



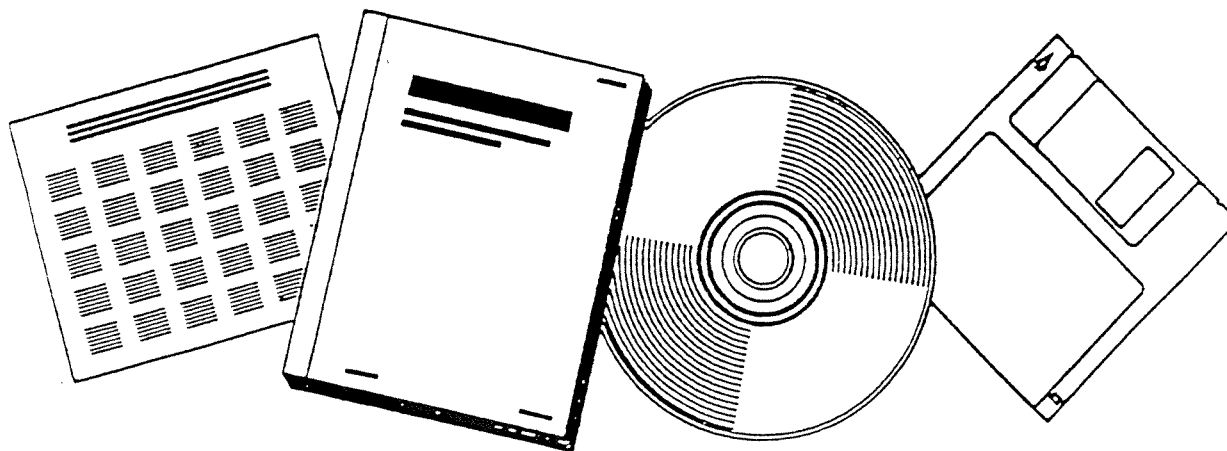
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DERIVATION OF HOST AND PATHOGEN GENOTYPES IN THE FUSIFORM RUST PATHOSYSTEM ON SLASH PINE USING A COMPLIMENTARY GENETICS MODEL AND DIALLEL DATA

H. E. Stelzer¹, R. L. Doudrick² and T.L. Kubisiak²

Abstract:-- Seedlings from 20, full-sib families of a five-parent slash pine diallel were inoculated using two, single urediniospore-derived cultures of the fusiform rust fungus on two different dates during the 1994 growing season. Presence or absence of fusiform rust galls was recorded for each inoculated seedling at nine months post-inoculation and percent infection levels for each family:inoculum:date combination were calculated. The complementary genetics model normally requires clonal material of either the host or pathogen in order to assign genotypes to identified complementary gene pairs. Diallel data, however, allows these assignments to be made even when both the host and pathogen populations are segregating. For the July data, three pairs of complementary genes are thought to explain the observed percent infection levels among the host families and fungus cultures. The putative genotypes of the fungus cultures WLP-10 and CCA-2 are $A_1A_1:A_2a_2:a_3a_3$ and $A_1a_1:A_2a_2:A_3A_3$, respectively. The respective host genotypes for parents 8-7, 9-2, 18-26, 18-62, and 18-27 are $R_1R_1:r_3r_3$, $R_1r_1:r_3r_3$, $r_1r_1:R_2r_2/r_2r_2:r_3r_3$, $r_1r_1:r_2r_2/R_2r_2:r_3r_3$, and $R_1r_1:R_3r_3$. The homozygous dominant reaction gene of parent 8-7 at locus 1, complemented by the homozygous dominant avirulence gene in culture WLP-10, is thought to be the reason that families with 8-7 as one of the parents appear to be so "resistant" when challenged with WLP-10. Two additional complementary gene pairs can be hypothesized if the May data is included for families 9-2 x 18-26 and 18-27 x 18-26. The identification of these gene pairs using both the May and July inoculation data suggest the existence of temperature-sensitive genes in this pathosystem. A Chi-square analysis of the July inoculation data using a complementary model with four gene pairs indicate a good fit between expected and observed percentage infection levels. The implications of these findings on rust screening and deployment are discussed.

