. Okeola & J. Machuka, unpublished data). This estimate is not surprising, considering that lectins alone account for up 50% of the total seed protein in some plant species (Sharon & Lis, 1990; Van Damme et al., 1998). Hence 35.0% dietary levels were used in order to find out the effect of a dose that approximates estimated physiological concentrations, on development of the pod borer. Surprisingly, even this high dose did not significantly affect survival of pod borer larvae. However, lack of sufficient protein from Enugu 95-3 meant that the experiment at 35.0% level had to be performed using Umuahia 98-3-2 and Enugu 98-2. We are not aware of

Table 2
Effect of *Sphenostylis* lectins on survival and weight of *Maruca vitrata* in artificial diets[a]

Lectin source	% Lectin[b]	% Survival		Weight (mg)	
		Day 7	Day 10	Larvae	Pupa
Enugu 95-3	5.0	88.89 ± 6.27a	77.78 ± 7.49ab	67.42 ± 5.74a	51.50 ± 2.24b
Enugu 95-3	20.0	77.78 ± 6.27a	69.44 ± 7.63b	NC[c]	52.25 ± 1.62b
Enugu 98-2	35.0	87.50 ± 6.53a	83.33 ± 9.40ab	13.13 ± 3.25c	52.20 ± 2.64b
Umueze 98-3-2	35.0	84.72 ± 6.63a	76.11 ± 6.61ab	11.36 ± 1.85c	60.83 ± 6.00a
Enugu 97-1	10.0	94.44 ± 3.75a	84.72 ± 5.60ab	35.87 ± 3.34b	46.33 ± 2.79b
Umuahia 97-1	10.0	80.56 ± 6.10a	69.44 ± 6.10b	62.40 ± 7.05a	47.56 ± 3.36b
BSA[d]	35	90.58 ± 3.45a	88.01 ± 4.15ab	59.32 ± 4.59a	61.75 ± 1.89a
Diet + water	0.0	89.77 ± 3.80a	89.77 ± 3.80a	62.58 ± 3.75a	48.84 ± 1.93b

[a] Means in the same column with the same letter are not significantly different ($p < 0.05$).
[b] Percent (wt/wt) lectin incorporated into artificial diets.
[c] NC, data not collected.
[d] BSA, bovine serum albumin.

experiments where such high lectin doses have been tried on insects in artificial diet insect bioassays, perhaps because such experiments have little value except, among other things, to show that high levels of lectin can be ingested without adversely affecting insect development. It was, therefore, concluded that the AYB lectin does not significantly affect survival of pod borer larvae in artificial diets. Moreover, among the plant lectins tested so far, relatively few are toxic to lepidopteran insects (Czapla, 1998), even when they have been found to be stable to proteolysis by enzymes in the insect gut (Gatehouse, Powell, Peumans, Van Damme & Gatehouse, 1995).

The feeding deterrence score (FDS) is a visual assessment based on a score of 0–3 to indicate the extent of inhibition of larval feeding by the diet. A score of 0 indicates no feeding deterrence, that is, larvae are alive and feeding well. A score of 3 indicates high mortality with little or no survival, and little or no feeding, while scores of 1 and 2 indicate low and moderate feeding deterrence, respectively. Based on the FDS, significant interference of feeding by pod borer larvae was observed only when lectins were incorporated in diets at 35.0% level (Fig. 2). The FDS scores were identical (1.8) in diets containing lectins from Enugu 98-2 and Umuahia 98-3-2. At this dietary level, the presence of bovine serum albumin (BSA) did not significantly deter larval feeding. Ideally, high FDS should be correlated with reduced survival and diminished larval weights. This relationship held true only when lectin was administered in diets at 35.0% level. Moreover, differences in larval weight were not reflected in corresponding pupal weights, although percentage pupation was lower (between 33.3% and 58.8%), in diets containing lectins than in BSA (88.9%) and water (83.3%) containing diet controls. With the exception of three treatments (Enugu 95-3, 5.0%; Enugu 97-1, 10.0% and water control), the percentage of adult insects that emerged from the pupal stage was lower than the percentage that pupated. It is likely that the yam bean lectin inhibits pupation, but not necessarily adult emergence, since reduction in

