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Cyanide in the Chemical Arsenal of Garlic Mustard, *Alliaria petiolata*

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Abstract Cyanide production has been reported from over 2500 plant species, including some members of the Brassicaceae. We report that the important invasive plant, *Alliaria petiolata*, produces levels of cyanide in its tissues that can reach 100 ppm fresh weight (FW), a level considered toxic to many vertebrates. In a comparative study, levels of cyanide in leaves of young first-year plants were 25 times higher than in leaves of young *Arabidopsis thaliana* plants and over 150 times higher than in leaves of young *Brassica kaber*, *B. rapa*, and *B. napus*. In first-year plants, cyanide levels were highest in young leaves of seedlings and declined with leaf age on individual plants. Leaves of young plants infested with green peach aphids (*Myzus persicae*) produced just over half as much cyanide as leaves of healthy plants, suggesting that aphid feeding led to loss of cyanide from intact tissues before analysis, or that aphid feeding inhibited cyanide precursor production. In a developmental study, levels of cyanide in the youngest and oldest leaf of young garlic mustard plants were four times lower than in the youngest and oldest leaf of young *Sorghum sudanense* (cv. Cadan 97) plants, but cyanide levels did not decline in these leaves with plant age as in *S. sudanense*. Different populations of garlic mustard varied moderately in the constitutive and inducible expression of cyanide in leaves, but no populations studied were acyanogenic. Although cyanide production could result from breakdown products of glucosinolates, no cyanide was detected *in vitro* from decomposition of sinigrin, the major glucosinolate of garlic mustard. These studies indicate that cyanide produced from an as yet unidentified cyanogenic compound is a part of the battery of chemical defenses expressed by garlic mustard.

Keywords Allelopathy · *Alliaria petiolata* · Cyanide · Invasive plants · Plant defenses · *Sorghum sudanense*

Introduction

Cyanide is a well-known inhibitor of respiratory electron transport that is produced by over 2500 plant species including many food plants (Jones 1998). Although substantial debate

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exists on the role of cyanogenic compounds and cyanide in plants (Jones 1998; Gleadow and Woodrow, 2002), cyanide production has been invoked as an herbivore- and pathogen-resistance mechanism in many plants including clover, black cherry, and sorghum (Lindroth et al., 2000; Busk and Møller, 2002; Fitzgerald et al., 2002; Gleadow and Woodrow, 2002). In addition, cyanogenic compounds in plants like cassava appear to have roles in storage and mobilization of nitrogen (Gleadow and Woodrow, 2002; Jorgensen et al., 2005). Cyanide production in each of the aforementioned species typically reaches levels that can impact animal or human health (Jones 1998). Cyanide production has been reported in some members of the Brassicaceae, including *Brassica oleraceae*, *B. juncea*, *B. rapa*, *B. napus*, and *Raphanus sativus* (Sexton and Howlett, 2000; Chandra et al., 2004), but levels found thus far in plants in this family have been minimal and generally well below those considered to affect plant or animal health. In turn, the source of the detectable cyanide in brassicaceous plants is unknown, although the decomposition of glucosinolates, a ubiquitous group of defensive secondary compounds in this family, is one candidate (Sexton and Howlett, 2000).

Garlic mustard *Alliaria petiolata* [(M. Bieb) Cavara and Grande; Brassicaceae] is a Eurasian biennial herb that is an important invasive plant in North America. This plant has several known or presumed impacts on the flora and fauna in its invaded range that are apparently chemically mediated. These include allelopathic interactions with plants (Prati and Bossdorf, 2004), mycorrhizal fungi (Roberts and Anderson, 2001; Stinson et al., 2006), and herbivorous insects (Porter 1994; Haribal and Renwick, 1998; Haribal et al., 2001). Garlic mustard is also grazed little by mammalian or avian herbivores (D. Cipollini, personal observation). Like all brassicaceous plants, garlic mustard produces glucosinolates and defense proteins in both a constitutive and inducible fashion (Vaughan and Berhow, 1999; Cipollini et al., 2005). Garlic mustard is also rich in flavone gycosides, including isovitexin 6″-*O*-β-D-glucopyranoside, and it produces a novel cyanoallyl glycoside, alliarinoside; both have demonstrable effects on insect resistance (Haribal and Renwick, 1998; Haribal et al., 2001; Cipollini et al., 2005). It is likely that this suite of defenses contributes to the invasive success of this plant in novel habitats.

