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Foliar Phenolic Variation in Wild Tomato Accessions

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Plants contain numerous non-nutritive, bioactive compounds known as "phytochemicals". Many of these components are oxidative in nature and include phenolic compounds (Shahidi 2000). Phenolics in plants include simple phenols, flavonoids, anthocyanins, lignans and lignin, stilbenes and tannins. Many natural materials not only possess antifeedant activity rendering plants unattractive or unpalatable to insects, but also possess potential growth regulating properties (Saxena 1989). Recent reports support the concept that the breakdown products of tannins (phenols) behave as toxins and feeding deterrents, particularly for phytophagous insects that do not typically feed on diets rich in tannins (Gibson et al. 1995; Antonious et al. 1999). Phenols are often connected with plant defense against predators, bacteria and fungi (Daniel et al. 1999). Protection against fungi and microorganisms may have played a major role in the evolution of tannins in plants (Ayres et al. 1997). Many of the biological effects of tannins have been reviewed (Swain 1979; Liener 1980; Beier and Nigg 2000) for protein, carbohydrate, and phospholipid binding, nutritional effects, and antifeedant activity. Tannins can be stored in high concentrations in vacuoles of plant cells, and intense deposits have been found in the epidermis of leaf trichomes (Scalbert 1991; Li et al. 1996).

Phenols and polyphenols also constitute main classes of compounds responsible for health promotion ascribed to plant foods (Ramarathnam et al. 1995). These phenolics not only protect the plant from injurious damage under photosynthesis, attack by herbivores and assist in the wound healing process, they also act as antioxidants in the body to prevent damage to DNA, proteins, sugars and lipids and thus control degenerative diseases of aging such as cancer, cardiovascular diseases, cataract, immune system decline and brain dysfunction (Shahidi 1997; 2000).

Currently, there is an increasing interest in the use of natural plant products for insect control (Antonious 2001; Antonious et al. 2001 a & b). Knowledge of the toxicological effects of plant secondary metabolites is limited compared to pesticides and other anthropogenic chemicals (Daniel et al. 1999). Some research articles have indicated that resistance of plants against pests depends partially or completely on the presence of phenolic compounds (Gibson et al. 1995;

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Mallampalli et al. 1996). Tomato leaf phenols for example, may cause the leaves to be less suitable for insect growth and may influence leaf palatability (Gibson et al. 1995). The cotton leafworm (*Spodoptera littoralis* Boisduvar) is one of the most destructive insect pests of tomato. The larvae can cause substantial defoliation, resulting in decreased tomato yield. A significant positive correlation was found between total phenol concentration in cultivated tomato leaves and mortality of the cotton leaf worm indicating that phenols might render the leaves less suitable for larval growth (Antonious et al. 1999).

Botanical insecticides are the subject of intense interest for use in crop protection because their toxicological properties permit control of certain insect species at low application rates. Materials of natural origin are also biodegradable and could serve as environmentally safe pesticides. The use of synthetic pesticides on cultivated crops has led to residue problems on the treated products, soil, surface and groundwater. The challenge in plant protection programs is to seek management alternatives that mitigate environmental degradation (Antonious 2000; Antonious et al. 2001a & b), human health and farm-worker safety (Antonious 2002) while maintaining agricultural productivity and profitability.

The present study is a continuation of previous work on botanical insecticides and biochemical composition of wild tomato leaves (Antonious 2001). The objectives of this study were 1) to determine phenol content in the leaves of eleven wild tomato accessions; 2) to determine monthly variation of phenols concentration in wild tomato leaves; and 3) to identify wild tomato accessions with high levels of phenols for future research on insecticidal efficiency of wild tomato leaf extracts.

