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Inheritance of Resistance to *Colletotrichum acutatum* Simmonds on Runners of Garden Strawberry and its Backcrosses

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Abstract. **Two half diallel mating designs were conducted to study the inheritance of resistance to *Colletotrichum acutatum* Simmonds on runners of strawberry. The main design included six genotypes representing a range of responses to the pathogen: 'Chandler' (very susceptible); FL 87-210 (tolerant); MS/US 541 (very resistant); NC 92-01 (*Fragaria chiloensis* Duch.) (resistant); NCH 87-10 (tolerant-susceptible); and NCC 89-39 (susceptible). The cross 'Chandler' x MS/US 541 was absent. The secondary test included 'Chandler' and selections FL 87-210 and NC 85-01 (*Fragaria virginiana* Duch.) (very resistant) as parents. Griffing's methods 4 and 2, model I, were used to test for combining ability in the main and secondary tests, respectively. General combining ability and specific combining ability were highly significant in all analyses. This study indicated that nonadditive effects are more important than additive effects in the inheritance of resistance of runners to anthracnose. The frequency distribution of lesion lengths within progenies suggests that resistance to *C. acutatum* on runners is quantitative. Therefore, breeding for resistance should be accomplished using progeny testing followed by individual selection within progenies.**

Anthracnose caused by *Colletotrichum* spp. is one of the most important factors limiting strawberry production in many southeastearn states of the United States (Ballington and Milholland, 1993); and became a problem in North Carolina during the mid-1970s. *Colletotrichum acutatum* Simmonds is now the main cause of anthracnose in North Carolina (Grand et al., 1990; Smith and Black, 1985).

The genetics of resistance to anthracnose is complicated. Differential reactions of cultivars over time and across locations have been found by several authors (Ballington and Milholland, 1993; Delp and Milholland, 1981; Gupton and Smith, 1991; Horn et al., 1972; Smith and Black, 1985, 1987, 1990).

Inheritance of resistance to anthracnose in strawberry has been addressed by only a few authors. Gupton and Smith (1991) evaluated progenies of 40 parents crossed in a Comstock and Robinson Design II Mating scheme to determine the relative importance of the different components of genetic variance in the inheritance of resistance to *Colletotrichum* spp. Petiole inoculation was used for disease rating. Estimates of dominance variance were 6–10 times higher than those for additive variance. Epistatic variance accounted for 35% to 38% of the total genetic variance. The frequency distribution of disease severity "ratings" appeared to be bimodal, suggesting major gene action may also be involved. Broad sense heritability estimates were 0.87 and 0.85 and narrow sense heritability estimates were 0.37 and 0.26 for females and males respectively, which indicates the possibility of genetic gain from recurrent selection. Faedi and De Clauser (1991) and Winterbottom et al. (1991), working with selections and commercial cultivars, reported that plant resistance to *C. acutatum* appeared to be conferred by a major gene. Neither of these studies specified which isolates (races) of *C. acutatum* were used in screening for resistance. In the latter study, runner inoculation was utilized. The inheritance of resistance was determined by screening parents, S_1 and reciprocal F_1 progenies obtained from crosses between susceptible and resistant parents. This inconclusive information about resistance to anthracnose justifies more research. There is no information in the literature about estimates of combining ability for resistance to *Colletotrichum acutatum*. This study was undertaken to obtain more information about inheritance of resistance and estimates of general and specific combining ability in the resistance to *C. acutatum* on runners of strawberry.

