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The Feeding Effect of Polyphenolic Compounds on the Colorado Potato Beetle [*Leptinotarsa decemlineata* (Say)]

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ABSTRACT

Colorado Potato Beetle, *Leptinotarsa decemlineata* (Say) is a serious pest of potato plants and devastates additional crops such as eggplant, tomato, pepper, and tobacco by its voracious feeding. Plants of the *Lamiaceae* family produce a large number of polyphenols with insecticidal activity. In this study we tried to identify if some of these compounds could be used as antifeedants and if there is any influence of these compound on food conversion by the Colorado Potato Beetle whose 3rd instar larvae were fed food contaminated with quercetin, naringenin, vanillin, p-coumaric acid, catechin, caffeic acid, vanillin acid, rutin and phloroglucinol in 1.33, 0.66 and 0.11 mg/cm^{-2} doses. Several compounds remarkably decreased larval growth and inhibited their feeding. The flavonoids were almost ineffective, having little or no effect on growth and/or food deterrence of the larvae, whereas phenols and phenolic acid demonstrated a very high efficiency.

Keywords: botanical insecticides, flavonoids, insecticidal activity, phenols, polyphenols

INTRODUCTION

Plant secondary metabolites are an enormously variable group of phytochemicals in terms of their number, structural heterogeneity and distribution. Polyphenolic compounds constitute one of the most numerous and widely-distributed group of substances in the plant king-dom, with more than 8,000 phenolic structures currently known. Polyphenols are present in a variety of plants utilized as important components of both human and animal diets (Bravo 1998; Chung *et al*. 1998; Donoval *et al*. 1999).

Polyphenols exhibit a wide range of biological effects as a consequence of their antioxidant properties (Frankel *et al*. 1993; Furman *et al*. 1995; Nigdikar *et al*. 1998). In addition, flavonoids have antithrombotic and anti-inflammatory effects (Gerritsen *et al*. 1995; Muldoon and Kritchevsky 1996). The antimicrobial property of polyphenolic compounds has been well documented (Chumg *et al*. 1998). Several types of polyphenols (phenolic acids, hydrolysable tannins, and flavonoids) show anticarcinogenic and antimutagenic effects. Polyphenols might interfere in several of the steps that lead to the development of malignant tumors, inactivating carcinogens, inhibiting the expression of mutant genes and the activity of enzymes involved in the activation of procarcinogens and activating enzymatic systems involved in the detoxification of xenobiotics (Bravo 1998).

Our knowledge about the different aspects of insect-plant interactions with polyphenolic compounds has been expanding, but not as far as our knowledge of the distribution and diversity of these compounds within differ-rent plant species. This is partially because the majority of recent work on polyphenols relates to their benefits to human health and/or to their utilization in phylogenetic studies (Simmonds 2003).

Polyphenols are a group of plant compounds involved in a large range of physiological and ecological processes, providing resistance to fungal, bacterial and viral infections (Harbone 1980; Middleton *et al*. 1986; Selway 1986; Martins *et al*. 1992). They reduce damage caused by insects through their deterrent and/or antifeedant effects (Echeverri and Suarez 1989; Simmonds *et al*. 1990; Echeverri *et al*. 1991). A large range of insects belonging to different orders appear to be highly sensitive, including aphids (Dreyer and Jones 1981), Lepidoptera (de Paula *et al*. 1997), Orthoptera (Owen Smith 1995), Diptera (Capasso *et al*. 1994) and Coleoptera (Regnault-Roger *et al*. 2004). For example Regnault-Roger *et al*. (2004) tested polyphenols from five aromatic plants (Lamiaceae) against *Acanthoscelides obtescus* (Say) (Coleoptera: Bruchidae). A bioassay to assess the effect on the behaviour and survival of beetles was conducted with 17 polyphenolic compounds (phenols, phenolic acids and flavonoids). Tested polyphenols were toxic to the beetles in different degrees. Caffeic and ferulic acids, vanillin and luteolin-7-glucoside induced a knockdown effect on the first day, garlic acid on second day. Quercetin significantly decreased natural mobility from the first day, naringin, syringaldehyde, vanillic acid and garlic acid after the 4th day. On the 8th day all compounds caused significant mortality. Rosmaric acid and luteolin-7-glucoside were the most active compounds. An attractive effect enhanced the toxicity.

The majority of phytophagous insects are monophagous or oligophagous, feeding on a very narrow range of plant species or families. Their host range is limited, in part, by the chemical complexity of the plants they encounter: host-plants being selected because they either contain stimulants or lack deterrents. Giving compounds to these plants plays such a major role in the selection behavior of the insects that the ability of the insects to perceive and discriminate among compounds becomes very profound.

