

Ecological Importance of Cyanogenesis and Extrafloral Nectar in Invasive English Laurel, *Prunus laurocerasus*

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Ecological Importance of Cyanogenesis and Extrafloral Nectar in Invasive English Laurel, *Prunus laurocerasus*

Abstract

English laurel (Rosaceae: *Prunus laurocerasus* L.) is an evergreen shrub to small tree that is native to Asia but widely used in landscaping in temperate zones. In the Pacific Northwest this plant has become an important invasive species. However, surprisingly little is known about its ecology—in particular, its chemical defenses and interaction with herbivores and organisms of higher trophic levels. In the present field study, we quantitatively measured cyanogenesis (a constitutive direct chemical defense) and secretion of extrafloral nectar (EFN; an inducible indirect defense), and analyzed the effects of these defenses on insect herbivores (black vine weevil [*Otiiorhynchus sulcatus*]) and predators (sugar ants [*Tapinoma sessile*]). To induce EFN production, English laurel leaves were mechanically damaged at different intensities and in a way to mimic feeding damage by the black vine weevil, which represents the only observed insect herbivore feeding on this plant at our study site near Portland, OR. While cyanogenesis was expressed at homogeneously high levels ($> 80 \mu\text{mol CN}^- \text{g}^{-1}$ leaf fresh weight) among all experimental plants and was not affected by leaf damage, we found a significant positive correlation between damage, EFN production, and presence of ants. The number of vine weevils observed on experimental plants was significantly negatively correlated with EFN secretion and ant attraction. Our findings suggest that English laurel—beyond expression of cyanogenesis—efficiently utilizes indirect defense through ants in its invasive range. This protective mutualism may significantly contribute to the success of English laurel as an invasive species in native ecosystems of the Pacific Northwest.

Keywords: direct defense, indirect defense, Rosaceae, Pacific Northwest, black vine weevil

Introduction

Prunus laurocerasus (Rosaceae), commonly known as the cherry or English laurel in the United States, is a fast-growing shrub or small tree native to Asia minor. Up to eight meters in crown diameter and 10 meters in height, *P. laurocerasus* exhibits a quick, dense, evergreen growth habit and is hardy to -20°C . Combined with its shade tolerance and ease of propagation, these features have made English laurel a popular horticultural screening plant—however, these same features have allowed English laurel to naturalize in forest understories to such an extent as to be considered invasive in the Pacific Northwest, where it out-competes native plant species (Swearingen, 2006).

Like many other rosaceous plants, English laurel is cyanogenic and secretes extrafloral nectar (EFN)

as defenses. Widely distributed in about 10% of all vascular plants (Gleadow and Woodrow 2002), cyanogenesis is considered a constitutive direct chemical plant defense mostly against generalist herbivores (Ballhorn et al. 2005, Ballhorn et al. 2013a,b). Cyanogenic plants have the capacity to release toxic gaseous hydrogen cyanide (HCN) from endogenous cyanide-containing compounds, generally cyanogenic glycosides (Selmar et al. 1989, Poulton 1990, Seigler 1998). Hydrogen cyanide toxicity comes from its affinity to the terminal cytochrome oxidase in the mitochondrial respiratory pathway, and a number of other reactions particularly with metal-containing enzymes (Solomonson 1981, Brattsten 1992).

Extrafloral nectar—nectar not involved in pollination, typically secreted from plant parts other than flowers—represents one of the most widely distributed indirect defenses in the plant kingdom (Janzen 1966, Bentley 1977). More than 8000 species from 745 genera contain plants with extrafloral nectaries, including angiosperms, gym-

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nospersms, and ferns, which provide evidence for EFN origins being more ancient than floral nectar (Weber and Keeler 2012). In particular, EFN is frequently found in rosids (> 2000 species) (Weber and Keeler 2013), and belongs to the inducible defenses. Most plants can adjust nectar quantity depending on abiotic factors, such as time of day and relative humidity, but also biotic factors, such as consumer identity, consumption rate, and leaf damage (Ness et al. 2009). Additionally, the composition of EFN varies from species to species, but generally consists of modified phloem sap: mostly water and sucrose, with low amounts of amino acids, tannins, and enzymes involved in anti-microbe defense. EFN can be secreted from all parts of the plant except the roots, and acts as an indirect defense via the attraction of carnivorous arthropods, particularly ants, which are one of the most dominant insect groups in most terrestrial ecosystems. As ants frequently act as voracious predators, EFN production is considered an effective indirect defense (Godschalx et al. 2015, Grasso et al. 2015).

