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Genetics and Nature of Resistance to Powdery Mildew in Crosses of Butternut with Calabaza Squash and 'Seminole Pumpkin'

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Abstract. **The inheritance of resistance in butternut squash (*Cucurbita moschata* Poir) to powdery mildew (*Erysiphe cichoracearum* DC) was studied under greenhouse and field conditions using F_1, F_2, and BC_1 generations derived from crosses of 'La Primera' (resistant) (calabaza type) with 2 butternut cultivars 'Ponca' (susceptible), and 'Waltham' (susceptible), and 'Ponca' x 'Seminole Pumpkin' (intermediate resistance). The breath-blowing method of inoculation was effective in greenhouse tests. Petiole and stem reactions of plants in both field and greenhouse trials and leaf-blade reactions of plants in the field tests indicated that 3 alleles at a single locus determined resistance reactions in crosses involving 'Ponca', 'Waltham', and 'La Primera'. Proposed genotypes for the susceptible 'Ponca' and 'Waltham' butternut squash are $pm-1^P$ $pm-1^P$ and $pm-1^W$ $pm-1^W$, respectively, and for the resistant 'La Primera', $pm-1^L$ $pm-1^L$. The partial and complete dominance relations of these alleles for the leaf-blade (field) and petiole/stem reactions (field/greenhouse), respectively, are $pm-1^P>pm-1^L>pm-1^W$. A different gene, $pm-2^s$, controlled the intermediate resistance of 'Seminole Pumpkin'. However, a quantitative inheritance pattern of disease reactions of leaf blades was observed in the greenhouse trials in all crosses, presumably because of the higher inoculum load and the increased intermediate ratings of the heterozygotes under these conditions. Leaf-blade and stem/petiole resistance was completely associated in segregating progenies in the field but not in the greenhouse because of the intermediate susceptibility of the leaf blades of heterozygotes under those conditions. Scanning electron micrographs of powdery mildew on compatible and incompatible hosts showed that differential compatibility occurred in conidial germination. Resistance in 'La Primera' involved delayed conidia germination, retarded hyphal growth, shorter conidiophores, and weak sporulation.**

Butternut squash is one of the most popular types of winter squash grown in many parts of North America and there is a need to develop improved cultivars with resistances to pests and pathogens. Powdery mildew is one of the more serious diseases of cucurbits (12, 15, 21, 24, 29) and is usually incited by either of 2 organisms, *Erysiphe cichoracearum* and *Sphaerotheca fuliginea* (Schlecht. ex. Fr.) Poll. (4, 5, 11, 14, 15, 20). Both pathogens may occur in the same locality and on the same plant (4). In the absence of cleistothecia, the perfect stages of the causal organisms, conidial characteristics and differential hosts have been used to distinguish between the 2 pathogens (4, 11, 13). The nature of host-pathogen interactions, using scanning or transmission electron microscopy, have been investigated with the powdery mildew pathogen of cereals (8, 9, 18). Resistance was expressed in an incompatible cereal host genotype after penetration by the pathogen into the leaf (25).

Control of powdery mildew on all of the cultivated *Cucurbita* species by fungicide applications has been less than satisfactory, and various investigators have conducted studies to identify sources of resistance (1, 3, 7, 17, 20, 26, 27, 28, 29), to study the associations between the resistances in different plant organs (6, 7), to determine the inheritance of resistance (6, 7, 10, 19, 20, 22, 23), and to develop resistant cultivars (H.M. Munger, personal communication). High resistances to both pathogens were found in *C. lundelliana* Bailey (6, 7, 27, 29). Resistance to *E. cichoracearum* was found in both *C. martinezii* Bailey (*C. okeechobeensis* Bailey) (6, 7) and *C. moschata* (20). A single dominant gene determined resistance to both pathogens in crosses of the first 2 species (6, 7, 20). Nevertheless, no butternut squash cultivar resistant to powdery mildew has been released yet.

Two *C. moschata* cultivars, 'La Primera' (calabaza type) and 'Seminole Pumpkin', were observed by us to have high and intermediate resistance to powdery mildew in field plantings, respectively, in Nebraska. A low frequency of a 'flecking' type reaction, not previously reported, occurred on the leaves of 'La Primera' late in the season (August). *C. lundelliana* and *C. martinezii* had not exhibited any symptoms in these plantings. This paper reports on comparison of inoculation methods, inheritance of different levels of resistance, allelic tests, and the nature of the host-pathogen interaction.

Materials and Methods

Pathogen identification and use. The pathogen used in these experiments was identified as *E. cichoracearum* on the basis that it produced unbranched germ tubes after conidial germination and the conidia showed no fibrosin bodies upon staining in 3% aqueous potassium hydroxide (4, 25).

Leaves of powdery mildew-susceptible butternut 'Ponca', which showed abundant sporulating colonies, were collected in the field, Lincoln, Neb. in October 1979. The fungus was main-

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tained on greenhouse-grown plants of 'Ponca' for use in all subsequent experiments.

Evaluation of inoculation methods. Four methods of inoculation were tested using greenhouse-grown 'Ponca' seedlings (experiment 1): 1) dusting, 2) spraying, 3) breath-blowing, and 4) open-air or natural inoculation (Table 1). Plants were inoculated at cotyledon expansion and again at first true leaf expansion. In method 1, seedlings were dusted with spores from infected leaves. For method 2, conidia were suspended in water and applied to the seedlings with an atomizer. In method 3, conidia from heavily infected leaves were applied by 'blowing' over the test plants via the human breath. Noninoculated plants were used as a control.

A randomized complete block design, with 3 replications of each of 4 pots (2 plants/pot) per treatment was used in each experiment. The seeds were sown in 15-cm pots under 21.1°C (day)/15.6° (night), November 25, 1979 and January 18, 1980, respectively. Photoperiod was about 10 hr. No supplementary light was used. Since similar results were obtained for each test, the data were pooled.

