Use of Race- and Cultivar-Specific Elicitors from Intercellular Fluids for Characterizing Races of *Cladosporium fulvum* and Resistant Tomato Cultivars

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ABSTRACT

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Intercellular fluids obtained by an in vacuo infiltration technique were used to compare and to characterize European and North American races of *Cladosporium fulvum*. In previous studies, it was found that such fluids contained specific elicitors of chlorosis and/or necrosis. The races of North American origin tested included race 0 with cycloheximide tolerance (C+), a race 4(C+) mutant derived from the race 0 (C+) isolate, race 2.3 (C+), race 4 with benlate tolerance (B+), and two isolates of race 2.3.4 (#3 and #78). Races 4, 5, and 2.4.5.9 originating from the Netherlands were also tested. Intercellular fluids obtained from all compatible race-cultivar combinations showed race- and cultivar-specific elicitor activity when injected into tomato cultivars with genes conferring high phenotypic resistance (*Cf*2, *Cf*4, *Cf*5, and *Cf*9) and also when tested in a cultivar with gene *Cf*3 which allegedly confers only partial resistance. A cultivar with the gene *Cf*1, also giving partial resistance, behaved in a manner similar to cultivars with no known genes for resistance, i.e., none of the elicitor preparations induced chlorosis or necrosis. Fluids obtained from races with the same virulence spectrum but originating from different continents or by artificial means showed identical race and cultivar specificity. Fluids obtained by using the complex race 2.4.5.9, which is virulent on all European commercial tomato cultivars but not on two recently released Ontario hybrids, showed activity only in the hybrids. Polyacrylamide gel electrophoresis of intercellular fluids confirmed that preparations with necrosis activity on cultivar Sonatine contained a fast-moving basic peptide. The mannose/glucose ratios of fluids obtained from previously designated compatible and incompatible interactions had ranges of 1.45 to 3.10, and 0.50 to 0.71, respectively. In interactions in which partial resistance due to genes *Cf*1 or *Cf*3 was originally expected, the ratios ranged from 0.52 to 3.92, but for each interaction the ratio corresponded to the estimated degree of colonization based on elicitor activity and histological examination.

Additional key words: *Fulvia fulva*, gene-for-gene interaction, leaf mold of tomato, *Lycopersicon esculentum*.

deWit and Spikman (6) showed that race- and cultivar-specific elicitors could be isolated from intercellular fluids during compatible race-cultivar interactions of *Cladosporium fulvum* Cooke [syn. *Fulvia fulva* (Cooke) Ciferri] and tomato. These elicitors when injected into the interveinal area of tomato leaflets induced necrosis and/or chlorosis in cultivars that were resistant to the races involved, but not in cultivars susceptible to these races. An additional study (4) showed that the specificity of the elicitors was not determined by the gene(s) for resistance present in the cultivar from which the intercellular fluids were isolated but was determined by the race of the invading fungus. More recently, the elicitor that induces necrosis on cultivar Sonatine was characterized as a small basic peptide (5).

A criticism of studies on specificity is that often too few isolates are tested and that all isolates tested originate in the same geographic region. Studies to date on this system have involved only races originating in the Netherlands and other European countries. In characterizing various strains of *C. fulvum* that had been developed by mutagenesis and other selection methods (V. J. Higgins and H. J. Hollands, *unpublished*) (10), we saw an opportunity to test the "universality" of the specific elicitors by using races that had originated naturally in North America or via mutagenesis and possibly by recombination in the laboratory. Of particular interest was a comparison of a North American race 0, a race 4 that had been derived from the race 0 by mutagenesis, and naturally occurring isolates of race 4 from North America and Europe. In addition, it was not clear from the initial study (6), whether the resistance genes *Cf*1 and *Cf*3, both considered to give an incomplete form of resistance (1,8), behaved the same as the commercially useful genes, *Cf*2, *Cf*4, *Cf*5, and *Cf*9. This question was reexamined by using cultivars Sterling Castle (with gene *Cf*1) and cultivar V121 (with gene *Cf*3). As numerous race-cultivar combinations with varying degrees of compatibility were to be examined, this study also presented an opportunity to determine whether monosaccharide levels, particularly those of mannose and glucose, in the intercellular fluids were a useful indication of the degree of compatibility of an interaction. Previous studies (P. J. G. M. deWit, *unpublished*) had suggested that mannose increased to unusually high levels in many compatible interactions.

