Domestication in Murtilla (\textit{Ugni molinae}) Reduced Defensive Flavonol Levels but Increased Resistance Against a Native Herbivorous Insect

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ABSTRACT Plant domestication can have negative consequences for defensive traits against herbivores, potentially reducing the levels of chemical defenses in plants and consequently their resistance against herbivores. We characterized and quantified the defensive flavonols from multiple cultivated ecotypes with wild ancestors of murtilla, \textit{Ugni molinae} Turcz, an endemic plant from Chile, at different times of the year, and examined their effects on a native insect herbivore, \textit{Chilesia rudis} Butler (Lepidoptera: Arctiidae). We hypothesized that domestication results in a decrease in flavonol levels in \textit{U. molinae} plants, and that this negatively affected \textit{C. rudis} performance and preference. Ethanolic extracts were made from leaves, stems, and fruit of murtilla plants for flavonol analysis. Flavonols identified were kaempferol, quercetin, rutin, and quercetin 3-D-β-glucoside, the last two being the most abundant. More interestingly, we showed differences in flavonol composition between wild and cultivated \textit{U. molinae} that persisted for most of the year. Relative amounts of all four flavonols were higher in wild \textit{U. molinae} leaves; however, no differences were found in the stem and fruit between wild and cultivated plants. In choice and no-choice assays, \textit{C. rudis} larvae gained more mass on, and consumed more leaf material of, wild as compared with cultivated \textit{U. molinae} plants. Moreover, when applied to leaves, larvae ate more leaf material with increasing concentrations of each flavonol compound. Our study demonstrates that domestication in \textit{U. molinae} reduced the amount of flavonols in leaves as well as the performance and preference of \textit{C. rudis}, indicating that these compounds stimulate feeding of \textit{C. rudis}.

KEY WORDS domestication, HPLC, choice assay, nochoice assay, \textit{Chilesia rudis}

The domestication of plants is a process of artificial selection in which wild plants are modified to meet human needs (Meyer and Purugganan 2013, Cornille et al. 2014, Gepts 2014). As a result, plant domestication may generate a so-called “domestication syndrome” (Hammer 1984, Evans 1993, Abbo et al. 2014), where the domesticated plants have features useful for human consumption such as increases in yield, fruit size, number of seeds, and plant growth (Wink 1988). However, certain plant traits such as those associated with defense against herbivores can be negatively affected (Hammer 1984). This may occur particularly in plants domesticated for high yield where a pool of available resources is allocated to fruit production instead of defense (Herns and Mattson 1992, Davila-Flores et al. 2013). Consequently, domesticated plants may be less defended against their enemies as compared with their wild ancestors (Rosenthal and Dirzo 1997, Rodriguez-Saona et al. 2011, Chen and Bernal 2011, see review by Chen et al. 2015). For example, in a recent study, Altesor et al. (2014) found that cultivated potato (\textit{Solanum tuberosum} L.) plants have lower levels of glycoalkaloids and were more susceptible to attack by two generalist herbivores, the green peach aphid, \textit{Myzus persicae} Sulzer, and the potato aphid, \textit{Macrosiphum euphorbiae} Thomas, as compared with their wild ancestors.

Flavonoids are an important group of plant defensive compounds (Harborne 1988, 2000), which can be affected by domestication. For instance, Mikulic-Petkovsek et al. (2012) analyzed several species of wild and cultivated berries and found higher levels of the flavonoid quercetin in wild plants than in cultivated ones. Similarly, Giovanelli and Buratti (2009) analyzed four varieties of cultivated blueberries and a wild counterpart and showed that the total phenols and anthocyanin concentrations in wild fruit were two- and threefold higher, respectively, than in cultivated fruit. Flavonoids are known to affect insect feeding behavior (Harborne 1988). For example, Simmonds (2001) reported that rutin, a commonly studied flavonol glycoside, is a

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phagostimulant to many polyphagous insects such as Schistocerca americana Drury and Heliotis virescens F.; however, high levels of this compound were deterrent to the noctuids Helicoverpa zea Boddie, Spodoptera littoralis Boisd. sp., Spodoptera exigua Hübner, and Spodoptera exempta Walker. Some flavonoids are also known to reduce larval performance (Elliger et al. 1990). For example, rutin caused 50% mortality and reduced the relative growth rate of Spodoptera eridania Cramer (Lindroth and Peterson 1988). Also, Beninger and Abou-Zaid (1997) showed that rutin, quercetin, and a glucoside quercetin isolated from four pine species decrease larval mass and increase mortality of Lymnaea dispar L.

