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Feeding Preferences of Dogwood Sawfly Larvae Indicate Resistance in *Cornus*¹

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Abstract

Dogwood sawfly (*Macremphytus tarsatus* Say) is a native, phytophagous insect that relies on *Cornus* sp. host plants for larval development. Feeding injury by dogwood sawflies is primarily aesthetic and seldom results in host plant death. Still, native and non-native dogwoods have not been evaluated for susceptibility to larval feeding by this aesthetically damaging wasp. Ten species or cultivars of dogwoods that are either naturalized native plants or economically significant landscape plants were assayed for host suitability to dogwood sawfly larvae in no-choice and choice experiments. Flowering, kousa and corneliancherry dogwoods were consistently ranked among the least susceptible host plants while 'Sibirica' tatarian, gray, and 'Flaviramea' golden-twigg dogwoods were highly preferred hosts. Preliminary GC/MS comparisons of foliar metabolite extracts from all 10 species have identified five peaks of interest that varied between resistant and susceptible hosts. These results suggest that certain chemical constituents in foliage of dogwood species may be important predictors of host palatability. More research is needed to confirm this hypothesis before crossbreeding for sawfly resistance can proceed.

Index words: host plant resistance, integrated pest management, Hymenoptera, Tenthredinidae.

Species used in this study: 'Sibirica' tatarian dogwood (*Cornus alba* L. 'Sibirica'); silky dogwood (*C. amomum* Mill.); roughleaf dogwood (*C. asperifolia* Michx. var. *drummondii* (C.A. Mey); flowering dogwood (*C. florida* L.); kousa dogwood (*C. kousa* (Buerger ex Miq.) Hance); corneliancherry dogwood (*C. mas* L.); gray dogwood (*C. racemosa* Lam.); bloodtwig dogwood (*C. sanguinea* L.); redosier dogwood (*C. sericea* L.), and 'Flaviramea' golden-twigg dogwood (*C. sericea* L. 'Flaviramea').

Significance to the Nursery Industry

Dogwood sawflies (*Macremphytus tarsatus*) are seldom recognized as pests of dogwood in the southeastern United States, where native flowering dogwoods and non-native kousa dogwoods predominate for landscape use. Yet, the host range of this wasp extends across the eastern U.S. Aesthetic injury from larval sawfly feeding activity is more prevalent in northern states where native gray, redosier, and golden-twigg dogwoods and non-native tatarian dogwoods are more commonly encountered and planted. Landscape design and grounds-management professionals in the southeastern U.S. can expect to encounter more larval feeding injury if 'Sibirica' tatarian, gray, and 'Flaviramea' golden-twigg preferred dogwood host plants increase in popularity and use. Flowering, kousa, and corneliancherry dogwoods appear to have foliar chemical constituents that render them unpalatable as *M. tarsatus* host plants.

Introduction

Cornus sp. trees and shrubs are host plants for four sawfly species in the genus *Macremphytus* (Hymenoptera: Tenthredinidae) (8, 10). Smith (10) provides descriptions of adults and larvae of four *Macremphytus* sawfly species, including *M. lovetti* Macgillivray, *M. semicornis* (Say), *M. tarsatus* (Say), and *M. testaceus* (Norton) that utilize *Cornus* hosts. *Macremphytus* sawflies are not prone to population outbreaks and unlike outbreak species (e.g., *Neodiprion* spp. sawflies (Hymenoptera: Diprionidae) on pine (*Pinus* sp.) hosts); *Macremphytus* sawfly feeding is seldom sufficient to

kill the host plant (4, 7, 11). Still, dogwood sawflies, *M. tarsatus* Say, are commonly encountered pest defoliators of dogwoods in northeastern and midwestern U.S. landscapes (4) and periodic pests in the southeastern U.S. (12) causing extensive aesthetic injury to some species of dogwoods in the landscape.

