Variation in Pathogenicity and Aggressiveness of *Erysiphe necator* from Different *Vitis* spp. and Geographic Origins in the Eastern United States

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ABSTRACT

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Eastern North America is considered the center of diversity for many *Vitis* spp. and for the grape powdery mildew pathogen, *Erysiphe necator*. However, little is known about populations of *E. necator* from wild *Vitis* spp. We determined the phenotypic variation in pathogenicity and aggressiveness of *E. necator* among isolates from wild and domesticated *Vitis* spp. from diverse geographic regions in the eastern United States. To test pathogenicity, we inoculated 38 *E. necator* isolates on three wild *Vitis* spp., two commercially grown hybrids and the European wine grape, *Vitis vinifera*. *V. rotundifolia* (muscadine grape) was the only host species on

which complete host specialization was evident; it was only susceptible to isolates collected from *V. rotundifolia*. All isolates, regardless of source host, were pathogenic on the other *Vitis* spp. We found no differences in components of aggressiveness latent period and lesion size among isolates from different source hosts when inoculated on *V. vinifera*, which is highly susceptible to powdery mildew. However significant variation was evident among isolates on the more resistant *V. labruscana* 'Niagara'. Isolates from the wild species *V. aestivalis* were the most aggressive, whereas isolates from *V. vinifera* were not more aggressive than isolates from other source hosts. Greater aggressiveness was also detected among isolates from the southeastern United States compared with isolates from the northeastern United States.

Additional keywords: durability, *Uncinula*.

Adaptation and fitness of individual pathogen genotypes is partially determined by two distinct factors: pathogenicity (i.e., the ability to infect particular host species or genotypes) and aggressiveness (i.e., the quantitative component of pathogenicity). In previous studies, large variation in pathogenicity and aggressiveness has been detected within and between pathogen populations. Such variation could arise due to the agroecological environment, host diversity, cultural practices, and sexual recombination.

The grape powdery mildew fungus, *Erysiphe necator* (syn. *Uncinula necator*), is an obligate biotroph on host species in the family Vitaceae, particularly in the genus *Vitis* but also in *Parthenocissus* and *Ampelopsis* (2,20). Although powdery mildew is one of the most economically important diseases of grape worldwide (39), many commercially grown grape cultivars are highly susceptible to *E. necator* because they are derived from the European wine grape, *Vitis vinifera*, which was not exposed to this pathogen during its evolution (38). Eastern North America is the center of diversity for many wild species of *Vitis*, with relatively high levels of resistance to some of the most notorious diseases and pests of grapevines, including powdery mildew. Native grapevine species such as *V. labrusca*, *V. rupestris* (12), *V. aestivalis* (24), and *V. rotundifolia* (syn. *Muscadinia rotundifolia*) (35), which probably coevolved with *E. necator* in North America, possess varying levels of resistance to powdery mildew. Resistance to powdery mildew in North American *Vitis* spp. is determined by diverse mechanisms. For example, resistance in *V.*

rotundifolia is associated with *Run1*, a dominant, complex locus that contains two families of resistance gene analogs. Resistance in plants with *Run1* is mediated through a hypersensitive response (11). Alternatively, resistance may be associated with constitutive expression of pathogenesis-related (PR) genes, such as genes encoding β -1,3-glucanases (PR-2) and chitinases (PR-3), in addition to other quantitative resistance mechanisms found in North America *Vitis* spp. (18,24).

Few studies have been conducted to study variation in aggressiveness in *E. necator*. In Europe, these efforts have focused on comparisons between genetic groups A and B (sampled only from *V. vinifera*), which represent distinct lineages of *E. necator* derived from populations in North America (3). Some studies found significant differences between groups A and B in infection efficiency, sporulation, latent period, and colony expansion rates (33,41). In contrast, much less effort has been devoted to studying variation in aggressiveness in North America. In a comprehensive study of host specialization, Gadoury and Pearson (20) tested 35 *E. necator* isolates from different *Vitis* spp. on two experimental hosts: *V. vinifera* and labrusca hybrid cv. Katawba. They found no differences among isolates in latent period or colony expansion rates on *V. vinifera*. In contrast, they did find significant differences in colony expansion rates when they inoculated the more resistant 'Katawba' with the same isolates. However, their sampling of isolates was mostly done in the northeastern United States (New York and New England), with a few isolates from the southeastern United States. In addition, they focused mostly on isolates sampled from *V. vinifera* and hybrids of *V. vinifera* with North American species from commercial vineyards. As a consequence, no information, to our knowledge, is available on the aggressiveness of populations from wild *Vitis* spp. such as *V. aestivalis* or from cultivated (or wild) muscadine grape (*V. rotundifolia*). These latter two species are potentially important

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hosts of *E. necator* because *V. rotundifolia* (36) and cultivars of predominantly *V. aestivalis* ancestry (e.g., cv. Norton) (44) are grown commercially in the southeastern and central United States, sometimes sympatrically with *V. vinifera*.