MATERIALS AND METHODS

Fungi. Isolates of races 4, 5, and 2.4.5.9 (previously designated as 2.4.5.x) of *C. fulvum* were used in the earlier studies (4,6) and originated in the Netherlands. Race 0(C+) was produced from a wild type race 0 isolate originating in southern Ontario and was selected for cycloheximide tolerance (C+) following UV-irradiation. Race 4(C+) was a race 4 isolate with cycloheximide tolerance and was isolated from a Purdue 135 (with gene *Cf*4) plant inoculated with conidia of race 0(C+) that had been treated with *N*-methyl-*N*-nitrosoguanidine. The data (V. J. Higgins and H. J. Hollands, *unpublished*) indicate that race 4(C+) resulted by mutation of 0(C+). Races 2.3(C+) and 4(B+) were developed (10) from wild type isolates of race 2.3 and race 4 originating in southern Ontario and Ohio (2), respectively, and subjected to UV-mutagenesis during selection for cycloheximide or benlate tolerance (B+). Races 2.3.4(#78) and 2.3.4(#3) were identified on differentials as race 2.3.4 and were isolated from cultivar

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Vinequeen (with genes *Cf*2 + *Cf*4) in two experiments in which strains 2.3(C+) and 4(B+) were first grown together on cultivar Potentate (*Cf*0) and the resulting conidia were then inoculated onto Vinequeen. Although it is suspected that these 2.3.4 isolates resulted from asexual recombination, the absence of the markers precludes a positive conclusion (10). All isolates were grown on PDA as described before (3,6).

Plants. Table 1 includes the cultivars used, their genes for resistance to *C. fulvum*, and their interactions with the races used. Sonato and Sonatine are commercially available cultivars used in the Netherlands. Near isogenic lines of Moneymaker with genes *Cf*4 and *Cf*5 were developed by I. Boukema (Institute for Horticultural Plant Breeding, Wageningen, the Netherlands) by backcrossing five times to Moneymaker and selfing three times. Seeds of cultivars Potentate, Vetomold, V121, Purdue 135, Vinequeen, Ontario Pink 774, and Ontario Red 775 were obtained from E. Kerr (formerly at the Horticultural Experiment Station, Simcoe, Ontario). The hybrids Ontario Pink 774 and Ontario Red 775, commercially available from Stokes Seeds Ltd. (St. Catharines, Ontario), are resistant to all known races of *C. fulvum*.

Inoculation. The plants were inoculated and incubated as described before (3,6). These procedures ensured optimal infection with almost 100% colonization of leaves that were fully expanded at the time of inoculation.

Histological examination. To estimate the degree of colonization of tomato leaves by a given isolate, the lower epidermis of representative leaflets was removed with fine forceps, and mounted in lactophenol cotton blue for immediate observation. The extent of growth from the site of ingress was visually rated on a scale in which "−" represented the localized resistant response (9) and "±" to "++++" represented varying degrees of growth up to the maximum possible.

Isolation of elicitors. From 10 days postinoculation, plants were visually examined each day, and leaves of susceptible plants were harvested when sporulation appeared almost maximal, or with certain isolates, when it appeared that the leaves would become wilted or necrotic under further incubation. Harvesting of leaves from incompatible interactions was done on day 14.

Leaves were infiltrated with distilled water in vacuo, and the intercellular fluids were obtained by centrifugation at 2,400 *g* for 30 min in special centrifuge holders as described previously (6). Intercellular fluids were immediately stored at −20 C. Prior to use, fluids were thawed and immediately centrifuged at 1,400 *g* for 10 min to remove precipitates. In the initial experiment, all fluids were then sterilized by membrane filtration (Millex-GS; 0.22 μm), but in subsequent experiments this step was omitted without affecting activity.

