

Research Article

Southern Blight (*Sclerotium rolfsii* Sacc.) of Cowpea: Genetic Characterization of Two Sources of Resistance

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Field studies were conducted to characterize the genetic nature of resistance to southern blight (caused by *Sclerotium rolfsii* Sacc.) exhibited by the cowpea [*Vigna unguiculata* (L.) Walp.] cultivars Carolina Cream and Brown Crowder and to determine if a genetic relationship exists for this resistance between the two cultivars. Examination of the comparative frequency distributions of the parental and progeny populations of the “Carolina Cream” x “Magnolia Blackeye” and “Brown Crowder” x “Magnolia Blackeye” crosses and the corresponding segregation data indicates that the southern blight resistances exhibited by “Carolina Cream” and “Brown Crowder” are conditioned by single dominant genes. Examination of the segregation data from the parental and progeny populations of the “Carolina Cream” x “Brown Crowder” cross suggests that the two resistance genes are not allelic. The availability of each of the resistance genes in cultivar-type genetic backgrounds should allow for rapid incorporation of southern blight resistance genes into other cowpea cultivars by the application of conventional plant breeding methodologies.

1. Introduction

Southern blight, a stem disease caused by *Sclerotium rolfsii* Sacc., is a common disease of cowpea [*Vigna unguiculata* (L.) Walp.] worldwide. Aycock [1] described the general effects of *S. rolfsii* in causing southern blight on numerous species of host plants. The first observed symptoms are usually a general wilting and yellowing of plants, which is soon followed by drying of foliage and plant death. Plants with advanced disease development characteristically exhibit tan to brown sclerotia and white mycelial growth on the stem epidermis at the soil surface [2]. The disease is best known by its girdling stem lesions near the soil line. Many researchers use the frequency of plant mortality to estimate the impact of southern blight on crop yields. Toler et al. [3], for example, assayed the economic importance of various diseases of cowpea in Georgia, concluding that southern blight, although a widespread problem, was responsible for an economic loss of less than 1%. More recently, Fery and Dukes [4] observed that the impact of southern blight on cowpea yield may be more attributable to reduced plant vigor

than to plant mortality *per se*. They reported that the disease can cause dry-seed yield loss of up to 53.4%.

Although southern blight has long been considered an important disease of cowpea, there is only limited information in the scientific literature that deals with resistance in cowpea. Muquit et al. [5] evaluated 20 cowpea lines for their reactions to *S. rolfsii*, and they reported that “BARI Felon-1” was moderately susceptible to the pathogen and the other tested lines were susceptible. Karat et al. [2] tested eight cowpea cultivars for their reactions to *S. rolfsii* in a replicated field trial and observed that southern blight symptoms were severe on C-152 and virtually nonexistent on NP-3. Nwakpa and Ikotun [6] screened 20 cowpea cultivars against *S. rolfsii*. They concluded that none of the cultivars were immune to the disease, but noted that the plant reactions ranged from resistant to highly susceptible. Fery and Dukes [4] noted significant variability between cowpea germplasm lines in their reactions to *S. rolfsii*. They reported that two horticultural-type cultivars, Brown Crowder and Carolina Cream, exhibited promising levels of resistance in two years of testing. The availability of southern

blight-resistant cowpea germplasm is of particular interest to plant breeders wanting to develop southern blight-resistant cowpea cultivars. The breeding value of any southern blight-resistant cowpea germplasm would be enhanced greatly if the mode of inheritance was understood. To address this need, we investigated, under field conditions, the mode of inheritance of the southern blight resistance exhibited by the cowpea cultivars Brown Crowder and Carolina Cream.

