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Biogeographic Variation in Resistance of the Invasive Plant, Alliaria Petiolata, to a Powdery Mildew Fungus and Effect of Resistance on Competitive Dynamics

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ORIGINAL PAPER

Biogeographic variation in resistance of the invasive plant, *Alliaria petiolata*, to a powdery mildew fungus and effect of resistance on competitive dynamics

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Abstract Garlic mustard is an invasive Eurasian biennial that has spread throughout the eastern United States and southern Canada. Populations of this plant vary in their susceptibility to *Erysiphe cruciferarum*, a causal agent of powdery mildew disease in Brassicaceous plants. We examined whether there were biogeographic patterns in the distribution of resistance in invasive North American and native European populations of this plant. We grew plants from 78 invasive and 20 native populations and screened them for powdery mildew resistance in the greenhouse. We found that populations were mostly monomorphic for either resistance or susceptibility but that some polymorphic populations were found from both continents. The proportion of populations showing resistance versus susceptibility was similar in both Europe and North America. Within continents, the spatial distribution of resistant and susceptible populations did not deviate significantly from random. We also examined whether the possession of the resistance trait alter intraspecific competitive dynamics. In two trials, we competed plants from resistant and susceptible populations in a target-neighbor design in the presence and absence of powdery mildew inoculum and examined the growth of the target plant. Target plants from resistant populations were overall larger than target plants from susceptible populations. Target plants were overall larger when grown in competition with susceptible neighbors. Further, resistant target plants showed a greater degree of release from competition when grown with a susceptible neighbor versus a resistant neighbor than the degree of release shown by susceptible target plants. This suggests a benefit of possessing the resistance trait with little apparent costs which should promote selection for this trait within plant populations.

Keywords Costs of resistance · Fungal pathogens · Invasive plants · Resistance · Spatial distribution · Susceptibility

Introduction

Invasive plants can be good models for the study of the ecology and evolution of plant resistance to herbivores and pathogens (Orians and Ward 2010) because they are often removed from their normal complement of natural enemies during the invasion process. This can provide a framework for natural experiments on the effects of the loss of historical enemies or the gain of novel interactions on the expression and distribution of plant resistance traits.

Several hypotheses have been proposed about how defense traits should respond to a change in pressure

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from natural enemies in invasive plant populations. Blossey and Notzhold (1995) introduced the evolution of increased competitive ability hypothesis (EICA), positing that lack of enemies in an introduced range should result in evolutionary changes in allocation of resources away from costly and unneeded defenses toward growth and fitness (e.g. increased vigor and fecundity). For this hypothesis to be supported, the defenses in question must be significantly costly to fitness, presumably expressed in a quantitative fashion, and enemy escape must be demonstrated. Tests of the EICA hypothesis have produced mixed results at best (Van Kleunen and Schmid 2003; Franks et al. 2008; Felker-Quinn et al. 2013), spawning more refined hypotheses, such as the shifting defence hypothesis (SDH). The SDH predicts the loss of some defenses in invaders that have escaped their historical enemies, coupled with maintenance or gains in other defenses, depending upon the herbivore or pathogen community to which the invader is newly exposed (e.g., prevalence of generalist versus specialist enemies) (Doorduin and Vrieling 2011). Regardless of enemy presence or absence, however, founder effects, genetic drift, and dispersal patterns could all influence the geographic distribution of resistance traits among populations across an introduced range, especially for plants that exhibit variation in their defensive phenotype in the native range and for which multiple introductions have been documented (Ward et al. 2008). Finally, competitive interactions between resistant and susceptible individuals within populations could affect the frequency and local distribution of resistance traits (Cipollini 2004), the outcome of which can be contingent upon enemy pressure.

Garlic mustard (*Alliaria petiolata*, Brassicaceae) is a Eurasian biennial herb that is expanding rapidly in forests across the U.S. and in southern Canada (Nuzzo 1999). This plant has largely escaped its historical complement of enemies from its home range (Blossey et al. 2001; Cipollini 2001; Lewis et al. 2006; Evans and Landis 2007), yet it is faced with some novel enemies in the introduced habitat, such as slugs (Hahn et al. 2011) and lepidopteran larvae (Davis and Cipollini 2014). It possesses quantitative (i.e., having continuous variation) chemical defenses that are costly to growth and fitness when expressed (Cipollini and Lieurance 2012), but only minor support for EICA has been found in this plant thus far (Bossdorf et al. 2004a, b; Cipollini et al. 2005; Hillstrom and Cipollini 2011; Cipollini and Lieurance 2012). At least two studies examining genetic variation in this plant have indicated that it was likely introduced multiple times to North America, and that it exhibits greater genetic diversity among populations than within them (Meekins et al. 2000; Durka et al. 2005).

