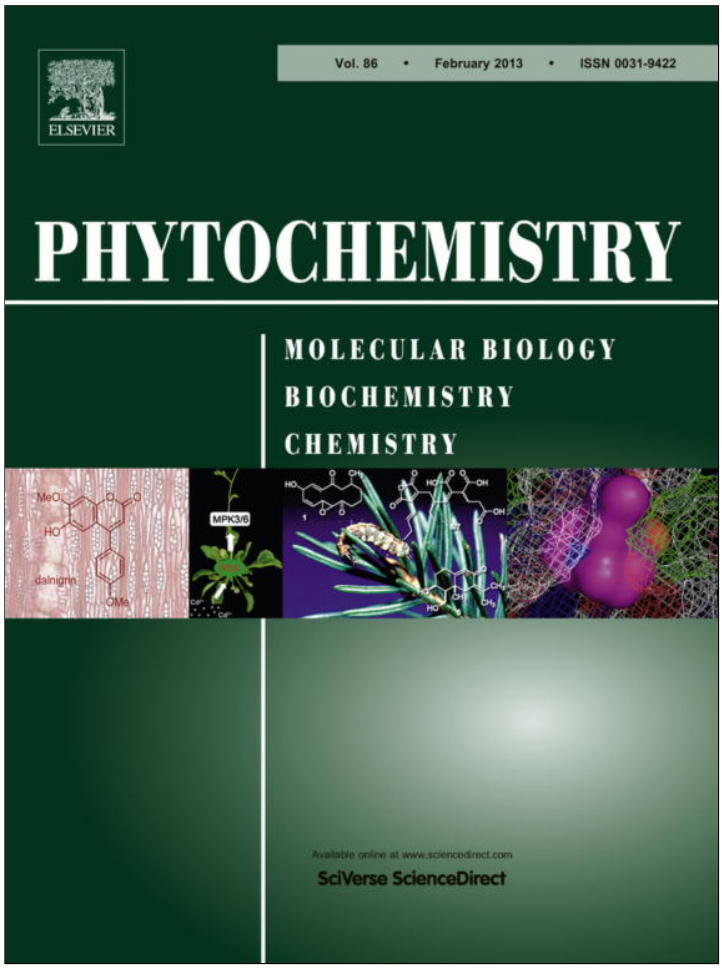
Provided for non-commercial research and education use.
Not for reproduction, distribution or commercial use.



This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

http://www.elsevier.com/copyright

Author's personal copy

Phytochemistry 86 (2013) 57–63

Contents lists available at SciVerse ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Iridoid and secoiridoid glycosides in a hybrid complex of bush honeysuckles (*Lonicera spp.*, Caprifolicaceae): Implications for evolutionary ecology and invasion biology

Susan R. Whitehead *, M. Deane Bowers

Ecology and Evolutionary Biology and Museum of Natural History, University of Colorado at Boulder, UCB 334, Boulder, CO 80309, USA

ARTICLE INFO

Article history:
Received 30 January 2012
Received in revised form 6 September 2012
Available online 7 December 2012

Keywords:
Lonicera × *bella*
Lonicera tatarica
Lonicera *morrowii*
Caprifoliaceae
Hybridization
Fruit secondary metabolites
Evolution of invasiveness
Plant defense
Iridoid glycosides
Secoiridoid glycosides

ABSTRACT

Interspecific hybridization among non-native plant species can generate genotypes that are more reproductively successful in the introduced habitat than either parent. One important mechanism that may serve as a stimulus for the evolution of invasiveness in hybrids is increased variation in secondary metabolite chemistry, but still very little is known about patterns of chemical trait introgression in plant hybrid zones. This study examined the occurrence of iridoid and secoiridoid glycosides (IGs), an important group of plant defense compounds, in three species of honeysuckle, *Lonicera morrowii* A. Gray, *Lonicera tatarica* L., and their hybrid *Lonicera* × *bella* Zabel. (Caprifoliaceae), all of which are considered invasive in various parts of North America. Hybrid genotypes had a diversity of IGs inherited from both parent species, as well as one component not detected in either parent. All three species were similar in that overall concentrations of IGs were significantly higher in fruits than in leaves, and several compounds that were major components of fruits were never found in leaves. However, specific patterns of quantitative distribution among leaves, unripe fruits, and ripe fruits differed among the three species, with a relatively higher allocation to fruits in the hybrid species than for either parent. These patterns likely have important consequences for plant interactions with antagonistic herbivores and pathogens as well as mutualistic seed dispersers, and thus the potential invasiveness of hybrid and parental species in their introduced range. Methods established here for quantitative analysis of IGs will allow for the exploration of many compelling research questions related to the evolutionary ecology and invasion biology of these and other related species in the genus *Lonicera*.

© 2012 Elsevier Ltd. All rights reserved.

1. Introduction

Hybridization between plant species has been implicated as an important mechanism that can underlie the evolution of invasiveness (Ellstrand and Schierenbeck, 2000; Schierenbeck and Ellstrand, 2009). A large source pool of genetic variation in hybrid genotypes provides increased raw material on which natural selection can act, potentially leading to evolutionary novelty in life history, morphology, phenology, or secondary metabolite chemistry that can make some hybrid populations better adapted to new environments (e.g. Geiger et al., 2011; Oberprieler et al., 2010; Schweitzer et al., 2002). However, despite the importance of interspecific hybridization in invasion biology, plant evolution, and the structuring of ecological communities (Barton, 2001; Hegarty and Hiscock, 2005; Martinsen et al., 2001; Schierenbeck and Ellstrand, 2009; Whitham et al., 1999), there are still many unanswered questions about patterns of trait introgression in hybrids, particularly for secondary metabolite chemistry (Orians, 2000). Hybrids can differ chemically from the parental species both qualitatively and quantitatively—they may have chemical compounds typical of one or both parents, fail to express certain compounds produced by parents, or have novel compounds not typical of either parent (Cheng et al., 2011; Orians, 2000; Orians and Fritz, 1995; Rehill et al., 2006; Rieseberg and Ellstrand, 1993). Because plant chemistry has important consequences for species interactions and, therefore, the reproductive success of plants (Coley and Barone, 2001; Eisner and Meinwald, 1995), a better understanding of the chemical variation among hybrids may provide important insights into why certain hybrids become invasive in introduced ranges while others never establish viable populations (Ellstrand and Schierenbeck, 2000; Fritz, 1999; Fritz et al., 1999; Strauss, 1994; Whitney et al., 2006).

Exotic bush honeysuckles (*Lonicera spp.*, Caprifoliaceae) are some of the most problematic invasive species in the eastern and mid-western United States (Nyboer, 1992; Webster et al., 2006). Most species fruit in abundance and are thought to be dispersed primarily by birds (Bartuszevige and Gorchov, 2006; Ingold and Craycraft, 1983), although white-tailed deer may also be important

* Corresponding author. Tel.: +1 434 841 1512; fax: +1 303 492 8699.
E-mail address: susan.whitehead@colorado.edu (S.R. Whitehead).