Keywords: *Pinus elliottii*, *Cronartium quercuum* f.sp. *fusiforme*, complementary genetics, diallel data

¹USFS Southern Institute of Forest Genetics, P.O. Box 1387, Normal, AL 35762.

²USFS Southern Institute of Forest Genetics, 23332 Hwy. 67, Saucier, MS 39574.

INTRODUCTION

Although phenotypic selection for disease resistance and breeding among recognized resistant trees (Zobel and Talbert 1984) have increased the supply of resistant seedlings, the genetic basis of the host-pathogen interaction in the slash pine (*Pinus elliottii* Engelm. var. *elliottii*) : fusiform rust fungus (*Cronartium quercuum* [Berk.] Miyabe ex Shirai f. sp. *fusiforme*) pathosystem is not understood despite 37 years of research (Powers 1991). Efforts to characterize this genetic basis have resulted in the hypothesis that the interaction may conform to a complementary genetic system (Griggs and Walkinshaw 1982; Kinloch and Walkinshaw 1990; Nelson et al. 1993). In such a system, the genetics of both the host and the pathogen must be considered (Figure 1). Resistance is considered dominant in the host and avirulence is dominant in the pathogen. Any combination of a particular pair of complementary genes where the complementary gene from one organism is homozygous recessive will result in a high infection type (e.g., a gall). In the case where two or more pairs of complementary genes are present, the gene pair that imparts the low infection type (e.g., no gall) is epistatic to all other gene pairs.

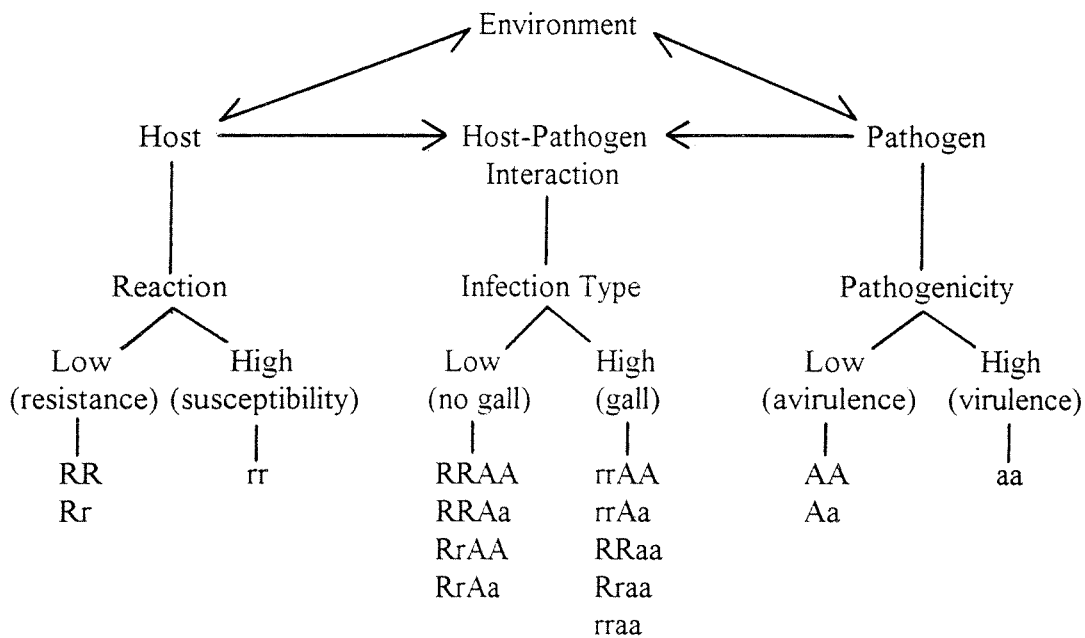


Figure 1. Simplified schematic of the complementary genetics model.

To demonstrate the operation of a complementary genetic system, the ideal model would involve two symbionts in which classical diploid inheritance occurs and that can be propagated as clones (Loegering 1984). This would make it possible to observe the phenotype of all possible combinations of the F₂ individuals of both symbionts. Of the models worked with to date, the one that best approximates the ideal is flax rust. The pathogen, *Malampsora lini* (Pers.) Lev., has

diploid inheritance and the uredial cultures are clones. It is genetically stable and is easily maintained. Flax, Linum usitatissimum L., is self-pollinated and has normal diploid inheritance. While it is difficult to propagate as a clone, sequential inoculation of a given plant with different cultures of the pathogen is possible.

