Inheritance of Resistance to *Uromyces vignae* in Cowpea and the Correlation Between Resistance and Sensitivity to a Cultivar-Specific Elicitor of Necrosis

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ABSTRACT

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Microscopic examination of rust-inoculated progeny of a cross between a resistant and a susceptible cowpea cultivar revealed infection sites in F_1 and F_2 plants that differed in fungal growth and plant responses from those observed in the parents. Frequencies of different categories of infection sites and the general correlation in each plant of the categories recorded for both monokaryotic and dikaryotic infections suggested that resistance to both the monokaryon and dikaryon were governed primarily by two, partially dominant, resistance genes. Six of the possible nine genotypes in F_2 plants could be distinguished microscopically in contrast to only two by macroscopic assessment. Correlations between cytological phenotypes and predicted genotypes were generally confirmed by selected selfings and backcrosses to the susceptible parent, although a few discrepancies suggested phenotype modification by other factors. The number of dead cells induced in F_2 plants by injection of intercellular washing fluids from monokaryon-infected, susceptible tissue was similar for all plants predicted from their cytological phenotypes to have one or more genes for resistance and was higher than that induced by water injection. The frequency of plants that showed significant sensitivity to the fluids differed between cytological phenotypes and declined with increasing susceptibility to the fungus.

Uromyces vignae Barclay (the cowpea rust fungus) is a macrocyclic, autoecious rust fungus that can complete its monokaryotic and dikaryotic stages of growth in susceptible cowpea plants. Although urediospores and basidiospores of the fungus penetrate cowpea leaves in different ways, tested cowpea cultivars exhibit the same relative degree of resistance or susceptibility to both stages of the fungus (7). This finding led to the suggestion that, in each cultivar, the two fungal stages activate the same gene(s) for resistance, assuming that a gene-for-gene relationship exists between host and pathogen (7). However, the genetics of host resistance and pathogen avirulence has not been studied yet for this plant-parasite combination.

If the same resistance genes are effective against both the monokaryon and the dikaryon, both fungal stages may express the same recognition signals (elicitors) (7). We have previously shown that exudates from basidiospore germlings, or intercellular washing fluids (IWFs) from basidiospore-infected susceptible cowpea leaves, contain an elicitor(s) that induces cell necrosis in certain resistant, but not susceptible, cowpea cultivars (1,2). In contrast, the dikaryon of the fungus may not produce this elicitor, or more likely, production may be restricted to the highly specialized intracellular haustorium (2). If this elicitor is important in the determination of cultivar specificity, the sensitivity of progeny from crosses of resistant and susceptible cultivars should segregate with resistance. Therefore, this study was initiated to investigate: 1) the number of genes conferring resistance to race 1 of the cowpea rust fungus in an elicitor-sensitive cowpea cultivar, 2) whether the same gene(s) is responsible for resistance to both stages of the fungus, and 3) whether sensitivity to the elicitor is linked to resistance.

MATERIALS AND METHODS

Pathogen. Urediospores of race 1 of the cowpea rust fungus used in this and previous studies (1,2,7) were produced on the susceptible cultivar California Blackeye, harvested with a cyclone collector about 10 days after inoculation, and stored at −20 C until use. To produce teliospores, from which basidiospores are obtained, plants inoculated with urediospores were kept for 40–50 days. Leaves bearing teliospores were then harvested, air-dried after removal of all the urediospores from the pustules by vacuum, and kept in foil bags for at least another 2 mo at room temperature (a procedure that enhanced subsequent teliospore germination).

Production of basidiospores. After surface sterilization for about 30 s in 3% sodium hypochlorite solution and several rinses in sterile, double-distilled water, teliospores were scraped from leaves with a sterile knife. Teliospores were placed on the surface of 2% water agar in petri dishes that were then sealed with Parafilm and incubated in a growth chamber for about 2 days, by which time the teliospores had begun to germinate to produce basidiospores (2).

Inoculation techniques. Water agar, bearing germinating teliospores, was cut into 1.5-cm^2 blocks and placed on the upper surface of primary leaves of 9 to 10-day-old plants with the spore side facing the leaf. Inoculated plants were kept in a dark, humid chamber at 22 C for 24 h, then all the agar blocks were removed, and the plants were returned to a growth room and maintained at 20–24 C with a 16-h photoperiod per day at about 250 μmol m^{-2} s^{-1} (2) for three additional days.