MATERIALS AND METHODS

Three wild tomato accessions of *Lycopersicon hirsutum f. typicum* (PI-308182, PI-127826, and PI-127827); six accessions of *L. hirsutum f. glabratum* (PI-251304, PI-251305, LA-407, PI-134417, PI-134418, and PI-126449); one accessions of *L. pennellii* (PI-414773); and one accession of *L. pimpinellifolium* (PI-1335) were chosen. The plants were obtained from seeds received from the USDA/ARS, Plant Genetic Resources Unit, Cornell University Geneva, NY, USA. A commercial tomato variety, *Lycopersicon esculentum* cv. Fabulous (Holmes Seed Co., Canton, OH) was also grown from seeds and used as control for comparison. The seeds were germinated in the laboratory on moistened filter paper in petri dishes kept in the dark. After sprouting of leaves, plants from each accession were transferred to 20 cm diameter plastic pots containing Pro-Mix soil (Premier Horticulture, Red Hill, PA) and transported to the greenhouse at Kentucky State University Research Farm. All plants were grown under natural daylight conditions, supplemented with sodium lamps, irrigated daily, and fertilized twice a month with Peters (Scotts Co., Marysville, OH) as a general purpose fertilizer containing 20:20:20 NPK. The average temperature and humidity in the greenhouse were 30 ± 3.9°C and 49.5 ± 11.8%, respectively.

When plants were 3 months old, leaves were chosen from the fourth, fifth, and sixth pair of leaves from the apex. Extracts were prepared by shaking 5 g of leaflets taken from each accession with 80% ethanol for 1 h using an electric shaker. The solvent rinse was decanted through a Whatman 934-AH glass microfibre filter (Fisher Scientific, Pittsburgh, PA), and the filtrate was evaporated under vacuum using a rotary vacuum evaporator (Buchi Rotovapor Model 461, Switzerland) at 35°C. The extract was then reconstituted in 5 mL of 80% ethanol for phenols determination. At maturity, all plant leaves from each accession were harvested and weighed. Representative sub-samples were collected at random and analyzed for total phenols. Determination of total phenols was performed using the Folin-Ciocalteu colorimetric method (McGrath et al. 1982; Hagerman et al. 1997).

A standard calibration curve was obtained using chlorogenic acid (Sigma Chemicals, St. Louis, MO) in the range of 1.2 to 14.0 µg/mL ethanol and used to determine phenols as chlorogenic acid equivalent. Standards solutions ranging from 1.0 to 10.0 µg/mL were prepared and used to spike tomato leaflets for evaluating the analytical procedures. Recovery values ranged from 91.0 to 95.6%. All phenol concentrations detected in tomato leaves were adjusted for efficiency of recovery. Phenols in 5 g samples and in total plant leaves were analyzed by accession using ANOVA procedure. Means were analyzed and separated using Duncan's LSD test (SAS Institute, 1999).

RESULTS AND DISCUSSION

Concentration of total phenols in wild tomato accessions tested during one year of sampling and analysis averaged from 12 to 39.3 mg/100g fresh leaves. Of the eleven accessions PI-134417, PI-251304, PI-126449, PI-134418, and LA-407 contained the highest amount of phenols (Figure 1) with maximum concentration during the month of September (Figure 2). Over all the accessions analyzed, amount of total phenols obtained from whole plant leaves were higher in three *L. hirsutum f. glabratum* accessions (PI-134417, PI-126449, and PI-134418) than *L. esculutum* cv. Fabulous, *L. hirsutum f. typicum* (PI- 308182, PI-127826, and PI-127827), *L. pennellii* (PI-414773); and *L. pimpinellifolium* (PI-1335) accessions (Table 1).

Leaves of *L. hirsutum f. glabratum* accessions also contain considerable amounts of methyl ketones (Antonious 2001) having insecticidal efficiency. Concentration of total phenols per plant fresh leaves (Table 1) indicates that five accessions of *hirsutum f. glabratum* (PI-251304, PI-126449, PI-134417, PI-134418, and LA-407) contain high concentrations of methyl ketones. Four of these accessions (PI-251304, PI-126449, PI-134417, and PI-134418) contain considerable concentrations of both total phenols and methyl ketones. These four *glabratum* accessions can be used as suitable candidates for plant breeders to develop insect resistant commercial tomato cultivars from wild tomato accessions by genetic improvements. These data provide a possible explanation for the variation observed between wild tomato accessions and insecticidal efficiency of crude extracts prepared from the leaves of the eleven accessions. PI-251304, PI-126449,