Materials and Methods

Plant material. Six parents that varied in reaction to *C. acutatum* were crossed in a half diallel design and the resulting progenies were used in this experiment. The parents included in the main test were *F. ×ananassa* genotypes, except where indicated and included: MS/US 541 (very resistant), NC 92-01 (*F. chiloensis*) (resistant), FL 87-210 (resistant-tolerant), NCH 87-10 (tolerant-susceptible), NCC 89-39 (susceptible-tolerant), and 'Chandler' (very susceptible). The *F. chiloensis* parent (NC 92-01) originated as an open-pollinated seedling from a mixed planting of collected wild clones of the species on a farm near Panguipulli, Chile. The total number of crosses was 15 [equal to p (p-1)/2, with p being the number of parents]. Due to a mistake in handling the seeds, the cross 'Chandler' x MS/US 541 was improperly labeled. Therefore, it was eliminated from the analyses and its effects were considered equal to zero in the combining ability analysis. In a secondary test, the only cross combinations successfully accomplished were with the parents NC 85-01 (*F. virginiana*) (very resistant), FL 87-210 (resistant-tolerant), and 'Chandler' (very susceptible). NC 85-01 originated from open-pollinated seed collected from a wild *F. virginiana* population in Wake County, N.C. Crosses were made during Spring 1994; and five hundred seeds from each cross were put under mist in 13-cm pots on sand in a greenhouse in Dec. 1994. Once seedlings reached the two true leaf stage, they were transplanted to 40-cell plug trays containing peat substrate (Fafard Mix No.4-P; Conrad Fafard, Agawam, Mass.) and placed on greenhouse benches. Dates of transplanting depended on the rate of plant development, beginning on 25 Jan. and finishing on 20 Feb. 1995. A total of 80 seedlings from each cross and 25 plants from each parent were transplanted. Plants were watered daily and fertilized weekly with 20–20–20 (3 $g \cdot L^{-1}$ of water) to ensure proper growth and enhance runner formation. Flowers were completely removed from parent plants to avoid competition with runners. Seedlings were inoculated when they were ≈4 months old and runners were at least 15 cm long. Seedlings and plants of the parents were grown in the Horticulture Greenhouse facilities on the North Carolina State Univ. (NCSU) campus, Raleigh.

Inoculation. Milholland's technique was used to screen progeny and parents for resistance to anthracnose (Ballington and

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Milholland, 1993). The most virulent isolate of *C. acutatum* (CA-1) evaluated in a previous study (Ballington and Milholland, 1993) was grown on potato dextrose agar (PDA) medium in petri dishes for 10 d at room temperature (25 °C) under continuous fluorescent light. Conidia were washed from the surface of the colony, filtered through cheese cloth and suspended in sterile distilled water. Two drops per liter of the surfactant Tween 20 were added to the conidial suspension. Concentration of the inoculum was adjusted with a hematocytometer to 1×10^6 conidia/mL. Runners of the seedlings were carefully sprayed to runoff using a hand sprayer. Once the inoculum dried, inoculated plants were placed in a wet chamber at 28 °C and 100% relative humidity for 48 h and then returned to a greenhouse held between 21 and 28 °C. Twenty seedlings of each cross and 5 plants of each parent were inoculated each time and the test was repeated four times during Summer 1995 (80 plants/cross; 25 plants/parent). Disease development in each of two runners/plant was recorded 11 d after inoculation. A total of 1280 seedlings from crosses and 175 plants of the parents were screened for resistance, including the main and secondary tests with four replications. Inoculations and incubation in the moist chamber were conducted at the Castle Hayne Research Station, Castle Hayne, N.C. Incubation and disease readings were done in the Horticulture Greenhouses on the NCSU Campus.

Disease index. Disease severity was rated according to the length of lesion development on the runner: 0.0–1.9 cm = resistant; 2.0–2.9 cm = intermediate; and >3.0 cm = susceptible (Ballington and Milholland, 1993).

Statistics. Analysis of variance (ANOVA) was performed using the general linear models procedure (PROC GLM, SAS Institute, Cary, N.C.). Estimates of general and specific combining ability were determined using the standard methodology for analyzing data from diallel crosses (Griffing, 1956). Method 4, model I (fixed) based on F_1's, and Method 2, model I (fixed), based on parents and F_1's (half diallel) was used for the analysis of main and secondary test respectively. These methods and models for combining ability analysis were selected because of the characteristics of the experiments of this study (i.e. a reduced number of parents were used without reciprocal crosses). When a limited number of parents are evaluated (<10) and the selection of these parents cannot be considered random, then a fixed model is recommended (Baker, 1978; Christie and Shattuck, 1992). Griffing's analysis of combining ability requires no special (genetic) assumptions beyond those necessary for the ANOVA and has been used to convey reliable information on the combining potential of parents (Baker, 1978; Christie and Shattuck, 1992). Baker (1978) concluded that diallel analyses should be used only to estimate combining ability, and to attempt to interpret diallel statistics in terms of components of genetic variance is subject to failure in either of both assumptions of independent distribution of genes in the parent and absence of epistasis, unless parents have been produced by a process of random mating and nonselective inbreeding.