Bioassays for elicitor activity. Intercellular fluids were injected into fully expanded leaflets of 6- to 8-wk-old tomato plants by using a 1.0-ml disposable syringe fitted with a fine (26–30 gauge) needle. Generally, each leaflet was injected at four sites (one interveinal area per quadrant of the leaflet) and the treatment was replicated on another plant of the same cultivar. Plants were incubated in the greenhouse as described previously (3,6), and the leaves were assessed for necrosis and chlorosis from 1 to 7 days after injection. Although leaves were rated for necrosis and chlorosis by the system described before (4,6), this paper uses "N" to indicate necrosis that developed within 24 or 48 hr and "+" to indicate chlorosis developing within 4–7 days. Each injection site was rated separately, but as there was little variation between sites this information is not presented. All experiments were repeated at least once.

Sugar determinations. Free monosaccharides in the intercellular fluids were quantitatively determined as their alditol acetates by the method of Jones and Albersheim (7). Gas-liquid chromatography (glc) of alditol acetates was carried out on a 250 cm × 2 mm (i.d.) glass column packed with 0.6% OV275 and 0.8% XF1150 on diatomite CQ 100–120 mesh (Chrompack). Nitrogen was used as the carrier gas at a flow rate of 40 ml·min^{-1}, and detection was by flame ionization. Operating temperatures were as follows: injection port, 250 C; detector, 250 C; and column, 190 C. Inositol was used as an internal standard.

Polyacrylamide gel electrophoresis. Samples (0.5 ml) of each fluid were precipitated by the addition of 4.5 ml of cold acetone. Precipitates were collected by centrifugation at about 4,000 *g* and washed once with 5 ml of 90% acetone in water before freeze-drying. The freeze-dried material was taken up in 125 μl of a solution containing 34.2% (w/v) sucrose and 0.005% (w/v) Pyronin Y as a marker. Samples (50 μl) were subjected to electrophoresis under low pH conditions by a method described by Reisfeld et al (11) on 10% (w/v) polyacrylamide gels. Gels were stained for 2 hr in a solution containing 180 ml of ethanol, 420 ml of water, 100 ml of 35% (v/v) formaldehyde, and 0.8 g Coomassie Brilliant Blue R250 and were destained overnight in a solution containing 250 ml of ethanol, 750 ml of water, and 10 ml of 35% formaldehyde as described by Steck et al (12).

TABLE 1. Necrosis (N)-eliciting and/or chlorosis (+)-eliciting activity of intercellular fluids from compatible race/cultivar combinations of *Cladosporium fulvum* and tomato tested by injection into the interveinal area of leaflets on cultivars with different genes for resistance to *C. fulvum*

Race/ cultivar combination[a]	Potentate *Cf*0	Sterling Castle *Cf*1	Vetomold *Cf*2	V121 *Cf*3	Purdue *Cf*4	Vinequeen *Cf*2+*Cf*4	Near-isogenic *Cf*4	Near-isogenic *Cf*5	Sonato *Cf*2+*Cf*4	Sonatine *Cf*2+*Cf*4+*Cf*9
0(C+)/ *Cf*0	−[b](S)[c]	−(R)	+(I)	+(R)	N(I)	N(I)	+(I)	+(I)	+(I)	N(I)
4(C+)/ *Cf*4	−(S)	−(R)	+(I)	+(R)	−(S)	+(I)	−(S)	+(I)	+(I)	N(I)
2.3(C+)/ *Cf*2	−(S)	−(R)	−(S)	−(S)	N(I)	N(I)	+(I)	+(I)	+(I)	N(I)
4(B+)/ *Cf*4	−(S)	−(R)	+(I)	+(R)	−(S)	+(I)	−(S)	+(I)	+(I)	N(I)
2.3.4(78)/ *Cf*2+*Cf*4	−(S)	−(R)	−(S)	−(S)	−(S)	−(S)	−(S)	+(I)	−(S)	N(I)
4/ *Cf*4	−(S)	−(?)	N(I)	+(R)	−(S)	+(I)	−(S)	+(I)	+(I)	N(I)
5/ *Cf*0	−(S)	−(?)	−(I)[d]	−(?)[d]	N(I)	N(I)	+(I)	−(S)	+(I)	N(I)
2.4.5.9/ *Cf*2+*Cf*4[e]	−(S)	−(?)	−(S)	−(?)	−(S)	−(S)	−(S)	−(S)	−(S)	−(S)

