Effects of New Cultivars of Ninebark on Feeding and Ovipositional Behavior of the Specialist Ninebark Beetle, Calligrapha spiraeae (Coleoptera: Chrysomelidae)

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Abstract. Two newly developed cultivars of ninebark [Physocarpus opulifolius (L.) Maxim.], a yellow-leaved cultivar called ‘Dart’s Gold’ and a purple-leaved cultivar called ‘Monlo’, were compared with the native for resistance to feeding and oviposition by the specialist ninebark beetle [Calligrapha spiraeae (Say)]. When offered the choice between two types of ninebark, beetles statistically preferred native (P < 0.0001) and ‘Dart’s Gold’ (P < 0.0001) over ‘Monlo’, but showed no preference between native and ‘Dart’s Gold’ (P = 0.0743). Long-term performance bioassays showed significantly more eggs on ‘Dart’s Gold’ than ‘Monlo’ (P = 0.0468). ‘Monlo’ contained the most anthocyanins (P < 0.0001) and chlorophyll B (P < 0.0001) and the least total nitrogen (P = 0.00283) and Kjeldahl nitrogen (P = 0.0014). Anthocyanins are known to act as feeding deterrents and bind with nitrogen, which may explain why beetles avoided feeding on ‘Monlo’ in preference tests.

The nursery industry is interested in producing plants resistant to insects to reduce pesticide use and prevent economic loss. Programs range from traditional breeding (Painter, 1951) to mechanisms of induced defense (Zavala and Baldwin, 2006) to the roles of genes in plant resistance (Smith, 2005; Smith and Boyko, 2007). Studies on plant–insect coevolutionary theory may help explain what types of phytochemicals may be investigated in traditional breeding programs to develop plants resistant to specialist leaf-feeding beetles (Coleoptera) and moths (Lepidoptera). Coevolutionary theory argues that new phytochemicals in a plant may permit the plant to escape from herbivory by confusing the insect with the novel chemical. Specialist herbivores use chemicals as behavioral, feeding, and oviposition cues (Ehrlich and Raven, 1969; Futuyma, 2000). The theory speculates that herbivores are deterred by qualitative compounds that are toxic (less than 2% dry weight) such as alkaloids, phenolic glycosides, and furanocoumarins (Rhoades, 1979). However, specialist insects become physiologically (cytochrome P-450 systems for detoxifying phytochemicals) and behaviorally (feeding, oviposition) adapted to these toxins (Berenbaum and Zangerl, 1993). In contrast, quantitative compounds (5% to 20% dry weight) such as anthocyanins, polyphenols, and tannins are more difficult for specialists to adapt to because they often bind to nitrogen, reduce nitrogen availability, and slow insect development (Feeny, 1970). Data support the premise that specialist insects use qualitative compounds as behavioral, feeding, and ovipositional cues. Bronze birch borer (Agrilus anxius Gory) used rho-dodendrol in bark of birch (Betula sp.) as an attractant (Santamour, 1999). The willow leaf beetle (Chrysomela aeneicollis Schaeffer) was attracted to salicylate in Sierra willow (Sulfa oreestra Schneid.) (Rank, 1992). Black cottonwood (Populus trichocarpa Torr. & Gray) contained salicin, which was sequestered and transformed into the larval defensive chemical salicylaldehyde in cottonwood leaf beetle (Chrysomela scripta F.) (Warren et al., 2002).

Quantitative compounds are considered in plant insect coevolutionary theory to be feeding deterrents, even to specialist insects (Rhodes and Cates, 1976). In quaking aspen (Populus tremuloides Michx.), higher tannin levels decreased cottonwood leaf beetle larval growth rate by 30% (Donaldson and Lindroth, 2004). In white oak (Quercus alba L.) and black oak (Q. velutina Lam.), fewer specialists such as weevils (Attelabus sp.) (Curculionidae), southern oak dagger moth [Acronicta ineata Grote (Noctuidae)], and the moth Chionodes pereyra Clarke (Gelechiidae) were found on individual trees with higher tannin concentrations in the canopies (Forkner et al., 2004). In cotton (Gossypium hirsutum L.), chrysanthemum (anthocyanin), gossypol (polyphenol related to tannins), and other anthocyanins decreased feeding and survival of tobacco budworm [Heliothis virdecens (F.)] (Hedin et al., 1983).