We have observed populations of garlic mustard in Ohio, Michigan, and Pennsylvania that exhibit infection by *Erysiphe cruciferarum* Opiz ex. L. Junell (Koch and Slusarenko 1990), the causal agent of powdery mildew disease in this plant (Ciola and Cipollini 2011). This pathogen is an obligate biotrophic fungus that specializes on Brassicaceous species and is distributed worldwide (Koch and Slusarenko 1990). The origin of the powdery mildew strain(s) that we have observed is unknown, but there are brief reports and observations of *E. cruciferarum* infecting garlic mustard populations in both the native and invaded range of this plant (e.g., Ellis and Ellis 1997; Blossey et al. 2001; Newsletter of the Warwickshire Fungus Survey 2004; D. Cipollini, pers. obs.; K. Morris, pers. obs.). However, no reports of powdery mildew infection on garlic mustard in the invaded range were made until recently, despite this plant being the subject of numerous field studies in the introduced range going back to the early 1970s (Trimbur 1973). For example, (Evans and Landis 2007) reported no powdery mildew infection on garlic mustard plants from a thorough survey of natural enemies of this plant on numerous populations in Michigan. For this reason, we assume that garlic mustard has largely escaped from this enemy during its invasion until fairly recently. Interestingly, observations of plants growing in the same greenhouse conditions indicate that populations of garlic mustard vary in their resistance phenotype to this pathogen in a qualitative fashion, that is, being either resistant (showing no symptoms of successful fungal infection) or susceptible (showing substantial fungal infection), with a small number of populations having a mixture of individuals with these two resistance phenotypes (D. Cipollini, pers. obs.). This kind of resistance is likely based on resistant individuals recognizing the pathogen and rapidly inducing defenses via a resistance (R)-gene-like mechanism that has been observed in related species (e.g. *Arabidopsis thaliana* (Xiao et al. 2001)). In susceptible garlic mustard individuals, powdery mildew disease can reduce survival, growth and reproduction, and interspecific competitive ability

(Enright and Cipollini 2007; Cipollini and Enright 2009; Enright and Cipollini 2011). Fitness benefits of possessing resistance to powdery mildew disease are clearly evident in observations of the survival, growth and reproduction of resistant individuals in the presence of disease inoculum (D. Cipollini, pers. obs.). Before qualitative resistance to powdery mildew disease was recognized, Ciola and Cipollini (2011) reported variation in infection rates in the field among garlic mustard populations in southwestern Ohio, attributing most of this variation to variation in disease pressure and environmental conditions. Aside from this study, spatial patterns in the distribution of powdery mildew resistance in either the native or invaded range have never been examined.

In the first part of this study, we screened populations of garlic mustard from throughout its native and invaded ranges for their resistance to a local (Dayton, OH) strain of *E. cruciferarum* under controlled conditions in the greenhouse. We asked whether there were any patterns in the spatial distribution of resistance to powdery mildew disease among these populations that deviated from random, and more specifically, if the ratio of resistant to susceptible populations within continents supported the predictions of EICA (e.g., a higher prevalence of resistance in native populations than in invasive populations). In the second part of this study, we examined whether the presence of powdery mildew disease and competition with other plants could be a selective force on the frequency of the resistance trait within populations that possess both phenotypes. We addressed this question by placing individuals of susceptible and resistant populations in direct competition with each other in the presence and absence of the powdery mildew inoculum and examining their growth.

Methods

Spatial distribution of resistance

We examined 20 native populations of garlic mustard from throughout the native range in Europe, and 78 populations from throughout the introduced range in North America (Table 1). Seeds were acquired from these populations through either direct collection by us or acquired from collaborators. Populations were chosen for collection largely based on accessibility, but attempts were made to sample a large portion of the range of the plant. In general, seeds were collected from several individual plants (usually at least 10) from each population and bulked. Due to different collection schedules, seed stratification requirements, and space constraints, populations were screened for disease resistance over the course of 4 years, always during the late spring and early summer months conducive to powdery mildew disease development. We have found no temporal changes in the expression of qualitative resistance of individual plants over a single season and the expression of qualitative resistance has never changed through time for any population that was tested repeatedly (i.e., no populations were found susceptible in one trial and resistant in another).

During each screening trial, seeds of different populations were stratified, germinated, and planted in potting soil in pots in the greenhouse, as in Cipollini and Lieurance (2012). Seeds were generally collected in the summer and fall, stratified for 3 months and then screened, but there was no standard time that seeds were stored before they stratified. Seeds were stored ranging from 1 month to 2 years, but length of storage does not affect the expression of qualitative resistance in garlic mustard (D. Cipollini, pers.obs.). Typically, between 3 and 10 plants per population, depending on availability, were screened for resistance by inoculating them with conidia from *E. cruciferarum* grown on a susceptible population of garlic mustard collected from the Wright State University Woods and grown in the greenhouse. This fungus has been maintained in the greenhouse since 2005, when we first worked with this pathogen in a controlled fashion (Enright and Cipollini 2007). Test plants were inoculated when they were between 4 and 6 weeks old. Plants were inoculated by holding a heavily infected garlic mustard plant above flat of plants holding up to 18 plants and knocking the conidia off of the infected plant onto the plants below. Using the methods of Wright and Heale (1984), we previously determined that this inoculation procedure delivered $\sim$ 150 condia per mm^2 on average to leaves of target plants. After intentional inoculation, we also placed infected garlic mustard plants from a local susceptible population adjacent to flats of plants being screened so that an additional inoculum source was always present throughout the trial. Two weeks after inoculation, we assessed whether plants were susceptible, as evidenced by

