

Genotype, environment, and genotype by environment interactions determine quantitative resistance to leaf rust (*Coleosporium asterum*) in *Euthamia graminifolia* (Asteraceae)

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Summary

- The strength and consistency of genotypic differences in disease resistance determine the potential for resistance evolution in host populations that rely on vegetative reproduction. Here we surveyed infection intensity of host genotypes across space and time to estimate genotypic and environmental effects on quantitative disease resistance.
- Cloned fragments of 12 *Euthamia graminifolia* genotypes were grown in unweeded experimental fields and outdoor pots. Infection intensity was surveyed during 2 yr of natural infection by the non-systemic rust pathogen, *Coleosporium asterum*.
- Five of six surveys detected infection intensity differences among genotypes, despite substantial variation in mean infection intensity across surveys. When resistance was defined relative to local pathogen density, 10–40% of resistance variation was due to host genotype. Although two genotypes exhibited greater resistance across environments, G × E interactions in resistance were common. Furthermore, infection intensity was unrelated to host size.
- We conclude that quantitative resistance level can evolve in this system and show how logistic analysis (relative to local pathogen density) can provide insight into the mechanism(s) responsible for G × E interactions in infection intensity.

Key words: clonal plant, environmental variation, fungal pathogen, genotypic basis, logistic analysis, quantitative disease resistance, *Euthamia graminifolia* (= *Solidago graminifolia* (Goldenrod)), *Coleosporium asterum*.

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Introduction

Most of what we know about the evolution of plant resistance to pathogens comes from studies of agricultural species or their wild relatives that address race specific, qualitative, gene-for-gene resistance mechanisms (Flor, 1971; Burdon, 1987; Simms, 1996). In these systems, host genotypes are either resistant to a specific pathogen race because they can recognize and eliminate it via the hypersensitive response, or susceptible because the pathogen is not recognized until after the disease is established, if at all (Staskawicz *et al.*, 1995). Inoculation of test plants with one or more pathogen races provides a resistance profile for each genotype. Annual changes in the frequency of host individuals

resistant to particular pathogen races and of races capable of infecting particular host genotypes have provided evidence of co-evolution of resistance and virulence (McDonald *et al.*, 1989; Thompson & Burdon, 1992).

Although there is increasing evidence that race specific resistance operates in natural pathosystems (Burdon *et al.*, 1996; Espiau *et al.*, 1998), it is likely to be difficult to generate (Clarke, 1997), and its effects short-lived (Wolfe & McDermott, 1994). Furthermore, gene-for-gene mechanisms may be of limited importance in natural populations where resistance is expressed as a quantitative trait (Barrett, 1985; Parker, 1992; Burdon, 1997). Quantitative resistance reduces the rate and extent of pathogen development and is characterized by a continuum

of disease severity across host individuals. Despite the recognition that this race non-specific form of resistance occurs in all plant species (Burdon, 1997), little is known about its genetic control (Burdon, 1987) or impact on fitness (Alexander, 1992).

Quantitative resistance to pathogens results from polygenic traits that affect the frequency of host penetration, the rate of development from spore to lesion, and/or the fecundity of those lesions in a race non-specific manner (Parlevliet, 1979). Frequency of host penetration can be influenced by cuticle thickness (Hooker, 1967), trichome and/or stomatal density (Shaik, 1985; Zaiter *et al.*, 1990), and stomatal structure and function (Romig & Caldwell, 1964). To our knowledge, traits that slow the rate of disease development and reproduction have not been explicitly identified, but may be associated with constitutive secondary compounds or rates of nutrient transfer. The effects of these traits, however, have been frequently observed and utilized in breeding for durable resistance (Szteinberg & Wahl, 1976; Milus & Line, 1980; Lee & Shaner, 1984; Mehan *et al.*, 1994).

Mechanisms of quantitative, race non-specific resistance may be of exceptional importance when gene-for-gene resistance mechanisms are less effective and/or less likely to develop. Long-lived clonal species attacked by heteroecious (host-alternating) rust fungi are one potential example. Selection on obligate alternate hosts (Agrios, 1997) and/or dispersal from great distances may make pathogen virulence structure extremely unpredictable (Roelfs, 1986). Even in cases where pathogen populations are localized, clonal species that depend on vegetative reproduction for establishment and perpetuation of their populations (initial seed recruitment species: 60% of clonal species, Eriksson, 1989, including *Euthamia graminifolia*, see Price, 2003) may be unable to respond to changes in pathogen racial structure due to limited sexual recombination. Thus we might expect generalized, quantitative resistance to be of greatest importance in long-lived clonal species that host heteroecious pathogens. It is especially important to understand the effects of disease on clonal plant species because they dominate many terrestrial plant communities (Silander, 1985).

In order to assess the importance of quantitative resistance in natural populations of clonal plants, resistance must be measured on randomized replicates of genotypes in ecologically relevant conditions (Alexander, 1992). Quantitative resistance level is often defined in terms of infection intensity: plants with lower infection intensity are characterized as possessing greater resistance. This definition is appropriate when pathogen pressure is equal for all plants, but is of less value when there are microsite differences in disease pressure, which appear to be common for fungal pathogens in field settings (Dinoor & Eshed, 1990; Jarosz & Davelos, 1995; Giesler *et al.*, 1996; Morrison, 1996).

One way to control for this variation is to inoculate plants with large equal doses of the pathogen (Morrison, 1996). Although such studies are ideal for identifying qualitative

resistance, quantitative responses to natural infection are often uncorrelated to those observed after inoculation (Hooker, 1967; Alexander, 1989; de Nooij *et al.*, 1995). Inoculation may overwhelm or circumvent quantitative resistance traits that affect host penetration (e.g. Wynn, 1976), especially since it is often performed under ideal disease establishment conditions. An alternative method of eliminating the effects of variation in disease pressure in quantification of resistance is to allow for variation in spore abundance among individuals, but to define resistance relative to that variation. We used this approach, allowing for natural establishment and spread of disease in experimental field populations, and defining resistance relative to neighbour infection intensity.

In this study, we examined whether *Coleosporium asterum* rust infection intensity varies among genotypes of *E. graminifolia* (Asteraceae). Replicates of 12 *Euthamia* genotypes were grown in experimental fields and in pots outdoors. These plants experienced natural disease spread over sequential 2-yr periods. We asked the following questions: (i) Do genotypes differ in rust infection intensity? (ii) How consistent are resistance levels among clonal replicates within individual sites? (iii) Are genotypic differences in resistance level consistent across space and time? Answers to these questions will allow assessment of phenotypic variation in resistance and its genotypic basis: two of the three components necessary for evolution of resistance in the *Coleosporium/Euthamia* pathosystem. For an assessment of the third component, fitness effects of infection, see Price (2003).

Study system

E. graminifolia L. Nutt. (Asteraceae), the flat-topped goldenrod, is a rhizomatous perennial herb that was formerly classified as a member of the genus *Solidago* (formerly *Solidago graminifolia*, Gleason & Cronquist, 1991). Unlike the common goldenrod (*Solidago canadensis*), *E. graminifolia* is a guerilla-type species (*sensu* Lovett Doust, 1981), characterized by rapid clonal spread via rhizomes and almost no recruitment from seed in established populations (Price, 2003). It prefers moist rich soil, but is found in almost all soil types, especially along roadsides, fallow fields and open woodlands (Deam, 1940). *E. graminifolia* stems senesce in late autumn, leaving rhizomes to overwinter below ground. New stems emerge from rhizome buds in spring, and new rhizomes are initiated over the next few months. All rhizome connections to the base of the prior year's stems break down by mid-summer and aboveground growth ceases as flowering begins in late July. Infection of leaves by the goldenrod rust fungus (*C. asterum*) can occur as early as June, but usually does not spread in the host population until August. Thus, the fungus spreads through the population after stems have become disconnected individuals and have reached their final size.