For inoculation with urediospores, the spores were first washed (5) and then applied to primary leaves of 9 to 10-day-old plants using a soft, moist brush. The inoculated plants were sprayed with double-distilled water, incubated in the humid chamber for 24 h, and then returned to the same growth room described above for another 3 days.

Preparation and bioassay of IWFs. IWFs from basidiospore-inoculated leaves were isolated and prepared as described previously (2). They were bioassayed either at their original concentration or reconstituted to a desired volume with sterile, double-distilled water after lyophilization. IWFs were injected into primary leaves of 9-day-old plants using a 1-ml syringe with a 30-gauge needle as described previously (2).

Plants. Cowpea (*Vigna unguiculata* (L.) Walp.) cultivars Dixie Cream and California Blackeye were used as parentals in this study. Dixie Cream is resistant to race 1 of *U. vignae* and Cali-

fornia Blackeye is susceptible. Plants were grown in a growth room as described elsewhere (6) and, when necessary, transferred when several weeks old to a greenhouse to produce seed. To investigate the inheritance of resistance, one Dixie Cream plant (female parent) was crossed with one plant of California Blackeye. Eight F_1 plants were examined for their responses to the monokaryon and dikaryon of the fungus, and selfed seeds from two of the plants were used to produce F_2 progeny. Selected F_2 plants were selfed or backcrossed to California Blackeye, and progeny were inoculated and/or injected with IWF as described above. Some the these plants were again selfed or backcrossed, and the progeny tested with the fungus.

For plants inoculated simultaneously with both the monokaryon and the dikaryon of the fungus, one primary leaf was inoculated with urediospores while the other was inoculated with germinating teliospores. For experiments involving IWFs, one primary leaf of each plant was injected with water on one side of the main vein and with IWF on the other. The injected areas were harvested 24 h after injection. The remaining primary leaf of each plant was then inoculated either with germinating teliospores only or with urediospores on one side of the main vein and with germinating teliospores on the other. Pieces of inoculated tissue were harvested 4 days after inoculation. Remaining infected areas of leaves were examined for signs of sporulation 3–6 days later.

Light microscopy. Tissue pieces injected with IWF or inoculated with the fungus were decolorized, cleared, and mounted in modified Hoyer's medium as described elsewhere (6). Fungal growth and plant responses were examined with a Reichert Polyvar light microscope using differential interference contrast optics. Data were collected from 50 infection sites per plant. Tissue pieces injected with IWFs were examined for cell autofluorescence (a sign of cell death) using epifluorescence and blue light irradiation (6). The number of autofluorescent, necrotic cells were counted in two 1-cm^2 areas of leaf tissue per plant. Differences between values for IWF- and water-treated areas of the same plant were analyzed using Student's *t*-test.

RESULTS

Urediospore inoculation of parental and F_1 plants. In the parental plant of susceptible California Blackeye, and in its tested selfed progeny ($n = > 20$), over 90% of infection sites in each plant had healthy-looking, growing fungal colonies with few or no collapsed or brown plant cells (similar to that shown in Fig. 1). In contrast, no growing colonies were seen in leaves of Dixie Cream or any of its selfed progeny ($n = > 20$). Over 80% of infection sites in each plant consisted of a single, intercellular-infection hypha and a single, intracellular haustorium in a brown, autofluorescent, plant mesophyll cell (Fig. 2). The remaining sites were characterized by a cluster of two to four brown cells, some of which did not contain visible haustoria and probably represented cells that had died in response to the death of the neighboring haustorium-containing cell. In other cases, cells were too brown and/or collapsed to allow detection of any haustoria; however, the lack of any visible intercellular fungus, other than the infection hypha, suggested that fungal growth was not extensive.

Eight F_1 plants from the California Blackeye × Dixie Cream cross were inoculated, and all were resistant to the fungus (i.e., no sporulation at 7–10 days after inoculation). Microscopically, 58% ± 10 (SD) of infection sites had a single infection hypha terminating in one brown cell. The remainder either had two to three brown cells where the fungus was difficult to see or, at less than 5% of infection sites, had small, compact fungal colonies with about five haustoria. One or two of these usually were in brown cells, and often some haustoria were encased in refractive, calloselike material (similar to the infection site shown in Fig. 3). Occasionally, we saw infection sites with one or two encased haustoria or with one encased haustorium and one unencased haustorium in a brown cell (similar to that in Fig. 4). Encased haustoria were not usually in brown cells (Fig. 4) and they commonly autofluoresced, indicating that they were dead (6).