The fusiform rust pathosystem on slash pine presents some challenges to the ideal model. First and foremost, the non-repeating spermatial-aecial stage of the fungus is the one that causes the economic damage we wish to control. As a result, specific cultures of the fungus cannot be clonally propagated. The basidiospores infecting the slash pine have been shown to be haploid products of meiosis and hence represent a segregating population (Doudrick et al. 1993a,b). Efforts have been made to develop in vitro inoculation techniques using single genotype haploid cultures of the fungus, but they have been largely unsuccessful (Frampton 1984; Hu 1990). Significant problems with the host include the long time it takes to generate F₁ and F₂ populations (compared to agronomic species), and the inability to clonally propagate large numbers of selected host genotypes. Rooted cuttings are currently the most efficient means of vegetative propagation. Rooting success however, is still very much dependent upon the genotype of the individual. Hence, it is difficult to reliably produce the genetic array needed.

One possible way to circumvent these problems is to study the genetics of one member of the relationship in detail. Moseman (1966) working with barley mildew (Hordeum vulgare L.:Erysiphe graminis D.C. f.sp. hordei Em. Marchal) has clearly demonstrated that the genetics of one member of the relationship can be determined by studying the genetics of the other member. A recent study conducted at the Southern Institute of Forest Genetics however, suggests that by inoculating families of slash pine from a diallel mating scheme using single-urediniospore derived cultures of the rust fungus, the problem of segregating host and pathogen populations can be mitigated. The objective of this paper is to further develop hypothetical genotypes for individual parents of slash pine and single-urediniospore derived cultures of the rust fungus using a complementary genetics model and this diallel data.

MATERIALS AND METHODS

Host seedlings. Five, first-generation selections of P.e. elliottii were control-pollinated in a full-diallel crossing design. All five selections were chosen from the U. S. Forest Service's Harrison Experimental Forest in Harrison County, MS (Griggs et al. 1983, unpublished USFS SRS Study Plan No. 3.48 available on request). Previous research has classified these selections using bulk inocula of the rust fungus as follows: parent 8-7, resistant; parents 9-2 and 18-27, moderately resistant; and parents 18-26 and 18-62, susceptible (Jewell and Mallett 1967; Snow et al. 1975). Twenty of the 25 full-sib families (selfs excluded due to insufficient seed) were propagated from seed (Doudrick et al. 1996). Seed germination was staggered so that the seedlings were eight weeks old at the time of each inoculation series.

Pathogen cultures. Two single urediniospore-derived cultures of C.g. fusiforme were used in deriving the hypothetical genotypes: CCA-2, WLP-10 (G. Snow. 1984, unpublished USFS SRS

Study Plan No. 20.55 available upon request. Both cultures originated from aeciospore collections made in 1984: CCA-2 on planted Livingston Parish (LP) loblolly pine (*P. taeda*) in Madison County, FL; WLP-10 on planted LP loblolly pine in Livingston Parish, LA. Single urediniospore-derived cultures were then developed from the aeciospore collections (Doudrick et al. 1993b; Powers 1980). This analysis is part of a larger study that included two additional cultures of the rust fungus. They have been excluded for brevity and because they did not provide any additional information.

Artificial inoculations. Seedlings of each full-sib family were inoculated using each of the four pathogen cultures on two different dates, May 23 and July 12, in 1994. For each inoculation date and pathogen culture, 16 to 80 seedlings from each family were randomly divided into four replications and inoculated. In each replication, the families were inoculated in random order using a forced-air apparatus (Snow and Kais 1972). The succulent terminal shoot of each seedling was inoculated at a density of 12 to 18 spores per mm², and the inoculum density was verified after every tenth seedling inoculated.

After inoculation, the infected seedlings were incubated in the dark at 20 to 22 °C and 100% relative humidity for 24 hours. After incubation, the seedlings were returned to the greenhouse and grown under an 18-hour-light photoperiod provided by 1,000-W metal halide lamps. Two weeks after inoculation and every second week thereafter, the seedlings were fertilized using 20-20-20 (200 ppm N).

