2004

Chemical Defenses in Garlic Mustard (Alliaria petiolata) and their Potential Role in Species Interactions in Forest Understories

Don Cipollini

Wright State University - Main Campus, don.cipollini@wright.edu

Repository Citation

Gottschalk, Kurt W., ed. Proceedings, XV U.S. Department of Agriculture interagency research forum on gypsy moth and other invasive species 2004; 2004 January 13-16; Annapolis, MD. Gen. Tech. Rep. NE-332. Newtown Square, PA: U.S. Department of Agriculture, Forest Service, Northeastern Research Station: 15-17

CHEMICAL DEFENSES IN GARLIC MUSTARD (*ALLIARIA PETIOLATA*) AND THEIR POTENTIAL ROLE IN SPECIES INTERACTIONS IN FOREST UNDERSTORIES

Don Cipollini

Wright State University, Department of Biological Sciences, 3640 Colonel Glenn Highway, Dayton, OH 45435

Garlic mustard [*Alliaria petiolata* [(M. Bieb) Cavara & Grande; Brassicaceae] is a European native biennial herb, first recorded on Long Island, NY in the 1860s, and is expanding rapidly in northeastern and midwestern forests in the U.S. and in southern Canada. Garlic mustard flourishes in moist woodlands with moderate exposure to light, but it can grow in a diversity of other habitats. It is found in natural areas, woodlots, and along edges of agricultural fields and lawns throughout North America. Several life history traits likely contribute to the invasiveness of this species. It has a high inbreeding rate and can produce numerous seeds. It exhibits remarkable morphological plasticity to local environmental conditions. It can exude allelopathic chemicals (glucosinolates and their hydrolysis products) that can reduce seed germination and growth of some species, and that can affect mycorrhizal potential of soils. Garlic mustard has been shown to outcompete some ecologically and commercially important hardwoods in short-term experiments, and its presence in natural areas is associated with reduced native herb abundance and diversity. Garlic mustard can also negatively impact salamander populations that rely on litter dwelling animals for food, and it can endanger populations of the rare butterfly *Pieris virginiensis* by serving as an oviposition site by adults on which larvae can not survive. Because of its known or potential negative impacts in natural and agricultural ecosystems, garlic mustard is an important target for chemical and biological control efforts.

The Evolution of Increased Competitive Ability (EICA) Hypothesis predicts that invasive plants in novel habitats lacking substantial pressure by natural enemies will evolve reduced expression of costly, unneeded chemical defenses to the benefit of growth and reproduction. We tested predictions of this hypothesis in garlic mustard, a European native that lacks substantial specialist herbivory in North America, where it is also largely resistant to generalist herbivores (Figure 1). We grew plants from four North American populations from Ohio and Pennsylvania, and seven European populations from the United Kingdom and the Netherlands in the greenhouse from field-collected seed. Plants were grown for 35 days, at which time length and width of the third true leaf were taken and half of the plants were treated with a foliar spray of jasmonic acid (JA). Jasmonic acid is a wound-related hormone involved in the induction of several chemical defenses associated with insect and pathogen resistance. Exogenous treatment of this hormone can induce defenses in a manner similar to herbivore attack, without the confounding effects of leaf damage. Four days later, samples from the fourth true leaves were harvested for the analysis of several constitutive and JA-inducible defense proteins and secondary metabolites that range from general resistance factors to defenses that are unique to garlic mustard. Levels of some of these defenses have been shown to vary among populations in the field, which may explain variation in herbivore resistance among natural populations in the field (Figure 2). Data were analyzed with mixed model ANOVA with continent, population within continent, and JA treatment as main effects.

