SPECIFICITY OF HOST:PATHOGEN GENETIC INTERACTION FOR FUSIFORM RUST DISEASE ON SLASH PINE[1]

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Abstract.-- Multiple stems on individual ramets (rooted cuttings) of 60 slash pine (Pinus elliottii Engelm. var. elliottii) clones were artificially inoculated using 2 single urediniospore-derived cultures of the fusiform rust fungus, Cronartium quercuum (Berk.) Miyabe ex Shirai f. sp. fusiforme. Presence or absence of fusiform rust galls were recorded for each inoculated branch at 6 and 9 months post-inoculation. Using disease phenotypes, the cultures were distinguished from each other by several pairs of clones, and the clones could be sorted into distinct groups. Several phenotypic groups appeared to represent specificity between corresponding gene pairs (CGPs) in the host and pathogen, suggesting the presence of a gene-for-gene interaction. Variation in disease phenotypes within clone:cultures combinations was interpreted as segregation at the pathogenicity loci of the CGPs. To utilize this additional information, a modified gene-for-gene analysis was developed and applied to the data. The analysis suggested the presence of four CGPs and clone and culture genotypes were postulated for each CGP. Further inoculation experiments involving these clones and cultures and genetic test crosses of both will be required to verify the hypothetical CGPs and clone and culture genotypes.

Keywords: gene-for-gene interaction, rust resistance, Pinus elliottii, Cronartium quercuum.

INTRODUCTION

Inoculation studies between members of the Pinus subsection Australes and various collections of Cronartium quercuum have shown host specialization, resulting in the taxonomic distinction of formae speciales within C. quercuum (Burdsall and Snow 1977). Cronartium quercuum f. sp. echinata cultures differentially infect Pinus echinata Mill. but not P. taeda L., P. elliottii Engelm. or P. palustris Mill., while the opposite holds for cultures of C. quercuum f. sp. fusiforme. An effort to further characterize resistance and pathogenic variability in P. e. elliottii and C. q. fusiforme (Griggs and Walkinshaw 1982), resulted in the hypothesis that the interaction may conform to a gene-for-gene system (Kinloch and Walkinshaw 1990). The hypothesis could not be rigorously evaluated because several assumptions about the experimental materials were necessary and experiments to check the assumptions were not conducted.

The literature provides opposing views on the likelihood of a gene-for-gene system between P.e. elliottii and C.a. fusiforme. Arguments against this system suggest that gene-for-gene specificity is an artefact of plant breeding and thus is very unlikely in a natural system such as the P. elliottii:C. q. fusiforme pathosystem (Barrett 1985). However Burdon and Jarosz (1988) have shown gene-for-gene specificity in wild populations of Glycine canescens and G.

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[1] Paper presented at the 22nd Southern Forest Tree Improvement Conference, June 14-17, 1993, Atlanta, GA.

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arm-ea and the rust pathogen Phakopsora pachyrhizi. Additionally Loegering (1984) argues that much of what is called general resistance is due to specificity and it can be explained by the interaction of corresponding gene pairs (CGPs) in a gene-for-gene system.

Tests of the assumptions made by Kinloch and Walkinshaw (1990) have not been possible due to technical problems in efficiently cloning and breeding both the host and pathogen species. Recent improvements in these technologies have allowed us the opportunity to begin testing the hypothesis of gene-for-gene interaction in the P. e. elliottii:C.q. fusiforme pathosystem. In the present study, we inoculated several clones of slash pine with basidiospore progenies of two single urediniospore derived cultures of the fusiform rust fungus. Because basidospores represent a segregating population of the single (dikaryon) urediniospore derived culture (Doudrick et al. 1993), basidiospore inoculations present a problem in data analysis under the gene-for-gene model (Flor 1956, 1971; Person 1959; Loegering 1974, 1984). However, given several opportunities to infect each host genotype with each pathogen culture progeny, we reasoned that under certain inoculation conditions we may be able to identify segregating alleles for pathogenicity in each culture. Under these conditions, a more powerful gene-for-gene analysis would result, because three interaction phenotypes would be apparent instead of two. The objective of this paper is to present the experimental data and our modified gene-for-gene analysis. Additionally, inoculation and genetic experiments designed to test the assumed gene-for-gene model were derived, and some implications of the model to breeding slash pine for fusiform rust resistance were considered.