Results and Discussion

Main test. The ANOVA for the main test showed highly significant differences among genotypes (Table 1). The general combining ability (GCA) and specific combining ability (SCA) mean squares were highly significant (Table 1), suggesting that additive and nonadditive effects were involved in the inheritance of resistance to isolate CA-1 of *C. acutatum* on runners in these populations. Parent means for mean lesion length confirmed the resistance designations of these parental genotypes (Table 2). GCA effects for the parents refers to the average performance of a parent in hybrid combinations (Baker, 1978; Griffing, 1956; Sprague and Tatum, 1941) (Table 2). Negative values of GCA for a parent indicate that the hybrids involving that particular parent had shorter lesion lengths than the average of all hybrids. In contrast, positive values of GCA for a parent indicate that the hybrids of this parent had longer lesions than the average of all hybrids. Those parents with high negative GCA value represent a useful source for runner resistance. This resistance is very important to prevent plant and fruit infection in the field (Ballington and Milholland, 1993). Therefore, based on GCA the best parents for resistance were MS/US 541 and FL 87-210. The highest GCA for susceptibility was recorded for 'Chandler'.

The SCA for mean lesion length of each hybrid combination measured whether the performance of a progeny is superior or inferior than the average performance of the parents involved (Table 3) (Sprague and Tatum, 1941; Griffing, 1956; Baker, 1978). Therefore, a negative value of SCA for a particular combination indicates a shorter mean lesion length for that progeny than would be expected based on the average performance of the parents. Overall, there did not appear to be much relationship between SCA values and the number of resistant seedlings (Table 3). The largest deviation from expected for shorter mean lesion was obtained with NCH 87-10 x NCC 89-39 and FL 87-210 x NC 92-01. Thus, these crosses are good hybrid combinations for runner resistance to anthracnose.

These two progenies also had the shortest mean lesion lengths, however, this did not translate into higher numbers of resistant seedlings than in the other parental combinations. The largest deviation for longer mean lesion was observed on FL 87-210 x NCC 89-39 and Chandler x NCH 87-10 crosses. Again, one of these crosses, FL 87-210 x NCC 89-39, performed quite well for number of resistant seedlings in spite of high positive SCA and mean lesion length.

Secondary test. The ANOVA for the half-diallel design using only 'Chandler', FL 87-210 and NC 85-01 also showed highly significant differences among genotypes (data not shown—for details see Giménez, 1997). Both GCA and SCA mean squares were highly

Table 1. Analysis of variance of differences among genotypes and combining analysis for resistance to anthracnose in the main test.

Source	df	MS	F
Rep	3	17.46	16.4**
Crosses	13	18.22	17.1**
GCA	5	1.03	4.3**
SCA	8	1.50	6.3**
Error	39	0.24	
Error	39	1.06	

**Highly significant ($P < 0.01$).

Table 2. Parent mean of lesion length and general combining ability (GCA) for resistance to anthracnose on runners of strawberry in the main test.

Genotype	Lesion length (cm)	GCA
'Chandler'	8.95	0.49
NCC 89-39	4.42	0.01
NC 92-01	1.52	–0.04
NCH 87-10	3.19	–0.05
FL 87-210	2.77	–0.16
MS/US 541	1.10	–0.25
SE		0.22

Table 3. Specific combining ability (SCA), mean lesion length (cm) and runner reaction to anthracnose for each strawberry hybrid combination in the main test.