Table 1 Collection information and resistance phenotype of garlic mustard populations from North America (NA) and Europe (EU) screened for resistance to a North American strain of *Erysiphe cruciferarum*

Origin	ID	Lat	Long	Resistance phenotype
NA	ONT1	43.262	− 79.073	R
NA	IL3	40.1384	− 88.0365	R
NA	IL5	40.0786	− 88.2111	R
NA	IL6	42.2623	− 87.8543	R
NA	IL7	40.1519	88.1631	R
NA	IL13	42.2093	− 88.1347	R
NA	IL18	42.1805	− 90.2584	R
NA	IL19	41.9602	− 89.3584	R
NA	IL21	42.1039	− 88.2215	R
NA	IL24	40.6759	− 89.5012	R
NA	IL25	42.1399	− 90.164	R
NA	IL26	40.8369	− 89.5841	R
NA	IL28	42.1358	− 88.0919	R
NA	IN1	39.5186	− 84.9974	R
NA	IN2	39.6141	− 84.9503	R
NA	MD2	39.174	− 77.272	R
NA	MI2	41.9125	− 86.0125	R
NA	MI3	41.9802	− 83.9328	R
NA	MI4	42.0435	− 84.153	R
NA	MI6	42.1106	− 85.97025	R
NA	MI8	42.7301	− 85.8045	R
NA	NJ1	39.0287	− 74.9385	R
NA	NJ2	40.8739	− 73.9665	R
NA	NY3	42.4434	− 76.4611	R
NA	NY4	42.699	− 76.41	R
NA	NY5	43.362	− 77.9597	R
NA	NY8	40.8445	− 73.8642	R
NA	NY9	41.1583	− 73.8616	R
NA	NY12	42.7173	− 78.8313	R
NA	OH1	40.805	− 82.009	R
NA	OH2	40.0966	− 83.0176	R
NA	OH3	39.9622	− 83.0007	R
NA	OH4	40.223	− 82.979	R
NA	OH5	40.016	− 83.5469	R
NA	OH8	39.536	− 83.977	R
NA	OH10	39.3864	− 83.903	R
NA	OH11	40.1057	− 84.6898	R
NA	OH12	39.9424	− 83.4008	R
NA	OH13	40.3044	− 83.0845	R
NA	OH14	39.6487	− 84.4166	R
NA	OH15			R
NA	OH16	40.1504	− 83.0381	R
NA	OH17	39.5827	− 84.7477	R
NA	OH19	40.9428	− 83.9572	R

Table 1 continued

Origin	ID	Lat	Long	Resistance phenotype
NA	OH21	40.0849	− 84.2102	R
NA	OH22	39.1609	− 83.2503	R
NA	OH24	39.2797	− 84.4038	R
NA	OH26	41.6897	− 81.0537	R
NA	OH27	39.44	− 83.818	R
NA	OH29	39.3996	− 82.9776	R
NA	OH30	39.2227	− 82.973	R
NA	OH32	41.6006	− 82.7085	R
NA	OH33	39.016	− 84.135	R
NA	OH34	41.6075	− 83.5667	R
NA	PA1	40.2626	− 79.282	R
NA	PA3	40.84	− 76.03	R
NA	PA5	41.58	− 79.46	R
NA	PA6	41.7886	− 76.9458	R
NA	PA9	40.4459	− 78.4233	R
NA	PA18	41.3031	− 75.1936	R
NA	PA19	41.8133	− 77.1667	R
NA	VA3	38.2588	− 78.8425	R
NA	VT1	43.2892	− 72.4603	R
NA	WI1	43.0859	− 89.49222	R
NA	WV1	40.0919	− 80.5355	R
NA	IL14	42.1806	− 89.18075	S
NA	MI7	42.8858	− 84.0461	S
NA	MI9	42.3545	− 83.1006	S
NA	NY11	41.1265	− 73.714	S
NA	NY13	42.6	− 78.05	S
NA	NY15	42.06	− 78.77	S
NA	OH9	39.8763	− 84.0925	S
NA	OH23	39.3033	− 84.3141	S
NA	OH31	39.7939	− 83.8815	S
NA	OH35	39.8645	− 84.166	S
NA	OH36	39.7825	− 84.0561	S
NA	PA8	41.4753	− 77.0369	S
NA	VA1	38.8471	77.30594	S
NA	NY6	43.1987	− 77.7405	RS
NA	NY7	41.4198	− 73.9549	RS
NA	NY10	43.1839	− 77.8907	RS
NA	OH7	40.0743	− 83.6596	RS
NA	OH18	39.857	− 84.0742	RS
NA	OH20	40.4665	− 83.888	RS
NA	OH25	39.1216	− 84.191	RS
NA	OH28	39.371	− 83.382	RS
NA	PA10	41.7322	− 77.6447	RS
NA	PA15	41.1233	− 75.2844	RS
EU	BE1	50.93	4.324	R