M. tarsatus is a univoltine, native sawfly occurring throughout the eastern United States, south to Florida and west to Texas (10). Adult female wasps emerge late May through July and lay 100 eggs or more on abaxial leaf surfaces. Dyar (2) collected larvae from *Cornus alternifolia* L. (Pagoda Dogwood) and described 9 larval stadia for *M.* (as *Harpiphorus*) *tarsatus* Say based on head capsule sizes. Newly eclosed dogwood sawfly larvae superficially resemble 'slug' sawfly larvae (e.g. oak slugs (*Caliroa* sp.) and rose slug [*Endelomyia aethiops* (Fabr.)]. Early instar *M. tarsatus* feed gregariously and skeletonize dogwood leaf surfaces. After the second molt, *M. tarsatus* larvae develop a powdery white coating across the integument. Cloaked larvae resemble bird droppings (4). When disturbed, larvae curl into a tight spiral, protecting the black-brown head capsule beneath the terminal abdominal segments. With the final juvenile molt, mature larvae are about 25 mm long and tan to cream-colored with brown-black reticulation. Prepupal larvae mobilize, searching for decomposing wood, clapboard siding, pressboard and landscape timbers that they bore into and use as overwintering sites (1, 2, 4, 6, 7, 10).

Gray (*C. racemosa* Lam.) and redosier dogwoods (*C. sericea* L., formerly *C. stolonifera*) are common landscape plants in northern latitudes and are purported to be preferred hosts for sawfly feeding (4, 7). Redosier and several other dogwood types are extensively planted in eastern U.S. landscapes extending into the southeastern U.S. Yet, in southern states, flowering (*C. florida* L.) and kousa (*C. kousa* (Buerger ex Miq.) Hance) dogwoods predominate in landscape use. Susceptibility among dogwood species to larval feeding in-

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jury by *M. tarsatus* has not been experimentally determined. Therefore, the objectives of this research were to identify potential host plant resistance in *Cornus* spp. by examining larval sawfly feeding preferences among ten dogwood species or cultivars commercially available to the landscape industry and to examine chemical constituents of *Cornus* foliage that may be related to host plant resistance.

Materials and Methods

In July 2005, penultimate instar *M. tarsatus* were collected from a mixed species collection of dogwoods (Table 1) at the research nursery of The University of Tennessee in Knoxville (35°58'N × 83°55'W) and subjected to host plant choice and no-choice lab assays using leaf disks of known diameter (5). Experimental leaf disks were obtained from foliage collected from nine different, 4-year-old, container-grown tree- or shrub-form dogwood species and from 8-year-old, field-grown corneliancherry dogwoods (*C. mas* L.) (Table 1). No pesticides had been applied to the plants. Throughout the trials, experimental arenas for both choice and no-choice assays were maintained on a lab bench under ambient environmental conditions (22 ± 1C and 25 ± 5% RH). Supplemental bench lighting sustained a 16:8 L:D photoperiod. Prior to introduction into experimental arenas, larvae were provided a cotton ball saturated with DI water, but held without food for 24 h.

No-choice assays were conducted by exposing individual larvae to three 20 mm (0.79 in) diameter leaf disks placed on the surface of a moistened 90 mm (3.5 in) diameter Whatman No. 1 filter paper (Whatman International, Maidstone, UK) in a 15 cm (6 in) diameter plastic dish (Pioneer Plastics, North Dixon, KY). Thus, 9.43 cm² (1.46 in²) of leaf tissue area was presented to larvae for 48 h, after which leaf disks were replaced and left for another 48 h. Larval consumption of leaf disk area was visually estimated to the nearest 10 percent 12, 24 and 48 hours after new leaf disks were provided. For the no-choice tests, feeding activity on dogwood host plants was examined with 7 replicates.

