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COMPARATIVE EFFECTS OF TWO PLANT SECONDARY METABOLITES ON HOST–PARASITOID ASSOCIATION

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Abstract—Two plant-derived allelochemicals, berberine and α-terthienyl (α-T), were tested for their effects on the European corn borer, *Ostrinia nubilalis*, and its endoparasitoid *Diadegma terebrans*. The compounds were administered to the host insect in meridic diets, and the responses of the host larvae and parasitoids reared from treated hosts were measured in terms of growth parameters and survival. In *O. nubilalis*, survival to pupation and adult emergence were reduced significantly by the inclusion of berberine and α-T in larval diets at a concentration of 100 μg/g. However, in the parasitoid, adverse effects were much more apparent with the α-T treatment than with the berberine treatment. α-T and one of its metabolites were found in host larvae and in emerged adult parasitoids and their cocoons. Berberine residues were not detected. The implications of these responses to compounds of widely differing physiological properties are discussed with reference to host-plant resistance and biological control.

Key Words—Third trophic level interaction, *Ostrinia nubilalis*, Lepidoptera, Pyralidae, *Diadegma terebrans*, Hymenoptera, Ichneumonidae, α-terthienyl, berberine, allelochemicals.

INTRODUCTION

The effects of secondary plant substances on the parasitoids of phytophagous insects have been the subject of few publications. Thurston and Fox (1972) demonstrated that nicotine in the diet of the tobacco hornworm, *Manduca sexta* (L.), reduced the emergence of *Cotesia congregata* (Say) (=*Apanteles congregatus*). Campbell and Duffey (1979, 1981) demonstrated that α-tomatine in the host

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diet had negative effects on *Hyposoter exiguae* (Viereck), a parasitoid of the tomato fruitworm, *Heliothis zea* (Boddie). Apparently, α-tomatine was not directly toxic, but rather produced these effects by imparing sterol utilization.

The effects of nicotine on two parasitoids—*C. congregata* (a parasitoid of *M. sexta*) and *Hyposoter annulipes* (the parasitoid of the fall armyworm, *Spodoptera frugiperda* [Smith],—were studied in detail by Barbosa et al. (1986). As found by Thurston and Fox (1972), they observed reduced emergence for both parasitoid species. They also reported that cocoon formation was inhibited. In *H. annulipes*, the toxic effects of nicotine were more severe; sublethal effects were also apparent, including prolonged larval development and reduced adult size.

Toxic effects on the third trophic level were also recently reported for the tomato phenolic rutin (Duffey et al., 1986). In this case, the lethal and sublethal effects of *H. exiguae* depended on the nutritional quality of the host's diet, particularly with respect to levels of protein. For each of the two noctuid hosts *H. zea* and *Spodoptera exigua* (Hbn.), specific levels of protein in host diets were found to enhance the expression of toxicity in the parasitoids.

Nicotine (Barbosa et al., 1982, 1986) and α-tomatine (Campbell and Duffey, 1979) were shown to persist in the host and were detected in emerged parasitoids reared from alkaloid-fed hosts. Carotenoids (Rothschild et al., 1977) and pyrrolizidine alkaloids (Benn et al., 1979) from food plants have also been found to persist in and travel through herbivorous insects into their parasitoids.

Investigations of three trophic level interactions have focused on the effects of one particular toxin within these host–parasitoid food chain models. We have examined the physiological effects of two secondary plant substances with different physiological properties and biological sites of action on the European corn borer, *Ostrinia nubilalis* (Hübner) (Pyralidae), and its endoparasitoid *Diadegma terebrans* (Gravenhorst) (Ichneumonidae). *O. nubilalis* is a polyphagous species (Caffrey and Worthley, 1927; Hodgson, 1928), and as such has been exposed to a wide range of allelochemicals during its evolutionary past.

The two compounds used in this study were berberine (Figure 1), an iso-

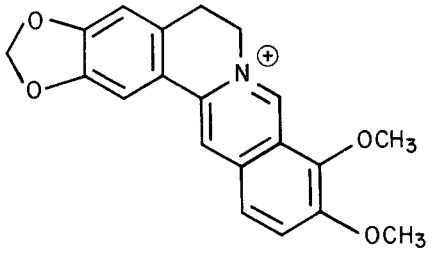

FIG. 1. Molecular structure of berberine.