C. asterum (Diet.) Syd. (Uredinales: Coleosporiaceae) is a non-systemic, heteroecious (host-alternating), macrocyclic

(five spore stage) rust fungus whose spermagonial and aecial (alternate) hosts are two-needle *Pinus* species (Hedgecock, 1928). In early summer, aeciospores colonize populations of a number of species in the Asteraceae (Cummins, 1978). Disease is then manifested in mid- to late summer as a few initial point infections amplify through short-distance dispersal of urediospores among neighbouring plants. It is this bright orange uredial (asexual, repeating) stage, which allows for exponential growth of the pathogen population and can reveal patterns of host quantitative resistance. Infection of *E. graminifolia* individuals differs in degree rather than by presence vs absence. By the end of the growing season, almost all plants in diseased populations are infected, but a great deal of variation in the intensity of disease among individuals is common (Guba, 1937).

Materials and Methods

Experimental design

Four populations (> 100 ramets each: Hardin Ridge [39°05'85" N, 86°25'35" W], Griffy Lake [39°12'06" N, 86°30'27" W], Friendship Road [39°09'16" N, 86°24'71" W], and Kent Farm [39°09'00" N, 86°23'72" W]) of *E. graminifolia* in Monroe Co., IN, USA, separated by a minimum of 1.5 km, served as sources of genotypes for this study. In September 1995, three clumps of stems (each ≤ 60 cm diameter) were collected from each population. Within a population, clumps of stems were assumed to represent different genotypes because they were separated by distances of > 5 m, although connections within clumps had disintegrated by the time they were collected. Isozyme and AFLP gels have confirmed genetic differences among some of the putative genotypes (LeMaster & Price, unpublished data), but comprehensive tests of all 12 were not performed due to problems with stability of the plant extract. In addition, repeated occurrence of infection intensity differences based on randomly placed replicates of each clump in our experimental populations support the assumption that these clumps were indeed distinct genotypes.

Rhizomes from each clump were cut into 10-cm segments and repotted into 12.5-cm diameter pots in October 1995 and again in 1996. By spring 1997, this procedure produced more than 40 replicates of each of the 12 genotypes. Hereafter, each of these 480 units or its clonal progeny is referred to as a fragment, i.e. one of many vegetatively propagated fragments of a genet. Each fragment was made up of one to many clustered but often unconnected stems (a.k.a. ramets) derived from a single 10-cm piece of rhizome at the beginning of the study. Thus, each fragment is a replicate of a single genotype in a distinct location.

In the spring of 1997 two 18 × 18 m experimental fields owned by Indiana University Bloomington (IUB, Monroe Co., IN, USA) were ploughed and disced. The fields are more than 4 km apart and have different soil types, use histories, and vegetation. The IUB Tenth Street botany experimental field

([39°10'58" N, 86°30'31" W], hereafter Hilltop field) contains heavy clay soil and has been used for research for decades. It is maintained as a regularly mowed herbaceous community when not in use. After ploughing, its seed bank produced a relatively dense flora, dominated by *Setaria*, *Daucus*, *Rumex*, *Plantago* and *Trifolium* spp. The IUB Bayles Road botany experimental field ([39°13'24" N, 86°32'47" W] hereafter Bayles field) is an agricultural field of silty clay loam, which was cultivated regularly until the beginning of this study. The vegetation that emerged after ploughing was relatively sparse with *Sorghum*, *Solidago*, and *Erigeron* as the dominant genera.

A set of 20 fragments of each of the 12 genotypes ($n = 240$) was planted into each field (Hilltop & Bayles) as juveniles (single stems *c.* 25 cm tall). Fragments were planted in a hexagonal array using a randomized block design such that fragments were 90 cm away from their nearest neighbours. Each field consisted of five blocks, each of which contained four fragments of each of the 12 genotypes. Transplants were watered for 2 weeks after planting, and bindweed (*Convolvulus* spp.) was removed from their stems throughout the season. The fields were otherwise left unweeded for the next 3 yr to mimic natural levels of interspecific competition and humidity. The latter is necessary for spore germination and hyphal penetration by *C. asterum* (Heath, 1992).

In 2000, after further vegetative propagation of the 12 genotypes, they were used in a complementary design in the fenced enclosure at Hilltop field (hereafter Hilltop pots). Single growing stems (*c.* 60 cm tall) and 10 cm of associated rhizome of 12 fragments of each genotype ($n = 144$) were transplanted into 3.8-l round pots in July 2000. Pot location was randomized within a single rectangular block (*c.* 6.75 m²); infectious spread of *C. asterum* was facilitated by leaving no space between pots. After most fragments were infected, pots were separated to allow for data collection (20–50 cm to nearest neighbour). These plants were watered one to two times per day and fertilized with Peters 20-20-20 fertilizer in early August each year. After overwintering in a cinderblock cold frame covered with straw, plants were repotted into 7.6-l round pots (May 2001), and exposed to another season of natural infectious spread, as before.

Quantifying infection intensity and resistance

Rust infection intensity scores for each stem were based on a visual estimate of the photosynthetic leaf area covered by orange rust pustules (0–10 scale). Estimates made by two people during the first season confirmed that relative differences among stems were consistent across observers (data not shown). All future infection intensity measures were made by the first author (JP), and fragment genotype was recorded elsewhere to avoid bias.

Before analysis, infection intensity scores were converted to percent leaf area infected (% LAI) using a modified logarithmic scale (Horsefall–Barrett system, Horsefall & Cowling, 1978).

The Horsefall–Barrett system is based on the Weber–Fechner law, which states that visual acuity is proportional to the logarithm of the intensity of the stimulus, and on the realization that the eye perceives the area of the colour that is in the minority. Thus, the classes diverge from 50% with a ratio of two. This results in the following classes: 0 = 0%, 1 = 0–3%, 2 = 3–6%, 3 = 6–12%, 4 = 12–25%, 5 = 25–50%, 6 = 50–75%, 7 = 75–88%, 8 = 88–94%, 9 = 94–97%, 10 = 97–100% (Horsefall & Cowling, 1978). Operationally, infection intensity scores were converted to percentage LAI by assigning the mean percentage of each class to stems that received the corresponding score.

Any measurement of levels of quantitative resistance requires measurement of levels of natural enemy damage (Berenbaum & Zangerl, 1992). For non-systemic foliar pathogens, resistance level is directly inversely proportional to infection intensity when pathogen propagules are uniformly distributed throughout the population. However, uniform distribution is very unlikely under conditions of natural disease spread when leaf area is not a limiting factor of infection (Jarosz & Davelos, 1995). In order to address resistance more directly in the presence of significant epidemiological variation within and between sites and years, we attempted to exclude variation due to the location of initial disease foci and the resistance level of neighbouring plants. We operationally defined fragment resistance level as its percent leaf area infected (% LAI) minus the average percentage LAI of its neighbour fragments. Data from a small spore trap survey suggest that most *C. asterum* urediospores fall within 1 m of the source plant in the field. No spores were found 1.5 m from source plants, while a mean of 5 spores cm⁻² was found at 0.5 m ($n = 6$ plants, $t = 3.26$, $P = 0.022$; Lasky & Price, unpublished data). Thus neighbours were defined as all fragments within 1 m of the focal fragment. For potted surveys, mean percentage LAI of the block was used as a surrogate of mean neighbour infection intensity since plants were packed close together during disease spread. Thus both infection intensity and resistance level were measured in terms of percentage LAI: infection intensity is an absolute measure, whereas resistance level is defined relative to neighbour infection intensity (i.e. an estimate of local spore density).