Basidiospore inoculation of parental and F_1 plants. In the susceptible California Blackeye parent and most of its tested selfed progeny ($n = 9$), over 90% of infection sites in each plant had growing fungal colonies associated with few or no brown plant cells. By 4 days after inoculation, many had begun to form pycnia (similar to the one in Fig. 5). In one plant from the selfed parent, however, about 30% of infection sites had rather little fungal growth and some browning of invaded cells. In the parent Dixie Cream and its selfed progeny ($n = 10$), over 90% of infection sites in each plant consisted of a short (<20 μm) brown primary hypha in a brown epidermal cell (similar to that in Fig. 6). At the remaining sites, the primary hypha was longer and often branched, and sometimes hyphae extended into one or two adjacent epidermal cells; however, both fungus and invaded cells usually were brown and autofluorescent, or the fungus was encased after it entered cells other than the first-invaded cell (similar to the site shown in Fig. 7).

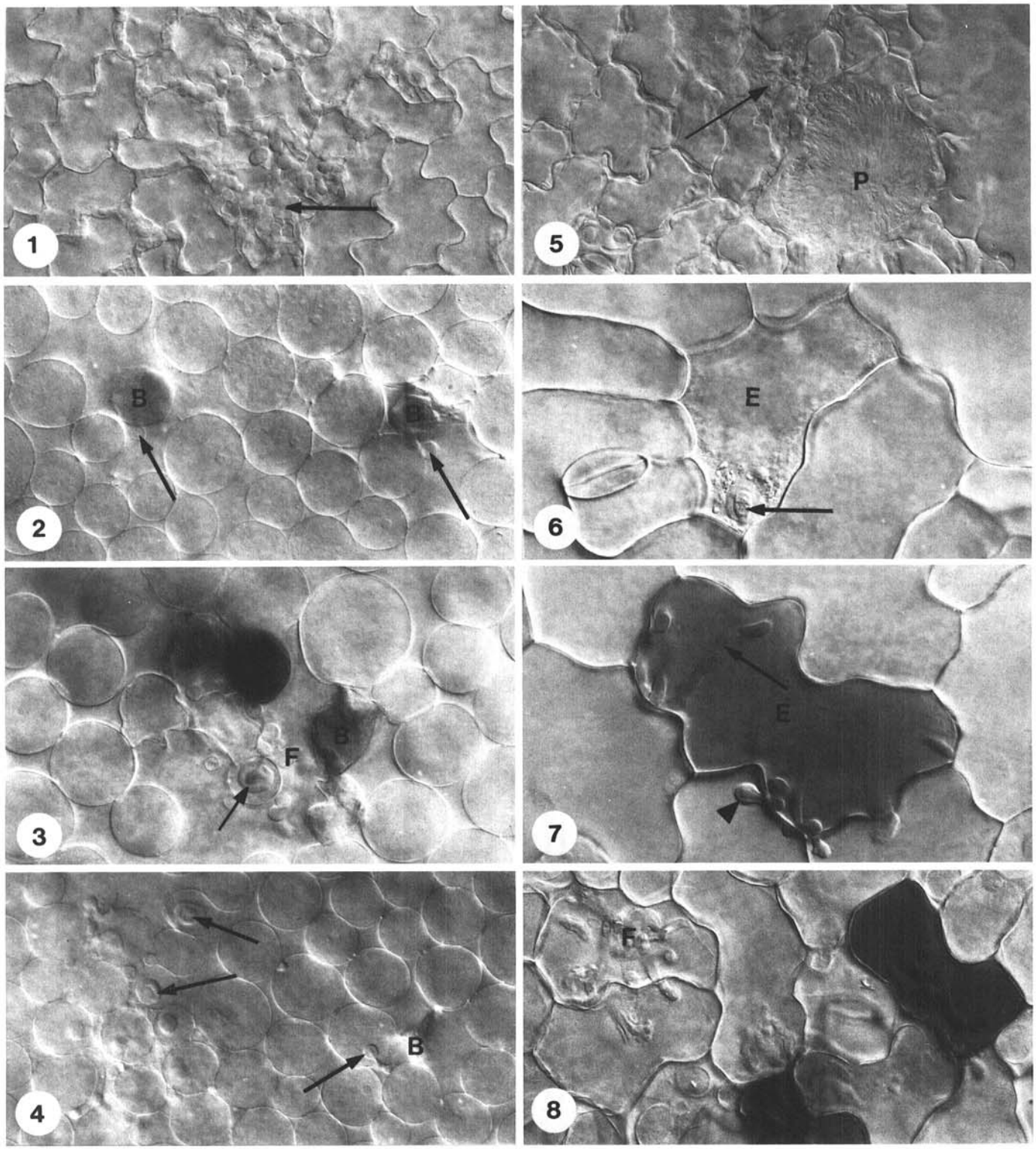
In the eight F_1 plants examined, infection sites from basidiospore inoculations were rare because of poor teliospore germination; accurate numerical data could not be obtained. However, in each plant, several sites were seen where the fungus had formed a small, apparently still-growing, colony that was not associated with brown or collapsed plant cells (similar to the sites shown in Fig. 8).