Although it is known that English laurel is cyanogenic and produces extrafloral nectar, no information exists on the quantitative expression of both traits and their relevance in interaction with herbivores and predators in nature, either in its native or invasive range. To better understand the ecology of this plant species in its invasive range, we measured both cyanogenesis and EFN production in English laurel plants under field conditions in the Pacific Northwest (Portland area, Oregon) and conducted field experiments to analyze the importance of EFN secretion on the attraction of ants and co-occurrence of herbivores. In this study, we aimed at answering these specific questions:

1. How cyanogenic is English laurel, how much EFN does this species secrete, and is the expression of both traits affected by leaf damage?
2. Does induced EFN secretion effectively attract ants?
3. Does variation in cyanogenesis, EFN secretion, and ant attraction affect plant resistance to the herbivorous black vine weevil *Otiorhynchus sulcatus*?

Methods

Study Area

English laurel seeds were collected in 2011 from an invasive population in the understory of big leaf maple (*Acer macrophyllum*) and Douglas fir (*Pseudotsuga menziesii*) dominated forest in Raleigh Hills, Portland, Oregon, (45°30'24.2"N 122°44'43.5"W) near highway 26. Subsequent experiments using plants grown from these seeds were conducted in the same location.

Black Vine Weevil

At the study site, the black vine weevil (Coleoptera: Curculionidae: *Otiorhynchus sulcatus* F.) was the only herbivore observed on English laurel. While the insects caused damage to English laurel foliage, when compared to neighboring evergreen shrubs in the area, such as *Camelia* spp., *Rhododendron* spp., and *Mahonia* spp., English laurel plants (both in naturalized and in garden sites) showed less leaf damage. Overall, the frequent signs of black vine weevil herbivory observed on English laurel and other evergreen plant species in the area indicated an abundant occurrence of the insects. Some degree of damage was observed on > 90% of surveyed plant individuals ($n = 122$ plants).

The black vine weevil was first reported in 1835 in the United States and by 1871 was a noted pest in Missouri. Adult weevils are all females and reproduce by thelytokous parthenogenesis (Smith 1932), where unfertilized eggs develop into females and the daughters are genetically identical to mothers. Adults can lay between 200 to 1000 eggs. Larvae are generally white but can have a yellow to pink cast, legless, and generally crescent-shaped with tan head capsules. Adults are 8 to 10 mm long and are jet black, with golden metallic patches of elongated hair and beads arranged lengthwise in rows on the elytra, the modified front wings which cover the back of the insect (Bruck 2004). In the Pacific Northwest, they have a single generation per vegetation period (person. observation). Notching on evergreen leaves is specific to adult feeding behavior, while larvae feed on roots.

Predatory Insects on English Laurel

At our study site, sugar ants, or odorous house ant, (*Tapinoma sessile*) were by far the most abundant ants on English laurel. *Tapinoma sessile* is a generalist ant and very common in the Pacific Northwest (Buczowski and Bennett 2006).

Plant Material for Field Experiments

Seed-grown, three-year-old English laurel plants were used for the experiments ($n = 130$ plants). Seeds were collected from an invasive English laurel population (see “Study Area” above). Experimental plants were cultivated in plastic pots (20 cm in diameter) filled with standard substrate (Sunshine Mix #1, LC1, SunGro Horticulture®, Bellevue, WA). At the time of the experiments plant size ranged between 40 and 50 cm and plants had developed 15 to 20 leaves. English laurel plants were grown under natural outdoor conditions at the same location where the seeds were collected and protected by anti-aphid netting against damage by herbivores. During the summer months (Jul–Sep), plants were watered every second day and fertilized with a NPK fertilizer (MicroPack 5-5-5™, 10 granules per pot) twice a month. During the rest of the year plants were watered as needed and fertilized every six weeks.