Murtilla, Ugni molinae Turcz (Myrtaceae), an endemic plant from Chile, is a highly polymorphic perennial shrub reaching heights of over 3 m (Valdebenito et al. 2003, Hoffmann 2005). Researchers at the Experimental Station of the Instituto de Investigaciones Agronómicas and the Station-Tranapuente in the Región de La Araucanía, Chile, have been domesticating this species for the past 20 yr for high productivity (Seguel and Torralbo 2004), as well as on their agronomic characteristics such as size, growth, and productivity (Seguel and Torralbo 2004), as well as the availability of their wild parents in the original collection areas. The ecotypes were sampled from two geographical regions: five ecotypes were originally sampled from the Región de La Araucanía (ecotypes 08-1, 12-1, 14-4, 18-1, and 19-1), and the other two ecotypes were originally sampled from the Región de Los Ríos (ecotypes 22-1 and 23-2). Fertilizer was applied annually according to soil analysis, and consisted of 80, 44, and 49 g per plant of nitrogen, P₂O₅, and K₂O, respectively. Pest control was carried out using Karate (lambda-cyhalothrin; Syngenta, Greensboro, NC) at a dose of 1 to 2 ml/liter of water or Lorsban 4E (chlorpyrifos; Dow AgroSciences, Indianapolis, IN) at a dose of 1 ml/plant (one to two applications during the year), according to the incidence of cutworms. To avoid residual toxicity, all samples for chemical and biological assays (see below) were collected at least 7 d after insecticide applications.

Each cultivated ecotype was paired with its wild ancestor, which was located in the same geographical area where its cultivated counterpart originated. For the wild plants, the following sampling areas were used: Caburga (39° 11′ S, 71° 49′ W), Pucón (39° 17′ S, 71° 55′ W), Manzanal Alto (38° 03′ S, 73° 10′ W), Soloyo (35° 35′ S, 72° 34′ W), and Porma (39° 08′ S, 73° 16′ W) from the Región de La Araucanía; and Mehuin (39° 26′ S, 73° 12′ W) and Queule (39° 23′ S, 73° 12′ W) from the Región de Los Ríos.

Therefore, the following seven cultivated, wild ecotypes, geographical areas were paired for chemical and biological assays: Eco 08-1, Caburga, Eco 12-1, Pucón, Eco 14-4, Manzanal Alto, Eco 18-1, Soloyo, Eco 19-1, Porma, Eco 22-1, Mehuin, and Eco 25-2, Queule.

**Plant Material and Insects.** Leaves, stems, and fruits (when available) of U. molinae were sampled monthly (from December 2012 until October 2013) from both cultivated and wild plants. Five plants were sampled for each cultivated ecotype and at each wild site (N = 70 plants). Samples were taken from all four cardinal directions and at different heights of each plant, and were standardized by age to control for pheno logical variation. After this, samples were stored in paper bags, placed in a cooler, and then transported to the Laboratorio de Química Ecológica, Universidad de La Frontera (Temuco, Chile). Samples were stored at −20°C (Zeraik 2010, Yi et al. 2012) until used in chemical analysis (see below), while samples for bioassays were used within 24 h after collection.

C. rudis larvae (older instars; size = 30–40 mm) used for bioassays were collected manually in late spring (December) from grasses in Temuco, Padre Las Casas, southern Chile. Through the process of domestication, U. molinae cuttings were first grown in greenhouses for 10 yr and then transplanted to the field (INIA Experimental Station-Transapuente in the Región de La Araucanía [south of Chile, 38° 45′ S, 73° 21′ W] until now. In Chile and worldwide, there is a strong economic interest in the production of U. molinae fruit due to its high antioxidant content. This antioxidant activity is attributed to the presence of flavonoid compounds (Avello and Pastene 2005; Rubilar et al. 2006, 2011). For example, several flavonoids such as quercetin, kaempferol, rutin, and myricetin, and their corresponding glycosides, have been identified from U. molinae fruit and leaves (Shene et al. 2009, 2012).