0031-9422/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.phytochem.2012.10.012

Author's personal copy

as dispersers (Vellend, 2002; Whitehead, personal observation). Their introduction and spread have led to altered plant communities and reduced native plant diversity in many areas (e.g. Collier et al., 2002; Woods, 1993), which may be due to competitive (Gorchov and Trisel, 2003) or allelopathic effects (Cipollini et al., 2008). High densities of honeysuckle shrubs can also have cascading effects in ecosystems, including alteration in resource availability for birds (Bartuszevige and Gorchov, 2006; Ingold and Craycraft, 1983), declines in amphibian communities due to high levels of allelochemicals produced by the plants (Watling et al., 2011), and even increased disease risk for humans through indirect effects on deer populations that serve as reservoirs for parasites and pathogens (Allan et al., 2010). Some of the most invasive species include *Lonicera tatarica* L., *Lonicera morrowii* A. Gray, and their hybrid progeny *Lonicera* × *bella* Zabel., which form hybrid swarms throughout much of the introduced range (Barnes and Cottam, 1974; Nyboer, 1992; Webster et al., 2006). The hybrid species appears to be more successful in North America than either parent, as evidenced by the wide variety of habitats that the hybrid inhabits, its higher abundance relative to the parent species, and the high frequency of hybrid individuals that exhibit morphological traits intermediate to the parents (Barnes and Cottam, 1974; Whitehead, personal observation).

The *L.* × *bella* hybrid complex provides an intriguing system for phytochemical research. A comparison of secondary metabolites produced in parental and hybrid species would add an important new component to a growing literature on the chemical consequences of hybridization and establish analytical methods that will allow researchers to address many questions related to the evolutionary ecology and invasion biology of these species. The phytochemistry of *Lonicera* has been previously investigated due to the importance of various species in traditional pharmacopeias, and the genus contains at least two classes of secondary compounds with known ecological and economic importance: iridoid and secoiridoid glycosides (IGs) and phenolics (Chen et al., 2007; Cipollini et al., 2008; Ikeshiro et al., 1992; Li et al., 2003; Song et al., 2006; Svobodova et al., 2008; Wang et al., 2003; Zadernowski et al., 2005). This study focuses on IGs, which are an important class of plant defensive compounds found in over 50 plant families (Bowers, 1991), but have not been previously investigated in the context of plant hybridization. Souzu and Mitsuhashi (1969, 1970) and Ikeshiro et al. (1992) have provided initial descriptions of six IGs in fruits and leaves of *L. morrowii*, and there is one report of secologanin (**6**) (Fig. 1) in *L. tatarica* (Hermanslokkerbol and Verpoorte, 1987). However, to our knowledge, there is no information of the role of IGs in the ecology and evolution of these species, and there are no studies that have described the occurrence of IGs in the hybrid species, *L.* × *bella*.

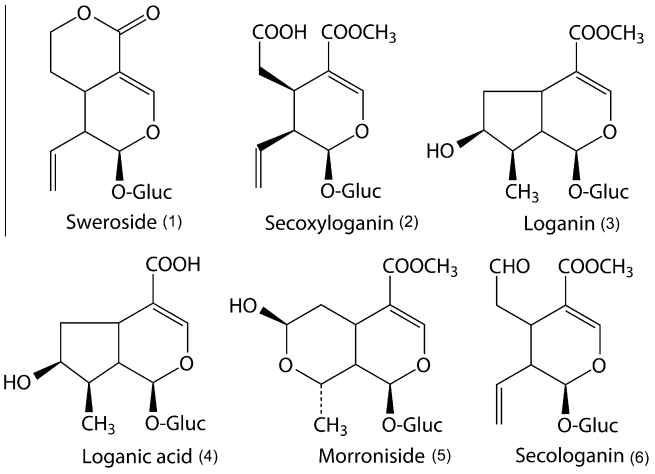


Fig. 1. Structures of major iridoid and secoiridoid glycosides from *Lonicera* × *bella*, *L. morrowii*, and *L. tatarica*.

IGs play an important ecological role in plant defense against both herbivores and pathogens (Bowers, 1991; Marak et al., 2002a, b). Their composition and concentration can vary considerably between species, among individual plants and plant organs, and throughout plant development (Jamieson and Bowers, 2010; Penuelas et al., 2006; Quintero and Bowers, 2011a). This spatial and temporal variation has direct implications for plant antagonists as well as cascading effects that can influence interactions with higher trophic levels (Dyer and Bowers, 1996; Jamieson and Bowers, 2010; Lampert et al., 2011; Lindstedt et al., 2010; Penuelas et al., 2006; Quintero and Bowers, 2011b; Reudler et al., 2011). Although past work on the ecological role of IGs has focused primarily on leaves; these compounds have also been found in the fruits of various species (Ikeshiro et al., 1992; Makarevich et al., 2009; Ono et al., 2005). Fruit secondary compounds may function to defend fruits against antagonists (e.g. insect seed predators and fungal pathogens) and/or to regulate the foraging and feeding behaviors of vertebrate seed dispersers (Cipollini and Levey, 1997a; Herrera, 1982; Levey et al., 2007; Tewksbury et al., 2008); however IGs have not been examined in this context. The potential role of IGs in fruit/frugivore interactions is of particular interest in the context of invasion biology, because the spread of an invasive species can be greatly accelerated by an effective dispersal mechanism in the novel habitat (Gosper et al., 2005; Higgins and Richardson, 1999; Richardson et al., 2000).

The objectives of this study were to: (1) provide a detailed method for extraction and quantification of IGs in *Lonicera* species that will be useful to ecologists and evolutionary biologists; (2) describe the occurrence of IGs in the hybrid honeysuckle *L.* × *bella* and its parental species *L. tatarica* and *L. morrowii*; and (3) compare the composition and concentration of IGs among leaves, unripe fruits, and ripe fruits in these three species. Results are discussed in the context of their potential implications for the evolution of chemical traits in hybrid genotypes, species interactions with herbivores and seed dispersers, and invasion biology.

2. Results and discussion

Leaf and fruit samples of *L. morrowii*, *L. tatarica*, and *L.* × *bella* were obtained from the living collections at the Arnold Arboretum of Harvard University (Cambridge, MA, USA). *L. morrowii* was originally wild-collected from the Honshu Provenance in Japan in 1984, *L. tatarica* was wild-collected from Tajikistan in 1978, and the hybrid species, *L.* × *bella*, is of cultivated origin and was received at the arboretum in 1919 from Boston, MA (BG-BASE, 2011). In addition, samples were collected from three wild populations of *L.* × *bella* growing near Boulder, CO, USA. The identification and quantification of IGs was carried out using gas chromatography with mass spectrometry detection (GC–MS).

Six major IG components (on average representing 89.1% of the estimated total IGs) were identified by comparison to authentic reference standards (Table 1). One other presumably related major component (Unknown G, 10.0% of estimated total IGs) and six minor components (totaling <1% of estimated total IGs) were also detected and provisionally characterized as IGs based on characteristic fragmentation patterns in mass spectra as described in detail in Inouye et al. (1976) and Popov and Handjieva (1983). Although there is no spectral peak associated with the molecular ion for silylated iridoids, several peaks associated with the aglycone portion of the molecule are very informative, and, in combination with peaks originating from the sugar moiety, served as a means for positive identification of previously characterized IGs.