In this paper, we report that garlic mustard produces substantial quantities of cyanide from its tissues, an as yet undescribed component of the chemical arsenal of this plant. We first examined cyanide production in leaves and roots of young plants from a single population of garlic mustard, and then compared levels found in shoots to those of several members of the Brassicaceae. Second, we compared temporal variation in cyanide production in the youngest and oldest leaves of garlic mustard to that of a known cyanide producer, *Sorghum sudanense* (Sudan grass). Third, we examined leaf age-specific cyanide production in garlic mustard in the presence and absence of aphid herbivory. Fourth, we examined variation among garlic mustard populations in constitutive and inducible cyanide production. Finally, we examined whether the decomposition of sinigrin, the primary glucosinolate in garlic mustard (Vaughan and Berhow, 1999), could be the source of cyanide produced by this plant.

Methods and Materials

Experiment 1: Demonstration of Cyanide in Roots and Shoots of Garlic Mustard Seeds of garlic mustard were collected from plants growing naturally in the Wright State University forest preserve, cold stratified, and grown in the greenhouse in Pro Mix BX potting

medium, as described by Cipollini et al. (2005). Most experiments were conducted with the Wright State population, although experiment 5 includes several other populations. In this experiment, four plants were grown for 3 wk at which time all stems, leaves, and petioles present on each plant were collected and pooled for cyanide analysis, as described below. Roots of each plant were carefully extracted from the soil at the same time and rinsed free of soil with distilled water before cyanide analysis. Cyanide production by above-ground tissues was compared to that of roots with one-way analysis of variance (ANOVA) on SAS (version 9.1). Means were compared using Tukey's test in this and all other experiments.

Experiment 2: Comparisons of Cyanide Production in Leaves of Garlic Mustard with Other Brassicaceous Plants Seedlings of *Arabidopsis thaliana* (ecotype Columbia), *Brassica kaber* (wild collected), *B. rapa* (wild collected), and *B. napus* (cv. Westar), were grown from seed as described above, but without cold stratification. Garlic mustard was grown from cold-stratified seed as described above. All leaf material present on five 3-wk-old plants of each species was collected and pooled for analysis of cyanide. Cyanide production was compared among species with one-way ANOVA.

Experiment 3: Temporal Variation in Cyanide Production in Leaves of Garlic Mustard in Comparison to Sudan Grass This experiment was designed to compare variation in cyanide production in garlic mustard to a known cyanide-producing plant. Garlic mustard and Sudan grass [*S. sudanense* (cv. Cadan 97)] were grown from seed individually in pots in Pro Mix BX, as described above. Starting 2 wk after germination, the youngest fully expanded leaf and the oldest fully expanded leaf were collected from five plants of each species for cyanide analysis. Harvests continued in this manner from a subset of previously unsampled plants once per week for the next 8 wk, although the oldest leaves on each plant could be sampled for only 4 wk until they were too senescent to sample. This collection scheme isolates the effect of plant age on cyanide production in leaves of two distinct developmental stages. Cyanide production through time was compared between garlic mustard and Sudan grass with repeated-measures ANOVA, with time as the within-subject effect and species as the between-subjects effect. As the youngest leaves on each plant were sampled for 8 wk, and the oldest for only 4 wk, cyanide production was statistically examined separately for the youngest and oldest leaves.

Experiment 4: Leaf-specific Cyanide Production in the Presence of Aphid Herbivory This experiment examined within-plant variation in cyanide production among leaves in garlic mustard, but the plants were impacted by an outbreak of green peach aphids (*Myzus persicae*) in our greenhouse. When plants grown as described above were 40-d-old, we classified subsets of them as noninfested or infested (~20–30 aphids per plant). Infested plants were affected by aphids for approximately 2 wk at the time they were sampled. Fully expanded third (old), fourth (intermediate), and fifth (young) true leaves of garlic mustard plants were collected from 10–13 plants in each aphid infestation group for analysis of cyanide. Cyanide production was compared among aphid exposures and leaf ages by using two-way ANOVA, with aphid exposure and leaf age nested within aphid exposures as effects.

