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# Feeding Responses of Japanese Beetle to Naturally Occurring Metabolites Found in Rosaceous Plants<sup>1</sup>

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## Abstract

Endogenous allelochemicals can have a profound influence on host plant resistance to arthropod pests. This study evaluated 22 naturally-occurring compounds found in Rosaceous trees for their influence on feeding of adult Japanese beetles [*Popillia japonica* (Newman)]. Individual compounds were incorporated into artificial diets at concentrations from 0 to 100 mM. No-choice feeding trials were conducted over 24 hours. Four general trends were recognized among the dose responses: 1) *no response*: benzaldehyde, calcium oxalate, tannic acid, 2) *stimulatory*: rutin, 3) *optimal peak*: benzoic acid, phloridzin, quercetin, catechin, geraniol, arbutin, caffeic acid, chlorogenic acid, and 4) *inhibitory*: p-coumaric acid, eugenol, amygdalin, phloretin, naringenin, o-coumaric acid, arginine, asparagine, oxalic acid, and gallic acid. These results suggest that variation in chemical constituents and concentrations may have a strong influence on host plant resistance to Japanese beetles among Rosaceous plants.

**Index words:** Rosaceae, *Popillia japonica* Newman, allelochemicals, host plant resistance, pest resistance, antixenosis.

## Significance to the Nursery Industry

As public concerns about pesticides increase and restrictions on pesticide use become greater, alternative methods of pest control need to be developed. Long-term integrated pest management (IPM) programs emphasize pest-resistant plants in developing more sustainable landscapes. Identification of specific chemical constituents that influence pest resistance enhances our abilities to evaluate and select plants with natural pest resistance. Ultimately, this information provides the foundation for developing plants and plant protectants that incorporate naturally occurring antifeedants.

## Introduction

The Rosaceae contains many species that are commercially important and commonly grown in the landscape. It is well known that different genera of trees in the Rosaceae vary in their natural resistance to insect pests. Some cultivars of Rosaceous plants are completely skeletonized by Japanese beetles while others are extremely resistant, even under no-choice conditions. Hawley and Metzger (8) documented considerable variability in natural resistance to Japanese beetle attack among different genera and species within the Rosaceae. In confirmation of these findings, recent studies have indicated that a wide range of resistance to Japanese beetle feeding exists among crabapple and cherry taxa (21).

In spite of the fact that the resistance of some varieties has been known for over 50 years, the nature of the resistance mechanism(s) has not been determined. Natural resistance can be the result of many plant characteristics. However, defensive chemicals that are produced within the plant as

primary or secondary metabolites have been shown to be an integral part of natural control of many insect pests (27, 22). In particular, phenolics, amino acids, and cyanogenic glycosides have been found to be important defensive chemicals for a variety of plants and pests. Therefore, the objective of this study was to evaluate and determine the potential antifeedant properties of 22 naturally occurring chemical compounds found in Rosaceous plants.

## Materials and Methods

**Insects.** Adult Japanese beetles were collected in the morning at the Mountain Horticultural Crops Research and Extension Center in Fletcher, NC. All beetles were obtained from smartweed (*Polygonum spp.*). Only female beetles were used for feeding trials because they tend to feed for longer periods of time and are less likely to be disturbed during feeding than male beetles (24). Before initiating feeding trials, beetles were held in a growth chamber lit with 75–85 mmol·m<sup>-2</sup>·s<sup>-1</sup> photosynthetically active radiation (PAR) for 24 hours at 25C (77F) on moist paper toweling without food.

**Artificial diet.** Artificial diet was prepared using a method similar to that of Hsiao and Fraenkel (9). Agar [4 g (0.14 oz)] and cellulose [4 g (0.14 oz)] were added to 100 ml (3.38 oz) of boiling water while stirring vigorously. Sucrose [3.42 g (0.12 oz)] as well as the appropriate amount of a given test compound were then added to the cooling diet. Each of the 22 test compounds with the exception of tannic acid and phloretin were added to the standard diet described previously to yield concentrations of 0.001, 0.01, and 0.1 molar (M). Tannic acid was tested at concentrations of 0 and 0.001 M because higher concentrations were incompatible with the artificial diet. Phloretin was only tested at concentrations of 0, 0.001 and 0.01 M due to the high cost of the compound. The control treatment consisted of the basic diet. The warm diet mixtures were poured into plastic petri dishes to a depth of approximately 1 cm.