Anthocyanins, one class of flavonoids, produce red and purple pigments and were reported to reduce herbivory (Close and Beadle, 2003; Harborne and Williams, 2000; Kursar and Coley, 1992; Simmonds, 2003). Correlations were reported between anthocyanin and total phenols (Close et al., 2001; Lee and Lowry, 1980); low levels of chlorophyll (Dodd et al., 1998; Krause et al., 1995), and low levels of nitrogen (Skirkanich et al., 1996). Feeding by the pyralid moth eggplant borer (Leucinodes orbonalis Gueneé) was lower on cultivars of eggplant (Solanum melongena L.) with higher levels of anthocyanins, phenolic compounds, and glycoalkaloids (Bajaj et al., 1989). In arabi-dopsis [Arabidopsis thaliana (L.) Heynh.], feeding by fall armyworm [Spodoptera frugiperda (JE Smith)] was lower on purple leaves [0.38 ± 0.03 absorbance units (AU) of anthocyanins] compared with green leaves with purple veins (0.29 ± 0.03 AU) or green leaves (0.023 ± 0.03 AU) (Johnson and Dowd, 2004). Bronze wild radish (Raphanus sativus L.) flowers containing anthocyanins were fed on less than white or yellow flowers by imported cabbageworm [Pieris rapae (L.)], beet armyworm [Spodoptera exigua (Hübner)], cabbage aphid [Brevicoryne brassicae (L.)], western flower thrips [Frankliniella occidentalis (Pergande)], and grey field slug [Agriolimax reticulatus (Müller)] (Irwin et al., 2003).

Breeding resistant plants to specialist insects should involve the development of cultivars with novel qualitative chemicals or increased levels of quantitative chemicals that bind with nitrogen and reduce its availability in food. Ninebark (Physocarpus opulifolius) (Rosales: Rosaceae) is a hardy shrub ranging from Quebec south to Tennessee that grows along riverbanks in sandy soil (Wheeler and Hoebeke, 1979, 1985). Nurseries breed ninebark for atypical leaf color: a purple-leaved cultivar called ‘Mono’ and yellow-leaved cultivar called ‘Dart’s Gold’. The purplish leaves of ‘Mono’ may be higher in anthocyanins, which can be feeding deterrents (Harborne and Williams, 2000; Simmonds, 2003) to the specialist ninebark beetle (Calligrapha spiraeae) (Coleoptera: Chrysomelidae). The objectives were to evaluate among two cultivars and native: 1) herbivore leaf preference, 2) herbivore fecundity in long-term rearing, and 3) leaf chemistry.

Materials and Methods

Plant material. Native ninebark was purchased from Outback Nurseries, Hastings, MN. ‘Dart’s Gold’ (yellow-leaved) and ‘Mono’ (purple-leaved) were purchased from Bailey Nurseries, St. Paul, MN. Shrubs were planted in 11.4-L containers containing Sunshine Professional Growing Mix (Sungro Horticulture, Seba Beach, Canada). Soil was
fortified with 35 g Osmocote 14N–4.2P–11.6K (Scotts–Sierra Horticultural Products Co., Marysville, OH) and fertilized weekly at 350 ppm with Peters 20N–8.4P–16.6K (Scotts–Sierra Horticultural Products Co.). Plants were grown outdoors in coldframes (University of Minnesota, St. Paul Campus) to simulate landscape conditions.

Insect material. Beetles were collected from the landscape and raised year-round in the greenhouse on mesh-covered native ninebark, because the beetle is multivoltine. Adults lay egg clusters (5.9 eggs) on the underside of leaves. In the laboratory, development time was 5 d for eggs and 18.9 d for larvae (four instars) at 22 °C with a natural photo period (Wheeler and Hoebeke, 1979). Additionally, beetles were raised on ninebark shoots irrigated in 25-mL plastic water tubes (Syndicate Sales, Kokomo, IN) contained in 14-L rectangular plastic boxes and stored in an incubator at 23 °C with 16 h of light.