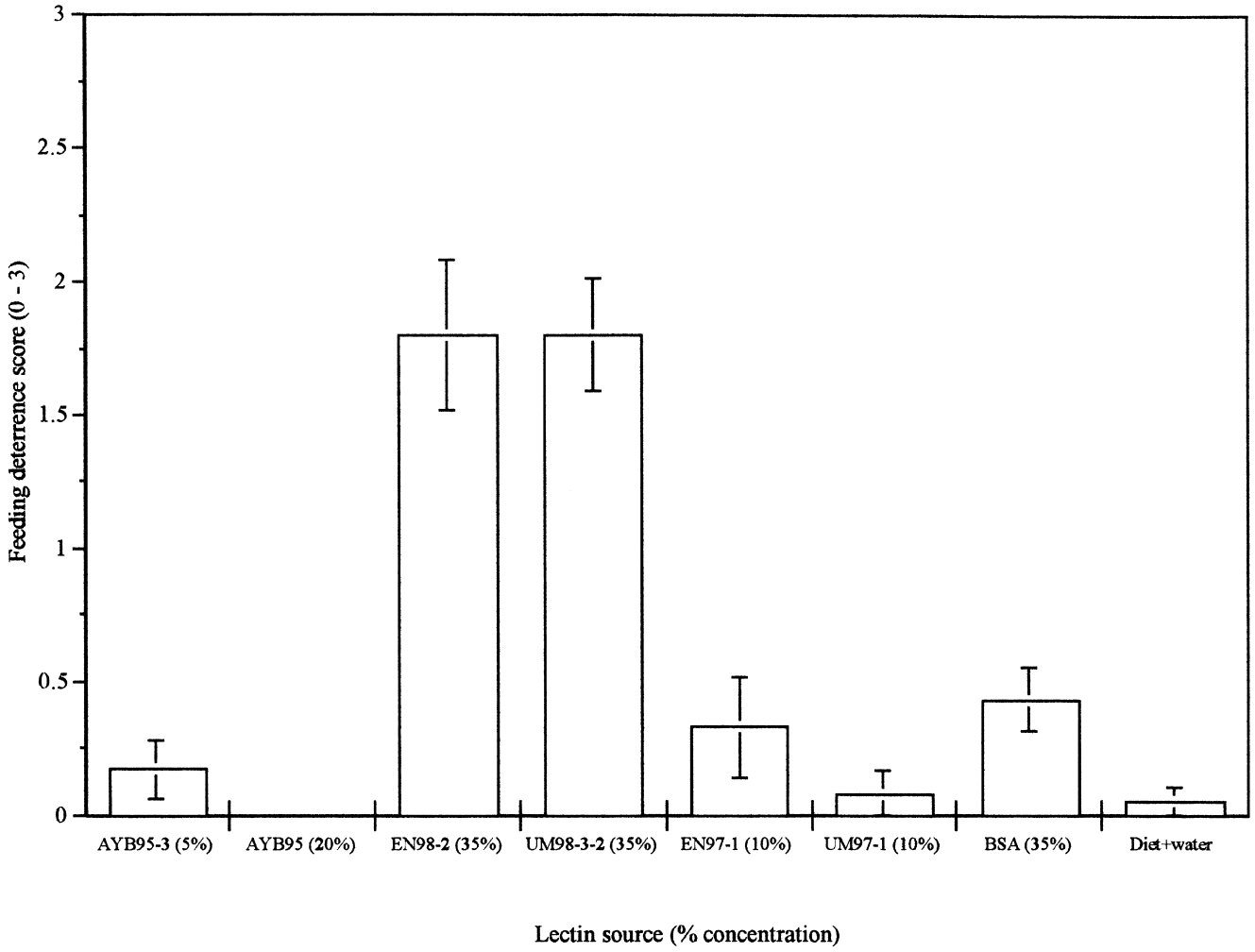

Fig. 2. Feeding deterrence of *Maruca vitrata* neonates fed on artificial diets containing *Sphenostylis* lectins.

adult emergence also occurred on diets containing BSA (Fig. 3).

The physiological level of lectins in AYB seeds is likely to account for the observed resistance of this semi-cultivated crop to *C. maculatus*. To date, lectins from several other plant species have been tested for their abilities to confer resistance against this pest (Gatehouse et al., 1984; Murdock et al., 1990; Gatehouse et al., 1991; Heusing, Murdock & Shade, 1991; Zhu, Huesing, Shade, Bressan, Hasegawa & Murdock, 1996). The list of potent lectins includes two (osage orange lectin and peanut agglutinin) *N*-acetylgalactosamine/galactose-specific, and six (wheat, rice, stinging nettle, potato, jimson weed and *Griffinia* lectins) *N*-acetylglucosamine-specific-, lectins (Murdock et al., 1990; Huesing et al., 1991a; Heusing et al., 1991; Zhu et al., 1996). In comparison to other major pests, artificial diet bioassays against the pod borer have been few, owing to the non-commercial value of the pulse crops attacked by this insect in tropical Africa and Asia (Sharma, 1998). Although the affinity purified, galactose-specific, AYB lectin inhibits *C. maculatus* development, it does not affect the development of pod borer larvae at concentrations that make it viable to use this lectin in genetic engineering approaches, as suggested previously based on bioassays that utilized ammonium sulphate, seed protein fractions (Omitogun et al., 1999). The example in which resistance to *C. maculatus* was mistakenly attributed to phytohemagglutinin rather than α-amylase inhibitor illustrates the importance of using pure protein preparations in artificial insect diet/seed bioassays (Gatehouse et al., 1984; Huesing, Shade, Chrispeels & Murdock, 1991b). Furthermore, further characterization of the ammonium sulphate fraction, including N-terminal sequencing, has shown that AYB seeds contain high concentrations of trypsin and chymotrypsin inhibitors, a pathogenesis-related protein, an acidic chitinase, and various lectin-