**Feeding study.** Diet that had been cooled overnight at 5C (41F) was cut into plugs with a cork borer (1.5 × 1 cm). Individual plugs of diet were placed into plastic petri dishes. A single, starved beetle was placed into each dish. The dishes

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were covered with clear petri dish lids and placed into a growth chamber at 25C (77F) and with 75–85 mmol·m<sup>-2</sup>·s<sup>-1</sup> PAR. Beetles were allowed to feed for 24 hours after which they were removed from the dishes. Fecal matter was dried at 68–70C (154–158F) for 24 hours and weighed. Feeding intensity was compared based on fecal dry weight produced as a percentage of the control.

**Statistics.** Each treatment (diet) concentration had ten replications. All concentrations of a given compound were tested on the same day and petri dishes were arranged in a completely randomized design. Data were subjected to linear and non-linear regression analyses (TableCurve 2D, Jandel Scientific, San Rafael, CA). Selection of appropriate models was based on the significance of the F-statistic and the degree of freedom adjusted R<sup>2</sup>, given that the model and all individual terms of the model were significant (p < 0.05). The effective dose that reduced feeding by 25% (ED<sub>25</sub>) was calculated from regression equations to provide a convenient way to compare efficacy among compounds.

## Results and Discussion

Four general trends were recognized among the dose responses based on curves fitted with statistical significance at p < 0.05 (Fig. 1—Please note that the data is presented on a log scale and the shapes of the curves will reflect this).

- **No response.** Benzaldehyde (Fig. 1A), calcium oxalate (Fig. 1B) and tannic acid (Fig. 1C) did not significantly inhibit or stimulate feeding of adult Japanese beetle over the range tested.
- **Stimulatory.** The flavonoid phenolic rutin (Fig. 1D) stimulated feeding significantly as dose increased.
- **Optimal peak.** Any compound which initially stimulated feeding at low concentrations, but inhibited feeding at higher concentrations was included in this category. These compounds were benzoic acid (Fig. 1E), phloridzin (Fig. 1F), quercetin (Fig. 1G), catechin (Fig. 1H), geraniol (Fig. 1I), arbutin (Fig. 1J), caffeic acid (Fig. 1K), and chlorogenic acid (Fig. 1L). This feeding trend indicates the possible existence of an apparent threshold level needed for antifeedant activity to be conferred by these compounds.
- **Inhibitory.** The remainder of the test compounds including p-coumaric acid (Fig. 1M), eugenol (Fig. 1N), amygdalin (Fig. 1O), phloretin (Fig. 1P), naringenin (Fig. 1Q), o-coumaric acid (Fig. 1R), arginine (Fig. 1S), asparagine (Fig. 1T), oxalic acid (Fig. 1U), and gallic acid (Fig. 1V), inhibited feeding as the dose increased.

Benzaldehyde, calcium oxalate, tannic acid, rutin, quercetin, and catechin all failed to reduce feeding by 25% and therefore no ED<sub>25</sub> is presented for these compounds. P-coumaric acid, eugenol and amygdalin reduced feeding by 25% at concentrations <2 mM indicating these particular compounds were very effective as antifeedants (Fig. 2). Arbutin, phloretin, geraniol, phloridzin, naringenin, and o-coumaric acid were effective in reducing feeding by 25% at moderately low concentrations between 5.9 and 14.6 mM. The remaining six compounds, arginine, benzoic acid, asparagine, chlorogenic acid, oxalic acid, caffeic acid and gallic acid all reduced feeding by 25% but only at medium (41.2 mM) to high (81.8 mM) concentrations.