Inheritance—crosses. During Summer 1978, plants of 'Ponca' (P_1) (susceptible), 'Waltham' (P_2) (susceptible), 'Seminole Pumpkin' (P_4) (moderately resistant), and 'La Primera' (P_3) (highly resistant) were grown in the field and the following crosses were made: $P_3 \times P_1$, $P_3 \times P_2$, and $P_1 \times P_4$. The crosses $P_1 \times P_3$ and $P_2 \times P_3$ included reciprocals. Several F_1 plants of each cross were grown subsequently in greenhouse and field plantings to produce F_2 and certain backcross seed. A cross between 'La Primera' (P_3) and 'Seminole Pumpkin' (P_4) was also made in the greenhouse.

Experimental design. A randomized complete block design (except experiment 12, observation trial) with 3 replications (except experiments 12, 14, and 15, 4 replications) was used in each greenhouse and field experiment, with each one containing the parents and progeny of a particular cross as described later. In the field experiments, each replicate consisted of rows of 7 single-plant-hills (except experiment 13, 8 hills) spaced 1.8 m apart within rows and 2.4 m between rows. Each replicate was separated by a border row of single hills of susceptible crookneck summer squash.

Greenhouse. Parental, F_1, F_2, and backcross generations of the crosses $P_3 \times P_1$ (experiment 2), $P_3 \times P_2$ (experiment 3), and $P_1 \times P_4$ (experiment 4) were evaluated in the greenhouse for reaction to powdery mildew. In all experiments, seeds were planted in flats (Jiffy 7's). Seedlings were inoculated using the "breath-blowing" method at the first true leaf stage. Three days later they were transplanted, 2 plants per pot, into 15-cm pots containing equal parts of soil, sand, peat, and vermiculite. Two more inoculations were made at weekly intervals. Photoperiod and temperatures were about the same as described previously.

Table 1. Comparison of methods for inoculating butternut 'Ponca' cultivar with *Erysiphe cichoracearum* (experiment 1).

Inoculation method	Rating disease reaction[z]
Dusting	5.0a[y]
Spraying	2.8c
Breath-blowing	3.6b
Open-air	1.7d

[z]Rating scale: 0 = no visible infection; 5 = entire leaf surface covered with dense mildew, plant dying or dead.
[y]Mean separation by Duncan's multiple range test, 5% level.

Field. Seeds of the parental, F_1, and F_2 generations of the crosses $P_3 \times P_1$ and $P_3 \times P_2$, and BC_1 $P_1 \times (F_1\ P_3 \times P_1)$ and $BC_1\ P_2 \times (F_1\ P_3 \times P_2)$ were planted in Jiffy 7's in the greenhouse (30°C day/22° night) on May 9, 1980, and transplanted in the field, Lincoln, Neb. on May 22, 1980. The herbicide trifluralin .0011 g m^{-2} (1.12 kg/ha) was soil-incorporated a day before transplanting (Experiments 5, 6, and 7). Seedlings were inoculated at the cotyledonary and first true leaf stages by the "breath blowing" inoculation method. Four single-row plots of each F_2 population and one single-row plot of each of the other populations were used in each replication.

Because of trifluralin injury to plants in experiments 5, 6, and 7, 3 additional, similar field-experiments involving parents and progeny from the crosses $P_3 \times P_1$ (experiment 8), $P_3 \times P_2$ (experiment 9), and $P_1 \times P_4$ (experiment 10) were conducted. The seed were planted June 28, 1980, in the greenhouse and transplanted to the field July 10, 1980. The herbicide amiben .0022 g m^{-2} (2.24 kg/ha) was sprayed over the plants post-transplanting in these and all subsequent experiments. The transplants were covered with milk cartons to protect the plants only during application. Sprinkler irrigation was used as needed in all field experiments.

Six separate experiments designated as experiments 11 through 15 were conducted in the field in 1981. Transplants, when used, were raised and inoculated as in the 1980 experiments. Seed of parents, F_1, F_2 $P_3 \times P_1$, BC_1 $P_1 \times (F_1\ P_3 \times P_1)$, and selfed BC_1 $P_1 \times (F_1\ P_3 \times P_1)$ generations were direct-seeded on May 22, 1981 (experiment 12). Greenhouse-raised seedlings (sown May 8, 1981) of the same populations as in experiment 12, except the backcross populations, were transplanted in the field May 21, 1981 (experiment 11). Trifluralin at the rate of .0011 g m^{-2} (1.12 kg/ha) was soil-incorporated a day before direct-seeding or transplanting (experiments 11 and 12). Both degree of powdery mildew reaction and trifluralin injury (1, 2) were recorded but only the powdery mildew data will be reported here.

Plants of parents, F_1, F_2, and second BC to $P_2 \times (F_1\ P_3 \times P_2)$ of the cross $P_3 \times P_2$ (experiment 12) were transplanted at the same time as those in experiment 11. The parents, F_1, and F_2 seeds of the crosses $P_4 \times P_3$ (experiment 14) and $P_1 \times P_2$, along with BC_1 $P_1 \times (F_1\ P_2 \times P_1)$ of the latter cross (experiment 15) were sown in Jiffy 7 pellets on June 16 and July 23, 1981, and were transplanted into the field, July 2 and August 4, 1981, respectively (Table 4). Ratings for the powdery mildew reaction were recorded at the end of September.