Data collection and initial analysis. The presence or absence of fusiform rust galls on the seedlings was recorded nine months after inoculation. A non-galled seedling received a score of 0, while a galled seedling received a score of 1. The percentage infection was then determined for each replication within a given full-sib family x inoculation date x inoculum treatment combination.

The data were initially analyzed using the SAS general linear model procedure (GLM, 6th ed., SAS Institute Inc., Cary, NC) to test for significant effects. Because percentage infection data are based upon a binomial response and some data points lie outside the stable variance range of 30 to 70 percent, all data were transformed to the arc sin of the square root of the percentage infection (Anderson 1974). Family, inoculum, and inoculation date were all considered to be fixed effects. Reciprocal cross effects were treated as a nested effect within family and considered fixed as well. The replication effect was nested within inoculation date and regarded to be random.

Derivation of hypothetical genotypes. Within a particular inoculation date, a significant family x inoculum interaction effect suggests that differential interactions exist between two or more fungus cultures and two or more host families. These differential interactions are equivalent to the interactions that were developed by Loegering and Burton (1974) and suggest that two pairs of complimentary genes could be operating in the host and pathogen. For each inoculation date, differential interactions were visually identified by sequentially stepping through the table one pair of host families at a time across all possible pairs of the inocula. Once a differential interaction was found involving two full-sib families and two fungus cultures, where percentage infection

levels either increased or decreased by at least 12 percent ($\geq 2 \times \text{SD}$), pairwise comparisons between the four proportions were made in order to test for statistical significance (Neter et al. 1982).

Having identified the potential number of complementary gene pairs, the percentage infection data were examined for a unique combination of host pedigree, fungus culture, and inoculation date that could serve as a starting point for assigning hypothetical genotypes. The process then involved a series of repetitive iterations, using deductive reasoning within the constraints of the complementary genetics model. In the interest of presenting the concepts of the complementary genetics model in a clear and concise format, we will forego the use of the nomenclature that has been proposed by Doudrick et al. (1994). Subsequent manuscripts will include proper nomenclature.

RESULTS

Analysis of variance revealed no significant reciprocal cross effects ($P=0.1118$), therefore, forward and reciprocal cross data were pooled. Likewise, the replication effect was not significant ($P=0.0901$). Pooling the replications for a given family:inoculum:date combination increased the average sample size from 9 to 72 and hence greatly increased the power of estimating percentage infection levels. Family, inoculum, and family \times inoculum effects were all significant ($P=0.0001$) and accounted for 49 percent of the observed variation. These data suggest that infection type reversals exist. While the date main effect was not significant ($P=0.0833$), the two-way interactions involving date implied that date did play some role in the development of disease symptoms.

Differential interactions. Percentage infection data for all family:inoculum:date combinations are given in Table 1. Several significant ($P=0.0500$) differential interactions among particular pairs of host families and fungus cultures were identified in both the May and July inoculation series. These interactions are delineated in Table 1 by lower- and upper-case letters for the May and July inoculation dates, respectively. Four significant differential interactions were found among the July inoculations. All four differential interactions shared the common family, 18-27 \times 18-62. The other host families involved were 8-7 \times 9-2, 8-7 \times 18-26, 8-7 \times 18-62, and 9-2 \times 18-26. Only two differential interactions were found among the May inoculations.

Inoculation date effects. Changes in percentage infection data between the May and July inoculations were observed in several families for the two fungus cultures (Table 1). When challenged using the WLP-10 inoculum, all host families having 8-7 as one of the parents exhibited a significant reduction in infection levels from the May to July inoculations. For CCA-2, the percentage infection increased from May to July regardless of host pedigree.

Three significant differential interactions involving inoculation dates were found by looking either within a given fungus culture for infection type reversal among two host families, or within a given host family for infection type reversal between the fungus cultures. These interactions are

identified in Table 1 by superscript Roman numerals. The first two interactions involved culture WLP-10 and host families 8-7 x 9-2, 18-27 x 18-26, and 9-2 x 18-26. The third differential inoculation date interaction involved both fungus cultures and host family 9-2 x 18-26.