Many of the chemicals tested are simple phenolics having aromatic ring structures. Phenolics have been shown in numerous studies to be involved in insect resistance mecha-

nisms and are generally considered to be defense chemicals. Isman and Duffey (11) reported that phenolics in tomato foliage contributed substantially to antibiosis against the corn earworm [*Helicoverpa zea* (Boddie)]. Woodhead et al. (29) reported that high concentrations of phenolic acids could be correlated with reduced feeding of several grasshopper species (*Acrididae* spp.). Pree (20), working with apple maggot [*Rhagoletis pomonella* (Walsh)], indicated that total phenolic content was higher in resistant compared to susceptible crabapple varieties. Pree also showed in the same study that gallic, tannic and o-coumaric acid, as well as the flavonoid phenolics quercetin, naringenin and catechin, all inhibited apple maggot pupation and larval development at a concentration of 1000 ppm. However, our results demonstrate that not all phenolics inhibit feeding and some are stimulatory to feeding of adult Japanese beetle. Catechin, quercetin, rutin and tannic acids failed to substantially reduce the feeding of Japanese beetle and in some cases were very stimulatory. Other phenolics and flavonoids, however, such as p-coumaric acid, eugenol, phloretin, phloridzin, geraniol, o-coumaric acid and naringenin were effective at reducing feeding of Japanese beetle by 25% at low concentrations. Certain compounds may be involved in host plant resistance of different genera. Phloretin and its 2'-glucoside phloridzin may be particularly important in the host plant resistance of *Malus* taxa. Phloretin has been found at concentrations up to 0.025% dry weight and is known to be repellent to elm bark beetles [*Scolytus multistriatus* (Marsham.)] (18). Phloridzin constitutes as much as 3 to 7% of the leaf dry weight in some apple cultivars (10). Similarly, the compound arbutin is specific to the genus *Pyrus* and is possibly a factor in resistance to the woolly pear aphid [*Eriosoma pyricola* (B. and D.)], (3, 4).

The antifeedant activity of eugenol and geraniol is interesting considering that these compounds are known attractants to the Japanese beetle (14, 16). Odor appears to be an important factor in the selection of a host plant by Japanese beetle (7). Both eugenol and geraniol are highly volatile compounds and at the higher concentrations used in this study certainly have the potential to impact beetles confined to such a small area. Another factor to consider is that all attractants are not feeding stimulants. Alonso-Amelot et al. (1) found that eugenol at moderate to high concentrations elicited antifeedant behavior in the rust-red flour beetle [*Tribolium castaneum* (Herbst.)].

These results indicate that phenolics, including flavonoids, vary in their activity and effectiveness as antifeedants depending on concentration and test organism. It is also important to note that phenolic compounds can have several different modes of action. Phenolics can bind with proteins (25), depriving the pest of nitrogen, and thus function as an antinutritional compound (6, 5, 15). This mode of action probably relates to antibiosis and inhibition of growth by phenolics. However, since our studies were short-term feeding trials, the mode of action contributing to antifeedant properties was more likely antixenosis based on taste or gustatory responses.

Other compounds tested in this study included the cyanogenic diglucoside amygdalin which was very effective at reducing feeding of adult Japanese beetle. This compound is commonly found in seeds of Rosaceous species such as apple (*Malus* spp.), cherry, peach and almond (*Prunus* spp) (17). Amygdalin has the potential to degrade and release hydrocyanic acid (HCN) and benzaldehyde through an enzymatic