Aguilera et al. (2009) reported, for the first time, larvae of Chilesia rudis Butler (Lepidoptera: Arctiidae) attacking U. molinae. C. rudis is a polyphagous, univoltine insect, native to Chile (Vargas and Parra 2003), and one of the most serious pests of grasslands, acting as a severe defoliator of several plants (Angulo and Ruiz 1974). The life cycle of C. rudis in the Región de La Araucanía, Chile, has been described by Angulo and Ruiz (1974): the larval stage lasts 6–8 mo, from May until December, while the pupal stage lasts 2 mo; the adults emerge in February and live for 8–14 d. The larvae feed on different plant parts but prefer the leaves.

In this study, we hypothesized that U. molinae domestication has decreased chemical defenses and resistance against herbivores. Specifically, we compared the levels of four major flavonoids—quercetin, kaempferol, quercetin 3-D-β glucoside, and rutin—in wild and domesticated U. molinae plants. We also determined seasonal differences in flavonol content as well as differences among various plant tissues. Finally, we studied C. rudis larval growth and feeding on wild and domesticated U. molinae leaves, and tested the effects of each of the four identified flavonols on larval leaf consumption.

**Materials and Methods**

**Sampling Area.** Seven cultivated ecotypes, i.e., geographically distinct populations, of U. molinae from the INIA Experimental Station-Transapuente were used for experiments. These ecotypes have been maintained in a field at the Experimental Station for almost 10 yr, as indicated above. We selected these ecotypes based on their agronomic characteristics such as size, growth, and productivity (Seguel and Torralbo 2004), as well as the availability of their wild parents in the original collection areas. The ecotypes were sampled from two geographical regions: five ecotypes were originally sampled from the Región de La Araucanía (ecotypes 08-1, 12-1, 14-4, 18-1, and 19-1), and the other two ecotypes were originally sampled from the Región de Los Ríos (ecotypes 22-1 and 23-2). Fertilizer was applied annually according to soil analysis, and consisted of 80, 44, and 49 g per plant of nitrogen, P₂O₅, and K₂O, respectively. Pest control was carried out using Karate (lambda-cyhalothrin; Syngenta, Greensboro, NC) at a dose of 1 to 2 ml/liter of water or Lorsban 4E (chlorpyrifos; Dow AgroSciences, Indianapolis, IN) at a dose of 1 ml/plant (one to two applications during the year), according to the incidence of cutworms. To avoid residual toxicity, all samples for chemical and biological assays (see below) were collected at least 7 d after insecticide applications.

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C. rudis larvae (older instars; size = 30–40 mm) used for bioassays were collected manually in late spring (December) from grasses in Temuco, Padre Las Casas,
Chile (35° 46′ S, 72° 36′ W); thus, these larvae likely had no prior experience on \textit{U. molinae}. Larvae were deprived of food for 3 d before the experiments.

**\textit{U. molinae} Extract.** Throughout a year, 735 samples from leaf, stem, and fruit of both cultivated and wild plants were collected. Samples were rapidly frozen in liquid nitrogen for 5 s (Mihalje-Petkovsek et al. 2012), and then milled in a grinder. After this, samples (5 g) were placed in a flask where ethanol HPLC grade (Sigma-Aldrich, St. Louis, MO) was added to the samples (50% v/v in water, solvent-to-solid ratio of 5:1). These flasks were placed in a magnetic stirrer for 20 min at 30°C and 170 rpm. After this time, samples were filtered in darkness through a Whatman number 1 filter paper (Whatman International Ltd., Maidstone, United Kingdom). The filtrate was concentrated in a rotary evaporator at 45°C and lyophilized for 8 h (Rubilar et al. 2011). Finally, each sample was suspended in 10 ml of ethanol and left for 5 min in a Branson 3510 sonicator. Samples were stored at −20°C in amber flasks (25 ml) until their use in High Performance Liquid Chromatography (HPLC) analysis.

