Using half-normal probability plot and regression analysis to differentiate the disease response of resistant and susceptible tomato genotypes to multiple isolates of *Phytophthora infestans*

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ABSTRACT

Late blight resistant tomato lines were created in independent breeding programs using the accession *L. pimpinellifolium* L3708 as the source of the resistance. However, initial field observation suggested that the late blight resistance in the lines produced by two different breeding programs differed. To address the possibility of a partial transfer of the late blight resistance derived from *L. pimpinellifolium* L3708 and to examine the possibility of race specificity of this resistance, laboratory analyses were performed of the responses of nine tomato genotypes against five *Phytophthora infestans* isolates. Prior analysis by standard ANOVA revealed significant differences across lines but could not determine whether the disease responses in the AVRDC lines were different from those of the heterozygous F_1 hybrids, created by crossing susceptible tomatoes with the Cornell resistant fixed lines. A different analytical method was needed. Therefore, sporangia numbers/leaflet and diseased area data were analyzed using a half-normal probability plot and regression analysis. The results of this analysis show its utility for genetic or pathological studies. Considering only the uniform genotypes, this method confirms the results obtained by using a standard ANOVA, but provides a clearer demonstration of the distributions of the individuals within the populations and how this distribution impacts variance and the difference among the populations. This method also allows a joint analysis of the uniform genotypes with an addition population that is less uniform, because it is segregating. Such an analysis would be invalid using a standard ANOVA. The results of this joint analysis determined that the additional population was divergent from the Cornell fixed line, and, against some isolates, against the AVRDC lines as well. These implications of the results obtained for use of this late blight resistance are also discussed.

INTRODUCTION

Late blight caused by *Phytophthora infestans* (Mont.) de Bary, causes severe loss of tomato production when the environment is favorable to the pathogen. The control of this disease is increasingly difficult due to changes in pathogen virulence and increased chemical resistance of the pathogen (Fry and Goodwin 1997a; 1997b; Goodwin *et al.* 1998; Kato *et al.* 1997). Currently, late blight is controlled in tomato production fields by the use of fungicidal sprays; in some regions, the timing of these sprays is guided by blight forecasts based upon current weather conditions. (Davis *et al.* 1996; 1998; Raposo *et al.* 1993) Even with the monitoring of environmental conditions to forecast blight-favorable conditions and the utilization of controlling chemicals, losses in tomatoes due to late blight can be very high. Furthermore, many countries cannot afford the application of heavy loads of chemicals to their tomatoes. An incorporation of resistance could be a useful addition to an integrated late blight control strategy.

Researchers at the AVRDC found that *L. pimpinellifolium* accession L3708 (*a.k.a.* LA1269, NSL116890 and PI365957) is a strong resistance source to late blight in tomatoes (AVRDC 1994; Chunwongse *et al.* 2002) and generously provided this accession to other breeding programs, each of which proceeded to transfer the resistance. However, when the resulting late blight resistant lines were grown together under natural infestation, the degree of resistance appeared to differ among lines from the different programs (R. Gardner, Pers. Comm.). Therefore, lines with resistance derived from L3708 bred at Cornell and at AVRDC were tested against a series of *P. infestans* isolates to test for differences in disease

response among lines across isolates. Standard ANOVA analysis revealed that the lines produced by the two programs were significantly different, with the set of lines bred at Cornell all resistant across the 5 isolates used and the other set of lines bred at AVRDC all showing resistance to high levels of the disease against some of the 5 isolates (Kim, 2003, Kim and Mutschler, submitted) Heterozygous F_1 hybrids produced by crosses of susceptible lines with the homozygous Cornell lines also showed resistance to high levels of disease against some of the 5 isolates. That analysis, however, could not fully determine whether the disease responses among all of the populations of interest. Using a standard ANOVA method for data analysis would not be appropriate if any of the lines/populations were segregating. Segregation was a possible explanation for the lower level of late blight resistance in the AVRDC lines than that in highly resistant Cornell lines bred from the same resistance source (Kim, 2003, Kim and Mutschler, submitted). Segregation was also a distinct possibility for some low- resistance selections from the Cornell program. Therefore, an alternative analytical method was needed for analysis of data including these populations.

Half-normal probability plot and regression analysis could be utilized to analyze a data set possibly including segregating populations. The half-normal probability plot method was conceived by Daniel (1959) and further developed by Birnbaum (1959) and Krane (1963). It is a procedure to determine if a set of observations are members of a single distribution, or if there are outliers present. The n observations are ranked from 1 to n (highest). The ordered values of $P_k = (2k - 1)/2n$, k = 1, 2, ..., n, are computed. Using half-normal probability plot graph paper, the values of P_k, as ordinate values, are plotted against the response Y_k as the abscissa values. The values of Y_k falling on a straight line are considered to belong to the same distribution. Those not falling on the line are omitted

and the P_k values are recomputed for the reduced set of observations.   The values are then re-plotted to determine if additional observations will be considered as outliers.

Originally, the half-normal probability plot was used to identify important vs. unimportant factors on effect (expressed as orders) in singly replicated factorial design experiments (Daniel 1959).   If some combination of factors contributed differently from the combined majority of the other factors, similarly contributed factors are found on a common line, and the different contributing factors are found off this common line.   If the data are normally distributed, the data will be on the line. Important factors will not be in a normally distributed data set and will be off this line, forming a different line.   Therefore, if the factors have different effects, data will be grouped differently, affecting the lines drawn.