Although populations of *E. necator* in Europe and Australia are composed of two genetically distinct lineages (groups A and B) (10,42), the structure of North American populations is markedly different. Brewer and Milgroom (3) recently reported that populations of *E. necator* in the eastern United States are genetically diverse, as expected in a native population, and are not structured by distinct lineages. Our overall hypothesis is that, because the populations of *E. necator* in the eastern United States are diverse with respect to genotypes, wild *Vitis* host species (and powdery mildew resistance mechanisms among them), geography, and climate, we expect to find large variation in pathogenicity and aggressiveness on different host species. Our specific aims were to quantify the variation in *E. necator* populations from different *Vitis* hosts and regions in the eastern United States with respect to their pathogenicity and aggressiveness.

MATERIALS AND METHODS

Collection and maintenance of fungal isolates. We collected *E. necator* in 2007 to 2009 and concentrated our search on the more common host species in the eastern United States (we refer to these as “source hosts”) (Table 1). We collected *E. necator* from (i) *V. riparia*, which is mostly found in the northeastern United States; (ii) *V. aestivalis*, which can be found across the eastern United States but is most common at high elevation in the southeast; (iii) *V. labrusca*, which is more common near coastal areas of New England and in the mid-Atlantic states, but can be also found at high elevations in the southeast; (iv) *V. rotundifolia* (muscadine grape), which is grown commercially and grows wild in the southeastern United States, from the coastal areas to the lower slopes of the Appalachian mountains; and (v) *V. vinifera*, the European wine grape, which is grown commercially across the eastern United States (21). Because powdery mildew cannot be easily detected on wild species, we sampled wherever we or our colleagues encountered it in various locations in the following northeastern states: Massachusetts (MA), New Hampshire (NH), Connecticut (CT), New York (NY), New Jersey (NJ), Ohio (OH), and Michigan (MI). In the southeast, we concentrated our sampling in two distinct areas. First, we sampled at low elevations in North Carolina (NC), South Carolina (SC), and Georgia (GA), where powdery mildew could only be found on *V. vinifera* and *V. rotundifolia*. Second, we sampled at higher elevations in the Appalachian Mountains in GA, NC, and Virginia (VA) from all five *Vitis* spp. In addition, we sampled isolates randomly from commercial vineyards of *V. vinifera* in NC and GA, where mildew was more common. Approximate collection sites are presented in Figure 1.

TABLE 1. Sampling of *Erysiphe necator* from diverse hosts in the northeastern (New York, Pennsylvania, Ohio, Michigan, Massachusetts, and New Hampshire) and southeastern (Georgia and North Carolina) United States

Source host	No. of isolates	Northeastern United States	Southeastern United States
<i>Vitis riparia</i>	13 ^a	11	1
<i>V. aestivalis</i>	16 ^a	3	11
<i>V. labrusca</i>	8	4	4
<i>V. rotundifolia</i> ^b	10	0	10
<i>V. vinifera</i>	13	6	7
Total	60	24	33

^a One isolate from *V. riparia* was collected in Kansas and two isolates from *V. aestivalis* were collected in Virginia and Kansas; these isolates were not included in either the northeastern or southeastern United States samples.

^b Among the 10 isolates collected from *V. rotundifolia*, 8 were from wild plants and 2 from commercial vineyards (*V. rotundifolia* ‘Carlos’).

Isolates were maintained as described by Evans et al. (16). Briefly, *V. vinifera* ‘Cabernet Sauvignon’ was grown in the greenhouse to produce leaves for culturing *E. necator*. The fourth youngest leaves were collected and surface sterilized in 0.6% sodium hypochlorite for 2 min, rinsed twice in sterile distilled water, and dried in a sterile laminar flow hood. Leaves were placed in petri dishes containing 20 ml of 2% water agar to maintain sufficient moisture. Mass transfers of conidia and mycelium from colonies on infected leaves collected in the field were made to surface-sterilized leaves under a dissecting microscope (×200) using a sterile pipette tip. Another transfer was made from the resulting colony at least once to avoid contamination. After at least two transfers, an individual chain of conidia was transferred from each mildew colony, with a sterile pipette or with an eyelash fastened to a Pasteur pipette, to a new leaf, as described by Gadoury and Pearson (20). Resulting single-conidial-chain isolates were maintained on leaves in a growth room at 20 to 25°C in 12-h cycles of light and darkness. Colonies were transferred to new leaves every 18 to 24 days.