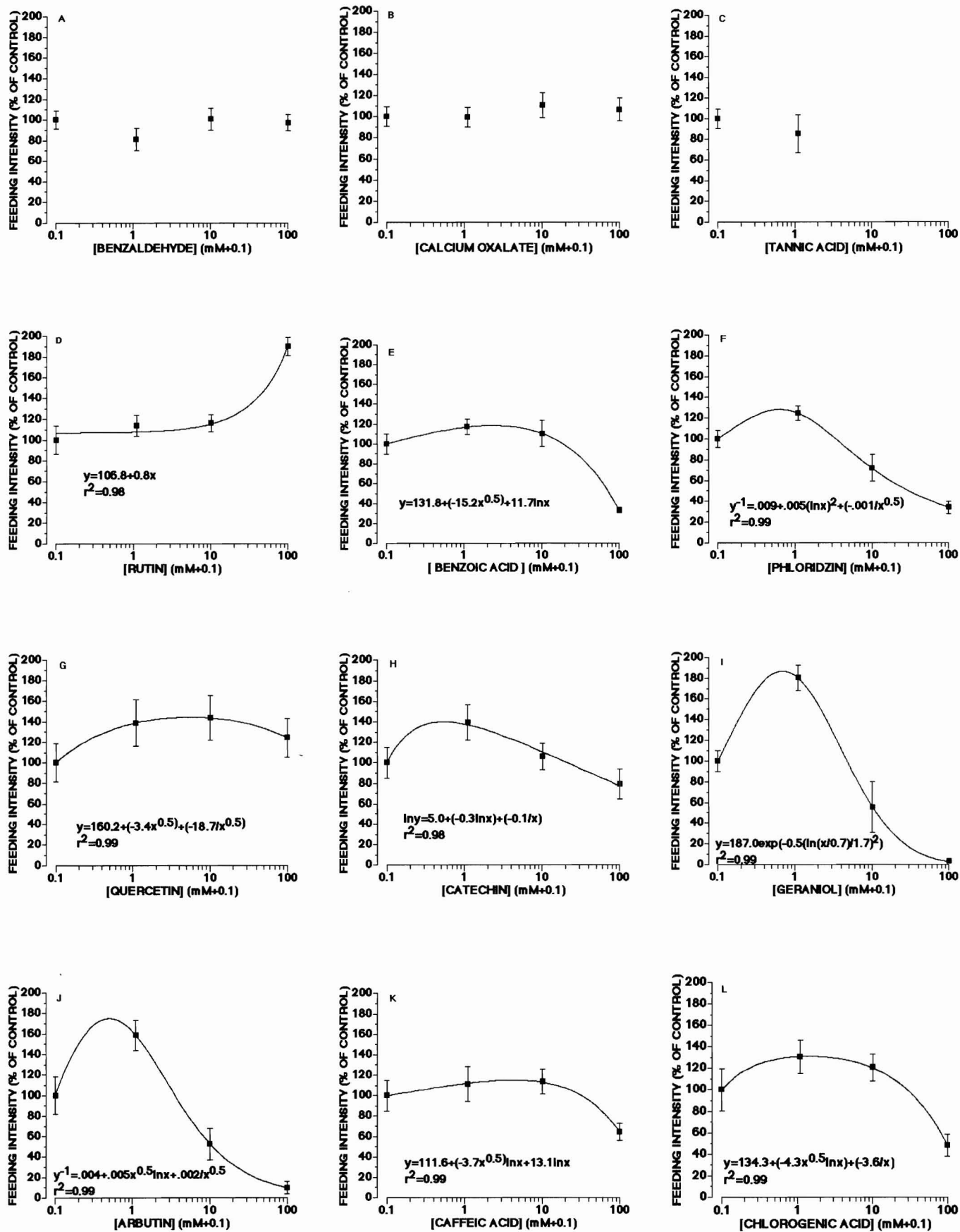


Fig. 1, A-L. Influence of chemical dose on feeding intensity of adult Japanese beetles for 22 test compounds. Symbols represent means  $\pm$  1 SEM, n = 10. Please note that the data are presented on a log scale and the shapes of the curves will reflect this.