**Chromatographic Separation and Quantification by HPLC Analysis.** The ethanolic extracts obtained from leaves, stems, and fruits were filtered through a 0.22 µm membrane and these were analyzed by HPLC. Twenty microliter of each sample was injected into a Shimadzu HPLC (Model LC-20A Prominance, Kyoto, Japan) equipped with a C-18 column (150 × 4.6 mm I.D.; particle size 5 mm) maintained at 40°C. The analysis was performed using a linear solvent gradient consisting of 1% formic acid (A) and acetonitrile (B) as follows: 0–5 min, 5% A/95% B; 5–10 min, 30% A/70% B; 10–20 min, 55% A/45% B; 20–30 min, 5% A/95% B at a flow rate of 1 ml/min (Simirgiotis et al. 2013). Flavonols were monitored at 280 nm; UV spectra from 190 to 400 nm were used for peak characterization. The identification of flavonols was based on the peak retention time in comparison with that of a standard.

To construct calibration curves for flavonols, standard solutions were dissolved in methanol (Sigma-Aldrich) at 1,000 mg/liter. The stock solutions of each standard were used to prepare a serial concentration between 0.05 to 500 mg/liter (Kumar et al. 2009). All the standards were stored at 4°C until their injection into the HPLC (Zu et al. 2006). To determine the limits of detection (LOD) and quantification (LOQ), the stock solution of each standard was diluted in MeOH to provide serial dilutions. Each solution was injected in the HPLC until obtaining the 3-σ signal to noise (S/N) ratio for LOD of each flavonol and a value of 10-σ for LOQ (Olszewska 2008).

**No-Choice Bioassay.** This study evaluated \textit{C. rudis} larval performance on cultivated versus wild \textit{U. molinae} leaves. One \textit{C. rudis} larva was placed in a Petri dish (94 mm in diameter by 16 mm high) containing either a cultivated or a wild \textit{U. molinae} leaf. The bioassay lasted 2 d, and leaves were replaced after 24 h. Ten replicates were performed for each of the seven cultivated ecotypes and their wild counterparts. The amount of feeding was measured in cm² by scanning each leaf and then measuring the area consumed using the ImageJ 1.42j software (Wayne Rasband National Institutes of Health). In addition, fresh larval mass was obtained prior to the bioassay and after 48 h, as described in Carpinella et al. (2003), and the mass gained was calculated.

**Choice Bioassay.** We also conducted experiments using a leaf choice test (Carpinella et al. 2002) to determine \textit{C. rudis} larval preference for cultivated versus wild \textit{U. molinae} leaves. Two leaves, one of a cultivated and one of a wild \textit{U. molinae} plant, were placed in a 10-mm-diameter Petri dish, with two 1-cm-diameter holes on the top covered by a fine mesh. Larvae of \textit{C. rudis} were placed in a position equidistant from both leaves and allowed to feed for 48 h. Ten replicates were run for each of the seven cultivated ecotypes and their wild counterparts. Larval consumption was measured as described for no-choice bioassays. Relative amounts (in percentages) of leaf area eaten for each cultivated ecotype and wild location were calculated based on a feeding index, \textit{FI}=-(W−C)/(C+W)×100, where C and W represent consumption on cultivated and wild leaves, respectively (Mazor et al. 2008).

**Bioassay with Individual Flavonols.** This experiment was conducted to determine if the identified flavonols act as phagostimulants, and also to rule out whether the observed effects were due to environmental factors such as pesticides. A cultivated \textit{U. molinae} leaf (~1.2 cm²) was placed on a Petri dish, as described above, and 0.5 ml of pure compound of quercetin, kaempferol, quercetin 3-D-glucoside, or rutin at concentrations of 0.1, 1, 5, 50 mg/liter were applied over each leaf using a micropipette. Leaves for this experiment were collected in January from one of the cultivated ecotypes (Eco18-1), which contains higher flavonol levels than the other ecotypes (see results). To test for concentration-dependent effects, we used a range of flavonol concentrations that were comparable with those found in the leaves. All compounds tested were dissolved in ethanol (solvent), purchased from Sigma-Aldrich, and had purities > 98%. Controls (0 mg/liter) had ethanol only. One \textit{C. rudis} larva was then placed inside the Petri dish. After 24 h the consumed area was recorded using the ImageJ 1.42j software. This experiment was replicated 10 times for each flavonol concentration and controls.