MATERIALS AND METHODS

Host clones.-- Individuals from eight full-sib families of P. e. elliottii were propagated by rooting cuttings. The cloned individuals were part of a study conducted the year before in which seedlings from 31 full-sib families were inoculated with 2 C. a. fusiforme cultures. Each cloned individual was infected during the previous study and had been saved for evaluations of gall development. At the time of initial propagation the donor plants were approximately 14 months from seed and had been hedged two times. Following a second cutting and propagation, 60 clones were represented by 4 or more rooted cuttings. The rooted cuttings from these clones were up-potted to 3.78-L pots and hedged to promote the regrowth of multiple, succulent shoots for subsequent inoculation.

Pathogen cultures.-- Two single urediniospore derived cultures of C. a. fusiforme were used in this study, CCA-2 and WLP-10. The cultures were developed with the procedure described by Doudrick et al. (1993), which is a modification of Power's (1980) method for developing single aeciospore derived cultures. Both cultures originated from aeciospore collections made in 1984 from Livingston Parish (LA) loblolly pine (P. taeda)— CCA-2 in Madison County, FL, and WLP-10 in Livingston Parish, LA.

Artificial inoculations.-- The forced air apparatus of Snow and Kais (1972) was used to inoculate the rooted cuttings. For each cutting, the 1 to 7 (mostly 2 to 4) most succulent terminal shoots were inoculated at a density of 12 to 18 spores per mm^2. The inoculum density was verified after every tenth cutting inoculated. For each clone, two to eight rooted cuttings were inoculated with each culture. The cuttings were inoculated in random order, half the cuttings with CCA-2 and the other half with WLP-10, resulting in two completely randomized experimental designs.

Following inoculations, the rooted cuttings were incubated in the dark at 20 to 22 °C and 100% relative humidity for 24 hours. After incubation, the cuttings were returned to the greenhouse and grown under an 18-hour photoperiod provided by 1,000-W metal halide lamps. Two weeks after inoculation, the cuttings were fertilized once with 9-45-15(liquid N-P-K)then

every second week thereafter with 20-10-20 (both, 200 ppm N). The presence or absence of fusiform rust galls were recorded 6 and 9 months after inoculation. For this analysis, a cutting was considered infected if at least one of the inoculated shoots was galled at the 9-month scoring.

Data analysis.-- To be included in the analysis, a clone must of had at least three rooted cuttings inoculated with each culture. Of the 60 clones inoculated, 19 met this criterion. A non-galled cutting was interpreted to represent the low infection type (L) of the union phenotype (Nance et al. 1992), whereas a galled cutting represented the high infection type (H). To account for variation between the infecting spores (basidiospores) within a clone:culture combination, we assumed that for a given host clone, any result other than 100% non-galled or 100% galled indicated heterozygosity for the culture's corresponding gene. Thus for each clone:culture combination, we observed one of three classes of union phenotypes— all low (L), all high (H), or low and high (L,H)— instead of the usual two classes (L and H).

Because clear interactions (i.e., infection type reversals) were evident between several pairs of clones and the two cultures, the infection type data were analyzed under the assumption of a gene-for-gene model (Loegering 1984). A modification in the analysis was necessary to utilize the three phenotypic classes. In the modified analysis, we assumed that both the L and L,H union phenotypes were definitive because L were observed in both, but different because H were observed in L,H only (Table 1). The resulting analysis procedure was as follows:

(1) sort the clones into union phenotype groups;
(2) try to fit the current model (initially a one CGP model) to a group;
(3) if it fits, designate the culture and clone genotypes and move to the next group; if it does not fit then (a) add a CGP, (b) designate culture and clone genotypes, and (c) move back to previous groups and designate clone genotypes for the new CGP; and
(4) move to the next union phenotype group and proceed with step 2.