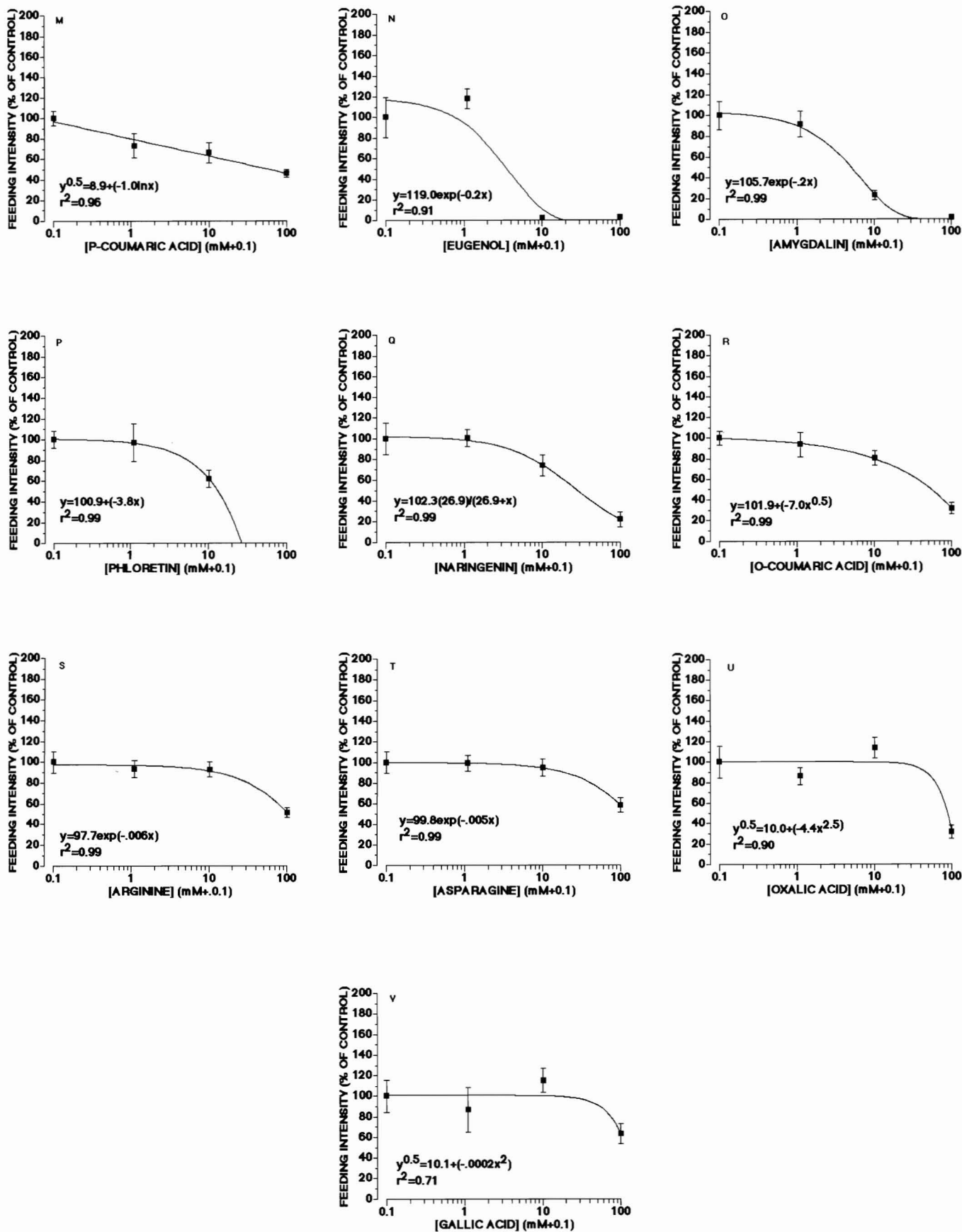


Fig. 1, M-V. Influence of chemical dose on feeding intensity of adult Japanese beetles for 22 test compounds. Symbols represent means  $\pm$  1 SEM, n = 10. Please note that the data are presented on a log scale and the shapes of the curves will reflect this.