Cross	SCA	Lesion length (cm)	Seedling response n[z]	R[y]	I	S
FL 87-210 x NCC 89-39	1.00	6.6	76	13	3	60
'Chandler' x NCH 87-10	0.52	6.7	76	4	3	69
NCH 87-10 x NC 92-01	0.38	6.0	80	9	10	61
NCC 89-39 x NC 92-01	0.18	5.9	78	13	3	62
MS/US 541 x NC 92-01	0.18	5.6	78	11	8	59
MS/US 541 x NCH 87-10	0.17	5.6	79	6	9	64
MS/US 541 x NCC 89-39	–0.04	5.4	76	12	8	56
'Chandler' x FL 87-210	–0.05	6.0	80	4	7	69
FL 87-210 x NCH 87-10	–0.15	5.3	80	11	9	60
'Chandler' x NC 92-01	–0.20	6.0	80	10	3	67
'Chandler' x NCC 89-39	–0.27	6.3	38	4	1	33
FL 87-210 x MS/US 541	–0.31	5.0	80	17	1	62
FL 87-210 x NC 92-01	–0.54	4.6	80	15	3	62
NCH 87-10 x NCC 89-39	–0.92	4.8	79	13	7	59
SE	0.38					

[z]n = Number of seedlings screened.

[y]R, I, S = Number of resistant, intermediate and susceptible seedlings, respectively.

significant, and GCA variance was much greater than SCA variance. However, the small number of parents probably influenced the results, especially SCA effects. Small partial diallels give reasonable GCA estimates but are less efficient in detecting SCA effects (Dhillon and Singh, 1978), and are subject to large sampling errors (Christie and Shattuck, 1992). Based on GCA effects, NC 85-01 (GCA = –1.65) appeared to be a superior parent for reduced lesion length, and the combination, NC 85-01 x FL 87-210, yielded twice as many resistant seedlings (30 out of 90) as the best of any other combination (Table 3).

Overall, these results emphasize the importance of additive and nonadditive variance in the inheritance of resistance to anthracnose caused by *C. acutatum* on strawberry runners, which agrees with Gupton and Smith's (1991) conclusions. The GCA/SCA variance ratio can be useful to compare the relative importance of the variance components. A relatively high ratio suggests the importance of additive gene effects, a low one implies the presence of nonadditive (dominant, epistatic, or both) gene effects (Griffing, 1956). In the main experiment, although both combining abilities were significant, the SCA mean square was greater than GCA mean square, suggesting that nonadditive inheritance is important for anthracnose resistance. Various authors have reported that nonadditive variance is important for several horticultural characteristics of strawberry, including disease resistance (Aalders and Craig, 1968; Comstock et al., 1958; Hansche et al., 1968; Spangelo et al., 1971; Watkins and Spangelo, 1971; Watkins et al., 1970). This fact should be considered in breeding for resistance to anthracnose on runners of strawberry. When nonadditive variance is present, the most appropriate breeding method is the one that exploits all the genetic