Statistical analysis

Although a number of studies of clonal species have used individual ramets/stems as their unit of replication (Swedjemark *et al.*, 1998; Piqueras, 1999; Cronin & Abrahamson, 2001), stems in the same clonal fragment are unlikely to be statistically independent. Even when they have no physical connection to other stems, as is the case for *E. graminifolia* during disease spread, the spatial structure of the biotic and abiotic microenvironment prevents independence due to the co-location of stems of the same fragment. As such, all analyses of genotypic variation were performed based on

fragment infection intensity (often mean values from a number of stems). Recall that a fragment is a replicate of a single genotype in a distinct location in a field or enclosure.

Variation in infection intensity among genotypes Random effects nested analyses of covariance were used to assess the role of within- and among-population genotypic variation in determining infection intensity. Population was treated as a random factor because we had no reason for choosing these specific populations other than that we knew of their existence (Underwood, 1997). Genotype nested within population was treated as a random factor because the genotypes were chosen without regard to specific characteristics and because we wished to make inferences about the effect of within-population genotypic variance in other populations. Total stem length (i.e. the sum of the height of all stems in a fragment) was included as a covariate because a fragment's size could affect the probability that it would be colonized.

PROC GLM in SAS (SAS Institute, 1988) was used to test the hypotheses that block, total stem length, origin population, and genotype (nested within population) explained a significant amount of the variance in percent leaf area infected. This test utilizes Type III sums of squares and accommodates unbalanced datasets by adjusting the weights of the components of the error mean squares. In addition, percent leaf area infected was arcsine square root transformed to increase homogeneity of variance. Transformed infection intensity means were back-transformed before reporting. The proportion of variance accounted for by each main effect was determined using the VARCOMP procedure in SAS. Variance components for the total stem length covariate were calculated by dividing its Type III sum of squares (SS) by the corrected total SS from each overall model. Ryan–Elliot–Gabriel–Welch (REGWQ) post-hoc tests, which limit Type I experiment-wise error (Lindman, 1992), were used for each survey to identify the origin populations whose genotypes varied in infection intensity.

In order to determine whether a single infection survey effectively represented relative infection intensity over an entire season, we used Kendall's coefficient of concordance (W_c). W_c assesses the degree of agreement among rankings, and is related to the mean value of all possible Spearman rank correlations between variables (Zar, 1999). This statistic provided a conservative test of the hypothesis that there is no association among the infection intensity rank of genotypes on different dates during a single season.

Genotypic basis of resistance level In order to address the genotypic basis of resistance level both within and among site/year combinations, we calculated the infection intensity of each fragment relative to the average infection intensity of its neighbours: our operational definition of resistance (see above). This definition removes the effect of chance differences in location and timing of initial disease foci from our measure

Table 1 Summary of sampling history and peak *Coleosporium asterum* infection intensity on *Euthamia graminifolia* plants over a period of 5 yr on weeded experimental fields or outdoor pots

Year	Site	Survey date	Overall stem density	Mean infection intensity [CV(%)]	n fragments surveyed	Portion of fragment surveyed
1997	Hilltop field	20 September	1.0 m ⁻²	8.6% [68.9]	237	All (1 stem)
	Bayles field	–	1.0 m ⁻²	0	0	–
1998	Hilltop field	15 September	5.5 m ⁻²	9.6% [65.3]	112	All (1–19 stems)
	Bayles field	28 July	10.6 m ⁻²	53.0% [37.6]	238	Part (1–25 stems)
1999	Hilltop field	–	36.2 m ⁻²	14.4% [92.0]	n/a	n/a
	Bayles field	25 September	2.1 m ⁻²	5.0% [93.3]	181	Part (1 stem)
2000	Hilltop pots	9 August	23.2 m ⁻²	54.1% [35.5]	145	All (1 stem)
2001	Hilltop pots	15 August	88.5 m ⁻²	45.4% [56.7]	120	All (1–15 stems)

of resistance, in addition to taking into account the large differences in overall infection intensity among surveys.

We calculated the clonal repeatability of resistance for each of the six surveys using one-way ANOVAS (r_{approx} , see Lessels & Boag, 1987). Because some portion of the environmentally induced differences in resistance could be transmitted to all clonal descendants (i.e. V_{Eg}), clonal repeatability should be regarded as an upper bound of the degree of genetic determination of a trait (Lynch & Walsh, 1998, but see Dohm, 2002). We minimized the size of V_{Eg} by using clonal fragments that had been reared in the greenhouse for two generations, which greatly decreases maternal environmental effects (Schwaegerle *et al.*, 2000).

We assessed the effects of seasonal and spatial variation on resistance level separately, with one nested ANOVA comparing the two experimental field sites in the same year, and three nested ANOVAS comparing the 2 yr of data from each site. Site and year are commonly included as factors in an overall ANOVA, but we could not use this approach because site and year could not be treated as independent factors. They were confounded because no data were collected from Bayles field in 1997 or Hilltop field in 1999, due to absence of infection and intermingling of genotypes, respectively (see Results section). For the between site and year ANOVAS, all effects were treated as random factors, as before. The SAS random *var*/test command was used to test for main effects of origin population, genotype (nested within population), and site or year, and for the appropriate interactions. No transformations of resistance level values were necessary.

Results

Infection history of study populations

Mean infection intensity in the four experimental field surveys varied from 5 to 53% (Table 1). In 1997, Hilltop field (HF) had low overall infection while Bayles field (BF) field was not infected by rust. Conversely in 1998, Bayles field experienced epidemic disease conditions, but infection at

Hilltop field was quite low. Both fields were infected in 1999, but growth of fragments at Hilltop field had resulted in intermingling of genotypes, making identification of each stem's genotype impractical. Fragments at Bayles field were less extensive in 1999, allowing for genotype-based intensity estimates there. As a result, experimental field infection intensity surveys were performed in overlapping (but not simultaneous) 2-yr periods (HF97-98, BF98-99, Table 1).

Although they fell within the range of field values, potted plants generally had higher infection intensities than field plants (Hilltop pots: HP00-01: 45–54% LAI). This was expected given their high stem density during infectious spread (23–89 stems m⁻²), and because plant foliage was wetted during watering at least once per day.

The six surveys can be divided into two groups: low stem density, low infection (HF97-98, BF99; $7.7 \pm 1.4\%$ LAI [$\bar{x} \pm \text{SE}$]), and high stem density, high infection (BF98, HP00-01, $50.8 \pm 2.7\%$ LAI [$\bar{x} \pm \text{SE}$]). Because ambient humidity, soil texture, and soil moisture also varied widely among these surveys, the estimates of the strength of association between genotype and infection intensity reported here should encompass the range of possible values.

These survey data suggest that *C. asterum* populations have little continuity across years. For one, there was large year-to-year fluctuation of infection intensity at Bayles field (greater than 10-fold, Table 1). In addition, even though Hilltop field 1997 had considerable infection, pathogen levels did not increase substantially in 1998, despite weather conditions that lead to an epidemic at Bayles field. Furthermore, infection intensity was not easily predictable from stem density in a given year, as might be inferred from the Bayles field data: Hilltop field 1999 had by far the highest density (among fields), but only a moderate level of infection (Table 1).