Urediospore inoculation of F_2 plants. A total of 56 F_2 plants were inoculated and 10 of these eventually developed at least one sporulating pustule. However, microscopically, only three of these plants appeared like the susceptible California Blackeye parent, with over 90% of infection sites having diffuse, growing, fungal colonies and no brown plant cells. Assuming that only these three plants were fully susceptible, the numbers of resistant and susceptible plants fit a 15:1 ratio ($X^2 = 1.68$, $P = 0.9$–0.5) more closely than a 3:1 ratio ($X^2 = 11.54$, $P = 0.05$–0.025), suggesting independent segregation of two genes for resistance (designated *Uv1* and *Uv2*).

Microscopic examination of inoculated leaves showed a wide range of types of infection sites (Figs. 1, 3, and 4) not only between plants but also within each leaf (Fig. 4). Although in some cases it was difficult to decide where to assign a particular plant, in general, plants could be divided into six categories according to the frequencies of specific types of infection sites (Table 1). Possible genotypes were ascribed to these categories: 1) from their frequencies (Table 1), 2) on the assumption that the microscopic phenotype of F_1 plants represents the genotype *Uv1uv1 Uv2uv2*, and 3) from the results of further crosses described later. *Uv1* appeared to have a greater effect on resistance than *Uv2* because large fungal colonies formed in all plants predicted to contain only the latter gene for resistance. At 7–10 days after inoculation, a few sporulating pustules were visible on plants in categories D, E (predicted to contain gene *Uv2*), and F (containing no resistance genes). All resistant plants had a few visible brown flecks; light microscopy showed many of these were caused by the coalescence of several infection sites, each with only a few brown cells.

Basidiospore inoculation of F_2 plants. A total of 118 F_2 plants were inoculated, and 32 developed at least one pycnium. However, only seven of these plants were microscopically similar to the susceptible California Blackeye and had over 80% of infection sites showing no signs of plant resistance. Assuming that only these seven plants were fully susceptible, the numbers of resistant and susceptible plants fit a 15:1 segregation ratio ($X^2 = 0.86$, $P = 0.975$–0.9), suggesting the presence of two genes for resistance against the monokaryon in Dixie Cream.

As for plants infected with the dikaryon, a wide variety of types of infection sites were observed both within plants (Fig. 8) and between plants (Figs. 5–8). Six categories of microscopic phenotypes could be recognized from the frequencies of different infection sites. The best fit between the potential genotypes and cytological phenotypes (Table 2) resulted in the same distribution of genotypes between categories as was suggested for the dikaryon



Figs. 1–8. Types of infection sites of the cowpea rust fungus seen in parental plants and F_2 progeny using light microscopy and differential interference contrast optics. **1–4,** Urediospore-derived infections. **5–8,** Basidiospore-derived infections. **1,** Large fungal colony in a fully susceptible F_2 plant. Urediospore initials (arrow) are beginning to form just under the plant epidermis (×472). **2,** Two infection sites in the resistant parent, Dixie Cream, where intercellular infection hypha (arrows) terminates in a single, brown mesophyll cell (B) (×463). **3,** A small, compact fungal colony (F) in a F_2 plant. Some haustoria are in brown cells (B) while others are encased (arrow) (×344). **4,** Two infection sites in a F_2 plant. (Left arrows) fungus formed a small colony with a high frequency of encased haustoria. (Right arrow) fungus formed one encased haustoria and one in a brown cell (B) (×229). **5,** Growing fungal colony in a fully susceptible F_2 plant. Developing pycnium (P) and extensive mycelium (arrow) just under the epidermis (×472). **6,** Infection site in a F_2 plant identical to that typical of the resistant parent. A short primary hypha (arrow) is present in an epidermal cell (E) with granular, brown cytoplasm (×463). **7,** F_2 plant infection site where a branched primary hypha (arrow) has formed in an epidermal cell (E) that has turned brown. Hyphae grown into neighboring epidermal cells are short and encased (arrowhead) (×344). **8,** A group of infection sites in a F_2 plant. Some consist of branched primary hyphae primarily restricted to a brown epidermal cell; others are small growing colonies (F) eliciting little detectable plant response (×229).

