Species Identification and Pathogenicity Study of French *Colletotrichum* Strains Isolated from Strawberry Using Morphological and Cultural Characteristics

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ABSTRACT

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This study reports for the first time the identification and characterization of *Colletotrichum* spp. of anthracnose isolates originating from strawberry grown in France. Sixteen French isolates of *Colletotrichum* and six North American isolates (also from strawberry) representing *C. acutatum*, *C. gloeosporioides*, and *C. fragariae* were compared with respect to morphological and cultural criteria. Fourteen of the French isolates were identified as *C. acutatum*, characterized by acute conidia and low growth rates. The remaining two isolates were identified as *C. gloeosporioides* (teleomorph, *Glomerella cingulata*), characterized by cylindrical conidia, production of perithecia, and high growth rates. *C. fragariae* was not found among the French isolates. Pathogenicity of *C. gloeosporioides* and *C. acutatum* isolates was evaluated on five strawberry cultivars: cvs. Elsanta and Valeta (susceptible), Addie (intermediate), and Sequoia and Dover (resistant). Isolates of *C. gloeosporioides* had low pathogenicity while *C. acutatum* isolates varied from slightly to very pathogenic. Some [isolate x cultivar] specificity was detected, and based on this interaction *C. acutatum* was classified into two groups. Isolates in group 1 caused a similar disease severity on Addie, Sequoia, and Dover, whereas those of group 2 were virulent on Addie but nonvirulent on Sequoia and Dover.

Additional keywords: resistance, *Fragaria* × *ananassa*.

The occurrence of anthracnose on strawberry (*Fragaria* × *ananassa*) in France was first reported on fruit in 1981 (23). Damage was later recorded on stolons, petioles, and foliage. In 1985, the first symptoms of crown rot were reported. In 1988 and 1989, a combination of warm weather and an increased area of production of the everbearing-type strawberry led to an increase in the occurrence of anthracnose. During these 2 yr fruit and crown rot were prevalent, reducing yields by as much as 80% in some fields (23). At the present time, the disease is prevalent in southwest France, an area representing 55% of the total production in France (source from SCEES, Service Central d'Etudes Economiques et de Statistiques) and where the humid climate favors the spread of this disease during spring and summer.

Four species of *Colletotrichum* have been reported as causal agents of strawberry anthracnose, *C. fragariae* Brooks, *C. acutatum* J. H. Simmonds, *C. gloeosporioides* (Penz.) Penz. & Sacc., and *C. demiatum* (Pers.) Grove. *C. fragariae*, the first of these species to be reported, causes a girdling of runners (7), spotting of petioles, crown rot (8), fruit rot (15), and black leaf spot (17). *C.* acutatum (24), described originally as *Gloeosporium* sp. (28,29), causes fruit rot. This species has caused a recent anthracnose outbreak in Brazil (14) and has become increasingly serious in many areas of the United States (19). *C. gloeosporioides* (teleomorph, *Glomerella cingulata* (Stoneman) Spauld. & H. Schrenk) was isolated for the first time from fruit in Florida in 1980 and 2 yr later from Florida-grown plants (18). *C. dematium* has been occasionally described as a pathogen of strawberry fruit (5).

Anthracnose disease can cause damage on any organ of the strawberry plant: crown, fruit, stolon, petiole, leaf, flower, bud, or root (3). In France, disease control may be achieved by spraying with the fungicide dichlofuanide, by using certified plants, and through the use of various cultivation techniques. In order to use resistant cultivars, *Colletotrichum* spp. present in France must be considered when choosing isolates for the screening test in a breeding program, whereas cultivars resistant to the predominant pathogen may be susceptible to other isolates in another area (19).

The taxonomy of *Colletotrichum* has been largely concerned with classical descriptive criteria such as conidial shape (13,20,24, 26,30), conidial size (30), and temperature response (13,20,24,26). The presence of perithecia is an additional criterion for characterizing *C. gloeosporioides* (18,26).

The objectives of this study were 1) to identify the *Colletotrichum* spp. present on strawberry in France using the combination of four criteria (conidial shape, conidial size, temperature responses, and the presence of perithecia), and 2) to study the pathogenicity of French isolates on five strawberry host cultivars known to differ in their susceptibility to these isolates.

MATERIALS AND METHODS

Collection and maintenance of *Colletotrichum* spp. Among the French collection of species of *Colletotrichum* obtained from strawberry plants in various locations, 22 isolates were investigated for their morphological and cultural characteristics. Six isolates were from North America: four isolates of *C. fragariae*, one isolate of *C. acutatum*, and one isolate of *C. gloeosporioides*. Sixteen unidentified *Colletotrichum* isolates were from France (each one representing a single spore isolate).