Disease reaction ratings. Disease reaction was assessed using the following rating scale: 1 = no infection; 2 = visible infection, chlorotic areas with no sporulation; 3 = few isolated sporulating colonies on less than 5% of leaf surface; 4 = numerous isolated sporulating colonies on 5–30% of leaf surface; 5 = many isolated sporulating colonies on more than 30% of leaf surface; 6 = coalescing colonies, with heavy sporulation on more than 30% of leaf surface; 7 = entire leaf surface covered with dense mildew, plant dying or dead. The rating scale is similar to Ballantyne's (4), but contains classes 2 and 7 for additional information since a rating of 2 indicates chlorotic areas with no sporulation and 7 indicates that the entire leaf is covered with dense mildew. The presence or absence of powdery mildew on stem and petioles was also determined. Ratings were recorded at the end of August in all experiments except for the late-planted experiments 8, 9, 10, 14, and 15 when the ratings were recorded at the end of September.

Host-pathogen interaction study. The host reaction to the pathogen causing powdery mildew was investigated using the cultivars 'La Primera' (P_3), 'Waltham' (P_2), 'Ponca' (P_1), and

Table 2. Frequency distributions (continuous) for leaf reaction and discrete segregation for petiole and stem reaction to powdery mildew in parents, F_1, F_2, and BC_1 generations derived from 3 *Cucurbita moschata* crosses (greenhouse, 1980).

Experiment Generations	Leaf reaction[z] (No. plants in disease reaction classes) Class 1	2	3	4	5	6	7	Total no. of plants	Mean	No. of plants with mildew-free petioles/stems	No. of plants with mildewed petioles/stems
Experiment 2											
Ponca (P_1)						2	6	8	6.8		8
La Primera (P_3)	1	5						6	1.8	6	
F_1 (P_3 x P_1)				9	6			15	4.4		15
F_2 (P_3 x P_1)		5	19	27	26	19	3	99	4.4	28	70
										(1:3, χ^2 = 0.67, P = .25–.50)	
BC_1 P_1 x (F_1 P_3 x P_1)					4	1	1	6	5.5		6
Experiment 3											
Waltham (P_2)						5	4	9	6.4		9
La Primera (P_3)	2	5						7	1.7	7	
F_1 (P_3 x P_2)			6	4				10	3.4	10	
F_2 (P_3 x P_2)	2	13	17	36	16	24		108	4.1	74	34
										(3:1, χ^2 = 2.42, P = .10–.25)	
Experiment 4											
Ponca (P_1)						3	3	6	6.5		6
Seminole Pumpkin (P_4)				4	5			9	4.6		9
F_1 (P_1 x P_4)					7	9		16	5.6		16
F_2 (P_1 x P_4)			22	23	22	20	24	111	5.1	22	79
										(1:3, χ^2 = 1.41, P = .10–.25)	

[z]Leaf reaction ratings: 1 = no infection; 2 = visible infection, chlorotic areas with no sporulations; 3 = few isolated sporulating colonies on less than 5% of leaf surface; 4 = numerous isolated sporulating colonies on 5–30% of leaf surface; 5 = many isolated sporulating colonies on more than 30% of leaf surface; 6 = coalescing colonies, with heavy sporulation on more than 30% of leaf surface; 7 = entire leaf surface covered with dense mildew, plant dying or dead.

'Seminole Pumpkin' (P_4) (experiment 16). Three 15-cm pots (2 plants/pot) of each cultivar were randomly arranged in a Kysor-Sherer growth chamber, temperature 21.1°C (day)/15.6° (night) and 12-hr photoperiod. The pots were seeded on April 14, 1981. A light intensity of 0.18 μ E $cm^{-2}sec^{-1}$ (at the rim of the pots, 87 cm below the light) was provided by two 100-watt incandescent bulbs and sixteen 1.83 m VHO white fluorescent tubes in the chamber. The potting mixture was 1 sand:1 soil:1 sphagnum moss:1 vermiculite (by volume). Plants were inoculated at the first true leaf stage, May 1, 1981, by breath-blowing spores from infected 'Ponca' leaves. Two 1 cm^2 leaf tissue samples containing fungus structures occurring on the adaxial leaf surface were obtained per plant. Leaf samples were collected at 6, 12, 18, 24, 36, 48, 60, and 72 hr and at 4, 7, 14, and 28 days after inoculation. Immediately after collection, leaf samples were fixed overnight at room temperature (20°) in formalin-acetic ethyl-alcohol (FAA) fluid described by Sass (21). Tissues were then rinsed with water and dehydrated for 30 min in each of the following series: 30, 50, 70, 90, and 100% acetone. Specimens were then dried to a critical point (with CO_2 at 74.9 kg/cm^2 and 42°) in a Denton critical point drying apparatus (DCP-1). After specimens were mounted on stubs, they were coated with 200 Å of gold-palladium in a Technic's Hummer II sputtering apparatus. Specimens were then observed with a Cambridge S4-10 stereoscan operated at 20KV.

Results

Evaluation of inoculation methods. The mean differences between inoculation methods for the leaf reaction of the 'Ponca' cultivar were significant, with the dusting method causing the most severe reaction (Table 1), followed by the breath-blowing and spraying methods, respectively. With the dusting method, plants suffered severe tissue damage due to abrasion from leaf contacts. In addition, clumps of mycelia and spores were deposited on leaf surfaces, resulting in too high an inoculum pressure on the host. Both the spraying and the breath-blowing methods provided an even distribution of spores on leaf surfaces, with the latter being more effective. Spore concentration on leaf surfaces was not quantified in any of the 4 methods. When spores were breath-blown from infected leaves, a cloud of spores settled on the leaf surfaces, giving a dusty-white appearance. The inoculum concentration in this method appeared to be higher than the inoculum load on leaf surfaces prevalent under field situations. Some fungal development occurred on the noninoculated plants but it was very slow and nonuniform. Inoculum probably came from adjacent inoculated plants.

