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THE EFFECT OF CONDENSED TANNINS IN LOTUS SPP. ON THE LESSER MIGRATORY GRASSHOPPER

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ABSTRACT

Hatchling grasshoppers (*Melanoplus sanguinipes* Fab.) fed one accession of field grown big trefoil (*Lotus uliginosis* Schkuhr) containing a tannin content of 56 mg. g^{-1} FW, had a significantly lower mean weight than hatchlings fed field grown *Lotus* species and accessions with lower tannin content. Adult female grasshoppers fed *L. uliginosis* foliage with 32.3 mg.g^{-1} FW tannin also ate more feed and had a 2-fold higher gut trypsin and chymotrypsin activity than adults fed low tannin *L. corniculatus* foliage (0.13 mg.g^{-1} FW tannin). In more controlled experiments, growth at 20^{o} C or 30^{o} C was used to increase tannin in rooted cuttings of *L. uliginosis* to concentrations ranging from 7 to 195 mg.g^{-1} FW. Hatchling weight decreased as a function of dietary tannin concentration when fed these clones. Both hatchling weight and survival were affected when hatchlings were fed pelleted wheat seedlings supplemented with purified *L. uliginosis* tannin preparations.

KEYWORDS

condensed tannins, *Lotus* spp., grasshopper, *Melanoplus sanguinipes*

INTRODUCTION

Consensed tannins are polymeric flavan-3-ols which often have detrimental effects on insects (Klocke and Chan, 1982). Their major effect has been attributed to a non-competitive association with enzymes and dietary protein (Butler et al., 1984; Griffiths, 1986). Many insects have evolved mechanisms which could counter the effects of condensed tannins on their digestive physiology. These include an alkaline pH in the midgut (Berenbaum, 1980), surfactants in gut fluid (de Veau and Schultz, 1992; Martin et al., 1985), high cation concentrations (Martin et al., 1985), extreme reducing conditions in the midgut (Appel and Martin, 1990) and increased production of peritrophic membrane (Barnehenn and Martin, 1992; Bernays et al., 1981). Here, we present the response of an important Canadian prairie insect, the polyphagous grasshopper, *Melanogaster sanguinipes*, to dietary condensed tannins.

MATERIALS AND METHODS

Plant growth and assays for condensed tannin content: Field grown, mature *L. uliginosis* accessions were grouped into high, intermediate and low tannin plants. Ten representative clones of each group were grown from root cuttings in a growth chamber at 20^{o}C/15^{o}C or 30^{o}C/25^{o}C (light/dark;16h/8h) and under identical light conditions. Condensed tannin was measured by the vanillin-HCl and butanol:HCl methods, using (+)-catechin or a condensed tannin polymer purified from *L. uliginosis* as standards, respectively (Dalrymple *et al*., 1984; Watterson and Butler, 1983). A 200 mg ground composite of leaves picked from the upper half of shoots from each plant was assayed. Condensed tannin was extracted from 200 g fresh *L. uliginosis* foliage using 70% aqueous acetone-0.1% ascorbic acid. The acetone fraction was reduced to the aqueous phase under vacuum, and partitioned against petroleum ether and ethyl acetate. The remaining aqueous was mixed with an equal volume of methanol and applied to a Sephadex LH20 column (15 cm x 4 cm, Pharmacia). The column was washed with 50% acetone until the eluate was clear, then condensed tannins eluted using 70% acetone.

Behavior and digestive physiology of grasshoppers exposed to dietary condensed tannins: In an initial experiment, the behavioral response of 2nd instar grasshoppers from a non-diapausing laboratory strain (Hinks and Erlandson, 1994) was monitored in choice and no-choice tests when fed Diet A, consisting of a five day regime of freshly cut foliage from August field plots of either *L. uliginosis* and *L. corniculatus*. The response was compared to a diet of 16 cm wheat seedlings cv. Katepwa (zero tannin performance control). Subsequently, 1st, 3rd or 5th instar nymphs in groups of 25 were subjected to one of two five-day no-choice diets, B or C, using protocols developed according to Westcott et al. (1992). [Diet B: foliage of one of the *L. uliginosis* growth chamber grown clones fed daily in cages, each clone with a different content of tannin. Diet C: 8-mm diameter pellets prepared from 80 mg aliquots of lyophilized, milled 16 cm wheat seedlings supplemented with *L. uliginosis* purified condensed tannin, fed in Mason 1qt. canning jars.] At the end of the 5th day, grasshoppers were frozen, counted, dried and weighed. Whole guts of 1st, 3rd and 5th instar grasshoppers undergoing diet B were cut open and washed free of gut contents. Tannin in guts and frass was assayed qualititatively using butanol-HCl.

Groups of 10 freshly moulted adult grasshoppers were fed for five days on either growth chamber grown *L. uliginosis* (high tannin) leaves, field grown *L. corniculatus* (low tannin) leaves or on 16 cm wheat or oat seedlings (high and low protein performance controls, respectively), then killed by freezing. Trypsin and chymotrypsin activities were measured at 30^{o} C on midgut dissected from each grasshopper, using α-*N*-benzoyl-$_{DL}$-arginine-p-nitroanilide and *N*-benzoyl-$_{L}$-tyrosine ethyl ester as artificial substrates, respectively (Hinks et al., 1991). The consumption ratio (amount consumed as a function of remaining feed), the % gain in body weight and the weight and condition of the ovaries were recorded. The % N was determined in both the frass and the *Lotus* diet, then used to calculate % leaf nitrogen (protein) utilized by the adult grasshoppers. The feeding behavior and size of reproductive organs in adults was also observed.