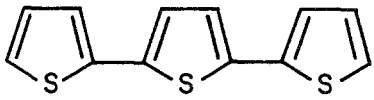

FIG. 2. Molecular structure of α-terthienyl.

quinoline alkaloid found in at least 26 genera of 10 plant families (Suffness and Cordell, 1985), and α-terthienyl (α-T) (Figure 2), a thiophene characteristic of numerous genera of the Asteraceae (Bohlmann et al., 1973), including most genera of the subtribe Pectinidae (Downum et al., 1985). The two compounds are different with respect to polarity: berberine is a quaternary ammonium compound (cationic), while α-T is highly lipophilic (McLachlan, 1984). Berberine-containing plants are not normally attacked by *O. nubilalis*, while plants containing thiophenes are among those preferentially attacked by the insect (Caffrey and Worthley, 1927; Hodgson, 1928). The very different physical properties of berberine and α-T provide a basis for studying a range of responses possible in one host–parasitoid system.

METHODS AND MATERIALS

Growth and Survival Studies with Ostrinia nubilalis. A laboratory colony of *O. nubilalis* was maintained by using the methods of Guthrie et al. (1971) except that corncob grits were added to the diet used for routine culture maintenance. The diets used in the experiments were prepared by mixing berberine (dissolved in water) or α-T (95% ethanol solution added to α-cellulose powder and solvent evaporated) to warm, unsolidified meridic diet at concentrations of 10, 31, and 100 μg/g (wet weight). These concentrations are realistic with respect to natural concentrations in plants and were selected after preliminary feeding tests in which chronic effects on *O. nubilalis* could be demonstrated. Control diets were prepared with an appropriate aliquot of water (for the berberine experiments) or α-cellulose (for the α-T experiments). Corncob grits were not included in these diets in order to ensure uniform distribution of the compounds throughout the media.

Experimental larvae were reared in a growth chamber under the following conditions: photoperiod 18:6 hr (light–dark) with a day–night temperature regime of 26.5°C/19°C and a constant relative humidity of 85%. Because both of the experimental compounds are potentially phototoxic, conditions of subdued photosensitizing ultraviolet (UV) illumination were used. Lighting within the environmental chamber was provided by four 20-W solar-simulating flu-

orescent lamps (Vitalight 48T12 U.H.O.) separated from the chamber by a 3-mm sheet of Plexiglas. The resultant light intensity was measured to be 7.6 W/m, with a UV cutoff at 390 nm.

Neonate *O. nubilalis* were placed on the appropriate treated or control diets in glass scintillation vials plugged with cotton wool. After eight days, 10 surviving larvae were randomly chosen from each treatment and placed in individual vials with fresh diet. Subsequently, every four days, vials and diets were changed. The larvae were observed daily and growth parameters were recorded. This procedure was replicated three times, such that data was generated from 30 individuals per treatment.

Growth and Survival with Diadegma terebrans. *O. nubilalis* were reared from hatching on the variously treated and control diets until they were of the instar and weight range of control larvae at age 9 days. At this time, 100 second-instar larvae ranging in weight from 2 to 7 mg were selected from each treatment and placed on 25–30 1 cm pieces of chopped corn stalk (Silver Queen, Stokes Seeds Ltd.) in a round plastic rearing box (18.3 cm diameter, 7.8 cm high). These larvae were allowed to feed and burrow into the corn overnight. The presence of host-plant tissue provided necessary visual and chemical cues for parasitization by *D. terebrans*.

The following morning, the dish with the corn and larvae was placed in the *D. terebrans* rearing cage for 1 hr, which enabled the female wasps to "saturate" the larvae and parasitize all acceptable hosts. The parasitization occurred within a controlled environment chamber (25°C and 70% relative humidity), with a high light intensity (8 W/m^2) provided by six 20-W solar-simulating fluorescent lamps (Vitalight 48T12 U.H.O.). The *O. nubilalis* larvae were then removed from the corn and returned to their appropriate diets in vials. A 5-cm square of paper towelling was placed in each vial above the diet cube. This provided a dry place within which the emerging *D. terebrans* could pupate. Pupae were held in gelatin capsules until adult emergence. Growth parameters were recorded.