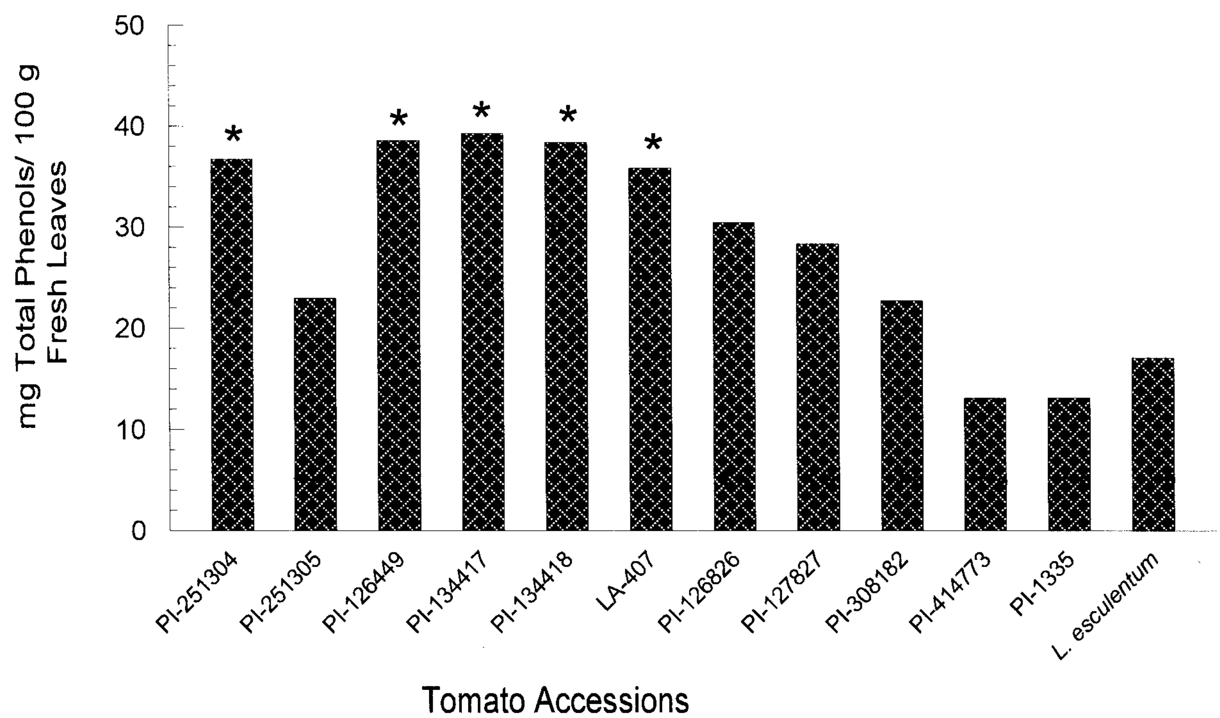

Figure 1. Average concentrations of total phenols (determined as chlorogenic acid equivalent) in the leaves of eleven wild tomato accessions and one commercial tomato cultivar (*L. esculentum* cv. Fabulous) grown under greenhouse conditions during January to December, 2001 at Kentucky State Universty Research Farm, Franklin County, KY. Bars accompanied by asterisks indicate significant differences ($P > 0.05$; Duncan's multiple range test, SAS Institute, 1999) between accessions.