Inheritance-Greenhouse. Continuous distributions for number of plants in different disease-reaction classes of leaf-blades were observed in the F_2 generations of 3 crosses (Table 2), indicating quantitative inheritance patterns. The mean disease reaction of all the F_1s approached the midparent means with additive gene action in F_1 (P_1 x P_3) and F_1 (P_1 x P_4) and with some partial dominance for resistance in P_2 x P_3. Moreover, transgressive segregation for increased levels of resistance was observed in the F_2 (P_1 x P_4).

Plants were classified as resistant or susceptible if mildew was absent or present on stems and petioles of plants (Table 2). A good fit to a 3:1 segregation ratio of susceptible to resistant plants was observed in the F_2 'La Primera' (P_3) x 'Ponca' (P_1) and F_2 'Ponca' (P_1) x 'Seminole Pumpkin' (P_4), indicating that a recessive gene primarily determined resistance of stems and petioles of plants to powdery mildew in these crosses. In the cross between 'La Primera' (P_3) and 'Waltham' (P_2), a different type of F_2 segregation pattern was observed. There was a sat-

Table 3. Frequency distributions and segregation for leaf reaction plus petiole and stem reactions to powdery mildew in parents, F_1, F_2, and BC_1 generations derived from crosses involving 'Ponca' (P_1), 'Waltham' (P_2), 'Seminole Pumpkin' (P_4) and 'La Primera' (P_3) (field, 1980).

	Frequency distributions and segregation for leaf reaction											Petiole and stem reaction	
	No. plants in disease reaction classes[z]									Observed no. of plants			
Experiment Generations	Class 1	2	3	4	5	6	7	Tot. No. of plants	Mean	Res.	Sus.	No. of plants with mildew-free petioles/stems	No. of plants with mildewed petioles/stems
Experiment 5													
Ponca (P_1)							14	14	7.0		14		14
La Primera (P_3)		23						23	2.0	23		23	
F_1 (P_3 x P_1)					9	4		13	5.3		13		13
F_2 (P_3 x P_1)		6	21			9	45	81	5.5	27	54	27	54
								(1:3, χ^2 = 3.00, P = .05–.10)				(1:3, χ^2 = 3.00, P = .05–.10)	
BC_1 P_1 x (F_1 P_3 x P_1)					6	2	9	17	5.6		17		17
Experiment 6													
Waltham (P_2)							22	22	7.0		22		22
La Primera (P_3)		9						9	2.0	9			
F_1 (P_3 x P_2)				15	8			23	4.4			23	
F_2 (P_3 x P_2)		8	12	38		8	18	84	4.5	58	26	57	27
								(3:1, χ^2 = 1.59, P = .10–.25)				(3:1, χ^2 = 2.29, P = .10–.25)	
BC_1 P_2 x (F_1 P_3 x P_2)			2	7			13	22	5.7	9	13	8	14
								(1:1, χ^2 = 0.73, P = .25–.50)				(1:1, χ^2 = 1.64, P = .10–.25)	
Experiment 7													
Ponca (P_1)							23	23	7.0		23		23
Seminole Pumpkin (P_4)				15	7			22	4.3	15	7	15	7
F_1 (P_1 x P_4)						4	6	10	6.6		10		10
F_2 (P_1 x P_4)				12	14	23	35	84	6.0			16	68
					(Continuous distribution observed)							(1:3, χ^2 = 1.58, P = .10-.25)	
Experiment 8													
Ponca (P_1)							16	16	7.0		16		16
La Primera (P_3)		12						12	2.0	12		12	
F_1 (P_3 x P_1)						14		14	6.0		14		14
F_2 (P_3 x P_1)		2	10	11		30	34	87	5.7	23	64	21	66
								(1:3, χ^2 = .10, P = .75–.90)				(1:3, χ^2 = .03, P = .75–.90)	
Experiment 9													
Waltham (P_2)							15	15	7.0		15		15
La Primera (P_3)		15						15	2.0	15		15	
F_1 (P_3 x P_2)				15				15	4.0	15		15	
F_2 (P_3 x P_2)		23	36	10		15	13	97	4.0	69	28	69	28
								(3:1, χ^2 = 0.77, P = .25–.50)				(3:1, χ^2 = 0.77, P = .25–.50)	
Experiment 10													
Ponca (P_1)						1	6	7	6.9		7		7
Seminole Pumpkin (P_4)				5	1			6	4.2	5	1	6	
F_1 (P_1 x P_4)					6	1		7	5.1		7		7
F_2 (P_1 x P_4)			1	9		14	14	38	5.8	10	28	7	31
								(1:3, χ^2 = .04, P = .75–.90)				(1:3, χ^2 = 0.88, P = .25–.50)	

[z]Leaf reaction ratings: 1 = no infection; 2 = visible infection, chlorotic areas with no sporulation; 3 = few isolated sporulating colonies on less than 5% of leaf surface; 4 = numerous isolated sporulating colonies on 5–30% of leaf surface; 5 = many isolated sporulating colonies on more than 30% of leaf surface; 6 = coalescing colonies, with heavy sporulation on more than 30% of leaf surface; 7 = entire leaf surface covered with dense mildew, plant dying or dead.

isfactory fit to a 3:1 ratio of resistant to susceptible plants, indicating that a single major dominant gene controlled resistance of stems and petioles to the fungus.

