DEVELOPMENTAL EFFECTS OF FEEDING TOBACCO BUDWORM LARVAE SALT-TOLERANT COTTON CALLUS TISSUE

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Abstract

Cotton callus was determined to be a suitable bioasay for the tobacco budworm, *Helicopvera virescens*. *H. virescens* larvae were fed one of three treatments: commercial diet (control), salt-sensitive cotton callus (0 mM NaCl), or salt-tolerant cotton callus (150 mM NaCl). The larvae feeding on either variety of callus showed a significant reduction in developmental and survival rates when compared to larvae fed the commercial diet. The larvae fed salt-tolerant callus also showed significant reduction in development and survivability when compared to the larvae fed salt-sensitive callus. The negative effects could be due to higher concentrations of antioxidant enzymes or the salt content.

Introduction

Most plants produce insufficient amounts of antioxidants to overcome oxidative damage caused by reactive O_2 species when under stress (Harper and Harvey, 1978; Dhindsa and Matowe, 1981; Rabinowitch and Fridovitch, 1983; Wise and Naylor, 1987; Monk and Davies, 1989; Spychalla and Desborough, 1990; Cakmak and Marschner, 1992; Polle et al., 1992; Asada, 1994; Krause, 1994). Oxidative damage has been shown to be decreased in plants which have high levels of antioxidants (Harper and Harvey, 1978; Dhindsa and Matowe, 1981; Wise and Naylor, 1987; Monk and Davies, 1987; Spychalla and Desborough, 1990; Mandamanchi and Alscher, 1991; Polle and Rennenberg, 1994). A salt-tolerant cell line of Coker 312 cotton, developed for areas with high saline soil and irrigation source, has been characterized at LSUS (Gossett *et al.*, 1994a, 1994b, 1996). This salt-tolerant cell line shows 0% growth reduction when grown at 150 mM salt concentration; the salt-sensitive variety of Coker 312 shows a 96% growth reduction when grown with 150 mM salt concentration (Gossett *et al.*, 1996). The cotton has been shown to overcome salt stress because of increased levels of antioxidants: catalase (341%); peroxidase (319%); glutathione reductase (287%); ascorbate peroxidase (450%); γ - glutamylcysteine synthase (224%); and glutathione S-transferase (500%) (Gossett *et al*, 1996). This salt-tolerant variety also appears to have developed a cross tolerance to paraquat, a bipyridinium herbicide that produces strong oxidants (Gossett *et al*, 1996). Soybean plants have been shown to have a reduction in nutritive value when stressed by wounding. The reduction of a soybean protein results in 24-63% decrease growth in *Helicopvera zea* as well as caused oxidative damage to their midgut (Felton *et al*, 1994). It was also shown that *H. zea* consume less valuable nutrients because the plant expends more energy forming defensive proteins than forming nutrients (Bi *et al*, 1994). Plants have also been shown to increase allelochemical production in the presence of reactive O_2 species (Sequiera, 1983; Neupane and Norris, 1990). Burden and Norris (1994) showed that iodoacetic (IAA), a stress factor for *Glycine max*, induced the production of allelochemicals. Neupane and Norris (1992) reported that applications of antioxidants induce production of allelochemicals. It was our purpose to determine if cotton callus could be used as a screening method and if salt-tolerant callus would effect the development of the tobacco budworm.

Materials and Methods

Maintenance of the Tobacco Budworm

Eggs were obtained from a field colony at the Red River Research Station (Bossier Parish, LA). One neonate larva was transferred to a 1-ounce plastic cup (Solo Cup Co.) half filled with commercial diet, prepared as suggested by the manufacturer without mold inhibitor added (Southland Products). Each cup was covered with a 4 cm paper lid (WMLA Inc.). The larvae were placed in a growth chamber set at 88°F. They were left untouched until pupation, at which time, they were sexed to ensure mating. The pupae were placed in a 1 gallon paper container . The lids were replaced with cheese cloth as a substrate for egg-laying. The adults were given a 8.5% sucrose/ water solution. The cheese cloth was replaced each day eggs were laid. The same commercial diet used for the maintenance was used as the control. The 1 ounce diet cups were half filled and covered with paper lids.