Variation in infection intensity among genotypes

To determine whether there were differences in infection intensity among genotypes under any disease or environmental conditions,

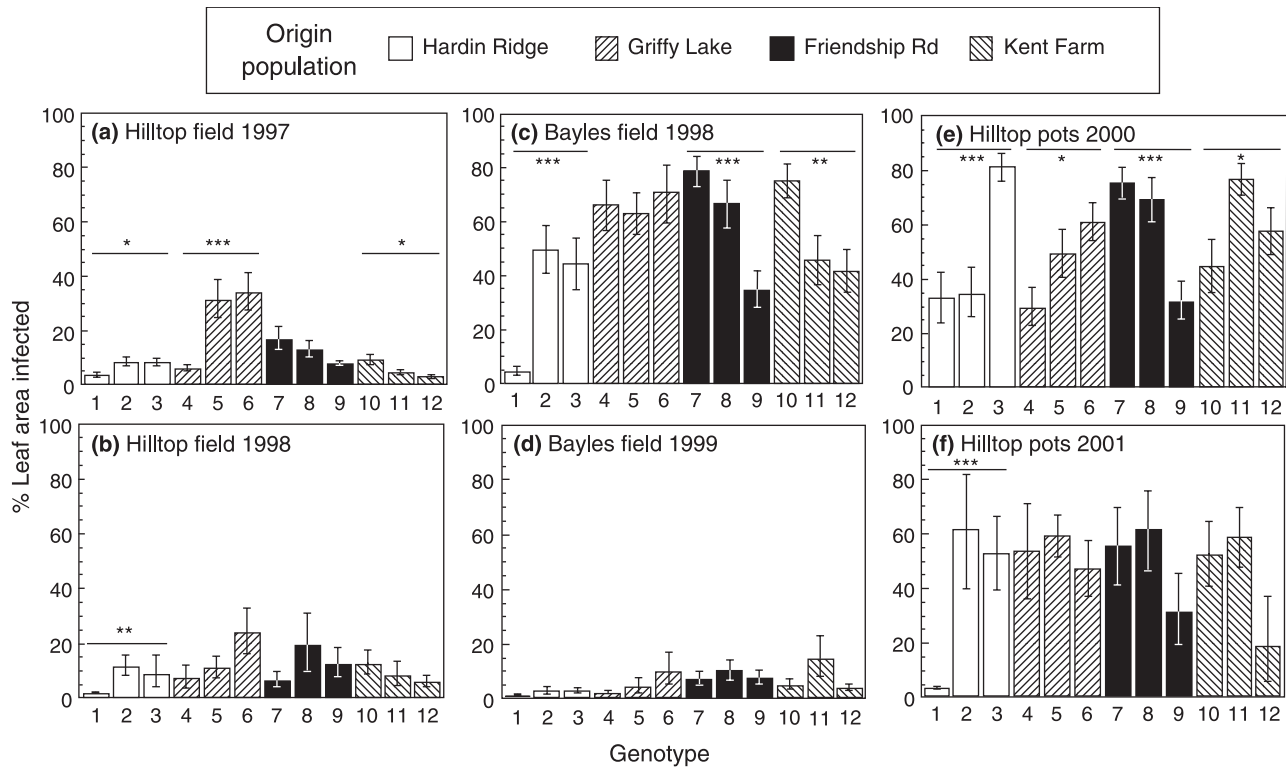


Fig. 1 Back-transformed means \pm SE of *Coleosporium asterum* infection intensity of 3 genotypes of *Euthamia graminifolia* from each of 4 origin populations ($n = 6.20$ replicates per bar). (a–d) Surveys of clonal fragments from two experimental fields in overlapping, but not simultaneous years. (e–f) Surveys from potted replicates of the same genotypes over 2 yr. Asterisks indicate significant differences among genotypes within populations: * $P = 0.05$; ** $P = 0.01$; *** $P = 0.001$. No significant differences were detected among populations (Table 2).

we analysed data from each particular site/year combination separately. The number of fragments per survey varied greatly because in 1998, half of the fragments at Hilltop field were used for a fungicide treatment in a fitness assay, and a quarter of the Bayles field fragments died. Mortality also resulted in a loss of 16% of the potted fragments between years. In addition, the number of individual stems that comprised a fragment varied markedly as stem number increased within fragments: some surveys involved only single-stem fragments, while in other surveys, fragment size ranged from one to 25 stems (Table 1).

By site-year combination Infection intensity was affected by genotype (nested within population) in three of the four experimental field surveys (Fig. 1a–d). The absence of a significant difference for Bayles field 1999 may have been due to the low overall infection intensity in this survey ($5.0 \pm 4.6\%$ LAI \pm SE). Genotype accounted for 9–22% of the variance in infection intensity in these experimental field surveys (Table 2). By contrast, population of origin had no detectable effect in any site year combination ($0.08 < P < 0.72$). On average, genotypic differences within populations explained three times more of the variance in infection intensity than origin population. The within-population effect was present in multiple origin populations in two of the three surveys in which it was significant (Fig. 1a,c).

Potted fragments surveyed in 2000 and 2001 showed a similar strong effect of genotype nested within population (Fig. 1e,f). Origin population did not influence infection intensity in either year ($P = 0.76$, Table 2), whereas genotype explained 33% and 21% of the variance in 2000 and 2001, respectively ($P = 0.004$). Potted plant infection intensity varied among genotypes within all four origin populations in 2000 (Fig. 1e), and among genotypes from one population in 2001 (Fig. 1f).

There was no effect of the fragment size covariate (total stem length) in three of the four experimental field surveys ($P > 0.18$) or in either of the potted surveys ($P > 0.24$). For the one case where total stem length was significant, it explained only a small amount of the variance in infection intensity (6.6%, HF98, Fig. 2). Furthermore, the effect was negative, contrary to the expectation that bigger fragments would have higher infection intensities. These results show that the effect of genotype on infection intensity is largely independent of fragment size.

Within a season After finding that infection intensity differed among genotypes based on surveys taken at the seasonal infection peak, we wanted to determine whether a single survey adequately characterized infection intensity over an entire season. Fragment infection intensity was recorded every

Table 2 Individual nested ancovas and variance components of *Coleosporium asterum* infection intensity on *Euthamia graminifolia*. Infection intensity values were arcsine square-root transformed and converted to degrees before analysis

Location & Year	Source	d.f.	MS	F	P	Variance component (%)
Hilltop field 1997	Block	4	155.8	1.12	0.347	0
	Total stem length	1	104.6	0.75	0.386	0
	Population	3*	2205.2	3.24	0.081	12.3
	Genotype(pop)	8	701.1	5.09	< 0.001	13.2
	Error	210	138.5			
Hilltop field 1998	Block	4	447.7	3.21	0.016	3.4
	Total stem length	1	1259.5	9.04	0.003	6.6
	Population	3*	156.9	0.47	0.714	0
	Genotype(pop)	8	346.9	2.49	0.017	9.3
	Error	95	139.4			
Bayles field 1998	Block	4	8370.2	27.12	< 0.001	27.2
	Total stem length	1	173.2	0.56	0.455	0
	Population	3*	5231.8	1.75	0.234	4.5
	Genotype(pop)	8	3119.2	10.10	< 0.001	21.8
	Error	221	308.7			
Bayles field 1999	Block	4	42.8	0.29	0.881	
	Total stem length	1	266.0	1.83	0.178	
	Population	3*	631.6	2.57	0.126	
	Genotype(pop)	8	246.8	1.70	0.102	
	Error	164	145.4			
Hilltop pots 2000	Total stem length	1	400.2	1.42	0.236	0
	Population	3*	411.4	0.26	0.851	0
	Genotype(pop)	8	1574.6	3.58	< 0.001	33.1
	Error	132	282.3			
Hilltop pots 2001	Total stem length	1	629.6	1.06	0.305	0
	Population	3*	693.6	0.40	0.760	0
	Genotype(pop)	8	1779.4	3.00	0.004	20.5
	Error	107	592.4			

*Population was tested over genotype nested in population, resulting in denominator d.f. of \approx or \approx 8.