One insect pest that constantly frustrates farmers and agricultural specialists is the Colorado Potato Beetle *Leptinotarsa decemlineata* (Say). It is a North American native that has spread to many parts of the world. This species has had a long history of development of resistance to several insecticide classes, including carbamates, organophophates, organochlorines and pyrethoids (Metcalf 1983). Increasing resistance of the pest to toxic insecticides and their negative impact on natural enemies are the potential problems associated with their long-term us. In addition, the expanding documentation of the negative environmental and health im-

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pacts of synthetic toxic insecticides and the increasing stringent environmental regulations of pesticides have resulted in a renewed interest in the development and use of the botanical pest management products by the majority of agrochemical companies. Not only might certain secondary metabolites of plant origin be a source of new pesticides, but also botanical derivatives from these synthetic chemicals may be more environmentally considerate. Recently some of our research has documented the bioactivity of extracts from plant species of the *Lamiaceae* family against *L. decemlineata* (Pavela 2002, 2004).

Plants of the *Lamiaceae* family produce a large number of polyphenols (Barberan and Muñoz 1990; Regnault-Roger *et al*. 2004). In this study we tried to identify whether some of these compounds could act as antifeedants and/or have effects on the conversion of ingested food in Colorado Potato Beetle.

MATERIALS AND METHODS

Insects for the bioassays

Newly ecdysed 3^{rd} instar larvae of *L. decemlineata* were obtained from a colony reared on potato, *Solanum tuberosum*, cv. Agria, at 25 ± 2°C, 90 ± 10% RH and 16:8 h (L:D) photoperiod in a environmental chamber. This colony was renewed annually with wild adults collected from potato fields.

Polyphenolic compounds

The biological tests were conducted with commercially purified compounds (Sigma-Aldrich, Prague, Czech Republic). The structures of the polyphenols used in this study are shown in **Fig. 1**.

Feeding deterrence experiments

No-choice tests were performed in plastic Petri dishes (15 x 90 mm). The potato disks (4.52 cm^{-2}) were treated on the upper surface with 20 µl of an acetone solution containing 6.0, 3.0 and 0.5 mg (i.e. 1.33, 0.66 and 0.1 mg/cm^{-2}) of the test compound (**Fig. 1**) or the acetone alone (control). After complete evaporation of the solvent, newly emerged 3^{rd} instar larvae were starved for 6 h. In the no-choice assay, four treated and/or control disks were used in each arena.

The leaf disks were placed on glass plates and a digital picture was obtained using a scanner with a digital imaging system. Areas of the treated and control leaf disks were measured using Scion Image software. A Feeding Deterrence Index (FDI) was calculated using the formula

$$FDI = \left[\left(\frac{C - T}{C + T}\right)\right] * 100$$

where C and T are the Control and Treated leaf areas consumed by the insect (Sadek 2003).

Experiments on the conversion of the ingested food

The surface areas of the leaf disks used in tests were treated with chemicals as "feeding deterrence" experiments. The leaf disks were exchanged every 12 hours. The amount of the consumed food was calculated using a similar measuring method of the food consumption as in previous experiments. The following nutritional indices were evaluated: the Relative Growth Rate index (RGR_i = mg of weight gained per mg of the initial larval weight per day), the Relative Consumption Rate index (RCR_i = mg mg^{-1} day^{-1}) and the Efficiency of Conversion of Ingested food index (ECI = (weight gained/weight of food ingested) x 100) (Wheeler and Isman 2001).

All experiments were placed in the growth chambers at 22 ± 0.5°C, 85 ± 10% RH and with a photoperiod of 16:8 h (L:D).

