Seasonal Variation in Cuticular Waxes on *Hosta* Genotypes Differing in Leaf Surface Glaucousness

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Abstract. Cuticular waxes were analyzed on abaxial and adaxial leaf surfaces of three *Hosta* genotypes differing in leaf surface glaucousness; the glossy-leaved *Hosta plantaginea*, the glossy-leaved *Hosta lancifolia*, and the glaucous-leaved *Hosta* 'Krossa Regal'. All three *hosta* had their highest total leaf wax quantity in the spring soon after full leaf expansion. The major wax constituent class on these *hosta* was primary alcohols, comprising up to 84.6% of the total wax. Many *hosta* leaves had unusually high C_{24} length primary alcohols, especially in the spring. However, the dominant chain length in this alcohol class varied with development and genotype. A unique class of β -diketones were present on the glaucous 'Krossa Regal', comprising as much as 28.7% of the total waxes on abaxial leaf surfaces in the summer. Interestingly, these β -diketones were only 0.9% of total waxes on adaxial leaf surfaces of 'Krossa Regal' in the summer. Studies are under way to determine whether the dramatic seasonal changes in the waxy leaf coatings described in this report are associated with biotic and abiotic stress resistance in *hosta*.

Hosta are among the most important landscape plants due to their high esthetic value and tolerance to many kinds of environmental stress. Notwithstanding, *hosta* foliage can be seriously damaged by slugs and other foliar feeding insects (Still, 1993). The basis for *hosta* susceptibility to slugs and insects is complex. Previous research suggests that the degree of *hosta* leaf surface glaucousness or glossiness could be playing an important role (Jenks and Ashworth, 1999; Jenks, unpublished). Glaucousness refers to the visible coating of epicuticular wax crystals whose presence makes *hosta* leaf surfaces appear colors from bright white, whitish-gray, whitish-blue, light blue, to silver. Glossiness, by comparison, refers to the very smooth, noncrystalline deposition of epicuticular waxes that makes *hosta* leaves appear bright, shiny green. Glaucousness and glossiness are important traits for the development of more attractive *hosta* via traditional breeding strategies.

Our laboratory recently began work to dissect the role of these cuticular waxes in *hosta* resistance to biotic and abiotic environmental stress by exploiting the wide variation for this waxy trait within *hosta* species and cultivars. To provide a better foundation for understanding *hosta* wax biology, we report here a detailed analysis of seasonal changes in cuticular

wax total quantity and chemical composition on three *hosta* genotypes differing in leaf surface glaucousness; the glossy-leaved *Hosta plantaginea*, the glossy-leaved *Hosta lancifolia*, and the glaucous-leaved *Hosta* 'Krossa Regal'.

Materials and Methods

Plant material. Three *hosta* genotypes, *Hosta plantaginea* (Lam.) Asch. (Fragrant Plantain Lily), *Hosta lancifolia* Engl. (Narrow-leaved Plantain Lily), and *Hosta* 'Krossa Regal' Summers (Krossa Regal *Hosta*) were grown using a completely randomized design in field plots during the 1999 growing season at the Maxwell Tract on the Purdue Univ. campus. Plants were grown under 50% shade (polyethylene shade cloth) with three inches of hardwood mulch and drip irrigation.

Wax extraction. Cuticular waxes from adaxial and abaxial leaf blades were extracted separately from the seventh through tenth leaf of all three cultivars in the early spring during leaf expansion (expanding leaves) on 25–27 Apr. 1999; and from fully expanded leaves on 3–5 May 1999 (spring leaves); 15–17 July 1999 (summer leaves); and 26–29 Sept. 1999 (fall leaves). For fully expanded leaves, those that appeared most representative of leaves most recently reaching full expansion were selected. The exact age or sequence number of individual leaves was not determined. Leaf wax samples were collected using an extraction device as described in Premachandra et al. (1993) that utilized a 20-mL standard glass scintillation vial with a 2.27-cm² circular opening as the extraction surface. Ten to 25 circular

areas (depending on estimated wax quantity for each genotype) were extracted from each leaf with ≈ 10 mL of GC-grade chloroform added to the vial. The extraction device with leaf tissues inserted was agitated for 10 s per area extracted, being sure that the leaf surface was completely covered by solvent during those 10 sec. Extracts contained waxes from either the abaxial or adaxial blade surfaces, and showed little or no coloration due to chlorophylls or other internal lipids.