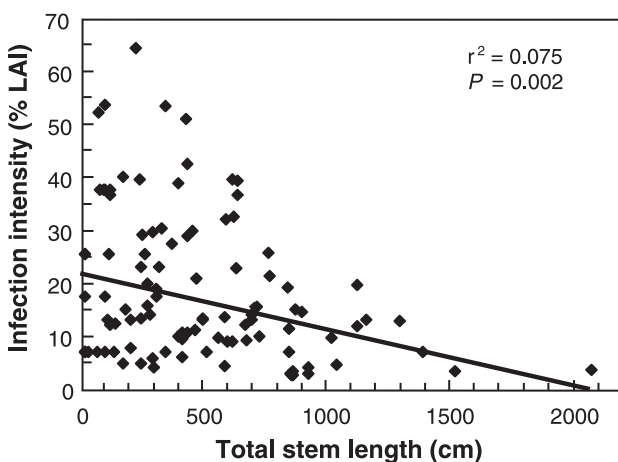


Fig. 2 Linear regression of *Coleosporium asterum* infection intensity vs size of *Euthamia graminifolia* fragments at Hilltop Field in 1998. Total stem length did not affect infection intensity in any of the other surveys (Table 2).

3 weeks at Hilltop field in 1997. Infection intensity was low during August and peaked in mid-September (Fig. 3). Similar phenological patterns occurred in the other surveys, although earlier infection peaks tended to coincide with higher infection intensity (see Table 1). Infection intensity ranks among genotypes were similar across the four sampling dates (Kendall's coefficient (\mathbf{W}_c) = 0.609, $\chi_{11}^2 = 26.8$, $P < 0.005$). When a comparison was made between the two samples taken after the disease had spread through the population (15 September and 6 October), genotype infection intensity rankings were even more similar ($r_{s,11} = 0.867$, $P < 0.001$). These results indicate that a single sample of infection intensity taken in the latter half of the season provides an adequate description of a genotype's infection intensity rank over the entire season.

Consistency of resistance level among genotypes

Genotypic variation in quantitative disease resistance may be an important underlying cause of the differences in infection

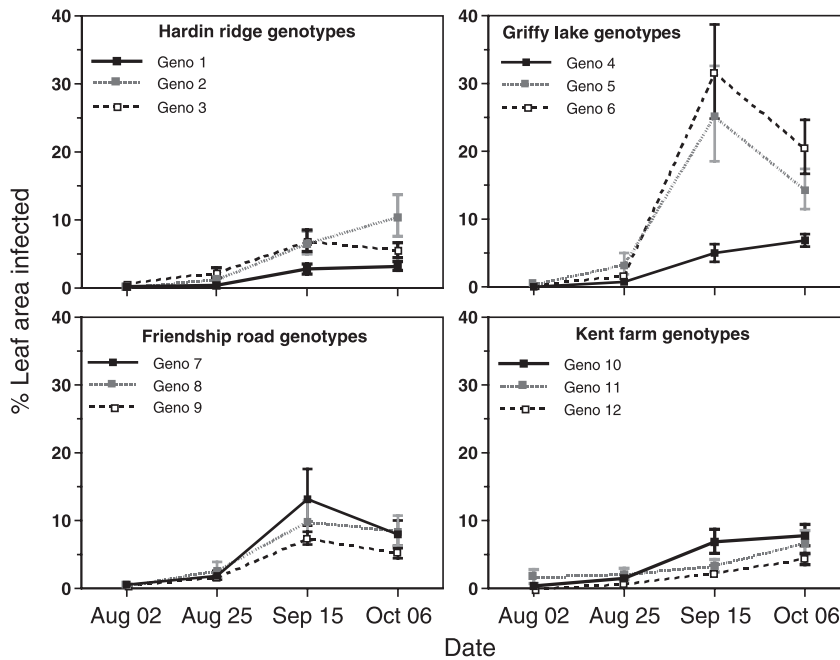


Fig. 3 *Coleosporium asterum* infection intensity of *Euthamia graminifolia* genotypes throughout the season at Hilltop Field in 1997. Each panel includes three genotypes from one origin population. Data points are means \pm SE of 17–20 replicates. Non-parametric analyses showed that infection intensity ranks were similar across sampling dates (see Results – within a season).

Table 3 Clonal repeatabilities of quantitative resistance to *Coleosporium asterum* in *Euthamia graminifolia* for experimental field and potted plant surveys (and their associated one-way ANOVAS of the effect of genotype on resistance)

Survey	Clonal repeatability	df Model	df Error	F	P
Hilltop field 1997	0.265	11	225	8.11	< 0.001
Hilltop field 1998	–	11	100	1.30	0.234
Bayles field 1998	0.389	11	226	13.65	< 0.001
Bayles field 1999	–	11	169	0.74	0.696
Hilltop pots 2000	0.276	11	138	5.78	< 0.001
Hilltop pots 2001	0.120	11	113	2.42	0.010

intensity among genotypes demonstrated above. Recall that we distinguish resistance from infection intensity by defining resistance as focal fragment infection intensity relative to neighbour infection intensity. First, we use repeatability estimates to assess the strength of the genotypic basis of resistance within each of the six site/year combinations. Then, to examine consistency across space and time, we combine resistance data across surveys to test the effect of site and year.

Within site/year combinations Estimates of the strength of the genotypic basis of resistance level (i.e. clonal repeatability) were significantly different from zero in four of the six surveys ($r_{\text{approx}} = 0.12\text{--}0.39$, Table 3). No genotypic basis for resistance was detected for two low disease pressure surveys of field plants with reduced numbers of replicates (due to an experimental treatment and fragment mortality at HF98 and BF99, respectively). The highest repeatability value came from a

high infection field survey (53% LAI, BF98), suggesting that genotypic effects are strongest under high disease pressure. The significant repeatability value for Hilltop field 1997, however, shows that genotypic variation in resistance level can be significant even when overall disease pressure is low (8.6% LAI).

Across sites and years Two genotypes were consistently more resistant than the other genotypes from their origin populations in high infection surveys. Genotype 9 averaged 10–20% less leaf area infected than its neighbours whereas the other two genotypes from Friendship Road averaged 10–20% greater leaf area infected than their neighbours (Fig. 4c, right half). Similarly, genotype 1 had an average intensity 20–50% below its neighbours, whereas the other two genotypes from Hardin Ridge averaged from 20% below to 25% above their neighbours (Fig. 4a, right half). Genotypes 1 and 9 were also on the resistant end of the spectrum in low disease pressure surveys (Fig. 4a,c, left halves). These patterns show that some genotypes were more resistant than others across a large range of environmental conditions.

On the other hand, the frequent crossing of resistance reaction norms suggests that genotype by environment interactions were also common (Fig. 4). Although we were unable to test for an overall genotype by site/year interaction (see Statistical analysis section), we did test for interactions of genotype with site or year independently. There was a significant site by genotype (nested within population) interaction among experimental fields (1998, $P < 0.001$, Table 4). In addition, the year by genotype (nested within population) interaction was significant at two of the three sites (BF: $P < 0.001$, and HP: $P = 0.029$, Table 4). Even given the large differences in disease pressure across sites and years, there was