(compare Tables 1 and 2). As seen after urediospore inoculations, brown flecks were macroscopically visible on all plants in categories A–E, and at least some spores were eventually produced in plants in categories D, E, and F, suggesting a lesser effect on resistance of gene *Uv2* than *Uv1*. At most infection sites in plants predicted to contain only *Uv2*, the fungus seemed to grow more slowly and often died and/or became encased in calloselike material. There was less browning and autofluorescence of invaded cells than there was in plants with *Uv1*, in which fungal growth was curtailed more rapidly and was associated with a high incidence of plant cell death.

Correlation between responses to the dikaryon and the monokaryon in F_2 plants. Eighty F_2 plants and their progeny (including those described in Table 3) were inoculated with both urediospores and basidiospores, and their microscopical phenotypes were ascribed to the categories described in Tables 1 and 2. All fully susceptible plants (cytological category F) were equally susceptible to both monokaryon and dikaryon. In only nine resistant plants were the categories for the monokaryon and dikaryon different, and in only three cases did the cytological phenotypes differ by more than one category.

Confirmation of genotype-phenotype correlations. F_2 and backcrossed plants with assumed genotypes, as predicted by their cytological phenotypes, were selfed or backcrossed to the susceptible parent to test the accuracy of using microscopic phenotypes for predicting genotypes (Table 3). Unfortunately, progeny numbers were low because of unexpected frost damage to seed-producing plants in the greenhouse. One F_2 plant with a cytological phenotype in category A for the monokaryon (dikaryon not tested) that was predicted to indicate homozygosity for *Uv1* was selfed. As expected, all progeny were in category A for both the monokaryon and the dikaryon. After testing with the dikaryon, another F_2 plant in category A was backcrossed and, as expected, all progeny were in category B for both the monokaryon and dikaryon. One of these progeny plants was backcrossed again and the resulting ratio of cytologically resistant and susceptible plants was as expected if the progeny plant had been heterozygous for both genes for resistance as predicted. Although no progeny

TABLE 1. Infection type categories derived from microscopic examination 4 days after inoculation with urediospores of F_2 progeny from California Blackeye × Dixie Cream

Category	Characteristics	Suggested plant genotype(s)	No. of plants	
			Expected	Observed
A	About 70% infection sites with infection hypha plus one brown cell, remainder with two to three brown cells. No compact or growing colonies.	*Uv1Uv1Uv2Uv2* *Uv1Uv1Uv2uv2* *Uv1Uv1uv2uv2*	14.0	17.0
B	50–70% infection sites with one brown cell, some sites with one or two encased haustoria only, a few sites with compact colonies associated with brown plant cells.	*Uv1uv1Uv2Uv2* *Uv1uv1Uv2uv2*	21.0	21.0
C	20–50% infection sites with one brown cell, some sites with one or two encased haustoria or compact colonies. No diffuse growing colonies.	*Uv1uv1uv2uv2*	7.0	8.0
D	Like C but with some diffuse growing colonies.	*uv1uv1Uv2Uv2*	3.5	2.0
E	Majority of infection sites with diffuse, small-large growing colonies, often with some encased haustoria or brown invaded cells. A few sites with a single infection hypha only plus one or two brown cells.	*uv1uv1Uv2uv2*	7.0	5.0
F	>90% of infection sites with diffuse growing colonies.	*uv1uv1uv2uv2*	3.5	3.0
			$X^2 = 2.07$, $P = 0.9$–0.5	

TABLE 2. Infection type categories derived from microscopic examination 4 days after inoculation with basidiospores of F_2 progeny from California Blackeye × Dixie Cream