Chemical analysis of cuticular waxes. The chloroform-soluble cuticular wax extracts were evaporated to dryness under a nitrogen stream and the dried residue prepared for gas chromatography by derivatization using BSTFA [*N,O*-bis(trimethylsilyl)trifluoroacetamide] for 15 min at 100 °C according to Jenks et al. (1995). After surplus BSTFA was evaporated under nitrogen, the sample was redissolved in chloroform for analysis with a Hewlett-Packard 5890 series II gas chromatograph (GC) (Avondale, Pa.) equipped with a flame ionization detector and automatic injector. The GC was equipped with a 12 m, 0.2 mm HP-1 capillary column with helium as the carrier gas. The GC was programmed with an initial temperature of 80 °C and increased at 15 °C min to 260 °C, where the temperature remained unchanged for 10 min. The temperature was then increased at 5 °C min to 320 °C, where the temperature was held for 15 min. Quantification was based on flame ionization detector peak areas and the internal standard, hexadecane, that was added to the original extract solution. Conversion factors for detector units to quantitative values were developed from external standards of the free fatty acids, primary alcohols, and alkanes representing short to long chain homologues from all classes. These were analyzed at four concentration levels (multi-level) representing the concentration range of sample peaks. For all other constituents, a factor of 1.03 was assigned (an average conversion factor for external standards at comparable concentrations). The total amount of cuticular wax was expressed per total abaxial or adaxial leaf blade area and included all identified and unidentified compounds whose individual amount represented at least 0.2% of the total quantity. All values represent the average of three to four replicate samples. Wax esters were identified using thin layer chromatography (TLC) and cleaved and derivatized with 10 methanol : 1 acetyl chloride (w/v) into fatty acid methyl esters and primary-alcohol products. Selected subsamples were injected into a gas chromatograph-mass spectrometer (GC/MS) using electron ionization to confirm the identification of the GC peaks. Capillary GC/MS analyses were carried out using a Thermospray GCQ (FinniganMAT/Thermospray Corp., San Jose, Calif.) ion trap mass spectrometer system. Electron energy was set at 70 eV, with the ion source temperature maintained at 200 °C. The individual components were separated using a 100 meter DB-1 capillary column. The initial column temperature was set at 100 °C and programmed to 310 °C at 10.0 °C min. The injector temperature was set at 250 °C.

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Results and Discussion

Our results revealed high variation in total wax quantity of both abaxial and adaxial leaf surfaces for these hosta genotypes at most developmental time points (Table 1). A general trend however appears clear from these results. Wax quantity on all three genotypes reached their maximum values during early spring soon after full leaf expansion. The expanding leaves had less wax, and it was assumed that this represented a period of rapid wax synthesis to cover an increasing leaf area. Interestingly, we recorded a large reduction in the amount of wax between spring (early May) and summer (mid-July) in all genotypes. Wax quantity dropped by 3.8-, 7.2-, and 2.5-fold on abaxial leaf surfaces of *H. plantaginea*, *H. lancifolia*, and 'Krossa Regal' respectively, between spring and summer. Wax quantity dropped by 3.0-, 4.5-, and 3.3-fold on adaxial leaf surfaces of *H. plantaginea*, *H. lancifolia*, and 'Krossa Regal' respectively, between spring and summer (Table 1). Many factors might explain this large reduction in leaf wax quantity. First, the spring plants did not experience precipitation from immediately after leaf expansion to the time of extraction. However, there were significant episodes of precipitation between the spring and summer extractions, and these appeared to degrade much of the visible waxy coating on the glaucous genotype 'Krossa Regal'. Other glaucous genotypes in our field plot also showed reduction in their visible wax coatings, due at least in part to precipitation. It is likely then that rainfall may have also degraded waxes on the more glossy genotypes. Why the glossy lines tended to have more wax on the adaxial than abaxial surfaces in the summer and fall (Table 1) is difficult to explain since rainfall might be expected to degrade wax on the upwardly exposed adaxial surface more. Besides potential effects of precipitation, air temperatures in the weeks preceding summer extractions were unseasonably high. Whether heat stress limited regeneration of new young leaves, or overall leaf wax metabolism, is uncertain. The leaves selected for analysis in the summer may thus have represented either older leaves whose waxes had been much degraded by wind and rain, and/or whose ability to synthesize wax was impaired by heat stress. In addition, flowering occurred in these genotypes between the spring and summer extractions. Previous studies showed that wax quantity on leaves of many plants decreased soon after flowering (Jenks et al., 1996; Rich 1996) and later in development (Freeman et al., 1979; Teusink et al., 2001), and thus post-flowering reduction in leaf wax quantity on hosta was comparable to previous reports. Further studies are needed to determine whether higher wax amounts in spring, and before flowering, are an adaptation to natural ecosystems that might play a role in protecting hosta from biotic and abiotic environmental stresses that limit reproductive success.