As the different treatment larvae were parasitized within one week of each other, separate control groups (100 larvae each) were run for each treatment. Control groups were parasitized one day prior to the treated groups. A full day was left between treatment and control parasitizations in order to allow the female wasps to feed and replenish their ovarioles with mature eggs. In this way, at least 30 larvae were parasitized in each of the treatment and control groups.

Analysis for Body Burden of Compounds in Host Larvae and Parasitoids. The analysis for body burden of berberine and α-T was performed on second- and third-instar *O. nubilalis* larvae which had been reared since eclosion on treated diets and then allowed to feed on fresh corn stalks for 24 hr in order to clear their guts of the compounds. *D. terebrans* adults and cocoons (including the fluid meconia) were collected on the day of adult emergence. Samples of

corn borer larvae, wasps, and cocoons were weighed and preserved in sample groups of three individuals in 2 ml HPLC grad methanol and held at −4 to −6°C until extraction. Three groups of each type of sample were analyzed.

The extraction protocol was as follows. Each sample was thoroughly ground using a 15-ml Wheaton homogenizer. An additional 3 ml methanol was added, and the slurry was futher homogenized. The suspension was refrigerated for two days at 4°C to allow the compounds to dissolve from the insect material. Samples were then vortexed and centrifuged for 10 min at 500*g* to remove macromolecular debris. The supernatant was evaporated to dryness under vacuum using a rotary evaporator. HPLC grade acetonitrile (0.5 ml) was added to the dried sample, and this was then sonicated for approximately 1 min to ensure that the compounds absorbed to the interior of the vials would be dissolved. Samples were then filtered using a millipore filter (Schleicher and Schuell, 0.45 μm), and 20-μl aliquots were analyzed by HPLC.

HPLC was performed using a Beckman system (Series 332), equipped with a model 165 variable wavelength UV–visible light detector. Reversed phase (C_{18}) columns, 25 cm × 4 mm internal diameter, packed with Ultrasphere octadecylsilicate (ODS), pore size 5 μm, were used for both compounds. The ion-pairing system for berberine consisted of 50% 0.1 M trichloroacetic acid (aqueous), buffered to pH 2.15 with saturated NaOH, and 50% acetonitrile, run isocratically at 1 ml/min. Berberine was monitored at 343 nm, one of the maximum absorbance wavelengths for the compound, and the minimum level of detection for this compound was 1.8 ng in a 20-μl injection. The retention time was 11.2 min.

Alpha-T was run isocratically with 85% acetonitrile and 15% water, and monitored at 350 nm. The minimum level of detection for this compound was 0.16 ng in a 20-μl injection, and the retention time under these conditions was 7.0 min. Insect extracts run in both solvent systems yielded very clean chromatograms (no more than two peaks), and the compound (if present) was readily distinguishable. Control larvae, wasps, and pupal cases were also extracted and analyzed in both solvent systems, to ensure that peaks in treated samples were not present in the controls. Untreated insects were also homogenized after injecting them with known amounts of berberine and α-T; the extraction efficiency was 80% for both compounds.

RESULTS AND DISCUSSION

The investigations with the two compounds under study demonstrate that the biological activity of secondary plant substances may be expressed differently in the host than in the parasitoids. The growth and survival studies with *O. nubilalis* alone on diets containing berberine did not demonstrate significant changes in the duration of the developmental stages (Table 1). A significant

TABLE 1. EFFECTS OF DIETARY BERBERINE ON *Ostrinia nubilalis*[a]

Berberine concentration (μg/g)	Growth parameters								Survival	
	Mean time (days) to pupation		Mean pupal weight (mg)		Mean time (days) to adult emergence		Mean adult weight (mg)		Pupation (%)	Adult emergence (%)
	M	F	M	F	M	F	M	F		
0	31.9	35.3	77.2a	100.4a	41.7	44.1	40.8a	53.3a	85	81
10	30.9	31.4	77.3a	93.6ab	41.6	40.9	40.8a	58.2a	89	82
31	34.9	36.8	74.8a	96.3ab	45.3	45.9	41.4a	60.2a	93	83
100	39.3	40.0	70.4a	85.3b	49.7	49.4	39.8a	52.8a	53*	45*

