Effects of synthesized tannin on the growth and survival of a generalist herbivorous insect, the common cutworm, *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae)

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Abstract

We examined the effects of tannin on a generalist herbivorous insect, the common cutworm (*Spodoptera litura*). The dosage effect was estimated by measuring the growth performance of the cutworm when the larvae were fed artificial diets containing different amounts of synthesized tannin. We used artificial diets to exclude the effects of non-tannin defense mechanisms in plant foliage. Indices of growth performance, number of dead larvae, pupal weight, total feeding amount and length of larval period were obtained. We found that tannin inhibited the growth of the cutworm larvae and that the inhibitory effect was proportional to the amount of tannin ingested. These results support the traditional theory that plant tannin is a defense agent against generalist herbivores, and that its effects are proportional to its concentration in foliage.

Key words: Anti-herbivory defense, common cutworm, generalist herbivore, tannin

INTRODUCTION

Many plants contain tannin (Bate-Smith, 1957, 1962; Harborne, 1976; Swain, 1976; Schoonhoven et al., 1998). Feeny (1968, 1969, 1970) suggested that oak tannin was important in the protection of foliage from caterpillar damage. After his work, the general significance of tannin in ecology received much attention, although some reviews challenged generalization (Rhoades and Cates, 1976; Scriber and Ayres, 1988; Bernays et al., 1989; Clausen et al., 1992; Feeny, 1992). The best known theory is that of constitutive chemical defense, which suggests a toxin like tannin differs from induced toxins in that its effectiveness against herbivores is less potent, more gradual, and more difficult to circumvent through adaptation. However, these predictions have not always held up to experimental study. Some studies have reported that tannin has no effect on certain herbivores (Bernays et al., 1980; Klocke and Chan, 1982; Manuwota and Scriber, 1986; Martin et al., 1987; Smith et al., 1992; McArthur and Sanson, 1993). One study has even suggested that tannin can be utilized by herbivores as a nutrient substrate (Bernays and Woodhead, 1982).

Although the constitutive chemical defense theory predicts that plant tannin is an anti-herbivory defense agent against generalist herbivores and that the effects of tannin are proportional to the levels of tannin present (Rhoades and Cates, 1976; Scriber and Ayres, 1988; Bernays et al., 1989; Schultz, 1989; Clausen et al., 1992; Feeny, 1992), only a few studies have measured the dosage response of herbivores to tannin (Feeny 1968; Ayres et al., 1997). Feeny (1968) measured the dosage effects of the oak tannin on the growth of a generalist herbivore, the winter moth (Lepidoptera: *Operophtera brumata* (L.)). He reported that the inhibitory effect of oak tannin on the growth of the winter moth was correlated with tannin levels. Ayres et al. (1997) measured the dosage effect of two kinds of tannins, *Populus tremuloides* Michx (Salicaceae) and *Betula resinifera* Britton (Betulaceae), on the growth of two herbivores, *Pyrrhalta lutea* (Müller) (Coleoptera) and *Chrysomela falsa* Brown (Coleoptera). *B. resinifera* tannin had a significant dosage effect on the growth of *C. falsa*, but no significant effect on the growth of *P. luteola*; conversely, *P. tremuloides* tannin had a significant dosage effect on the growth of *P. luteola*, but no significant effect on the growth of *C. falsa*.

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In this study, we measured the dosage effect of synthetic tannin on a generalist herbivorous insect, the common cutworm (*S. litura*), which is known to feed on at least 77 species of dicotyledonous plants (Okamoto and Okada, 1968). To measure the effect of tannin, we modified Feeny's method (1968); that is, we observed the inhibitory effect on the growth of the cutworm reared in the laboratory on an artificial diet with tannin. We used synthesized tannin to exclude the effects of other kinds of tannins found in plants, and to exclude the effects of non-tannin defense mechanisms.

MATERIALS AND METHODS

Insect. The common cutworm is a polyphagous herbivore and is widespread from Asia to Australia. The strain used in this study has been maintained for 25 years in the laboratory. Larvae and adults were reared at $28 \pm 1^\circ\text{C}$ and under a 16L : 8D photo regime. Larvae of the common cutworm are able to complete their growth even when they are fed a strictly artificial diet.