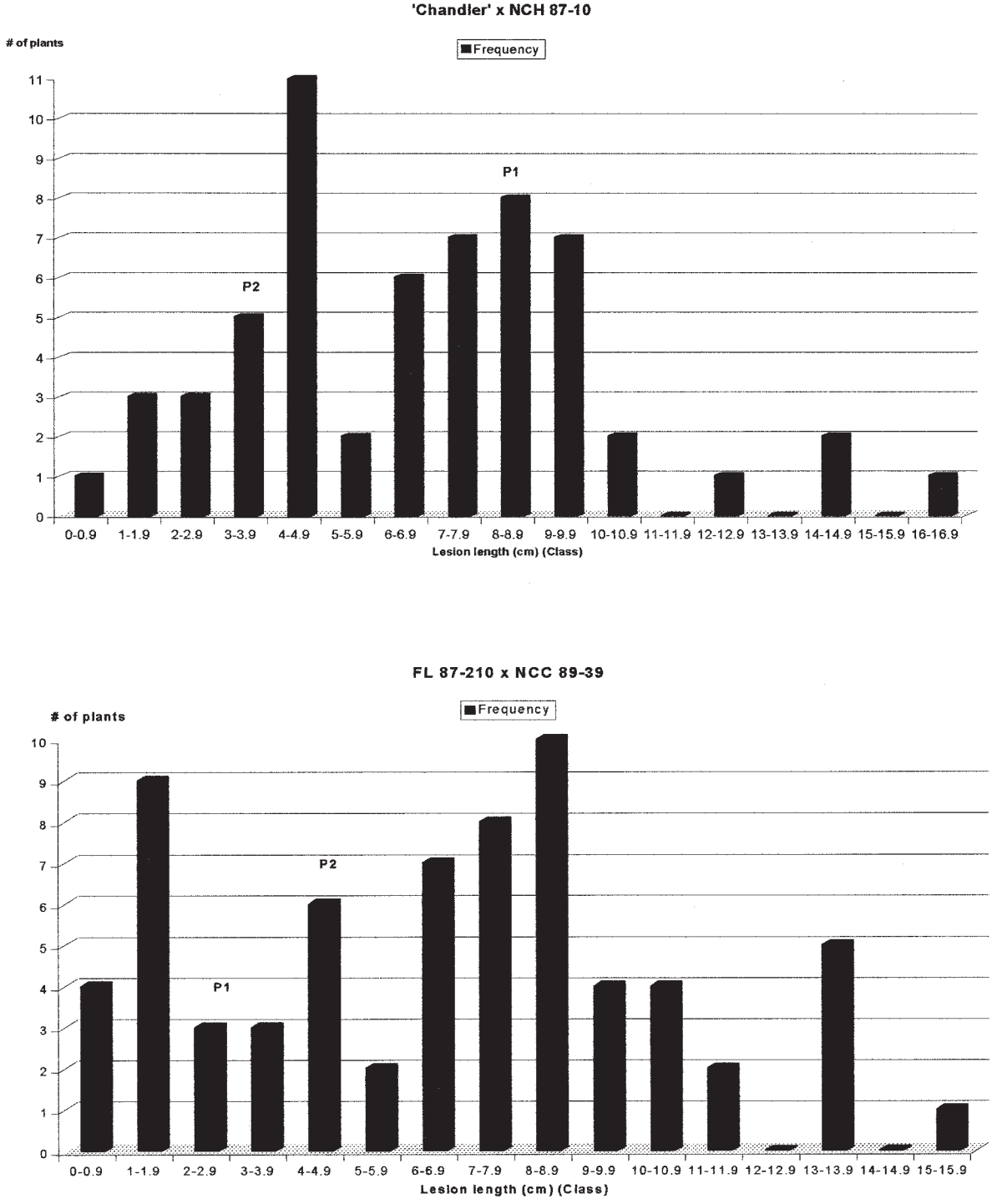

Fig. 1. Frequency distribution of *C. acutatum* lesion lengths on the runners of two strawberry progenies in the main test.

variance (Watkins and Spangelo, 1968). This could be accomplished by evaluating a large number of parental combinations (progeny testing) for identification and selection of the best combinations for resistance to anthracnose. In a second phase, these best crosses should be grown on a larger scale to select the best genotypes among them. Recombination of the best selections can also be done to obtain improved populations. Selection in a breeding program usually involves considering several characteristics, therefore, the best crosses should combine anthracnose resistance with other desirable horticultural traits.

Nature of gene action. The frequency distribution of lesion lengths for representative progenies indicate that variation was continuous (Figs. 1 and 2). Therefore, resistance to anthracnose caused by isolate CA-1 of *C. acutatum* on strawberry runners appears to be quantitative in nature. This differs from previous studies (Gupton and Smith, 1991; Faedi and De Clauser, 1991; Winterbottom et al., 1991), where major gene action was suggested for inheritance of resistance. It should be noted that mating scheme, method of genetic analysis, parents and isolate of *C. acutatum* used in this study were different from the previous ones, which could have led to different results.