Table 1. Percentage infection levels (percent \pm sd) of ten, full-sib families of slash pine inoculated using single urediniospore-derived cultures CCA-2 and WLP-10 of the fusiform rust fungus on May 23 and July 12, 1994. Average sample size per family:inoculum:date was 72 seedlings.

Family	Culture			
	CCA-2		WLP-10	
	May	July	May	July
8-7 x 18-27	20 \pm 5	30 \pm 5	11 \pm 4	1 \pm 1
8-7 x 9-2	39 \pm 5	57 \pm 6 ^B	16 \pm 4	3 \pm 2 ^B
8-7 x 18-26	44 \pm 7	50 \pm 6 ^A	40 \pm 7 ^I	4 \pm 2 ^{AI}
8-7 x 18-62	50 \pm 6 ^{a,b}	59 \pm 6 ^C	20 \pm 5 ^{a,b}	3 \pm 2 ^C
18-27 x 9-2	16 \pm 5	33 \pm 6	18 \pm 5	21 \pm 5
18-27 x 18-26	23 \pm 6	44 \pm 7	22 \pm 6 ^{LI}	50 \pm 7 ^{LII}
18-27 x 18-62	23 \pm 5	38 \pm 5 ^{A,B,C,D}	29 \pm 5	55 \pm 6 ^{A,B,C,D}
9-2 x 18-26	34 \pm 6 ^{a,III}	71 \pm 6 ^{D,III}	52 \pm 6 ^{a,II,III}	30 \pm 5 ^{D,II,III}
9-2 x 18-62	36 \pm 5	80 \pm 5	36 \pm 5	44 \pm 6
18-26 x 18-62	38 \pm 5 ^b	70 \pm 6	65 \pm 5 ^b	70 \pm 5

Note: Differential host and rust fungus pairs are identified by matching lower-case letters for the May inoculation, upper-case letters for the July inoculation, and Roman numerals for between inoculation dates.

Derivation of hypothetical genotypes. If one looks at the July inoculation, all families having 8-7 as a parent that were inoculated with the fungus culture WLP-10 exhibited extremely low levels of infection. Since the basidiospore is haploid, and by definition of the complementary genetics model, culture WLP-10 must be homozygous dominant for avirulence (A_1A_1) at this first locus. On the host side, one of the parents must be homozygous dominant for resistance (R_1R_1) at this locus. Otherwise, if both parents were heterozygous, R_1r_1 , then one should have observed an infection level of 25%. The odds are very low that four unique parents would all be R_1R_1 ; therefore, we conclude the common parent, 8-7, is R_1R_1 . Parents 9-2, 18-26, 18-62, and 18-27 could either be R_1r_1 or r_1r_1 .

As one proceeds down through the pedigrees for the July inoculation, no pattern emerges that is helpful in assigning host genotypes at locus 1 until family 18-26 x 18-62 at 70% infection is encountered. The only way one can observe an infection level greater than 50% is to assume that both the host gene and pathogen gene at that complementary locus are segregating. The pathogen's genotype must be Aa . Two scenarios can exist for the complementary host gene.

Either both parents are heterozygous Rr , or one parent is heterozygous Rr and the other parent is homozygous recessive rr . The former case would result in a 38% infection level and the latter case would generate a 75% infection level. Given the observed percentage infection of 70%, we conclude that one parent is heterozygous and the other parent is homozygous recessive for this gene pair.

But, we have already made the assumption that WLP-10 is A_1A_1 . Therefore, there must be another pair of complementary genes that are segregating. The putative genotype of the fungus culture WLP-10 is now A_1A_1, A_2a_2 , parent 18-26 is $r_1r_1, R_2R_2/r_2r_2$, and parent 18-62 is $r_1r_1, R_2R_2/r_2r_2$. At this time, we do not know which parent is heterozygous for the second complementary host gene. Furthermore, for this second gene pair to be expressed, by definition of the model, both host parents 18-26 and 18-62 must be homozygous recessive at the first locus.