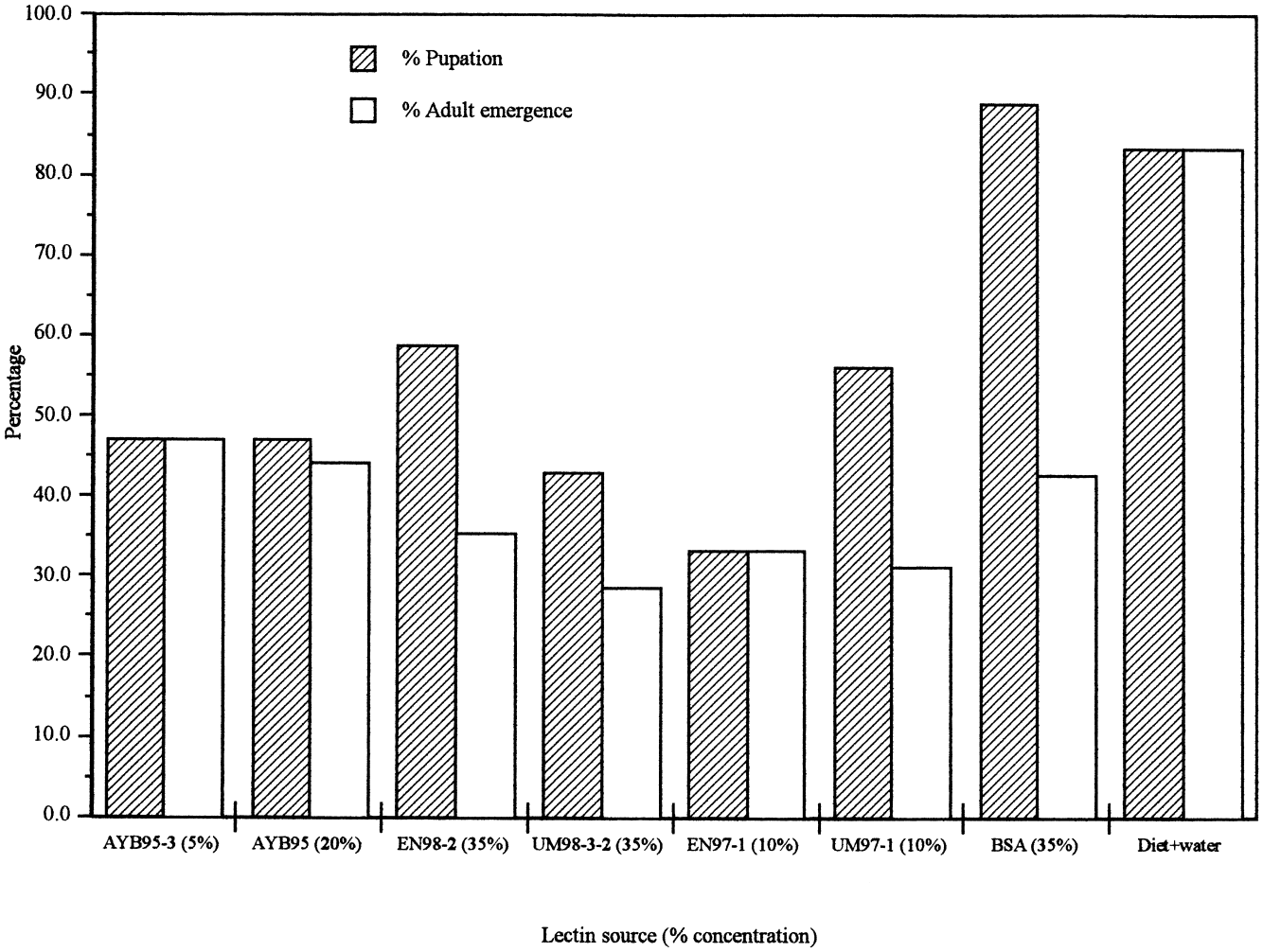

Fig. 3. Percentage pupation and emergence of *Maruca vitrata* juveniles (females only) maintained on artificial diets containing *Sphenostylis* lectins.

like proteins lacking hemagglutination activities (J. Machuka, unpublished). A full length cDNA encoding the acidic chitinase has recently been cloned (Colucci, Machuka & Chrispeels, 1999). It is likely that these proteins have a role in the resistance mechanisms of this species, both to insects and pathogens. Further characterization of defense proteins and the genes encoding them, combined with their deployment in artificial bioassays and expression in transgenic plants, should help clarify the existing scenario.