Feeding preference bioassays: Disc test. On the day of the bioassay, leaves were collected from outside plants (University of Minnesota, St. Paul Campus). Leaves were cut into 1-cm-diameter discs with a disc cutter and six discs were placed in 100 × 15-mm petri dishes lined with Fisherbrand P5 filter paper (7-cm-diameter; Fisher Scientific, Pittsburgh, PA). The filter paper was kept moist with water. Four beetles were placed in each dish for 24 h, after which each disc was divided into four quadrants and percent missing was quantified by adding the number of bites. Twenty dishes contained native and ‘Dart’s Gold’ discs, 20 contained native and ‘Monlo’, and 20 contained ‘Dart’s Gold’ and ‘Monlo’. The experiment was replicated six times and analyzed by one-way analysis of variance (ANOVA) (combined replicate experiments) (SAS Institute, 2005) and PROC GLM for treatment, replicate, and treatment by replicate interactions (SAS Institute, 2003). When variances were unequal and assumptions of homogeneity were not met (Levene test P < 0.05), data were analyzed by Welch ANOVA (SAS Institute, 2005). Means were compared using Tukey’s honestly significant difference test and transformed when necessary (SAS Institute, 2005).

Shoot preference bioassays: feeding and oviposition. Shoots of ‘Monlo’ and native were compared for leaf area consumed and number of eggs on intact shoots. For each replicate experiment, nine cages (30 cm × 30 cm; BioQuip, Rancho Dominguez, CA) were used, each containing 25 beetles. In each cage, two treatments were placed on opposite sides in plastic 25-mL water tubes (Syndicate Sales, Kokomo, IN). Shoots were added as needed. At 7 d, eggs were counted and percent leaf area consumed was visually quantified by adding the number of sections of each leaf per leaf to determine the percent leaf area removed per leaf and then calculating a mean for each shoot based on the total number of leaves. The experiment was replicated four times and analyzed by one-way ANOVA (combined replicate experiments) (SAS Institute, 2005) and PROC GLM for treatment, replicate, and treatment by replicate interactions (SAS Institute, 2003). When variances were unequal and assumptions of homogeneity were not met (Levene test P < 0.05), data were analyzed by Welch ANOVA (SAS Institute, 2005). Means were compared using Tukey’s honestly significant difference test and transformed when necessary (SAS Institute, 2005).

Leaf chemistry: anthocyanin, chlorophyll, and nitrogen. Anthocyanin and chlorophyll concentrations were measured with a spectrophotometer. Leaves from five plants of each treatment were collected, preserved on ice, and stored in an ultralow freezer (−20 °C) until extraction. Approximately 0.05 to 0.10 g of leaf tissue was placed in a homogenization tube and ground for 30 s in a solution of 1:9 (ammonium hydroxide: acetone) (Environmental Protection Agency, 1994). The extractions were chilled on ice for 2 h in 12-mL plastic centrifuge tubes and then centrifuged for 20 min at 270 × g. The supernatants were collected and measured with a spectrophotometer. Absorbencies at wavelengths 534, 643, and 661 nm were measured and used to determine the amounts of chlorophylls A and B and anthocyanins (Sims and Gamon, 2002).

Total nitrogen and Kjeldahl (usable) nitrogen were measured from five plants for each treatment. Leaves were dried in an oven at 65 °C until crisp, then ground with a 20-mesh sieve stainless steel grinder, redried at 65 °C for 2 h, cooled in a desiccator, and weighed. Total nitrogen concentration was measured using the Dumas method in which nitrogen contents were combusted with oxygen to form oxides. The oxides were then reduced to N2 by copper and a helium carrier stream measured thermocconductivity. For total nitrogen, 0.15 g of sample was placed in a gel capsule and analyzed on a LECO 528 analyzer (University of Minnesota Soil Testing Laboratory, St. Paul Campus).

For Kjeldahl nitrogen, 0.15 g of sample was mixed with 3.5 mL concentrated H2SO4, 1.5 g K2SO4, and 7.5 mg selenium. Samples were placed in a 400 °C heated aluminum box for 1 h and reacted with salicylate in the presence of hypochlorite and nitroprusside. Turbidity was measured at 660 nm on a Technicon AutoAnalyzer (University of Minnesota Soil Testing Laboratory, St. Paul Campus).