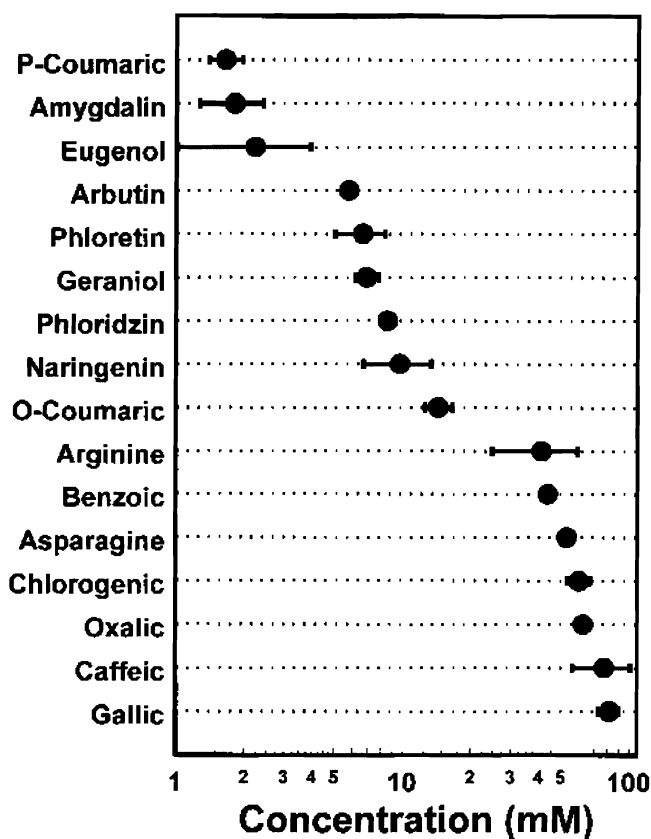


Fig. 2. Effective dosage of a test compound that reduced feeding of adult Japanese beetles by twenty five percent ( $ED_{25}$ ). Symbols are estimated values, based on regression analyses, bracketed by 95 percent confidence intervals.

process when acted on by herbivory (23). The known toxicity of HCN suggests it to be the major repellent factor, yet Peterson et al. (19) showed ants [*Myrmica americana* (Weber)] were tolerant of the odor of HCN and were repelled by the by-product benzaldehyde. In a review of cyanogenesis in animal-plant interactions, Jones (12) indicated the prime deterrents of foraging slugs to be the carbonyl compounds released in conjunction with HCN, not HCN itself. Conversely, Woodhead and Bernays (28) showed that protection in sorghum against locusts [*Locust migratoria* (L.)] was due to the release of HCN, not the cyanogenic glucoside or other by-products. In our study the diglucoside amygdalin was present in the diet without any catabolic enzymes. This indicates that either amygdalin itself was a deterrent to feeding of Japanese beetle or enzymes capable of catabolizing amygdalin were encountered at some point in the feeding process. Kaethler et al. (13) found that the action of enzymes in the gut of the obliquebanded leafroller [*Choristoneura rosaceana* (Harris)] were capable of hydrolyzing cyanogenic glycosides from peach leaves. In *Prunus* amygdalin appears only in the seed and the primary cyanogenic glycoside in leaf tissue is prunasin, the monoglucoside of amygdalin (17). Another study by (Patton, et al., in review) found that both amygdalin and prunasin had similar antifeedant properties.

The amino acids arginine and asparagine which were moderately effective at reducing feeding of Japanese beetle were found by Hsiao and Fraenkel (9) to have little or no effect on the biting and feeding responses of the Colorado potato beetle

[*Leptinotarsa decemlineata* (Say)]. In addition, they stated that as a group, amino acids were effective feeding stimulants. Beck and Hanec (2) showed that certain amino acids stimulated feeding of the European corn borer larvae [*Pyrausta nubilalis* (Hubn.)] while others, including arginine, were deterrent. Thorsteinson (26) showed that asparagine stimulated feeding of grasshopper.

The results of this study indicate a wide range of responses of the feeding of adult Japanese beetle to different chemical compounds. A variety of compounds known to exist in Rosaceous trees were effective feeding deterrents and may play an important role in host plant resistance. Potential difficulties lie in unraveling the complex chemistry in natural leaf conditions but this study constitutes an important first step in understanding the biology and chemistry of natural resistance to Japanese beetles. Further work is warranted to correlate resistance with concentration of key compounds *in situ*. More specific information on the chemical nature of host plant resistance will aid in the evaluation, selection, and development of more pest resistant plants. Identification of effective antifeedants may also have utility in the development of plant protectants that incorporate natural occurring compounds.

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