Author's personal copy

Table 1
Identifications, retention times, diagnostic MS peaks, and quantities (% dry wt) of iridoid and secoiridoid glycosides in three species of *Lonicera*. Quantities represent averages from 2–6 samples taken from a single individual of each species.

Compound identification	RT	MS	*Lonicera morrowii*			*Lonicera* × *bella*			*Lonicera tatarica*		
			Leaves	Unripe	Ripe	Leaves	Unripe	Ripe	Leaves	Unripe	Ripe
Unknown A	26.01	165, 139[a]	ND	ND	ND	ND	ND	ND	ND	ND	ND
Unknown B	28.87	301, 165, 139[a]	0.03[b]	ND	ND	ND	ND	ND	ND	ND	ND
Sweroside	29.38	179	0.21[b]	ND	ND	0.98	0.17[b]	0.20[b]	1.38	0.47	0.52
Secoxyloganin	29.77	297, 165, 139[a]	0.07[b]	ND	ND	0.37[b]	10.75	13.00	0.21[b]	5.90	2.22
Loganin	30.11	283, 165[a], 139[a]	0.06[b]	3.36	3.47	0.01[b]	0.10[b]	0.09[b]	ND	0.03[b]	ND
Unknown C	30.46	428[a], 339, 197	0.06	0.08[b]	0.03[b]	ND	ND	ND	ND	ND	ND
Unknown D	31.56	329, 165, 139	ND	0.47	ND	ND	0.03[b]	ND	ND	ND	ND
Unknown E	31.77	281	ND	0.06[b]	ND	ND	0.01	ND	ND	ND	ND
Loganic acid	32.05	341, 197, 165[a]	0.35	0.03[b]	ND	0.04[b]	ND	ND	ND	ND	ND
Morroniside	33.62	388[a], 299, 139	ND	2.05	1.58	ND	0.07[b]	ND	ND	1.20	0.36
Unknown F	35.06	314, 225, 165, 139	ND	0.16	0.07[b]	ND	ND	ND	ND	0.12[b]	ND
Unknown G	36.00	314, 225, 165, 139	ND	4.42	3.68	ND	0.50	0.21	ND	ND	ND
Secologanin	41.98	255[a], 165, 139	3.09	0.30[b]	ND	0.31[b]	1.77[b]	0.14[b]	0.65	0.61[b]	ND
Total			3.87	10.93	8.83	1.71	13.40	13.64	2.24	8.33	3.10

ND = not detected.
[a] Low intensity MS peak.
[b] Not detected in all samples.

The sugar moiety of IGs gives peaks at m/z 361 (usually the base peak), 271, 243, 217, 204, 191, 169, 147, 129, 103, and 73, all of which were present in the spectra of all detected IGs. Ions originating from the aglycone portion of the molecules are diagnostic of individual IGs and are presented in Table 1. Characteristic IG ion fragmentation patterns are shown in Fig. 3 and include Ion B (formed from the loss of the sugar moiety from the aglycone), Ion C (arising solely from the dihydropyran ring portion), and, for secoiridoids, Ion E (formed by McLafferty-type rearrangement of the dihydropyran ring). In a few cases, a peak was also observed due to Ion D (formed by the loss of the sugar moiety followed by the rearrangement of the TMSi group with Ion A). One unknown compound (Unknown E) appeared to be an iridoid of the oleuropein-type, with a characteristic peak due to Ion F, originating from a phenethyl alcohol side-chain at C-5 (Inouye et al., 1976). By comparing the observed diagnostic m/z peaks to those that would be predicted for known IGs occurring in *L. morrowii* and other closely related *Lonicera* species (Bailleul et al., 1981; Calis et al., 1984; Penuelas et al., 2006; Souzu and Mitsuhashi, 1969, 1970; Wang et al., 2003; Yu et al., 2011), it was possible to provisionally identify all but one of the putative IG components observed in our samples.

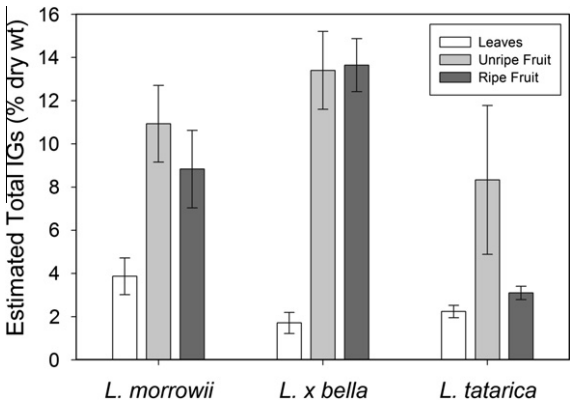


Fig. 2. Average (± SE) estimated total iridoid and secoiridoid glycoside (IG) concentration in leaves, unripe fruits, and ripe fruits of three species of *Lonicera*. Estimated totals include compounds 1–6 (89.1% of total) as well as Unknowns A–G that were provisionally identified as IGs. Averages are from 3–6 collections taken from a single individual of each species.

Both fruits and leaves from all three honeysuckle species contained IGs, the most abundant of which were sweroside (**1**), secoxyloganin (**2**), loganin (**3**), loganic acid (**4**), morroniside (**5**), and secologanin (**6**) (Fig. 1). On average, these six major components represented 89.1% of the total IGs in our samples (Table 1); however, a number of additional components were also detected. Of the three species sampled from the Arnold Arboretum, the parental species *L. morrowii* had the highest chemical diversity, with a total of 12 provisionally detected IGs. The chemical profiles of *L. tatarica* and *L.* × *bella* included only a subset of these compounds, with six individual IGs provisionally detected in *L. tatarica* and nine provisionally detected in *L.* × *bella* (Table 1). However, additional collections from wild populations of *L.* × *bella* in Colorado showed all of the 12 compounds detected in *L. morrowii* plus an additional minor unknown component not detected in either of the parental species (Table 2). Total quantities of IGs also differed among parental and hybrid species, but only for certain plant parts. Quantities were similar for all three species in leaves, but in fruits tended to be higher in the hybrid than for either parent (Fig. 2). However, this quantitative pattern among species was variable depending on the individual IG examined; for certain compounds the hybrid species contained more than either parent (e.g. secoxyloganin [**2**]) and for other compounds the hybrid contained less (e.g. morroniside [**5**]; Table 1).

The total concentration of IGs was significantly higher in unripe and ripe fruits than in leaves, but unripe and ripe fruits were not different from each other ($F_{2,28} = 23.95$, $p < 0.0001$; Fig. 2). This pattern supports the predictions of optimal defense theory, which suggests that fruits should be well defended due to their high reproductive value for the plant (McKey, 1974, 1979; Rhoades and Cates, 1976; Zangerl and Rutledge, 1996). However, it is notable that levels of IGs in fruits do not necessarily diminish with ripening, as one might expect for compounds that could be potentially toxic to seed dispersers. In fact, for the dominant compound in fruits of *L.* × *bella*, secoxyloganin (**2**), concentrations were substantially *higher* in ripe fruits than in unripe fruits collected from the same branch at the same time (Table 2).