Experiment 5: Population Variation in Constitutive and Salicylic Acid-induced Levels of Cyanide Seeds of five populations of garlic mustard collected in the wild from Dalsland, Sweden; Marmontweg and Elderveld, the Netherlands; Hanau, Germany; and Ligonier, Pennsylvania, were cold-stratified and grown as described above. When plants were 8 wk

old, five plants from each population were sprayed with a 0.05-M solution of salicylic acid, as described by Cipollini et al. (2004). Salicylic acid induces many defense-related genes in plants (Mauch-Mani and Metraux, 1998), but its effect on cyanide production has never been examined. Leaf material was collected from the fully expanded fourth true leaf of each of these plants along with each of five control plants. Cyanide production was compared among populations and salicylate treatments with two-way ANOVA.

Experiment 6: Assessment of Sinigrin as a Source of Cyanide In this experiment, we evaluated the ability of sinigrin, the primary glucosinolate in garlic mustard (Vaughn and Berhow, 1999), to liberate cyanide upon enzymatic decomposition with thioglucosidase. We incubated 0.1 ml of sinigrin (Sigma Chemical, St. Louis, MO, USA) at concentrations of 0.125, 0.25, 0.5, 1.0, and 2.0 mg/ml with 0.1 ml of 3 mg/ml thioglucosidase (E.C. 3.2.3.1 from *Sinapis alba*; Sigma Chemical) suspended in sodium phosphate buffer in 25 ml center-well incubation flasks, and followed the protocol for cyanide trapping and analysis described below.

Cyanide Analysis We analyzed endogenous cyanide production in our tissues using a method modified from Lambert et al. (1975). Briefly, a weighed amount of fresh plant material (~0.02–0.05 g depending on tissue and species) was ground in a cold mortar with 0.5 ml of ice-cold 0.1-M sodium phosphate buffer. The homogenate was quickly transferred to a 25-ml center-well incubation flask, and the mortar was rinsed into the flask with an additional 0.5 ml of phosphate buffer. To trap cyanide in the flask, 0.2 ml of 0.1 M NaOH was placed in the center well, and the flasks were sealed and incubated at room temperature for 18 hr. To quantify cyanide present in the trap solution, 0.1 ml of 1 M acetic acid were added to the 0.2-ml trap solution (which was diluted as needed), followed by 1 ml of 0.25% succinimide/0.025% *N*-chlorosuccinimide reagent and 0.2 ml of 3% barbituric acid–pyridine solution. After incubation at room temperature for 10 min, absorbance at 580 nm was recorded for each sample in a spectrophotometer. Cyanide levels were quantified relative to a standard curve made with potassium cyanide and are expressed as milligram cyanide per kilogram tissue fresh weight (=ppm FW).

Results

Experiment 1 Three-week-old garlic mustard plants produced a mean level of 44.4 (±2.17) ppm cyanide from their aboveground tissues (including stems, leaves, and petioles) and 5.4 (±2.13) ppm from their roots. These levels were significantly different from one another ($F_{1,\ 6}$=212.5, P<0.001).

Experiment 2 Leaf cyanide production varied among the five brassicaceous species examined ($F_{4,\ 14}$=526.0, P<0.001, Table 1). Levels of cyanide in leaves of 3-wk-old garlic mustard plants were 25 times higher than in leaves of 3-wk-old *A. thaliana* plants and over 150 times higher than in leaves of 3-wk-old *B. kaber*, *B. rapa*, and *B. napus* plants.

Experiment 3 Changes in cyanide production through time by the youngest fully expanded leaf varied between garlic mustard and Sudan grass (time × species: $F_{7,\ 2}$=54.02, P=0.018, Fig. 1). Cyanide production in the youngest leaf of Sudan grass was five times higher than

Table 1 Mean (±1 SE) cyanide levels in leaves of 3-wk-old seedlings of five brassicaceous species

Species	Cyanide (ppm)
Alliaria petiolata	108.9 (7.82)[a]
Arabidopsis thaliana	4.19 (2.83)[b]
Brassica kaber	0.733 (0.571)[c]
Brassica rapa	0.617 (0.562)[c]
Brassica napus	0.433 (0.096)[c]

Means followed by different letters are significantly different at $P=0.05$.

in garlic mustard on the first sampling date, and it steadily declined to minimal levels through the 8-wk-time course as plants aged. In contrast, cyanide production declined little through time in garlic mustard, and by the fifth week of the time course through the end, cyanide levels in the youngest leaves of garlic mustard plants were significantly higher than in Sudan grass. A similar pattern was observed in cyanide production in the oldest fully expanded leaf of garlic mustard and Sudan grass through the 4-wk-time course (time × species: $F_{3,\ 6}=150.54$, $P<0.001$, Fig. 2). The decline in cyanide production in the oldest leaves in Sudan grass through time was even more dramatic than in the youngest leaves.