Host specialization on *Vitis* spp. and hybrids. We determined the ability of *E. necator* isolates from different *Vitis* hosts to colonize leaf tissue (i.e., pathogenicity) of wild and domesticated *Vitis* spp. on the following six hosts (“inoculated hosts”): (i) *V. vinifera* Cabernet Sauvignon, (ii) *V. rotundifolia* var. *munsoniana*

Fig. 1. Approximate locations where we sampled *Erysiphe necator* from wild and commercially grown *Vitis* spp. in the eastern United States during 2007–2009. ○ = Sample from the northeastern United States ($n = 24$): New Hampshire (NH), Massachusetts (MA), Connecticut (CT), New York (NY), New Jersey (NJ), Ohio (OH), and Michigan (MI). ● = Samples from the southeastern United States ($n = 33$): North Carolina (NC) and Georgia (GA). □ = Samples from states which were excluded from the northeast versus southeast analysis (see Materials and Methods): Virginia (VA) and Kansas (Lawrence, KS, not shown on map).

(accession DVIT 2735; United States Department of Agriculture–Agricultural Research Service [USDA-ARS] National Clonal Germplasm Repository, Davis, CA), (iii) *V. aestivalis* (accession REM 59-77; USDA-ARS Plant Genetic Resources Unit, Geneva, NY), and (iv) *V. riparia* growing wild in Ithaca, NY. In addition, we used two hybrids: (v) cv. Niagara, which is a *V. vinifera* × *V. labrusca* hybrid (Double A vineyards, Fredonia, NY) and (vi) cv. Norton (syn. ‘Cynthiana’), which is a cultivar of predominantly *V. aestivalis* ancestry, probably derived by hybridization with *V. vinifera* (44). Both hybrids are commercially grown in the eastern United States and considered less susceptible to *E. necator* than *V. vinifera*. All *Vitis* spp. were grown from cuttings and received a cold treatment before being potted in 4-liter pots and grown at 25°C in the greenhouse. The only exception was *V. riparia*, whose leaves were collected from a single wild vine in May 2009. Fourth-youngest leaves from each of the six inoculated hosts were surface sterilized and placed in petri dishes as described above. We inoculated five to nine isolates collected from each of the five source hosts (total $n = 38$). Each inoculation was conducted six times with one inoculation per leaf. Inoculation was conducted by applying ≈ 200 conidia to the center of the leaf with a sterile pipette tip. Plates were wrapped with Parafilm and placed in growth chambers at 22°C under a regime of 12-h cycles of darkness and light. Leaves were inspected under a dissecting microscope ($\times 200$) to detect colony growth and sporulation beginning 5 days after inoculation and every 3 to 4 days thereafter until the maximum of 28 days post inoculation.

Aggressiveness components: latent period and lesion size. Aggressiveness was estimated on fourth-youngest leaves of *V. vinifera* cv. Cabernet Sauvignon and Niagara. ‘Cabernet Sauvignon’ is considered highly susceptible to powdery mildew, whereas labrusca hybrid cultivars are less susceptible (39). Gadoury and Pearson (20) demonstrated significant variation in aggressiveness of *E. necator* on labrusca hybrid cv. Katawba but not on *V. vinifera*. Therefore, we hypothesized that cv. Niagara, with its strong background from *V. labrusca*, might be comparable for showing differences in aggressiveness. We also attempted to use *V. aestivalis* as a host for estimating aggressiveness but, because many of the detached leaves of this species decomposed after <10 days in our experimental conditions, we excluded it from these experiments. Sixty isolates from the five source hosts were used in this study (Table 1). Aqueous suspensions of conidia were prepared by vortexing excised mildew colonies 8 to 10 days after inoculation in 1.5-ml microcentrifuge tubes with 1 ml of water and 0.05% Tween 20. After counting with a hemacytometer, the conidial suspension was adjusted to 1×10^5 conidia/ml and each leaf was inoculated with a 10- μ l droplet. Inoculated leaves were left in the laminar flow hood for 30 to 40 min until the droplet fully evaporated. Petri dishes were wrapped with Parafilm and placed in completely randomized locations in a growth chamber at 22°C under a 12-h regime of light and darkness. We were concerned that disease would develop too quickly under optimal conditions and, as a result, differences would not be distinguishable between isolates. Therefore, experiments were conducted at 22°C rather than the optimal temperature of 25°C to slow pathogen growth (9). Latent period (i.e., the time from inoculation until the appearance of the first conidia) was determined by daily inspection for conidia under a dissecting microscope ($\times 400$). Inspection took place each morning from day 4 to day 10. Lesion area was calculated from colony diameters measured in two perpendicular directions (a and b) 13 days after inoculation. Because the two diameters were often unequal, we calculated lesion area as an ellipse ($\pi ab/4$, in square millimeters). Each treatment (inoculated host species–isolate) had eight replicates and the experiment was conducted twice.

Statistical analyses were performed with the JMP 5.0 software for Windows (SAS Institute, Cary, NC). To conduct an analysis of variance on non-normally distributed data and unequal variances,

a rank transformation was applied to all results (6). We used the data from the 60 isolates to assess differences in aggressiveness based on source hosts by using a mixed model with the source host and inoculated host (*V. vinifera* or ‘Niagara’) as fixed effects, while isolates were treated as random effects. The experiment was considered as an additional fixed effect.