Glucosinolates, secondary compounds characteristic of the mustard family, are involved in numerous species interactions including specialist herbivore attraction, generalist herbivore resistance, and interactions with soil fungi. Total glucosinolates (of which sinigrin is a major component in garlic mustard) were assessed using the glucose release method. Total glucosinolate content differed significantly among the populations within continents (Figure 3a). In addition, the response of populations to JA treatment by continent was marginally significant, with North American populations tending to be more inducible by JA than European populations. Activity of the phenolic oxidizing enzyme, peroxidase, was assessed in soluble protein extracts using a spectrophotometric assay with guaiacol as a substrate. No variation among continents in peroxidase activity was found, although variation was found among populations within continents (Figure 3b). Although not significant, an interesting pattern was present in the response of populations to JA treatment. Two North American populations displayed higher peroxidase activities after JA treatment, and two populations displayed lower peroxidase activities. Six of seven European populations displayed lower peroxidase levels after JA treatment. Trypsin inhibitors, capable of competitively inhibiting digestive serine proteases of animals, were assessed in soluble protein extracts using a radial diffusion assay through a trypsin-containing agar. Garlic mustard expressed substantial activity of trypsin inhibitor. There was significant variation in trypsin inhibitor levels among the populations within each continent, but no trends could be significantly attributed to their continental origin (Figure 3c.) JA treatment significantly increased trypsin inhibitor expression, but there was no significant variation among populations in their response to JA. Unique secondary compounds of garlic mustard that have been shown to impart resistance to specialist herbivory include the cyanoallyl glucoside, alliarinoside, and the flavone glycoside, isovitexin 6″-O-B-D-glucopyranoside. Levels of these compounds in water-soluble fractions of ethanol extracts were analyzed by HPLC. A representative HPLC chromatogram is shown in Figure 4. North American populations had more variable amounts of alliarinoside (Figure 5a) and isovitexin 6"-O-B-D-glucopyranoside (Figure 5b) than European populations, and generally expressed higher amounts of isovitexin 6"-O-B-D-glucopyranoside. JA did not consistently induce higher expression of either compound. Due to low samples sizes, levels of these two compounds were not statistically analyzed. Length (Figure 6a) and width (Figure 6b) of the third true leaf, measured prior to JA treatment, significantly varied among populations within continents, but did not vary with continental origin. However, specific leaf weight of the fourth true leaf varied by continent, and among populations within each continent (Figure 6c). In particular, North American populations had higher specific leaf weight than European populations.

Our results provide mixed support for predictions of the Evolution of Increased Competitive Ability hypothesis in garlic mustard. Leaf growth traits, such as higher specific leaf weight, were suggestive of increased productivity in North American populations as predicted, but this must be verified with longer-term studies. In contrast to predictions, no evidence of reduced expression of chemical defenses was found in North American populations relative to European populations. In fact, greater inducibility of glucosinolates by JA and tendencies for peroxidase activity to be higher in North American populations suggest that the opposite may be true for some defenses. Invasive garlic mustard populations may both grow fast and defend well, despite the tradeoff typically posited between these traits. Future experiments will include a greater biogeographical representation of garlic mustard. In addition, chemical defenses will be assessed more thoroughly throughout the life cycle, and quantitatively related to herbivore resistance and seed production as determined in laboratory bioassays and field studies.

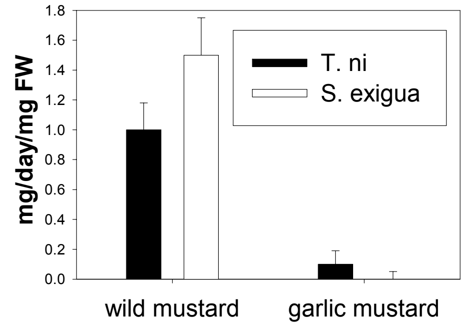

Figure 1. Relative growth rate of 7-day-old *Trichoplusia ni* and *Spodoptera exigua* on leaves of wild mustard and garlic mustard. N = 6-10.

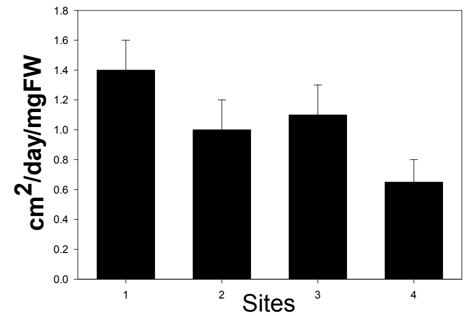

Figure 2. Relative consumption rate of *Trichoplusia ni* on garlic mustard leaves from different sites in the field. N = 9.