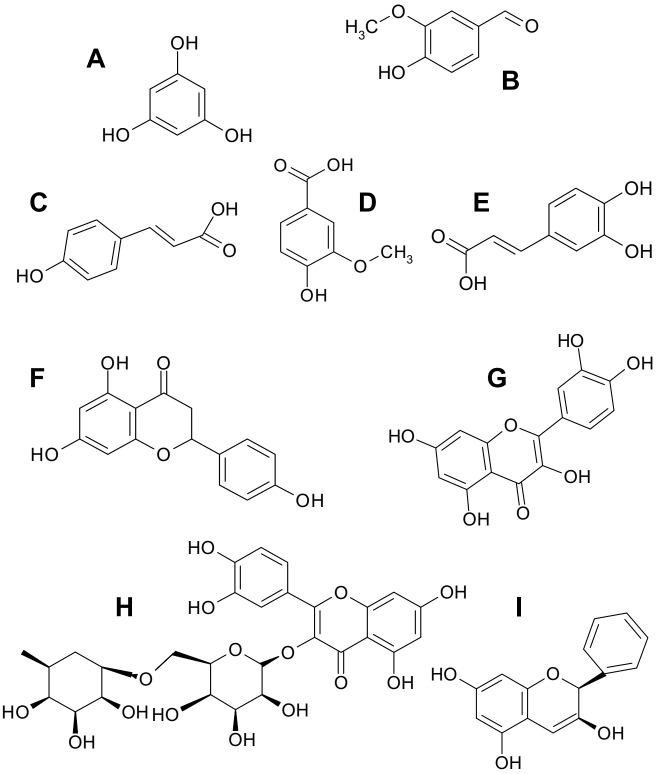

Fig. 1 Structure of polyphenol compounds used for tests, divided into three groups: phenols, phenolic acids, and flavonoids. Phenols: (**A**) phloroglucinol, (**B**) vanillin. Phenolic acids: benzoic acid, (**C**) cinnamic acid, (**D**) vanillic acid, (**E**) caffeic acid; Flavonoids: flavones, e.g. (**F**) naringenin, flavonols, e.g. (**G**) quercetin, and (**H**) rutin, flavanols, e.g. (**I**) catechin.

Statistical Analysis

Fifteen replications per treatment were used in all assays. The results are presented as means ± SE and were analyzed by the Student's *t* test and/or by the Tukey's test for the Feeding Deterrence Index. All tests were considered statistically significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

The tested polyphenols demonstrated various effects. Generally, flavonoids were the least effective or demonstrated no growth and/or food deterrence effects on the Colorado potato beetle larvae. In contrast, phenols and phenolic acid demonstrated a very high efficiency.

The most efficient substances were phenol (phloroglutinol) and phenolic acid (vanillic acid). The feeding deterrence (**Table 1**) of these substances was determined to be 40-55% when highest doses (1.33 and 0.66 mg/cm^{-2}) were used. The lowest dose (0.11 mg/cm^{-2}) resulted in less than 20% feeding deterrence. The results of the tests demonstrated a significantly ($P<0.001$) lower food consumption rate (**Table 2**) at high doses, whereas the lowest dose (0.11 mg/cm^{-2}) resulted in almost the same food consumption level as control larvae. Even though the conversion percentage of digested food (**Table 1**) was minimal (phloroglucinol; 8.0%) when the 0.11 mg/cm^{-2} dose was applied, or even though food utilization was negative (-3.8%) – measured by a reduction in larvae weight following the application of vanillic acid – both substances inhibited larval weight (**Table 2**).

The flavanol catechin demonstrated the best feeding deterrence from all the tested substances. The deterrence index ranged from 30-56%, depending on the dose (**Table 1**). This antifeedant effect was also evident in ensuing tests, where the food consumption rapidly decreased at all doses ($P<$ 0.001; **Table 2**). In the lowest concentration 0.11 mg/cm^{-2} was detected significantly lower growth of larvae ($P<0.01$; **Table 2**). It was probably caused by low insect feeding ($P<$

Table 1 The effect of polyphenols on food utilization and antifeedant activity (mean ± SE) by larvae *Leptinotarsa decemlineata*.

Compounds	**ECI (%)**			**FDI (%)**		
	1.33 mg/cm^{-2}	**0.66 mg/cm^{-2}**	**0.11 mg/cm^{-2}**	**1.33 mg/cm^{-2}**	**0.66 mg/cm^{-2}**	**0.11 mg/cm^{-2}**
quercetin	28.1 ± 11.3	29.2 ± 14.1	28.5 ± 4.55	2.3 ± 19.0^{a}	2.7 ± 15.5^{a}	–1.4 ± 6.5^{a}
naringenin	24.7 ± 11.0	29.3 ± 10.5	29.9 ± 9.14	3.2 ± 16.5^{a}	6.0 ± 10.3^{ab}	0.2 ± 19.6^{a}
vanillin	16.9 ± 14.5**	17.4 ± 12.3**	23.5 ± 11.29*	30.7 ± 10.0^{cd}	38.4 ± 12.8^{c}	21.7 ± 15.7^{bc}
p-coumaric acid	1.4 ± 13.1***	3.2 ± 17.9***	9.4 ± 16.35***	22.6 ± 15.3^{bc}	21.3 ± 13.0^{b}	3.9 ± 10.0^{ab}
catechin	–2.4 ± 24.8**	11.7 ± 19.2*	20.5 ± 30.16	56.1 ± 14.8^{e}	39.4 ± 16.3^{c}	29.9 ± 27.7^{c}
caffeic acid	17.3 ± 17.1**	22.8 ± 12.9*	28.4 ± 13.73	24.1 ± 17.2^{bc}	21.4 ± 14.8^{b}	7.7 ± 20.1^{ab}
vanillin acid	–27.4 ± 13.2***	–15.0 ± 13.8***	–3.8 ± 16.81***	46.1 ± 8.9^{de}	39.0 ± 7.8^{c}	21.1 ± 17.3^{bc}
rutin	3.2 ± 8.1***	5.7 ± 10.0***	6.0 ± 3.49***	7.3 ± 16.0^{ab}	0.9 ± 20.2^{a}	0.8 ± 13.3^{a}
phloroglucinol	–24.8 ± 16.8***	–33.3 ± 43.2***	8.0 ± 10.64***	55.7 ± 11.4^{e}	49.3 ± 18.0^{c}	16.2 ± 18.5^{abc}
control	32.5 ± 11.0					