To test whether aggressiveness differed between geographic regions, we divided isolates into those from the northeastern United States (NY, MA, NH, CT, NJ, OH, and MI; $n = 24$) and those from southeastern United States (GA and NC; $n = 33$). Three isolates collected in VA or Kansas were excluded from the analysis because they were not geographically and climatically aligned with either region. For this test, we used a second mixed model in which geographic region, inoculated host, and experiment were fixed effects and isolates were random effects. Multiple comparisons of the means were conducted with the Tukey-Kramer honestly significant differences (HSD) test ($\alpha = 0.05$).

RESULTS

We isolated *E. necator* from four wild species during the growing seasons of 2007–09 (Table 1; Fig. 1). Symptoms and signs of powdery mildew on *V. aestivalis*, *V. riparia*, and *V. labrusca* were very similar to those on *V. vinifera* and commercial hybrids. In the southeastern United States, source hosts were found from sea level at Myrtle Beach, SC up to 1,250 m in the Blue Ridge Parkway (Buncombe and Haywood Counties, NC). However, *E. necator* was only found on *V. riparia*, *V. aestivalis*, and *V. labrusca* at elevations of 875 to 1,180 m, and mostly in shaded areas. Interestingly, we frequently found severely diseased plants adjacent to plants of the same species with no symptoms of powdery mildew. Powdery mildew was found on *V. rotundifolia* growing wild on the lower slopes of the Appalachians (Panther Creek Falls, Clarksville, GA, elevation 450 m; and De Soto Falls, Lumpkin County GA, elevation 635 m) and from commercial vineyards in central NC (Hurdle Mills, elevation 187 m) and GA (Ringgold, elevation 237 m). Powdery mildew symptoms on *V. rotundifolia* differed from those on other *Vitis* spp. Colonies appeared mostly on the abaxial side of the leaves and lesions were generally smaller, with dense hyphal growth. In the northeastern United States, we found powdery mildew on plants at elevations as low as 15 m (*V. labrusca*, Portsmouth, NH) and up to 550 m (*V. riparia*, Sugar Hill State Forest, Schuyler County, NY). In the northeast, disease was less confined to shady areas than in the southeast, although no quantitative data were recorded. Cleistothecia were found in mildew colonies in most locations on all of the host species except for *V. rotundifolia*.

Host specialization on *Vitis* spp. and hybrids. All isolates, regardless of source host, formed mildew colonies and produced abundant sporulation on five of the inoculated hosts: *V. vinifera*, *V. riparia*, and *V. aestivalis* and the hybrids *V. labruscana* Niagara and cv. Norton (Table 2; Fig. 2). In contrast, only isolates collected from *V. rotundifolia* ($n = 10$) were pathogenic on *V. rotundifolia*. These isolates formed macroscopic colonies with abundant sporulation on *V. rotundifolia*, as well as on the other five inoculated hosts (Fig. 2). Isolates from other *Vitis* spp. ($n = 28$) produced only a few sparse hyphae, with no conidia or conidiophores, on *V. rotundifolia*. We observed necrotic epidermal cells below these hyphae, which we interpret as hypersensitive responses at the sites of attempted penetration (Fig. 2).

Variation in aggressiveness. We detected variation in latent period and lesion size among 60 isolates on both *V. vinifera* and ‘Niagara’. On *V. vinifera*, the average latent period among all 60 isolates was 6.0 days, ranging between 5.1 and 8.0 days (Fig. 3A). The average lesion size was 103.2 mm², ranging between 67.0 and 150.0 mm² (Fig. 3C). On ‘Niagara’, the average latent period among all 60 isolates was 6.5 days, ranging between 5.0 and

8.6 days (Fig. 3B). The average lesion size was 97.9 mm², ranging between 60.3 and 146.4 mm² (Fig. 3D).

Effect of original source hosts on aggressiveness. Linear models were used to compare rank transformations of latent period and lesion size among isolates from five source hosts (Table 3). Analysis of variance (ANOVA) models were significant ($P < 0.001$) and explained 29.6 and 22.8% of the observed variation of latent period and lesion size, respectively. The correlation between the means for latent period and lesion size was significant ($P = 0.01$) but weak ($r = 0.33$).

For both latent period and lesion size, the source host $\times$ inoculated host interactions were significant ($P = 0.003$ and 0.002 , respectively) (Table 3). Therefore, we analyzed the effect of source host on *V. vinifera* and ‘Niagara’ separately. The variance component of the random effect (isolates) on latent period was 40.5%. The experiment effect was not significant for latent period; therefore, we combined results of the two experiments. On *V. vinifera*, mean latent period varied from 5.9 to 6.2 days but no significant differences were detected among source hosts (Fig. 4A). In contrast, we found one significant difference in latent

period between source hosts on ‘Niagara’. Isolates from *V. aestivalis* had the shortest mean latent periods (6.1 days), which was significantly shorter than the mean latent period of isolates from *V. riparia* (6.7 days) (Fig. 4B). No significant differences were detected among means of isolates from *V. labrusca*, *V. vinifera*, and *V. rotundifolia* and any other source host (Fig. 4B).