Of the three genotypes examined, the most glaucous genotype 'Krossa Regal' had the highest wax quantity throughout development

Table 1. Seasonal changes in total cuticular wax quantity in $\mu\text{g}\cdot\text{dm}^{-2}$ ($\pm$ SD) of abaxial and adaxial leaf surfaces of three hosta genotypes differing in visible leaf glaucousness. Hosta genotypes included the glossy-leaved *Hosta plantaginea*, the glossy-leaved *H. lancifolia*, and the glaucous-leaved *H. 'Krossa Regal'*.

Genotype	Expanding	Spring	Summer	Fall
<i>H. plantaginea</i>				
Abaxial	80.7 $\pm$ 19.4	430.8 $\pm$ 71.2	111.8 $\pm$ 37.0	312.4 $\pm$ 36.3
Adaxial	80.6 $\pm$ 21.2	629.9 $\pm$ 107.3	211.8 $\pm$ 71.5	388.7 $\pm$ 74.6
<i>H. lancifolia</i>				
Abaxial	273.9 $\pm$ 81.6	552.2 $\pm$ 82.8.0	76.4 $\pm$ 36.6	414.0 $\pm$ 66.4
Adaxial	401.9 $\pm$ 131.8	747.7 $\pm$ 167.9	167.3 $\pm$ 66.2	677.3 $\pm$ 183.6
'Krossa Regal'				
Abaxial	1176.1 ^z	1763.6 $\pm$ 388.8	712.6 $\pm$ 158.1	919.6 $\pm$ 247.5
Adaxial	1123.4 $\pm$ 135.9	1669.9 $\pm$ 190.0	503.1 $\pm$ 47.7	686.9 $\pm$ 87.9

^zRepresents the average of two replicates.

(Table 1). The fully expanded leaves of 'Krossa Regal' had an abaxial wax quantity that was 4.1- and 3.2-fold higher, and an adaxial wax quantity that was 2.7- and 2.2-fold higher than *H. plantaginea* and *H. lancifolia*, respectively (Table 1). Although the high standard deviation means that differences between abaxial and adaxial leaf surface wax amounts could not be demonstrated at a $P < 0.05$ level, a clear trend toward more waxes on the adaxial than abaxial surfaces of *H. plantaginea* and *H. lancifolia* was evident throughout development (Table 1). In contrast, 'Krossa Regal' tended to have more total waxes on the abaxial than adaxial leaf surfaces throughout development (Table 1). Wax amounts on the leaves of all three genotypes examined in late September (fall leaves) were higher than wax amounts on leaves examined in the summer (although these differences were not significant ($P < 0.05$) for adaxial surfaces of *H. plantaginea* and both surfaces of 'Krossa Regal'). Whether increase in wax quantity in the fall was due to beneficial effects of cooler fall temperatures on wax synthesis and/or subsequent regeneration of leaves is uncertain. Nevertheless, these results together indicate that the wax quantity on abaxial or adaxial leaves of hosta vary dramatically during the season and are strongly effected by genotype, development, and likely environment, with the greatest wax amounts on abaxial and adaxial leaf surfaces of all three genotypes occurring in the spring.

Large variation existed within replicates of the same genotype grown under identical conditions. The basis for this variation may be that leaves selected for extraction were not of identical age, as the exact age of each leaf (in days) was not determined in this study. Also, variation in wax profiles may have been created by extracting from leaves having different orientation and exposure to the sun. For example, a shaded hosta leaf on the north side of a plant may have had different waxes than a leaf exposed to more sun on the south side of a plant, just as light levels have been shown to influence wax composition in other plants (Giese, 1975; Kasperbauer and Wilkinson, 1995). Variation between genotypes may also have been influenced by differences in leaf maturation and flowering time of the different cultivars. Further studies that examine more specifically the development and microenvironment of individual leaves may do much to explain the variation we report here.