Choice assays were conducted in a 28.5 cm (11.25 in) diameter Caterware® Eclipse® disposable serving tray (Pactive, Lake Forest, IL). Fifteen larvae were introduced into each of 10 (2005) and 9 (2006) replicated choice-test arenas and provided three 12 mm (0.47 in) diameter leaf disks per each of 10 dogwood types. Within arenas, leaf disks were mounted on 0.55 mm (0.02 in) diameter insect pins (Asta

Ento Pins, Pryn Whitecroft, Ltd., Lydney, England) and supported by 2 mm² overhead transparency plastic (3M Corp., St. Paul, MN) (3). In 2005, pins were inserted through a sheet of Reemay spun-bonded polyester row cover (Reemay, Inc., Old Hickory, TN) into a 50 mm (2 in) diameter styrofoam disk adhered to the tray surface with silicon adhesive (General Purpose Silicon Sealant, DAP Inc., Baltimore, MD). In 2006, organdy fabric replaced the Reemay polyester and pins without transparency plastic squares were inserted into a 22.9 cm (9 in) cardboard disk. Reemay and organdy surfaces were misted with about 2.5 ml distilled water to maintain humidity in experimental arenas, slowing leaf disk desiccation. Leaf disks were replaced three times at 24 h intervals, thus larvae were provided 10.2 cm² (1.58 in²) total leaf area during the 72 h experiment. In choice tests, larval consumption of leaf disk area was assessed as previously described 2, 4 and 24 hours after new leaf disks were provided.

For choice and no-choice studies, estimated feeding injury values were converted to mm² leaf tissue consumed from the total available leaf area and adjusted by arcsine transformation prior to statistical analyses. Data were subjected to analysis of variance (ANOVA) using PROC GLM in SAS (9). Means among statistically significant variables were separated using Fisher's least significant difference procedures (LSD, *P* ≤ 0.05). Untransformed data are presented.

Once differences in *M. tarsatus* feeding activity were substantiated, approximately 10 fully expanded, feeding injury- and disease symptom-free leaves were taken from several branches per dogwood type, frozen in liquid nitrogen, and stored at -70C until samples were processed. Leaf powder (100 mg) from each dogwood species or cultivar was mixed with 1 mL methyl tert-butyl ether (MTBE) to extract hydrophobic compounds from dogwood samples. Solutions were shaken at room temperature for 2 h then centrifuged at 13,000 rpm for 2 min. After centrifugation, hydrophobic compounds of MTBE supernatants were analyzed using a Shimadzu gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a SHR5XLB capillary column (30 m × 0.25 mm, thickness 0.25 mm) and a mass spectrometer (Shimadzu, Kyoto, Japan) with, 1-octanol used as an internal standard. Oven temperature was programmed to increase from 60 to 300C (140 to 572F) by 10C/min, after which temperatures were held at 60C (140F) for 2 min and at 300C (572F) for 4 min. Injector and ion source temperatures were 250C (482F) and 280C (536F), respectively. Detector voltage was 70 eV and MS spectra were recorded in the mass range of *m/z* 43–350. He-

Table 1. Relative susceptibility and origin of dogwood species or cultivars assayed for *M. tarsatus* feeding preferences.

Species/Cultivar (Taxonomic authority) ^a	Common name	Native plant range	Relative susceptibility 'Top (Bottom) 3' Tally ^b
<i>Cornus alba</i> 'Sibirica' L.	Tatarian dogwood	Eastern Asia	8 (0)
<i>C. amomum</i> Mill.	Silky dogwood	Eastern USA	2 (0)
<i>C. asperifolia</i> Michx. var. <i>drummondii</i> (C.A. Mey)	Roughleaf dogwood	Eastern USA	0 (0)
<i>C. florida</i> L.	Flowering dogwood	Eastern & Central USA	0 (8)
<i>C. kousa</i> (Buerger ex Miq.) Hance	Kousa dogwood	Eastern Asia	0 (8)
<i>C. mas</i> L.	Corneliancherry dogwood	Europe & Western Asia	0 (8)
<i>C. racemosa</i> Lam.	Gray dogwood	Eastern & Central USA	6 (0)
<i>C. sanguinea</i> L.	Bloodtwig dogwood	Europe	1 (0)
<i>C. sericea</i> L. (formerly <i>C. stolonifera</i> Michx. f.)	Redosier dogwood	Widespread USA	0 (0)
<i>C. sericea</i> 'Flaviramea' L.	Golden-twig dogwood	Cultivated US native variety	6 (0)

^aDirr, M.A. 1998. Manual of Woody Landscape Plants, 5th Ed., Stipes Publ., Champaign, IL.