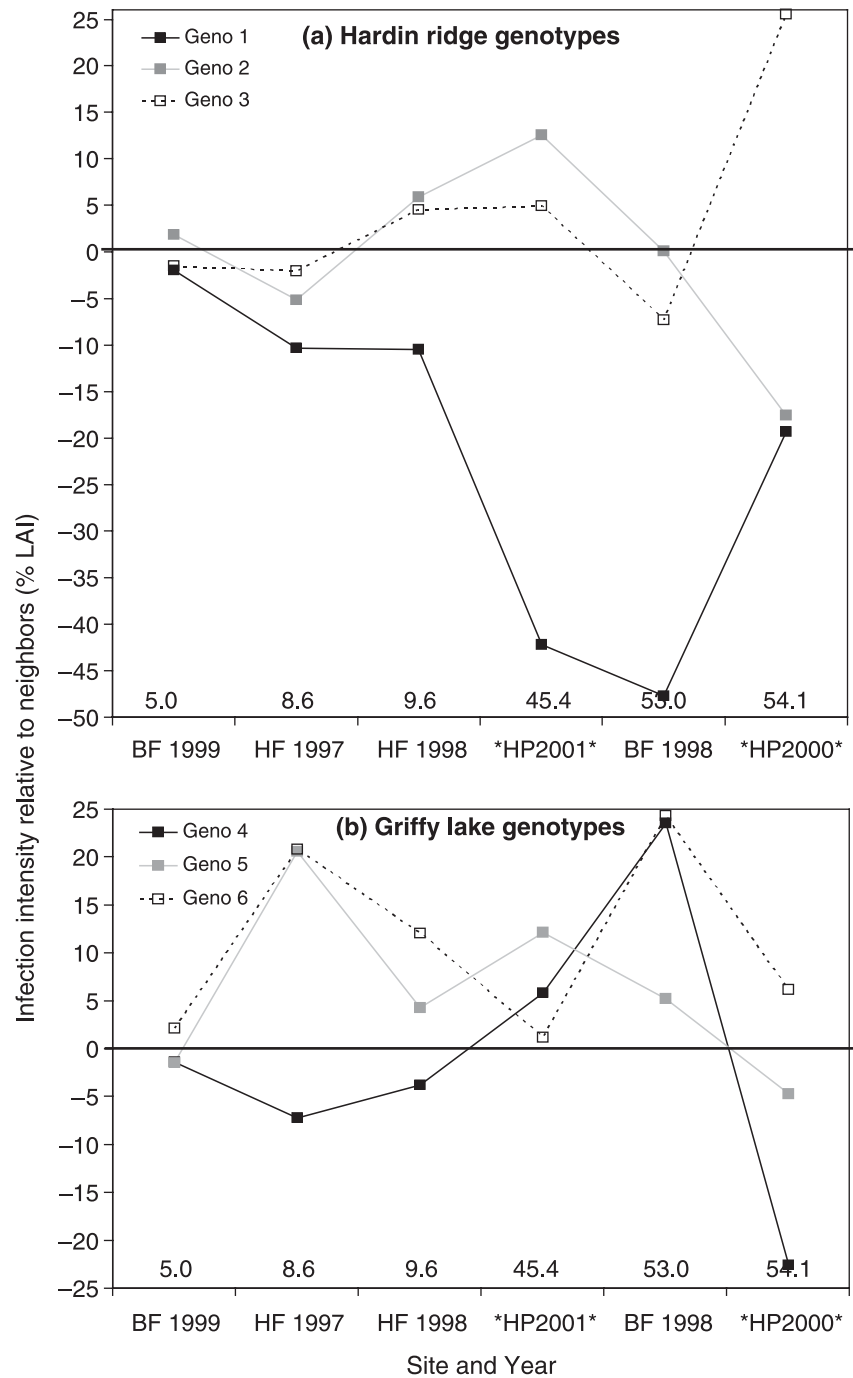


Fig. 4 Quantitative resistance reaction norms of *Euthamia graminifolia* genotypes to *Coleosporium asterum* in experimental fields (HF and BF) and outdoor pots (HP). (a–d) Mean resistance levels (y-axis: infection intensity–neighbour intensity) of genotypes from the four origin populations across six surveys. Surveys are ordered on the x-axis by increasing overall infection intensity (overall mean percentage LAI values for each survey are immediately above the x-axis). Genotype means falling near the zero line reflect the infection intensity of their neighbouring fragments, indicating no effect of resistance. Points falling below the line indicate a survey wherein replicates of that genotype were more resistant than their neighbours, and those falling above, more susceptible.

no detectable main effect of site or year in these analyses ($P > 0.81$) because the relative nature of our resistance measure removed these differences. In summary, while some genotypes appear to be resistant under a variety of conditions, resistance levels of others differed significantly across surveys.

Discussion

Many inoculation studies of non-agricultural species have revealed genotypic differences in infection intensity (Burdon,

1980; Burdon & Marshall, 1981; de Nooij & van Damme, 1988; de Nooij *et al.*, 1995; Ericson *et al.*, 2002). Our results add to others that verify that genotype is also commonly a factor in determining infection intensity under conditions of natural disease spread (Fig. 1; Alexander, 1989; Schmid, 1994; Davelos *et al.*, 1996). One way to attribute genotypic differences in infection intensity to genetic differences in resistance is to survey infection intensity of the same host genotypes over a number of locations and years. Evidence for resistance exists when the same genotypes have the lowest infection intensities

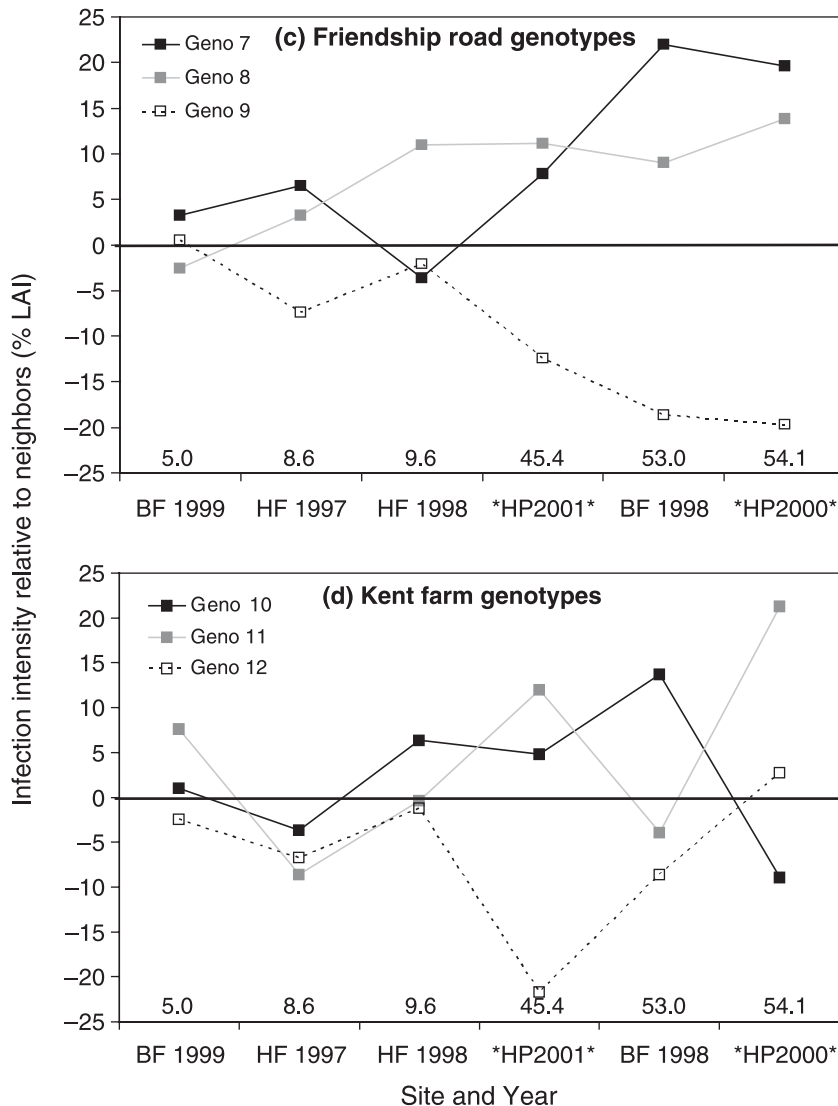


Fig. 4 continued

across surveys. Our results show that genotypes 1 and 9 regularly have lower infection intensity, than other genotypes (Fig. 1). This approach to assessing resistance is robust to among-survey differences in initial pathogen density, genetic makeup and environmental conditions. Stochastic epidemiological vary among locations and/or years. However, it can underestimate the magnitude of genotypic differences in resistance because it includes within-survey pathogen-based spatial effects in the unexplained variation in infection intensity.

