Phenolic Compounds in Foliage of Commercial Tomato Cultivars as Growth Inhibitors to the Fruitworm, Heliothis Zea

M. B. Isman and S. S. Duffey
Department of Entomology, University of California, Davis, CA 95616

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Abstract. Semi-purified extracts of phenolics from foliage of tomato (Lycopersicon esculentum Mill.) inhibit larval growth of the fruitworm, Heliothis zea (Boddie), when added to artificial diets for this insect pest. The degree of inhibition of growth (dose-response) is directly related to the quantity of total phenolics in extracts added to diets, whether the extracts are of equivalent amounts of foliage from different cultivars or of foliage pooled from several cultivars added in serial dilution. Dose-responses for extracts were equal to those obtained with pure chlorogenic acid or rutin, major phenolic constituents of tomato foliage. Also, equivalent quantities of phenolics from 5 different cultivars inhibited larval growth equally when added to diets. These 3 sets of observations show that isolated tomato foliar phenolics affect H. zea larvae quantitatively, with no measurable qualitative differences between cultivars. When 2nd instar larvae were reared on excised leaflets from several cultivars of field-grown tomatoes, significant differences in larval growth between cultivars were obtained, which were consistent through two years. However, significant relationships between foliar phenolic content and larval growth were not obtained, partially because of the highly variable nature of phenolic content within and between plants. Our results suggest that phenolics in tomato foliage at the minimum contribute a substantial background level of antibiosis to H. zea.

The fruitworm, Heliothis zea, is a key pest of market and processing tomatoes in California and other growing areas in North America (13, 24). As the early instars of this insect feed on foliage prior to boring into immature fruits, several investigators have sought to find chemical factors in tomato foliage which are capable of inhibiting H. zea larval growth (7, 8, 23). Among the most prevalent secondary compounds in tomato foliage are the low molecular weight phenolics (viz. chlorogenic acid and rutin) which have recently been shown to be effective growth inhibitors to larval H. zea in artificial contexts (7, 10). Phenolics like chlorogenic acid and rutin, which could have anti-nutritional effects on humans, degrade naturally in maturing tomato fruits (9), ensuring no deleterious changes in the quality or desirability of the harvested crop. Tomato phenolics added to artificial diets inhibit growth of not only the fruitworm, but also that of the beet armyworm, Spodoptera exigua Hubn. (unpubl. results), another economically important pest of tomatoes in California (13).

We present findings which show that the degree of inhibition of larval growth of H. zea reared on artificial diets impregnated with foliar extracts for phenolics is directly related to the total quantities of phenolics present in diets, irrespective of the cultivar from which the extracts were obtained. We attempt to relate foliar phenolic content to growth of larvae on excised leaflets from field grown plants from several cultivars. Our results point out some of the difficulties in comparing artificial diet studies with measurement of antibiosis in planta.

Materials and Methods

Larvae of H. zea were reared as previously described (10). Tomato plants of 5 commercial cultivars (‘Royal Flush,’ ‘Ace 55,’ ‘VF 315,’ ‘VFN Bush,’ and ‘UC 134’) were field grown at Davis, California in the summer of 1979 with ‘Castlemart’ added in 1980.

To assess toxicity of foliar phenolics of tomato to H. zea larvae, we extracted and purified phenolics from ‘Royal Flush,’ ‘Ace 55,’ ‘VF 315,’ and ‘VFN Bush’. Young foliage of each tomato cultivar (25 g fresh weight) was extracted with 250 ml of hot 50% aqueous methanol (aqMeOH) and filtered. The filtrate was defatted via extraction with petroleum ether then the aqueous layer was reduced to a minimal volume by evaporation in vacuo (50°). A purified phenolic fraction was obtained by running the extract through a 1.5 × 25 cm Sephadex LH-20 column eluted sequentially with 20%, 50% and 100% aqMeOH. The purified phenolic fraction was then added to an artificial diet on which neonate larvae were allowed to feed for 8 days and then weighed (10). To determine the phenolic content of the resultant diets (each impregnated with an extract from a different cultivar), aliquots of diet (ca. 100 mg) were extracted with 2.5 ml of 50% aqMeOH for 24 hr, 1.0 ml of which was used to determine phenolic content via the ammonium molybdate colorimetric method (14).

To determine if qualitative differences in phenolics from different tomato cultivars gave rise to differences in toxicity to H. zea larvae, 5 g dry weight of lyophilized foliage from each of 5 cultivars grown in 1979 was extracted with 50 ml of boiling water and filtered. To the filtrate, cholesterol was added to remove the glycoalkaloid α-tomatine from the extract, as previously described (10). After evaporating each extract to 10 ml, aliquots of each were added to artificial diets such that each diet contained 5.6 mmole of chlorogenic acid equivalents per kg wet weight of
diet. Neonate larvae were allowed to feed on these diets for 8 days and then weighed.