To our knowledge, the half-normal probability plot and regression analysis has not previously been used in genetic or pathological studies.   The goals of this work is to apply the half-normal probability plot and regression analysis to the sporangia number and disease area data in order to test the utility of this method on host/pathogen interaction data, and to attempt to determine more completely the differences in disease response across isolate among the tomato genotypes tested against a series of 5 isolates of *P. infestans*.

MATERIALS AND METHODS

Tomato Lines Tested

Nine tomato genotypes were tested against five *Phytophthora infestans* isolates in this study. The late blight susceptible control genotypes used were the open-pollinated freshmarket tomato line NC215E (Dr. Randolph Gardner, North Carolina State University)

and the open-pollinated processing tomato line E6203 (LA4024, available from C. M. Rick Tomato Genetics Resource Center, Davis, CA).

Two AVRDC late blight resistance lines, CLN 2037 B and CLN 2037 E (developed and provided by Dr. Hanson and Dr. Black of the AVRDC Tainan, Taiwan), which also carry resistance from L3708 were also used in the replicated test.

Two late blight resistant lines bred at Cornell (Kim, 2003, Kim and Mutschler, submitted) carrying the resistance from *L pimpinellifolium* L3708 (AVRDC 1994; Chunwongse *et al.* 2002) were used in this analysis. The *P. infestans* isolates US-7 and US-17 were used in screening late blight resistance during the breeding program; the resulting fixed lines were uniformly resistant to both of these isolates (Kim, 2003, Kim and Mutschler, submitted). The line 993104-10 was derived after one backcross to freshmarket tomatoes followed by one backcross to processing tomatoes, and line 993111-7 was derived after one backcross to freshmarket tomatoes followed by two backcrosses to processing tomatoes. Pollinating the susceptible genotypes, E6203 and NC215E, with pollen from the Cornell late blight resistant homozygous line, 993104-10 produced experimental hybrids that were heterozygous for the late blight resistance gene(s) carried by 993104-10.

In the course of breeding the Cornell late blight resistant fixed lines, selections had also been made for plants that appeared to have lower levels of resistance to US-7 and US-17. Self-progeny of one of these selections, designed low-R (982067-3) was also tested.

Pathogen Isolates Used

Phytophthora infestans isolates US-7 (940330), US-11 (980066), US-17 (970001),

NC-1 (980003), and DR4B (DR990004) were obtained from Dr. William Fry (Dept. of Plant Pathology, Cornell University) for use in these tests. US-7 was previously a dominant isolate in US, and US-11 is still a major isolate in California. NC-1 has been a dominant isolate in North Carolina, and US-17 was what was called "a tomato-specified isolate" in the southeast US. DR4B was collected from the Dominican Republic. Culture maintenance and inoculum preparation were as described in (Kim, 2003, Kim and Mutschler, submitted)

Inoculation and Data Collection

The detached leaflet droplet test method (Legard *et al.* 1995) was used to test resistance and susceptibility level. These experiments were replicated 3 times with 6 plants per genotype except the low resistance selection population that was tested 10 plants each. In all, 174 leaflets were tested. Assays were performed, and diseased leaflet area and sporangia produced per leaflet data were collected as described in (Kim, 2003, Kim and Mutschler, submitted)

Data Analysis

The collected spore number, diseased leaflet area and calculated sporangia number/unit diseased area (cm^2) data were analyzed both by half-normal probability plot and by regression analysis. Three rep average values of individual plants were calculated and analyzed by a half-normal probability plot analysis. Data were ranked from 0 to high value then P_k values were calculated according to the rank as following equation.

$P_k = ((2 \times rank)\text{-}1)/2n$

Data were plotted average sporangia No. and diseased area as X-axis and P_k value as Y- axis and regression lines were plotted.

RESULTS AND DISCUSSION

The trends are apparent in the differences among genotypes for average sporangia number and diseased leaflet area (cm^2) by some of the genotypes against the 5 isolates (Table 1 and 2). The average sporangia numbers indicate that the susceptible lines produced numbers of sporangia that were high, while in contrast the average sporangia numbers of the two Cornell homozygous lines were very low, demonstrating that these lines were resistant to all of these pathogen isolates (Table 1). Therefore, very little of the variability among genotypes or isolates in the experiment was generated from these homozygous lines. The average sporangia numbers of the heterozygous hybrids depended on the pathogen isolate used. The average sporangia numbers of the heterozygous hybrids were similar to those of the homozygous fixed lines against US-11, but were between those of the resistant fixed lines and susceptible controls against the isolates US-17, NC-1 and DR4B (Table 1). Furthermore, the heterozygous hybrids did not suppress sporangial production of US-7 although the parental homozygous fixed line was resistant to this isolate.

The AVRDC lines, CLN 2037 B and CLN 2037 E, which were also supposed to be fixed for late blight resistance from L3708, produced very different average sporangia numbers against the 5 isolates than did the two Cornell homozygous lines. The resistance

of the AVRDC lines was not effective across all five isolates used. Indeed, the average sporangial numbers of the AVRDC lines against US-7 followed a pattern that was more similar to that of the heterozygous F_1s than of the Cornell fixed lines.