Compositional analysis revealed that the major identifiable class of waxes (excluding the total unknowns as a separate class) on all genotypes, at all developmental time points, and both abaxial and adaxial surfaces (except abaxial 'Krossa Regal' in summer), was primary alcohols (Tables 2–4). Primary alcohols made up between 27.0% and 86.4% of the total wax quantity in all lines, except in 'Krossa Regal' during the summer when primary alcohols were only 11.7% of the total quantity

Table 2. Seasonal changes in the amount of cuticular wax in each wax class in $\mu\text{g}\cdot\text{dm}^{-2}$ ($\pm$ SD) of both abaxial and adaxial leaf surfaces of *Hosta plantaginea*.

Wax class	Expanding	Spring	Summer	Fall
Primary alcohols				
Abaxial	31.2 $\pm$ 10.2	333.1 $\pm$ 62.5	58.5 $\pm$ 21.9	221.8 $\pm$ 31.7
Adaxial	34.1 $\pm$ 17.1	531.4 $\pm$ 95.4	128.7 $\pm$ 54.2	198.4 $\pm$ 44.8
Alkanes				
Abaxial	19.1 $\pm$ 2.1	13.9 $\pm$ 0.9	5.7 $\pm$ 2.6	22.1 $\pm$ 2.2
Adaxial	11.1 $\pm$ 2.2	13.0 $\pm$ 1.4	27.0 $\pm$ 6.2	109.4 $\pm$ 39.5
Free fatty acids				
Abaxial	1.9 $\pm$ 0.7	2.2 $\pm$ 0.3	1.1 $\pm$ 0.8	4.3 $\pm$ 0.9
Adaxial	1.6 $\pm$ 0.4	1.4 $\pm$ 0.1	2.5 $\pm$ 0.8	3.9 $\pm$ 1.4
Aliphatic esters				
Abaxial	1.3 $\pm$ 0.3	4.8 $\pm$ 1.1	0.5 $\pm$ 0.2	1.2 $\pm$ 0.4
Adaxial	1.3 $\pm$ 0.3	4.7 $\pm$ 2.1	0.7 $\pm$ 0.3	2.1 $\pm$ 0.8
β -diketones				
Abaxial	nd*	nd	nd	nd
Adaxial	nd	nd	nd	nd
Unknowns				
Abaxial	27.3 $\pm$ 9.2	76.8 $\pm$ 10.9	46.0 $\pm$ 12.7	63.1 $\pm$ 19.1
Adaxial	32.6 $\pm$ 9.3	77.4 $\pm$ 7.7	52.8 $\pm$ 8.0	74.9 $\pm$ 19.1

*Not detected..

Table 3. Seasonal changes in the amount of cuticular wax in each wax class in $\mu\text{g}\cdot\text{dm}^{-2}$ ($\pm$ SD) of both abaxial and adaxial leaf surfaces of *Hosta lancifolia*.

Wax class	Expanding	Spring	Summer	Fall
Primary alcohols				
Abaxial	201.5 $\pm$ 86.8	454.1 $\pm$ 64.8	41.9 $\pm$ 17.2	300.3 $\pm$ 56.7
Adaxial	334.6 $\pm$ 109.9	616.2 $\pm$ 151.4	104.3 $\pm$ 61.2	337.5 $\pm$ 114.6
Alkanes				
Abaxial	24.9 $\pm$ 6.9	45.7 $\pm$ 17.8	4.3 $\pm$ 2.2	76.9 $\pm$ 16.8
Adaxial	23.1 $\pm$ 7.9	58.1 $\pm$ 19.0	23.2 $\pm$ 7.1	201.7 $\pm$ 48.7
Free fatty acids				
Abaxial	2.6 $\pm$ 1.3	4.5 $\pm$ 1.2	1.2 $\pm$ 0.3	2.7 $\pm$ 1.1
Adaxial	2.6 $\pm$ 1.4	2.9 $\pm$ 1.1	1.0 $\pm$ 0.6	3.4 $\pm$ 1.6
Aliphatic esters				
Abaxial	0.2 $\pm$ 0.0	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1	1.5 $\pm$ 0.9
Adaxial	0.5 $\pm$ 0.3	0.1 $\pm$ 0.0	0.4 $\pm$ 0.4	1.1 $\pm$ 0.5
β -diketones				
Abaxial	nd	nd	nd	nd
Adaxial	nd	nd	nd	nd
Unknowns				
Abaxial	44.5 $\pm$ 13.1	45.9 $\pm$ 15.3	28.8 $\pm$ 11.9	32.6 $\pm$ 5.0
Adaxial	41.2 $\pm$ 14.1	70.5 $\pm$ 14.2	38.5 $\pm$ 6.7	43.4 $\pm$ 7.1

ndNot detected.