Category	Characteristics	Suggested plant genotype(s)	No. of plants	
			Expected	Observed
A	Short (<20 μm) primary hypha in brown epidermal cell at >65% of infection sites. Longer primary hyphae usually restricted to one epidermal cell.	*Uv1Uv1Uv2Uv2* *Uv1Uv1Uv2uv2* *Uv1Uv1uv2uv2*	29.5	23.0
B	10–60% of infection sites with a short primary hypha in a brown cell, about 10–30% of sites with branched primary hypha restricted to one brown epidermal cell, <2% infection sites with growing colonies associated with little plant response.	*Uv1uv1Uv2Uv2* *Uv1uv1Uv2uv2*	44.4	48.0
C	Only a few sites with short primary hyphae in brown cells, fungus usually spread from epidermal cell, fungus often brown or encased, invaded cells often brown, a few growing colonies associated with little plant response.	*Uv1uv1uv2uv2*	14.8	14.0
D	Like C but with small growing colonies with little sign of plant resistance at >20% of infection sites.	*uv1uv1Uv2Uv2*	7.4	9.0
E	40–80% of infection sites with small-large growing colonies associated with little or no signs of plant resistance.	*uv1uv1Uv2uv2*	14.8	16.0
F	>80% of infection sites with growing colonies and no signs of plant resistance.	*uv1uv1uv2uv2*	7.4	7.0
			$X^2 = 2.26$, $P = 0.9$–0.5	

TABLE 3. Confirmation of predicted genotypes by selected selfings (Self) or backcrosses (BC) to susceptible California Blackeye

Plant source and phenotype category[a]	Possible predicted genotypes	Cross[b]	Number and phenotype categories of progeny		X^2	P
			Expected	Observed		
F_2, A (N)	*Uv1Uv1Uv2Uv2*	Self	All A	17A	0	...
	Uv1Uv1Uv2uv2		All A		...	...
	Uv1Uv1uv2uv2		All A		...	...
F_2, A (2N)	*Uv1Uv1Uv2Uv2*	BC (no. 1)	All B	12B	0	...
	Uv1Uv1Uv2uv2		1B:1C		...	...
	Uv1Uv1uv2uv2		All C		...	...
BC No. 1, B	*Uv1uv1Uv2uv2*	BC	1B:1C:1E:1F	0B:2C:4E:2F	4.0	0.5–0.1
			3R[c]:1S	6R:2S	0	...
F_2, B (2N)	*Uv1uv1Uv2uv2*	BC (no. 2)	1B:1C:1E:1F	0B:5C:1E:3F	6.55	0.1–0.05
			3R:1S	6R:3S	0.33	0.9–0.5
	Uv1uv1Uv2Uv2		1B:1E		...	...
BC No. 2, C	*Uv1uv1uv2uv2*	Self	1A:2C:1F	3A:6C:1F	1.2	0.9–0.5
BC No. 2, C	*Uv1uv1uv2uv2*	BC	1C:1F	7C:7F	0	0.995–0.98
BC No. 2, E	*uv1uv1Uv2uv2*	Self	1D:2E:1F	3B:2C:2D:3F (N)	...	...
				5B:1C:1D:3F (2N)	...	...
			3R:1S	7R:3S	0.13	0.9–0.5
BC No. 2, E	*uv1uv1Uv2uv2*	BC	1E:1F	10C:4F	...	...
F_2, E (N)	*uv1uv1Uv2uv2*	Self	1D:2E:1F	6D:9E:6F (N)	0.43	0.9–0.5
				7C:8D:6F (2N)	...	...
			3R:1S	15R:6S	0.14	0.9–0.5

[a]Phenotypes designated (N) were tested with the monokaryon only; those designated (2N) were tested with the dikaryon only. Other phenotypes identical for monokaryon and dikaryon.
[b]BC = backcrosses.
[c]R = showing microscopic signs of resistance: S = fully susceptible (phenotype category F).

plant had a cytological phenotype of category B, despite the fact that 25% should have had this phenotype, the probability of obtaining this result, given the small sample size, was 0.5–0.1 (Table 3).

When an F_2 plant in category B (predicted to be either heterozygous for both resistance genes or heterozygous for *Uv1* and homozygous for *Uv2*) was backcrossed, fully susceptible plants were observed, indicating heterozygosity in resistance genes in the parent F_2 plant. However, again, no plants in category B were found, and the probability of obtaining the observed ratios by chance was low (P = 0.1–0.05). Most progeny plants were in category C, predicted to be heterozygous for *Uv1* and lacking *Uv2*. Selfing and backcrossing one of these plants confirmed this prediction. Only one progeny individual from the backcrossed F_2 plant was in category E. Selfing this plant produced cytologically resistant and fully susceptible plants as expected for heterozygosity in any resistance gene. However, resistant progeny in categories B and C were found when expected only in categories D and E, and categories for the monokaryon and dikaryon did not always match. As expected, backcrossed seed from this plant produced some fully susceptible plants, but the remainder were in category C rather than category E. This observation suggests that the original plant might have been heterozygous for *Uv1* rather than *Uv2* and should have been assigned to category C rather than category E. However, if this were the case, some backcross progeny should have been in category A rather than category B.

