Interaction of Genes for Pathogenicity and Virulence in Trichometasphaeria turcica with Different Numbers of Genes for Vertical Resistance in Zea mays

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ABSTRACT

Differences in pathogenicity and virulence among isolates of *Trichometasphaeria turcica* (*Helminthosporium turcicum*) to maize were demonstrated by comparing qualitative and quantitative capacities to cause disease on four inbred lines of maize differing in number of chromosome arms with genes for vertical resistance. The increased virulence of isolates with more genes for pathogenicity suggests that virulence is conditioned both independently of and in part by genes for pathogenicity. The results indicate that genes for vertical resistance not only govern qualitative resistance to some isolates, but also quantitatively condition the amount of disease. Phytopathology 60:1250-1254.

The leaf blight disease of maize (*Zea mays* L.), caused by *Trichometasphaeria turcica* Lutt. (*Helminthosporium turcicum* Pass.), appears to be a suitable host-pathogen model for evaluating the genetic systems controlling pathogenicity and virulence in the pathogen and the nature of disease resistance in the host. The identification of chromosome arms carrying genes for resistance and susceptibility has been determined for several inbred lines ranging in reaction to the pathogen from a high degree of susceptibility to a high degree of resistance (1, 2). Van der Plank has used the term "vertical" to denote genes for resistance that react differentially to isolates of a pathogen; i.e., resistant to some isolates and susceptible to others or more resistant to some than to others (5). A previous report of the differential interaction between inbred lines of maize carrying genes for resistance on different numbers of known chromosomal arms and isolates of *T. turcica* (4) can now serve as the basis for concluding that these genes for resistance are vertical in nature by Van der Plank's criteria. Recent studies, a portion of which is included herein, substantiate this conclusion.

T. turcica is a heterothallic Ascomycete whose sexual stage can be produced readily in artificial culture (3), permitting genetic crosses and analyses of hybrid progenies. The recovery of hybrid ascospore isolates pathogenic to different combinations of one or more genetically identified host lines provides genetic stocks necessary for the study of specific host-pathogen interactions (4).

Pathogenicity is the ability of an entity to incite disease on given members of a host species. An entity is nonpathogenic when it is unable to incite disease on given members of a host species. A species is not an entity, but rather an abstraction. Isolates, strains, or races of a species are entities, and pathogenicity is their individual ability to incite disease on a given host genotype. Pathogenicity is not an attribute of a species. Pathogenicity is considered in this paper as the specific ability of a specific isolate of *T. turcica* to incite disease on a specific inbred line of maize.

The extent to which isolates incite disease on a specific inbred line, as evaluated herein by number and size of lesions, is a measure of their virulence. Virulence is relative. The virulence of one isolate pathogenic to a specific inbred line cannot be measured. Virulence can be measured only when two or more isolates pathogenic to a specific inbred line are compared for the relative amount of disease that each incites. Virulence of an isolate is not determined by the relative susceptibility or resistance of its host. Although virulence is relative, there can be no zero virulence or avirulence. An isolate inciting zero disease is nonpathogenic rather than avirulent. The opposite of pathogenic is nonpathogenic, not avirulent. Isolates characterized herein as nonpathogenic failed to incite disease, at least macroscopically. Nonpathogenic isolates did not incite a fleck or hypersensitivity reaction. If they did they would have been pathogenic.

Although we have not as yet identified specific genes for pathogenicity to specific inbred lines with differing numbers of chromosome arms carrying genes for vertical resistance, we believe that it is reasonable to assume that pathogenicity is under genetic control and, further, that an isolate of *T. turcica* pathogenic to a specific inbred line has genes for pathogenicity that are not present in isolates that are nonpathogenic to the same inbred line. We have observed that isolates that are pathogenic to a greater number of resistant inbred lines than other isolates are also more virulent on those inbreds that are susceptible to both kinds of isolates. We have observed further that isolates with similar pathogenic abilities to certain inbred lines vary in their virulence to those inbreds. However, it has become apparent that isolates differing in virulence on certain inbreds exhibit reasonably similar virulence to inbred lines with greater numbers of chromosome arms carrying genes for vertical resistance. This paper presents evidence to illustrate these kinds of variation in pathogenicity and virulence, and offers an interpretation for the differences and similarities.