To account for small-scale spatial heterogeneity and in pathogen pressure, resistance was defined as focal fragment infection intensity minus the mean infection intensity of its nearest neighbours. Given local dispersal of the repeating stage of the rust (see Materials and Methods section), this approach takes into account microsite differences in pathogen density and virulence (i.e. infectivity). Clonal repeatability of resistance defined in this manner (i.e. infection intensity relative to neighbours) accounted for twice the proportion of variance

in infection intensity explained by genotype (HF97, 0.27 vs 0.13; BF98, 0.39 vs 0.22). This shows that studies that do not account for microsite variation in disease pressure can underestimate the influence of genetically based resistance on infection intensity.

The significant clonal repeatability values for resistance (Table 3) show that genotypes have the potential to respond to natural selection for resistance if there are significant fitness costs of infection. For *E. graminifolia* and other early successional clonal species that show very limited seed recruitment in established populations (Meyer & Schmid, 1999; Price, 2003), vegetative reproduction will be of paramount importance. If disease results in reduced survival or vegetative reproduction of less resistant genotypes, especially during genet expansion (when the size of genets can increase exponentially and many die off, Hartnett & Bazzaz, 1985), it should cause an evolutionary increase in the average resistance level of future ramet generations (Van Kleunen & Fischer, 2003).

Table 4 Nested ANOVAS of year and site effects on *Euthamia graminifolia* resistance to *Coleosporium asterum* (infection intensity relative to neighbours)

Year	Source	Approximate error MS	df*	MS	F	P
1998	Population	genotype(pop) + year(pop) – year * genotype(pop)	3 (4.8)	4493	0.95	0.483
	Genotype(pop)	year * genotype(pop)	8	3722	2.14	0.151
	Site	year * pop	1	18	0.01	0.941
	Site * pop	year * genotype(pop)	3	2782	1.63	0.256
	Site * genotype(pop)	error	8	1739	3.48	< 0.001
	Error		320	500		
HF	Population	genotype(pop) + year(pop) – year * genotype(pop)	3 (5.5)	1339	0.80	0.541
	Genotype(pop)	year * genotype(pop)	8	1046	2.61	0.099
	Year	year * pop	1	73	0.07	0.808
	Year * pop	year * genotype(pop)	3	1043	2.62	0.120
	Year * genotype(pop)	error	8	401	1.44	0.178
	Error		333	279		
BF	Population	genotype(pop) + year(pop) – year * genotype(pop)	3 (3)	5594	0.94	0.519
	Genotype(pop)	year * genotype(pop)	8	3422	1.22	0.392
	Year	year * pop	1	29	0.01	0.946
	Year * pop	year * genotype(pop)	3	5311	1.91	0.207
	Year * genotype(pop)	error	8	2799	6.87	< 0.001
	Error		395	407		
HP	Population	genotype(pop) + year(pop) – year * genotype(pop)	3 (5.4)	1043	0.19	0.901
	Genotype(pop)	year * genotype(pop)	8	6164	3.15	0.063
	Year	year * pop	1	0	0.00	0.997
	Year * pop	year * genotype(pop)	3	1430	0.74	0.558
	Year * genotype(pop)	error	8	1958	2.19	0.029
	Error		251	895.2		

*df values in parentheses are denominators of the compound error mean squares.

Furthermore, when genotypic effects on resistance can be inherited by sexual offspring, resistance levels in other populations are also likely to be affected (Pan & Price, 2001).

Our observations also show that disease pressure varied widely over space and time. Overall infection intensity varied 10-fold across sites and years (Table 1). These differences are likely to be due to variation in pathogen virulence (i.e. infectivity), initial pathogen density, weather, and/or host density. Pathogens often vary in their ability to infect host populations and genotypes (Burdon, 1987; Alexander, 1992). Although this is of greater import in qualitative forms of resistance/virulence, it may also have contributed to variation in overall infection intensity among site/year combinations. Pathogen pressure can also vary due to initial aeciospore density, which apparently varies on a scale of kilometres in our system, since in 1997 no infection occurred at Bayles field, whereas, 3 km away, Hilltop field had *c.* 9% infection, even though weather and host density were similar at these sites. Much of the observed annual variation in overall infection intensity may be the result of stringent weather conditions necessary for growth of the fungal population. Nevo *et al.* (1991) found that significant rust infection of wheat requires a minimum number of degree days with high humidity. These conditions were guaranteed for our potted plant surveys, both of which had high overall infection intensity, because regular watering kept

their foliage damp. Finally, high host density seemed to increase the likelihood of an epidemic occurring, although the highest density field survey (HF99) had relatively low infection intensity, perhaps because this was a very dry year (the total rainfall deficit for July–September 1999 was 19.8 cm: plants received only a third of the normal rainfall amount; NOAA, 1999). Although it is likely that many (if not all) of these sources of variation contributed to differences in peak infection intensity among surveys, our results show that infection intensity differs among genotypes despite these significant sources of variation.

Because of the strong effects of environmental variation on infection intensity from year to year, it is crucial to measure multiple years of natural infection when assessing the genotypic contribution to fungal pathogen resistance. If there is a cost of resistance, this extensive variation will preclude consistent selection for increased disease resistance. This does not prevent persistent evolutionary effects on host resistance level, however. Effects of disease on vegetative reproduction in a single year could have long-lasting effects on host populations.

In addition to the independent effects of genotype and environment, interactions between these factors also affected resistance level (Fig. 4). Genotypic effects were not detected when tested over the significant genotype by site or year interactions (Table 4), suggesting that G × E interactions are more