Relative antibiosis of the different cultivars was assessed by rearing 2nd instar larvae (5–8 mg) on intact, excised leaflets from field-grown plants. We chose to begin with 2nd instar larvae because there is considerable (50+%) mortality of 1st instars attributable to antixenosis (3) and we specifically wanted to determine antibiotic potential of the foliage to *H. zea*. Larvae were provided with fresh young leaflets daily and allowed to feed for 10 days at room temperature and then weighed. Total phenolic content and catechol content were determined on fresh or frozen foliage using the leaf disc method previously described (4) or by extracting whole leaflets with boiling water. Estimations of foliar phenolic content were based on single determinations of pooled foliage of each cultivar in 1979, and on 5–7 leaflets per cultivar in 1980. Leaflet bioassays were performed in both 1979 and 1980.

**Results**

The semi-purified fractions obtained from equivalent amounts of foliage of different cultivars gave rise to significant differences in the inhibition of larval growth, directly proportional to their phenolic content, when added to an artificial diet for *H. zea*. The dose-response obtained is equal to that for serial dilutions of a pooled phenolic extract added to artificial diets and fed to larval *H. zea* (10) (Fig. 1). Neither the ED$_{50}$ (dose reducing growth to 50% of controls), 5.7, and 5.0 mmoles/kg diet respectively, nor the slopes, $-321$ and $-427$ respectively, differed significantly ($P > 5\%$). In the present study, extracts which had the highest phenolic contents, those from cultivars 'VFN Bush' and 'VF 315' (0.68% and 0.63% wet weight respectively) were the most inhibitory to larval growth (24% and 34% relative to controls respectively), whereas the extract of 'Royal Flush,' containing only 0.20% wet weight phenolics was significantly less inhibitory (64% of controls). In addition, when phenolic-rich extracts of different cultivars were added to artificial diets at equimolar concentrations (5.6 mmoles/kg) and fed to *H. zea* larvae, all diets were equally inhibitory relative to controls (Fig. 2).

Differences between the cultivars for antibiosis to *H. zea* larvae were consistent for both years based on excised leaflet bioassays (Fig. 3). In both years 'Royal Flush' and 'UC 134' were the least antibiotic and 'VF Bush' and 'VF 315' the most. At the end of each bioassay (10 days), mean larvae weights for larvae reared on the former 2 cultivars were double those of larvae reared on the latter 2. Foliage of 'Ace 55' appeared to give rise to moderate relative antibiosis in both years. However, within-plant variability with respect to foliar phenolic content was great enough to mask any significant relationship between larval antibiosis and phenolic content between this and the other cultivars (Fig. 4). It is important to note that all larvae reared on leaflets weighed from 30 to 150 mg after 10 days; whereas larvae reared on control diets for the same period weigh from 200–300 mg (unpublished data).

**Discussion**

The commercial tomato has recently received considerable attention with respect to potential resistance factors against several key insect pests, including the fruitworm, *H. zea* (3, 7, 8), the tomato pinworm *Keiferia lycopersicella* (Walsingham) (18), the tobacco hornworm *Manduca sexta* (L.) (11) and the Colorado potato beetle *Leptinotarsa decemlineata* (Say) (20). It is noteworthy that the search for, and development of, resistance in tomatoes to insect pests has lagged far behind other genetic developments.
of this crop (fruit size, texture, uniformity, color, solids content; synchrony of fruit set, and growth determinance of the plant) (15, 16). Some workers have demonstrated resistance to insects in accesses of L. hirsutum L. glabratum Mull. (8, 11, 23). Although it is possible to interbreed this species with commercial tomato cultivars, transfer of the genes for resistance would probably be time consuming and expensive. We have concentrated our efforts on investigating potentially antibiotic factors already present in some commercial cultivars currently in use in California, which might be causally related to resistance.

In this and a previous report (10), we have demonstrated that phenolic compounds of tomato foliage, either singly or in combination, are toxic to larvae of H. zea (i.e., inhibit early larval growth) when added to an artificial diet for this insect (also see ref. 7). Our earlier results showed that chlorogenic acid and rutin act in an additive fashion, such that larval growth is negatively related with phenolic content in the diet. In this report we corroborated this finding using foliar extracts from phenolics either in serial dilution (pooled extracts) or with extracts from different cultivars. This test precludes any significant qualitative difference in toxicity of phenolics from different cultivars.