Conclusions

The results of this study indicate that nonadditive and additive effects are important in the inheritance of resistance of strawberry runners to *C. acutatum*. However, since the estimate of SCA variance was greater than GCA variance, nonadditive inheritance appears to be more important. The frequency distribution of lesion lengths within progenies suggests that the inheritance of resistance to *C.*

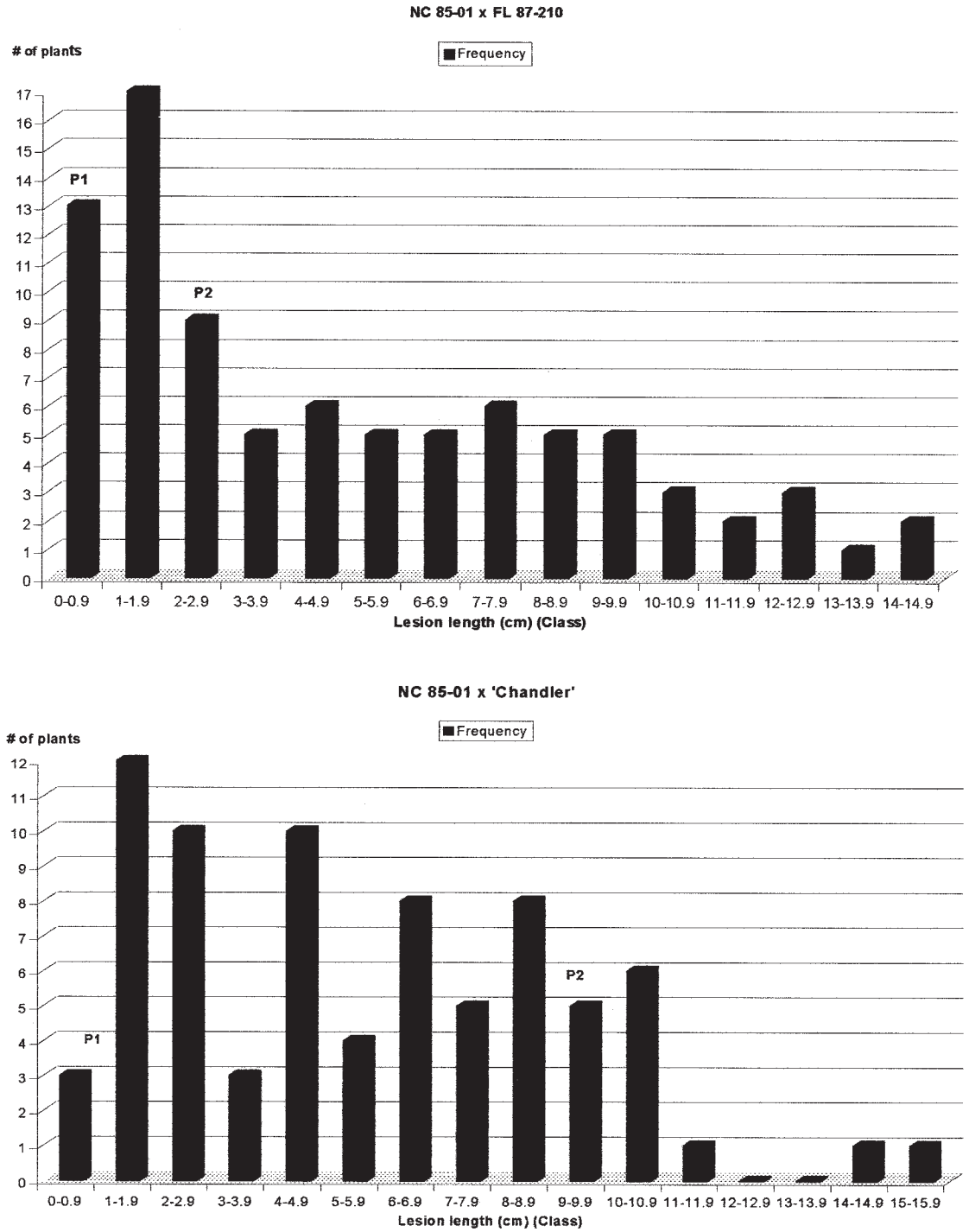

Fig. 2. Frequency distribution of *C. acutatum* lesion lengths on the runners of two strawberry progenies in the secondary test.

acutatum on strawberry runners is quantitative. Therefore, genetic progress for anthracnose resistance can be made using progeny testing for selection of the best parental combinations followed by individual selection within the best progenies.

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