Table 1 continued

Origin	ID	Lat	Long	Resistance phenotype
EU	CZ1	50.072	14.422	R
EU	GB2	53.2383	− 0.5464	R
EU	GE1	47.83	7.567	R
EU	GE4	51.911	7.565	R
EU	NE1	52.015	5.677	R
EU	NE2	51.9678	5.857687	R
EU	NE3	52.085	5.3147	R
EU	SL2	46.054	14.492	R
EU	SL3	45.78	14.39	R
EU	SW3	58.7135	12.35507	R
EU	SW4	58.5947	16.18361	R
EU	GB1	51.475	− 0.9698	S
EU	GE7	53.7999	8.5716	S
EU	GE10	50.1382	8.897552	S
EU	SL1	46.382	15.512	S
EU	SL4	46.37	14.102	S
EU	SW2	46.981	7.451	S
EU	UR1	47.58	34.906	S
EU	GE11	49.6592	10.04906	RS
EU	GE12	48.3978	9.976959	RS

R, all resistant; S, all susceptible; RS, mix of resistant and susceptible individuals

vigorous colony development with conidia formation, or resistant, as evidenced by a lack of visible disease development. As has been observed in *Arabidopsis* in response to powdery mildew inoculations, resistant individuals will often display small hypersensitive response-like lesions indicative of rapidly mounted defenses, but no disease development (Xiao et al. 2001).

A small number of populations were found to consist of a mixture of susceptible and resistant individuals. To simplify analysis, we considered these polymorphic populations to be susceptible. We determined if there was a difference in the proportion of susceptible and resistant populations between Europe and North America by using a Chi square contingency test. We tested for spatial association between susceptible and resistant garlic mustard populations using Ripley's cross K function (Cressie 1991). Given the geographic separation, North American and European populations were tested separately. We calculated cross K in 301 distance steps from 0° to 3° and determined 95% confidence intervals with 1000 simulations randomly reassigning the susceptible and resistant labels. We determined the spatial domain for all populations within which the cross K was calculated using the Ripley-Rasson estimator (Ripley and Rasson 1977) and applied Ripley's edge correction (Ripley 1988) to avoid edge effects. For variance stabilization and ease of interpretation we transformed K to L ($L = (K/\pi)^{1/2}$) for display on the graphs (Ripley 1979). L is expected to be zero under full spatial randomness.

Influence of disease susceptibility on the outcome of intraspecific competition

In two experimental trials using individuals from different North American populations, we examined the outcome of competition between individuals from resistant (R) and susceptible (S) populations in the presence and absence of powdery mildew inoculum. Populations were chosen randomly based on seed availability and resistance status. Because the experiments were done with different population pairs and at different times when disease severity may have varied, data from the two experiments were considered blocks in the experimental design. The first block utilized plants from a resistant population from Niagara on the Lake, Ontario, Canada and from a susceptible population from the Wright State University Woods, Dayton, Ohio. The second block utilized plants from a resistant population from Rossford, Ohio and from a susceptible population from Taylorsville, Ohio. We grew two plants from germinated seed together in pots in the greenhouse using a target-neighbor design, as in Cipollini (2007). In each experimental block, there were four combinations of treatments where the identity of the target and neighbor were manipulated. In the first combination, both the target and the neighbor were an individual from the same resistant population. In the second combination, both the target and the neighbor were an individual from the same susceptible population. In the third combination, the target was an individual from the susceptible population and the neighbor was an individual from the resistant population. The fourth combination was the reciprocal of the third. Due to differences in plant availability, there were 14–18 replicates of each target-neighbor combination treatment. Half of these replicates were were exposed to powdery mildew inoculum at 6 weeks, as above, while the other half were sprayed with an aqueous solution of Daconil fungicide on the leaves once weekly from this point onwards to inhibit disease development. Six weeks after inoculation, all plants were removed from the pots, separated into roots and shoots, and dried to constant mass at 60 °C for 48 h and weighed. Total mass was calculated as the sum of root and shoot mass and is a good indicator of fitness in this species (Cipollini and Lieurance 2012). Mass of the individual considered the target plant in each pot was compared across treatments using ANOVA with the main factors of block, target phenotype (R or S), neighbor phenotype (R or S), exposure to mildew inoculum (yes or no), and all interactions.

Results

When grown and screened under common greenhouse conditions, garlic mustard populations varying in resistance to powdery mildew disease from a North American inoculum source were found in both Europe and North America. In general, populations consisted of individuals of only one phenotype (either resistant (71%) or susceptible (17%), with a few (11%) populations being polymorphic (Table 1). Nine of 21 European populations were found to be susceptible, while 23 of 78 North American populations were found to be susceptibile. While this indicated a tendency for a higher proportion of North American populations to be resistant to powdery mildew disease, these proportions did not differ significantly ($p = 0.13$) (Table 2). We found little indication of either a positive or negative spatial association between susceptible and resistant populations in either North America or Europe (Fig. 2). The high values of L between North American populations are explained by spatial clustering in the sample locations (Fig. 1a), not spatial association between susceptible and resistant populations, as the line is fully contained within the 95% confidence intervals derived from simulations (Fig. 2). Thus, the distribution of resistance among populations that we observed in our screening trials did not differ significantly from random in either North America or Europe.