Treatment of Plants and Experimental Setup

Five levels of damage were applied to study the effect of leaf damage on the expression of defense traits in English laurel, and to quantify the subsequent occurrence of insect herbivores and predators on the treated plants. We punched leaf discs of 6 mm from the edges of the leaves (Figure 1), mimicking the typical feeding damage caused by black vine weevils. Damage was applied only to the distal part of the leaf to avoid leaf veins connected to the nectaries, which are located near the base of the leaves (Figure 1). To guarantee a similar level of damage, all leaves were measured with a tape measure and, starting from the leaf tip in steps of 10, 20, 30, 40 and 50% of the leaf length, pairs of leaf punches were removed (Figure 1). The same level of damage was applied to all fully developed leaves of an

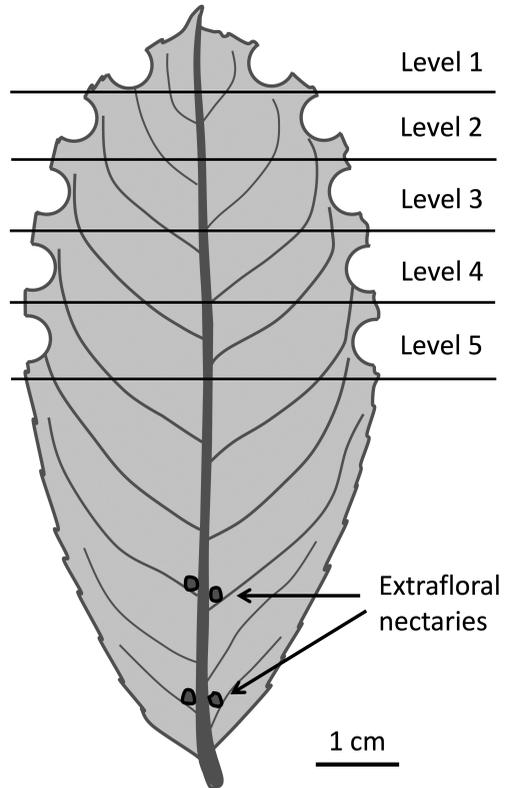


Figure 1. Experimental leaf damage. English laurel leaves were damaged by removing leaf tissue with a cork borer (6 mm in diameter) from the edge of the leaves mimicking damage by black vine weevils. Different degrees of damage were applied (Level 1: two punches; Level 2: four punches; Level 3: six punches etc.). All damage was applied in the distal half of the leaf not to damage leaf veins connected to the typically four extrafloral nectaries located at the base of the leaf.

individual experimental plant. The mechanical damage was applied in the morning (03:00–05:00 am) before sunrise according to the feeding activity of black vine weevils, with undamaged plants serving as the control.

Since EFN can only be quantified under insect exclusion, subsets of identically treated plants ($n=10$ plants per treatment group) were each subjected either to quantification of extrafloral nectar or to experiments on attraction of weevils and ants. Cyanogenic potential (HCNp) of English laurel plants was measured in leaves from plants

used in the insect attraction experiment. The EFN quantification as well as sampling of leaf material for HCNp analysis was conducted after the weevil and ant survey so as not to disturb the insects. The experiments took place in June 2014 (weather conditions: cloudy, day high: 22 °C, night low 12 °C, air–humidity 80%, wind: NW 10–13 kph).

Plants used for collection of EFN included plants of all five damage levels (Level 1–5) as well as undamaged controls (Control 1). All plants were protected by anti-aphid net against flying nectar consumers (bees, wasps, moths). In addition, we applied a ring of Tangle Trap® around the top of the pots and the stem to exclude any nectar-consuming ants.

In contrast, the plants used for the insect attraction experiment and HCNp quantification were openly exposed to incoming insects; their pots were dug 4/5 into the soil and the top of the pots was covered with dry leaves, with three sticks per pot collected from the vicinity to provide for easy access of weevils and ants. In this experiment, we added a further control group (Control 2) represented by undamaged plants with experimentally-covered nectaries (with aluminum foil strips attached with Wollwachs) to evaluate the effects of complete EFN exclusion from the system on insect attraction.