MATERIALS AND METHODS.—One susceptible inbred, R4, and three resistant maize inbreds, C.I. 28A, C.I. 42A, and C.I. 64, comprised the host germplasm used in the present studies. Studies on the inheritance of resistance to *T. turcica* suggest that inbred R4 has no genes for resistance, but rather has what is termed

a major gene for susceptibility (2). Inbreds C.I. 28A, C.I. 42A, and C.I. 64 have been shown by translocation studies to have genes for resistance associated with 3, 5, and 6 chromosome arms, respectively (1). The fact that these three resistant inbreds react differentially to isolates of *T. turcica* (4) indicates that the resistance genes are vertical sensu Van der Plank.

The pathogenicity of a total of 161 ascospore isolates obtained from three different crosses to the four inbreds was evaluated in field studies. One parental isolate in each cross was pathogenic to all four inbreds, while the other parental isolate was pathogenic only to R4 and C.I. 28A. The methods used in making crosses and isolating ascospore progeny have been described previously (3).

The pathogenicity of each of the isolates was evaluated in a separate plot consisting of three plants each of inbreds R4, C.I. 28A, C.I. 42A, and C.I. 64. Inoculum was prepared by mixing petri-dish cultures of the isolates, including the agar, with cold distilled water in a Waring Blendor for approximately 2 min. Equal amounts of an inoculum were poured into the leaf whorls of all plants in each plot, a method which appeared to eliminate inoculum drift. Plants were 8 weeks old when inoculated. Although spore loads among isolates were not standardized, cultures of all isolates were sporulating well. Lesions from primary infections were collected 16 days after inoculation, before secondary infections appeared. All lesions occurring on plants of inbred C.I. 28A, C.I. 42A, and C.I. 64 were collected, while only a representative sampling of lesions on plants of susceptible inbred R4 was obtained.

RESULTS.—Of the 161 ascospore isolates studied, 33 were pathogenic to the four inbreds and 36 were pathogenic only to susceptible inbred R4 and resistant inbred C.I. 28A. The results and discussion presented herein are concerned primarily with these two groups of isolates, since from the standpoint of pathogenic capacities they collectively represent the two extremes observed. The remaining 92 isolates exhibited intermediate pathogenic capacities as measured by their pathogenicity on different combinations of two of the three resistant inbreds. All isolates were pathogenic to inbred R4, but none was pathogenic only to inbred R4. Sixty-one of 92 isolates were pathogenic to C.I. 28A and C.I. 42A, while the remaining 31 isolates were pathogenic to C.I. 28A and C.I. 64. All isolates pathogenic to C.I. 42A or C.I. 64 were pathogenic also to C.I. 28A. The occurrence of these pathogenic types agrees with the data concerned with chromosome arms and genes for resistance (3). All three resistant inbreds have genes located on the long arm of chromosome 3 and 5 and the short arm of chromosome 7. In addition, C.I. 42A has resistance genes on the long arm of chromosomes 2 and 8, and C.I. 64 has additional resistance genes located on the short arm of chromosome 4 and the long arm of chromosomes 4 and 9. Thus, it is expected that certain isolates would be pathogenic either to C.I. 42A or C.I. 64 but not to the other inbred.

Data on the virulence to inbreds R4 and C.I. 28A of isolates pathogenic to all four inbreds are summarized in Fig. 1, and those pathogenic only to inbreds R4 and C.I. 28A in Fig. 2. The isolates will be referred to hereafter as "Fig.-1 isolates" and "Fig.-2 isolates" for purposes of convenience and reference. Both sets of isolates are ranked in descending order from left to right on the basis of average size of lesions produced on the susceptible inbred R4.

Although the number of genes conditioning pathogenicity of the isolates has not been determined, it is assumed that Fig.-1 isolates have more genes for pathogenicity than Fig.-2 isolates, since the latter are nonpathogenic on inbreds C.I. 42A and C.I. 64. This conclusion is pertinent to later discussion.