In the competition experiment, there were significant effects of the resistance phenotype of the target plant on the mass of the target plant (Table 1); target plants from resistant populations were generally larger than plants from the susceptible populations overall (Fig. 3). There was a significant effect of the resistance phenotype of the neighbor on target plant biomass as well (Table 1). Biomass of the target plant was larger when the neighbor was susceptible than when the neighbor was resistant, across both target plant resistance phenotypes (Fig. 3). The mass of the target plant was also affected by the interaction of the target plant phenotype with the neighbor plant phenotype (Table 1). Resistant target plants showed a greater degree of release from competition when grown with a susceptible neighbor versus a resistant neighbor than shown by susceptible target plants grown with a susceptible neighbor versus a resistant neighbor (Fig. 3). No other factors significantly affected target plant biomass.

Discussion

Studies of the interaction of garlic mustard with a powdery mildew fungus have revealed qualitative

Table 2 Results of ANOVA analysis for competition experiments

Source	DF	F value	*P* value
Block	1	15.55	<0.001
Target phenotype	**1**	**99.57**	**< 0.001**
Neighbor phenotype	**1**	**17.19**	**< 0.001**
Protection	1	0.01	0.926
Block*target phenotype	1	44.82	<0.001
Block*neighbor phenotype	1	12.57	0.001
Block*protection	1	4.32	0.040
Target phenotype*neighbor phenotype	**1**	**13.65**	**< 0.001**
Target phenotype*protection	1	1.45	0.231
Neighbor phenotype*protection	1	0.63	0.429
Block*target phenotype*neighbor phenotype	1	2.65	0.106
Block*target phenotype*protection	1	2.18	0.143
Block*neighbor phenotype*protection	1	0.00	0.998
Target phenotype*neighbor phenotype*protection	1	0.18	0.675
Block*target phenotype*neighbor phenotype*protection	1	0.49	0.485
Error	107		

Bold text indicates significant effects not related to block effects

variation in resistance to this fungus among populations, with most populations being monomorphic for resistance or susceptibility and with a few populations being polymorphic. Using an inoculum source from North America, we found a similar proportion of populations exhibiting resistance to powdery mildew disease in both the native and invaded range of this plant. This finding fails to support predictions of the EICA hypothesis (e.g., Blossey and Notzhold 1995), which would be supported if the invaded range harbored a lower proportion of resistant populations, but this hypothesis has never been tested using a qualitative resistance trait that is presumably controlled by one or a few genes. Qualitative resistance to powdery mildew disease in garlic mustard appears to be governed by an R-gene-mediated mechanism, as in related mustards, where possession of appropriate R genes by a plant leads to rapid induction of defense against recognized pathogens (Xiao et al. 2001). While possession of R-genes can be beneficial in the presence of disease and costly in its absence, stable polymorphism in the expression of R genes can be maintained in wild plant populations through a variety of means (Karasov et al. 2014). This suggests that even if temporary escape from European strains of powdery mildew disease had occurred in garlic mustard, other community interactions (such as the presence of other strains or other pathogen species) may have selected for maintenance of this trait in many invasive populations.

The spatial association of resistant and susceptible populations within continents was generally random across distances. In North America where garlic mustard is not native, this pattern would appear to result from multiple introductions of the resistance trait from distant locations, rather than a single introduction followed by incremental dispersal of the resistance (or susceptibility) trait across the invaded range. The fact that populations were often monomorphic for resistance to powdery mildew across native and invaded ranges is indicative of strong founder effects as this plant has expanded its range. In self-compatible plants such as garlic mustard, a single viable seed can found an entire population, and one major genotype may come to dominate certain habitats. The finding that garlic mustard typically exhibits low genetic diversity within, but not necessarily among, populations in its invaded range suggest that new populations are often founded by a few individuals sharing similar genotypes (Meekins et al. 2000; Durka et al. 2005). The lack of large scale spatial patterning also suggests that new populations are not necessarily founded by individuals from nearby populations, otherwise more clusters of populations sharing similar phenotypes should be apparent. As spatial patterns in the possession of the resistance trait by populations appears to be largely random across