For all leaf chemistry, experiments were replicated twice and analyzed by one-way ANOVA (combined replicate experiments) (SAS Institute, 2005) and PROC GLM for treatment, replicate, and treatment by replicate interactions (SAS Institute, 2003). When variances were unequal and assumptions of homogeneity were not met (Levene test P < 0.05), data were analyzed by Welch ANOVA (SAS Institute, 2005). Means were compared using Tukey’s honestly significant difference test and transformed when necessary (SAS Institute, 2005).

Results

Feeding preference bioassays: disc test. Beetles statistically preferred native (P < 0.0001) and ‘Dart’s Gold’ (P < 0.0001) over ‘Monlo’. However, there was no preference between native and ‘Dart’s Gold’ (P = 0.0743) (Table 1).

Shoot preference bioassays: feeding and oviposition. There was no significant difference in percent leaf area consumed (P = 0.4822) or number of eggs laid (P = 0.6223) on native or ‘Monlo’ at 7 d (Table 2).

Long-term performance bioassays: fecundity. Beetles laid significantly less eggs on ‘Monlo’ than ‘Dart’s Gold’ at 44 d (P = 0.0468) (Table 2).

Leaf chemistry: anthocyanin, chlorophyll, and nitrogen. ‘Monlo’ statistically contained the most anthocyanins compared with native and ‘Dart’s Gold’ (P < 0.0001). ‘Monlo’ contained the most chlorophyll B and ‘Dart’s Gold’ contained the least (P < 0.0001). ‘Monlo’ and native contained significantly more chlorophyll A than ‘Dart’s Gold’ (P < 0.0001).
Table 1. Percentage of ninebark leaf disc area consumed in 24 h by adult ninebark beetles.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Native/Mono</th>
<th>Monlo/Dart’s Gold</th>
<th>Native/Dart’s Gold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native</td>
<td>23.1 ± 1.4 a</td>
<td>22.1 ± 1.4 a</td>
<td></td>
</tr>
<tr>
<td>Monlo</td>
<td>12.5 ± 1.2 b</td>
<td>15.0 ± 1.2 b</td>
<td>18.7 ± 1.2 a</td>
</tr>
<tr>
<td>Dart’s Gold</td>
<td>—</td>
<td>25.7 ± 1.5 a</td>
<td>18.7 ± 1.2 a</td>
</tr>
<tr>
<td>F (df), P one way</td>
<td>35.0 (1, 230), &lt; 0.0001</td>
<td>29.9 (1, 232), &lt; 0.0001</td>
<td>3.2 (1, 226), 0.0743</td>
</tr>
<tr>
<td>F (df), P Welch</td>
<td>Levene test &gt; 0.05</td>
<td>Levene test &gt; 0.05</td>
<td>Levene test &gt; 0.05</td>
</tr>
<tr>
<td>F (df), P model</td>
<td>11.0 (11, 220), &lt; 0.0001</td>
<td>9.5 (11, 222), &lt; 0.0001</td>
<td>7.0 (11, 216), &lt; 0.0001</td>
</tr>
<tr>
<td>F (df), P treatment</td>
<td>45.5 (1), &lt; 0.0001</td>
<td>37.7 (1), &lt; 0.0001</td>
<td>3.7 (1), 0.0569</td>
</tr>
<tr>
<td>F (df), P replicate</td>
<td>13.2 (5), &lt; 0.0001</td>
<td>11.3 (5), &lt; 0.0001</td>
<td>9.6 (5), &lt; 0.0001</td>
</tr>
<tr>
<td>F (df), P trt*rep</td>
<td>2.0 (5), 0.0865</td>
<td>2.1 (5), 0.0657</td>
<td>5.0 (5), 0.0002</td>
</tr>
</tbody>
</table>

*Values with different letters are significantly different, Tukey-Kramer honestly significant difference, α = 0.05.