RESULTS

Data for 9-month post-inoculation observations are summarized in Table 2. Based on the pattern of union phenotypes, the clones were sorted into like groups (i.e., outcomes). With three phenotypes (u=3) and two cultures (c=2) a maximum of nine groups (u`) and four corresponding gene pairs [(u- 1)c, u=2 or 3 and c>1] could potentially be identified. Eight of the nine phenotypic groups are represented. The only group not represented is low CCA-2 and low, high WLP-10 (L/L,H).

Results from our modified gene-for-gene analysis of the data in Table 2 are presented in Table 3. Given the 2 cultures and 3 phenotypic classes, the maximum number of CGPs were identified with this set of 19 clones. With these two cultures, the missing group (L/L,H), could not differentiate another CGP. However, its postulated genotype can be determined and it is shown in the last row of Table 3. (Note that if the L/H clone is homozygous for the definitive allele at CGP4 (1h4) and the H/L,H clone is homozygous for the definitive allele at CGPl (1 hl) then a cross of these clones would yield all L/L,H progeny). Also, the complete genotype of the H/H clones can be postulated (Table 3, first row), since the postulated genotypes of both cultures contain at least one definitive allele for each CGP.

The data analysis proceeded as follows: Observing that the H/H group provides no information, because the clones and the cultures could be either 0 or 1 at each CGP, we moved

on to the H/L,H group. Here we found the first CGP and designated the H/L,H clone 1h13 and the CCA-2 and WLP-10 cultures 0pl and 1,0p1, respectively. Moving to the H/L group, we first attempted to explain the data with CGPl . This simply involved trying I hl and Ohl for the H/L clone. Neither possibility fit, so we added a second CGP and designated the clone ?hl 1h2 and the cultures 0pl 0p2 and 1,0p1 1p2. The clone genotype at CGPl is designated ?hl because it could be either Ohl or I h 1. Moving back to the H/L,H clone, we observed that it must be 1 hl Ohl. We then proceeded to the L,H/L,H group, continuing this procedure on through to the last group. Finally, the completed table contains postulated genotypes for both cultures and all the clones.

Table 1. Union phenotypes and associated host clone and pathogen culture genotypes for one corresponding gene pair.

Urediniospore (n+n)				
Culture Genotype		AA	Aa	as
Boolean Code		1	1p0p	0p0p
		1	1	1
Basidiospore (n)				
Progeny Genotype		A	A,a	a
Boolean Code		I p	1 p,0p	0p
		Union Phenotypes		
Host Clone				
(2n) Genotype	R_	L	L,H	H
Boolean Code	1h	lu	1 u,0u	0u
Host Clone				
(2n) Genotype	rr	H	H	H
Boolean Code	0h	0u	0u	0u

Notes: Gene symbols in the cultures are A (avirulent) and a (virulent) and in the clones R (resistant and dominant) and r (susceptible and recessive). The L and L,H union phenotypes are assumed to be definitive, thus the L union results from the R_ clone and the AA culture only and the L,H from the R_ clone and the Aa culture only. All other clone:culture combinations result in the H union phenotype, i.e., the non-definitive. The Boolean codes are adopted from Loegering (1978, 1984) and are useful for data analysis when more than one CGP is involved. The Boolean rule for determining union phenotypes and clone and culture genotypes is:

1h + 1p = lu = L; and 1h + 0p = 0h + 1p = 0h + 0p = 0u = H.