Experiment 4 Cyanide production varied among aphid-infested and noninfested plants ($F_{1,\ 64}=25.56$, $P<0.001$) and among leaf ages within each aphid treatment ($F_{4,\ 64}=10.72$, $P<0.001$, Table 2). Cyanide production was higher in young leaves than in older leaves in general and was lower in aphid-infested plants across all leaf ages.

Experiment 5 Significant variation in constitutive and salicylic acid (SA)-inducible cyanide production in leaves of 8-wk-old garlic mustard plants was detected among populations (pop: $F_{4,\ 40}=4.21$, $P=0.006$; pop × SA: $F_{4,\ 40}=8.4$, $P<0.001$, Table 3). Constitutive cyanide production was higher in plants from Dalsland, Marmontweg, and Elderveld than

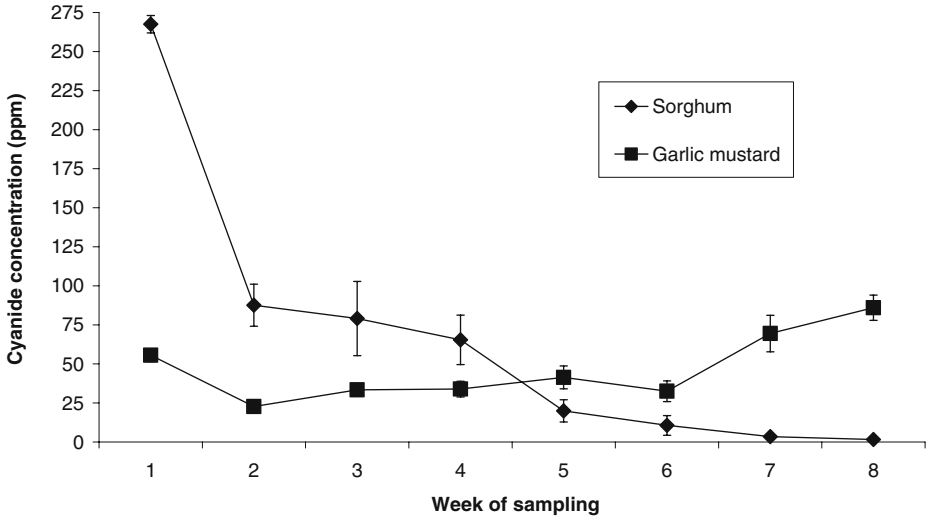

Fig. 1 Mean (±1 SE) cyanide levels in the youngest fully expanded leaf of garlic mustard and Sudan grass (*Sorghum sudanense*) through an 8-wk time course as plants aged. Plants were 2 wk old at the first sampling

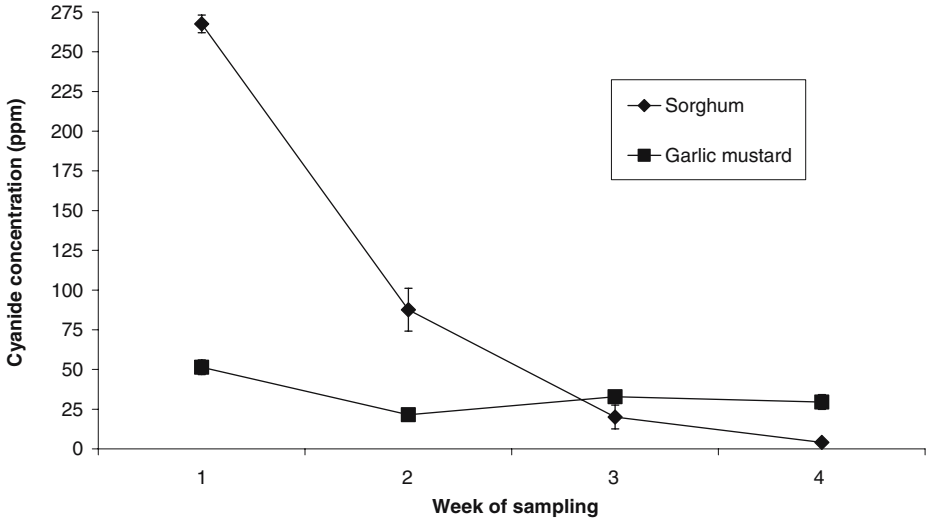

Fig. 2 Mean (±1 SE) cyanide levels in the oldest fully expanded leaf of garlic mustard and Sudan grass (*Sorghum sudanense*) through a 4-wk time course as plants aged. Plants were 2 wk old at the first sampling

in plants from Hanau and Ligonier. Although SA had little effect on cyanide production in plants from Dalsland, Marmontweg, and Ligonier, it strongly induced cyanide production in plants from Hanau and strongly reduced production in plants from Elderveld.