Field. Partial dominance for susceptibility were observed in the F_1 (P_1 x P_3) and F_1 (P_1 x P_4) and partial dominance for resistance in the F_1 (P_2 x P_3) (Tables 3, 4). A bimodal distribution of disease reaction classes was observed in each field-grown F_2 population, indicating that the inheritance of resistance to powdery mildew was determined primarily by major genes (Tables 3, 4). There was no evidence of transgressive segregation for disease resistance in any crosses. All BC_1 progeny of the cross P_1 x F_1 (P_3 x P_1) were susceptible, indicating dominance for susceptibility. Disease reaction of the different populations were similar over the 2 years. No differences were observed in

Table 4. Frequency distributions and segregation for leaf reaction to powdery mildew in parents and progeny derived from 4 *C. moschata* crosses grown in the field (1981).

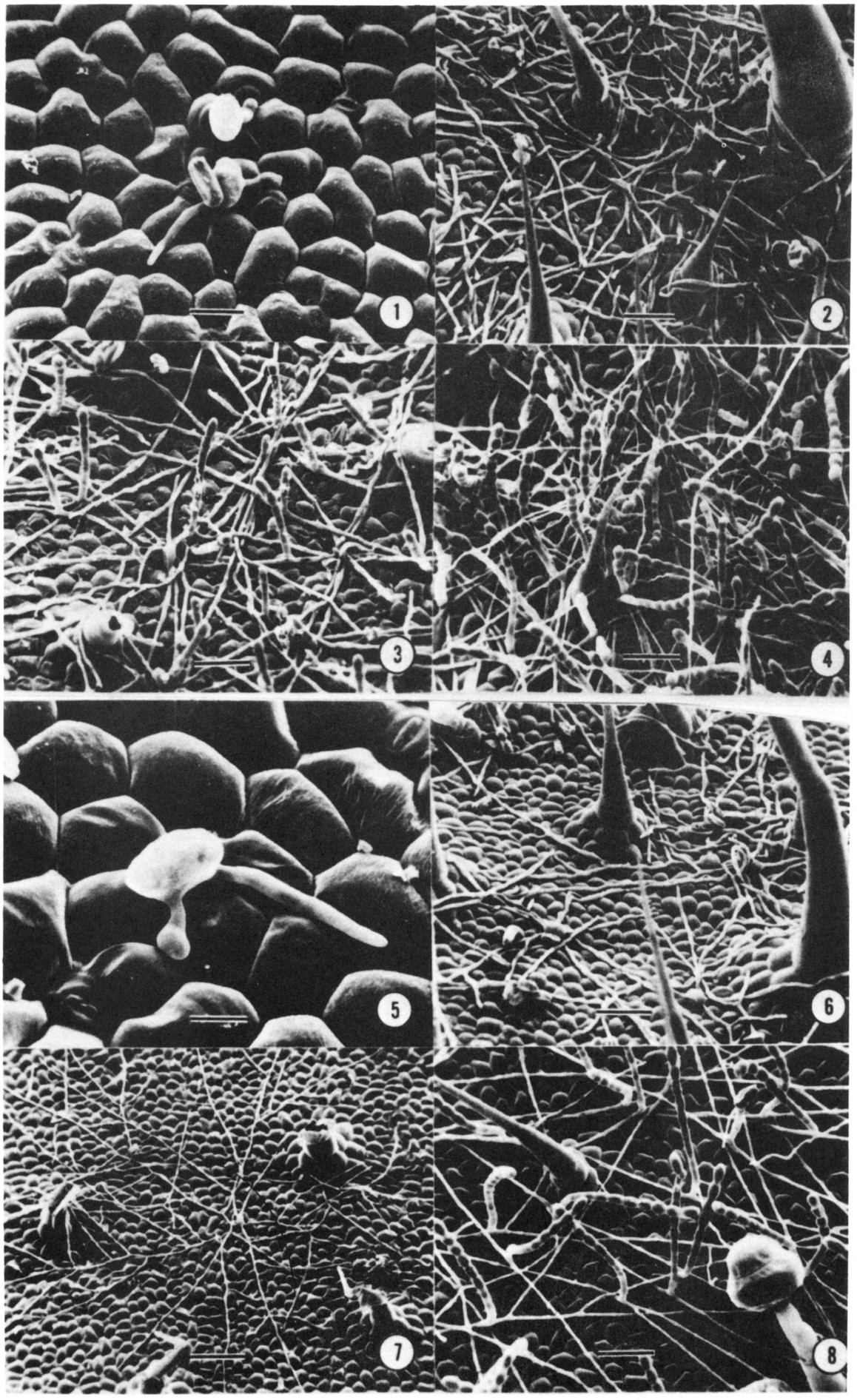
Experiment Generations	No. plants in disease reaction classes[z] Class 1	2	3	4	5	6	7	Total no. of plants	Mean	Observed no. plants Resistant	Susceptible
Experiment 11											
Transplants											
Ponca (P_1)							21	21	7.0		21
La Primera (P_3)		21						21	2.0	21	
F_1 (P_3 x P_1)						15	4	19	6.2		19
F_2 (P_3 x P_1)		3	11	5		29	21	69	5.5	19	50
										(1:3, χ^2 = 0.24, P = .50–.75)	
BC_1 [P_1 x F_1 (P_3 x P_1)]					15	21	4	40	5.7		40
Experiment 12											
Direct-seeding											
Ponca (P_1)							13	13	7.0		13
La Primera (P_3)		17						17	2.0	17	
F_1 (P_3 x P_1)						4	9	13	6.7		13
F_2 (P_3 x P_1)		8	9	3		33	17	70	5.3	20	50
										(1:3, χ^2 = 0.48, P = .25–.50)	
BC_1 [P_1 x F_1 (P_3 x P_1)]					12	8	6	26	5.8		26
selfed BC_1 [P_1 x F_1 (P_3 x P_1)]		2	2			9	10	23	5.8	4	19
										(1:3, χ^2 = 0.71, P = .25–.50)	
Experiment 13											
Waltham (P_2)							8	8	7.0		8
La Primera (P_3)		8						8	2.0	8	
F_1 (P_3 x P_2)			8					8	3.0	8	
F_2 (P_3 x P_2)		22	10	4		5	2	43	3.1	36	7
										(3:1, χ^2 = 1.74, P = .10–.25)	
BC_2 [P_2 x F_1 (P_3 x P_2)][y]		6	8	8		16	10	48	4.9	22	26
										(1:1, χ^2 = 0.33, P = .50–.70)	
BC_2 [P_2 x F_1 (P_3 x P_2)][x]						13	35	48	6.7		48
Experiment 14											
Seminole Pumpkin (P_4)			4					4	3.0		
La Primera (P_3)		5						5	2.0		
F_1 (P_4 x P_3)		11						11	2.0		
F_2 (P_4 x P_3)		53	31		15	4		103	2.9		
Experiment 15											
Ponca (P_1)						7	21	28	6.8		
Waltham (P_2)						9	19	28	6.7		
F_1 (P_2 x P_1)					5	9	13	27	6.3		
F_2 (P_2 x P_1)					54	18	39	111	5.9		
BC_1 [P_1 x F_1 (P_2 x P_1)]					3	6	18	27	6.6		