The results of our leaflet bioassays indicate, at least amongst the cultivars investigated, that the antibiotic properties of the foliage (measured as the inhibition of larval growth) are not attributable to foliar phenolic content. Within year and between year variability in foliar phenolic content amongst the cultivars may have obscured a significant dose-response in the larvae. From the results of our leaflet bioassays at present, we do not imply that phenolics play only a trivial role in antibiosis of the tomato to H. zea. At the minimum, we can conclude that the foliar phenolic levels within the range seen in the cultivars examined (0.2–0.6% fresh weight) constitute an important background level of antibiosis. We have good evidence that ingested tomato phenolics from glandular trichomes on leaves give rise to an increased level of antibiosis to larvae of H. zea (4). Total foliar levels of phenolics might be increased through selective breeding, which may enhance the antibiotic potential of some other chemical or physical resistance factor(s).

The fact that the relative antibiotic differences between cultivars were consistent from year to year (Fig. 3) but were not corre-

lated with either phenolic content or tomatine content (also 5, 6) suggests that some other chemical and/or physical factor(s) either override the biological activities of phenolics and glycoalkaloids or mask their expected antibiotic effect on H. zea. The salient point is that antibiosis of isolated compounds or extracts in artificial diets may not always be an indicator of antibiosis in situ. The unclear relationship between phenolic content and larval antibiosis amongst different tomato cultivars via the leaflet bioassay, compared with that clearly seen in the artificial diet studies, is not an unusual finding. There are many crops from which chemical factors have been isolated and shown to inhibit larval growth in laboratory bioassays (e.g., 2, 7), but corroborative evidence of the effectiveness of these factors in situ is often, unfortunately, lacking. In making the necessary link between the isolation of a biologically active compound and the demonstration of that compound’s contribution to host resistance, the investigator must traverse the confusing area in which variability of both the pest and host are among the hazards. There are few documented cases of chemical resistance factors in crop plants wherein the degree of antibiosis or insect resistance is relatable to quantitative chemical differences in cultivars, lines or genotypes of the crop (but see 12, 19). Chemically-based resistance can be a multi-faceted phenomenon, superimposed on the physical nature of the plant. Hence the approach of attempting to correlate relative antibiosis or resistance with one chemical or class of chemicals alone may be prone to producing ambiguous results. Other than vertical interactions between the plant and the pest (17), resistance should not be experimentally reducible to one key factor.

Our findings suggest that the identification of insect growth inhibitors from crop plants, defined by their biological activities in artificial diet bioassays, constitutes only the first step in determining chemical bases of resistance to insect pests, and that comparisons of biological activity in an artificial context and in the actual agricultural milieu should be made with considerable caution.

Literature Cited

Net Photosynthesis, Dark Respiration, Transpiration, and Stomatal Resistance of Young and Mature Apple Trees as Influenced by Summer or Dormant Pruning1

Richard P. Marini and John A. Barden2

Department of Horticulture, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061

Abstract. Young container-grown apple (Malus domestica Borkh.) trees and mature bearing trees were summer pruned either before or after shoot extension had ceased. Net photosynthesis, dark respiration, and transpiration of shoot leaves were increased by summer pruning, while stomatal resistance was decreased as compared to dormant pruned controls. These effects were more pronounced and of longer duration in basal leaves of container-grown trees and interior leaves of orchard trees than in distal or peripheral leaves, respectively.

European orchardists summer prune apple trees to maintain high density plantings and improve fruit quality (19), but little information exists on physiological effects of summer pruning. Productivity of fruit trees is influenced by photosynthesis, which may be altered by cultural practices such as pesticide application (1), tree nutrition (4), stem ringing (7), and summer pruning (18).

Photosynthetic rates of apple leaves vary with location in the tree. Mika and Antoszewski (13) found that interior leaves had lower photosynthetic rates than peripheral leaves, and attributed the results to low light levels at interior positions during photosynthetic measurements. Barden (2) found that even under saturating light, shade-grown leaves had lower net photosynthesis (Pn) and dark respiration (Rd) and that specific leaf weight (SLW) was lower than for comparable sun-grown leaves. Spur leaves sampled from interior positions of apple trees, where photosynthetically active radiation (PAR) was limited, had lower Pn, Rd, and SLW than well-illuminated peripheral leaves (14).

Tree size and shape influence PAR penetration into tree canopies, and both may be altered by pruning. Penetration of PAR in apple trees was improved immediately after summer pruning, but not in the year following treatment (12, 15). Apple leaves acclimate physiologically and morphologically to changing light conditions (2). Because summer pruning improves light penetration in apple trees, changes in leaf physiology may be expected. The purpose of this study was to compare Pn, Rd, transpiration (Tr) and stomatal resistance (Rs) of apple leaves on shoots that were headed during the summer or the dormant period.

Materials and Methods

Greenhouse experiment. ‘Topred Delicious’/Malling 9 trees were planted in 10 liter plastic pots on Jan. 21, 1980, and grown in...