2. Materials and Methods

2.1. Field Studies. The data reported are from three field studies conducted in 1987 at the U.S. Vegetable Laboratory in Charleston, SC, USA. Plants of the parental, F_1 , F_2 , and backcross generations of the crosses “Carolina Cream” x “Magnolia Blackeye” (*Inheritance study I*) and “Brown Crowder” x “Magnolia Blackeye” (*Inheritance study II*) were evaluated for resistance to southern blight. Additionally, plants of the parental, F_1 , and F_2 generations of the cross “Carolina Cream” x “Brown Crowder” were also evaluated for resistance (*Allelism study*). “Carolina Cream” and “Brown Crowder” have consistently exhibited significant levels of resistance and “Magnolia Blackeye” has consistently exhibited susceptibility to southern blight in repeated tests at Charleston, SC, USA [4]. The severity of southern blight symptoms in all of the studies was assessed by assigning each plant a subjective score based on the severity of stem lesion development. The following scale was used to score severity of stem lesions: (1) no stem lesion, (2) small stem lesions ($\leq 25\%$ of the stem circumference), (3) moderate stem lesions (26–50% of the stem circumference), (4) large stem lesions ($> 50\%$ of the stem circumference), and (5) dead plant (stem completely girdled).

All three studies were planted on 16 July, and all plants were inoculated with *S. rolf sii* sclerotia on 13 August. A modified randomized complete block design with four replications was used for each study. Each replicate contained one plot of each parental population, 1 plot of the F_1 population, 1 plot of the reciprocal F_1 population, 4 plots of the F_2 population, and 4 plots of each backcross population (*inheritance studies only*). The plots were located in the center of raised beds spaced 102 cm apart. Each plot contained a maximum of 15 plants; the plants were spaced 91 cm apart. Starting in early October, each plant was removed from its planting site and the stem scored for degree of lesion development (2 October—*Inheritance study I*, 9 October—*Allelism study*, and 13 October—*Inheritance study II*).

2.2. Inoculum Preparation and Inoculation Procedures. The isolate of *S. rolf sii* used in these studies was collected at Charleston, SC, USA, from a field-grown pepper plant using locally prepared, acidified potato dextrose agar (APDA) medium. The isolate was subsequently maintained as dry sclerotia stored in glass vials at room temperature. The inoculum for inoculating plants was prepared using a four-step procedure. First, dry sclerotia were surface sterilized (dipped in 95% ethanol and flamed) and placed in the center of APDA plates and incubated at 30°C until the

resulting cultures produced new sclerotia. Second, 9 mm diameter plugs of APDA media containing both sclerotia and mycelium were used to inoculate 2.8 L Fernbach flasks (10 plugs per flask) containing an autoclaved mixture of 1000 g of clean sharp sand (air dry), 200 g of yellow corn meal, and 300 mL of distilled water. Third, the infested corn meal-sand media in the Fernbach flasks were incubated at 30°C for 20 days. Fourth, the corn meal-sand medium containing high concentrations of newly developed, mature sclerotia was removed from the Fernbach flasks and mixed with sufficient quantities of additional clean sharp sand to produce inoculum with the desired concentration of sclerotia. Individual plants were prepared for inoculation by removing all the top soil within ≈ 5 cm of the stem to a depth of ≈ 2 cm. One tablespoon (≈ 15 cm³) of inoculum containing approximately 34 sclerotia was placed in direct contact with the entire circumference of the exposed stem of each plant. Finally, the inoculum in the disturbed area around each stem was lightly covered with top soil.

2.3. Weighting Procedures to Correct for Misclassifications in Segregating Populations. Preliminary evaluation of the data showed variation in symptom expression in the nonsegregating parental and F_1 populations. Although this variation was expected, it is a problem when classifying individual plants with sufficient accuracy for genetic analysis. De Jong and Honma [7] encountered a similar problem in accurately classifying tomato plants for reaction to *Corynebacterium michiganense* ((E. F. Sm.) H. L. Jens). They used weighting procedures to correct the F_2 and backcross populations for misclassifications in the parental and F_1 populations. Fery and Dukes [8], who classified pepper plants for their reactions to *S. rolf sii*, used a similar type of weighting procedure to correct F_2 and backcross populations for misclassification in the nonsegregating parental and F_1 populations. We developed the following equation to correct the F_2 population and the F_1 x resistant parent and F_1 x susceptible parent backcross populations for misclassifications in the resistant parent population, the susceptible parent population, and resistant F_1 population:

$$R_w = R_o + \left(\frac{A}{B} \times C \times D \right) - \left(\frac{E}{F} \times G \times D \right) + \left(\frac{H}{I} \times J \times D \right), \quad (1)$$

where R_w is the weighted number of resistant plants in the segregating population, R_o is the observed number of resistant plants in the segregating population, A is the number of susceptible plants in the resistant parent population, B is the total number of plants in the resistant parent population, C is the expected frequency of plants with the resistant parent genotype in the segregating population, D is the total number of plants in the segregating population, E is the number of resistant plants in the susceptible parent population, F is the total number of plants in the susceptible parent population, G is the expected frequency of plants with the susceptible parent genotype in the segregating population, H is the number of susceptible plants in the F_1 population, I is the total number of plants in the F_1 population, and J

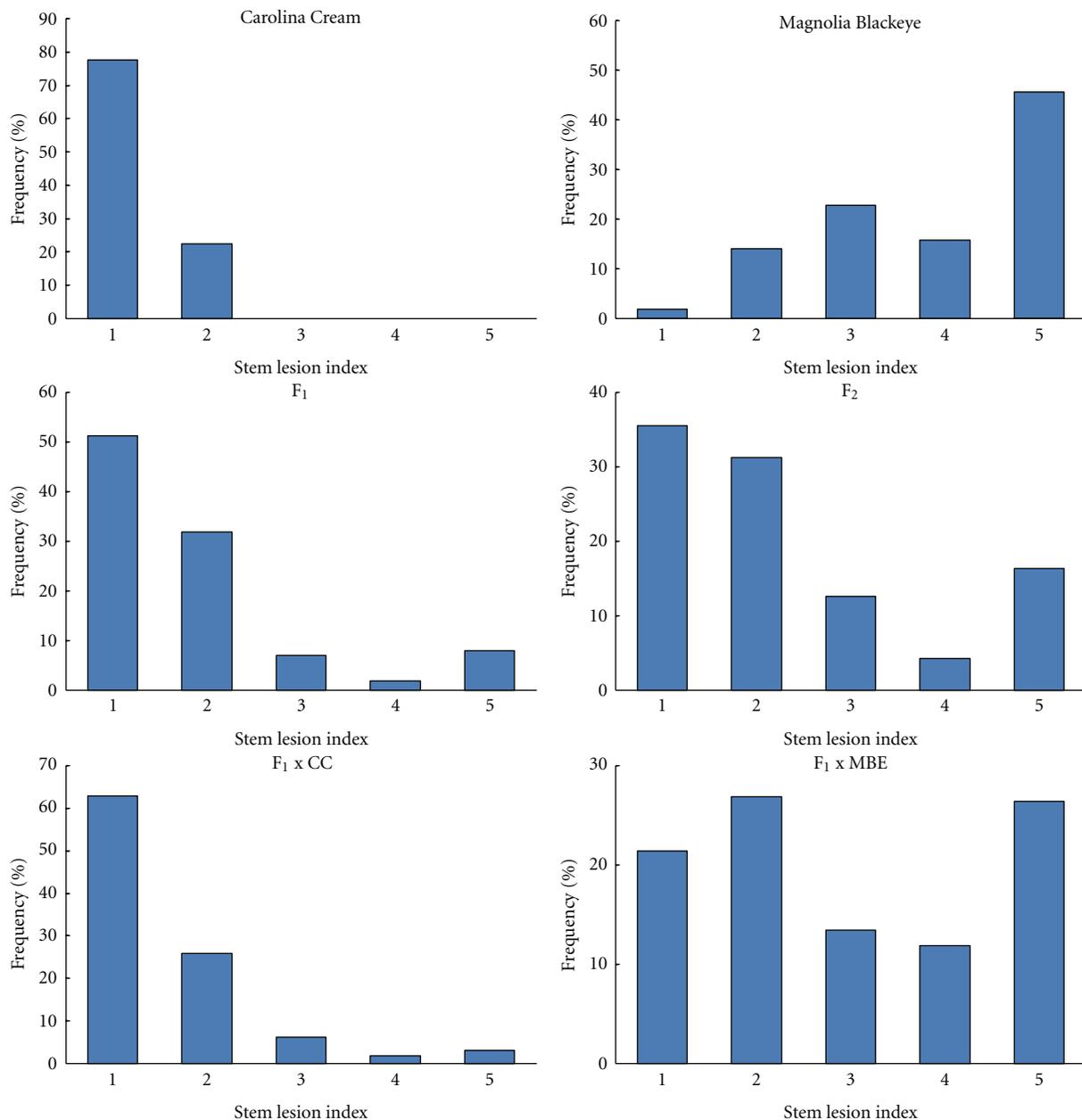


FIGURE 1: Comparative frequency distributions of reactions (1 = no stem lesions; 5 = dead plant or stem completely girdled) of field-grown plants from the parental, F₁, F₂, and backcross populations of the cross “Carolina Cream” x “Magnolia Blackeye” inoculated with *Sclerotium rolfsii* (inheritance study I).

is the expected frequency of plants with the F₁ genotype in the segregating population. Chi-square tests for goodness of fit were used to test genetic hypotheses (weighted observed segregation versus expected segregation).

3. Results

3.1. Reciprocal F₁ Populations. Preliminary analyses revealed no significant differences between any of the reciprocal F₁ populations evaluated in the three studies. As a result, the data from the reciprocal F₁ populations were pooled for all genetic analyses.

3.2. Inheritance Study I. Examination of the comparative frequency distributions of observed stem lesion reactions of the “Carolina Cream,” “Magnolia Blackeye,” F₁, F₂, F₁ x “Carolina Cream,” and F₁ x “Magnolia Blackeye” populations illustrated several aspects of the genetic system conditioning southern blight resistance in “Carolina Cream” (Figure 1). First, all of the plants in the “Carolina Cream” population exhibited a resistant reaction (stem lesion score ≤2). Second, the majority of the plants in the “Magnolia Blackeye” population exhibited a susceptible reaction (stem lesion score ≥3). Third, the majority of plants in the F₁ population