^bNumber of times, in 8 statistically distinct experimental assays, that each dogwood type or species was ranked as one of the three most (least) susceptible hosts, based on mean leaf area consumed by individual *M. tarsatus* larvae after 72 h no-choice assays.

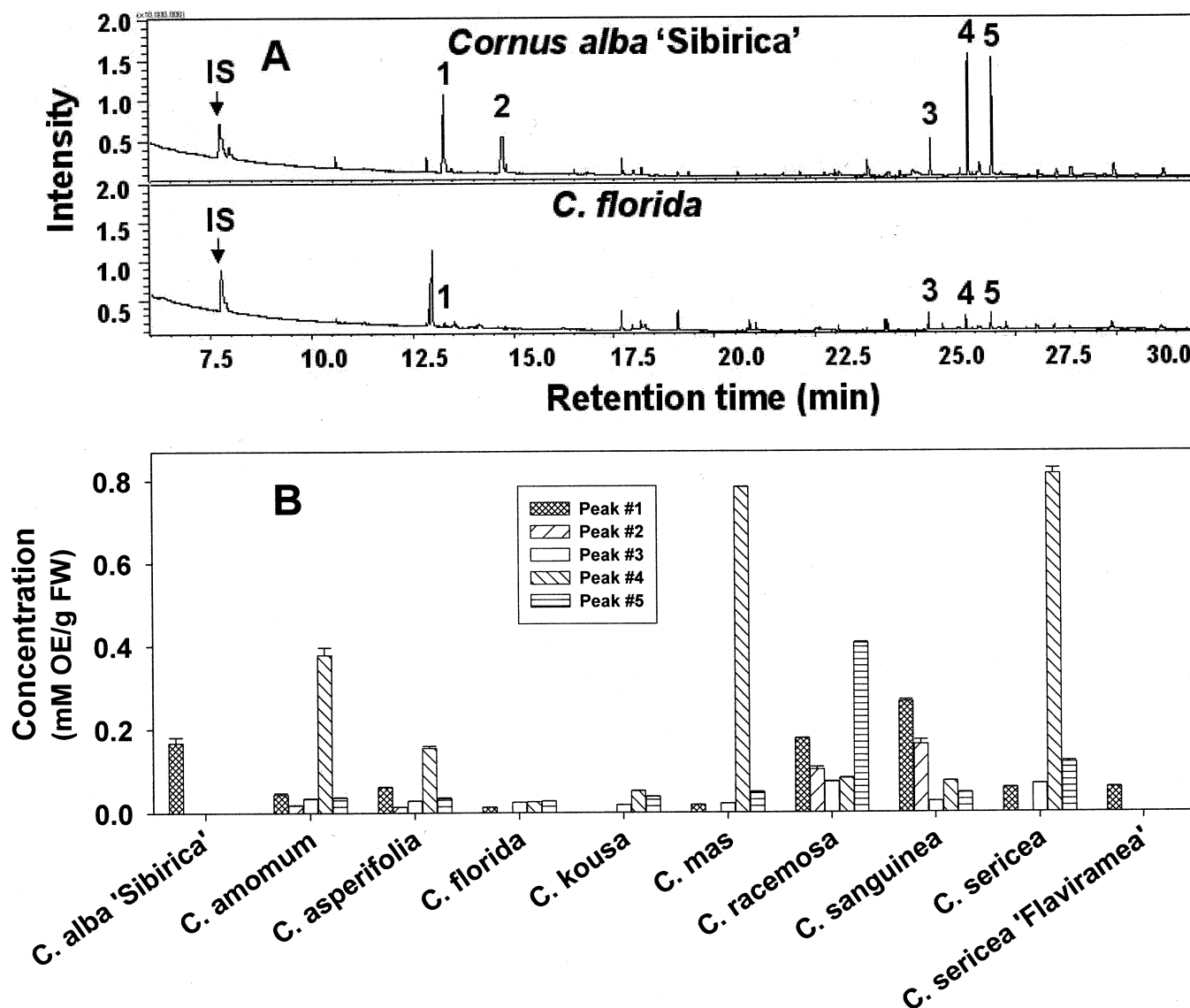


Fig. 1. A) Gas chromatographic separation of hydrophobic compounds present in dogwood leaves that were most (*C. alba* 'Sibirica', top) and least (*C. florida*, bottom) susceptible to dogwood sawfly feeding injury. Peak numbers correspond to tentative identities of target peaks presented in Table 4. IS indicates 1-octanal internal standard. B) Relative concentrations, compared to a 1-octanal internal standard, of five target peaks that varied among 10 tested dogwood species.