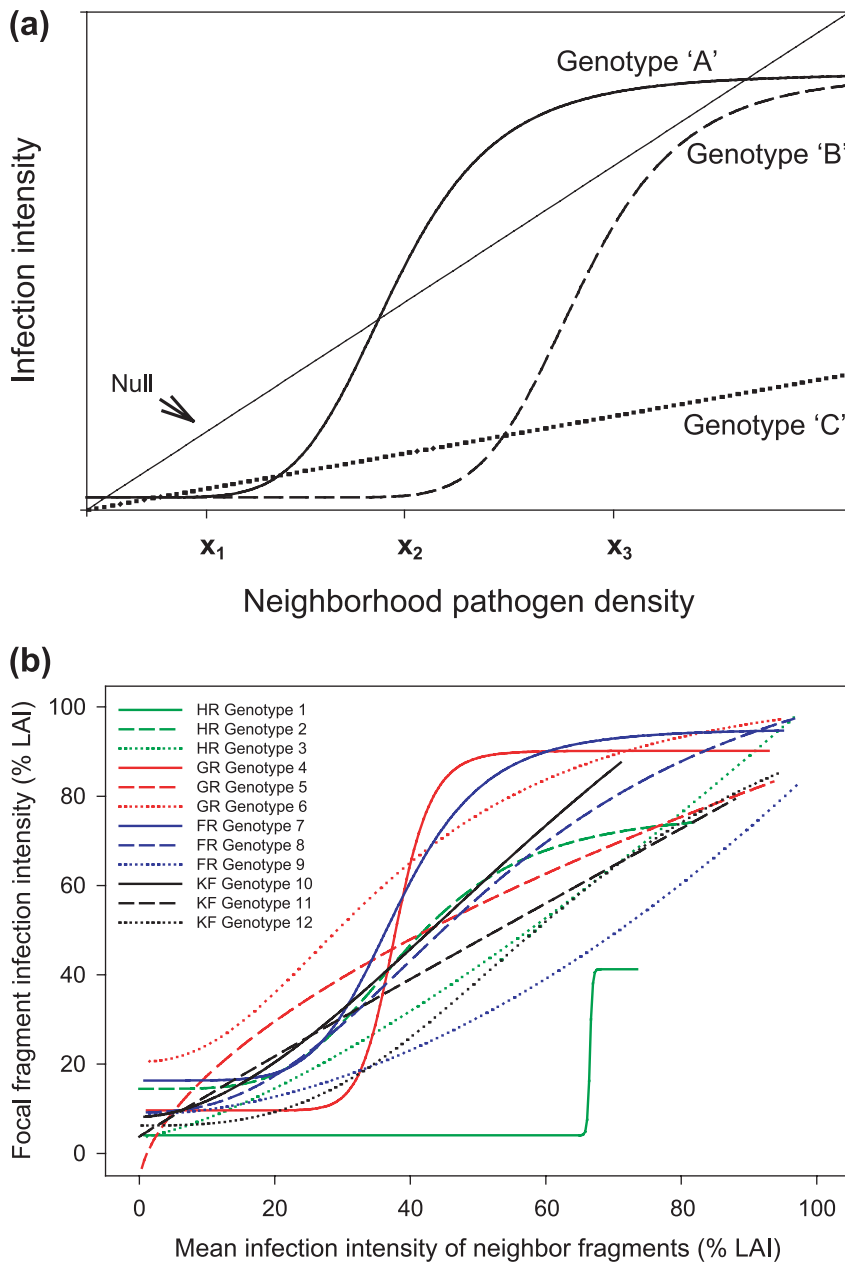


Fig. 5 (a) Differences in threshold and shape of reaction norms can indicate the mechanism(s) responsible for genotype by environment interactions in infection intensity. Hypothetical genotypes A and B have similar shapes but different thresholds, suggesting variation in effectiveness of the same resistance mechanism. Genotype C differs in shape from the other two, potentially reflecting the action of a different resistance mechanism. (b) Logistic curve reaction norms of resistance to *Coleosporium asterum* infection in *Euthamia graminifolia*. Each line represents replicates of one host genotype surveyed across four experimental field surveys at two sites over 3 yr. Only fragments with > 2 living neighbours were included ($n/\text{genotype} = 29\text{--}50$). Both axes are in units of percent leaf area infected. Lines of the same colour represent genotypes from the same origin population (HR, Hardin Ridge; GR, Griffy Lake; FS, Friendship Road; KF, Kent Farm).

important that genotype *per se* in determining resistance levels across space and time. Such interactions appear to be common in transplant studies (Schmid, 1994; Davelos *et al.*, 1996; Morrison, 1996; Pinnschmidt & Hovmoller, 2002) and their importance for evolution over the long-term should not be underestimated.

Understanding $G \times E$ effects as variation in quantitative resistance

One way to synthesize the effects of genotype, environment and their interaction on resistance is to envision infection level

as a function of pathogen density. This can be accomplished through a comparison of scatter plots that illustrate realized infection intensities of replicates of each genotype across a range of pathogen densities. The null expectation is for individual infection intensity to be a direct reflection of neighbour pathogen density (straight solid line in Fig. 5a). Genotypes with quantitative resistance will have infection intensity reaction norms (where neighbour pathogen density is the environmental variable) that can be fitted to logistic curves. The first portion of the curve will be flat, showing a minimal increase in infection intensity while local pathogen density is low. The curve accelerates as

pathogen density increases and the threshold of quantitative resistance is reached. The curve then includes an asymptote as remaining leaf area available for infection approaches zero (e.g. Genotype A, Fig. 5).

The position and shape of these genotype-specific curves can provide insight into the level of complexity of the genotype by environment interaction in resistance. The simplest pattern occurs when genotypes differ only in resistance threshold. In this circumstance, the interaction arises from variation in the degree of infection intensity difference among genotypes across a range of pathogen densities. For example, Genotype A and B are indistinguishable at one pathogen density (e.g. x_1 , Fig. 5) and differ at another (e.g. x_2). Although there is an interaction, the similarity in the shape of the reaction norms suggests that the population contains a single quantitative resistance mechanism that varies in effectiveness, as reflected by differences in curve *position*. Additionally, the interaction could be due to opposite 'direction' of infection intensity differences. When genotype B is less infected than genotype C at one pathogen density (e.g. x_2), but more infected at another (e.g. x_3), their reaction norms cross because of differences in curve *shape*. Large differences in the shape of reaction norms might indicate that there are multiple resistance mechanisms at work.

We generated genotype-specific logistic curves based on data from all field surveys combined (Fig. 5b, Four parameter logistic function fit of Sigmaplot 9.0, SPSS Inc. 2001). These curves are defined by their inflection point (x_0), y -intercept (y_0), slope (r), and upper asymptote (K) from the logistic equation:

$$y = y_0 + \left(\frac{K}{1 + \left(\frac{x}{x_0} \right)^r} \right)$$

where y = focal fragment intensity and x = mean neighbour fragment intensity; x_0 defines curve position; and y_0 , r and K describe aspects of curve shape.

Our data provide evidence that curve shape differs among some genotypes from these populations. Specifically, the upper asymptote (K) of genotype 1 was significantly lower than that of 4 and 7, indicating that its resistance is more effective than that of other genotypes under high disease pressure conditions (Fig. 5b; non-overlapping 95% confidence intervals of estimates of K – solid green line: genotype 1 < 50% LAI vs solid red and blue lines: genotype 4 and 7 > 64% LAI). While no other statistically significant differences were detectable, further examination of parameter values can be used to illustrate other ways in which the curves might differ. The position of genotype 1's curve (i.e. its inflection point, x_0) is higher than that of 4 or 7 (genotype 1: 66% vs 38% LAI for genotype 4 or 7). One potential explanation for greater resistance in genotype 1 is that it possesses major gene resistance to

infection, which is largely effective in preventing infection of this genotype. Position differences among genotypes from Friendship Road (blue lines) show how thresholds of quantitative resistance may vary as well: x_0 coefficients of genotypes 7 and 8 (solid and broken blue lines) differed by 18% (38% vs 56% LAI). r -values (slopes) could also differ among lines, as might be typified by genotypes 4 and 5 (red solid and broken lines: $r = 14$ and 2, respectively).

We believe this approach has the potential to reveal the mechanism(s) responsible for genotype by environment interactions in quantitative disease resistance. For instance, a range of slopes with some not differing from genotype 1 would indicate the absence of resistance in some genotypes. Other curve parameters could indicate that some genotypes resist initial establishment of fungal colonies, but are poor at slowing colony growth, while others exhibit the opposite strategy. Such patterns would specify those genotypes that should be examined to identify the physiological characters responsible for quantitative resistance variation. If curves are similar in shape, those with the greatest slope would be the best candidates for determining the mechanism of resistance. When dramatically different curve shapes are revealed, there may be more than one resistance mechanism acting in the population, and the extremes of the curve types should be examined. This type of logistic analysis would presumably be most effective for a non-clonal or clonal phalanx species where stem density is more consistent within and among years. For these systems, neighbour infection intensity would be an accurate predictor of neighbourhood pathogen density. Since our curves were generated without regard to the size of each neighbour, they provided only a rough estimate of the pathogen density each focal fragment had to contend with.

Conclusions

E. graminifolia genotypes differed in *C. asterum* infection intensity in the experimental field partly because of resistance level differences among genotypes, demonstrating that two of the three components necessary for the evolution of resistance are present in this system (phenotypic variation and genetic variation). However, the factors determining infection intensity in the field are complex. Our study concurs with others that show that environmental factors and $G \times E$ interactions are of equal or greater importance in determining infection intensity of individual plants (de Nooij & van Damme, 1988; Jarosz & Levy, 1988; Morrison, 1996). Logistic analysis can be used to describe the pattern of infection intensity among replicates of genotypes relative to their neighbourhood pathogen densities across a range of physical and disease environments. Further application of logistic analysis to cases of quantitative resistance should provide insight into its effect on disease expression in natural plant populations.

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