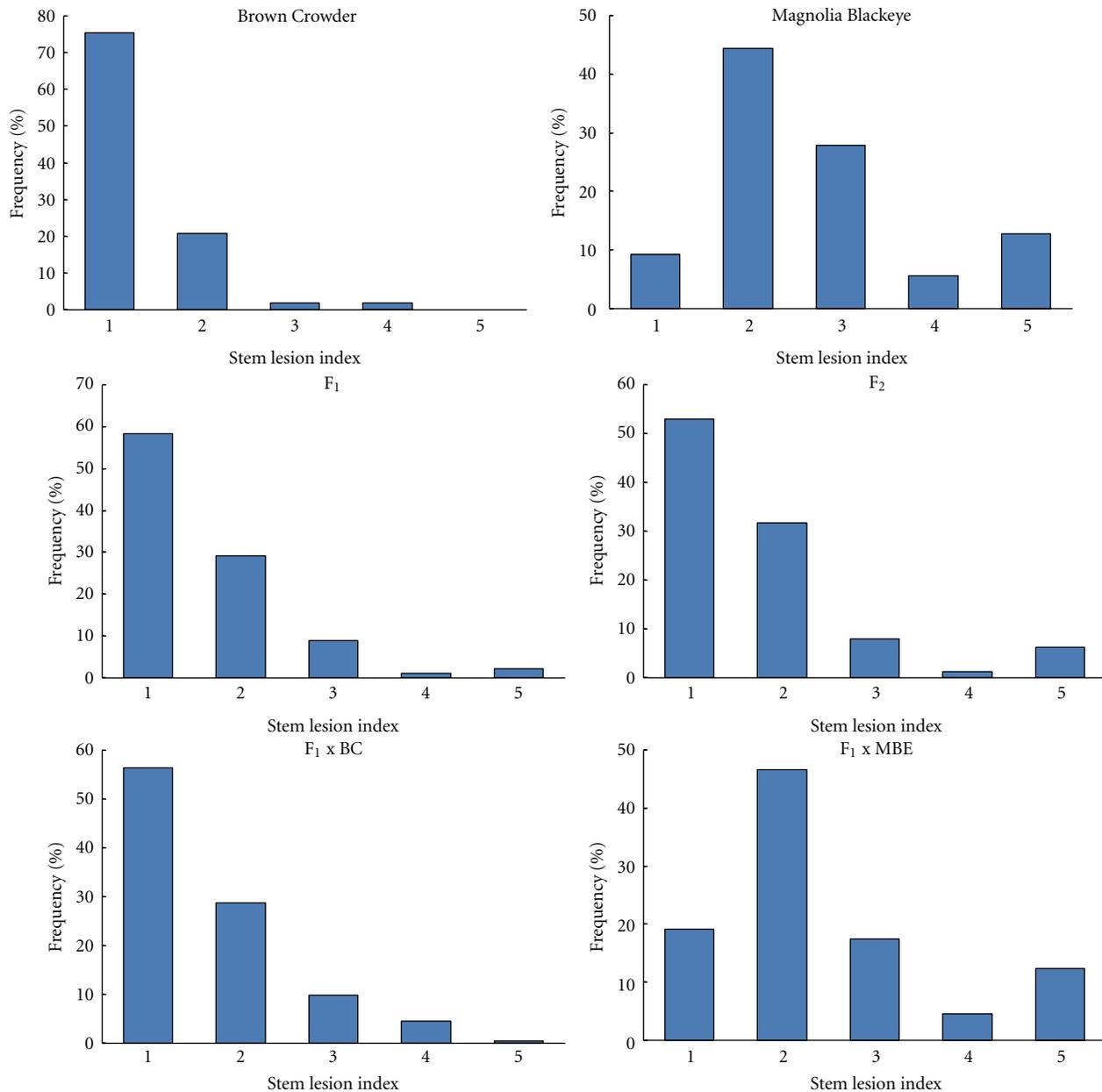


FIGURE 2: Comparative frequency distributions of reactions (1 = no stem lesions; 5 = dead plant or stem completely girdled) of field-grown plants from the parental, F₁, F₂, and backcross populations of the cross “Brown Crowder” x “Magnolia Blackeye” inoculated with *Sclerotium rolfsii* (inheritance study II).

exhibited a resistant reaction, indicating that the southern blight resistance is inherited as a dominant trait. Fourth, the distribution frequencies in the F₂ population and F₁ x “Magnolia Blackeye” backcross population appeared to be bimodal, which indicated that southern blight resistance is conditioned by a qualitative rather than a quantitative genetic system. Examination of weighted segregation data indicated that southern blight resistance in “Carolina Cream” is conditioned by a single dominant gene (Table 1). The F₂ population segregated 3 resistant: 1 susceptible, the F₁ x “Magnolia Blackeye” backcross population segregated 1

resistant: 1 susceptible, and all but 6 of the 224 plants in the F₁ x “Carolina Cream” backcross population were resistant.

3.3. Inheritance Study II. Examination of the comparative frequency distributions of observed stem lesion reactions of the “Brown Crowder,” “Magnolia Blackeye,” F₁, F₂, F₁ x “Brown Crowder,” and F₁ x “Magnolia Blackeye” populations illustrated several aspects of the genetic system conditioning southern blight resistance in “Brown Crowder” (Figure 2). First, most of the plants in the “Brown Crowder” population exhibited a resistant reaction (stem lesion score ≤ 2). Second,

TABLE 1: Segregation for resistance to southern blight (*Sclerotium rolfsii*) in parental, F₁, F₂, and backcross populations of the cross “Carolina Cream” x “Magnolia Blackeye” (*inheritance study I*).