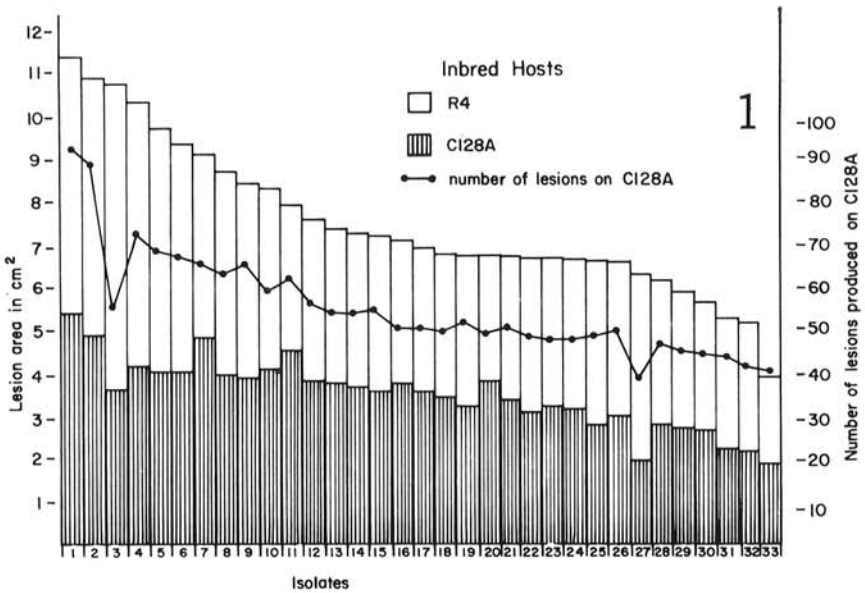
Differences in virulence, as measured by lesion size on inbred R4, exist among Fig.-1 and Fig.-2 isolates and between isolates of the two groups. Approximately threefold differences in lesion size are evident between the most and least virulent isolates of each group. Twofold differences exist between the most and least virulent isolates of Fig. 1 and Fig. 2. Six Fig.-2 isolates are more virulent than the least virulent Fig.-1 isolate.

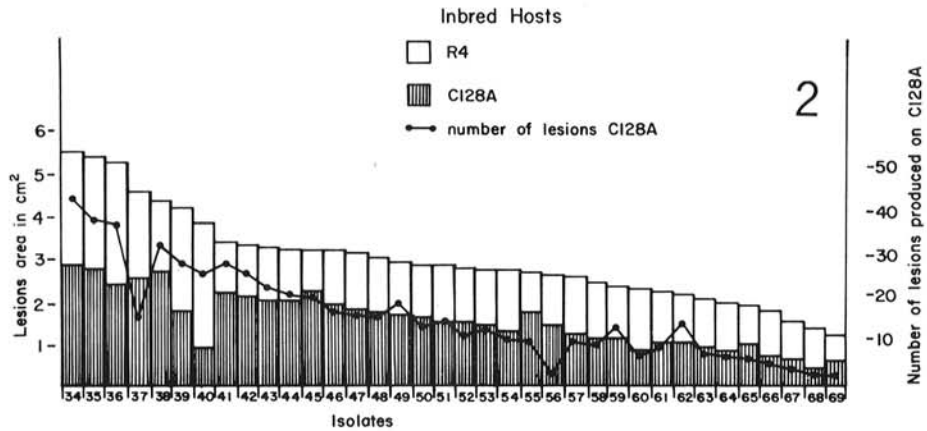
On inbred C.I. 28A, differences in virulence also exist among isolates within Fig. 1 and Fig. 2 and between isolates of the two groups. In general, the differences within and between groups follow a pattern similar to that observed on R4; i.e., isolates most and least virulent on R4 are similarly most and least virulent on C.I. 28A. If isolates were ranked in order of lesion size on C.I. 28A, no major shifts in the relative virulence of the isolates would be evident.

The virulence of Fig.-1 isolates to inbreds C.I. 42A and C.I. 64 is summarized in Fig. 3. Although differences in virulence among isolates are evident on C.I. 42A, no consistent trend or obvious relationship to virulence on inbreds R4 and C.I. 28A is apparent. All isolates exhibit a reasonably similar and a relatively low level of virulence on inbred C.I. 64. Increased (or decreased) virulence of Fig. 1-isolates on R4 and C.I. 28A does not equate to their increased (or decreased) virulence on C.I. 42A or C.I. 64.

By the criterion of number of lesions produced on C.I. 28A, differences in virulence are evident among isolates within each group as well as between the two groups. In general, the number of lesions incited on C.I. 28A follows the same patterns exhibited by isolates for lesion size on both C.I. 28A and R4; i.e., more virulent isolates incite larger and more lesions than less virulent isolates. The several exceptions to this association may be due to differences in inoculum load or may merely reflect variables frequently encountered in quantitative assessments.