Experiment 6 Cyanide production was not detectable from the decomposition of sinigrin at any concentration in our experimental setup.

Discussion

Garlic mustard, an important invasive plant of forest understories throughout North America, produces a wealth of chemical defenses (e.g., Cipollini et al., 2005). We demonstrate for the first time here that garlic mustard produces substantial amounts of cyanide from its tissues.

Table 2 Mean (±1 SE) cyanide levels in young, intermediate, and old leaves of 5-wk-old seedlings of garlic mustard in the presence and absence of green peach aphids

Treatment	Leaf age	Cyanide (ppm)
Aphids absent	Young	66.2 (8.74)[a]
	Intermediate	38.7 (5.39)[b]
	Old	40.4 (5.00)[b]
Aphids present	Young	47.9 (6.68)[a]
	Intermediate	14.9 (2.13)[b]
	Old	16.9 (2.1)[b]

Means within an aphid treatment with different letters are significantly different at P=0.05.

Table 3 Mean (±1 SE) cyanide levels in leaves of 8-wk-old plants of five garlic mustard populations treated with 0.05 M salicylic acid or a control solution

Population	Salicylate Treatment	Cyanide (ppm)
Dalsland, Sweden	−	30.0 (4.2)[a]
	+	25.4 (4.3)
Elderveld, Netherlands	−	32.0 (4.6)[a]*
	+	11.4 (4.0)
Marmontweg, Netherlands	−	27.0 (4.7)[a]
	+	23.1 (4.1)
Hanau, Germany	−	14.4 (4.1)[b]*
	+	36.7 (4.3)
Ligonier, Pennsylvania	−	17.2 (4.0)[b]
	+	23.4 (4.6)

Means followed by different letters differ significantly at $P=0.05$ in the absence of salicylic acid. Means followed by asterisks indicate populations where salicylic acid significantly affected cyanide levels.

Cyanide contents reached by garlic mustard in some experiments were over 100 ppm FW in leaves, a level considered to be acutely toxic to humans (and other vertebrates) for plants like cassava (Jorgensen et al., 2005). In other experiments, levels in leaves ranged from 20 to 85 ppm, levels that are still considered to be moderately toxic (Jorgensen et al., 2005). As cyanide has known biological activity against animals, plants, and microbes (Jones 1998; Blenis et al., 2004; Gonzalez and Sotomayor, 2005), cyanide may contribute to some of the reported allelopathic effects of garlic mustard on other plants, insects, and mycorrhizal fungi (e.g., Porter 1994; Prati and Bossdorf, 2004; Stinson et al., 2006), and to its known deterrence of mammalian or avian herbivores (D. Cipollini, personal observation). It should be noted that we expressed cyanide content in garlic mustard per unit fresh weight, although many authors express cyanide content per unit dry weight (e.g., Lindroth et al., 2000). Expressing cyanide content in garlic mustard per unit dry weight would increase levels substantially, accounting for the average moisture content of leaves.

Within each plant, cyanide production was much higher in leaves than in roots and generally declined with leaf age. These findings are consistent with patterns of cyanide production in the foliage and roots of many other cyanogenic plants (e.g., Vickery et al., 1987; Busk and Møller, 2002; Fitzgerald et al., 2002; Jorgensen et al., 2005). In plants where the roots contain high levels of cyanide, like cassava, cyanide production by leaves is thought to be the source of cyanogenic compounds that are subsequently transported to roots (Jorgensen et al., 2005). Unlike *S. sudanense* studied here, cyanogenic potential in the youngest leaf of garlic mustard did not decline as plants aged. This indicates that garlic mustard consistently maintains the potential for cyanide toxicity in young leaves throughout much of its first season of growth, a pattern also seen in some other cyanogenic plants (e.g., Gleadow and Woodrow, 2000).