All plants (from the different treatment groups and the different experiments) were randomly placed among the invasive English laurel population. The distance between plants was 1 m.

Ant Attraction to Experimental Plants

Number of ants on the experimental plants was evaluated 24 hours after mechanically-induced extrafloral nectar (EFN) production. Ants per plant were counted only once to avoid pseudo-replication by counting individual ants multiple times and to prevent excessively disturbing the EFN consumers.

Weevil Occurrence on Experimental Plants

The occurrence of weevils on the experimental plants was recorded 24 hours after mechanically damaging the leaves. According to the weevil's night active behavior, the evaluation was conducted

in the early morning (03:00–05:00). Plants were carefully screened using a dim flashlight as beetles tend to drop themselves when disturbed.

Measurement of Extrafloral Nectar

EFN was collected from aphid net-protected plants 24 hours after mechanical damage was applied. One fully developed leaf per plant was sampled; this developmental stage represented the majority of leaves on the experimental plants. EFN production was quantified according to Ballhorn et al. (2014a) and Godschalx et al. (2014). Extrafloral nectar production was quantified as amounts of soluble solids by using microcapillaries (PCR Micropipettes 1–10 µl; Drummond) for determination of volume and a brix refractometer for determination of sugar concentration.

Cyanogenic Potential

The cyanogenic potential (HCNp) of a plant species describes the amount of cyanogenic precursors per gram plant tissue (Ballhorn et al. 2016a). Within a given plant species, the HCNp typically varies depending on plant genotype and environmental factors, while in individual plants variation further occurs depending on plant part analyzed as well ontogenetic development of the plant or plant part. Thus, for comparable analyses of foliar HCNp (as well as extrafloral nectar, EFN; see below) plants and leaves of a similar developmental stage were used for the analyses. Furthermore, great care was taken to avoid premature release of cyanide due to leaf damage during sampling. Cyanogenic plants generally accumulate cyanogenic glycosides in the vacuoles; in the intact plant, these precursors are separated from one or more specific β -glucosidases that are localized in the apoplast (Poulton 1990). In the case of injury, the β -glucosidase is brought into contact with its substrate and hydrolyzes the cyanogenic glycosides, forming α -hydroxynitriles that are relatively unstable and dissociate—either spontaneously or by enzymatic acceleration by α -hydroxynitrile lyase—into HCN and an aldehyde or a ketone (Selmar et al. 1989). In this study, leaves were individually placed in labeled Ziploc® bags and stored in a cooler on ice until processed in the laboratory (within 8 h after sampling). Ex-

traction of cyanogenic glycosides, their enzymatic decomposition, and quantification of released cyanide was conducted according to Ballhorn et al. (2014b). In short, the central vein of leaves was removed with a razor blade, with the remaining leaf material weighed to the nearest 0.001 g and ground with liquid nitrogen in a cooled mortar and pestle at 4 °C. Five mL ice-cold Na₂HPO₄ buffer (67 mmol L⁻¹) were added and the samples were quantitatively analyzed for HCNp by complete enzymatic hydrolysis of cyanogenic precursors with β-glucosidase (Emulsin from almonds, EC 3.2.1.111, Merck). We used closed glass vessels (Thunberg vessels) for incubation of leaf extracts together with enzyme solution adjusted to an activity of 20 nkat. Quantitative detection of the released HCN was carried out spectrophotometrically at 585 nm using the Spectroquant® cyanide test (Ballhorn et al. 2005).

Statistical Analysis

One-way analysis of variance (ANOVAs), Tukey's posthoc analysis, and Pearson correlations were used to determine statistical significance of variation within and interactions among variables.

Results

Plants showed homogeneous leaf HCNp among all control and treatment groups (Figure 2A). In contrast to HCNp, the amount of secreted EFN showed significant variation among the experimental groups (one-way ANOVA: $F_{(5, 53)} = 73.906$; $P < 0.001$) (Figure 2B). Plants with high levels of experimental damage produced significantly more EFN than plants with lower damage or undamaged control plants (Control 1). Plants with the lowest level of damage (treatment group 1), however, did not secrete significantly higher amounts of EFN than the undamaged control plants (Control 1). Plants with covered nectaries (Control 2) were not included in this analysis of variance as EFN was experimentally removed from the system (Figure 2B).