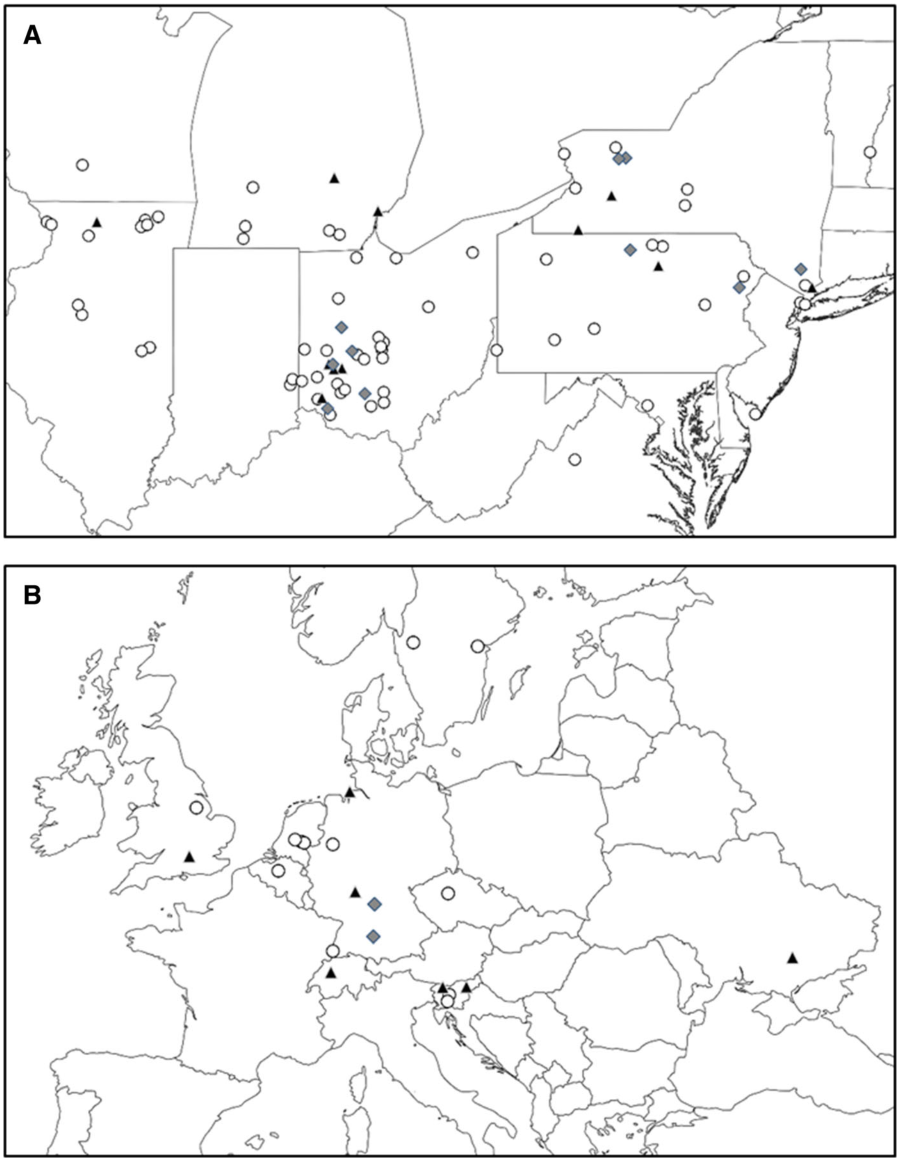

Fig. 1 Distribution of resistance phenotypes of garlic mustard populations to a powdery mildew fungus in **a** North America and **b** Europe. Open circles represent resistant populations; gray diamonds represent populations that had both resistant and susceptible individuals; black triangles represent susceptible populations

both the native and invaded ranges of this plants, the role of this resistance trait in the invasion process of this plant is not clear.

In our competition experiments, individuals from resistant populations were generally larger than individuals from susceptible populations. This effect was especially noticeable when they were competing with plants from susceptible populations. There was a tendency in one experiment for this effect to be stronger in the presence of powdery mildew disease, but there was no significant effect of exposure to inoculum in this study. This result is evidence of an ecological benefit of possession of the mildew resistance trait (Cipollini and Heil 2010), and could influence the frequency of the resistance trait within populations that harbor both genotypes and that are regularly exposed to powdery mildew inoculum. It should be noted that individuals from susceptible

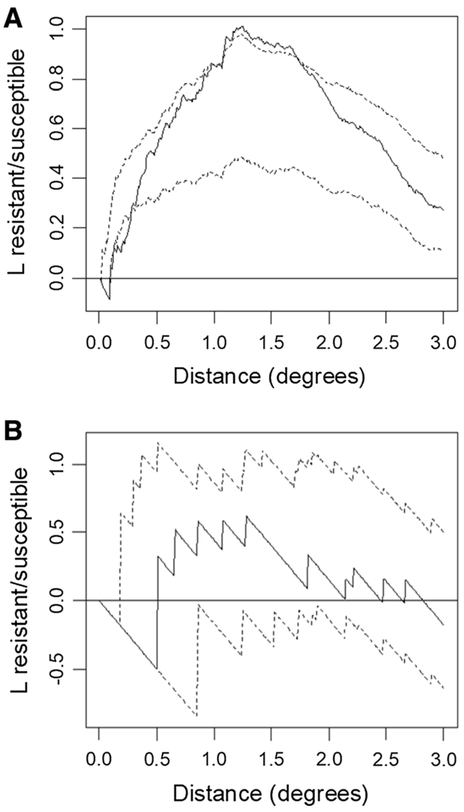

Fig. 2 The spatial association of resistant and suceptible populations calculated using Ripley's cross K as a function of distance for populations of garlic mustard in **a** North America and **b** Europe. The solid line represents the modelled function and the dashed lines represent the 95% confidence interval