Population	Observed segregation				Expected segregation		Chi-square ^c	P
	Unweighted R ^a	S ^b	Weighted R	S	No. of plants R	S		
Carolina Cream (P ₁)	58	0			58	0	1:0	
Magnolia Blackeye (P ₂)	9	48			0	57	0:1	
F ₁	94	19			113	0	1:0	
F ₂	154	77	164	67	173	58	3:1	1.87
F ₁ x P ₁	199	25	218	6	224	0	1:0	
F ₁ x P ₂	97	104	98	103	101	100	1:1	0.18

^aR: number of resistant plants (stem lesion index ≤ 2); ^bS: number of susceptible plants (stem lesion index ≥ 3); ^cweighted observed segregation versus expected segregation.

TABLE 2: Segregation for resistance to southern blight (*Sclerotium rolfsii*) in parental, F₁, F₂, and backcross populations of the cross “Brown Crowder” x “Magnolia Blackeye” (*inheritance study II*).

Population	Observed segregation				Expected segregation		Chi-square ^c	P
	Unweighted R ^a	S ^b	Weighted R	S	No. of plants R	S		
Brown Crowder (P ₁)	51	2			53	0	1:0	
Magnolia Blackeye (P ₂)	29	25			0	54	0:1	
F ₁	78	11			89	0	1:0	
F ₂	192	35	178	49	170	57	3:1	1.50
F ₁ x P ₁	172	30	188	14	202	0	1:0	
F ₁ x P ₂	117	61	80	98	89	89	1:1	1.82

^aR: number of resistant plants (stem lesion index ≤ 2); ^bS: number of susceptible plants (stem lesion index ≥ 3); ^cWeighted observed segregation versus expected segregation.

a large portion of the plants in the “Magnolia Blackeye” population exhibited a susceptible reaction (stem lesion score ≥ 3). Third, the majority of plants in the F₁ population exhibited a resistant reaction, suggesting that the southern blight resistance is inherited as a dominant trait. Fourth, the distribution frequencies in the F₂ population and F₁ x “Magnolia Blackeye” backcross population appeared to be bimodal, which indicated that southern blight resistance is conditioned by a qualitative rather than a quantitative genetic system. Examination of weighted segregation data indicated that southern blight resistance in “Brown Crowder” is conditioned by a single dominant gene (Table 2). The F₂ population segregated 3 resistant: 1 susceptible, the F₁ x “Magnolia Blackeye” backcross population segregated 1 resistant: 1 susceptible, and all but 14 of the 202 plants in the F₁ x “Brown Crowder” backcross population were resistant.

3.4. Allelism Study. The parental lines used in the cross “Carolina Cream” x “Brown Crowder” reacted to *S. rolfsii* as expected (Table 3). Fifty-seven of the 58 plants evaluated in the “Carolina Cream” population and 52 of the 56 plants evaluated in the “Brown Crowder” population exhibited resistant reactions. However, the frequencies of susceptible plants in the F₁ and F₂ populations are much greater than would be expected if the same gene conditions resistance in both “Carolina Cream” and “Brown Crowder.” The 19.7% frequency of susceptible plants in the F₁ population

and the 14.5% frequency of resistant plants in the F₂ population are considerably greater than the 1.7% observed in the parental “Carolina Cream” population and the 7.1% observed in the parental “Brown Crowder” population. The apparent segregation observed in the F₂ population of the “Carolina Cream” x “Brown Crowder” cross suggests that the dominant resistance genes in these resistant cultivars are not allelic. Additionally, the high frequency of susceptible plants observed in the F₁ “Carolina Cream” x “Brown Crowder” population is similar to the responses of the F₁ populations evaluated in the “Carolina Cream” and “Brown Crowder” inheritance studies discussed earlier; this type of response is indicative of a degree of partial dominance when the resistance genes are in the heterozygous condition and would not be expected if the F₁ “Carolina Cream” x “Brown Crowder” plants received the same dominant resistance allele from both parents.