RESULTS AND DISCUSSION

In initial feeding behavior trials, weights of 2nd instar grasshoppers fed low tannin field grown *L. corniculatus* cv. Empire (SL-644) or *L. uliginosus glabrusculus* (SL-7569) foliage, i.e. <40 mg.g^{-1} DW tannin, or zero tannin wheat seedlings were not significantly different in choice feeding tests. However, weights of grasshoppers were significantly lower when fed the higher tannin field grown *L. uliginosis* (SL-7569, 56 mg.g^{-1} DW tannin). As well, grasshoppers preferred to feed on the latter species. Hatchling surival on *Lotus* foliage ranged from 84-92%, compared with 100% survival on wheat seedlings. Newly moulted adult females fed *L. uliginosis* foliage (32.3 mg.g^{-1} FW tannin) for five days utilized 26.5% of the leaf nitrogen (i.e. protein), compared with 30.9% of N from *L. corniculatus* foliage (0.13 mg.g^{-1} FW tannin). The lower N utilization by adult females fed the *L. uliginosis* diet was accompanied by a 1.2-fold increase in the consumption ratio , as well as a two-fold increase in total midgut trypsin and chymotrypsin activity. Similarly, a 1.8-fold increase in adult gut tryptic activity was observed with a diet of high protein wheat seedlings (cv. Katepwa) compared with low protein oats. The *Lotus* field diet results imply that the grasshoppers compensated for low available protein caused by sublethal concentrations of tannin in their diet. They may explain why condensed tannins do not always appear to negatively affect protein digestion in insects. Since dry, hot field conditions are the major factors which contribute to high grasshopper damage on the Canadian prairies, first instar grasshoppers were subjected to feeding trials using leaves from *L. uliginosis* clones grown in a growth chamber either at 20°C or 30°C. Under these conditions, the condensed tannin content rose as a function of both temperature and plant development, resulting in clones with tannin levels from 7 to 195 $mg.g^{-1}$ DW. Tannin content in many of these clones was 4-fold higher than in the harvested field grown plants. Red patches of anthocyanin were observed in some clones exposed at length to the higher temperature. Anthocyanin-containing foliage was not selected for use in feeding trials. Initially, the 1st instar nymphs tended to eat more of the low tannin clones. However, when the tannin concentrations exceeded typical field plant levels, the hatchlings tended to eat less foliage. Mean hatchling weight declined significantly with increasing tannin content in the foliage, regardless of the plant growth temperature (Fig. 1). Survival and mean weight were also determined for grasshoppers fed milled wheat seedlings supplemented with a high content of purified *L. uliginosis* condensed tannins (Fig. 2). Dry weight of 1st instar nymphs decreased as a function of dietary tannin concentration without affecting survival up to a threshold of 10% tannin. At 20% dietary tannin, both weight and survival of 1st instar nymphs decreased to 60%, compared with the wheat diet alone. When a similar content of tannin was included in the wheat diet, a mucous-like material was found lining the midguts of 3rd instars. Tannin was not observed in the washed gut, but was found in the frass when 1st, 3rd and 5th instars were tested. Our results outline the effect of dietary tannins on Canadian grasshopper digestive physiology at both lethal and sublethal concentrations. They indicate that crop plants which contain tannin and which can induce high concentrations under conditions which bring on grasshopper infestations will deter grasshopper populations from multiplying.

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Figure 1
The relationship between hatchling grasshopper weight after five days feeding and the concentration of condensed tannin in *Lotus uliginosis* diets. Error bars indicate standard error, N=6. Where no error bars, error was within the data point boxes. Each replicate contained 25 insects.

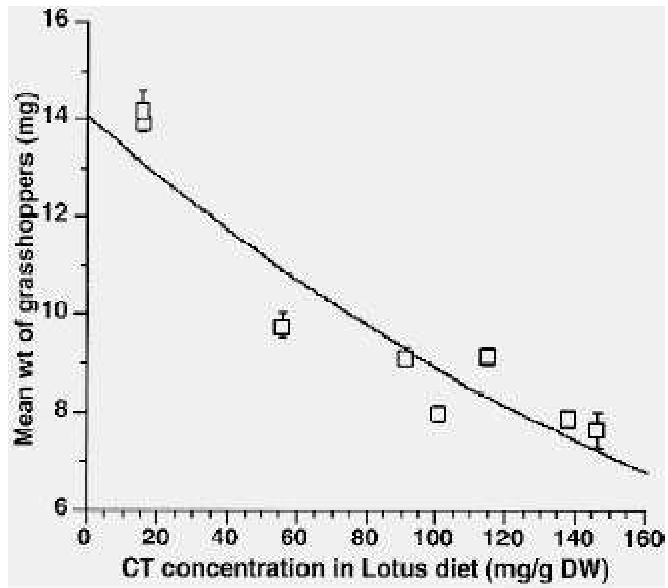

Figure 2
The relationship between survial (filled box) and mean weight (open box) of hatchling grasshoppers after five days feeding as a function of a wheat diet supplemented by purified *L. uliginosis* condensed tannin. Error bars indicate standard error, N=6. Where no error bars, error was within the data point boxes. Each replicate contained 25 insects.