Interestingly, cyanide production was lower in aphid-infested plants across all leaf ages than in noninfested plants. Because we classified plants as infested or noninfested after the aphids had time to choose plants, it is possible that the patterns we observed resulted from aphid selection of the more weakly cyanogenic individuals in our experiment. However, as can be seen in the mean cyanide contents of the noninfested plants, individuals do not vary to a large extent in cyanide production in this population, which is known to be relatively genetically homogenous for other growth and defense characteristics (Cipollini 2002). Thus, it is more likely that aphid feeding somehow caused the differences in cyanide contents that we observed. For example, aphid feeding may have led to the volatilization of

cyanide from intact tissues before their analysis by enabling cyanide precursors and enzymes to mix *in planta*, thus lowering the cyanogenic potential of the tissue at the time of analysis. Alternatively, aphid feeding may have decreased cyanide precursor production either directly by downregulating gene expression or indirectly through nutrient limitation of precursor production. As cyanide production is increased by nitrogen fertilization in many plants (e.g., Busk and Møller, 2002; Jorgensen et al., 2005), aphid feeding may have reduced cyanide content by lowering tissue nitrogen content.

Populations varied to some extent in the constitutive and inducible production of cyanide, similar to other cyanogenic plant species (e.g., Lindroth et al., 2000; Schappert and Shore, 2000), but none of the six total populations studied here was acyanogenic. We have observed differences in defense production among populations of garlic mustard (Cipollini et al., 2005), but not enough populations were sampled in the current study to place our observations in an evolutionary or ecological context. Salicylic acid increased, decreased, or had no effect on cyanide production, depending upon the population studied. Salicylic acid is involved in the induction of numerous defenses in plants in response to biotic stimuli (Mauch-Mani and Metraux, 1998) and is known to induce cyanide-resistant respiration in plants (Kapulnik et al., 1992). Its effect on cyanide production has not been examined in any plant to our knowledge, but it has been shown to suppress the expression of dhurrinase mRNA in *Sorghum bicolor* (Salzman et al., 2005), which would presumably decrease cyanide production from the cyanogenic glycoside, dhurrin, found in these plants.

Garlic mustard produces substantially more cyanide than any brassicaceous plant studied to date (e.g., Sexton and Howlett, 2000; Chandra et al., 2004), including *A. thaliana* and the three *Brassica* species examined in this study. This appears to make garlic mustard unique among members of this family, which are characterized by their production of glucosinolates; cyanide production, however, has not been widely surveyed in this family. Only a few plant species are known as producers of both glucosinolates and cyanogenic glycosides, including *Carica papaya* in the Caricaceae family (Bennett et al., 1997), which is a member of the Capparales order along with members of the Brassicaceae family (Rodman et al., 1998). It has been suggested that glucosinolate production arose in an already cyanogenic species (Rodman et al., 1998; Halkier and Gershenzon, 2006), and it is perhaps surprising that so few members of the Brassicaceae have been confirmed to be cyanogenic.

Regardless of origin, cyanide production by garlic mustard may contribute to its unpalatability toward mustard specialist herbivores that typically prefer glucosinolate-containing plants (Porter 1994; Haribal and Renwick, 1998; Haribal et al., 2001) and some of its other allelopathic effects. We have noted anecdotally that treatment of garlic mustard leaves by freezing followed by subsequent thawing and incubation at room temperature makes them substantially more palatable and fosters better growth of larvae of the cabbage looper, *Trichoplusia ni* (D. Cipollini, unpublished data). This effect may be explained, in part, by the freezing-induced loss of cyanide from tissues. Interestingly, garlic mustard is also distinguished among members of its family by a characteristic garlic odor when its leaves are crushed (thus its common name). To our knowledge, the chemical profile and significance of this volatile blend has not been examined, but cyanide is certainly a part of it.

The source of cyanide in garlic mustard could not be determined in this study. The decomposition of glucosinolates has been suggested as a possible source in brassicaceous plants, but this does not appear to be the source in garlic mustard. Garlic mustard contains sinigrin (2-propenyl glucosinolate) as its primary glucosinolate (Vaughan and Berhow, 1999), but neither we nor Sexton and Howlett (2000) found that the thioglucosidase-

catalyzed hydrolysis of this compound could liberate cyanide *in vitro*. Moreover, the *Brassica* species and *A. thaliana* studied here are each well known as producers of glucosinolates (e.g., Cipollini et al., 2003, 2004; Chandra et al., 2004), yet each gave rise to negligible levels of cyanide in this study. The conversion of 1-amino-cyclopropane 1-carboxylic acid (ACC) into ethylene results in some cyanide production in plants, but levels seen here are much higher than those noted to be produced in this manner in other plant tissues (Yip and Yang, 1998). Although not initially considered a cyanogenic compound, garlic mustard contains a novel cyanoallyl glycoside, alliarinoside, with a –CN group as part of the molecule (Haribal et al., 2001). It is possible that either the enzymatic or nonenzymatic decomposition of this compound could yield volatile cyanide. If this compound is not the source of cyanide, then either other glucosinolates or an as yet undescribed cyanogenic compound may be the source.