4. Discussion

The procedures used to evaluate cowpea plants in this study for reaction to *S. rolfsii* were not ideal for an inheritance study because the plants within all three nonsegregating populations (the resistant parent, the susceptible parent, and the F₁) displayed a range of reactions. However, this was not unexpected because we have seen similar responses in virtually all cowpea cultivars evaluated in field trials

TABLE 3: Segregation for resistance to southern blight (*Sclerotium rolfsii*) in parental, F₁, and F₂ populations of the cross “Carolina Cream” x “Brown Crowder” (*Allelism study*).

Population	Number of resistant plants ^a	Number of susceptible plants ^b	Total number of plants	Frequency of susceptible plants (%)
Carolina Cream (P ₁)	57	1	58	1.7
Brown Crowder (P ₂)	52	4	56	7.2
F ₁	61	15	76	19.7
F ₂	201	34	235	14.5

^aResistant plant (stem lesion score ≤ 2).

^bSusceptible plant (stem lesion score ≥ 3).

conducted over multiple years. Aycock [1] cited a substantial body of published *S. rolfsii* literature about “the erratic distribution of diseased plants in the field.” He noted that “it is characteristic that all plants even in a uniformly infested area do not become infected.” Aycock [1] cited work by Higgins [9] noting that the distribution of southern blight “on pepper was quite unlike that of diseases caused by wilt *Fusaria* in which a high percentage of plants in an infested area succumb.”

The high levels of variation in the reactions to *S. rolfsii* exhibited by the parental and F₁ hybrid populations used in the studies and the categorical system of rating individual plants (versus a true metric rating system) placed severe limitations on the effective use of any quantitative type of analysis. Our rationale for using a weighting procedure to correct the segregating F₂ and backcross populations using the misclassification frequencies observed in the resistant parent populations, the susceptible parent populations, and the resistant F₁ populations is based on the following assumptions: (1) the underlying genetic system conditioning the resistance is qualitative rather than quantitative in nature, (2) the parental populations are not heterogeneous for resistance or susceptibility to *S. rolfsii*, and (3) the homozygous resistant genotypes (e.g., *RR*), homozygous susceptible genotypes (e.g., *rr*), and heterozygous resistant F₁ genotypes (e.g., *Rr*) respond in the segregating populations in the same manner as in the nonsegregating parental and F₁ populations.

5. Conclusions

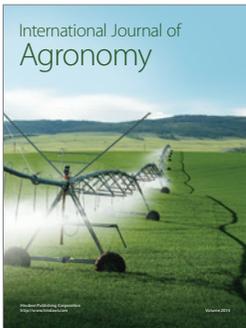
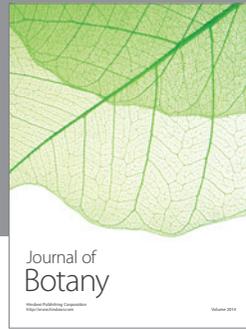
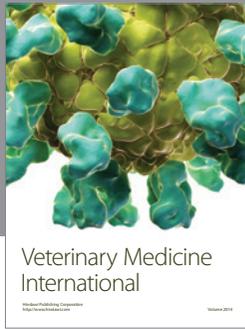
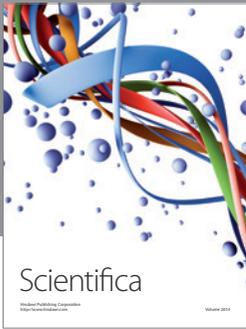
The results of these studies indicate that single dominant genes condition southern blight resistance in both “Carolina Cream” and “Brown Crowder.” The resistance genes do not appear to be allelic. The level of resistance conditioned by each of the genes is high enough to recommend them for use in cowpea breeding programs. The availability of each of these genes in cultivar-type genetic backgrounds should make the incorporation of southern blight resistance genes into cowpea cultivars by the application of conventional plant breeding methodologies a readily achievable objective.

Acknowledgments

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