lium was the carrier gas (flow rate of 1 mL/min) and split injection was used (split ratio of 2 and injection volume of 2 μ L). Foliage from each dogwood species was analyzed three times. Compounds were tentatively identified using the National Institute of Standards and Technology (NIST) mass spectral database and their concentrations in dogwood leaves were presented as μ M 1-octanol equivalent (OE) per g fresh weight (FW).

Results and Discussion

Geographic origin of *Cornus* species does not appear to reliably predict native *M. tarsatus* wasp host preferences (Table 1). *Cornus alba* 'Sibirica', native to eastern Asia was most susceptible to dogwood sawfly feeding, after which U.S. native *C. racemosa* and *C. sericea* 'Flaviramea' were most

preferred. Greater larval sawfly preference for 'Flaviramea' golden-twist dogwood foliage (*C. sericea* 'Flaviramea') than for leaves of the red-stemmed redbud dogwood (*C. sericea*) is not readily explained, for example by hydrophobic compound concentrations in leaf tissues (Fig. 1B). While southeastern U.S.-native pagoda dogwoods (*C. alternifolia* L.) and giant dogwoods (*C. controversa* Hemsl.), which are native to Japan and China, are recommended for use in landscape plantings, neither tree species were available at the time our study was initiated, thus were not included. Susceptibility of giant dogwoods to sawfly larvae has not been reported, but pagoda dogwood has been listed as a species from which *M. tarsatus* sawflies have been collected (2).

In both years, *M. tarsatus* sawfly larvae demonstrated differences in feeding among tested dogwood host plants. Differences were consistent for no-choice and choice tests us-

Table 2. Average leaf tissue area consumed (mm²) by individual dogwood sawfly larvae 12 and 24 hours after introduction (HAI) during 2005 and 2006 no-choice laboratory assays.

Species/Cultivar ^a	Leaf area consumed (mm ²)					
	2005 ^y					
	12 HAI		24 HAI			
<i>Cornus alba</i> 'Sibirica'	406a ^x		589ab			
<i>C. amomum</i>	157cd		260cd			
<i>C. asperifolia</i> var. <i>drummondii</i>	200bcd		339bcd			
<i>C. florida</i>	51d		85d			
<i>C. kousa</i>	86cd		204cd			
<i>C. mas</i>	104cd		169cd			
<i>C. racemosa</i>	362ab		653a			
<i>C. sanguinea</i>	227bcd		317cd			
<i>C. sericea</i>	217bcd		329bcd			
<i>C. sericea</i> 'Flaviramea'	302abc		412bc			

Species/Cultivar ^a	2006					
	Run 1		Run 2		Run 3	
	12 HAI	24 HAI	12 HAI	24 HAI	12 HAI	24 HAI
<i>Cornus alba</i> 'Sibirica'	380ab	600a	694a	760a	470a	599a
<i>C. amomum</i>	224bc	488a	446c	558bc	426a	613a
<i>C. asperifolia</i> var. <i>drummondii</i>	138c	283b	344c	448c	217b	341b
<i>C. florida</i>	0d	5c	3d	6d	2c	3c
<i>C. kousa</i>	3d	27c	47d	58d	20c	30c
<i>C. mas</i>	3d	8c	71d	93d	42c	58c
<i>C. racemosa</i>	393a	597a	506bc	570abc	432a	562a
<i>C. sanguinea</i>	349ab	597a	457c	558bc	383a	540a
<i>C. sericea</i>	330ab	597a	424c	518bc	350ab	462ab
<i>C. sericea</i> 'Flaviramea'	465a	628a	633ab	705ab	336ab	515ab