The average sporangia numbers of the low-R progeny against US-7 was also similar to that of the AVRDC lines (Table 1). This presentation of the data provides the means and a measure of the variance around the means, but is not informative regarding the distribution within a group.

The averages diseased area results of the susceptible lines and the Cornell fixed lines were very similar to those for the average sporangia results for these two genotypes (Table 2). The results for the average diseased area indicate that the susceptible lines were all highly susceptible to all isolates, with diseased areas often extending throughout the entire leaflet. The results for the average diseased area of the two Cornell homozygous lines showed similar strong resistance to all of the isolates. Therefore, very little of the variability among genotypes or isolates for disease area was generated from these late blight resistant entries (Kim, 2003, Kim and Mutschler, submitted). The results for the average diseased area were different than the sporangia results for the heterozygous hybrids. The average diseased areas in the heterozygous hybrids were generally much closer to those of the susceptible lines than was seen in the sporangia results. The diseased area data of the heterozygote were particularly close to susceptible lines when challenged with US-7 (Table 2).

The disease expression of the two AVRDC lines and the low resistance population inoculated with the five isolates was similar to that of the heterozygous F_1s. The response pattern for the average diseased area results for heterozygous hybrids were similar to

sporangia results for the AVRDC lines, as far as general ranking of the virulence of the isolates. When US-7 is used, the AVRDC lines, low resistance selection population, and the heterozygous F_1's all have disease expression closer to that of the susceptible lines (Table 2).

Simply by observing the averages presented in Table 1 and 2, it is clear that the AVRDC lines showed different responses against the 5 isolates, in terms of average sporangia numbers and average disease area, than the Cornell late blight resistant lines. However, a statistical test of the data is required to show that the differences are significant. To choose the appropriate method of testing, one must consider the natures of the lines being tested and of the L3708-derived resistance as transferred into the AVRDC lines vs. the Cornell lines. If a single gene controls the L3708 resistance and this resistance was transferred to the fixed lines bred in both breeding programs, then these fixed lines should all perform similarly. Prior field observations with the AVRDC lines suggested that this might not be the case. One explanation is that the full resistance derived from L3708 is controlled by more than one gene and that AVRDC lines are either not homozygous for, or are missing at least one of, the resistance genes. If either the AVRDC lines or the low resistance Cornell selection were heterozygous for a resistance gene and produced segregating progeny, then comparisons of averages and variations with the other non-segregating lines would be inappropriate. Therefore, we employed the half-normal probability plot and regression analysis (Birnbaum 1959; Daniel 1959; Krane 1963) instead of a more typical ANOVA analysis.

It is a procedure to determine whether sets of observations are members of a single distribution or if there are outliers present. The half-normal probability plot method has

been criticized because a precise rule for omitting observations has not been formulated. Usually, however, one only omits observations with large divergences from the line. The process is then repeated to determine which additional observations are likely outliers. The process of repeating the procedure until all remaining observations are approximately on the same line is sufficient to detect outliers. Often, the experimenter will have a reason for an observation being an outlier. In our situation, we minimized the problem since we were able to detect an entire group that was divergent, such as resistant versus susceptible. The procedure is also useful for detecting divergent observations in a segregating group. In addition, the procedure allows for an estimate of the experimental error variance. From the final set of n observations considered to have the same distribution, compute $m = 0.683n + 0.5$. The value of Y_m is the estimated experimental variance. An eye-fitted line rather than a computed linear regression line is usually sufficient.

There are advantages in using the half-normal probability plot method for analysis to detect heterogeneous components. In a homogeneous fixed line population, each plant will be considered as a factor combination and will be in a commonly distributed data set. In a segregating population, the population is heterogeneous and each plant is considered as a different factor combination; susceptible plants and resistant plants will not be in the same distributed data set. However, if the contribution to an effect is similar, the plants will be in commonly distributed data set. If we expand the concept that resistant gene combinations are factor combinations, the contribution of the same resistant gene combinations will be in the same commonly distributed data set. In other words, the same resistant gene(s) combination will lie on the same or a similar slope line. If the data of two populations were distributed in same range, the slopes of regression lines would be similar because the P_k

value would be similar too. If the range of the Y_k values of one population was small and the range of another population was wide, the slopes of the distributions would be different. With this method, we could also compare individual plants in a low resistance population which might be segregating.

Data were grouped as Cornell fixed lines, the low resistance selection population, the heterozygous F_1s, and the AVRDC lines, and then analyzed by the half-normal method described. Rather than using half-normal probability graph paper, we used Microsoft Excel to obtain the graphs. Excel uses equally-spaced values of P_k, which has the effect of flattening the slopes. This, however, does not affect the ability to observe discrepant observations. The data points located on Y-axis are all zero, even though they have different rank and P_k value. For the purpose of graphing, theses zero points were all given different ranks so the points would be visually distinguishable. This would not affect regressions of non-zero containing data sets and regressions would not be calculated on all zero data sets. The sporangia number plots for 5 different isolates are summarized in Figures 1 with the regression line slopes summarized in Table 3.

Sporangia half-normal probability results against US-11 indicated that the Cornell homozygous lines, the low resistance selection population, the heterozygous F_1s and the AVRDC lines were all resistant against this isolate. The slope of regression lines indicated that a few plants of the F_1s and AVRDC lines were outliers, but these were probably experimental error (Fig. 1A, Table 3). The susceptible lines were a discrete group with a line that differed in its slope and placement from all of the other genotypes.