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References

BENNETT, R. N., KIDDLE, G., and WALLSGROVE, R. M. 1997. Biosynthesis of benzylglucosinolate, cyanogenic glucosides and phenylpropanoids in *Carica papaya*. *Phytochemistry* 45:59–66.

BLENIS, P. V., CHOW, P. S., DUNCAN, I., and KNOWLES, N. R. 2004. Cyanide levels near fairy rings affect the growth of grasses and fungi. *Can. J. Bot.* 82:1324–1329.

BUSK, P. K. and MØLLER, B. L. 2002. Dhurrin synthesis in sorghum is regulated at the transcriptional level and induced by nitrogen fertilization in older plants. *Plant Physiol.* 129:1222–1231.

CHANDRA, A. K., MUKHOPADHYAY, S., LAHARI, D., and TRIPATHY, S. 2004. Goitrogenic content of Indian cyanogenic plant foods and their in vitro anti-thyroid activity. *Indian J. Med. Res.* 119:180–185.

CIPOLLINI, D. F. 2002. Variation in the expression of chemical defenses in *Alliaria petiolata* in the field and common garden. *Am. J. Bot.* 89:1422–1430.

CIPOLLINI, D. F., BUSCH, J., SIMMS, E., STOWE, K., and BERGELSON, J. 2003. Genetic variation and relationships of constitutive and induced trypsin inhibitor activity, glucosinolate content, and biological resistance in *Brassica rapa*. *J. Chem. Ecol.* 29:285–302.

CIPOLLINI, D. F., ENRIGHT, S., TRAW, B., and BERGELSON, J. 2004. Salicylic acid inhibits jasmonic acid-induced resistance of *Arabidopsis thaliana* to *Spodoptera exigua*. *Mol. Ecol.* 13:1643–1653.

CIPOLLINI, D., MBAGWU, J., HILLSTROM, C., BARTO, K., and ENRIGHT, S. 2005. Expression of constitutive and inducible chemical defenses in native and invasive populations of *Alliara petiolata*. *J. Chem. Ecol.* 31:1243–1255.

FITZGERALD, T. D., JEFFERS, P. M., and MANTELLA, D. 2002. Depletion of host-derived cyanide in the gut of the eastern tent caterpillar, *Malacosoma americanum*. *J. Chem. Ecol.* 28:257–268.

GLEADOW, R. M. and WOODROW, I. E. 2000. Temporal and spatial variation in cyanogenic glycosides in *Eucalyptus cladocalyx*. *Tree Physiol.* 20:591–598.

GLEADOW, R. M. and WOODROW, I. E. 2002. Constraints on the effectiveness of cyanogenic glycosides in herbivore defense. *J. Chem. Ecol.* 28:1301–1313.

GONZALEZ, E. and SOTOMAYOR, C. 2005. Allelopathic affects of cyanogenic glucosides on Nemaguard peach seedlings. *Cien. Inv. Agr.* 32:11–15.

HALKIER, B. A. and GERSHENZON, J. 2006. Biology and biochemistry of glucosinolates. *Annu. Rev. Plant Biol.* 57:303–333.

HARIBAL, M. and RENWICK, J. A. A. 1998. Isovitexin 6-*O*-β-D-glucopyranoside: a feeding deterrent to *Pieris napi oleracea* from *Alliaria petiolata*. *Phytochemistry* 47:1237–1240.

HARIBAL, M., YHANG, Z., ATTYGALLE, A. B., RENWICK, J. A. A., and MEINWALD, J. 2001. A cyanoallyl glucoside from *Alliaria petiolata* as a feeding deterrent for larvae of *Pieris napi oleraceae*. *J. Nat. Prod.* 64:440–443.

JONES, D. A. 1998. Why are so many food plants cyanogenic? *Phytochemistry* 47:155–162.