It is also notable that there was considerable qualitative and quantitative variation in IGs *within* individuals and species. Fruits and leaves were sampled from 2–6 branches of each individual, and often compounds detected in one collection were not detected in other branches from the same shrub, especially for minor components (Table 1). This suggests that abiotic and/or biotic factors that

Author's personal copy

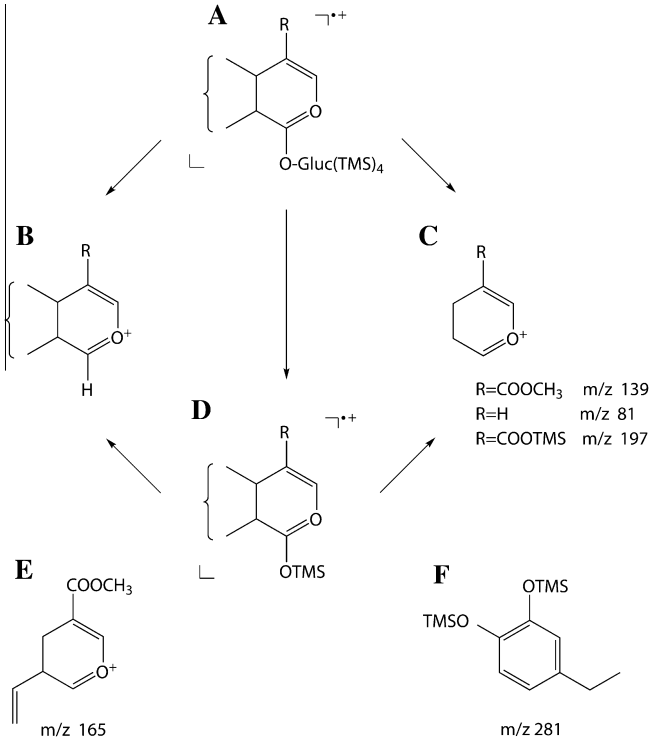


Fig. 3. Fragmentation patterns of silylated iridoid and secoiridoid glycosides in GC-MS analysis. Figure redrawn from Inouye et al. (1976).

influence IG composition and concentration may be localized within a plant and that additional sampling of these and other individuals may show considerable variation and additional minor components not detected here. For the hybrid species, *L.* × *bella*, a total of four different individuals were sampled, and the chemical composition was highly variable. In particular, there were four compounds present in our collections from Colorado that were not present in the collections from the Arnold Arboretum. Even within Colorado, different individuals had different compositions, e.g. secoxyloganin (**2**), which is the dominant IG in fruits for most individuals, was not detected in the Gregory Canyon samples (Table 2).

These results are similar to those reported previously for *L. morrowii* showing different patterns of IG occurrence in fruits and leaves. Compounds occurring in *L. morrowii* fruits, but not detected in leaves, included morroniside (**5**), as well as one unidentified major component (Unknown G) and three minor components provisionally characterized as IGs (Unknowns D–F; Table 1). Past work has reported two IGs unique to fruits in this species, morroniside (**5**) and kingiside (Ikeshiro et al., 1992; Souzu and Mitsuhashi, 1969, 1970). Kingiside was not definitively identified in this study, but has predicted MS fragmentation patterns consistent with Unknown G (Fig. 3; Table 1). Other compounds occurred in leaves of *L. morrowii*, but were not detected in fruits, including sweroside (**1**) and secoxyloganin (**2**); however, interestingly, these compounds were major components of both fruits and leaves of *L. tatarica* and *L.* × *bella* (Table 1). Various other compounds occurred in both fruits and leaves, including loganin (**3**), loganic acid (**4**), and secologanin (**6**) (Table 1).

This study provided the first examination of IGs in the hybrid species, *L.* × *bella*. Hybrid individuals collected from the Arnold Arboretum had chemical traits that were intermediate between the two parents (Table 1), but some collections from Colorado had higher chemical diversity and higher overall concentrations than either parent (Table 2). These differences may be due to the variable genetic background of hybrids collected from wild populations, which readily backcross with parental species and form hybrid swarms throughout much of the introduced range in North America (Barnes and Cottam, 1974; Hauser, 1966). Morphological characters that clearly differentiate the two parental species, such as leaf pubescence and peduncle length (Weber and Wittmann, 2001), are highly variable in Colorado populations, and appear to form a continuum between the two parental phenotypes (Whitehead, personal observation). Results presented here include only samples taken from individuals with intermediate phenotypes (e.g. pink flowers and sparse pubescence on the leaves and peduncles), but an examination of how the variation in chemical and morphological traits correlate with the patterns of genetic introgression in hybrid swarms would be enlightening and have important consequences for our understanding of the adaptive radiation of *Lonicera* species. Thorough studies of this type should include an examination of compounds in different plant parts, since our results show that it can be in the distribution of compounds among leaves and fruits that the hybrid origin of chemical traits is most apparent.

Because the fruits of *Lonicera* are vertebrate-dispersed, IGs in ripe fruits likely have important implications for the palatability of fruits to mutualists and therefore the reproductive success of the hybrid species. Although birds and other frugivores do tolerate

Table 2
Comparison of iridoid and secoiridoid glycoside quantities (% dry wt) in four individuals of *L.* × *bella*. Quantities represent averages from 2–6 samples taken from a single individual from each location.

Compound Identification	Arnold Arboretum			CO-Skunk Canyon			CO-Gregory Canyon			CO-Bluebell Canyon		
	Leaves	Unripe	Ripe	Leaves	Unripe	Ripe	Leaves	Unripe	Ripe	Leaves	Unripe	Ripe
Unknown A	ND	ND	ND	0.37	0.61	0.39	0.70	0.24	0.11	0.88	0.15	0.21
Unknown B	ND	ND	ND	0.19	0.21	ND	0.23	0.15	ND	0.18	0.15	ND
Sweroside	0.98	0.17	0.2	3.92	3.78	3.50	3.92	1.20	1.26	2.64	1.75	1.98
Secoxyloganin	0.37	10.75	13	0.30	8.53	13.74	ND	ND	ND	0.71	2.57	7.48
Loganin	0.01	0.1	0.09	0.25	0.35	0.21	1.42	11.34	7.04	0.19	0.39	0.33
Unknown C	ND	ND	ND	0.11	0.19	0.07	0.10	ND	ND	0.16	0.16	0.10
Unknown D	ND	0.03	ND	0.28	1.25	0.66	0.10	0.45	0.70	0.11	0.62	0.19
Unknown E	ND	0.01	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Loganic acid	0.04	ND	ND	0.06	0.18	0.18	0.09	0.24	0.06	0.16	0.11	0.06
Morroniside	ND	0.07	ND	ND	3.04	2.06	ND	4.53	1.90	ND	0.51	0.47
Unknown F	ND	ND	ND	ND	0.20	0.12	ND	0.17	0.03	ND	0.06	0.04
Unknown G	ND	0.5	0.21	ND	ND	ND	ND	0.02	ND	ND	0.97	0.93
Secologanin	0.31	1.77	0.14	3.44	6.05	2.38	2.12	1.65	0.09	1.61	1.70	0.48
Total	1.71	13.40	13.64	8.92	24.38	23.31	8.68	20.00	11.21	6.65	9.14	12.28

ND = not detected.
CO = Colorado.