^a'Sibirica' tatarian dogwood (*Cornus alba* L. 'Sibirica'); silky dogwood (*C. amomum* Mill.); roughleaf dogwood (*C. asperifolia* Michx. var. *drummondii* (C.A. Mey)); flowering dogwood (*C. florida* L.); kousa dogwood (*C. kousa* (Buerger ex Miq.) Hance); corneliancherry dogwood (*C. mas* L.); gray dogwood (*C. racemosa* Lam.); bloodtwig dogwood (*C. sanguinea* L.); redosier dogwood (*C. sericea* L.), and 'Flaviramea' golden-twig dogwood (*C. sericea* L. 'Flaviramea').

^yColumn means per dogwood type per run based on (n = 14 *Macremphytus tarsatus* larvae; 2005) and (n = 10 *M. tarsatus* larvae; 2006).

^xWithin years, means within columns followed by the same letter(s) are not significantly different at the *P* = 0.05 level by Fisher's least significant difference test.

ing leaf disks in laboratory bioassays (Tables 2, 3). Leaf areas consumed by individual sawfly larvae differed between years for no-choice assays ($F = 8.04$; $df = 1, 390$; $P < 0.005$). Larval sawfly preference for individual dogwood cultivars also differed by year ($F = 4.67$; $df = 9, 390$; $P < 0.0001$), thus observations on choice of host plant species could not be pooled for both years.

In 2005, larvae responded similarly to cultivars during the experimental runs ($F = 0.52$ and 0.59 ; $df = 9, 120$; $P < 0.80$ to 0.85), thus run data were pooled. Differences in leaf disk consumption during no-choice trials were also apparent within 12 h in 2005 ($F = 3.18$; $df = 9, 120$; $P < 0.0002$). Differences persisted through 24 h ($F = 3.46$; $df = 9, 390$; $P = 0.0008$), after which *C. racemosa* leaf disks had the heaviest foliar consumption while *C. florida* disks had the least missing tissue (Table 2). Observations of larval feeding activity in 2006 no-choice trials differed among dogwood types across all three experimental runs ($F = 2.25$; $df = 18, 569$; $P = 0.0023$). As a result, data could not be pooled and untransformed data are presented for individual runs (Table 2). Differences observed between runs in 2006 are readily explained by variation in feeding activity on dogwood types for which sawfly larvae demonstrated intermediate levels of leaf disk consumption (Table 2).

Despite variation at intermediate levels, sawfly larvae showed clear preference and aversion toward certain dog-

wood types. *Cornus florida*, *C. kousa*, and *C. mas* leaf disks incurred only light feeding activity while *C. alba* 'Sibirica', *C. racemosa*, and *C. sericea* 'Flaviramea' were heavily consumed, regardless of year or experimental run (Tables 1, 2).

These findings were consistent when *M. tarsatus* larvae were presented a choice among the 10 dogwood host resources (Table 3). Though differences in larval feeding activity on dogwood hosts were apparent between year ($F = 5.90$; $df = 9, 931$; $P < 0.0001$), *C. florida* and *C. kousa* leaves consistently incurred the least feeding injury after 24 h exposure to 15 larvae, while *C. alba* 'Sibirica' and *C. racemosa* were both heavily consumed (Table 3). Reliability of these results across both years and experimental runs provides a tally measure by which *C. alba* 'Sibirica', *C. racemosa*, and *C. sericea* 'Flaviramea' are scored most susceptible to *M. tarsatus* larval feeding activity (Table 1).

By comparison, *C. florida*, *C. kousa*, and *C. mas* appear to be resistant to *M. tarsatus* larval feeding. Indeed, these dogwood species are seldom observed to support sawfly populations in the southeastern U.S. While the mechanism of resistance or susceptibility remains unclear, evidence from preliminary metabolic profiling suggests that relative presence and quantity of certain hydrophobic compounds in leaves of these species may be important. GC/MS analysis of the 10 dogwood types led to tentative identification of five principal peaks of interest (Table 4). Peak profiles varied between

Table 3. Mean leaf tissues of each dogwood type consumed by dogwood sawfly larvae (n = 15) 24 hours after introduction (HAI) during 2005 and 2006 choice laboratory assays.