JORGENSEN, K., BAK, S., BUSK, P. K., SORENSEN, C., OLSEN, C. E., PUONTI-KAERLAS, J., and MOLLER, B. L. 2005. Cassava plants with a depleted cyanogenic glucoside content in leaves and tubers. Distribution of cyanogenic glucosides, their site of synthesis and transport, and blockage of the biosynthesis by RNA interference technology. *Plant Physiol.* 139:363–374.

KAPULNIK, Y., YALPANI, N., and RASKIN, I. 1992. Salicylic acid induces cyanide-resistant respiration in tobacco cell-suspension cultures. *Plant Physiol.* 100:1921–1926.

LAMBERT, J. L., RAMASAMY, J., and PAUKSTELLS, J. V. 1975. Stable reagents for the colorimetric determination of cyanide by modified König reactions. *Anal. Chem.* 47:916–918.

LINDROTH, R. L., HOFMANN, R. W., CAMPBELL, B. D., MCNABB, W. C., and HUNT, D. Y. 2000. Population differences in *Trifolium repens* L. response to ultraviolet-B radiation: Foliar chemistry and consequences for two lepidopteran herbivores. *Oecologia* 122:20–28.

MAUCH-MANI, B. and METRAUX, J.-P. 1998. Salicylic acid and systemic acquired resistance to pathogen attack. *Ann. Bot.* 82:535–540.

PORTER, A. 1994. Implications of introduced garlic mustard (*Alliaria petiolata*) in the habitat of *Pieris virginiensis* (Pieridae). *J. Lepid. Soc.* 48:171–172.

PRATI, D. and BOSSDORF, O. 2004. Allelopathic inhibition of germination by *Alliaria petiolata* (Brassicaceae). *Am. J. Bot.* 91:285–288.

ROBERTS, K. J. and ANDERSON, R. C. 2001. Effect of garlic mustard [*Alliaria petiolata* (Bieb. Cavara & Grande)] extracts on plants and arbuscular mycorrhizal (AM) fungi. *Am. Midl. Nat.* 146:146–152.

RODMAN, J. E., SOLTIS, P. S., SOLTIS, D. E., SYTSMA, K. J., and KAROL, K. G. 1998. Parallel evolution of glucosinolate biosynthesis inferred from congruent nuclear and plastid gene phylogenies. *Am. J. Bot.* 85:997–1006.

SALZMAN, R. A., BRADY, J. A., FINLAYSON, S. A., BUCHANAN, C. D., SUMMER, E. J., SUN, F., KLEIN, P. E., KLEIN, R. R., PRATT, L. H., CORDONNIER-PRATT, M.-M., and MULLET, J. E. 2005. Transcriptional profiling of sorghum induced by methyl jasmonate, salicylic acid, and aminocyclopropane carboxylic acid reveals cooperative regulation and novel gene responses. *Plant Physiol.* 138:352–368.

SCHAPPERT, P. J. and SHORE, J. S. 2000. Cyanogenesis in *Turnera ulmifolia* L. (Turneraceae): Developmental expression, heritability, and cost of cyanogenesis. *Evol. Ecol. Res.* 2:337–352.

SEXTON, A. C. and HOWLETT, B. J. 2000. Characterization of a cyanide hydratase gene in the phytopathogenic fungus *Leptosphaeria maculans*. *Mol. Gen. Genet.* 263:463–470.

STINSON, K. A., CAMPBELL, S. A., POWELL, J. R., WOLFE, B. E., CALLAWAY, R. M., THELEN, G. C., HALLETT, S. G., PRATI, D., and KLIRONOMOS, J. N. 2006. Invasive plant suppresses the growth of native tree seedlings by disrupting belowground mutualisms. *PLOS Biol.* 4/5: e140. DOI: http://dx.doi.org/10.1371/journalpbio.0040140.

VAUGHN, S. F. and BERHOW, M. A. 1999. Allelochemicals isolated from tissues of the invasive weed garlic mustard (*Alliaria petiolata*). *J. Chem. Ecol.* 25:2495–2504.

VICKERY, P. J., WHEELER, J. L., and MULCAHY, C. 1987. Factors affecting the hydrogen cyanide potential of white clover (*Trifolium repens* L.). *Aust. J. Agric. Res.* 38:1053–1059.

YIP, W. K. and YANG, S.-F. 1998. Ethylene biosynthesis in relation to cyanide metabolism. *Bot. Bull. Acad. Sin.* 39:1–7.