on the abaxial surface (Tables 2–4). Likewise, leaves of *Simmondsia chinensis* (Link) C.K. Schneid (Gulz and Hangst, 1983), *Triticum aestivum* L. (Bianchi et al., 1980), *Fagus sylvatica* L. (Prasad and Gulz, 1990a), *Juglans regia* L. (Prasad and Gulz, 1990b), and *Quercus robur* L. (Gulz and Muller, 1992) had primary alcohols as their major wax component class. Unique relative to other plant species (Jenks and Ashworth, 1999) was that the C_{24} primary alcohol was highly abundant on many hosta samples (Figs. 1–3). On abaxial leaves of *H. plantaginea*, the C_{28} alcohol was the dominant constituent on expanding and fully expanded leaves in the spring (Fig. 1). In the summer and fall, abaxial leaves of *H. plantaginea* were dominated by C_{30} and C_{32} alcohols. Adaxial leaf surfaces of *H. plantaginea* were dominated by either C_{24} or C_{26} alcohols in the spring, and C_{28} and longer chain alcohols in the summer and fall (Fig. 1). In the summer and fall, both abaxial and adaxial surfaces of *H. plantaginea* had significant reductions in the proportional amounts of C_{24} alcohols. Abaxial surfaces of *H. plantaginea* likewise displayed reduced C_{26} alcohols in the summer and fall (Fig. 1). The abaxial surface of *H. lancifolia*

possessed similar proportional amounts of all of its primary alcohol homologues, with the C_{32} alcohols by comparison being much higher than those on abaxial surfaces of *H. plantaginea* expanding and expanded leaves (Figs. 1 and 2). As in *H. plantaginea*, the adaxial waxes of *H. lancifolia* contained greater amounts of the shorter chain length alcohols than the abaxial surface throughout development (Fig. 2). Neither *H. lancifolia* nor ‘Krossa Regal’ showed as great a reduction in the C_{24} alcohols later in development as did *H. plantaginea* (Figs. 1–3). Similar to *H. plantaginea* and *H. lancifolia*, ‘Krossa Regal’ had longer chain length alcohols on the abaxial than adaxial surface, with the amount of the C_{24} alcohol decreasing considerably more on the abaxial than on the adaxial surface after leaf expansion in the spring (Fig. 3). The C_{28} primary alcohol was the dominant constituent throughout development on ‘Krossa Regal’ (Fig. 3). In general, both abaxial and adaxial surfaces of all three hosta genotypes examined showed increasing amounts of the longer chain length primary alcohols during development, similar to *Coffea arabica* L. (Stocker and Wanner, 1975) and *Fagus sylvatica* L. (Gulz et al.,

1992). Many plants display increasing chain length distribution in other wax classes during organ development (Herbin and Robins, 1969; Salasoo, 1983; Stocker and Wanner, 1975; Faboya et al., 1980; Jenks et al., 1996; Gulz et al., 1991; Jenks et al., 2001). The metabolic and ecological significance for this phenomenon is presently unclear.

The most notable change in alkanes with development was that total amount of alkanes on leaf surfaces in the fall was higher than on surfaces of all younger organs, except for ‘Krossa Regal’ whose abaxial fall alkane amounts were not significantly different from spring alkane amounts (Tables 2–4). Whereas the amount of alkanes on fully expanded abaxial leaves of all genotypes was lowest in the summer, alkanes on the adaxial leaves of *H. plantaginea* and ‘Krossa Regal’ increased continuously during development (Tables 2–4). By comparison, adaxial leaf alkanes of *H. lancifolia* decreased in the summer, similar to all abaxial leaves (Table 3). By comparison, alkane amounts increased on *Solandra grandifolia* leaves throughout development (Herbin and Robins, 1969). The alkanes were 25, 27, 29, and 31 carbons long, with the major alkane being either the 29 or 31 carbon homologue, and like alcohols, there was a proportional increase in the amount of longer chain length alkanes throughout development on surfaces of all genotypes (data not shown). Whereas the C_{29} alkane was the dominant homologue in the spring and summer, the C_{31} homologue dominated the alkane class in the fall on all genotypes (data not shown). The major fatty acids identified on hosta were the C_{16} and C_{18} homologues. The C_{20} , C_{22} and C_{24} acid homologues were detectable using mass spectrometry but not accurately quantifiable for most extractions (and thus not included in acid total quantity). The total and relative amounts of C_{16} and C_{18} acids showed only small changes during development (Tables 2–4). The wax esters were minor constituents on hosta, and like the acids, changed only little during development (Table 2–4). Notable however was that ‘Krossa Regal’ had significantly more esters than the two glossy genotypes throughout development (Tables 2–4).