Sporangia half-normal probability results against US-17, DR4B and NC-1 indicated that the Cornell fixed lines were a unique group with the greatest resistance. The

low resistance selection population and the AVRDC lines grouped together and showed similar patterns of distribution and slopes indicating a resistance less than the Cornell resistant fixed lines. (Fig. 1B, C, D, Table 3).   The heterozygous F_1s were in a group of their own between a group with AVRDC lines and the low resistance selection population and the group of susceptible lines.

Sporangia half-normal probability results using US-7 were different than those obtained with any of the other isolates.   The US-7 results separated the genotypes into three distinct grouping.   Most resistant were the Cornell homozygous fixed lines, which had sporangia numbers of zero.   The heterozygous F_1s, AVRDC lines and the low resistance selections all had similar slopes of regression lines and had sporangia number ranges greater than the homozygous Cornell lines but lower than that of the last group composed of the susceptible lines (Fig. 1E, Table 3).

Against US-7, the group composed of the AVRDC lines, the heterozygous F_1s, and the low resistance selection population were in same range of distribution and had regression line slopes that were similar to that of the susceptible lines.   This result clearly suggested that the heterozygotes and AVRDC lines were not resistant to US-7 even though they were resistant to US-11.   The results across isolates indicated that the AVRDC lines and the low resistance selection were more susceptible to US-7 than to US-17, NC-1 and DR4B.   These results indicate that the susceptibility level of the two AVRDC lines was similar to that of the heterozygous F_1s and the low resistance selection, rather than the Cornell fixed lines.

Considering the preceding results, it is unlikely that resistance, transferred to the

AVRDC and the Cornell lines, is controlled by single, completely dominant gene. The results of the heterozygous F_1s were clearly different from their fixed line parent against the 4 isolates other than US-11.

The alternative hypothesis, suggested by Chunwongse *et al* (2002), is that resistance is due to a single incompletely dominant gene, and so lower levels of resistance could be attributed to the heterozygous condition. However, the data from the less resistant AVRDC and high resistant Cornell fixed lines do not support this hypothesis. The responses across isolates of the AVRDC and Cornell fixed lines are very different. The levels of resistance of the of the AVRDC lines for some isolates does have similarities to that of the heterozygous F_1 hybrid created using the Cornell fixed lines, however there was no evidence that these less resistant AVRDC lines or their selfed progenies segregate for resistance. If only one incompletely dominant gene controlled the resistance, and a population is not fixed and uniform for the resistance (due to segregation and/or assortment), then the progeny of at least some of these lines should include plants with the higher resistance against all 5 isolates and/or plants that are fully susceptible to all 5 isolates. Such off-type plants were not observed in the progeny of the AVRDC lines. Therefore one cannot attribute the lower levels of resistance to heterozygousity.

Diseased area data were analyzed with the half-normal probability plot method (Figures 2, Table 4). The half-normal probability plot results of the diseased area data had similarities and differences with the results of the sporangia number analysis. Diseased area half-normal probability results of the homozygous Cornell fixed lines against the isolates US-17, DR4B, NC-1 indicate that these lines grouped together and have strong resistance against all of these isolates (Fig. 2B to D, Table 4). Comparatively, no other

plant genotypes groups with these lines. These results were very similar to those concerning sporangia numbers. The US-11 diseased area results were also the same as US-11 sporangia results. Homozygous fixed lines, low resistance selection population, F_1s, and AVRDC lines were all grouped together and resistant against US-11 (Fig. 2A, Table 4).

Diseased area half-normal probability results of F_1s, the low resistance selection population and the two AVRDC lines against US-17, DR4B, NC-1 (Fig 2B to D, Table 4) were very similar, and more similar to that of the susceptible than to the resistant Cornell lines. This stands in contrast to the sporangia number analysis, in which a group with AVRDC lines and the low resistance selection population were more resistant than the heterozygous F_1's, and closer to the Cornell lines.

Diseased area half-normal probability results against US-7 are perhaps the most extreme. Most resistant were the homozygous fixed lines, which are a discrete class. All of the other genotypes fall in different, though overlapping, ranges on or very near a common line, indicating a lack of significant difference among these genotypes against US-7 (Fig. 3E, Table 4).

The combined sporangia results and diseased area results indicates that the AVRDC-developed resistant lines showed wider diseased area than sporangia production, like heterozygous F_1s. A model that would fit the data would postulate that the resistance is controlled by a major gene, which provides resistance to US-11, and in combination with one (or more) additional gene(s), provides the resistance to the other isolates. The Cornell resistant fixed line results were completely different from the AVRDC line results. This difference would be explained if other minor gene(s), fixed in the Cornell lines, was/were recessive and supports major gene action to provide the wider range of resistance. The

existence of the low resistance selection, which was derived from the same base population as the homozygous Cornell fixed lines, also fits the hypothesis that the full resistance, as expressed in the Cornell fixed lines, involves more than one gene. Progeny test results of CLN 2037 B and CLN 2037 E against US-17 support that two AVRDC lines are homozygous and do not segregate (Kim, 2003, Kim and Mutschler, submitted). If all of the populations are indeed fixed, one cannot explain the differences in response of the Cornell fixed lines and the AVRDC lines if the resistance was controlled by single gene.