[a] Cultivars used for obtaining the fluids were those of North American origin, i.e., Potentate (*Cf*0), Vetomold (*Cf*2), Purdue (*Cf*4), and Vinequeen (*Cf*2 + *Cf*4).
[b] The reactions are indicated by "N" for necrosis within 48 hr of injection, "+" for chlorosis within 4–7 days after injection, and "−" for no effect.
[c] The expected degree of resistance of the cultivar injected to the race used to obtain the fluid is shown in parentheses; S = fully susceptible, I = a high degree of resistance, R = a low degree of resistance, and ? = not previously tested.
[d] Intercellular fluids obtained from better infections with race 5 gave activity on both Vetomold and V121.
[e] This fluid was active on Ontario Red 775 and Ontario Pink 774.

RESULTS

Race and cultivar specificity of intercellular fluids from fully compatible interactions. Intercellular fluids obtained when each of the races of *C. fulvum* was grown on a cultivar known to be compatible to that race gave the predicted (4,6) reactions when injected into a differential set of cultivars (Table 1). The only exceptions to the expected pattern were the lack of activity on Sterling Castle and the behavior of race 5 fluids on Vetomold and V121. Subsequent tests of more active intercellular fluids obtained when race 5 was grown on the near-isogenic line with gene *Cf*5 gave chlorosis-inducing activity on both Vetomold and V121.

Fluids from the Potentate/0(C+) interaction were particularly active on both Purdue and Vinequeen; necrosis appeared within 24 hr following injection. Nevertheless, the mutant race 4(C+) derived from race 0(C+) yielded fluid that produced no reaction on Purdue and thus was similar to the two naturally occurring isolates of race 4.

In the series involving races 2.3(C+), 4(B+), and the putative recombinant 2.3.4 (#78), the pattern of activity was again as expected based on the difference in genes for avirulence (Table 1). The other putative recombinant 2.3.4 (#3) gave the same results as strain 2.3.4(#78), so data are not shown.

Activity of intercellular fluids from interactions on cultivars with genes *Cf*1 and *Cf*3. Intercellular fluids obtained from Sterling Castle (*Cf*1) plants inoculated with any of the races (except race 2.4.5.9) showed necrosis-inducing activity when injected into Sonatine but the greatest dilution giving activity varied among the races (Table 2). These fluids (with the predictable exceptions of those from race 5 and 2.4.5.9) also showed chlorosis-inducing activity on the near-isogenic line with gene *Cf*5 (Table 2). Activity of fluids from interactions involving Sterling Castle and race 2.3(C+) or 4(B+) was particularly weak with little or no activity detected on the near-isogenic line with gene *Cf*5 in the second inoculation series. Brief histological examination of these interactions showed that Sterling Castle was colonized by all races but that in no case was colonization as extensive as in a fully compatible interaction (Table 2).

Intercellular fluids obtained from V121 (*Cf*3) plants inoculated with each race, showed necrosis and chlorosis inducing activity on Sonatine and on the near-isogenic line with gene *Cf*5 only for interactions involving 2.3(C+) and 2.3.4(#3 and #78), races which were fully virulent on V121 (Table 2). Histological observations showed that colonization by the other races (except for 2.4.5.9) was generally similar to that seen in fully incompatible interactions (Table 2).

Elicitor activity of intercellular fluids obtained from compatible interactions involving race 2.4.5.9 were tested by injection into the resistant hybrids Ontario Red 775 and Ontario Pink 774, because all of the other cultivars used are susceptible to that race. Fluids obtained from Vinequeen, V121, and Sterling Castle inoculated with race 2.4.5.9 showed activity on both hybrids within 24–48 hr. Such activity, in combination with the histological observations, suggests that the race 2.4.5.9/Sterling Castle and 2.4.5.9/V121 interactions are relatively compatible.