Author's personal copy

high levels of secondary metabolites in certain fruits (Barnea et al., 1993; Cipollini and Levey, 1997b; Filardi and Tewksbury, 2005; Levey and Del Rio, 2001), the little existing evidence for the effects of IGs on birds suggests that some of these compounds can be strongly deterrent and have emetic properties (Bowers, 1980). One possible explanation for the maintenance of high levels of IGs in the ripe fruits of the hybrid may be that these compounds function to defend fruits against antagonistic seed predators and fruit pathogens, leading to longer persistence times on the plant and higher overall levels of fruit removal over time (Cipollini and Levey, 1997a; Cipollini and Stiles, 1992). Long persistence time of ripe fruit may be especially beneficial for plants in an introduced range, since often non-native fruits (including those of *Lonicera*) are not removed until late in the season once high-quality native fruits are no longer available (White and Stiles, 1992).

3. Conclusions

This study demonstrates that the hybrid honeysuckle, *L.* × *bella*, exhibits a complex composition of IGs that is variable among individuals and reflects contributions from both parental species as well as some patterns of distribution that are not typical of either parent. Concentrations of IGs are higher in fruits than in leaves for both parents and the hybrid species, and in the hybrid species concentrations remain high even in ripe fruits. These patterns have important implications for plant defense as well as mutualistic interactions with seed dispersers, and may influence the reproductive success of the hybrid species. The characterization of compounds and methods for quantitative analysis established in this study will provide essential tools for future work in this system, which offers an excellent model within which ecologists and evolutionary biologists can explore questions related to the chemical ecology of plant/herbivore and fruit/frugivore interactions, the patterns of trait introgression during hybridization, and the invasion biology of a widespread and problematic group of non-native shrubs in North America.

4. Experimental

4.1. Plant material

Samples of leaves, unripe fruits, and ripe fruits were obtained from the living collections of the Arnold Arboretum (Harvard University, Cambridge, MA, USA) for all three species examined in this study: *L. tatarica* (Acc. #299–78), *L. morrowii* (Acc. #525–84), and *L.* × *bella* (Acc. # 10087 and its asexual propagule, Acc. #392–92). Dried specimens of these accessions are also available from the Herbarium of the Arnold Arboretum at Harvard University. To obtain material for chemical analysis, two to six branches were clipped from each individual (depending on availability) when the fruits on that plant were in mid-ripening (between June 26 and July 12, 2011), thus obtaining leaves, unripe, and ripe fruits from the same branch at the same time.

Additional samples of *L.* × *bella* were collected during July 2008 in the same manner from three populations occurring near Boulder, CO, in Bluebell Canyon (39.99135 N, −105.28568 W), Gregory Canyon (39.99727 N, −105.2940 W), and Skunk Canyon (39.98611 N, −105.27660 W). Identification as *L.* × *bella* was confirmed by two local authorities (Tim Hogan and Dina Clark, Herbarium COLO, University of Colorado Museum of Natural History). Voucher specimens from the Colorado collections used in this study were deposited at the Herbarium COLO at the University of Colorado (Acc. #'s 543191, 543192, and 543193).

Plant samples were field-collected as clippings of entire branches and stored in coolers until they arrived at the laboratory (within 24 h of collection). For each sample, leaves, unripe fruits, and ripe fruits were separated, retaining a minimum of 20 leaves, 10 unripe, and 10 ripe fruits. Only fruits that were completely unripe (green) or completely ripe (red or orange) were retained for analysis; those at intermediate stages were discarded. All samples were then oven-dried for 48 h at 50 °C (as is typical for IG analysis; see Gardner and Stermitz, 1988; Jamieson and Bowers, 2010; Lampert and Bowers, 2011; Quintero and Bowers, 2011b).

4.2. Extractions of IGs

Methods for extraction of IGs were modified from previously published studies (Bowers and Stamp, 1993; Gardner and Stermitz, 1988). The dried fruits and leaves were ground to a fine homogenous powder using a mortar and pestle, first removing all seeds from the fruit samples by grinding fruits through a wire mesh strainer with the pestle. Seeds were discarded, leaving only pulp and skin, which was further ground to a fine powder in the mortar and pestle. For chemical analysis, aliquots (25 mg) were taken from fruit samples and aliquots (50 mg) were taken from leaf samples, weighed to the nearest 0.01 mg. A smaller mass of fruit material (25 mg) was used because preliminary analyses showed high levels of IGs in fruit samples, leading to overloaded chromatograms. Each sample was placed in a test tube with MeOH (5 ml), tightly capped, vortexed, and left overnight for extraction. All samples were then filtered, and the extracts evaporated to dryness. Extracts were then re-suspended in H_2O (3 ml), and an internal standard (phenyl-β-D-gluco-pyranoside) was added to each. Samples were then partitioned three times against equal volumes of Et_2O. The Et_2O fractions were discarded, and the H_2O fractions, containing mostly IGs and sugars, were evaporated to dryness. Each residue was then re-suspended in MeOH (1 ml) and left overnight to allow complete dissolution of IGs. Samples were then vortexed and aliquots (100 μL) were transferred to micro-inserts for GC vials and evaporated to dryness at 50 °C.

4.3. Identification and quantification of IGs using GC–MS

IGs were converted to their trimethylsilane (TMSi) analogs by adding Tri-Sil-Z derivatizing reagent (Thermo-Scientific, Waltham, MA, USA) (100 μl) to the evaporated sample and heating for 20 min at 70 °C in a mineral oil bath. After derivatization, each sample (0.2 μl) was injected onto an HP Agilent 6890 N GC coupled with an Agilent 5975C inert mass selective detector with an ion source of 70 eV at 230 °C and equipped with a DB-1MS capillary column (30 m × 0.25 mm i.d.,0.1 μm film thickness; Agilent Technologies, Santa Clara, CA, USA). Ultra-pure He was used as carrier gas at a flow rate of 2 ml min^{-1}, a split flow ratio of 100:1, and a front inlet temperature of 275 °C. The following oven conditions were employed: initial temperature 180 °C, initial hold time 1 min; ramp 1: 5 °C min^{-1} to 200 °C, hold time 11 min; ramp 2: 2 °C min^{-1} to 260 °C, hold time 0 min; ramp 3: 30 °C min^{-1} to 320 °C, hold time 0 min; for a total run time of 48 min. These oven conditions were modified from previously described methods (Gardner and Stermitz, 1988) to ensure adequate peak resolution of IGs while minimizing the run time for each sample. A blank sample (Tri-Sil-Z only) was run after every five samples to ensure there was no carryover between runs. Data were recorded and processed using MSD ChemStation software (version D.02.00.275).