Considered together, the results of these experiments show that the resistance of L3708 can be transferred to create lines that have a full level of resistance, such as those released by the Cornell program. The results also show the possibility that a very good breeding program could transfer only partial resistance due to isolates used in a selective screen, producing lines with resistance that was weak and narrow in its protection. The weaker or partial resistance may be due to the absence of the other gene(s).

Control of resistance by more than one gene is not unusual. In a study of resistance to late blight in potatoes, race-specific resistance required more than a single dominant R gene for expression of the dominant suppressor (El-Kharbotly *et al.* 1996). The interaction of more than one dominant gene for a fully functional expression of resistance has also been seen in studies of other host plant/disease systems. *Cf-2*, which is derived from *L. pimpinellifolium*, required the unlinked *Rcr3* gene to be fully functional. Interestingly, *Rcr3* is allelic to *Ne* gene, which derived from *L. pimpinellifolium* and suppress *Cf-2*–dependent autonecrosis conditioned by its *L. esculentum* allele *ne* (Kruger *et al.* 2002). *Mla-12*, race-specific resistant gene to powdery mildew in barley, also required *Nar-1* and *Nar-2* loci for full functionality (Freialdenhoven *et al.* 1994). Resistance for rice blast was

found to be controlled by two dominant unlinked genes (Pan *et al.* 1996). A race non-specific resistant breeding effort is currently underway for rice blast resistance (Castano *et al.* 1989). The strategy in this program is to use pyramiding to obtain a broad spectrum of resistance (Li *et al.* 2001; Rao *et al.* 2002)

Considering the impact of the choice of isolate on the expression of the resistance (Kim, 2003, Kim and Mutschler, submitted), a likely cause for the difference in results between programs would be the type of isolates used for screening and selections in the course of the breeding program. The production of lines with partial resistance would be more likely if screening used only one of the weaker isolates, such as US-11, or was done using natural infection in a location which has only one or very few isolates of similar pathogenicity.

These results have important implications for the use of the resistance from L3708 in tomato cultivars. Release of varieties with the partial resistance would be risky as they would subject to failure in a commercial setting when isolates new to the region moved in to the area. It would also be misleading for a breeder to interpret the "failure" of a partially resistant variety as indicating that a more complete resistance was not attainable from L3708. When a breeding program succeeds in transferring the complete resistance, it should use the resistance in the homozygous condition to help maintain the stability of the resistance and prevent the selection for isolates with a response such as that seen with US-7.

REFERENCES

AVRDC (1994). 1993 Progress Report. Asian Vegetable Research and Development Center, Shanhua, Tainan, Taiwan: 201-203.

Birnbaum, A. (1959). On the analysis of factorial experiments without replication. Technometrics 1: 343-357.

Castano, J., Mackenzie, D. R.Nelson, R. R. (1989). Components Analysis of Race Non-Specific Resistance to Blast Disease of Rice Caused by *Pyricularia-Oryzae*. Phytopathology 127(2): 89-99.

Chunwongse, J., Chunwongse, C., Black, L.Hanson, P. (2002). Molecular mapping of the *Ph-3* gene for late blight resistance in tomato. Horticultural Science and Biotechnology 77(3): 281-286.

Daniel, C. (1959). Use of half-normal plots in interpreting factorial two-level experiments. Technometrics 1: 311-342.

Davis, R. M., Hamilton, G., Lanini, W. T.Spreen, T. H. (1996). The importance of pesticides and other pest management practices in U.S. tomato production. USDA, NAPIAP: Document No 2-CA-96.

Davis, R. M., Hamilton, G., Lanini, W. T., Spreen, T. H.Osteen, C. (1998). The Importance of pesticides and other pest management practices in U. S. tomato production. USDA, NAPIAP: Document No 1-CA-98.

El-Kharbotly, A., Pereira, A., Stiekema, W. J.Jacobsen, E. (1996). Race specific resistance against *Phytophthora infestans* in potato is controlled by more genetic factors than only R-genes. Euphytica 90(3): 331-336.

Freialdenhoven, A., Scherag, B., Hollricher, K., Collinge, D. B., Thordal, C. H.Schulze, L. P. (1994). *Nar-1* and *Nar-2*, two loci required for *Mla-12*-specified race-specific resistance to powdery mildew in barley. Plant Cell 6(7): 983-994.

Fry, W. E.Goodwin, S. B. (1997a). Re-emergence of potato and tomato late blight in the United States. Plant Disease 81(12): 1349-1357.

Fry, W. E.Goodwin, S. B. (1997b). Resurgence of the Irish potato famine fungus. Bioscience 47(6): 363-371.

Goodwin, S. B., Smart, C. D., Sandrock, R. W., Deahl, K. L., Punja, Z. K.Fry, W. E. (1998). Genetic charge within populations of *Phytophthora infestans* in the United States and Canada during 1994 to 1996: Role of migration and recombination. Phytopathology 88(9): 939-949.

Kato, M., Mizubuti, E. S., Goodwin, S. B.Fry, W. E. (1997). Sensitivity to protectant fungicides and pathogenic fitness of clonal lineages of *Phytophthora infestans* in the United States. Phytopathology 87(9): 973-978.