Discussion

Phytochemical analyses showed that purple-leaved ‘Monlo’ contained higher concentrations of foliar anthocyanins and lower concentrations of nitrogen than ‘Dart’s Gold’. In other studies, anthocyanins deterred feeding (Harborne and Williams, 2000; Simmonds, 2003), decreased nitrogen levels (Skillman et al., 1996), and were correlated to increased concentrations of condensed tannins from Alaska paper birch (Betula resinifera) and growth in specialist herbivores. Condensed tannins from Alaska paper birch (Betula resinifera Britton) were applied to leaves of felfleaf willow [Salix alaxensis (Anderss.)] and quaking aspen. Tannins significantly reduced growth rates of leaf beetles Calligrapha verrucosa (Suffrian) on felfleaf willow and Chrysomela falsa Brown and C. crochi Brown on quaking aspen (Ayres et al., 1997). In a study on Alaska paper birch, pupal weight of spear-marked black moth [Rheumaptera hastata (L.)] was 10 to 20 mg lower when larvae were fed from artificially defoliated trees, which contained volatile chemicals as intact shoots, which explained why beetles did not show a preference between native and ‘Monlo’ in shoot bioassays. However, in long-term rearing bioassays, beetles were given no choice and differences in fecundity probably reflected leaf quality.

Similar to our results, several studies reported that increased concentrations of quantitative compounds reduced feeding and growth in specialist herbivores. Condensed tannins from Alaska paper birch (Betula resinifera Britton) were applied to leaves of felfleaf willow [Salix alaxensis (Anderss.)] and quaking aspen. Tannins significantly reduced growth rates of leaf beetles Calligrapha verrucosa (Suffrian) on felfleaf willow and Chrysomela falsa Brown and C. crochi Brown on quaking aspen (Ayres et al., 1997). In a study on Alaska paper birch, pupal weight of spear-marked black moth [Rheumaptera hastata (L.)] was 10 to 20 mg lower when larvae were fed from artificially defoliated trees, which contained volatile chemicals as intact shoots, which explained why beetles did not show a preference between native and ‘Monlo’ in shoot bioassays. However, in long-term rearing bioassays, beetles were given no choice and differences in fecundity probably reflected leaf quality.

Table 2. Feeding (percent shoot leaf area consumed) and oviposition (number of eggs), and total fecundity (number of eggs per female) of adult ninebark beetles on ninebark.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percent consumed ± se (7 d choice)</th>
<th>No. of eggs ± se (7 d choice)</th>
<th>Eggs per female ± se (44 d no choice)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native</td>
<td>21.0 ± 2.4 a</td>
<td>57.4 ± 16.5 a</td>
<td>83.2 ± 9.0 ab</td>
</tr>
<tr>
<td>Monlo</td>
<td>18.6 ± 2.2 a</td>
<td>68.6 ± 15.3 a</td>
<td>66.8 ± 6.0 b</td>
</tr>
<tr>
<td>Dart’s Gold</td>
<td>—</td>
<td>68.6 ± 15.3 a</td>
<td></td>
</tr>
<tr>
<td>F (df), P one way</td>
<td>0.5 (1, 54), 0.4822</td>
<td>0.2 (1, 54), 0.6223</td>
<td>3.2 (2, 93), 0.0468</td>
</tr>
<tr>
<td>F (df), P Welch</td>
<td>Levene test &gt; 0.05</td>
<td>Levene test &gt; 0.05</td>
<td>Levene test &gt; 0.05</td>
</tr>
<tr>
<td>F (df), P model</td>
<td>5.4 (7, 48), 0.0001</td>
<td>18.4 (7, 48), &lt; 0.0001</td>
<td>1.8 (7, 88), 0.0938</td>
</tr>
<tr>
<td>F (df), P replicate</td>
<td>0.2 (1), 0.6484</td>
<td>0.5 (1), 0.4774</td>
<td>4.9 (2), 0.0093</td>
</tr>
<tr>
<td>F (df), P trt*rep</td>
<td>11.7 (3), &lt; 0.0001</td>
<td>42.1 (3), &lt; 0.0001</td>
<td>1.1 (2), 0.3534</td>
</tr>
<tr>
<td>F (df), P trt*rep</td>
<td>0.7 (3), 0.5682</td>
<td>0.6 (3), 0.6169</td>
<td>1.7 (3), 0.1723</td>
</tr>
</tbody>
</table>

*Values with different letters are significantly different, Tukey-Kramer honestly significant difference, α = 0.05.