The average number of ants per plant varied significantly among the treatment groups ($F_{(6, 62)} = 12.070$; $P < 0.001$) and increased with leaf damage (Figure 3A). The number of ants attracted

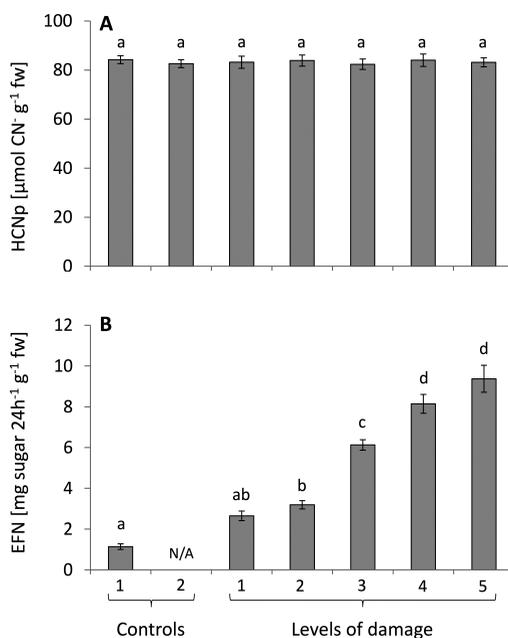


Figure 2. Cyanogenic potential and EFN secretion of English laurel leaves. Mature leaves from plants of different treatment groups were analyzed for A) cyanogenic potential (HCNp, amount of cyanogenic precursors) and B) secretion of extrafloral nectar (EFN). Control 1 represents undamaged leaves, Control 2 are undamaged leaves but with covered extrafloral nectaries (thus, Control 2 is not present in the EFN experiment), whereas damage levels 1–5 indicate different degrees of experimental leaf area removal with Level 5 being the most extensive damage. Bars are means \pm SE. Lowercase letters on top of columns represent statistically significant differences (according to post-hoc tests [Tukey's HSD; $P < 0.05$] after one-way ANOVA; $n = 10$ plants per treatment group).

to extensively damaged and EFN-secreting plants (treatment group 5) was significantly higher than for less damaged plants (groups 1 and 2), whereas the differences in ant attraction among the intermediate treatment groups were not significant. EFN secretion and ant visitation were significantly correlated (Pearson correlation: $r = 0.661$, $P < 0.01$).

Weevil occurrence on experimental plants was significantly different among plants of the treatment groups ($F_{(6, 62)} = 3.963$; $P < 0.01$) and

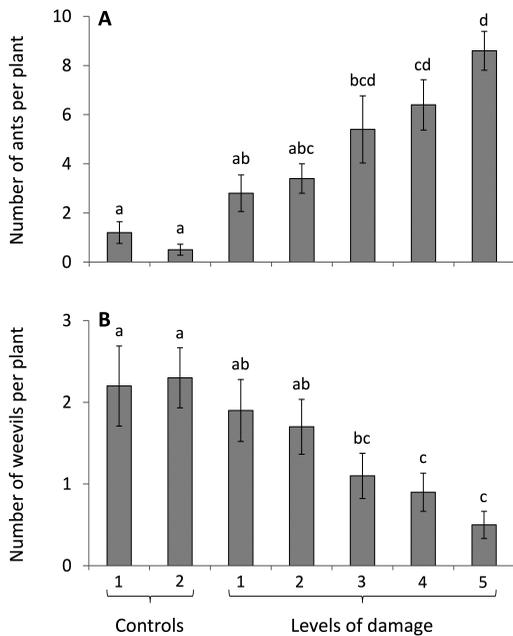


Figure 3. Ant and weevil occurrence on English laurel plants. The number of A) attracted ants and B) occurring beetles on plants of the different treatment groups was counted. Control 1 represents undamaged leaves, Control 2 are undamaged leaves but with covered extrafloral nectaries. Damage levels 1–5 indicate different degrees of experimental leaf area removal with Level 5 being the most extensive damage. Bars are means \pm SE. Lowercase letters on top of columns represent statistically significant differences (according to post-hoc tests (Tukey's HSD; $P < 0.05$) after one-way ANOVA; $n = 10$ plants per treatment group).