IGs were identified by comparisons of retention times and mass spectra with authentic standards, including sweroside (**1**), secoxyloganin (**2**), loganin (**3**), loganic acid (**4**), morroniside (**5**), and secologanin (**6**). Secoxyloganin (**2**) was provided by Søren R. Jensen (Technical University of Denmark), morroniside (**5**) was purchased from Tauto Biotech Co., Ltd. (Shanghai, China), and all other standards were purchased from Indofine Chemical Company

Author's personal copy

(Hillsborough, NJ, USA). On average, the IGs identified using reference standards represented 89.1% of the total IGs in our samples, and included all major components except for Unknown G.

Estimated quantities of all compounds were based on peak areas in total ion current chromatograms. For individual IGs for which reference standards were available (1–6), a six-point calibration curve ($R^2 > 0.99$) was created with concentrations ranging from 0.01–5 mg/ml. Secologanin (**6**) was observed as two well-resolved peaks that occurred in a regular ratio of 1:1.2; this occurred for both authentic standards and plant extracts containing secologanin (**6**). These likely represent stereoisomers; however their fragmentation patterns in mass spectra are indistinguishable and thus could not be definitively differentiated in the scope of this study. Thus, to determine total quantities of secologanin (**6**), the sum of the two peak areas was used; this method gave excellent linearity ($R^2 = 0.997$) in a six-point calibration curve with known concentrations of authentic secologanin (**6**). To approximate concentrations of IGs for which no reference standards were available, a response factor equivalent to that of our internal standard was assumed.

4.4. Statistical analysis

To determine whether IG concentrations differed among leaves, unripe fruits, and ripe fruits, a one-way analysis of variance was employed using the statistical software JMP v. 8 (JMP, 2009), with tissue type specified as a fixed effect and individual plant and branch as nested random effects included in the error term. This was followed by a Tukey HSD post hoc test for multiple comparisons to determine pairwise differences among tissue types. Data were arcsin-square root transformed prior to analysis to meet assumptions of normality. Meaningful statistical comparisons of the three species were not possible since all samples of the parental species, *L. tatarica* and *L. morrowii*, were obtained from a single individual of each species at the Arnold Arboretum.

Acknowledgements

We gratefully acknowledge the collections management staff at the Arnold Arboretum of Harvard University, especially M. Dosmann, K. Richardson, and S. Schmerler, for their assistance in the collection of plant material for this work. M.O. Krasnec, A. Trowbridge, and K. Keefover-Ring are thanked for technical support in GC–MS analyses. Prof. S.R. Jensen kindly provided a reference standard for secoxyloganin. K. Keefover-Ring, L.A. Dyer, C.S. Jeffrey, A. Trowbridge, N.S. Robinson, S. Song, and C.A. Kelley provided helpful feedback that greatly improved the manuscript. Funding for this work was provided by a Beverly Sears Graduate Student Research Grant and the Department of Ecology and Evolutionary Biology at the University of Colorado.

References

Allan, B.F., Dutra, H.P., Goessling, L.S., Barnett, K., Chase, J.M., Marquis, R.J., Pang, G., Storch, G.A., Thach, R.E., Orrock, J.L., 2010. Invasive honeysuckle eradication reduces tick-borne disease risk by altering host dynamics. Proc. Natl. Acad. Sci. U. S. A. 107, 18523–18527.

Bailleul, F., Leveau, A.M., Durand, M., 1981. A new iridoid of fruits of *Lonicera alpigena*. J. Nat. Prod. 44, 573–575.

Barnea, A., Harborne, J.B., Pannell, C., 1993. What parts of fleshy fruits contain secondary compounds toxic to birds and why. Biochem. Syst. Ecol. 21, 421–429.

Barnes, W.J., Cottam, G., 1974. Some autecological studies of the *Lonicera* × *bella* complex. Ecology 55, 40–50.

Barton, N.H., 2001. The role of hybridization in evolution. Mol. Ecol. 10, 551–568.

Bartuszevige, A.M., Gorchov, D.L., 2006. Avian seed dispersal of an invasive shrub. Biol. Invasions 8, 1013–1022.

BG-BASE, 2011. Arnold Arboretum of Harvard University Living Collections Database. <http://arboretum.harvard.edu/plants/plant-inventory/> (accessed 13.10.11.)

Bowers, M.D., 1980. Unpalatability as a defense strategy in *Euphydryas phaeton* (Lepidoptera, Nymphalidae). Evolution 34, 586–600.

Bowers, M.D., 1991. Iridoid glycosides. In: Rosenthal, M.R., Berenbaum, G.A. (Eds.), Herbivores: Their Interactions with Plant Secondary Metabolites, vol. 1. Academic Press, San Diego, pp. 297–325.

Bowers, M.D., Stamp, N.E., 1993. Effects of plant age, genotype, and herbivory on *Plantago* performance and chemistry. Ecology 74, 1778–1791.

Calis, I., Lahloub, M.F., Sticher, O., 1984. Loganin, loganic acid, and perclymenoside, a new biosidic ester iridoid glucoside from *Lonicera periclymenum* L. (Caprifoliaceae). Helv. Chim. Acta 67, 160–165.

Chen, X.G., Jiang, Z.L., Zhu, Y., Tan, J.Y., 2007. Use of two-dimensional liquid chromatography combined with diode-array and mass spectrometric detection for analysis of compounds in Flos *Lonicera*. Chromatographia 65, 141–147.

Cheng, D.D., Vrieling, K., Klinkhamer, P.G.L., 2011. The effect of hybridization on secondary metabolites and herbivore resistance: implications for the evolution of chemical diversity in plants. Phytochem. Rev. 10, 107–117.

Cipollini, D., Stevenson, R., Enright, S., Eyles, A., Bonello, P., 2008. Phenolic metabolites in leaves of the invasive shrub, *Lonicera maackii*, and their potential phytotoxic and anti-herbivore effects. J. Chem. Ecol. 34, 144–152.

Cipollini, M.L., Levey, D.J., 1997a. Secondary metabolites of fleshy vertebrate-dispersed fruits: adaptive hypotheses and implications for seed dispersal. Am. Nat. 150, 346–372.

Cipollini, M.L., Levey, D.J., 1997b. Why are some fruits toxic? Glycoalkaloids in *Solanum* and fruit choice by vertebrates. Ecology 78, 782–798.

Cipollini, M.L., Stiles, E.W., 1992. Relative risks of fungal rot for temperate Ericaceous fruits – effects of seasonal variation on selection for chemical defense. Can. J. Bot. 70, 1868–1877.

Coley, P.D., Barone, J.A., 2001. Ecology of defenses. In: Levin, S.A. (Ed.), Encyclopedia of Biodiversity, vol. 2. Academic Press, San Diego, pp. 11–21.

Collier, M.H., Vankat, J.L., Hughes, M.R., 2002. Diminished plant richness and abundance below *Lonicera maackii*, an invasive shrub. Am. Midl. Nat. 147, 60–71.

Dyer, L.A., Bowers, M.D., 1996. The importance of sequestered iridoid glycosides as a defense against an ant predator. J. Chem. Ecol. 22, 1527–1539.

Eisner, T., Meinwald, J., 1995. Chemical Ecology: The Chemistry of Biotic Interactions. National Academy Press, Washington, D.C.