For lesion size, the variance component of the random effect (isolates) was 15.5% (Table 3). Although the means for lesion size in the experiments were different, the relative differences among isolates from different source hosts were similar between experiments; therefore, we show the combined results of the experiments. Mean lesion size on *V. vinifera* varied from 97.5 to 107.8 mm² but no significant differences were detected among isolates from different source hosts (Fig. 4C). On ‘Niagara’, however, lesion sizes for isolates from *V. aestivalis* (108.9 mm²) and *V. riparia* (87.0 mm²) were significantly different. Isolates from *V. aestivalis* also had significantly larger lesions than those of isolates from *V. vinifera* and *V. rotundifolia* (Fig. 4D). Mean lesion size of isolates from *V. rotundifolia* was also significantly larger than the mean lesion size of isolates from *V. riparia*. No

TABLE 2. Pathogenicity of *Erysiphe necator* isolated from five *Vitis* source hosts inoculated on four *Vitis* spp. and two hybrids (‘Niagara’ and ‘Norton’)^a

Source hosts (origin of isolates) ^b	Inoculated hosts					
	<i>V. vinifera</i>	<i>V. riparia</i>	<i>V. aestivalis</i>	‘Niagara’	‘Norton’	<i>V. rotundifolia</i>
<i>V. vinifera</i> (n = 8)	+	+	+	+	+	–
<i>V. riparia</i> (n = 8)	+	+	+	+	+	–
<i>V. aestivalis</i> (n = 8)	+	+	+	+	+	–
<i>V. labrusca</i> (n = 5)	+	+	+	+	+	–
<i>V. rotundifolia</i> (n = 9)	+	+	+	+	+	+

^a Pathogenicity was defined as successful infection and colony establishment; + indicates compatible or pathogenic interaction and – indicates incompatible or nonpathogenic interaction.

^b Number in parenthesis indicates the number of isolates from each source host that were used in the experiment.

Fig. 2. Powdery mildew colonies resulting from inoculation of *Vitis vinifera* and *V. rotundifolia* with isolates of *Erysiphe necator* collected from *V. vinifera* or *V. rotundifolia*. Results for the isolate from *V. vinifera* are representative of those for isolates from the other *Vitis* spp. (i.e., *V. aestivalis*, *V. labrusca*, and *V. riparia*). Colony boundaries are outlined in black. A plus sign (+) indicates successful infection and colonization, whereas the minus sign (–) indicates no colonization.

significant differences were detected among isolates from *V. rotundifolia*, *V. vinifera*, and *V. labrusca* (Fig. 4D).

The ANOVA models comparing aggressiveness of isolates from two different geographic regions, northeastern United States ($n = 24$) and southeastern United States ($n = 33$), are presented in Table 4. ANOVA models were significant for latent period and lesion size and explained 29.2 and 22.8% of the observed variation, respectively. For latent period, only the main factor (inoculated host; *V. vinifera* versus ‘Niagara’) was significant (Table 4). The differences between the experiments were not significant and the results of the two experiments were analyzed together. No significant differences were detected for latent period between isolates from the northeastern and southeastern regions on either of the inoculated hosts (6.0 and 6.3 days, respectively, on *V. vinifera*; and 6.3 and 6.8 days, respectively, on ‘Niagara’) (Fig. 5A and B). For lesion size, the main factors, geographic region and inoculated host, were significant ($P = 0.027$ and 0.002 , respectively) but not their interaction ($P = 0.977$). The variance component of the random effect (isolates) was 14.7%. The isolates from the southeast had a significantly larger lesion size than isolates from the northeast (103.9 and 95.1 mm², respectively), regardless of which host was inoculated (Fig. 5B).

Sampling from source host was partially confounded with geographic region because isolates of *V. rotundifolia* were only found in the southeast and all but one isolate from *V. riparia* were found in the northeast. Therefore, testing the significance of the geographic region $\times$ source host interaction would not be meaningful. However, for the three source hosts (*V. aestivalis*, *V. vinifera*, and *V. labrusca*) for which we had reasonable sample sizes from both regions (Table 1), we performed *t* tests. Southeastern isolates of *V. aestivalis* had a significantly shorter mean latent period than northeastern isolates from *V. aestivalis*, regardless of which host was inoculated (5.9 versus 7.3 days on *V. vinifera* and 5.8 versus 7.1 days on ‘Niagara’; $P < 0.001$) and significantly larger mean lesion size than northeastern isolates (106.9 versus 81.2 mm² on *V. vinifera* and 116.5 versus 78.9 mm² on ‘Niagara’; $P < 0.001$). No significant differences were found between regions when comparing isolates from *V. vinifera* ($P > 0.09$) or *V. labrusca* ($P > 0.15$).