Chemical. Tannin ($\text{C}_6\text{H}_2(\text{OH})_3\text{COOC}_6\text{H}_2(\text{OH})_2\text{COOH}$; 85%) was purchased from Funakoshi Chemical Industries, Ltd. (Tokyo, Japan). Five different amounts of tannin and 2 ml of distilled water were mixed into 10 g of food (Insecta LF; Nihon Nosankougyou Co., Ltd., Japan). The mixture was stored in a plastic cup at 4°C until use to prevent desiccation and decay.

Assessment of the effects of tannin on the growth of cutworm larvae. To determine the effect of tannin on the common cutworm, we conducted a bioassay. We placed 10 first-stadium larvae within 12 h after hatching in each of 72 plastic boxes ($22 \times 14 \times 3.7$ cm). The 72 plastic boxes were assigned to six treatments (12 replications per treatment). Each box was supplied with an artificial diet containing tannin. Every two days, larval survival was checked and food was replenished. The larvae were reared at 28°C in the laboratory. Pupal weight was measured one day after pupation. The pupae were stored at $28 \pm 1^\circ\text{C}$ until eclosion.

The total amount eaten in each replication was also measured. Called the total feeding amount (TFA), this was calculated as follows:

$$\text{TFA} = \sum \{1 / N \times (D_{\text{initial}} - D_{\text{leftover}} \times k)\},$$

where N is the number of surviving insects in each replication each day, D_{initial} is the fresh weight of the diet just before feeding, D_{leftover} is the dry weight of the leftover diet, and k is the ratio of fresh weight to dry weight of the artificial diet containing tannin. Based on TFA, we calculated the mean amount of tannin eaten (MATE) as follows:

$$\text{MATE} = \text{TFA} \times \text{tannin concentration of artificial diet}$$

RESULTS

The effects of tannin on the total mortality of the larval stage, pupal weight, larval period, and TFA

Table 1. Effects of tannin on the growth performance of the common cutworm (*S. litura*)

Doses (mg/10 g)	Mean amount of tannin eaten ^a (mg/insect)	<i>N</i>	No. survivors until pupa	No. survivors until adult	Pupal weight (g $\pm$ SD)	Larval period (day $\pm$ SD)	Total feeding amount (g $\pm$ SD)
Control	0	120	118 a	109 a	0.35 ab $\pm$ 0.06	17.4 a $\pm$ 1.3	4.87 a $\pm$ 0.38
2	0.73 $\pm$ 0.07	120	109 a	84 b	0.34 bc $\pm$ 0.06	17.5 a $\pm$ 1.4	3.65 b $\pm$ 0.37
5	1.78 $\pm$ 0.20	120	106 a	73 b	0.35 ab $\pm$ 0.05	17.9 a $\pm$ 1.4	3.56 b $\pm$ 0.39
10	3.92 $\pm$ 0.13	120	100 a	70 b	0.37 b $\pm$ 0.06	18.9 b $\pm$ 1.5	3.92 bd $\pm$ 0.13
20	6.56 $\pm$ 0.34	120	101 a	69 b	0.32 c $\pm$ 0.05	20.0 c $\pm$ 0.8	3.28 c $\pm$ 0.17
40	17.20 $\pm$ 0.96	120	47 b	30 c	0.26 c $\pm$ 0.07	38.1 d $\pm$ 3.9	4.30 ad $\pm$ 0.74
Statistics from ANOVA							
<i>F</i>			24.5	26.6	23.5	1249.6	19.7
d.f.			5	5	5	5	5
<i>p</i>			<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

^a See text for the calculation of total amount of tannin eaten.

Means followed by the same letter are not significantly different (Scheffé's-*F* test at $p < 0.05$). Data are given as mean $\pm$ SD.

Fig. 1. The hazard curves of cumulative survival rates of the common cutworm (*S. litura*) larvae that fed on artificial diets containing various amounts of tannin: 2 mg (open circles); 5 mg (open triangles); 10 mg (open squares); 20 mg (solid circles); and 40 mg (solid triangles). Crosses indicate the hazard curve in control treatment (artificial diet without tannin). The hazard curves of the cumulative survival rates end on the day when all larvae were dead or reached the pupal stage.

were all significantly different among the six treatments (ANOVA, summarized in Table 1).