Krane, S. A. (1963). Half-normal plots for multi-level factorial experiments. Proceedings of the Eighth Conference on the Design of Experiments in Army Research Development and Testing. ARO-D Report 63-2. U. S. Army Research Office,

Durham: 261-285.

Kruger, J., Thomas, C. M., Golstein, C., Dixon, M. S., Smoker, M., Tang, S., Mulder, L.Jones, J. D. G. (2002). A tomato cysteine protease required for *Cf-2*-dependent disease resistance and suppression of autonecrosis. Science 296(5568): 744-747.

Legard, D. E., Lee, T. Y.Fry, W. E. (1995). Pathogenic specialization in *Phytophthora infestans*: Aggressiveness on tomato. Phytopathology 85(11): 1355-1361.

Li, Z. K., Sanchez, A., Angeles, E., Singh, S., Domingo, J., Huang, N.Khush, G. S. (2001). Are the dominant and recessive plant disease resistance genes similar?: A case study of rice R genes and *Xanthomonas oryzae* pv. *oryzae* races. Genetics 159(2): 757-765.

Pan, Q., Wang, L., Ikehashi, H.Tanisaka, T. (1996). Identification of a new blast resistance gene in the indica rice cultivar Kasalath using Japanese differential cultivars and isozyme markers. Phytopathology 86(10): 1071-1075.

Rao, K. K., Lakshminarasu, M.Jena, K. K. (2002). DNA markers and marker-assisted breeding for durable resistance to bacterial blight disease in rice. Biotechnology Advances 20(1): 33-47.

Raposo, R., Wilks, D. W.Fry, W. E. (1993). Evaluation of potato late blight forecasts modified to include weather forecasts: A simulation analysis. Phytopathology 83(1): 103-108.

Table 1. Average sporangia numbers and standard errors for nine tomato genotypes tested with five *P. infestans* isolates.

Genotype name and class		*P. infestans* isolate				
		US-11	US-17	DR4B	NC-1	US-7
E6203	S	67,812 ± 13,370	163,953 ± 49,056	256,078 ± 36,872	383,109 ± 86,844	683,655 ± 116,371
NC215E	S	125,891 ± 19,695	145,086 ± 43,315	196,279 ± 23,712	209,995 ± 31,532	518,186 ± 64,490
NC215E X 993104-10	F_1	1,172 ± 682	9,688 ± 7,034	18,984 ± 8,246	24,961 ± 8,865	158,273 ± 40,985
E6203 X 993104-10	F_1	312 ± 243	7,656 ± 2,540	4,063 ± 1,897	26,367 ± 7,973	186,832 ± 28,608
993104-10	R	0	0	0	0	820 ± 661
993111-7	R	0	0	0	0	0
CLN 2037 B	A	273 ± 273	9,648 ± 3,722	742 ± 510	9,766 ± 4,404	107,294 ± 25,924
CLN 2037 E	A	0	4,961 ± 2,439	0	1,211 ± 760	61,377 ± 21,843
992067-3	LR	0	2,273 ± 1,176	30,975 ± 13,812	2,391 ± 1,272	53,423 ± 15,924

N=30 for 992067-3 and all others, N=18

Class   S: Susceptible checks

R: Cornell resistant fixed lines

F_1: Heterozygous F_1 hybrid between Cornell resistant fixed lines X Susceptible checks.

A: AVRDC lines

LR: Cornell low resistant selection.

Table 2. Average diseased areas (cm^2) and standard errors for nine tomato genotypes tested with five *P. infestans* isolates

Genotype name and class		*P. infestans* isolate				
		US-11	US-17	DR4B	NC-1	US-7
E6203	S	11.19 ± 0.54	13.74 ± 1.06	9.42 ± 0.64	12.66 ± 0.82	14.07 ± 0.94
NC215E	S	7.12 ± 0.69	9.90 ± 0.94	8.44 ± 0.78	10.97 ± 0.63	13.09 ± 0.88
NC215E X 993104-10	F$_1$	0.58 ± 0.33	1.92 ± 1.06	1.27 ± 0.42	3.98 ± 0.90	8.11 ± 0.90
E6203 X 993104-10	F$_1$	0.27 ± 0.20	4.20 ± 1.07	0.71 ± 0.29	4.78 ± 0.98	9.59 ± 0.75
993104-10	R	0	0	0	0	0.42 ± 0.42
993111-7	R	0	0	0	0	0
CLN 2037 B	A	0.21 ± 0.21	2.57 ± 0.90	0.66 ± 0.49	2.86 ± 0.99	9.76 ± 1.45
CLN 2037 E	A	0	2.39 ± 1.00	0	0.39 ± 0.19	3.80 ± 1.15
992067-3	LR	0	0.84 ± 0.40	1.99 ± 0.57	1.07 ± 0.56	4.67 ± 0.73

N=30 for 992067-3 and all others, N=18

Class   S: Susceptible checks

R: Cornell resistant fixed lines

F$_1$: Heterozygous F$_1$ hybrid between Cornell resistant fixed lines X Susceptible checks.

A: AVRDC lines

LR: Cornell low resistant selection.

Table 3. Summary of regression and R^2 of P_k vs. average sporangia number by genotype.