DISCUSSION

Centers of diversity of native hosts often correspond with the centers of diversity of their pathogens. In many cases, these centers of diversity are studied to characterize important phenotypic

Fig. 3. Distribution of mean aggressiveness components estimated for 60 isolates of *Erysiphe necator*: **A and B**, Latent period; **C and D**, lesion size (13 days after inoculation). Isolates were inoculated on detached leaves of *Vitis vinifera* ‘Cabernet Sauvignon’ (**A and C**) and labrusca hybrid ‘Niagara’ (**B and D**). Detached leaves were maintained at 22°C under a 12-h regime of light and darkness. Mean values $\pm$ standard deviations over all 60 isolates are presented.

TABLE 3. Analysis of variance to determine the effect of source hosts on the aggressiveness (latent period and lesion size) of 60 isolates of *Erysiphe necator* inoculated on two hosts, *Vitis vinifera* ‘Cabernet Sauvignon’ and labrusca hybrid ‘Niagara’

Source	df	Latent period			Lesion size		
		SS	F ratio	Prob > F	SS	F ratio	Prob > F
Source host ^a	4	178,428	0.4883	0.7444	938,381	2.2075	0.0662
Inoculated host	1	6,071,478	66.4621	<0.0001	1,841,758	17.3304	<0.0001
Source host $\times$ inoculated host	4	1,447,361	3.9609	0.0034	1,804,593	4.2452	0.0020
Experiment	1	152,137	1.6654	0.1971	17,223,235	162.0653	<0.0001

^a Isolates of *E. necator* were collected from five source hosts: *V. riparia*, *V. aestivalis*, *V. labrusca*, *V. vinifera*, and *V. rotundifolia*.

traits of the host which have agricultural importance, such as disease resistance (27,30). In the same manner, exploring phenotypic variation in pathogenicity and aggressiveness of the natural pathogen populations may provide significant knowledge about these traits (49), as well as inform us about host resistance mechanisms and the potential breakdown of resistance.

In this study, we challenged several of the most common wild and domesticated species in the genus *Vitis* in the eastern United States with a collection of *E. necator* isolates from diverse hosts and geographic origins in the eastern United States. The only combination where complete host specialization was observed was between *V. rotundifolia* and *E. necator* isolates collected from this same host species. Interestingly, all 10 isolates from *V. rotundifolia* were pathogenic on *V. rotundifolia* as well as on all

other *Vitis* spp. we tested, whereas none of the isolates from the other *Vitis* spp. was pathogenic on *V. rotundifolia*, even though they were all pathogenic on the other *Vitis* hosts. *V. rotundifolia* (muscadine grape) differs from the other *Vitis* spp. in number of morphological, anatomical, and cytological characters (21). Those differences include having 40 chromosomes (whereas other *Vitis* spp. have 38 chromosomes) and having prominent lenticels, among other differences (21). Overall, *V. rotundifolia* is different enough from other *Vitis* spp. that it is sometimes considered as being in a different genus, *Muscadinia* (21,35). It is possible that *V. rotundifolia* has defense mechanisms that are different from those of the other *Vitis* spp. tested. One example is the dominant resistance locus *Run1*, which is present in some *V. rotundifolia* accessions. Analysis of the *Run1*-mediated resistance response

Fig. 4. Means of aggressiveness components **A and B**, latent period and **C and D**, lesion size for isolates of *Erysiphe necator* from five source hosts inoculated on two hosts, *Vitis vinifera* ‘Cabernet Sauvignon’ (**A and C**) and labrusca hybrid ‘Niagara’ (**B and D**). Lesion size was measured 13 days after inoculation. Vertical lines represent the standard errors and different letters represent significant differences (highly significant differences test, $\alpha = 0.05$).

TABLE 4. Analysis of variance to determine the effect of geographic origin on the aggressiveness (latent period and lesion size) of 57 isolates of *Erysiphe necator* inoculated on two hosts, *Vitis vinifera* ‘Cabernet Sauvignon’ and labrusca hybrid ‘Niagara’^a

Source	df	Latent period			Lesion size		
		SS	F ratio	Prob > F	SS	F ratio	Prob > F
Geographic region	1	245,183	2.6635	0.1029	530,607	4.9170	0.0268
Inoculated host	1	5,874,779	63.8202	<0.0001	1,059,327	9.8164	0.0018
Geographic region × inoculated host	1	278,734	3.0280	0.0821	3,691	0.0342	0.8533
Experiment	1	166,011	1.8034	0.1796	16,343,970	151.4540	<0.0001

^a Isolates were obtained from five *Vitis* spp. (source hosts) in two regions of the eastern United States: northeastern (Massachusetts, New Hampshire, Connecticut, New York, New Jersey, Ohio, and Michigan; $n = 24$) and southeastern (Georgia and North Carolina; $n = 33$).

indicates that it involves induction of programmed cell death specifically within the penetrated epidemic cell. Unfortunately, no information is available about resistance genes in the *V. rotundifolia* accession we inoculated in this study. Therefore, the *V. rotundifolia*–*E. necator* interaction might be a fertile ground for additional studies of host–pathogen interactions. Interestingly, we did not detect differences in aggressiveness (latent period and lesion size) between isolates from *V. rotundifolia* and those from *V. vinifera* or *V. labrusca*.