Callus Treatments

Both 0 mM and 150 mM NaCl Coker 312 cotton callus was obtained from Dr. Dalton Gossett (LSUS). New media was made and callus was transferred monthly. The callus generation media was prepared by adding: MS salts (Murashige and Skoog, 1962), 4.4 g/L Gamborg's vitamins (Gamborg, 1978), 0.75 g/L $MgCl_2$; 100ul/L,2,4-D; 100ul/L Kinetin; 30 g/L glucose; 2.2 g/L phytagel adjusted to pH 5.8 (Trolinder and Goodin, 1987). (All chemicals were purchased from Sigma). Salt-sensitive callus was transferred to media with a 0 mM NaCl concentration; salt-tolerant cell line was placed on media with a 150 mM NaCl concentration. The callus was grown in a lighted growth chamber set at 30°C. The amount of callus given to *H. virescens* was determined by the larval size. A piece of callus 5-8 times the size of the larva was given to each larvae. The callus was placed in a 1 oz plastic diet cup on top of moist filter paper (Fisherbrand). New callus was given daily; the filter paper was replaced as needed.

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Insect Measurements

For each of three treatments (commercial diet, 0mM NaCl, and 150 mM NaCl callus), the insects were handled in the same manner. Each treatment was carried out at room temperature with 15 neonate larvae for each of three replications. The larval weight was measured daily on an analytical balance to the nearest 0.0001 g. All data was collected every 24 hours, plus or minus three hours. The weight of the first instars was below the readability of the balance, the average weight of 800 larvae was calculated. Once the sixth instar showed a 0.0050 g or greater weight loss, the larva was allowed to pupate without further handling. The instar stage was also recorded. After pupation, a final weight was taken. The pupae were sexed to ensure mating could occur. Individual emergence of pupae into adults was also noted. The adults were housed in one gallon containers covered with cheesecloth. The cheese cloth was removed each day after eggs were observed. The adults were given an 8.5% sucrose/water solution.

Dry Weight of Grass

The frass was weighed and removed daily. Because of the high moisture of the callus, the dry weight was determined. The frass from each treatment was collected from the individual larva and weighed collectively. The frass was put in an oven set at 55°C to remove the moisture. The average percentage of drying was calculated and used to adjust the weight of the moist frass collected daily.

Results

The development of the first and second instars (Table 1) showed no significant difference between larvae fed diet and 0 mM NaCl callus. The development of the larvae fed 150 mM callus was greatly reduced in comparison. The larvae fed 150 mM did not survive past the second instar. The third instars fed diet and 0 mM callus also showed no significant difference in weight; however, there were significant differences (student's t-test, $p<0.05$) between the fourth, fifth, and sixth instars and pupae. Larvae raised on the diet had the fastest developmental rate. All of the diet larvae pupated within 40 days, compared to 65 days for complete pupation for the larvae raised on the salt-sensitive callus. The difference in developmental rates was obvious after the third stadium. The data for pupae fed 0 mM callus is representative of three pupae which had an average weight of 0.15 g. The average weight of the larvae fed diet was 0.25 g. Even though the 0 mM callus larvae had such a reduced development, all three adults emerged and were much smaller than adults fed diet. The percent survival (first instar larvae to adult emergence) of the tobacco budworms fed diet was 65% , those fed salt-sensitive callus had a 7% survival. There was a 100% mortality of larvae fed 150 mM callus during the second instar. The diet larvae had an average frass production of 0.015 g (dry weight) where as the salt-sensitive larvae produced only 0.004 g. Frass was observed from the salt-tolerant callus; however, it was not enough to weigh.

Discussion

A method for successfully feeding larvae cotton callus was achieved. The larva fed the salt-tolerant callus had a 100% mortality rate during the second instar. Those fed the salt-sensitive callus showed a decreased development, larval weight, pupal weight and survival and increased stadia periods when compared to those fed commercial diet. The cotton callus was not a quality diet for the larvae. The combination of lower frass production and the decreased weight and development of the larvae fed callus compared to those fed commercial diet suggests a nutrient deficiency associated with the callus.

Further investigations are being carried out to determine whether the increased levels of antioxidant enzymes, the salt content, or oxidative damage to the insects' midguts resulted in the a 100% mortality of the salt-tolerant callus.

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Table 1 Effect of Treatments on Weight (g±s.e.).

Stage	Diet	OmM Salt	150 mM Salt
Instar 1	.001±9.5E-5	.001±1.3E-4	2.6E-4±3.1E-5
Instar 2	.006±.001	.006±.001	.003±4.4E-4
Instar 3	.027±.002	.024±.002	0
Instar 4	.106±.004	.071±.002	0
Instar 5	.238±.011	.130±.005	0
Instar 6	.384±.005	.212±.004	0
Pupae	.246±.0001	.154±.006	0