The 61 isolates pathogenic to R4, C.I. 28A, and C.I. 42A, but nonpathogenic to C.I. 64 and the 31 isolates pathogenic to R4, C.I. 28A, and C.I. 64, but nonpathogenic to C.I. 42A exhibited levels of virulence on R4 and C.I. 28A intermediate to those exhibited by Fig.-1 and Fig.-2 isolates. Some general patterns are noteworthy. Within each group of isolates, approximately twofold differences existed between the most and least virulent isolates on both inbreds R4 and C.I. 28A. The levels and ranges in


Inbred Hosts
R4
CI28A
number of lesions on CI28A
1
Lesion area in cm²
Number of lesions produced on CI28A
Isolates


Inbred Hosts
R4
CI28A
number of lesions CI28A
2
Lesions area in cm²
Number of lesions produced on CI28A
Isolates

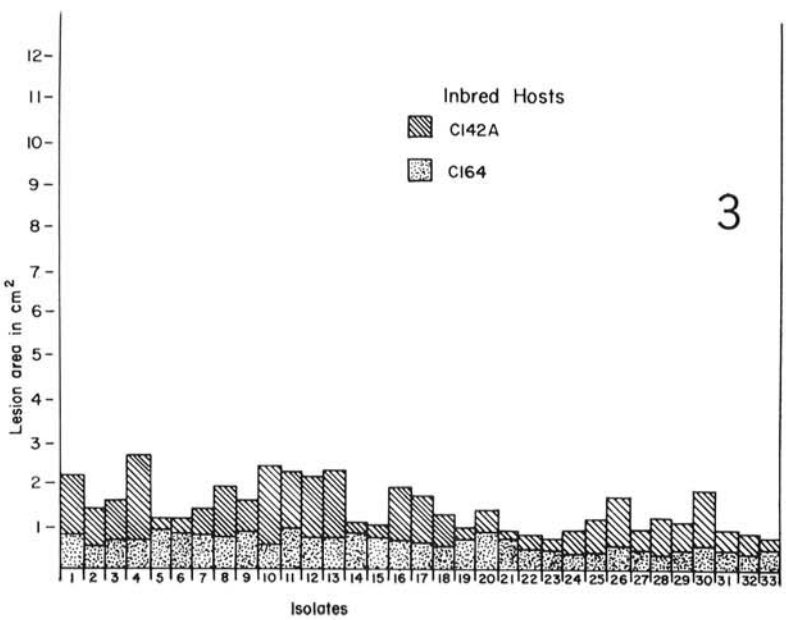

Inbred Hosts
CI42A
CI64
3
Lesion area in cm²
Isolates

Fig. 1-3. **1)** Differences in virulence on inbreds R4 and C.I. 28A among isolates of *Trichometasphaeria turcica* with genes for pathogenicity to maize inbreds with 0, 3, 5, and 6 chromosome arms with genes for vertical resistance. **2)** Differences in virulence on inbreds R4 and C.I. 28A among isolates of *T. turcica* with genes for pathogenicity to maize inbreds with no and three chromosome arms with genes for vertical resistance. **3)** Differences in virulence on inbreds C.I. 42A and C.I. 64 among isolates depicted in Fig. 1.

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virulence within both groups were more comparable to that of Fig.-1 isolates than of Fig.-2 isolates. Differences in virulence between the two groups of isolates were not marked, although isolates pathogenic to C.I. 64 usually were somewhat more virulent on R4 and C.I. 28A than were isolates pathogenic to C.I. 42A. The group of isolates pathogenic to C.I. 64 exhibited a more or less uniform and low level of virulence on C.I. 64 comparable to Fig.-1 isolates. Isolates pathogenic to C.I. 42A, but not to C.I. 64, exhibited similar but slightly decreased levels of virulence on C.I. 42A than did Fig.-1 isolates.

Discussion.—Van der Plank (5) contrasts genes for vertical resistance with genes for horizontal resistance, the latter conferring a level of resistance uniformly against all isolates or races of a pathogen. While vertical resistance reacts specifically against specific races, it is totally incorrect to assume that the gene-for-gene system must apply in cases of vertical resistance. Vertical resistance sensu van der Plank may operate on a one-for-oneness, and indeed does in some instances. However, the test for genes for vertical resistance is not a one-for-oneness but a differential reaction. Two points are worthy of discussion in considering horizontal resistance. Van der Plank illustrates the uniformity of host response by drawing a straight horizontal line. He has done so either to accentuate the differences between uniform and nonuniform (vertical) resistance or because he believes that the reaction is uniform. It is inconceivable that a given host genotype will react identically against all races pathogenic to the genotype with respect to intensity of disease. The different reactions of a host with genes for horizontal resistance to different pathogenic isolates or races should be of a considerably less magnitude than hosts with genes for nonuniform resistance. Horizontal or uniform resistance probably is controlled by many genes. Intensity usually is a quantitative trait. A quantitative trait is controlled by many genes because one gene can't accomplish the trait. The genes conditioning a quantitative trait are essentially polygenes in that each contributes something to a collective venture that none can accomplish alone. Removing a single gene from a many-gene system should have a relatively minor effect. How minor the effect depends on the relative importance of the gene in the collective venture. Greater fluctuations in the reaction of a host to different pathogenic races should occur as more and more polygenes are removed. As all but one gene are removed, the host would react quite differently to different isolates. It would react so differently, in fact, as to simulate a vertical reaction. The implication, thus, is that genes function vertically or nonuniformly when they are separate, and that the *same* genes function horizontally or uniformly when they are together.