[z]Leaf reaction ratings: 1 = no infection; 2 = visible infection, chlorotic areas with no sporulation; 3 = few isolated sporulating colonies on less than 5% of leaf surface; 4 = numerous isolated sporulating colonies on 5–30% of leaf surface; 5 = many isolated sporulating colonies on more than 30% of leaf surface; 6 = coalescing colonies, with heavy sporulations on more than 30% of leaf surface; 7 = entire leaf surface covered with dense mildew, plant dying or dead.

[y]Resistant BC_1 plant backcrossed to 'Waltham'.

[x]Susceptible BC_1 plant backcrossed to 'Waltham'.

Fig. 1–8. Stages of powdery mildew development on leaf surfaces of susceptible Butternut 'Ponca' (Fig. 1–4) and moderately resistant 'Seminole Pumpkin' (Fig. 5–8). **Fig. 1.** Germinated conidia (one with 2 germ tubes), at 24 hr after inoculation, showing appressoria and early hyphal growth (20μm). **Fig.2.** Young fungal colony with ramifying hyphae and early stages of conidiophore development along trichome bases 4 days after inoculation (50μm). **Fig. 3.** Ridges on newly formed conidial chains 7 days after inoculation (50μm). **Fig. 4**. Conidial chains of a sporulating colony 2 weeks after inoculation. Note ejected conidia (50μm). **Fig. 5.** Germinating conidium showing 2 germ tubes, appressorium and hyphal development, 24 hr after inoculation (10μm). **Fig. 6.** Ramifying hyphae 4 days after inoculation (50μm). **Fig. 7.** Young fungal colony at 7 days showing conidiophore initials (100μm). **Fig. 8.** Sportulating colony, 2 weeks after inoculation (50μm).



the reaction of plants direct-seeded or transplanted into the field (Table 4).

Plants with ratings of 4 and below were designated resistant plants and those with ratings of 5 and above were called susceptible. A satisfactory fit to a 3:1 ratio of susceptible to resistant plants was observed in the F_2 of the cross $P_3 \times P_1$ in all field experiments, indicating that a single major gene primarily controlled the disease reaction, but with high partial dominance for susceptibility (Tables 3, 4). Good fits to 3:1 and 1:1 ratios of resistant to susceptible plants were recorded in the F_2 and BC_1 generations, respectively, in the cross $P_3 \times P_2$, indicating that the disease reaction on leaf-blades was conditioned by a major gene, with low partial dominance for resistance. When a resistant BC_1 plant was backcrossed to the susceptible parent, 'Waltham' (P_2), a good fit to a 1:1 ratio of resistant to susceptible plants was observed in the BC_2 $P_2 \times F_1$ ($P_3 \times P_2$) progeny. Backcrossing a susceptible BC_1 plant to 'Waltham' (P_2) produced susceptible BC_2 [$P_2 \times F_1$ ($P_3 \times P_2$)] progeny (Table 4).

In the cross between 'Ponca' (P_1) and 'Seminole Pumpkin' (P_4), disease reactions on leaf-blades of F_2 plants showed a continuous distribution in the first field experiment, suggesting a quantitative inheritance pattern (Table 3). However, in the second 1980 experiment, a bimodal distribution of disease reaction classes was noted in the F_2 and a good fit to a 3:1 ratio of susceptible to resistant plants was obtained, indicating that a single major gene primarily controlled the disease reaction, with high partial dominance for susceptibility (Table 3). The divergent inheritance patterns obtained in the same year could be attributed to environmental variations influencing the disease reactions in the 2 experiments.

Petiole and stem reactions of F_2 ($P_3 \times P_1$) crosses showed a satisfactory fit to a 3:1 ratio of susceptible and resistant plants indicating a single major gene determined petiole and stem reaction, with susceptibility being dominant (Table 3). Good fits to 3:1 and 1:1 ratios of resistant to susceptible plants were observed in the F_2 and BC_1 generations, respectively, in the cross $P_3 \times P_2$ indicating that resistance to powdery mildew was conditioned by a single major gene, with resistance being dominant.

All plants of the F_1 ($P_2 \times P_1$), F_2 ($P_2 \times P_1$) and BC_1 [$P_1 \times (P_2 \times P_1)$] generations were susceptible, indicating that the same genes in P_1 and P_2 controlled susceptibility to the fungus (Table 4). Transgressive segregation for susceptibility was observed in F_2 ($P_4 \times P_3$) cross, supporting the hypothesis that different genes were involved in controlling disease resistant reactions in the 2 parents (Table 4). The F_1 and most of the F_2 plants showed a high level of resistance similar to 'La Primera'.