DISCUSSION

The application of a standard gene-for-gene analysis to these experimental data would have resulted in the detection of two corresponding gene pairs (CGP2 and CGP4). The clone:culture combinations producing both low and high infection types would have been discarded, attributing the anomaly to variation in inoculation technique or host tissue suitability. In the present study, we could not conclude that variation in technique or tissue suitability caused these results. Instead it appeared that the variation was caused by

[3] The trailing number in the genotype and phenotype codes identify the CGP.

segregation of heterozygous loci for pathogenicity in the cultures. Inoculating multiple shoots of at least three rooted cuttings per clone with a moderately low inoculum density, evidently provided this opportunity. The fact that in only 5 out of 17 cases of low and high infection types (L,H union phenotypes) the percent galled was less than 50 percent reinforced our tenet that variation was due to segregation. A straightforward test of the tenet can now be proposed— that the same experiment run with a high inoculation density (e.g., 120 to 150 spores/mm^2) would reduce to a two gene system (i.e., the L,H union phenotypes would convert to H phenotypes, while the L phenotypes would remain L).

Table 2. Summary of infection type data for 19 slash pine clones and 2 single urediniospore derived C.q. fusiforme cultures.

Clone	Family	CCA-2 #Gall/#Inoc	CCA-2 Interaction	WLP-10 #Gall/#Inoc	WLP-10 Interaction	Group
396	556	3/3	0	3/3	0	H/H
397	556	3/3	0	3/3	0	H/H
399	586	3/3	0	3/3	0	H/H
401	586	3/3	0	3/3	0	H/H
572	586	3/3	0	1/3	1,0	H/L,H
577	586	3/3	0	0/3	1	H/L
507	536	7/8	1,0	6/7	1,0	L,H/L,H
514	536	4/5	1,0	4/5	1,0	L,H/L,H
517	536	3/4	1,0	3/4	1,0	L,H/L,H
520	536	1/3	1,0	2/3	1,0	L,H/L,H
537	556	3/8	1,0	2/8	1,0	L,H/L,H
531	556	2/4	1,0	2/4	1,0	L,H/L,H
559	582	2/4	1,0	2/4	1,0	L,H/L,H
510	536	1/3	1,0	3/3	0	L,H/H
555	581	2/3	1,0	0/3	1	L,H/L
536	556	0/3	1	3/3	0	L/H
495	531	0/4	1	0/4	1	L/L
528	551	0/3	1	0/3	1	L/L
543	581	0/3	1	0/3	1	L/L
Totals		43/73		40/72		
		59 %		56 %		

Notes:

#Gall is the number of galled rooted cuttings.

#Inoc is the number of rooted cuttings inoculated.

Interaction is the union phenotype (Nance et al. 1992) using Loegering's (1978, 1984) symbolization, where 1 is definitive and 0 is non-definitive. A definitive union phenotype (L = 1u or L,H = 1,0u) results only from definitive host and pathogen genotypes (i.e., 1h + 1p = 1u or 1h + 1 p,0p = 1,0u). A non-definitive union phenotype (H = 0u) results from all other combinations of host and pathogen genotypes (i.e., 1 h + 0p = 0h + 1 p = 0h + 0p = 0h + I p,0p = 0u).

Group is a classification of the host clones according to their reaction to cultures CCA-2 and WLP-10, where L = low infection type (definitive), H = high infection type (non-definitive), and L,H = both low and high infection types (definitive and non-definitive).

Table 3. Postulated culture and clone genotypes for the four corresponding gene pairs detected in the modified gene-for-gene analysis.

			Corresponding Gene Pair (CGP)			
			1	2	3	4
			Pathogen Culture Genotypes			
		CCA-2	0p	0p	1,0p	1p
		WLP-1 0	1,0p	1p	0p	0p
Group CCA-2/WLP-10	Union Phenotypes CCA-2/WLP- 10	#Clones	Host Clone Genotypes			
H/H	0u/0u	4	0h	0h	0h	0h
H/L,H	0u/1,0u	1	1h	0h	0h	0h
H/L	0u/1u	1	?h	1	0h	0h
L,H/L,H	1,0u/1,0u	7	1 h	0h	1h	0h
L,H/H	1,0u/0u	1	0h	0h	1h	0h
L,H/L	1,0u/1u	1	?h	1h	1h	0h
L/H	1u/0u	1	0h	0h	?h	1h
L/L	1u/1u	3	?h	1h	?h	1h
L/L,H	1u/1,0u	0	1h	0h	?h	1h