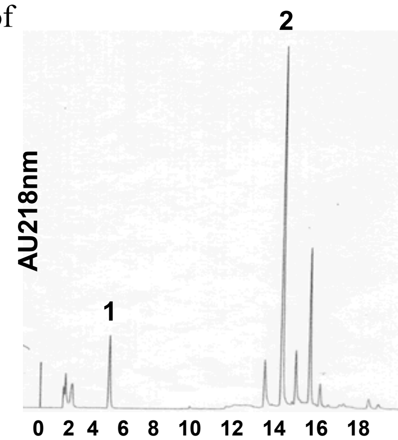

Figure 4. Representative HPLC trace of flavonoids isolated from garlic mustard leaves. Peak 1 = alliarinoside, Peak 2 = Isovitexin 6"-O-B-D-glucopyranoside

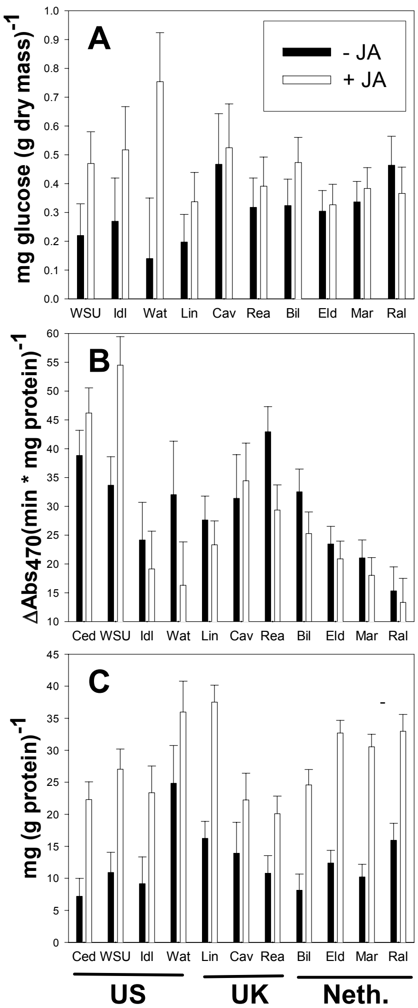

Figure 3. A. Glucosinolate, **B.** peroxidase and **C.** trypsin inhibitor levels in third true leaves of garlic mustard from the US, UK and Netherlands. N = 5-10.

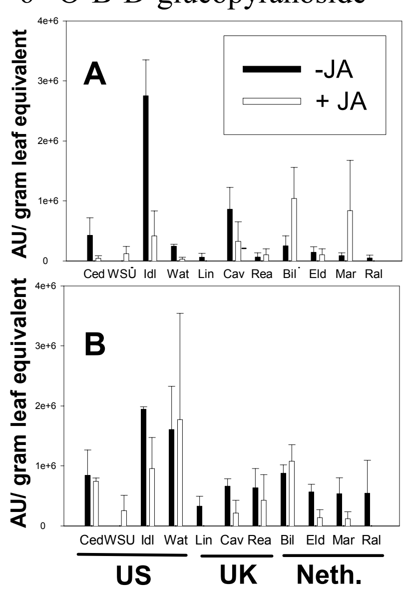

Figure 5. A. Alliarinoside and **B.** isovitexin-6"-O-B-D-glycopyraniside levels in third true leaves of garlic mustard from the US, UK and Netherlands. N = 5-10.

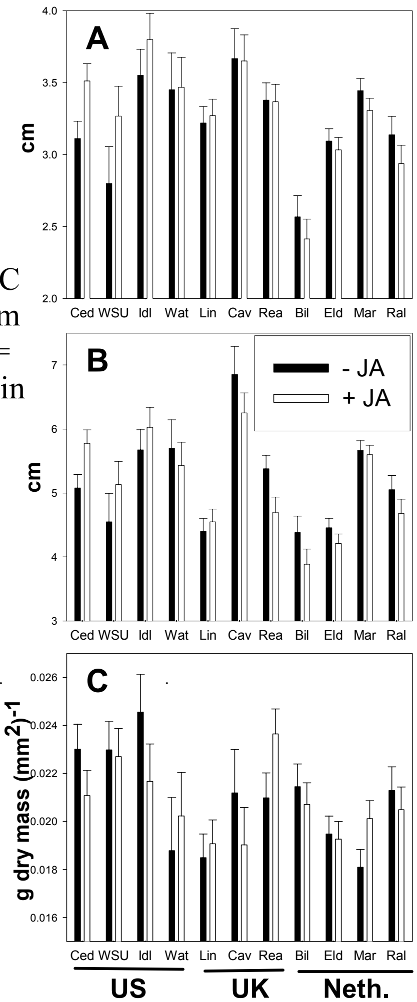

Figure 6. A. Leaf length, **B.** leaf width and **C.** specific leaf weight of third true leaves of garlic mustard from the US, UK and Netherlands. N = 5-10.