Free sugars in intercellular fluids. Mannose and glucose were the only monosaccharides present to any significant degree in intercellular fluids. The amount and ratio of these is given in Table 3 for all interactions discussed as well as for six incompatible interactions. A pattern for fully compatible and incompatible interactions is obvious. In incompatible interactions (or uninoculated controls) the level of glucose is higher than that of mannose, but in compatible interactions this relationship is reversed to varying degrees. In no case did a compatible interaction have a mannose/glucose ratio of <1.0, and in no case did an incompatible interaction have a ratio >0.71. The fluids from Sterling Castle did not give an obvious pattern, with ratios ranging from 0.65 to 3.92, but the higher ratios tended to be from the interactions which were judged by both colonization and elicitor activity to have had the most hyphal growth. In the case of interactions on V121, all races fitted the pattern discussed including race 2.4.5.9 if this race is assumed on the basis of the histological examination and by the elicitor activity on the Ontario hybrids to be fully compatible on V121.

Polyacrylamide gel electrophoresis. All intercellular fluids that exhibited strong elicitor activity (activity at dilutions of 1/16 to 1/64) on Sonatine had a distinct band which corresponded to the fast moving basic peptide shown (5) to be the necrosis elicitor on Sonatine (Table 3). Fluids with weaker activity generally exhibited a similar, but less distinct, band (Table 3). As expected from the results of deWit et al (5), this band was absent from fluids derived with race 2.4.5.9 and from fluids obtained from incompatible interactions. No other consistent electrophoretic differences were detected among races even when all races were grown on Potentate to ensure that the host contribution to the fluids was similar.

DISCUSSION

The race-cultivar specific elicitor system described by deWit and Spikman (6) proved to be highly reliable when tested with naturally occurring races of *C. fulvum* from North America, or with artificially induced races, and with tomato cultivars differing in genetic background from those previously used. All of the intercellular fluids gave the predicted results, although in the case of cultivar Sterling Castle (with gene *Cf*1) the degree of colonization had to be considered in reconciling the results with the specific elicitor system. Intercellular fluids from compatible race-cultivar combinations contained elicitors which specifically induced necrosis and/or chlorosis when injected into cultivars which gave an incompatible response to the race involved. Both race 4(C+), obtained by mutation from 0(C+), and race 2.3.4(#3 and #78), possibly derived by asexual recombination between race 2.3 and race 4 (10), behaved exactly as expected based on the

TABLE 2. Necrosis-eliciting and/or chlorosis-eliciting activity of intercellular fluids obtained from the tomato cultivars Sterling Castle (with gene *Cf*1) and V121 (with gene *Cf*3) inoculated with each race of *Cladosporium fulvum* and tested by injection of a dilution series into cultivar Sonatine or by injection at the original concentration into near-isogenic line with *Cf*5. Histological rating of the degree of colonization was made on epidermal peels from infected leaflets

	Sterling Castle inoculated with:			V121 inoculated with:		
Race or strain	Histological rating[a]	Fluids on Si[b]	Fluids on *Cf*5[c]	Histological rating	Fluids on Si	Fluids on *Cf*5
0(C+)	++	1/64,1/16[d]	+,+[d]	−	−,−[d]	−,−[d]
4(C+)	±	1/16,1/16	+,+	−	−,−	−,−
2.3(C+)	++	1/4,0	±,−	++++	1/64,1/64	+,+
4(B+)	+	1/4,0	±,−	−	−,−	−,−
2.3.4(78)	++	1/8,1/4	+,±	++++	1/32,1/64	+,+
4	++	1/8,1/8	+,+	−	−,−	−,−
5	+++	1/64,1/64	−,−	−	−,−	−,−
2.4.5.9	+++	−,−[e]	−,−	+++	−,−	−,−[e]

[a]Symbols: − = no colonization, ± to ++++ = increasing degrees of intercellular growth with ++++ equivalent to a fully compatible interaction.
[b]Highest dilution showing activity on Sonatine (Si).
[c]+ = chlorosis, ± = weak chlorosis, − = no activity on near-isogenic line with gene *Cf*5.
[d]Values separated by a comma are for two separate inoculation series.
[e]These fluids from race 2.4.5.9 interactions showed activity in tests on Ontario Red 775 and Ontario Pink 774.

observed changes in compatibility. Fluids from incompatible race-cultivar combinations did not exhibit activity on any cultivars, a result which deWit and Spikman (6) attributed to the very limited amount of fungal growth which occurs in an incompatible response. However, it is also possible that the specific elicitors are immediately bound by cells of the resistant cultivars.