**Statistical Analysis.** The statistical software R (R 3.0.2; the R foundation for statistical computing, Vienna, Austria) was used to analyze the data. The effects of domestication (i.e., wild versus cultivated plants) and location (i.e., cultivated ecotype, wild geographical area of collection) on total flavonol content and larval mass and consumption were analyzed using fully nested hierarchical random analysis of variance (ANOVA), with domestication nested within location and location used as a random effect. The effects of domestication, time of year, or plant part, and their interaction on total flavonol content were analyzed using a two-way ANOVA. ANOVA was also used to test for the effects of different concentrations of individual flavonols on leaf consumption. Scott–Knott tests were used for comparisons among groups. For each individual flavonol, t-tests were used
for paired comparisons between wild and cultivated plants. Data were natural-log transformed to meet the assumptions of normality and homogeneity of variance. We used arcsine square-root transformation for percent data. Values of $P < 0.05$ were considered as significant.

Results

Chromatographic Analysis. Overall, across all tissues (leaves, stems, and fruit), the total amount of flavonols in wild $U. \textit{molinae}$ plants was $\sim 20\%$ higher than in the cultivated plants, showing a significant domestication effect (Table 1A; Fig. 1A), which was dependent on the ecotype or geographical area (Table 1A; Fig. 1B), such that wild plants from Manzanal Alto, Caburgua, Mehuin, and Queule had significantly higher flavonol concentrations as compared with their cultivated counterparts (Scott–Knott test, $P < 0.05$), while other sites were not significant ($P > 0.05$).

There was temporal variation in flavonol content (significant time of year effect: $F_{6,84} = 15.41$, $P < 0.001$). For both wild and cultivated plants, total flavonol content increased from December to April (which coincides with adult emergence and oviposition), then decreased from April to June (coinciding with young larval development), and increased again from June to October (which coincides with older larval development; Fig. 2). Throughout the year, wild plants had higher levels of flavonols than cultivated plants (significant Domestication effect: $F_{1,84} = 33.66$, $P < 0.001$; Fig. 2). There was, however, no Domestication $\times$ Time of Year effect ($F_{6,84} = 4.69$, $P = 0.001$), indicating that the differences in flavonol content between wild and cultivated plants were consistent throughout the year. Within plants (spatial variation), $U. \textit{molinae}$ fruit had higher flavonol content than leaves and stems (significant Plant Part effect: $F_{2,168} = 63.163$, $P < 0.001$; Fig. 3). However, differences in flavonol content between wild and cultivated plants were significant only for leaves (significant Domestication $\times$ Plant Part interaction: $F_{20,168} = 1.869$, $P = 0.018$; Fig. 3). The analysis of $U. \textit{molinae}$ extract showed the presence of four flavonols—rutin, quercetin-3-D-glucoside, quercetin, and kaempferol. The amount of flavonol was higher in wild plants ($P < 0.05$) than cultivated plants (Table 2; Fig. 4).
No-Choice Bioassay. In no-choice tests, *C. rudis* larvae gained almost twice as much mass when fed foliage from wild *U. molinae* plants compared with those fed cultivated plants; however, the effect of domestication on larval mass gained depended on location (Table 1B; Fig. 5A). In all cultivated and ecotype and wild and geographical area combinations, except for Eco12-1, Pucon, *C. rudis* larvae gained more mass when they were fed wild *U. molinae* plants than those fed cultivated plants (Table 1C; Fig. 5D).

Choice Bioassay. *C. rudis* larvae consumed 61% more of the wild *U. molinae* leaves than the cultivated leaves (Table 1D; Fig. 5E). As in the no-choice test, there was a significant domestication nested within location effect (Table 1D), indicating that the effect of domestication on *C. rudis* leaf area consumption was affected by ecotype and geographical area (Fig. 5F).

Assays with Individual Flavonols. *C. rudis* larval consumption increased with increasing concentrations of all flavonols tested ($F_{3,64} = 38.207, P < 0.05$). However, there was no effect of flavonol type ($F_{3,64} = 1.454, P = 0.235$) or interaction between flavonol type and concentration ($F_{9,64} = 0.243, P < 0.987$), indicating that the effect of all flavonols on larval consumption was similar instead of all concentrations. *C. rudis* consumed 40% of cultivated *U. molinae* treated with a concentration of 0.1 mg/liter of any flavonol; however, when higher concentrations were applied to leaves, *C. rudis* larvae increased their consumption to 80–90% with increasing concentrations, indicating that these compounds acted as phagostimulants (Fig. 6).