diminished with increasing leaf damage (Figure 3B). On experimental plants of treatment groups 4 and 5, we observed significantly lower numbers of beetles than on plants of the treatment groups 1 and 2. Both ant visitation and beetle occurrence ($r = -0.291$, $P < 0.05$) as well as EFN secretion and beetle occurrence ($r = -0.501$, $P < 0.01$) were significantly negatively correlated.

Discussion

Our results show that English laurel expresses extensive cyanogenesis as well as damage-induced secretion of extrafloral nectar (EFN). While it has

been known that this plant species is cyanogenic and has extrafloral nectaries, quantitative studies of these traits were lacking so far. Furthermore, no studies exist on the importance of these traits for the interaction with herbivores and predators either in its native nor invasive range. Studying invasive English laurel in the Pacific Northwest, we show here for the first time that in this species, ant-attraction through EFN serves as an efficient defense against generalist black vine beetles, whereas cyanogenesis provides a rather weak defense against these herbivores. This is surprising, as at our study site English laurel expresses high levels of cyanogenic potential (HCNp), which typically indicates an efficient chemical defense against leaf-chewing herbivores. In fact, compared to other cyanogenic plants such as white clover (*Trifolium repens*; approx. $5\text{--}8 \mu\text{mol CN}^- \text{g}^{-1}$ leaf fresh weight; Ballhorn and Elias 2014), giant bamboo (*Cathariostachys madagascariensis*; approx. $70 \mu\text{mol CN}^- \text{g}^{-1}$ leaf fresh weight; Ballhorn et al. 2009, 2016b), and lima bean (*Phaseolus lunatus*; up to $80 \mu\text{mol CN}^- \text{g}^{-1}$ leaf fresh weight; Ballhorn et al. 2007), English laurel (consistently $> 80 \mu\text{mol CN}^- \text{g}^{-1}$ leaf fresh weight, Figure 2A) shows particularly high levels of HCNp.

Our study further suggests that the production of cyanogenic precursors is constitutive rather than inducible through damage (Figure 2B). However, recent studies on cyanogenic lima bean (Kautz et al. 2014) and rubber tree (*Hevea brasiliensis*) (Kadow et al. 2012) report that while the amount of cyanogenic precursors remained unchanged in damaged leaf tissue, the activity of enzymes (β -glucosidases) involved in release of free cyanide from these precursors can change in response to cell damage. In our study we focused on the HCNp rather than on the release of cyanide from these precursors per time (cyanogenic capacity, HCNc). Thus, it remains to be tested whether or not English laurel can respond with the activation of enzymes involved in the release of cyanide when damaged. Nevertheless, while such changes in enzyme activity and HCNc may affect herbivores, attraction of predatory ants through EFN generally is unaffected by cyanogenesis as these ants do not destroy cyanogenic precursor-containing cells and thus do not trigger cyanide release.

Although plant cyanogenesis has been reported as an efficient chemical defense against both generalist and specialist insect herbivores (Ballhorn and Lieberei, 2006; Ballhorn et al. 2007, Ballhorn et al. 2010), the black vine weevil feeds extensively on English laurel. According to our field observations—at least at our study site—this weevil represents the major insect herbivore on English laurel. These findings are in line with other studies showing that black vine weevils are extremely polyphagous with about 100 known food plant species in 46 families (Masaki et al. 1984). This high number of suitable host plants suggests a broad tolerance to many chemical plant defenses.