Pathogenicity of isolates from *V. rotundifolia* on all the *Vitis* hosts we tested and their equal aggressiveness as isolates from commonly cultivated source hosts imply that the population of *E. necator* on *V. rotundifolia* has the potential to be a source of inoculum for other *Vitis* spp. However, not a single isolate in our sample, including those from *Vitis* spp. in North Carolina (Hurdle Mills area) and Georgia (Ringgold area) sympatric with *V. rotundifolia*, was able to colonize *V. rotundifolia*, implying that gene flow from *V. rotundifolia* to other *Vitis* spp. is uncommon. This discrepancy has at least two possible explanations. First, the number of isolates we sampled from sympatric hosts was not large. By chance, we may not have sampled isolates from other *Vitis* spp. that could infect *V. rotundifolia*. Second, experimental inoculations in the laboratory (e.g., drop inoculations on detached leaves) do not always perfectly reflect pathogenesis in nature. For example, single-generation pathogenicity assays do not take into account other ecological factors which define fitness, such as differences related to overwintering survival, variation in sporulation and spore viability among isolates from different hosts (37), or epidemiological patterns on different host organs (34). Therefore, small differences in fitness among individuals measured in artificial conditions may translate into larger differences and niche partitioning in nature (34). To test these hypotheses, larger samples of isolates from sympatric hosts would need to be inoculated on *V. rotundifolia*. In addition, a complementary approach (also requiring larger samples) would be to genotype isolates with polymorphic genetic markers to test for genetic differentiation among *Vitis* spp. Estimating gene flow in field populations is likely to reveal more about the actual migration of *E. necator* under natural conditions, as opposed to the potential migration inferred from inoculations. This kind of population genetics approach would take into account multiple generations of selection acting on pathogen populations and avoid artifacts associated with inoculations done under artificial conditions (40).

In addition to complete host specialization on *V. rotundifolia*, we were able to detect significant variation in aggressiveness among isolates. We used the drop-inoculation technique described by Gadoury and Pearson (20) but we modified it for detached leaves, instead of in vitro plants and seedlings, which are harder to maintain and guard from contamination. Use of detached leaves also allowed us to assay a larger number of isolates than might be feasible with seedlings. Despite this modification, our results were consistent with those of Gadoury and Pearson (20) (i.e., we could detect significant differences among isolates on the resistant labrusca host, but not on the more susceptible host, *V. vinifera*). A major difference between our study and that of Gadoury and Pearson (20) is that we found significant differences in aggressiveness among isolates from different hosts and geographic origins. The most aggressive isolates were those from *V. aestivalis*, which produced larger lesions than isolates from the rest of the hosts (except for those from *V. labrusca*); their mean latent period was also significantly shorter than that of isolates from *V. riparia*. Although *V. aestivalis* is a common wild species in the eastern United States, most of the isolates we sampled were from the Appalachian Mountains, from relatively undisturbed and isolated environments. The distances between some sites where we sampled isolates from *V. aestivalis* in the southeast were more than 200 km (e.g., Blood Mt., GA and Marion, NC). In addition, isolates from *V. aestivalis* are not all clonal but belong to at least

two different multilocus haplotypes (3). These findings exclude the possibility that we tested a single aggressive clone of *V. aestivalis*, which could have biased our results.

Few reports have compared the selective effect of quantitative resistance on pathogen aggressiveness. Gandon and Michalakis (22) hypothesized in their model that increased quantitative host resistance will select for higher pathogen aggressiveness. Cowger and Mundt (8) showed in field trials that individuals of *Mycosphaerella graminicola* with the highest level of aggressiveness were found on the more resistant cultivars. This phenomenon was also observed in *Lettuce mosaic virus* in lettuce (43). Although little is known about the mechanisms of quantitative resistance in wild *Vitis* spp., Fung et al. (18,19) recently quantified the expression of defense-related genes in the hybrid cv. Norton (which they referred to as being *V. aestivalis*). Representative PR genes, including PR-2 and PR-3, were transcribed constitutively at higher levels and salicylic acid was found at higher levels in cv. Norton leaves than in *V. vinifera*. Additional expression profiling of different defense-related genes from labrusca hybrids, *V. riparia*, and *V. rotundifolia* and correlating them with aggressiveness measures of the pathogen populations might provide interesting findings on the possible connection between quantitative resistance in *Vitis* spp. and aggressiveness in *E. necator*.