Species/Cultivar ^a	Leaf area consumed (mm ²)	
	2005 ^b	2006
<i>Cornus alba</i> 'Sibirica'	279ab ^x	164a
<i>C. amomum</i>	255ab	156b
<i>C. asperifolia</i> var. <i>drummondii</i>	272ab	75c
<i>C. florida</i>	24e	3d
<i>C. kousa</i>	51d	16cd
<i>C. mas</i>	165c	38bc
<i>C. racemosa</i>	288a	154a
<i>C. sanguinea</i>	277ab	183a
<i>C. sericea</i>	279ab	167a
<i>C. sericea</i> 'Flaviramea'	251b	187a

^a'Sibirica' tatarian dogwood (*Cornus alba* L. 'Sibirica'); silky dogwood (*C. amomum* Mill.); roughleaf dogwood (*C. asperifolia* Michx. var. *drummondii* (C.A. Mey)); flowering dogwood (*C. florida* L.); kousa dogwood (*C. kousa* (Buerger ex Miq.) Hance); corneliancherry dogwood (*C. mas* L.); gray dogwood (*C. racemosa* Lam.); bloodtwig dogwood (*C. sanguinea* L.); redosier dogwood (*C. sericea* L.), and 'Flaviramea' golden-twig dogwood (*C. sericea* L. 'Flaviramea').

^bColumn means from observations per dogwood type (n = 30; 2005) and (n = 18; 2006).

^xFor each year, means within columns followed by the same letter(s) are not significantly different at the *P* = 0.05 level by Fisher's least significant difference test.

Results for *C. sericea* 'Flaviramea' suggest that other factors, including other types of metabolites such as hydrophilic compounds that were excluded in our analysis, may also have a role in determining palatability of dogwood species to *Macremphytus* sawflies. Moreover, effects of synergism between multiple compounds on susceptibility versus resistance in dogwood foliage should also be considered. Given limited resources, true identities of the five peaks of interest could not be reliably determined by searching the NIST database. Additional effort will be needed to assign chemical names and structures to these individual peaks, and to determine their possible roles in arthropod pathology. Regardless, our GC/MS results offer a potential pathway for identifying chemical constituents that contribute to sawfly resistance in *Cornus* sp. and additional work will direct future breeding efforts.

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Table 4. Tentative identifications of peaks of interest from the National Institute of Standards and Technology (NIST) mass spectral database.

Peak number	Compound identity ^a (MW)	Homology (%)
1	1'-hydroxy-4,3'-diethyl-bicyclohexyl-3,3'-dien-2-one (220)	84
2	2-bromo-N-(4-hydroxyphenyl)propanamide (243)	75
3	7-n-hexyleicosane (394)	94
4	trans-squalene (410)	97
5	11-decyltetracosane (478)	95

^aCompounds are tentatively identified in respect to homologous chemical similarity and comparable molecular weights (MW) to compounds present in the NIST database.

the most susceptible (*C. alba* 'Sibirica') and resistant (*C. florida*) dogwoods from our study (Fig. 1A). In particular, peaks 1, 2 and 5 appear to influence sawfly preference and were either absent, or present in smaller concentrations in resistant *C. florida*, *C. kousa*, and *C. mas* dogwoods than in susceptible *C. alba* 'Sibirica' and *C. racemosa* species (Fig. 1B). This trend was not consistent for *C. sericea* 'Flaviramea' leaves, which yielded smaller peaks 1 and 5 and no peak 2, yet were highly preferred by larval sawflies.

In the *C. florida* profile (Fig. 1A, bottom), two chemical components preceded peak 1 by approximately 20 s. Profiles of these earlier-emerging peaks, which were tentatively identified in the NIST database as 3-methyl-3-cyclohexen-1-ol followed by (2E)-3-ethyl-4,4'-dimethyl-2-pentene, with 83 and 85% homologies respectively, did occur in smaller concentrations in *C. kousa* leaf tissues but did not appear in *C. mas* nor any other analyzed dogwood foliage (data not shown).

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