3. Experimental

3.1. Purification of Sphenostylis lectins

Seed lectins from five AYB collections (Enugu 95-3, Enugu 97-1, Enugu 98-2, Umuahia 97-1 and Umueze 98-3-2) were purified using affinity chromatography on galactose Sepharose-4B according to a previously described protocol (Machuka et al., 1999), and were shown by electrophoresis to be free of contaminating proteins.

3.2. Insects

A culture of *M. vitrata* was maintained on a cowpea-wheatgerm artificial diet in the Entomology Unit at IITA. The composition of the diet and rearing conditions were as described (Jackai & Raulston, 1988). To minimize the negative effects of prolonged laboratory rearing, insects were regularly crossed to field caught adults and their behavior monitored to ensure that their performance was normal. The laboratory population of *C. maculatus* has been maintained at IITA since 1973, with regular crossing to insects obtained from local markets at Ibadan. The insects are cultured on seeds of susceptible cowpea cultivars (Ife Brown, IT82E-889 or IT84D-715) as described (Singh & Jackai, 1985). Laboratory maintenance conditions are 26°C ± 2 and relative humidity (RH) of 70–80% for most of the year except in the dry season (December to March) when the RH falls to 40–60%.

3.3. Insect bioassays

In the bioassays with *C. maculatus*, artificial cowpea seeds (ACS) were used as the delivery system. An aqueous solution containing 0.2%, 2.0% and 5.0% (wt/wt) of lectin was incorporated into the ACS, using the procedure described by Shade et al. (1986). This involved blending decorticated cowpea seed of a susceptible cowpea cultivar (Ife Brown) into base flour and adding an aqueous solution of the lectin to the flour. Seeds of Ife Brown and TVnu 72, a resistant *V. vexillata* accession, were used as normal seed controls. Prior to their use, the seeds were disinfected by being left in a freezer overnight. Five artificial seeds or control seeds were placed in a petri dish (35 mm × 10 mm) each with two adults (1 male and 1 female). The dishes were placed on a shelf at 26°C ± 2 and RH of 65 ± 2%, and left for 24 h to allow for oviposition, after which the insects were removed. The number of eggs on the seeds was counted 7 DAI, by which time the larvae had hatched and bored into the seeds as indicated by the cream color of the eggs. After 2 weeks, the various treatments were examined daily for adult emergence. Emerged adults were removed and counted daily. The total developmental time (TDT) was calculated and the sex and weight of each insect determined. Observations were terminated 2 weeks after the first adult emerged. Other variables scored were number of eggs per seed, percentage adult emergence (i.e. proportion of adults that emerged from number of eggs laid on seeds) and growth index (GI) which is expressed as $\ln S/T$, where S = % adult emergence and T = developmental time.

Maruca pod borer bioassays were performed in 24-well tissue culture trays (Nu-Trend Container, Corrrigan and Co. Inc., Jacksonville, FL, USA). Freshly prepared media (Jackai & Raulston, 1988) was dispensed into each well and allowed to cool prior to the addition of lectin solution, prepared in sterile water, onto the surface of the medium. The final concentration of lectin in the diet was 5.0%, 10.0%, 20.0% or 35.0% (wt/wt). As controls, diet was used with water or bovine serum albumin added instead of lectin solution. Once the solution had penetrated into the diet, each well was infested with three first instars. The entire tray was then sealed by a layer of Mylar film (Clear Lam Packaging Inc., Elk Grove Village, Illinois, USA) to prevent larval escape, as well as diet dessication. Holes were made into the film using a small insect pin (size 3) to facilitate air exchange. For each treatment, either 12 or 24 wells were used, with 2–3 replicates.

Larval mortalities were taken at 7 and 10 DAI. Larval weights were taken 10 DAI. Other parameters scored were number of pupae, time taken to pupation, time taken to adult emergence and number of emerged adults. Analysis for significant differences in TDT, GI, larval weights and survival were conducted using SAS GLM ($p < 0.05$) (SAS Users Guide, 1989).

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