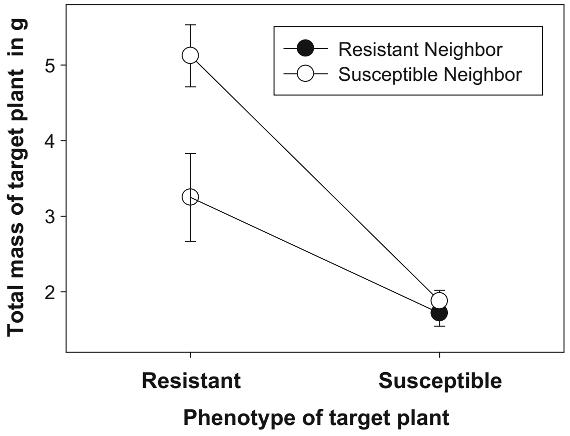

Fig. 3 Target plant biomass (mean ± SE) for resistant and suceptible target plants grown with either resistant or suceptible neighbors, across both blocks and inoculation treatments

populations did not grow larger than competing individuals from resistant populations when protected with fungicide against mildew infection. This would indicate that possession of this particular mildew resistance trait is not very costly (Cipollini and Heil 2010), at least when assessed in the relatively low-power (i.e., low number of replicates and low strength of competition) competitive scenario focusing on vegetative growth that we examined. Fitness costs of the possession of R genes have been observed in *Arabidopsis,* but the detection of such effects was benefited by the use of 500 replicates of experimental lines in field experiments (Tian et al. 2003). Fitness benefits of the possession of mildew resistance, coupled with minimal costs, may explain why the majority of populations were indeed found to be resistant to this fungus. Fitness benefits with minimal costs should also select for increases in the frequency of resistant individuals through time in polymorphic populations, especially if mildew inoculum is present for at least some of the time. However, other physiological or ecological costs of the possession of the resistance trait may exist that have yet to be discovered that may favor the persistence of susceptibility in some populations (Cipollini and Heil 2010; Karasov et al. 2014).

Despite the apparent advantages of the resistance trait, populations made up entirely of susceptible individuals were found relatively frequently, which may have resulted from founder effects. Exposure to the powdery mildew fungus should suppress population growth rates and competitive impacts of individuals in these populations. However, since powdery mildew infection is not necessarily lethal to garlic mustard (Enright and Cipollini 2007), and disease intensity can vary with environmental conditions (Enright and Cipollini 2011), susceptible populations might persist despite their susceptibility to infection. It is also possible that some individuals or entire populations might escape infection due to lack of exposure to inoculum despite being susceptible. Susceptible populations might be more likely to exhibit more substantial 'boom and bust' cycles (i.e., interannual variability in reproduction and subsequent population size) dependent on the extent of disease in a given year than resistant populations. If the resistance trait enters these populations through dispersal, however, it would be expected to increase in frequency through time.

In this study, we exposed variation among populations in the expression of a qualitative resistance trait to a powdery mildew fungus that was distributed largely randomly across European and North Amerian landscapes, and whose frequency within populations appears to be influenced by founder effects and competitive interactions among resistant and susceptible genotypes. It is important to note that our results are based on an inoculum that is prevalent on garlic mustard in North America. However, as yet unexplored variation in virulence of different mildew strains may mitigate the evolution, spatial patterning, and competitive effects of the resistance trait. While powdery mildew disease is likely ubiquitous and is known to affect garlic mustard in Europe, the use of European strains of this fungus in our screening and competition trials could produce different results.

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References

Blossey B, Notzhold R (1995) Evolution of increased competitive ability in invasive nonindigenous plants: a hypothesis. J Ecol 83:887–889

Blossey B, Nuzzo V, Hinz H, Gerber E (2001) Developing biological control of *Alliaria petiolata* (M. Bieb.) Cavara and Grande (Garlic Mustard). Nat Areas J 21:357–367

Bossdorf O, Prati D, Auge H, Schmid B (2004a) Reduced competitive ability in an invasive plant. Ecol Lett 7:346–353

Bossdorf O, Schröder S, Prati D, Auge H (2004b) Palatability and tolerance to simulated herbivory in native and introduced populations of *Alliaria petiolata* (Brassicaceae). Am J Bot 91:856–862

Ciola V, Cipollini D (2011) Distribution and host range of a powdery mildew fungus infecting garlic mustard, *Alliaria petiolata*, in Southwestern Ohio. Am Midl Nat 166:40–52

Cipollini DF (2001) Variation in the expression of chemical defenses in *Alliaria petiolata* in the field and common garden. Am J Bot 89:1422–1430

Cipollini D (2004) Stretching the limits of plasticity: can a plant defend against both competitors and herbivores? Ecology 85:28–37

Cipollini D (2007) Consequences of the overproduction of methyl jasmonate on seed production, tolerance to defoliation, and competitive effect and response of *Arabidopsis thaliana*. New Phytol 173:146–153

Cipollini D, Enright S (2009) A powdery mildew fungus levels the playing field for invasive garlic mustard (*Alliaria petiolata*) and a North American native plant. Invasive Plant Sci Manag 2:253–259

Cipollini D, Heil M (2010) Costs and benefits of induced resistance to pathogens and herbivores in plants. CAB Rev Pers Ag Vet Sci Nutr Nat Res. https://doi.org/10.1079/PAVSNNR20105005

Cipollini D, Lieurance DM (2012) Expression and costs of induced defense trails in *Alliaria petiolata*, a widespread invasive plant. Basic Appl Ecol 13:432–440