Given a two-gene model and the hypothetical genotypes of the fungus culture WLP-10 and parents 18-26 and 18-62, we need to develop the hypothetical genotypes of the remaining three parents before we proceed. The fact that a complementary gene pair that imparts a low infection type is epistatic to all other gene pairs and that parent 8-7 is homozygous dominant R_1R_1 prevents us from verifying that parent 8-7 possesses the second reaction gene.

The remaining two parents, 18-27 and 9-2, are also thought to be lacking the second reaction gene. If both of these parents are assumed to be R_1r_1 , then when they are crossed with either 18-26 or 18-62 (r_1r_1) the expected percentage infection among the progeny inoculated with fungus culture WLP-10 should be 50%. When 18-27 and 9-2 are crossed, the expected percentage infection would be 25%. With the exception of family 9-2 x 18-26, the observed infection levels corresponded to the expected values.

Let us further develop these hypothetical genotypes by incorporating the July inoculation data using the fungus culture CCA-2. The complementary gene pair at locus 1 can explain the observed infection levels for families 8-7 x 9-2, 8-7 x 18-26, and 8-7 x 18-62, if one assumes that instead of the fungus culture CCA-2 being homozygous dominant for avirulence it is in fact heterozygous A_1a_1 . In all of the cases, a low infection level would occur 50% of the time. If this assumption is made, then the only way to obtain a 30% infection rate in family 8-7 x 18-27 would be to invoke a third locus. The pathogen would be homozygous for avirulence, A_3A_3 , and the host genotypes for this family would be R_3R_3 and r_3r_3 , segregating in a 1:1 ratio. Parent 8-7 is hypothesized to be the homozygous recessive parent. Again, the key point to remember is that the gene pair invoking the low infection type is epistatic to all other gene pairs. In this family, the resulting offspring have the genotype R_{1-1} at locus 1. Since we have assumed the pathogen is heterozygous at locus 1, we would expect 50% infection. However, the epistatic effect of locus 3 would result in 50% of the 50% infected if only locus 1 were operating to be resistant; therefore, we would expect an overall infection of 25%.

Given that parent 8-7 is hypothesized to be r_3r_3 , parents 9-2, 18-26, and 18-62 must also be r_3r_3 in order for this locus not to be epistatic to locus 1 and yield expected infection levels comparable to the observed values. The theoretical infection level of 31.25% in family 18-27 (R_1R_1, R_3R_3) x 9-2

(R_1r_1, R_3r_3) is also a result of this epistasis and is comparable to the observed infection level. Likewise, similar epistatic effects are hypothesized for the infection levels observed in families 18-27 (R_1r_1, R_3r_3) x 18-62 (r_1r_1, r_3r_3), and 18-27 (R_1r_1, R_3r_3) x 18-26 (r_1r_1, r_3r_3). Family 9-2 (R_1r_1, R_3r_3) x 18-26 (r_1r_1, r_3r_3) and family 9-2 (R_1r_1, R_3r_3) x 18-62 (r_1r_1, r_3r_3) would both be expected to exhibit infection levels of 75% because the segregation is in both symbionts at the first gene pair. As in the case of family 18-26 x 18-62 inoculated using culture WLP-10, we hypothesize that the second complementary gene pair is responsible for the observed infection levels of 70% when this family is inoculated with culture CCA-2.

At this point, the theoretical expectations of only one family, 9-2 x 18-26, does not fit the observed infection levels. If we now include the May inoculation data however, a possible explanation comes to light. As noted earlier, a date-dependent differential interaction was observed within the WLP-10 culture for families 18-27 x 18-26 and 9-2 x 18-26. By invoking a fourth complementary gene pair that is homozygous avirulent, A_4A_4 , in either May or July on the pathogen side and segregating in a 1:1 ratio for R_4r_4, r_4r_4 on the host side, the theoretical infection level for the family 9-2 (R_1r_1, R_4R_4) x 18-26 (r_1r_1, r_4r_4) becomes 25% and now is comparable to the observed 30% infection level.

Table 2 summarizes the observed and expected percentage infection levels for the July inoculation. A Chi-square analysis to test the goodness-of-fit of this theoretical model to the observed percentage infection levels, yielded a test statistic of $\chi^2 = 6.63$. Since the test statistic is less than the critical $\chi^2_{(19, .05)}$ of 30.14 we conclude this complementary genetics model of four gene pairs adequately describes the observed results.