The tests with cultivars possessing resistance genes *Cf*1 and *Cf*3 indicated that these two genes are quite different, gene *Cf*1 being considerably less effective than gene *Cf*3. Resistance conferred by the *Cf*1 gene present in Sterling Castle has long been regarded as extremely weak and neither gene *Cf*1 nor gene *Cf*3 is used in differential cultivars in Europe. Under certain environmental conditions, such as the shorter photoperiod and lower light intensity in winter, the resistance conferred by gene *Cf*1 is completely lost (7). In this study, under the conditions used in the Netherlands, all of the isolates tested grew more extensively on Sterling Castle than was expected based on our experience in Ontario where it is still included in the differential set; it is possible that the very high inoculum load used in these experiments contributed to further weaken the resistance. Certainly, under the conditions of this study, gene *Cf*1 allowed sufficient intercellular growth of each isolate for detectable levels of elicitor to occur. With this degree of compatibility, it is not surprising that no intercellular fluid derived by using any of these races showed activity when injected into Sterling Castle.

In contrast, V121 (which is used as the gene *Cf*3 differential in Ontario) gave results similar to those for the highly effective resistance genes *Cf*2, *Cf*4, *Cf*5, and *Cf*9. Results of the initial tests with V121 by deWit and Spikman (6) were more like those with Sterling Castle. It is possible that some changes have occurred in the genetic background of V121; based on histological observations, plants from our present seedlot appear to allow less growth of avirulent races than noted previously (9). Nonetheless, these plants were still susceptible to only races 2.3, 2.3.4, and 2.4.5.9 (which may be 2.3.4.5.9) as would be expected for V121.

It should be noted that, because genes *Cf*1 and *Cf*3 are not included in the differential series used in Europe, European race designations do not indicate either the presence or absence of virulence on plants with genes *Cf*1 and *Cf*3. The results of this study further justify the deletion of the *Cf*1 gene from the differential series but suggest that the *Cf*3 gene still merits inclusion. It appears that if the *Cf*3 gene was added in the Netherlands series that race 2.4.5.9 would be changed to race 2.3.4.5.9 but that race 4 and race 5 would remain unchanged.

The analysis of sugars in the intercellular fluids indicated that mannose/glucose ratios might be a useful index for the degree of

TABLE 3. Mannose and glucose concentrations in intercellular fluids obtained from various race/cultivar interactions. All initial values are for one inoculation series and a second value is given only for interactions in which the result of the second inoculation series was considerably different. A qualitative assessment of the presence of the necrosis-eliciting basic peptide on polyacrylamide gels is also given