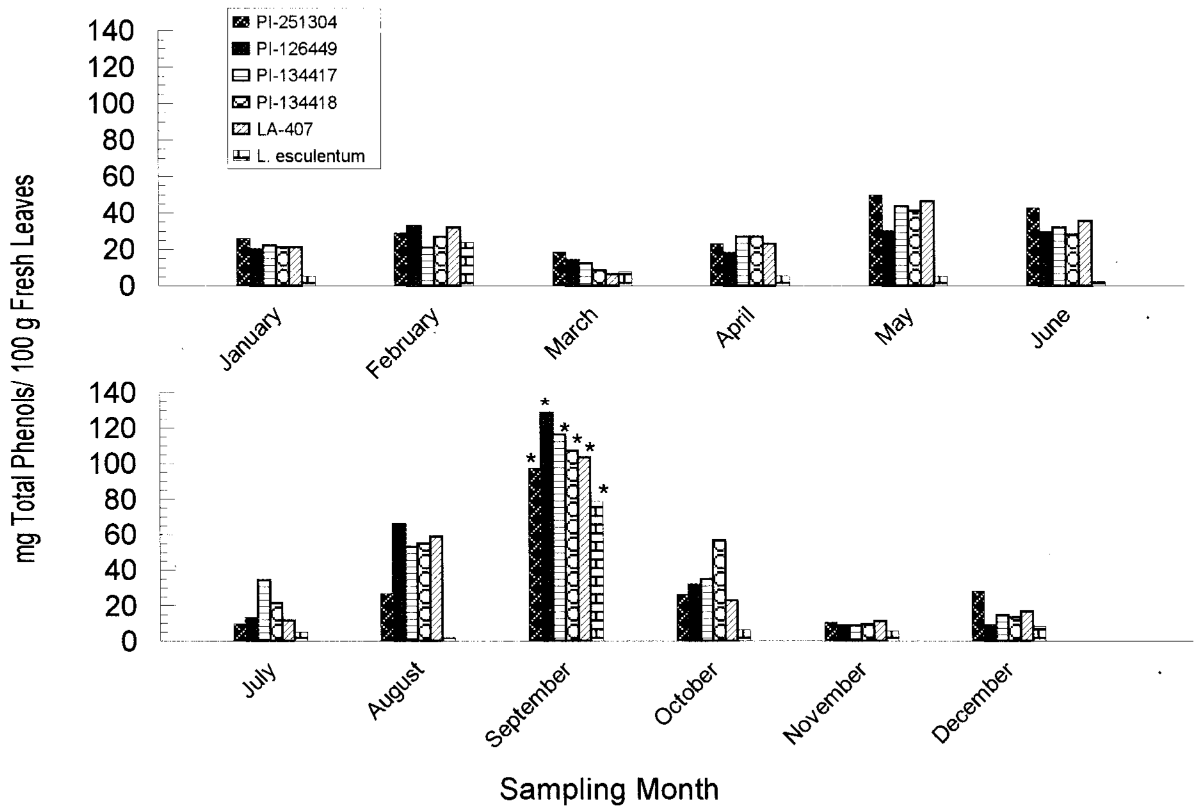

Figure 2. Concentrations of total phenols (determined as chlorogenic acid equivalent) in the leaves of five *L. hirsutum f. glabratum* wild tomato accessions and one commercial cultivar (*L. esculentum* cv. Fabulous) grown under greenhouse conditions (January - December, 2001) at Kentucky State University Research Farm, Franklin County, KY. Bars accompanied by asterisks indicate significant differences ($P > 0.05$; Duncan's multiple range test, SAS Institute, 1999) between accessions during sampling months.

Table 1. Concentrations† of total phenols and methyl ketones in the leaves of 11 wild tomato accessions and one cultivated tomato (*L. esculentum* cv. Fabulous) grown under greenhouse conditions at Kentucky State University Research Farm (Franklin County, Frankfort, KY).

Accessions	mg/ Fresh Leaves	
	Total Phenols	Total Methyl Ketones‡
PI-251304	2276.57 b	709.67 a
PI-251305	427.56 e	11.16 c
PI-126449	3428.83 a	478.90 b
PI-134417	3524.76 a	543.65 ab
PI-134418	3864.47 a	613.54 ab
LA-407	868.15 d	701.73 a
PI-127826	2180.07 b	12.71 c
PI-127827	1183.29 cd	76.53 c
PI-308182	140.18 e	4.08 c
PI-414773	127.64 e	5.63 c
PI-1335	76.99 e	7.79 c
L. esculentum	1442.06 c	17.74 c

† Each value in the table is an average obtained from weight of 9 tomato plants.
‡ Calculated using the methods described by Antonious 2001.
Values within a column having different letter(s) are significantly ($P < 0.05$) different from each other, using Duncan's LSD test (SAS Institute, 1999).

PI-134417, and PI-134418 were the most effective accessions against the tobacco hornworm (*Manduca sexta*) and the tobacco budworm (*Heliothis virescens*) (Hawkins and Antonious 2002). Accordingly, extracts from the foliage of PI-251304, PI-126449, PI-134417, and PI-134418 containing significant concentrations of phenolic compounds and methyl ketones (Table 1) may be beneficial as a source of botanical pesticides for use as foliar sprays for crop protection. Dissipation of phenols and methyl ketones on treated plant surfaces under field conditions and the impact of ultra-violet light on their persistence and efficiency remain to be answered.

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