Table 2. Observed and expected percentage infection levels of ten, full-sib families of slash pine inoculated using single urediniospore-derived cultures of the fusiform rust fungus, CCA-2 and WLP-10, on July 12, 1994.

Family	Culture			
	CCA-2		WLP-10	
	May	July	May	July
8-7 x 18-27	30	25	1	0
8-7 x 9-2	57	50	3	0
8-7 x 18-26	50	50	4	0
8-7 x 18-62	59	50	3	0
18-27 x 9-2	33	31.25	21	25
18-27 x 18-26	44	37.50	50	50
18-27 x 18-62	38	37.5	55	50
9-2 x 18-26	71	75	30	25
9-2 x 18-62	80	75	44	50
18-26 x 18-62	70	75	70	75

DISCUSSION

The results of this study clearly demonstrate the power of using diallel data not only to study the genetics of the host, but the pathogen as well in the fusiform rust pathosystem on slash pine. Clonal material of slash pine would have allowed us to sort the results into union phenotypes and derive hypothetical genotypes similar to Nelson et al. (1993). The diallel data however, did help mitigate the observed segregation. Even the most complex percentage infection levels could be explained using the complementary genetics model and the diallel data.

It was hypothesized that two parents, 9-2 and 18-27, did not possess the reaction gene associated with the second complementary locus. As this is a coevolved pathosystem, one would not expect every host genotype to possess the corresponding reaction gene for every complementary pair that exists in the pathosystem. Not all individuals will possess all the genes for every complementary pair, nor could any individual possess all alleles at these genes. For the cereal rust system, resistance genes have been shown to be members of small gene families (Loegering and Powers 1962). Mutations, insertions, deletions, and duplications in both the host and pathogen genome during sexual reproduction, as well as random drift, all drive the genetics of the pathosystem.

These data also show the potential pitfall of using bulk inocula to screen host genotypes. For example, if family 8-7 x 9-2 were screened with even a simple bulk inocula such as CCA-2 and WLP-10, one would falsely conclude that this family exhibited good "resistance" to the fungus. If this family is then deployed in an area where CCA-2 is the predominating inoculum however, the apparent resistance immediately would break down.

Another potential hazard in the screening process is that these evaluations might be temperature dependent. If temperature is important, then screening evaluations might not correlate well to field performance. For example, the good "resistance" based on a July screening of those families having 8-7 as a parent might easily appear to have broken down when those outplanted individuals are exposed to the same fungus in May. When in fact, other gene pairs are interacting and the "July interaction" would still be observed under appropriate environmental conditions.

It could be argued that the inoculation date effect is an artifact of the artificial inoculations. The warmer day/night temperatures of July may have so affected the physiology of either the host seedlings, or the fungus cultures, that the disease syndrome failed to develop. This hypothesis could account for the reduction in the July percentage infection levels for those pedigrees having 8-7 as a parent when they were challenged with fungus culture WLP-10. This host physiological resistance does not explain, however, the ability of those same families to exhibit higher percentage infection levels when inoculated using culture CCA-2 in July.

The environment is known to profoundly affect the expression of infection types (Waterhouse 1929). Temperature-sensitive genes in both the host and pathogen have been identified in the wheat stem rust pathosystem (Knott and Anderson 1956; Loegering and Geis 1957). Furthermore, it was demonstrated that these temperature-sensitive genes adhere to the

Furthermore, it was demonstrated that these temperature-sensitive genes adhere to the complementary genetic model (Loegering 1968). Similar temperature-sensitive genes could be at play in the differential reactions observed in this study.

A more definitive study that involves clonal lines of these host pedigrees and seven, single urediniospore-derived cultures of the rust fungus currently is being established. The results of the current study will enable us to focus our efforts on those host families and fungus cultures that were involved in differential interactions. In addition, the galled trees from the current study have been maintained and specific crosses of the fungus will be initiated this fall. These combined efforts will provide us with the opportunity to advance the knowledge on complementary genetic interactions in fusiform rust disease.

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