Isolate	Genotype							
	Susceptible		F$_1$		Low R		AVRDC	
	Regression	R^2	Regression	R^2	Regression	R^2	Regression	R^2
US-11	$y = 1.E-6x + 0.77$	0.82	$y = 1.E-4x + 0.33$	0.58	N.A.	N.A.	$y = 8.E-5x + 0.63$	0.35
US-17	$y = 9.E-7x + 0.74$	0.97	$y = 3.E-6x + 0.54$	0.38	$y = 3.E-5x + 0.30$	0.93	$y = 2.E-5x + 0.34$	0.71
DR4B	$y = 9.E-7x + 0.69$	0.94	$y = 2.E-5x + 0.40$	0.54	$y = 2.E-6x + 0.54$	0.33	$y = 6.E-5x + 0.38$	0.57
NC-1	$y = 5.E-7x + 0.75$	0.92	$y = 1.E-6x + 0.61$	0.86	$y = 2.E-5x + 0.34$	0.86	$y = 1.E-5x + 0.33$	0.69
US-7	$y = 5.E-7x + 0.72$	0.88	$y = 2.E-6x + 0.42$	0.98	$y = 3.E-6x + 0.31$	0.88	$y = 4.E-6x + 0.30$	0.78

Small values of slope are due to digit differences between P_k and sporangia No.
Regression and R^2 for resistant genotype couldn't be calculated.

Table 4. Summary of regression and R^2 of P_k vs. average diseased area by genotype.

Isolate	Genotype							
	Susceptible		F$_1$		Low R		AVRDC	
	Regression	R^2	Regression	R^2	Regression	R^2	Regression	R^2
US-11	$y = 0.026x + 0.66$	0.99	$y = 0.430x + 0.30$	0.83	N.A.	N.A.	$y = 0.125x + 0.63$	0.40
US-17	$y = 0.022x + 0.62$	0.88	$y = 0.051x + 0.39$	0.93	$y = 0.085x + 0.29$	0.93	$y = 0.076x + 0.30$	0.96
DR4B	$y = 0.039x + 0.55$	0.97	$y = 0.237x + 0.29$	0.91	$y = 0.085x + 0.44$	0.65	$y = 0.136x + 0.38$	0.83
NC-1	$y = 0.045x + 0.36$	0.98	$y = 0.050x + 0.37$	0.94	$y = 0.076x + 0.27$	0.90	$y = 0.049x + 0.38$	0.95
US-7	$y = 0.052x + 0.18$	0.98	$y = 0.081x + 0.11$	0.98	$y = 0.043x + 0.16$	0.95	$y = 0.056x + 0.12$	0.99

Small values of slope are due to digit differences between P_k and diseased area.
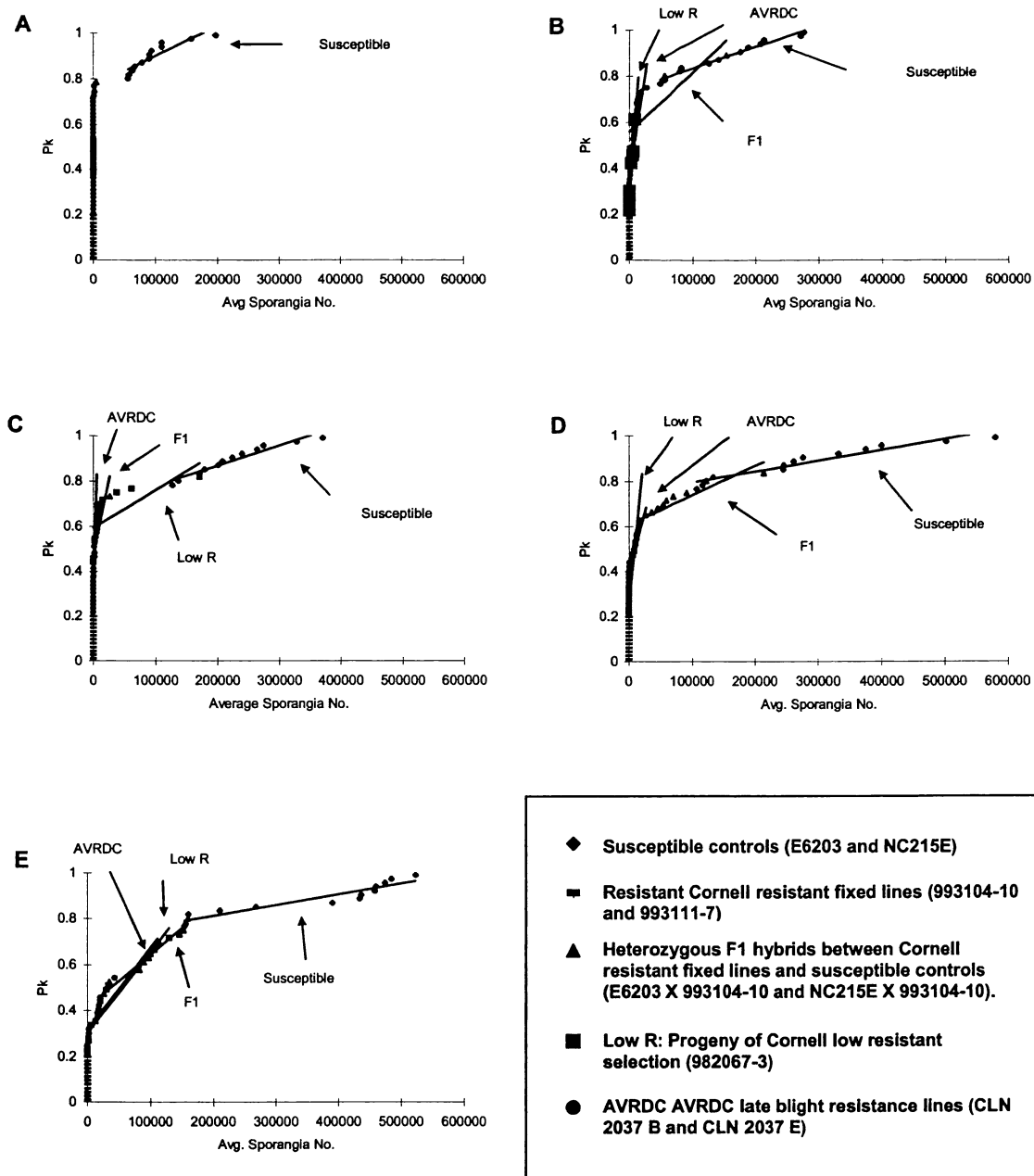Regression and R^2 for resistant genotype couldn't be calculated.