The hazard curves of the cumulative survival rates were significantly different among 6 treatments (Logrank-test, $\chi^2=35.02$, $p<0.0001$; Fig. 1). The total numbers of mortalities throughout the larval stage in the 20 and 40 mg tannin treatments were significantly higher than that in the control treatment (Scheffé's- F test at $p<0.05$; Table 1). The totals of mortalities throughout the larval stage in the 2, 5 and 10 mg tannin treatments were not significantly greater than that in the control treatment.

The pupal weights in the 20 and 40 mg tannin treatments were significantly less than those in the control treatment (Scheffé's- F test at $p<0.05$; Table 1).

The larval periods in the 10, 20 and 40 mg tannin treatments were significantly longer than those in the control treatment (Scheffé's- F test at $p<0.05$; Table 1).

The TFAs in the 2, 5, 10 and 20 mg tannin treatments were significantly less than that in the control treatment (Scheffé's- F test at $p<0.05$, Table 1).

The TFA in the 40 mg tannin treatment was not significantly less than that in the control treatment. Because the larval period in the 40 mg tannin treatment was much longer than that in the control treatment, larvae in the 40 mg tannin treatment ate more.

DISCUSSION

Research on tannin has been plagued by methodological difficulties. Although many studies measured the inhibition effects of tannin on the growth of herbivores when they were fed on plant leaf containing tannin, results from those studies could not separate the effects of tannin from the effects of non-tannin defense mechanisms. Moreover, many studies have not used purified tannin. In some studies, purified tannin was used; however, about the effects of tannin on herbivore growth differed (Feeny, 1968; Ayres et al., 1997).

In this study, we used synthesized tannin to minimize these methodological difficulties. We measured the effect of tannin on the growth of a generalist herbivore, the common cutworm. Tannin reduced the survival rate, pupal weight and TFA and extended the larval period (Table 1). It is well known that the pupal weight correlates with fecundity in many insect species (Scriber and Slamsky, 1981). In the common cutworm, as well, the pupal weight is positively correlated with the fecundity ($Y=1287X-1416$, $r=0.79$, $p<0.0001$, $n=40$, pupal weight range: 0.258–0.430 g; unpublished data). The cutworm fed a diet containing tannin might produce fewer offspring than that fed a diet without tannin. Moreover, the extended larval period may increase the risk of predation and parasitizing in the wild. Thus, tannin may decrease plant damage both directly, as a deterrent, and indirectly, in reducing herbivore populations. Our results corroborate the theory that tannin is an anti-herbivory agent (Rhoades and Cates, 1976; Scriber and Ayres, 1988; Bernays et al., 1989; Schultz, 1989; Clausen et al., 1992; Feeny, 1992).

It is widely assumed that the effect of tannin on herbivores is proportional to tannin level (Feeny, 1968, 1992). However, only a few studies have measured the dosage effect of tannin (Feeny, 1968; Ayres et al., 1997). Our study correlates the inhibition effect of tannin on the growth of the common cutworm with tannin amounts (Table 1). Our re-

sults support Feeny's conclusion that the effect of tannin is proportional to the tannin level (Feeny, 1968, 1992).

The common cutworm has a wide host range (Okamoto and Okada, 1968). Therefore, we assumed that the effect of tannin on cutworm growth represents the constitutive chemical defense of plants containing significant levels of tannin. However, some studies have reported that tannin has no effect on the growth of other generalist herbivorous insects (Bernays and Chamberlain, 1980; Ayres et al., 1997). Clearly, in the generalist herbivores, the reactions to tannin differ among species. The effects of tannin on individual herbivorous insects need to be clarified through further research. Moreover, some researches have shown that tannin structures differ among plant species and that different tannins play different ecological roles (Zucker, 1983; Marini Bettola et al., 1986; Clausen et al., 1990; Hagerman and Robbins, 1993; Mole, 1993; Ayres et al., 1997). The relationship between tannin structural variations and functions needs to be clarified through further research.

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