The present studies with isolates of *T. turcica* and inbred lines of maize support the above discussion. Inbreds with greater numbers of chromosome arms with genes for resistance tend to react more uniformly to different pathogenic isolates than inbreds with fewer chromosome arms with genes for resistance. Thus, the inbreds used in the present study react both vertically and horizontally to isolates of *T. turcica.* We submit that the same genes are involved in both reactions. It is possible that a gene in one genetic background will behave differently than it would in another genetic background. When each gene for resistance is separated into a different background, it will react differentially to races of a pathogen. When all are pooled into one background or one genotype, they will behave differently. In the present case, the difference is between differential and uniform behavior.

Our conclusion is that genes for vertical and horizontal resistance are the same genes. This is not to imply that all genes contributing to a horizontal resistance will by themselves function vertically. Certain single genes for resistance may not at the present time be able to react differentially to isolates with genes for pathogenicity. One could argue that the horizontal resistance of inbred C.I. 64 is not accomplished by the vertical genes for resistance present on six chromosome arms, but rather is effected by other horizontal genes. Such an argument can be challenged in part by the fact that genes for resistance on the three chromosome arms common to inbreds C.I. 28A, C.I. 42A, and C.I. 64 were derived from inbred MO. 21A. Any genes in MO. 21A present on those arms and conferring only horizontal resistance should occur with a similar frequency in all three inbreds, unless it were postulated that all horizontal genes occurred on the chromosome arms unique to C.I. 42A and C.I. 64. It is unlikely that all genes functioning in horizontal resistance would be present on one or a few chromosome arms. It is more plausible to us that the specific genes for vertical resistance in inbred C.I. 64 function collectively to confer a horizontal resistance to isolates that are pathogenic to the inbred.

We will now turn to a similar, but briefer, discussion of genes for pathogenicity and virulence. It has been assumed that certain genes contribute to the pathogenicity of an isolate and that different genes contribute to its virulence. Our data suggest that the expression of pathogenicity and virulence may be conditioned by the *same* genes. Considering only those genes for pathogenicity that confer a pathogenic attribute to inbred lines C.I. 28A, C.I. 42A, and C.I. 64, it is evident that more genes for pathogenicity are necessary to incite disease on C.I. 64 than on C.I. 28A, since all isolates pathogenic to C.I. 64 are also pathogenic to C.I. 28A, while the reverse is not the case. Isolates pathogenic to all three inbreds will have

"unnecessary" genes for pathogenicity in relation to inbred C.I. 28A. To assume that genes for pathogenicity function *only* to render an isolate pathogenic and have no influence on the intensity of the attack means that the unnecessary genes (in context with inbred C.I. 28A) are not only unnecessary, but also functionless. The present studies, however, clearly indicate that isolates with unnecessary genes for pathogenicity are more virulent than isolates without unnecessary genes for pathogenicity. Our conclusion is that genes for pathogenicity can contribute in a collective or polygenic fashion to the intensity of the attack. The variation in virulence among Fig.-1 isolates and among Fig.-2 isolates may be accounted for by environmental effects or by certain genes in the pathogen contributing at the present time only in a relative way to virulence. If individual genes in the complex of genes conditioning virulence function or functioned at one time to condition pathogenicity, it is possible that some of them may have functioned against a genotype of the pathogen long since removed from the parasite population. With current host and parasite genotypes, some single genes may not now function qualitatively or vertically. Evolution is a long-term process. It is perhaps even more plausible to consider that the parental isolate pathogenic to C.I. 28A, C.I. 42A, and C.I. 64 possessed additional genes for pathogenicity, and that these genes were segregated at random among the progeny pathogenic to the C.I. inbreds.

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