While cyanogenesis seems a weak defense against the black vine weevil in English laurel, the attraction of *Tapinoma* ants through the secretion of EFN significantly reduced weevil presence on the experimental plants. We found both significant effects of elevated EFN secretion on ant and beetle occurrence in response to leaf damage as well as effects of experimental EFN removal (Figures 3A,B). In particular, plants with covered nectaries showed the lowest numbers of ants but the highest numbers of weevils. Both findings indicate that ants efficiently defend their host plants against black vine weevils when attracted in sufficient numbers to extrafloral nectaries. In this ant-beetle interaction, the ants deter rather than prey on the large and heavily-shielded beetles. In fact, obser-

vations in nature as well as on the experimental plants frequently showed that beetles dropped themselves from the plant when attacked by the ants. Indirect defense through ants is well known in many plant species and ranges from obligate to facultative interactions (Kautz et al. 2012). However, the potential effects and consequences of indirect defense through ants for the success of invasive species are surprisingly little studied. Given the ubiquitous occurrence and often extreme abundance of ants in most terrestrial ecosystems (Andersen et al. 2004), this defensive mutualism is of broad ecological relevance—including the biology of invasive plant species.

Conclusion

Our study is the first we know of to demonstrate the inducibility of extrafloral nectar and to quantify direct chemical defense through cyanogenesis in English laurel. We further show that EFN-mediated ant attraction in English laurel significantly reduces occurrence of black vine weevils on the plants, thus providing an efficient indirect defense. We predict that ant-plant interactions contribute to the success of this invasive plant species in the Pacific Northwest.

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Literature Cited

- Andersen, A. N., A. Fisher, B. D. Hoffmann, J. L. Read, and R. Richards. 2004. Use of terrestrial invertebrates for biodiversity monitoring in Australian rangelands, with particular reference to ants. *Austral Ecology* 29:87-92.
- Ballhorn, D. J., R. Lieberei, and J. U. Ganzhorn. 2005. Plant cyanogenesis of *Phaseolus lunatus* and its relevance for herbivore-plant interaction: The importance of quantitative data. *Journal of Chemical Ecology* 31:1445-1473.
- Ballhorn D. J., and R. Lieberei. 2006. Oviposition choice of Mexican bean beetle *Epilachna varivestis* depends on host plant cyanogenic capacity. *Journal of Chemical Ecology* 32:1861-1865.
- Ballhorn D. J., A. Pietrowski, M. Heil and R. Lieberei. 2007. Quantitative effects of cyanogenesis on an adapted herbivore. *Journal of Chemical Ecology* 33:2195-2208.
- Ballhorn D. J., S. Kautz, and F. P. Rakotoarivelo. 2009. Quantitative variability of cyanogenesis in *Catharostachys madagascariensis*—the main food plant of bamboo lemurs in southeastern Madagascar. *American Journal of Primatology* 71:305-315.
- Ballhorn, D. J., S. Kautz, and R. Lieberei. 2010. Comparing responses of generalist and specialist herbivores to various cyanogenic plant features. *Entomologia Experimentalis et Applicata* 134:245-259.
- Ballhorn, D. J., S. Kautz, and M. Heil (2013a) Distance and sex determine host plant choice by herbivorous beetles. *PLoS ONE* 8:e55602.
- Ballhorn, D. J., A. L. Godschalx, and S. Kautz. 2013b. Co-variation of chemical and mechanical defenses in lima bean (*Phaseolus lunatus* L.). *Journal of Chemical Ecology* 39:413-417.