Cipollini D, Mbagwu J, Barto K, Hillstrom C, Enright S (2005) Expression of constitutive and inducible chemical defenses in native and invasive populations of *Alliaria petiolata*. J Chem Ecol 31:1255–1267

Cressie NAC (1991) Statistics for spatial data. Wiley, New York

Davis S, Cipollini D (2014) Do mothers always know best? Oviposition mistakes and resulting larval failure of *Pieris virginiensis* on *Alliaria petiolata*, a novel, toxic host. Biol Invasions 16:1941–1950

Doorduin LJ, Vrieling K (2011) A review of the phytochemical support for the shifting defence hypothesis. Phytochem Rev 10:99–106

Durka W, Bossdorf O, Prati D, Auge H (2005) Molecular evidence for multiple introductions of garlic mustard (*Alliaria petiolata*, Brassicaceae) to North America. Mol Ecol 14:1697–1706

Ellis MB, Ellis JP (1997) Microfungi on land plants. Richmond Publishing, Slough

Enright S, Cipollini D (2007) Infection by powdery mildew *Erysiphe cruciferarum* (Erysiphaceae) strongly affects growth and fitness of *Alliaria petiolata* (Brassicaceae). Am J Bot 94:1813–1820

Enright S, Cipollini D (2011) Overlapping defense responses to water limitation and pathogen attack and their consequences for resistance to powdery mildew disease in garlic mustard, *Alliaria petiolata*. Chemoecology 21:89–98

Evans JA, Landis DA (2007) Pre-release monitoring of *Alliaria petiolata* (garlic mustard) invasions and the impacts of extant natural enemies in southern Michigan forests. Biol Conserv 42:300–307

Felker-Quinn E, Schweitzer JA, Bailey JK (2013) Meta-analysis reveals evolution in invasive plant species but little support for evolution of increased competitive ability (EICA). Ecol Evol 3:739–751

Franks SJ, Pratt PD, Dray FA, Simms EL (2008) No evolution of increased competitive ability or decreased allocation to defense in *Melaleuca quinquenervia* since release from natural enemies. Biol Inv 10:455–466

Hahn PG, Draney ML, Dornbush ME (2011) Exotic slugs pose a previously unrecognized threat to the herbaceous layer in a Midwestern woodland. Restor Ecol 19:786–794

Hillstrom C, Cipollini D (2011) Variation in phenotypic plasticity among native and invasive populations of *Alliaria petiolata*. Int J Plant Sci 6:763–772

Karasov TL, Kniskern JM, Gao L, DeYoung BJ, Ding J, Dubiella U, Lastra RO, Nallu S, Roux F, Innes RW (2014) The long-term maintenance of a resistance polymorphism through diffuse interactions. Nature 512:436–440

Koch E, Slusarenko A (1990) Fungal pathogens of *Arabidopsis thaliana* (L.) Heyhn. Bot Helvet 100:257–268

Lewis KC, Bazzaz FA, Liao Q, Orians CM (2006) Geographic patterns of herbivory and resource allocation to defense, growth, and reproduction in an invasive biennial, *Alliaria petiolata*. Oecologia 148:384–395

Meekins JF, Ballard HE, McCarthy BC (2000) Genetic variation and molecular biogeography of a North American invasive plant species (*Alliaria petiolata*, Brassicaceae). Int J Plant Sci 162:161–169

Nuzzo V (1999) Invasion pattern of the herb garlic mustard (*Alliaria petiolata*) in high quality forests. Biol Invasions 1:169–179

Orians CM, Ward D (2010) Evolution of plant defenses in nonindigenous environments. Ann Rev Entomol 55:439–459

Ripley BD (1979) Tests of 'randomness' for spatial point patterns. J R Stat Soc B (Method) 41:368–374

Ripley BD (1988) Statistical inference for spatial processes. Cambridge University Press, Boston

Ripley BD, Rasson J-P (1977) Finding the edge of a Poisson forest. J Appl Prob 14:483–491

Tian D, Traw MB, Chen JQ, Kreitman M, Bergelson J (2003) Fitness costs of R-gene-mediated resistance in *Arabidopsis thaliana*. Nature 423:74–77

Trimbur TJ (1973) An ecological life history of *Alliaria officinalis*, a deciduous forest "weed". MS Thesis, The Ohio State University

Van Kleunen M, Schmid B (2003) No evidence for an evolutionary increased competitive ability in an invasive plant. Ecology 84:2816–2823

Ward SM, Gaskin JF, Wilson LM (2008) Ecological genetics of plant invasion: What do we know? Invasive Plant Sci Manag 1:98–109

Warwickshire Fungus Survey (2004) Newsletter of the Warwickshire Fungus Survey for 2004. Affiliated with the British Mycological Society. http://wfs.bnhsoc.org.uk/wfsNews.htm. Accessed 16 June 2019

Wright AJ, Heale JB (1984) Adult plant resistance to powdery mildew (Erysiphe graminis) in three barley cultivars. Plant Path 33:493–502

Xiao S, Ellwood S, Calis O, Patrick E, Li T, Coleman M, Turner JG (2001) Broad-spectrum mildew resistance in Arabidopsis thaliana mediated by RPW8. Science 291:118–120