Conclusion

Anthocyanins present in ‘Monlo’ ninebark reduce preference and fecundity in the specialist ninebark beetle. Understanding that specialist insects avoid feeding on and reduce fecundity in response to increased levels of quantitative chemicals, as well as novel qualitative chemicals, offers a hypothesis-based method for breeding resistant plants. Because humans do not ingest ornamental plants (unlike crop plants), it is possible to introduce genes for producing four times the amount of tannins and less than two-thirds the amount of nitrogen compared with trees that were not defoliated (Bryant et al., 1993). In a study on Viburnum sp. (a rosaceous relative of ninebark), the specialist viburnum leaf beetle [Pyrrhalta viburni (Paykull)] avoided blackhaw viburnum (Viburnum prunifolium L. ‘Early Red’), on which emerging leaves are reddish (Weston and Desurmont, 2002). Koreanspice viburnum (Viburnum carlesii Hemsl.), Fragrant snowball viburnum (V. × carcephalum Burkwood ex R.B.Pike), and Judd viburnum (V. × juddii Rehd.) display reddish leaves in the fall and are resistant to viburnum leaf beetle. Leaf chemical analysis revealed a resistant factor, but it was not determined (Weston et al., 2000).

In addition to breeding plants to have higher amount of quantitative chemicals, breeding plants to contain novel qualitative compounds will reduce feeding and increase mortality in specialist insects. Numerous papers reported that novel compounds not present in host plants of specialist insects reduce feeding and fecundity. For instance, the crucifer flea beetle (Phyllotreta tetragonia Com.), the turnip flea beetle (P. undulata L.), and the mustard beetle (Phaseolus vulgaris L.) refused to feed on cruciferous wallflowers (Erysimum and Cheiranthus sp.), which contained cardenoloids and cardiac glycosides. The yellow-striped flea beetle (Phyllotreta nemorum L.) refused to feed on candytuft (Iberis sp.), which contained cucurbitacin E and I (Nielsen, 1978; Nielsen et al., 1997). A crucifer specialist, imported bagworm, did not feed or oviposit on wormseed mustard (Erysimum cheiri (Huds.) (Dimock et al., 1991), a plant containing cardenoloids such as erysinoside and erychroside (Sachdev-Gupta et al., 1990).

Table 3. Anthocyanin, chlorophyll, and nitrogen contents of ninebark.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>mmol/g leaf ± se</th>
<th>Chlorophyll A</th>
<th>Chlorophyll B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native</td>
<td>0.4 ± 0.12 b</td>
<td>1.7 ± 0.07 a</td>
<td>0.4 ± 0.03 b</td>
</tr>
<tr>
<td>Monlo</td>
<td>1.6 ± 0.11 a</td>
<td>1.9 ± 0.06 a</td>
<td>0.6 ± 0.02 a</td>
</tr>
<tr>
<td>Dart’s Gold</td>
<td>0.2 ± 0.07 b</td>
<td>0.7 ± 0.09 a</td>
<td>0.1 ± 0.03 c</td>
</tr>
<tr>
<td>F (df), P one way</td>
<td>55.9 (2, 27), &lt; 0.0001</td>
<td>82.0 (2, 27), &lt; 0.0001</td>
<td>116.3 (2, 27), &lt; 0.0001</td>
</tr>
<tr>
<td>F (df), P Welch</td>
<td>Levene test &gt; 0.05</td>
<td>Levene test &gt; 0.05</td>
<td>Levene test &gt; 0.05</td>
</tr>
<tr>
<td>F (df), P model</td>
<td>27.0 (5, 24), &lt; 0.0001</td>
<td>29.8 (5, 24), &lt; 0.0001</td>
<td>41.8 (5, 24), &lt; 0.0001</td>
</tr>
<tr>
<td>F (df), P treatment</td>
<td>64.0 (2), &lt; 0.0001</td>
<td>74.3 (2), &lt; 0.0001</td>
<td>104.5 (2), &lt; 0.0001</td>
</tr>
<tr>
<td>F (df), P replicate</td>
<td>0.0 (1), 0.8499</td>
<td>0.0 (1), 0.9636</td>
<td>0.2 (1), 0.6995</td>
</tr>
<tr>
<td>F (df), P trt*rep</td>
<td>3.4 (2), 0.4888</td>
<td>0.2 (2), 0.8040</td>
<td>0.1 (2), 0.9548</td>
</tr>
</tbody>
</table>

*Values with different letters are significantly different, Tukey-Kramer honestly significant difference, α = 0.05.
novel toxins and increased polyphenols. Reducing insect damage and insecticide use will foster plant and urban ecosystem health.

Literature Cited