- Ballhorn D. J., and J. D. Elias J. D. 2014. Salinity-mediated cyanogenesis in white clover (*Trifolium repens* L.) affects trophic interactions. *Annals of Botany* 114:357-366.
- Ballhorn, D. J., J. Kay, and S. Kautz. 2014a. Quantitative effects of leaf area removal on indirect defense of lima bean (*Phaseolus lunatus*) in nature. *Journal of Chemical Ecology* 40:294-296.
- Ballhorn D. J., A. L. Godschalx, S. M. Smart, S. Kautz, and M. Schädler. 2014b. Chemical defense lowers plant competitiveness. *Oecologia* 176:811-824.
- Ballhorn, D. J., S. Kautz, and J. M. Laumann. 2016a. Herbivore damage induces a transgenerational increase of cyanogenesis in wild lima bean (*Phaseolus lunatus*). *Chemoecology* 26:1-5.
- Ballhorn D. J., F. P. Rakotoarivelo, and S. Kautz. 2016b. Coevolution of cyanogenic bamboos and bamboo lemurs on Madagascar. *PLoS ONE* 11:e0158935.
- Bentley, B. L. 1977. The protective function of ants visiting the extrafloral nectaries of *Bixa orellana* (Bixaceae). *Journal of Ecology* 65:27-38.
- Bruck, D. J. 2004. Stopping weevil deeds. *American Nurseryman* 199:43.
- Buczkowski, G., and G. W. Bennett. 2006. Dispersed central-place foraging in the polydomous odorous house ant, *Tapinoma sessile* as revealed by a protein marker. *Insectes Sociaux* 53:282-290.
- Gleadow, R. M., and I. E. Woodrow. 2002. Constraints on effectiveness of cyanogenic glycosides in herbivore defense. *Journal of Chemical Ecology* 28:1301-1313.
- Godschalx, A. L., M. Schädler, J. A. Trisel, M. A. Balkan, and D. J. Ballhorn. 2015. Ants are less attracted to the extrafloral nectar of plants with symbiotic, nitrogen-fixing rhizobia. *Ecology* 96:348-354.
- Grasso, D. A., C. Pandolfi, N. Bazihizina, D. Nocentini, M. Nepi, and S. Mancuso. 2015. Extrafloral-nectar-based partner manipulation in plant-ant relationships. *AoB Plants* 7:plv002.
- Janzen, D. H. 1966. Coevolution of mutualism between ants and acacias in Central America. *Evolution* 20:249-275.
- Kadow, D., K. Voß, D. Selmar, and R. Lieberei. 2012. The cyanogenic syndrome in rubber tree *Hevea brasiliensis*: tissue-damage-dependent activation of linamarase and hydroxynitrile lyase accelerates hydrogen cyanide release. *Annals of Botany* 109:1253-1262.
- Kautz, S., D. J. Ballhorn, J. Kroiss, J., S. U. Pauls, C. S. Moreau, S. Eilmus, E. Strohm, and M. Heil. 2012. Host plant use by competing acacia-ants: mutualists monopolize while parasites share hosts. *PLoS ONE* 7:e37691.
- Kautz, S., J. A. Trisel, and D. J. Ballhorn. 2014. Jasmonic acid enhances plant cyanogenesis and resistance to herbivory in lima bean. *Journal of Chemical Ecology* 40:1186-1196.
- Masaki, M., K. Ohmuri, and F. Ichinohe. 1984. Host range studies of the black vine weevil, *Otiiorhynchus sulcatus* (Fabricius) (Coleoptera: Curculionidae). *Applied Entomological Zoology* 19:95-106.
- Ness, J. H., W. F. Morris, and J. L. Bronstein. 2009. For ant-protected plants, the best defense is a hungry offense. *Ecology* 90:2823-2831.
- Poulton, J. E. 1990. Cyanogenesis in plants. *Plant Physiology* 94:401-405.
- Seigler, D. S. 1998. Cyanogenic glycosides and cyanolipids, pp. 273-296. *In* D. S. Seigler (editor), *Plant Secondary Metabolism*. Kluwer Academic Press, Boston. Pp. 273-296.
- Selmar, D., R. Lieberei, E. E. Conn, and B. Biehl. 1989. Alpha-hydroxynitrile lyase in *Hevea brasiliensis* and its significance for rapid cyanogenesis. *Physiologia Plantarum* 75:97-101.
- Smith, F. F. 1932. Biology and the control of the black vine weevil. US Department of Agriculture Technical Bulletin. 325.
- Solomonson, L. P. 1981. Cyanide as a metabolic inhibitor. *In* Vennesland, B., E. E. Conn, C. J. Knowles, J. Westby, and Wissing, F. (editors), *Cyanide in Biology*, Academic Press, London.
- Swearingen, J. 2006. WeedUS database, Alien plant invaders of natural areas. Plant Conservation Alliance, Alien Plant Working Group, Washington, DC.
- Weber, M. G., and K. H. Keeler. 2013. The phylogenetic distribution of extrafloral nectaries in plants. *Annals of Botany* 111:1251-1261.

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