Figure 1. Average sporangia No. by genotypes and isolates.

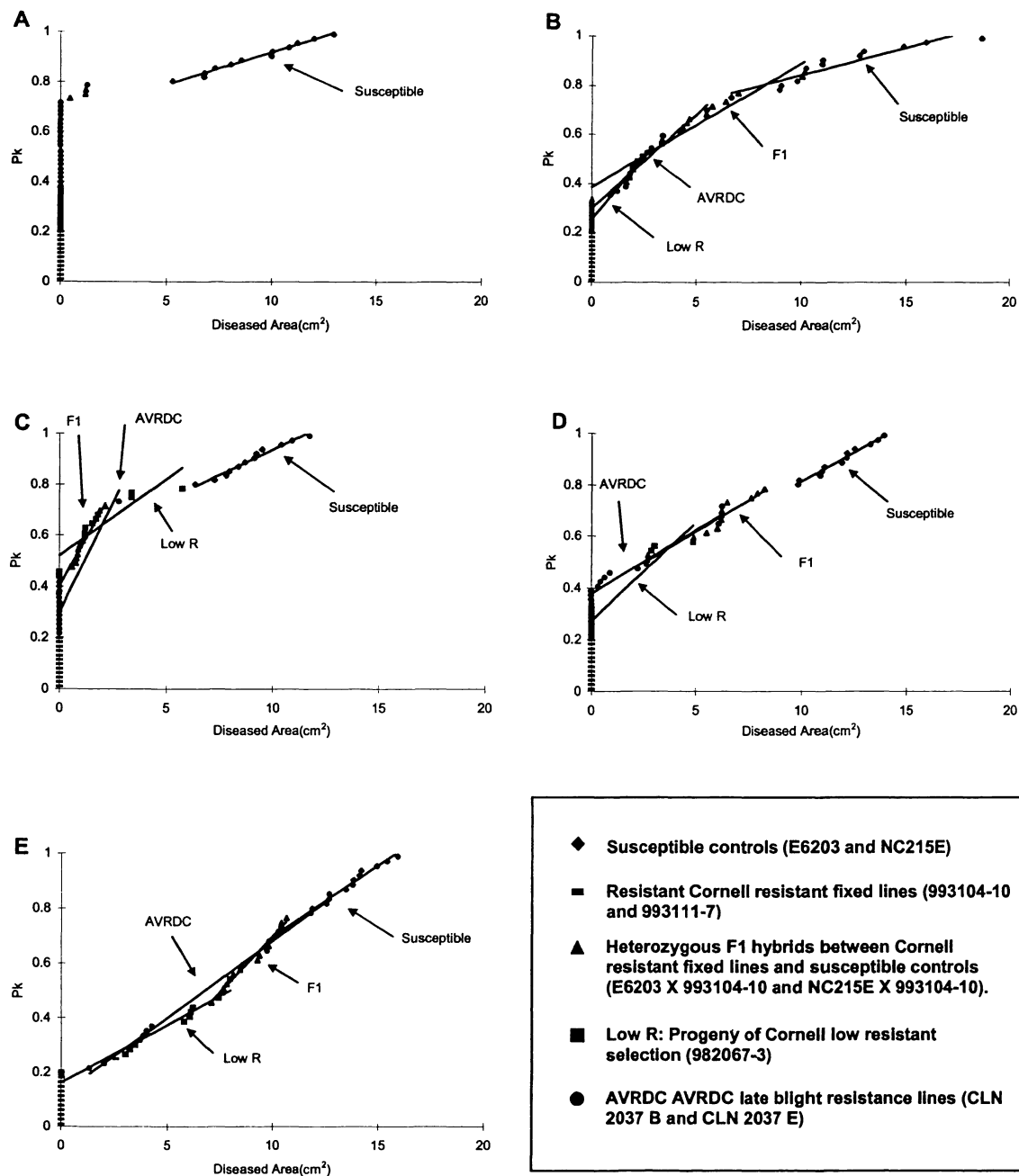A: US-11, B: US-17, C: DR4B, D: NC-1, E: US-7

Figure 2. Average diseased area by genotypes and isolates.
A: US-11, B: US-17, C: DR4B, D: NC-1, E: US-7