Ellstrand, N.C., Schierenbeck, K.A., 2000. Hybridization as a stimulus for the evolution of invasiveness in plants? Proc. Natl. Acad. Sci. U. S. A. 97, 7043–7050.

Filardi, C.E., Tewksbury, J., 2005. Ground-foraging palm cockatoos (*Probosciger aterrimus*) in lowland New Guinea: fruit flesh as a directed deterrent to seed predation? J. Trop. Ecol. 21, 355–361.

Fritz, R.S., 1999. Resistance of hybrid plants to herbivores: genes, environment, or both? Ecology 80, 382–391.

Fritz, R.S., Moulia, C., Newcombe, G., 1999. Resistance of hybrid plants and animals to herbivores, pathogens, and parasites. Annu. Rev. Ecol. Syst. 30, 565–591.

Gardner, D.R., Stermitz, F.R., 1988. Host plant utilization and iridoid glycoside sequestration by *Euphydryas anicia* (Lepidoptera, Nymphalidae). J. Chem. Ecol. 14, 2147–2168.

Geiger, J.H., Pratt, P.D., Wheeler, G.S., Williams, D.A., 2011. Hybrid vigor for the invasive exotic Brazilian peppertree (*Schinus terebinthifolius* Raddi., Anacardiaceae) in Florida. Int. J. Plant Sci. 172, 655–663.

Gorchov, D.L., Trisel, D.E., 2003. Competitive effects of the invasive shrub, *Lonicera maackii* (Rupr.) Herder (Caprifoliaceae), on the growth and survival of native tree seedlings. Plant Ecol. 166, 13–24.

Gosper, C.R., Stansbury, C.D., Vivian-Smith, G., 2005. Seed dispersal of fleshy-fruited invasive plants by birds: contributing factors and management options. Divers. Distrib. 11, 549–558.

Hauser, E.J.P., 1966. The natural occurrence of a hybrid honeysuckle (*Lonicera* × *bella*) in Ohio and Michigan. Mich. Bot. 5, 211–217.

Hegarty, M.J., Hiscock, S.J., 2005. Hybrid speciation in plants: new insights from molecular studies. New Phytol. 165, 411–423.

Hermanslokkerbol, A., Verpoorte, R., 1987. Purification of secologanin from *Lonicera tatarica* extracts using RLCC. Planta Med. 53, 546–548.

Herrera, C.M., 1982. Defense of ripe fruit from pests – its significance in relation to plant-disperser interactions. Am. Nat. 120, 218–241.

Higgins, S.I., Richardson, D.M., 1999. Predicting plant migration rates in a changing world: the role of long-distance dispersal. Am. Nat. 153, 464–475.

Ikeshiro, Y., Todo, Y., Mase, I., Tomita, Y., Tanaka, S., Herath, W., 1992. Iridoid glucosides from the fruits of *Lonicera morrowii*. Planta Med. 58, 109.

Ingold, J.L., Craycraft, M.J., 1983. Avian frugivory on honeysuckle (*Lonicera*) in southwestern Ohio in fall. Ohio J. Sci. 83, 256–258.

Inouye, H., Uobe, K., Hirai, M., Masada, Y., Hashimoto, K., 1976. Studies on monoterpene glucosides and related natural products XXXI: gas chromatography and gas chromatography-mass spectrometry of iridoid and seco-iridoid glucosides. J. Chromatogr. 118, 201–216.

Jamieson, M.A., Bowers, M.D., 2010. Iridoid glycoside variation in the invasive plant Dalmatian Toadflax, *Linaria dalmatica* (Plantaginaceae), and sequestration by the biological control agent, *Calophasia lunula*. J. Chem. Ecol. 36, 70–79.

JMP., 2009. Version 8. SAS Institute Inc., Cary, NC

Lampert, E.C., Bowers, M.D., 2011. A comparison of sample preparation techniques for quantifying iridoid glycosides sequestered by Lepidopteran larvae. J. Chem. Ecol. 37, 496–499.

Lampert, E.C., Dyer, L.A., Bowers, M.D., 2011. Chemical defense across three trophic levels: *Catalpa bignonioides*, the caterpillar *Ceratomia catalpae*, and its endoparasitoid *Cotesia congregata*. J. Chem. Ecol. 37, 1063–1070.

Levey, D.J., Del Rio, C.M., 2001. It takes guts (and more) to eat fruit: lessons from avian nutritional ecology. Auk 118, 819–831.

Author's personal copy

Levey, D.J., Tewksbury, J.J., Izhaki, I., Tsahar, E., Haak, D.C., 2007. Evolutionary ecology of secondary compounds in ripe fruit: case studies with capsaicin and emodin. In: Dennis, A.J., Green, R.J., Schupp, E.W., Westcott, D.A. (Eds.), Seed Dispersal: Theory and its Application in a Changing World. CABI Publishing, Oxon, pp. 37–58.

Li, H.J., Li, P., Ye, W.C., 2003. Determination of five maior iridoid glucosides in Flos *Lonicerae* by high-performance liquid chromatography coupled with evaporative light scattering detection. J. Chromatogr. A 1008, 167–172.

Lindstedt, C., Talsma, J.H.R., Ihalainen, E., Lindstrom, L., Mappes, J., 2010. Diet quality affects warning coloration indirectly: excretion costs in a generalist herbivore. Evolution 64, 68–78.

Makarevich, I.F., Kovalenko, S.N., Gusarova, T.D., Gubin, Y.I., 2009. Iridoids from *Symphoricarpos albus*. Chem. Nat. Compd. 45, 40–44.

Marak, H.B., Biere, A., Van Damme, J.M.M., 2002a. Systemic, genotype-specific induction of two herbivore-deterrent iridoid glycosides in *Plantago lanceolata* L. in response to fungal infection by *Diaporthe adunca* (Rob.) niessel. J. Chem. Ecol. 28, 2429–2448.

Marak, H.B., Biere, A., van Damme, J.M.M., 2002b. Two herbivore-deterrent iridoid glycosides reduce the in-vitro growth of a specialist but not of a generalist pathogenic fungus of *Plantago lanceolata* L. Chemoecology 12, 185–192.

Martinsen, G.D., Whitham, T.G., Turek, R.J., Keim, P., 2001. Hybrid populations selectively filter gene introgression between species. Evolution 55, 1325–1335.

McKey, D., 1974. Adaptive patterns in alkaloid physiology. Am. Nat. 108, 305–320.

McKey, D., 1979. The distribution of secondary compounds within plants. In: Rosenthal, G.A., Janzen, D.H. (Eds.), Herbivores: Their Interactions with Secondary Plant Metabolites. Academic Press, New York, pp. 55–133.

Nyboer, R., 1992. Vegetation managment guideline – bush honeysuckles – Tatarian, Morrows, Belle, and Amur honeysuckle (*Lonicera tatarica* L., *L. morrowii* Gray, *L.* × *bella* Zabel, and *L. maackii* Rupr Maxim). Nat. Areas J. 12, 218–219.