Besides the typical leaf wax constituents described above, ‘Krossa Regal’ leaf waxes included large amounts of two long chain aliphatic molecules identified as C_{29} and C_{31} β -diketones. These compounds fragmented via the loss of H_2O and decomposed slightly during gas chromatography, consistent with β -diketones (Tulloch and Hogge, 1978). Their ion mass spectra were consistent with these compounds being nonacosane-10,12-dione and hentriacontane-10,12-dione. β -diketones were not detected in *H. plantaginea* or *H. lancifolia*. The β -diketones have been reported on few other plants, these including *Hordeum vulgare* (L.) (Wettstein-Knowles, 1995), certain other grasses, *Eucalyptus* species, and *Acacia* species, with hentriacontane-14,16-dione and tritriacontane-16,18-dione being the most typical members (Baker, 1982). Although these hosta β -diketones are uncommon, similar compounds have been reported in other plants.

Table 4. Seasonal changes in the amount of cuticular wax in each wax class in $\mu\text{g}\cdot\text{dm}^{-2}$ ($\pm$ SD) of both abaxial and adaxial leaf surfaces of *Hosta* ‘Krossa Regal’.

Wax class	Expanding	Spring	Summer	Fall
Primary alcohols				
Abaxial	499.4 ^z	653.9 $\pm$ 122.9	80.9 $\pm$ 21.2	276.6 $\pm$ 106.9
Adaxial	515.9 $\pm$ 131.7	611.1 $\pm$ 99.1	143.5 $\pm$ 52.4	184.5 $\pm$ 19.7
Alkanes				
Abaxial	9.7 ^z	20.8 $\pm$ 3.0	14.9 $\pm$ 2.7	15.8 $\pm$ 7.6
Adaxial	5.3 $\pm$ 1.9	11.5 $\pm$ 2.4	20.9 $\pm$ 15.3	113.2 $\pm$ 33.6
Free fatty acids				
Abaxial	1.7 ^z	7.8 $\pm$ 1.1	3.9 $\pm$ 1.1	2.1 $\pm$ 1.2
Adaxial	4.5 $\pm$ 1.9	6.2 $\pm$ 5.1	3.6 $\pm$ 2.7	5.7 $\pm$ 1.6
Aliphatic esters				
Abaxial	7.7 ^z	43.7 $\pm$ 17.4	11.8 $\pm$ 7.3	15.1 $\pm$ 7.6
Adaxial	9.8 $\pm$ 2.0	16.4 $\pm$ 11.5	2.3 $\pm$ 0.2	11.4 $\pm$ 6.5
β -diketones				
Abaxial	340.4 ^z	242.7 $\pm$ 101.4	193.3 $\pm$ 120.6	72.7 $\pm$ 70.1
Adaxial	242.7 $\pm$ 101.4	401.6 $\pm$ 153.9	4.7 $\pm$ 3.9	7.7 $\pm$ 2.1
Unknowns				
Abaxial	317.3 ^z	768.5 $\pm$ 175.2	407.7 $\pm$ 39.6	540.2 $\pm$ 73.4
Adaxial	345.3 $\pm$ 68.5	623.3 $\pm$ 38.4	328.1 $\pm$ 33.7	457.7 $\pm$ 78.8

^zRepresents the average of two replicate abaxial leaf samples.

Hosta plantaginea

Fig. 1. Abaxial and adaxial leaf cuticular wax composition of primary alcohols on *Hosta plantaginea*. The amount of all identified primary alcohol constituents as a percent of the total primary alcohol quantity (see Table 2 for total alcohol quantity per leaf area) is labeled on the vertical axis. The chain length of each homologue is shown on the horizontal axis.

Hosta lancifolia

Fig. 2. Abaxial and adaxial leaf cuticular wax composition of primary alcohols on *Hosta lancifolia*. The amount of all identified primary alcohol constituents as a percent of the total primary alcohol quantity (see Table 3 for total alcohol quantity per leaf area) is labeled on the vertical axis. The chain length of each homologue is shown on the horizontal axis.