One F_2 plant in category E, predicted to be heterozygous for *Uv2*, was selfed. The ratio of cytologically resistant progeny to fully susceptible progeny confirmed that the parent plant was heterozygous for the resistance gene; the ratio of cytological infection types for the monokaryon was as predicted if only *Uv2* was present. However, cytological phenotypes for the dikaryon were in categories C and D rather than in categories D and E.

Responses of F_2 plants to IWF. A total of 117 F_2 plants were treated with IWFs in four experiments, each using a different IWF preparation. For two of the experiments, unconcentrated IWF was tested in 26 and 23 plants, respectively. In the other two experiments, the IWFs were reconstituted after lyophilization to one-third of their original volume and tested in 42 and 26 plants, respectively. The number of dead cells per leaf piece was highly variable for tissue pieces injected with IWFs or water, in part, because it was difficult to standardize any damage

TABLE 4. Response to intercellular washing fluids (IWFs) in F_2 plants

Phenotype category	Dead cells per 1 cm^2		Percent plants responding	
	IWF x1[a,b]	IWF x3	All experiments[c]	IWF x3
A	323 ± 84	832 ± 250	78 (23)	92 (13)
B	342 ± 141	753 ± 449	75 (48)	80 (35)
C	211 ± 40	926 ± 765	64 (14)	78 (9)
D	240 ± 125	734 ± 372	56 (9)	67 (3)
E	297 ± 227	488 ± 300	31 (14)	17 (5)
F	120 ± 89[d]	259 ± 104[d]	42 (9)	0 (4)

[a]IWFs either unconcentrated or reconstituted to one-third original volume. Unconcentrated IWFs tested in two experiments of 26 and 23 plants, respectively; concentrated IWFs tested in two experiments of 42 and 26 plants, respectively. Mean value for all water controls = 292 ± 229.
[b]Standard deviation.
[c]Numbers in parentheses are the number of plants in each category.
[d]Significantly different (P = 0.05) from values of category A but not water control.

produced by the injection process. However, in general, the average number of dead cells elicited by IWFs was higher than that elicited by water injection, and mean values for the more concentrated IWFs were more than twice as high as those elicited by the unconcentrated preparations (Table 4). For both concentrations of IWF, there was no significant difference between mean values obtained for plants in phenotype categories A–E, but values for plants in category F (no resistance genes) were significantly lower than those in category A (homozygous for both resistance genes) and were not different from those of water controls. Despite the similarity in numbers of dead cells between plants in the phenotype categories A–E, there were marked differences between categories in the proportion of plants that showed a statistically different response from that induced in the same plant by water injection (Table 4). These differences were more pronounced when only experiments using the concentrated IWF were considered (Table 4). The resulting higher numbers of dead cells made it more likely that values exceeded the range elicited by water injection.

One of the plants assigned to category A (predicted genotype *Uv1Uv1-*), in which injection of unconcentrated IWF elicited no more necrosis than water injection, was selfed and 17 of its progeny were tested with IWF concentrated to one-fifth its original volume.

Sixteen of these plants (94%) developed significantly more dead cells than plants injected with water. All showed cytological phenotypes in category A with both the monokaryon and dikaryon.

DISCUSSION

Using microscopic, rather than macroscopic, phenotypes, this study suggests that two genes, designated *Uv1* and *Uv2*, are involved in cowpea cultivar Dixie Cream's resistance to both the monokaryon and the dikaryon of the cowpea rust fungus. In F_2 plants, the nine possible genotypes could be assigned to six different cytological phenotypes. Crosses and backcrosses of F_2 plants and their progeny that were suggested by their cytological phenotypes to be homozygous for *Uv1*, or to contain only *Uv1*, produced progeny that segregated for cytological phenotypes as predicted. These results demonstrated that homozygosity in only *Uv1* was necessary for plants to show the same cytological phenotype as the Dixie Cream parent. However, similar crosses, involving plants predicted to contain *Uv2* and to have one or no alleles of *Uv1* often gave the predicted ratio of cytologically resistant progeny to susceptible progeny but not the predicted ratio of cytological phenotypes. Whether these discrepancies are due to the presence of other genes that can modify certain phenotypes or to the greater effects in plants lacking two alleles of *Uv1* of physiological variation in plant and fungus remains to be determined.