Host-parasite interaction. The stages of development of the powdery mildew on leaf surfaces of parents showing different reactions to the fungus are presented in Fig. 1–16. In the susceptible 'Ponca', the first germ tube emerged from a conidium within 24 hr after inoculation, then produced an appressorium and penetrated the host (Fig. 1). Successful infection resulted in further development of hyphae, producing a mat of mycelium. Within 4 days, conidiophore initials were produced. Shortly afterwards, the first crop of conidia became apparent (Figs. 2, 3). Sporulation intensified and sporulating colonies coalesced, forming a mass of mycelium on the entire leaf surface (Fig. 4). No unusual modification of host's epidermal cells was observed.

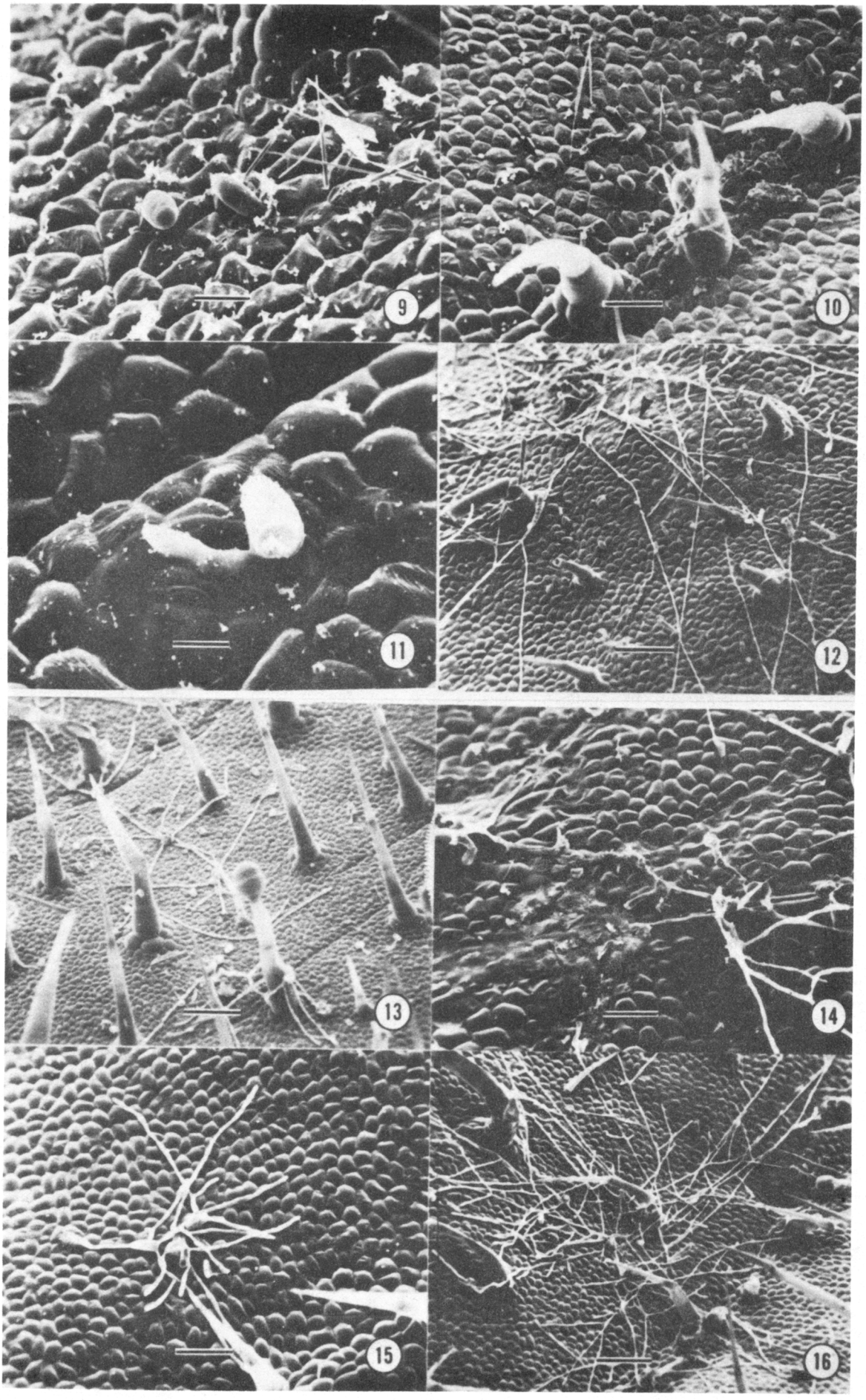
On the leaf blades of the intermediate-resistant 'Seminole Pumpkin', conidia germination and appressoria formation occurred within 24 hr (Fig. 5). Slower and reduced hyphal development and growth occurred following successful infection as compared to 'Ponca' on which conidiophore initiation was observed a week after inoculation (Figs. 6, 7). Several isolated sporulating colonies with a few conidia chains were observed 2 weeks after inoculation (Figs. 3, 8). There was no obvious visual change on the host surface. No conidial germination was observed on the leaf surface of the resistant 'La Primera' at 24 hr (Fig. 9) and 4 days (Fig. 10) after inoculation. Germinating conidia were observed at 7 days (Fig. 11) and 2 weeks after inoculation (Fig. 13); however, even at 2 weeks many nongerminating conidia were observed (Figs. 12–13). Some surface areas of the leaf showed possible collapse of epidermal cells at 3 weeks (Fig. 14). At 4 weeks, mycelium was limited, sporulation was low, conidiophores were sparse, and conidial chains were short (Figs. 15–16). These reactions of 'La Primera' indicated that several factors, acting at different stages of infection, conditioned the resistance of 'La Primera' to the pathogen.