Oberprieler, C., Barth, A., Schwarz, S., Heilmann, J., 2010. Morphological and phytochemical variation, genetic structure and phenology in an introgressive hybrid swarm of *Senecio hercynicus* and *S. ovatus* (Compositae, Senecioneae). Plant Syst. Evol. 286, 153–166.

Ono, M., Ueno, M., Masuoka, C., Ikeda, T., Nohara, T., 2005. Iridoid glucosides from the fruit of *Genipa americana*. Chem. Pharm. Bull. 53, 1342–1344.

Orians, C.M., 2000. The effects of hybridization in plants on secondary chemistry: implications for the ecology and evolution of plant-herbivore interactions. Am. J. Bot. 87, 1749–1756.

Orians, C.M., Fritz, R.S., 1995. Secondary chemistry of hybrid and parental willows–phenolic glycosides and condensed tannins in *Salix sericea*, *S. eriocephala*, and their hybrids. J. Chem. Ecol. 21, 1245–1253.

Penuelas, J., Sardans, J., Stefanescu, C., Parella, T., Filella, I., 2006. *Lonicera implexa* leaves bearing naturally laid eggs of the specialist herbivore *Euphydryas aurinia* have dramatically greater concentrations of iridoid glycosides than other leaves. J. Chem. Ecol. 32, 1925–1933.

Popov, S.S., Handjieva, N.V., 1983. Mass-spectrometry of iridoids. Mass Spectrom. Rev. 2, 481–514.

Quintero, C., Bowers, M.D., 2011a. Changes in plant chemical defenses and nutritional quality as a function of ontogeny in *Plantago lanceolata* (Plantaginaceae). Oecologia 168, 471–481.

Quintero, C., Bowers, M.D., 2011b. Plant induced defenses depend more on plant age than previous history of damage: implications for plant-herbivore interactions. J. Chem. Ecol. 37, 992–1001.

Rehill, B.J., Whitham, T.G., Martinsen, G.D., Schweitzer, J.A., Bailey, J.K., Lindroth, R.L., 2006. Developmental trajectories in cottonwood phytochemistry. J. Chem. Ecol. 32, 2269–2285.

Reudler, J.H., Biere, A., Harvey, J.A., van Nouhuys, S., 2011. Differential performance of a specialist and two generalist herbivores and their parasitoids on *Plantago lanceolata*. J. Chem. Ecol. 37, 765–778.

Rhoades, D.F., Cates, R.G., 1976. Toward a general theory of plant antiherbivore chemistry. Recent Adv. Phytochem. 10, 168–213.

Richardson, D.M., Allsopp, N., D'Antonio, C.M., Milton, S.J., Rejmanek, M., 2000. Plant invasions – the role of mutualisms. Biol. Rev. Camb. Philos. Soc. 75, 65–93.

Rieseberg, L.H., Ellstrand, N.C., 1993. What can molecular and morphological markers tell us about plant hybridization. Crit. Rev. Plant Sci. 12, 213–241.

Schierenbeck, K.A., Ellstrand, N.C., 2009. Hybridization and the evolution of invasiveness in plants and other organisms. Biol. Invasions 11, 1093–1105.

Schweitzer, J.A., Martinsen, G.D., Whitham, T.G., 2002. Cottonwood hybrids gain fitness traits of both parents: a mechanism for their long-term persistence? Am. J. Bot. 89, 981–990.

Song, Y., Li, S.L., Wu, M.H., Li, H.J., Li, P., 2006. Qualitative and quantitative analysis of iridoid glycosides in the flower buds of *Lonicera* species by capillary high performance liquid chromatography coupled with mass spectrometric detector. Anal. Chim. Acta 564, 211–218.

Souzu, I., Mitsuhashi, H., 1969. Structures of iridoids from *Lonicera morrowii* A. Gray. Tetrahedron Lett., 2725.

Souzu, I., Mitsuhashi, H., 1970. Structures of iridoids from *Lonicera morrowii* A. Gray II. Tetrahedron Lett., 191.

Strauss, S.Y., 1994. Levels of herbivory and parasitism in host hybrid zones. Trends Ecol. Evol. 9, 209–214.

Svobodova, A., Rambouskova, J., Zdaoilova, A., Vostdlova, J., 2008. Protective effect of phenolics from *Lonicera caerulea* fruits against UVB-induced damage to HaCaT keratinocytes. Planta Med. 74, 1125.

Tewksbury, J.J., Reagan, K.M., Machnicki, N.J., Carlo, T.A., Haak, D.C., Penaloza, A.L.C., Levey, D.J., 2008. Evolutionary ecology of pungency in wild chilies. Proc. Natl. Acad. Sci. U. S. A. 105, 11808–11811.

Vellend, M., 2002. A pest and an invader: white-tailed deer (*Odocoileus virginianus Zimm.*) as a seed dispersal agent for honeysuckle shrubs (*Lonicera L.*). Nat. Areas J. 22, 230–234.

Wang, Y.L., Wei, Q.Y., Yang, L., Liu, Z.L., 2003. Iridoid glucosides from chinese herb *Lonicera chrysatha* and their antitumor activity. J. Chem. Res., S676–S677.

Watling, J.I., Hickman, C.R., Lee, E., Wang, K., Orrock, J.L., 2011. Extracts of the invasive shrub *Lonicera maackii* increase mortality and alter behavior of amphibian larvae. Oecologia 165, 153–159.

Weber, W.A., Wittmann, R.C., 2001. Colorado Flora: Eastern Slope. University Press of Colorado, Boulder, CO.

Webster, C.R., Jenkins, M.A., Jose, S., 2006. Woody invaders and the challenges they pose to forest ecosystems in the eastern United States. J. Forensic 104, 366–374.

White, D.W., Stiles, E.W., 1992. Bird dispersal of fruits of species introduced into eastern North America. Can. J. Bot. 70, 1689–1696.

Whitham, T.G., Martinsen, G.D., Floate, K.D., Dungey, H.S., Potts, B.M., Keim, P., 1999. Plant hybrid zones affect biodiversity: tools for a genetic-based understanding of community structure. Ecology 80, 416–428.

Whitney, K.D., Randell, R.A., Rieseberg, L.H., 2006. Adaptive introgression of herbivore resistance traits in the weedy sunflower *Helianthus annuus*. Am. Nat. 167, 794–807.

Woods, K.D., 1993. Effects of invasion by *Lonicera tatarica* L. on herbs and tree seedlings in four New England forests. Am. Midl. Nat. 130, 62–74.

Yu, Y., Song, W.X., Zhu, C.G., Lin, S., Zhao, F., Wu, X.L., Yue, Z.G., Liu, B., Wang, S.J., Yuan, S.P., Hou, Q., Shi, J.G., 2011. Homosecoiridoids from the flower buds of *Lonicera japonica*. J. Nat. Prod. 74, 2151–2160.

Zadernowski, R., Naczk, M., Nesterowicz, J., 2005. Phenolic acid profiles in some small berries. J. Agric. Food Chem. 53, 2118–2124.

Zangerl, A.R., Rutledge, C.E., 1996. The probability of attack and patterns of constitutive and induced defense: a test of optimal defense theory. Am. Nat. 147, 599–608.