[a]For growth parameters, males (M) and females (F) were analyzed separately. Time measurements were subjected to the Kruskal-Wallis test ($P = 0.05$). Weight measurements were subjected to Duncan's multiple-range test: Means in columns followed by the same letter are not significantly different ($P = 0.05$). The chi square test was applied to data expressed as percentages: significant differences from the controls ($\alpha = 0.05$) are denoted by an asterisk.

reduction in female pupal weight was observed in the 100 μg/g berberine treatment compared to the controls. However, the weights of surviving adults were not significantly different between any of the concentrations tested. Although sublethal effects on growth parameters were not highly pronounced with berberine in the diet, survival to pupation and adult emergence was significantly decreased in the 100 μg/g treatment.

With α-T in the diet, sublethal effects on *O. nubilalis* alone were more obvious (Table 2). Mean time to adult emergence was significantly prolonged for females fed 31 μg/g α-T. With the 100 μg/g treatment, the duration of the developmental stages was increased significantly for both males and females. In addition, pupal and adult weights were significantly reduced with this treatment. These results confirm and extend observations first made by Champagne et al. (1986): the growth rate of *O. nubilalis* larvae was significantly reduced with 100 μg/g α-T in the diet. in the present study, survival to pupation and adult emergence of *O. nubilalis* was also significantly reduced with 100 μg/g α-T in the diet.

Parasitoids reared from hosts fed diets containing berberine did not exhibit sublethal effects in their growth (Table 3), except for an increase in mean pupal weight in the 31 μg/g treatment group. Although no significant differences from the controls were apparent in survival to pupation on any of the berberine treatments, survival to adult emergence was significantly decreased with 100 μg/g berberine in the host's diet. The parasitoids reared from hosts fed 100 μ/g berberine did not incur as much mortality (significantly different by the chi square test at $\alpha = 0.05$) as did the unparasitized host larvae (Table 1), which suggests that the parasitoids were less susceptible than their hosts to the effects of berberine.

Parasitoids reared from hosts fed α-T at concentrations of 10 and 31 μg/g in the diet were similar to the controls in the growth parameters of surviving insects (Table 4). However, the male parasitoids reared from hosts fed 100 μg/g α-T were significantly smaller than the controls. No females were produced in this treatment group. Mean time to pupation and adult emergence was not significantly affected by any concentration of α-T. Survival to adult emergence was somewhat reduced with the 31 μg/g treatment ($P > 0.90$ by the chi square test). However, significant mortality occurred with 100 μg/g α-T in the host diet. The mortality caused by 100 μ/g α-T was significantly higher (by the chi square test at $\alpha = 0.05$) in *D. terebrans* than in the unparasitized hosts. The parasitoid was apparently more susceptible to the thiophene than its host.

The chromatographic analyses for berberine did not show any evidence of the compound either in host larvae (removed to corn for 24 hr) or emerged wasps and cocoons. In contrast, α-T and/or a metabolite were detected in groups of each of the sample types analyzed (Tables 5 and 6). Despite the differences between the detection efficiencies of berberine and α-T (1.8 and 0.16 ng in a

TABLE 2. EFFECTS OF DIETARY α-TERTHIENYL ON *Ostrinia nubilalis*[a]

α − T concentration (μg/g)	Growth parameters								Survival	
	Mean time (days) to pupation		Mean pupal weight (mg)		Mean time (days) to adult emergence		Mean adult weight (mg)		Pupation (%)	Adult emergence (%)
	M	F	M	F	M	F	M	F		
0	23.7	24.8	77.7a	98.3a	32.9	31.2	36.7a	60.3a	93	93
10	22.2	23.5	76.0a	100.1a	30.9	31.9	35.8a	60.2a	93	90
31	22.7	24.7	71.9a	95.2a	31.3	33.3*	34.8a	54.9a	90	83
100	30.7*	37.7*	62.2b	65.5b	39.9*	46.0*	27.7b	27.7b	66*	66*

[a] For growth parameters, males (M) and females (F) were analyzed separately. Time measurements were subjected to the Kruskal-Wallis test: significant differences from the controls ($P = 0.05$) are denoted by an asterisk. Weight measurements were subjected to Duncan's multiple-range test: means in columns followed by the same letter are not significantly different ($P = 0.05$). The chi square test was applied to data expressed as percentages: significant differences from the controls ($\alpha = 0.05$) are denoted by an asterisk.