Discussion

Greenhouse-grown parents and progenies showed heavier fungal sporulation than in the field. It is thought that the low temperature regime, 21°C (day)/16° (night), was responsible for increased sporulation. Preliminary results from growth chamber studies (unpublished) indicated that plants grown in chambers maintained at 29° (day)/21° (night) showed less sporulation on leaf-blades. This is similar to the observations of Munger (16). Besides, the increased sporulation contributed to elevated spore load in the greenhouse, thus providing high inoculum pressure on both old and new leaves. The F_1 heterozygotes were more intermediate in susceptibility of leaf-blades under the higher inoculation load in the greenhoue than in the field where higher partial dominance for susceptibility, F_1 ($P_1 \times P_3$), or resistance, F_1 ($P_2 \times P_3$), was observed. The more intermediate classification of the heterozygotes in the greenhouse, combined with more variation in ratings, resulted in the quantitative inheritance pattern of the leaf-blade disease reactions in the F_2 generations. The segregation patterns of field-grown progenies of these crosses clearly showed bimodal distributions, indicating that major genes were involved and that the observed quantitative inheritance pattern in the greenhouse was due to the failure to clearly differentiate the heterozygotes from the homozygotes in the F_2 generations under a higher inoculum load. Under field conditions, segregates which had resistant leaves also had resistant stems and petioles but under greenhouse conditions, many F_2 segregates which were intermediate in resistance for leaf-blade reaction had resistant stems and petioles (mildew-free).

When segregating greenhouse-grown squash progenies were classified on the basis of presence or absence of mildew on stems and petioles, bimodal distributions of plants were ob-

Fig. 9–16. Stages of development of powdery mildew on leaf surfaces of the resistant host 'La Primera' at different times after inoculation. **Fig. 9.** Conidia showing no germination, 24 hr after inoculation (20μm). **Fig. 10.** Conidia with no sign of germination, 4 days after inoculation (50μm). **Fig. 11.** Germinating conidium with single germ tube showing hyphal growth at 7 days (10μm). **Fig. 12.** Sparsely distributed hyphae with the initiation of conidial chains at 2 weeks (100μm). **Fig. 13.** Germinated conidia with several germ tubes and hyphal growth 2 weeks after inoculation. Note numerous nongerminated conidia (100μm). **Fig. 14.** Collapse (presumptive) of epidermal cells of some leaf areas of host at 3 weeks. **Fig. 15.** Several hyphae develop from one conidium (4 weeks). Note collapse of conidium (50μm). **Fig. 16.** Part of a young fungal colony showing conidiophore initials 4 weeks after inoculation (100μm).



tained. The heterozygotes showed either complete dominance for resistance, F_1 (P_2 x P_3), or susceptibility, F_1 (P_1 x P_3). Resistance of stems/petioles to powdery mildew was determined by a single gene, with dominance for susceptibility in the crosses P_1 x P_3 and P_1 x P_4 and with dominance for resistance in the cross P_2 x P_3. Similar results were obtained in the field. This classification procedure, based on the reaction of stems and petioles, can be used for rapid evaluation of large numbers of seedlings under greenhouse conditions, thereby reducing space, time, and cost.

Overall, the segregation patterns for the petiole and stem reactions in F_1 and F_2 field and greenhouse-grown progenies, as well as leaf-blade reactions of F_1 and F_2 field-grown progenies, indicated that a multiple allelic series is involved in the crosses of 'La Primera' with both butternut cultivars. The 3 alleles involved are symbolized as $pm-1^P$, $pm-1^L$ and $pm-1^W$, with pm representing powdery mildew, *P* for 'Ponca', *L* for 'La Primera' and *W* for 'Waltham'. The allele $pm-1^P$ is highly partially dominant to $pm-1^L$ and $pm-1^L$ is slightly partially dominant to $pm-1^W$ for the leaf-blade reaction. Complete dominance for these alleles, respectively, was observed for the petiole and stem reactions. Thus, the proposed genotypes of the parents are as follows: $pm-1^P$ $pm-1^P$ for the susceptible 'Ponca' (P_1), $pm-1^L$ $pm-1^L$ for the resistant 'La Primera' (P_3), and $pm-1^W$ $pm-1^W$ for the susceptible 'Waltham' (P_2). Based on the behavior of 'Seminole Pumpkin' (P_4) in the 2 crosses, P_1 x P_4 and P_4 x P_3, it is hypothesized that a recessive gene, designated $pm-2^S$ (*S* representing 'Seminole Pumpkin'), conditions the 'Seminole Pumpkin' type of resistance. The genes controlling reaction to *E. cichoracearum* were not linked to genes controlling fruit color or trifluralin injury (1, 2) and so no difficulty should be involved in recombining those traits. A backcross breeding procedure should be effective in transferring genes for resistance to *E. cichoracearum* from 'La Primera' to butternut squash.

Differential host compatibility, using the scanning electron microscope, was first observed at conidial germination. With populations of conidia fairly synchronized for germination and subsequent development, spore germination was delayed on leaf surfaces of the resistant host. This is in contrast to Slesinski and Ellingboe's report (25) that no alteration of the infection process of *E. graminis* f. sp. *tritici* on wheat occurred until appressoria formation. Subsequent fungal interactions with host cytoplasm in hosts with resistance genes produced changes in host cells resulting in the reduction of parasitic units. It is evident that the gene for resistance in 'La Primera' may act at various stages of pathogen development. These multiple effects include reduced spore germination, retarded hyphal growth, shorter and reduced number of conidiophores, and reduced sporulation. Light and possibly transmission electron microscopy would be useful to study haustoria development and the number of elongating secondary hyphae to understand the nature of host-pathogen interaction within the host tissue.

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