Notes:
Genotypes for clones and cultures are designated as follows: 1 = definitive, 0 = non-definitive, and 1,0 = both definitive and non-definitive. Union phenotypes are a function of the clone and culture genotypes across the CGPs. For example,

		CGP			
		1	2	3	4
Culture	WLP-10	1,0p	1p	0p	0p
Clone	H/L,H	1 h	0h	0h	0h,

results in a 1,0 union phenotype (coded 1,0u1, where the trailing 1 indicates the definitive or expressed CGP), because the lowest infection type is "epistatic" to all higher infection types (Loegering 1984). In this example, for the segregation at CGP1 (1p) to be expressed, the interactions of CGPs 2-4 must all be non-definitive.

Some of the very high percent galled clone:culture combinations could be H union phenotypes instead of L,H, but this would not have qualitatively changed the outcome of our analysis, we simply would have had more clones in the H/H group and no clones in the L,H/L group. More problematic is the question of the non-definitive (H) union phenotypes. A failure to identify non-definitive phenotypes would certainly lead us to reject the gene-for-gene hypothesis. Currently, we have little power to determine the correctness of the H phenotypes, and, in several of these cases, fewer than half of the inoculated shoots were galled. However, we have observed substantial morphological variation among shoots within rooted cutting ramets of slash pine and it is generally thought that this variation is correlated with host tissue

suitability for infection[4]. Thus, it may not be surprising to find H union phenotypes with less than 50 percent galled shoots. Also note that the same conclusions and problems apply regardless of whether the low or the high infection type is the definitive.

Assuming that we have correctly classified the infection types, the postulated CGP genotypes of the clones and cultures should be valid. Genotypes postulated in this manner have proven to be at least as useful as those derived from genetic studies (i.e., inoculating polymorphic parents, and their F1 and F2 progeny) (Loegering and Burton 1974). However in either case, the postulated genotypes must be verified by studying the inheritance of the CGPs in controlled crosses of the clones and the cultures. For example, selfing an H/L,H clone and inoculating rooted cuttings of the progeny with WLP-10 would determine whether or not the clone was homozygous or heterozygous for 1 h 1. If the clone is heterozygous and the union phenotypes segregate 3 L:1 H, then resistance is dominant to susceptibility. Likewise, selfing WLP-10 and using clonal lines (single urediniospore derived cultures) of the progeny to inoculate the H/L,H clone should result in a I L:2 L,H:1 H ratio of union phenotypes. Many genetic tests can now be proposed by simply observing the postulated genotypes presented in Table 3.

The results of this study suggest that gene-for-gene specificity exists between slash pine and the fusiform rust fungus. With only 2 pathogen cultures and 19 host clones, we have hypothesized the presence of 4 genes controlling gall formation on slash pine rooted cuttings. That these genes control specificity during the initial point of interaction, suggests that they may have a major affect on the genetic structure of the pathogen population. Genes controlling different forms of specificity will no doubt be found, resulting in less severe consequences for the pathogen population. Arguments for finding and using the later type of genes in resistance breeding programs are good (Snow et al. 1975; Powers and Matthews 1979), because adequate resistance is accomplished without "forcing" the pathogen to mutate (Loegering 1984; Mundt 1990). Sources of resistance that place little selection pressure on the pathogen population will no doubt be more durable than the type of resistance investigated in this study.

ACKNOWLEDGEMENTS

We thank Larry Lott, Tom Caldwell, and Herb Wells for technical support during the inoculations and greenhouse assistance during the propagation and evaluation periods. We also thank Drs. Henry Amerson, Neil Anderson, Glenn Furnier, and Paula Spaine for useful comments during the review process. Finally, we thank Dr. Hans van Buijtenen for his valuable insight into the analysis of these data.

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