TABLE 3. EFFECTS OF BERBERINE ADMINISTERED TO HOST LARVAE ON *Diadegma terebrans*[a]

Berberine concentration (μg/g)	Growth parameters								Survival	
	Mean time (days) to pupation		Mean pupal weight (mg)		Mean time (days) to adult emergence		Mean adult weight (mg)		Pupation (%)	Adult emergence (%)
	M	F	M	F	M	F	M	F		
10	17.6	18.2	28.3	28.5	28.1	29.5	9.0	9.6	76	52
Control	16.7	16.8	29.2	32.2	27.4	28.4	8.9	10.7	82	69
31	16.0	17.2	31.0*	31.3	26.5	27.8	9.7	10.3	88	81
Control	16.9	18.0	25.8	32.3	27.3	28.6	8.3	10.0	94	76
100	18.4	18.8	27.9	31.2	28.9	30.4	8.6	9.6	77	61*
Control	17.7	19.6	26.6	28.1	28.1	30.1	8.4	9.2	88	81

[a] For growth parameters, males (M) and females (F) were analyzed separately. Time measurements were subjected to the Kruskal-Wallis test ($P = 0.05$). Weight measurements were subjected to t tests ($P = 0.05$). The chi square test was applied to the data expressed as percentages ($\alpha = 0.05$). An asterisk indicates that the treatment value is significantly different from the control.

TABLE 4. EFFECTS OF α-TERTHIENYL ADMINISTERED TO HOST LARVAE ON *Diadegma terebrans*[a]

α − T concentration (μg/g)	Growth parameters								Survival	
	Mean time (days) to pupation		Mean pupal weight (mg)		Mean time (days) to adult emergence		Mean adult weight (mg)		Pupation (%)	Adult emergence (%)
	M	F	M	F	M	F	M	F		
10	17.6	19.6	26.8	29.8	28.2	30.6	8.1	9.8	92	77
Control	18.1	18.3	29.9	32.9	28.6	29.4	8.9	10.2	89	74
31	18.2	17.8	26.0	31.6	28.3	28.6	8.2	10.5	76	65
Control	17.5	19.1	26.9	33.3	27.6	29.9	8.4	11.4	95	84
100	20.8	ND	22.1*	ND	30.8	ND	7.3*	ND	38*	25*
Control	17.8	19.1	31.5	31.6	28.4	30.3	9.5	9.9	88	76

[a] For growth parameters, males (M) and females (F) were analyzed separately. Time measurements were subjected to the Kruskal-Wallis test ($P = 0.05$). Weight measurements were subjected to t tests ($P = 0.05$). The chi square test was applied to the data expressed as percentages ($\alpha = 0.05$). An asterisk indicates that the treatment value is significantly different from the control. ND = no data available.

TABLE 5. QUANTIFICATION OF α-TERTHIENYL IN INSECT SAMPLES[a]

	Treatment concentrations (μg/g)		
	10	31	100
Host larvae	ND	0.31	0.05
		(0.26)	(0.074)
Adult parasitoids	0.53	0.20	ND
	(0.34)	(0.29)	
Cocoons + meconia	4.17	0.23	3.19
	(2.97)	(0.023)	(1.68)

[a] Units are in μg/g insect material. Values are means of three samples. Standard deviations are in parentheses. ND = not detected at lowest level of detection.

20-μl injection, respectively), an estimated minimum body burden of 1.9 μg berberine per gram insect material would be detectable by HPLC. This was not present even in the pupal cases, where waste products are often execreted as meconia by the metamorphosing parasitoids. The absence of berberine in insect samples is not surprising, considering that berberine is a quaternary ammonium compound and likely to be rapidly excreted (Bodor et al., 1981). The concentration of berberine in the host hemolymph would probably be much less than was originally ingested by the host.