ECI, efficiency of conversion of digested food, Student's *t* test: **P*<0.05; ***P*<0.01; ****P*<0.001; FDI, feeding deterrence index, Tukey's test (*P*≤0.001, Df = 134, F = 31, 35; *P*≤0.001, Df = 134, F = 22, 57 and *P*≤0.001, Df = 134, F = 6, 32 for 1.33; 0.66 and 0.11 mg/cm^{-2}, respectively) mean values with the same superscript letter are not significantly different (*P*<0.05).

Table 2 The effect of polyphenols on growth and food consumption (mean ± SE) by larvae *Leptinotarsa decemlineata*.

Compounds	**RGR (mg/mg/day)**			**RCR_i (mg/mg/day)**		
	1.33 mg/cm^{-2}	**0.66 mg/cm^{-2}**	**0.11 mg/cm^{-2}**	**1.33 mg/cm^{-2}**	**0.66 mg/cm^{-2}**	**0.11 mg/cm^{-2}**
quercetin	0.18 ± 0.05	0.17 ± 0.09	0.19 ± 0.03	0.68 ± 0.17	0.65 ± 0.15	0.66 ± 0.1
naringenin	0.16 ± 0.06	0.18 ± 0.06	0.16 ± 0.07	0.67 ± 0.16	0.62 ± 0.1	0.74 ± 0.16
vanillin	0.02 ± 0.06***	0.08 ± 0.07***	0.12 ± 0.06***	0.44 ± 0.14**	0.48 ± 0.16***	0.53 ± 0.12
p-coumaric acid	0.02 ± 0.07***	0.03 ± 0.09***	0.06 ± 0.1***	0.51 ± 0.14**	0.51 ± 0.12**	0.58 ± 0.11
catechin	0.02 ± 0.09***	0.06 ± 0.08***	0.1 ± 0.1**	0.26 ± 0.12***	0.37 ± 0.11***	0.44 ± 0.18***
caffeic acid	0.1 ± 0.1**	0.12 ± 0.06***	0.18 ± 0.09	0.51 ± 0.18*	0.5 ± 0.1**	0.63 ± 0.14
vanillin acid	–0.1 ± 0.04***	–0.07 ± 0.06***	–0.01 ± 0.08***	0.37 ± 0.09***	0.51 ± 0.13**	0.67 ± 0.19
rutin	0.02 ± 0.05***	0.06 ± 0.05***	0.05 ± 0.03***	0.71 ± 0.16	0.82 ± 0.22*	0.8 ± 0.14**
phloroglucinol	–0.07 ± 0.05***	–0.1 ± 0.15***	0.05 ± 0.04***	0.29 ± 0.07***	0.39 ± 0.15***	0.75 ± 0.21
control		0.2 ± 0.07			0.65 ± 0.12	

RGR, relative growth rate; RCR_i, relative consumption rate (mg/mg body weight/day); Student's *t* test: **P*<0.05; ***P*<0.01; ****P*<0.001

0.01; **Table 2**) even though no effect was found on the efficiency of food conversion digested by larvae (**Table 1**).

The phenol; vanillin and the phenolic acids; *p*-coumaric acid and caffeic acid demonstrated a relatively low (from 3.9 to 38.4%) efficiency in feeding deterrence (**Table 1**). A significant decrease in food consumption (*P*<0.01) was detected for 1.33 and 0.66 mg/cm^{-2} doses. The lowest dose did not demonstrate any decreasing effect (**Table 2**). We detected variability efficiency in food deterrence and larval growth in this group of compounds. *P*-coumaric acid significantly (*P*<0.001) inhibited conversion of digested food (**Table 1**). A less effective conversion of the food was reflected in the slow growth velocity of the larvae (*P*<0.001) at all tested doses. Vanillin decreased the efficiency of food conversion; however, the percentage efficiency decreased with a decrease in dose (**Table 1**). Yet, the relative growth rate of the larvae, at all tested doses, was rapidly slower than control larvae (**Table 2**). Caffeic acid also decreased the conversion of digested food; however, this efficiency decreased with a decrease in dose.