Although *V. vinifera* is severely affected by powdery mildew wherever it is grown, individuals we sampled from this species were not significantly different in aggressiveness than those from other host species. This result may be related to the fact that *V.*

Fig. 5. Mean aggressiveness components **A**, latent period and **B**, lesion size for isolates of *Erysiphe necator* from two geographic regions in the eastern United States: southeastern isolates ($n = 33$, solid bars) and northeastern isolates ($n = 24$, open bars) measured on two inoculated hosts: *Vitis vinifera* ‘Cabernet Sauvignon’ and labrusca hybrid ‘Niagara’. Lesion size was measured 13 days after inoculation. Vertical lines represent the standard errors and asterisks indicate significant differences between regions ($P < 0.01$).

vinifera is extremely susceptible to *E. necator*. The fact that no significant differences were detected between isolates from the different source hosts after inoculating them on *V. vinifera* supports the speculation that there is no significant selection on this host against individuals that were less aggressive on other *Vitis* spp. However, this hypothesis could be tested independently with genetic markers to assess the genetic structure of and estimate gene flow among populations of *E. necator* from *V. vinifera* compared with those from other *Vitis* hosts. Genetic markers and techniques for testing this type of hypothesis are currently being developed (*unpublished results*).

This research also found significant differences in aggressiveness between populations from different geographic regions because isolates from the southeast produced larger lesions than those from the northeast. This finding is consistent with inferences based on multilocus sequencing of restricted gene flow between populations of *E. necator* from commercial vineyards between these two regions (3). Diverse host–pathogen systems such as the plantain powdery mildew pathogen *Podosphaera plantaginis* and its host *Plantago lanceolata* (28), the yellow rust pathogen *Puccinia striiformis* f. sp. *tritici* and wheat in France, and the plant *Lithophragma parviflorum* and the floral-parasitic moth *Greya politella* in the northwestern United States (47) demonstrated that current co-evolutionary selection and interspecific interactions, including host–pathogen interactions, can be highly divergent across both narrow and wide geographic scales. Climatic factors differ markedly between the northeastern and southeastern United States. Many environmental factors such as summer and winter temperatures, wind, rain events, and UV radiation all potentially influence survival and dispersal of both sexual and asexual stages of *E. necator* (9,50). In addition, the influences of these environmental factors on aggressiveness are well documented in other pathosystems. Thus, environmental influences on the ecology of *E. necator* may be fertile ground for further research. One issue which must be taken into consideration is the fact that the composition of *Vitis* spp., both wild and cultivated, differs between the northeast and the southeast. Except for *V. labrusca* and *V. vinifera*, sampling from other host species was unbalanced because *V. rotundifolia* does not grow in the northeast and *V. riparia* was uncommon in the sampling areas in the southeast. Therefore, we must be cautious when making conclusions about the differentiation of populations based on geography because it might be confounded by source hosts. On the other hand, the fact that isolates from *V. aestivalis* from the northeast were less aggressive than those from the southeast, the relatively short-distance dispersal of the pathogen by wind and rain (50), and the potential for selection by environmental factors is consistent with the potential for such differentiation by restricted gene flow. This possible source of host $\times$ geographic region interaction will surely need to be explored further.

The wild *Vitis* spp. in the eastern United States have been used for many years for pest and disease resistance breeding programs. However, complementary research on the genetics and biology of wild pathogens, and especially *E. necator*, are rare (20). We believe that a greater understanding of wild populations of *E. necator* would be beneficial for improving the collection of wild *Vitis* spp., screening for resistance, and contributing to the assessment of durability. *E. necator* is present in diverse geographic areas and landscapes and on wild *Vitis* hosts. In many places, we detected single plants late in the growing season with high severity of powdery mildew while adjacent plants of the same species were mildew-free. Despite the possibility that microclimatic parameters may explain part of these observations, we suggest that collecting germplasm under these conditions would allow a collector to distinguish between putatively resistant and susceptible individuals. Results of our study suggest the importance of including isolates from *V. rotundifolia* in screening for powdery mildew resistance trials of new muscadine cultivars and, more

important, of new *V. vinifera* $\times$ *V. rotundifolia* hybrids. Similarly, cultivars being genetically modified with transgenes from *V. rotundifolia* should also be screened with isolates of *E. necator* from *V. rotundifolia* to test whether they have the ability to overcome resistance.

We also showed variation among *E. necator* isolates for two quantitative components of aggressiveness. Because quantitative resistance is likely to play a prominent role in future breeding programs (14), it might be important to assess resistance against diverse *E. necator* populations, including challenging breeding material with *E. necator* isolates collected from wild species such as *V. aestivalis*, because they are more aggressive than those from other host species and may include diverse mechanisms with the potential to break the qualitative host resistance.

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