The absence of berberine in insect samples is consistent with the data pertaining to the biological effects of berberine (Tables 1 and 3). Although survival was decreased for both host and parasitoid, the mortality in *D. terebrans* was not as severe as that observed in unparasitized *O. nubilalis*. In fact, mortality during the parasitoid's internal (feeding) stages was not significantly different

TABLE 6. RELATIVE QUANTITIES OF α-TERTHIENYL METABOLITE IN INSECT SAMPLES[a]

	Treatment concentrations (μg/g)		
	10	31	100
Host larvae	1.75	0.75	1.50
	(0.82)	(0.20)	(0.32)
Adult parasitoids	3.50	2.00	3.00
	(1.07)	(0.32)	(2.80)
Cocoons + meconia	9.75	12.75	8.25
	(6.72)	(8.17)	(6.10)

[a] Values are in absorbance units $\times 10^{-5}$/mg insect material, representing the mean of three samples. Standard deviations are in parentheses.

between the berberine treatments and the controls. This suggests that berberine may have indirectly affected *D. terebrans* by reducing the quality of the host as a food source (Duffey et al., 1986), but this was only manifested during metamorphosis in the group reared from hosts fed diets containing 100 μg/g berberine. The results with α-T demonstrate that this compound is clearly capable of accumulating in insect tissues, which is to be expected of a highly lipophilic xenobiotic. Although *O. nubilalis* larvae reared on diets containing 10μg/g α-T did not appear to sequester the thiophene, α-T was present in the larvae with 31 and 100 μg/g α-T. The level of α-T in the larvae fed 100 μg/g α-T was relatively low compared to the 31 μg/g samples. Apparently, less α-T was accumulating in the 100 μg/g-fed larvae, and this may have been the result of reduced feeding with this treatment. The 100 μg/g α-T has been previously shown to effectively deter feeding in *O. nubilalis* larvae (Champagne et al., 1986).

Alpha-T was recovered from parasitoid adults reared from hosts fed 10 and 31 μg/g α-T but not from hosts feed 100 μg/g α-T. Cocoons from all three groups contained α-T. The levels of α-T were relatively high in the cocoons of the parasitoids in the 10 and 100 μg/g treatments compared to the levels of the compound in the emerged wasps. This is similar to the findings of Barbosa et al. (1986) with nicotine: the parasitoids apprently dispose of such toxins via the cocoon silk and meconium.

The presence of α-T in the emerged parasitoids and cocoons suggests that the thiophene could have had a direct toxic effect on *D. terebrans*, in contrast to the indications of the data on berberine. Although the highest parasitoid mortality occurred with 100 μg/g α-T in the host diet, the surviving wasps did not contain detectable levels of α-T. This was a small and relatively resilient segment of the experimental population, which may have been inherently superior in their ability to metabolize α-T.

In addition to detecting the parent compound in the insects treated with α-T, a polar metabolite of α-T was found (Table 6). The compound has a typical thiophene spectrum, with a retention time of 1.7 min under the conditions of this HPLC system. As the structure and extinction coefficient of the metabolite have not yet been elucidated, absolute quantification was not possible. However, relative quantities (based on absorption at 350 nm) have been determined (Table 6). Relatively higher quantities of the metabolite were found in the cocoon samples than in the adult parasitoids. This is further evidence for the suggestion of Barbosa et al. (1986) that wastes from host-ingested allelochemicals are shunted by the parasitoid into the cocoon silk and meconia.

In the present study, the adverse effects on immature *D. terebrans* were much more severe with the highly lipophilic thiophene, α-T, than with the polar alkaloid, berberine, even though the parasitoid was capable of detoxifying and possibly metabolizing α-T. This suggests that pest management startegies which

take advantage of secondary plant substances for breeding resistance could run into problems with compounds which tend to be lipophilic (reminiscent of the pesticide bioaccumulation problem). On the other hand, an amphiphilic compound such as berberine may be relatively innocuous for the parasitoid, even though it can dramatically reduce host survival.

It is clear from this and other studies that allelochemical effects on herbivores do not necessarily reflect the consequences of these substances on the third trophic level. Novel allelochemicals with potential for agricultural applications should be examined for effects on natural enemies in order to assess their real usefulness for pest management strategies involving biological control.

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