Discussion

Crop domestication can affect plant defenses and resistance against herbivores in unpredictable ways (Meyer et al. 2012). For example, in a recent study, Turcotte et al. (2014) found that domestication across 29 crop species results in reduced resistance to a generalist leaf-chewing herbivore, *S. exigua*, but had no effect on the generalist aphid, *M. persicae*. In this area, except for Eco12-1, Pucon, ate more foliage when fed wild *U. molinae* plants as compared with those fed cultivated plants (Table 1C; Fig. 5D).
regard, our study demonstrates higher levels of flavonoids in wild as compared with cultivated *U. molinae* leaves. However, the performance and preference of the native caterpillar *C. rudis*, a generalist folivore, was higher on wild as compared with cultivated *U. molinae*, despite the fact that the former plants have higher amounts of flavonoids, indicating that these chemical compounds may be acting as phagostimulants in this insect–plant interaction.

Domestication and breeding for high-yielding crops are expected to reduce chemical defenses in plants because of potential trade-offs between growth or reproduction and defense (Wink 1988, Herns and Mattson 1992, Rodriguez-Saona et al. 2011). Domestication in *U. molinae* has focused mainly on selection of traits associated with increased productivity, such as bigger plants, more fruit, and larger fruit sizetraits associated with increased productivity, such as bigger plants, more fruit, and larger fruit size (Seguel and Torralbo 2004). As a result, we would expect that bigger plants, more fruit, and larger fruit size would be expected, because of potential trade-offs between growth or reproduction and defense. Indeed, despite its short history of domestication (< 20 yr), our study shows that domestication in *U. molinae* has led to decreases in flavonol levels, an important class of defensive secondary metabolites in plants. This is in accordance with our hypothesis that domestication has reduced chemical defenses in *U. molinae*. In fact, amounts of four flavonoids—rutin, kaempferol, quercetin, and quercetin 3-β-glucoside—were lower in cultivated *U. molinae* than in their wild ancestors. Although the trend was the same, i.e., reduction in flavonol levels in cultivated plants, the strength of the effect of domestication varied among populations (Fig. 1B), with some populations responding more strongly than others. Future common garden studies from our group will aim to separate the genetic from the environmental factors responsible for this population-level variation.

If cultivated plants are less defended (Chen et al. 2015), we predicted that domestication in *U. molinae* would make plants more susceptible to herbivores. In fact, some flavonoids found in *U. molinae* have been implicated with resistance against herbivores in other plant systems. For example, Todd et al. (1971) showed that quercetin, a constituent of barley leaves, was toxic to greenbugs, *Schizaphis graminum* (Rondani). Moreover, Dreyer and Jones (1981) reported increased resistance of wheat against *M. persicae* also due to quercetin. This was, however, not the case for the herbivore *C. rudis*, an important defoliator in the ecosystem (Ángulo and Ruiz 1974), which showed lower performance and preference for cultivated *U. molinae* plants than their wild counterparts. In fact, our study shows that flavonol content stimulates feeding in *C. rudis*. Takeamura et al. (2002) also reported increased susceptibility of *Vicia angustifolia* L. against the aphid *Megoura ccrassicuda* Mordvilko, and attributed it to the presence of flavonol glycosides. Diaz et al. (2010) reported that quercetin acts as a phagostimulant for the beetle *Epilachna paenulata* (Germar) (Coleoptera: Coccinellidae). Moreover, Lin and Mullin (1999) reported stimulant feeding activity by quercetin 3-D-glucoside in the western corn rootworm, *Diabrotica virgifera virgifera* LeConte. Wink (1988), Nielsen et al. (1998), and Bernays (1991) also reported stimulation of feeding and ovipositional activity by kaempferol, rutin, and glycoside compounds for other herbivores. Even though *C. rudis* is a generalist herbivore, preference for wild *U. molinae* might be due to the fact that both plant and insect are native to the region and it has likely evolved to exploit its host plant’s defenses. In the future, it will be interesting to test the effects of domestication on other native as well as non-native herbivores.