Resistance conditioned by both genes was only partially dominant. In plants heterozygous for *Uv1*, the presence of *Uv2* increased the proportion of infection sites per plant where fungal growth was significantly curtailed and where invaded plant cells were brown and necrotic. In the absence of *Uv1*, plants homozygous for *Uv2* had fewer growing colonies with little signs of plant resistance than were seen in plants heterozygous for this gene. Resistance genes that have additive effects at the macroscopic level have been reported before (10,11,13,14). However, although light microscopy of barley generations segregating for genes that control resistance to powdery mildew has been used to show the lack of involvement of certain genes in the formation of secondary hyphal initials (3), microscopic investigations combined with genetic studies are rare. In this study, we could have missed the presence of *Uv2* if we had examined only high inoculum levels and macroscopic phenotypes, because this gene allowed the fungus to sporulate at least a few infection sites when *Uv1* was absent.

In all plants showing microscopic signs of resistance, many infection sites were observed where fungal growth was less than that seen at almost all infection sites in the susceptible parent. In the F_2 progeny population, a continuum of infection sites was observed. Some had highly restricted fungal growth and rapid death of invaded cells, some had small colonies associated with many dead plant cells, some had few dead plant cells and a necrotic intracellular fungus often encased in calloselike material, and some had larger colonies with low incidences of encasement or plant necrosis. Different genotypes were recognized not by the different types of infection sites but by their different frequencies, as has also been shown for powdery mildew infections of wheat and barley (3). For the dikaryotic stage of the fungus, no particular type of individual infection site could be correlated with a particular resistance gene because at least some examples of each type occurred in all resistant F_2 progeny. Nevertheless, plants with gene *Uv2* always had a few infection sites where the fungus sporulated. This was not observed for plants with gene *Uv1*. For the monokaryon stage, *Uv1* seemed to govern more frequent death of invaded cells and more rapid containment of the fungus. *Uv2* seemed to allow greater fungal growth, and the most frequent plant response was encasement of the intracellular fungus in calloselike material, a response commonly elicited by fungal death (6).

For autoecious, macrocyclic rust fungi, such as the cowpea rust fungus, an interesting question first raised by Flor (4) is whether the same plant and/or fungal genes control compatibility and incompatibility with both the monokaryon and the dikaryon. Different fungal genes may be involved in the case of flax rust (4,12), but the same genes appear to control virulence and avirulence in the two stages of the bean rust fungus (9). In our study, only nine out of 80 F_2 plants and their progeny inoculated at the same time with both the monokaryon and the dikaryon had a genotype predicted by monokaryotic infection site types that differed from that predicted by dikaryotic types. Given that the boundaries between the different microscopic phenotypes were not well defined, and that fungal development and plant responses may be affected by physiological variations in either organism that may override the effects of genes for resistance or avirulence, such a frequency of discrepancies does not seem unexpected. However, at this point, we cannot rule out different, but closely linked, genes controlling resistance to the two different growth stages of the fungus.

As shown previously (1,2), the number of dead cells induced by injecting IWFs from monokaryon-infected susceptible tissue depended on the concentration of the IWF. Even with F_2 plants injected with a concentrated IWF preparation, those showing signs of resistance to the monokaryon did not always respond statistically with more necrosis than that elicited by water injection. In part, this was a consequence of both the high variability in the numbers of necrotic cells between leaf pieces and the small number of samples that could be obtained from each plant. Also, it could be argued that a single injection of elicitor does not mimic very well the more continual, intracellular supply released by the invading fungus. Significantly, the percentage of F_2 plants that showed a marked response to IWFs increased with the predicted presence, from the cytological phenotypes, of more resistance genes; plants predicted to have no resistance genes (cytological phenotype category F) resembled plants injected with water in terms of the number of dead cells elicited. Moreover, when an F_2 plant (predicted to be homozygous for *Uv1* but not responding significantly to IWF) was selfed, 94% of the progeny had more dead cells after injection with IWF than after injection with water. This value is similar to the proportion of all F_2 plants predicted to be homozygous for this gene that responded significantly to concentrated IWF. Taken as a whole, there appears to be a strong correlation between the presence of genes for resistance in the plant and the ability of IWF to elicit necrosis.

Although only a low percentage of plants with gene *Uv2* responded to IWF, the fact that some did suggests that sensitivity is not conditioned solely by *Uv1*. Assuming that the necrosis-inducing factor in the IWF is a product of a gene for avirulence and that a gene-for-gene relationship exists in this plant-parasite combination, then the currently popular elicitor-receptor interpretation of this relationship (8) predicts that the fungus should produce two elicitors, one "matching" each gene for resistance. The nature and number of elicitors present in IWF preparations are currently under investigation.

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