Nonacosane-10,12-dione was reported in *Dianthus caryophyllus* leaf wax (Baker, 1982) and hentriacontane-10,12-dione was reported in leaf waxes of *Andropogon* (Tulloch and Hoffman, 1979) and *Rhododendron* species (Evans et al., 1975).

On abaxial and adaxial leaves of 'Krossa Regal', the C₂₉ and C₃₁ β -diketones together comprised between 14.9% and 28.7% of total waxes during leaf expansion and on fully expanded leaves in the spring (Table 4). By comparison, β -diketones were only 0.9% and 1.0% of adaxial surface waxes in the summer and fall, respectively, but remained significant constituents on the abaxial surface, comprising 25.2% and 7.0% of abaxial waxes in the summer and fall, respectively (Table 4). The total amount of these β -diketones per abaxial leaf surface area dropped just 1.3-fold (242.7 to 193.3 $\mu\text{g}\cdot\text{dm}^{-2}$) between spring and summer, and then 2.7-fold (193.3 $\mu\text{g}\cdot\text{dm}^{-2}$ to 72.7 $\mu\text{g}\cdot\text{dm}^{-2}$) between summer and fall. Comparable reduction in the amount of visible leaf glaucousness and β -diketones, and the fact that glossy hosta cultivars had no β -diketones, raises the possibility that β -diketones may influence the visual intensity of leaf surface glaucousness. Evidence that

β -diketones were associated with glaucousness on *Hordeum vulgare* was presented by Wettstein-Knowles (1974). Moreover, whether the absence of these β -diketones on adaxial surfaces, but presence on abaxial surfaces, in the summer and fall was a result of these compounds being susceptible to degradation by precipitation is also unclear. Preliminary studies in our lab demonstrate that these β -diketones were present on many other glaucous hosta lines (data not shown). Whether all glaucous hosta possess β -diketones however is not known. Reputable garden magazines and nursery catalogues often advertise glaucous hosta as slug and insect resistant. Further studies are needed to determine whether β -diketones on glaucous hosta have any ecological role such as providing pest or other biotic or abiotic stress resistance.

The total of all unknown (not identified) constituents on these hosta surfaces was for some extractions quite high, comprising between 7.9% and 65.8% of the waxes on these hosta genotypes over the time course examined (Tables 2–4). Unknowns were more abundant on 'Krossa Regal' than the two glossy genotypes, comprising between 37.5% and

65.8% of the total 'Krossa Regal' leaf wax quantity after leaf expansion (Table 4). These unknowns represented roughly 30 to 40 unique GC peaks based on different retention times. None of these compounds alone represented more than 2.0% of the total wax quantity, and nearly all compounds were present at a much lower concentrations. In 'Krossa Regal', one other unknown had a mass spectral profile whose major ions were similar to those of the two β -diketones. Based on retention time, this compound could be the longer C₃₃ β -diketone in the series. Further studies are needed to determine whether this unknown or others on 'Krossa Regal' are members or derivatives of the β -diketone class.

Clearly, hosta leaf wax quantity and composition changed dramatically during a single growing season, and significant differences existed among these three hosta genotypes. Leaves of these hosta had their greatest wax quantity in the spring soon after full leaf expansion. It seems probable that other hosta likewise have their highest leaf wax amounts in the spring. Whether these waxes play an important role in hosta biotic or abiotic environmental stress resistance is still uncertain. Nevertheless, future studies to elucidate the

Hosta 'Krossa Regal'

Fig. 3. Abaxial and adaxial leaf cuticular wax composition of primary alcohols on *Hosta* 'Krossa Regal'. The amount of all identified primary alcohol constituents as a percent of the total primary alcohol quantity (see Table 4 for total alcohol quantity per leaf area) is labeled on the vertical axis. The chain length of each homologue is shown on the horizontal axis.

role of wax in plant stress physiology must take into consideration the dramatic variation in wax properties that occur during a single growing season. The presence of unique β -diketones on glaucous *hosta* genotypes, genotypes often advertised by nurseries and garden centers as pest resistant, raises some intriguing questions about their possible role. Further studies are needed to determine whether these β -diketones are present on all glaucous *hosta*, and whether they are in fact associated with resistance to problematic *hosta* pests like slugs, or whether they may play a more important role in resistance to other environmental stresses.

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