There was substantial temporal (monthly) and spatial (within plant) variation in flavonol content in *U. molinae* (Figs. 2 and 3). Concentrations of flavonols peaked in April and were lowest in June, and they were higher in the fruit and leaves than in the stem. Based on *C. rudis* life cycle (Ángulo and Ruiz 1974), larvae are present from May until December, which coincides with an increase in flavonol levels (Fig. 2). They also prefer to feed on leaves, which contain high quantities of these flavonols. This evidence suggests that the herbivore *C. rudis* is well adapted to feed on host plants at times of the year when concentrations of these secondary metabolites in tissues are high. This is further supported by the fact that wild plants, which are preferred by *C. rudis*, have greater amounts of these compounds throughout most of the year and in the leaves.

The performance and preference of *C. rudis* was, in general, consistent across most *U. molinae* populations—the herbivore grew and ate more foliage from wild hosts than in cultivated hosts—except for one geographic site (corresponding to the Eco 12-1,
Fig. 4. Representative HPLC chromatograms of synthetic standards (A) and wild (B) and cultivated extracts (C) of murtilla, *U. molinae*, leaves. 1) rutin, 2) quercetin-3-D-β-glucoside, 3) quercetin, and 4) kaempferol.
Pucon cultivated, wild pairing; Fig. 5), where the opposite was observed, i.e., C. rudis gained more mass and consumed more foliage from cultivated (Pucon region) plants as compared with its cultivated (ecotype Eco 12-1) counterpart. Many factors, both physical and chemical, often contribute to resistance of plants against herbivores, which may act alone or interact with each other (Agrawal 2007). In our study, we tested a single class of secondary metabolites, the flavonols, and the individual effects of some of them—rutin, kaempferol, quercetin, and quercetin 3-D-β-glucoside—on the feeding behavior of C. rudis, and found that all are feeding stimulants (Fig. 6). It is possible that other factors of resistance, unmeasured in this study, were responsible for the small inconsistencies reported here on the effects of domestication on C. rudis. The effects of domestication on other classes of secondary metabolites in U. molinae and their interactive effects on herbivores require future examination.

In conclusion, although domestication and selective breeding have had great positive influences on food

![Fig. 5. Results from no-choice bioassays (A–D): C. rudis larval mass gained (A) and food consumption (C) when fed wild and cultivated murtilla, U. molinae, leaves, and mass gained (B) and food consumption (D) based on ecotype, geographical area. Results from choice bioassays (E–F): C. rudis larval food consumption (E) when fed wild and cultivated U. molinae leaves, and food consumption (F) based on ecotype, geographical area. * Significant difference (Scott–Knott test, P ≤ 0.05).](image-url)
availability through increased crop yield and quality (Wink 1988), it has often had a cost for resistance against herbivores (Chen et al. 2015), which may lead to increased use of pesticides. While in a number of crop plants, domestication had reportedly led to lower levels of defensive compounds and therefore lower resistance to pests (e.g., Rosenthal and Dirzo 1997, Rodriguez-Saona 2011, Altesor et al. 2014), in the system studied here, domestication has led to lower levels of chemical defenses in the Chilean native crop *U. molinae* but increased resistance against one of its native herbivores, *C. rudis*. In fact, *C. rudis* uses these compounds as feeding stimulants. These findings show that we cannot generalize the effects of crop domestication on resistance to herbivores from a number of plant species studied so far to all systems. Moreover, our study highlights the fact that there is some specificity in the response of herbivores to domestication and that not only does herbivore identity matters in these types of studies but also the type of defense measured.

The results reported here have important implications for the cultivation of *U. molinae*, a crop that is highly valued due to the antioxidant activity of flavonols in its fruit (Rubilar et al. 2011, Alfaro et al. 2013). In our study, we showed that the levels of flavonols in the leaves were reduced due to domestication but levels in the fruit were not affected by this process; thus, domestication should not have jeopardized its economic value. However, if the focus of domestication in *U. molinae* shifts from higher productivity, i.e., increase in yield, to higher levels of antioxidant compounds, these berries could become more susceptible to certain native herbivores like *C. rudis*. Altogether, the reported findings provide first insights on the impact of domestication on plant defenses and resistance against herbivores in *U. molinae*. It may also guide future breeding programs by highlighting the potential risks of breeding for high flavonol content on susceptibility of fruit against native, adapted herbivores.

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