Race/cultivar combination[a]	Disease reaction[b]	Mannose[c] (μg/ml)	Glucose[c] (μg/ml)	Mannose/glucose[d]	Lowest dilution active on Sonatine[e]	Presence of basic peptide[f]
0(C+)/Pot	S	345	188	1.84	1/32	++
4(C+)/P	S	492	207	2.38	1/64	++
2.3(C+)/VM	S	2,273	1,563	1.45	1/32	++
4(B+)/P	S	135	83	1.63	1/16	++
2.3.4(78)/VQ	S	808	368	2.20	1/64	++
2.3.4(3)/VQ	S	769	290	2.65	1/64	++
4/P	S	333	170	1.96	1/32	++
5/Pot	S	291	104	2.80	1/64	+
2.4.5.9/VQ	S	304	146	2.08	–	–
0(C+)/SC	R	271	283	0.96	1/64	±
4(C+)/SC	R	123	96	1.28	1/16	±
2.3(C+)/SC	R	171	180	0.95	1/4	–
4(B+)/SC	R	231	317	0.73	1/4	–
2.3.4(78)/SC	R	216	259	0.83	1/8	±
2.3.4(3)/SC	R	121	92	1.31	1/16	±
4/SC	? (R)	324	424	0.76	1/8	±
5/SC(exper.1)	? (S)	81	22	3.68	1/64	+
5/SC(exper.2)	? (S)	718	702	1.02	1/64	+
2.4.5.9/SC(exper.1)	? (S)	102	26	3.92	–	–
2.4.5.9/SC(exper.2)	? (S)	343	222	1.55	–	–
0(C+)/V121	R	254	471	0.54	–	–
4(C+)/V121	R	155	333	0.46	–	–
2.3(C+)/V121	S	183	59	3.10	1/64	++
4(B+)/V121	R	110	212	0.52	–	–
2.3.4(78)/V121	S	666	287	2.32	1/32	++
2.3.4(3)/V121	S	952	415	2.30	1/64	++
4/V121	? (R)	78	149	0.52	–	–
5/V121	? (R)	44	76	0.58	–	–
2.4.5.9/V121(exp.1)	? (S)	444	415	1.06	–	–
2.4.5.9/V121(exp.2)	? (S)	435	242	1.80	–	–
4(C+)/VQ	I	94	137	0.69	–	–
4(C+)/VM	I	135	189	0.71	–	–
4(B+)/VQ	I	87	173	0.50	–	NT
4/VM	I	240	408	0.58	–	NT
4(B+)/VM	I	144	244	0.59	–	NT
5/P	I	177	338	0.52	–	NT

[a] Abbreviations for cultivars, Pot = Potentate, P = Purdue 135, VM = Vetomold, VQ = Vinequeen, and SC = Sterling Castle (see Table 1 for *Cf* genes present in each).

[b] Expected disease reaction, S = susceptible, I = highly resistant, R = low degree of resistance, ? = not previously tested but with reaction in brackets based on histological examinations during this study.

[c] As determined by gas-liquid chromatography.

[d] Average mannose/glucose ratios for different interactions: S = 2.22 ± 0.48 (n = 12); I = 0.59 ± 0.09 (n = 6); and R = 0.87 ± 0.29 (n = 9).

[e] The highest dilution showing necrosis on Sonatine is given.

[f] Rating of bands on electrophoresis, ++ = strong band, + = moderate band, ± = weak band, – = no band detected, and NT = not tested.

compatibility of an interaction. A ratio greater than 1 was always obtained for interactions known to be fully compatible, whereas incompatible combinations always had ratios well below 1.0. Based on the results with Sterling Castle, it appears that partially compatible interactions give more variable ratios, presumably because of varying levels of colonization. On the other hand, the absolute mannose and glucose concentrations did not seem to fit any established pattern; some fluids from fully compatible interactions had relatively high levels (e.g., up to 2.2 mg of mannose per milliliter) while others had much lower concentrations (e.g., 0.13 mg of mannose per milliliter).

All of the intercellular fluids showing high specific elicitor activity on Sonatine apparently contained the fast-moving basic peptide which was shown (5) to be the molecule that elicits the necrotic response in Sonatine. The failure of this electrophoretic method to designate any other bands that might be specific elicitors acting on other cultivars is not surprising; the method is designed to enhance the resolution of the *Cf*9-specific elicitor. Overall, however, results of this and the previous studies (4–6) lead invariably to the conclusion that each gene for avirulence leads to the production of a different elicitor.

This study has confirmed the value of using intercellular fluids to study race-cultivar interactions in the tomato leaf mold system. The universality of the race- and cultivar-specific elicitors was demonstrated, and these elicitors proved to be a useful supplement to pathogenicity tests in characterizing wild type and induced races of the fungus. The potential of the elicitors for use in defining different types of resistance genes was illustrated by the comparison of genes *Cf*1 and *Cf*3 with the more effective genes for resistance. The reproducibility of the method also suggests that intercellular fluids, rather than the live fungus, could be used as a screening tool by plant breeders.

LITERATURE CITED

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