Rutin demonstrated interesting test results. This flavonol did not decrease food consumption (**Tables 1, 2**) but rather increased it with a decrease in the dose of rutin in the food (*P*<0.05), relative to the control. The conversion of digested food was minimal (*P*<0.001) and the growth rate was also insignificantly retarded (**Tables 1, 2**).

Quercetin and naringin, two flavonoids, did not demonstrate any effect on feeding deterrence (**Table 1**) and did not influence larval growth or even food consumption or efficiency of conversion of digested food (**Tables 1, 2**). Colorado potato beetle is a good example of an insect that has developed resistance to most classes of chemicals (Georghiou 1990). Biochemical mechanisms of resistance in *L. decemlineata* have developed as a result of elevated polysubstrate monooxygenase (Rose and Brindley 1985; Ahammas-Sahib *et al*. 1994; Wierenga and Hollingword 1994), glutathione *S*-transferase (Ahammas-Sahib *et al*. 1994; Argentine *et al*. 1994), and esterase activities. Our finding is that food ingestion containing quercetin and naringin may be the cause of these test results, within the scope of the natural detoxification ability of the Colorado Potato Beetle, a natural pest of potato plants (*Solanum tuberosum*), and which usually contains quercetin and naringin (van Eldik *et al*. 1997; Lewis *et al*. 1998).

Rutin, one of the most widely studied flavonol glycosides, is a phagostimulant to many polyphagous insects including the locust *Schistocerca americana* (Bernays *et al*. 1991), and the caterpillar *Heliothis virescens* (Blaney and Simmonds 1983). The behavioural response of insects to rutin can vary depending on the concentration tested and the age of the insect tested. For example, rutin at concentrations greater than 10^{-3} M deterred the final stage of *Heliothis zea* and *Helicoverpa armigera* larvae from feeding, but at concentrations less than 10^{-4} M it stimulated feeding in final stage larvae (Simmonds 2001). Rutin also deterred second stage *H. zea* larvae from feeding (Isman and Duffey 1982). Once rutin is ingested the glycosidic bond is hydrolysed to release the aglycone quercetin. Quercetin can inhibit mitochondrial ATPase (Lang and Racker 1974). This inhibitory activity could explain why rutin has detrimental effects on the development of early instars larvae of *H. zea* (Isman and Duffey 1982), *Spodoptera litura* and gypsy moth, *Lymantria dispar* (Simmonds 2001), whereas the later and larger instars of these species consume rutin-enriched diets with no adverse effects.

In this study, rutin stimulated feeding of the 4th instar larvae of the Colorado Potato Beetle and subsequently inhibited their growth. Quercetin and naringenin, on the other hand, had no influence on the feeding and growth of the larvae, despite other researchers claiming that quercetin 3-*O*-glucoside and quercetin reduced the growth of second stadium gypsy moth larva (*Lymantria dispar*) when tested at 0.05 and 0.1%. These flavonoids also reduced growth and survival of the European corn borer, *Ostrina nubilalis* (Simmonds 2001).

Even though many researchers have studied the influence of phenols on insects, we still do not have enough documentation to decipher the complexity of these effects. The most important studies have been conducted on warehouse pests. For example, caffeic acid, vanillic acid, phloroglucinol and vanillin caused mortality or affected the mobility of *Acanthoscelides obtecus* adults (Regnault-Roger *et al*. 2004).

From previous experiments, it was found that the Colorado Potato Beetle is sensitive to extracts from plants of the Lamiaceae family. The result of this sensitivity is an antifeedant and repellent effect (Pavela 2002, 2004). Plants from the Lamiaceae family contain a high percentage of polyphenols (Regnault-Roger *et al*. 2004) that were also used in the tests in this study. Even though the antifeedant effect of several substances was documented it was never significant enough to determine whether this antifeedant effect was caused by any one of the substances only. It is likely that the insecticidal and antifeedant effects of the extracts from plants of the Lamiaceae family are caused by the synergistic effects of several substances and/or by the terpenoids, which these plants also contain.

In this study, in contrast, several purified polyphenols significantly decreased the feeding and growth of Colorado Potato Beetle larvae, with a subsequent decrease in weight and growth, and it is possible that a significant mortality could be caused by a long-term exposure. It is therefore important to study these substances as a means of developing new environmentally friendly or botanical insecticides.

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