The sources and designations of the isolates used in this study are given in Table 1. Stock cultures were maintained on silica gel at 4 C as described previously by Perkins (22). Cultures were initiated by transferring silica gel particles from the stock cultures to potato-dextrose agar (PDA, [Difco Laboratories, Detroit, MI]) plates.

Conidial shape and size. Conidial suspensions were prepared by flooding 5-day-old PDA cultures with sterile distilled water and lactophenol (1:1, v/v). Two droplets of suspension were deposited on two slides. Each droplet was covered with a coverslip and nail varnish applied to avoid desiccation. Slides were stored at 4 C.

Fifty arbitrarily chosen conidia per isolate were classified into one of three groups according to their shape: 1) cylindrical, with both ends rounded; 2) intermediate, with one end acute-angled and the other rounded; and 3) acute-angled at both ends (26). This experiment was performed twice.

Length and width of 100 conidia were measured using a eyepiece micrometer. The length/width ratio was calculated for each conidium.

Temperature responses on PDA. Cultures were obtained by transferring a 5-mm-diameter mycelial plug from a PDA culture to PDA in a 90-mm-diameter petri plate. Cultures were incubated in the dark at 10, 15, 20, 25, 28, and 30 C for 5 days. Five replicate plates were prepared for each isolate-temperature treatment. Growth was expressed as an increase in surface area of the cultures during the incubation period. Surface areas were determined with a digitizing tablet.

Sexual state and cultural aspect. Each isolate was examined weekly for 2 mo for the presence of perithecia on oatmeal agar (oatmeal 45 g, agar 13 g, qsp 1 L) or on PDA cultures grown at 25 C under a 12-h fluorescent light/12-h dark cycle.

Pathogenicity tests. To study the variability in pathogenicity of *Colletotrichum* spp. causing strawberry anthracnose in France, five strawberry cultivars were inoculated with 15 isolates of *C. acutatum* and three isolates of *C. gloeosporioides*. Four of the cultivars used represented a range of susceptibility/resistance to five French *Colletotrichum* isolates determined by preliminary test (B. Denoyes, *unpublished results*): cvs. Elsanta and Valeta (susceptible), cv. Addie (intermediate), and cv. Sequoia (resistant). In addition, cv. Dover was used as a very resistant cultivar (16).

Plants originating from micropropagation were transplanted to 200-ml pots containing steam-sterilized soil and were maintained in a glasshouse at 25 C. Plants were used for inoculation at the 6- to 7-wk-old stage.

Conidial suspensions were prepared from 4- to 6-day-old PDA cultures raised under a 16-h blue light (260 nm)/8-h dark cycle. The surface was scraped gently with a Pasteur pipette to remove conidia. Conidial suspensions obtained were filtered through gauze (40-μm mesh) to remove pieces of mycelium. Suspensions were then diluted with distilled water to give a final concentration of 2×10^6 conidia per milliter. The conidial suspensions used in different experiments were prepared from the same stock cultures in order to reduce intraisolate variation.

Conidial germination was carried out by placing droplets of the conidial suspension onto a microscope slide and incubated for 24 h under a 12-h fluorescent light/12-h dark cycle.

Six plants were used for each isolate. Experiments were performed twice, once under glasshouse conditions and once under controlled environment conditions. The foliage of plants was inoculated with a hand-pump sprayer held directly at 20 cm above the plant and the conidial suspension was applied until runoff. Control plants were similarly treated with sterile distilled water. Plants were transferred to plastic boxes to maintain a high relative humidity and to avoid cross-contamination between isolates. Incubation temperatures inside the plastic boxes were 25 C ±4 C and 29 ±1 C under glasshouse and controlled environment conditions, respectively.

The main visible symptom observed at the beginning of disease development was a foliar necrosis. Only a small amount of petiole necrosis was observed. When the disease was more advanced, wilting caused by crown rot occurred. We used a numerical scale, from 0 to 5, in which a disease severity from 0 to 2.5 represented petiolar or foliar necrosis and a range from 3.0 to 5.0 represented a wilting reaction due to crown rot. A full definition of the symptoms assigned to this scale is as follows: 0 = no lesion; 0.5 = lesion just visible on the petiole or foliage; 1.0 = a single developed lesion on the petiole or foliage; 1.5 = two lesions; 2.0 = at least two leaves with expanded lesions; 2.5 = stunted plant but not wilted; 3.0 = beginning of wilting; 3.5 = two wilted leaves; 4.0

TABLE 1. Morphological and cultural characteristics of 22 isolates of *Colletotrichum* spp.

Isolates[s]	Origin	Conidial shape[t]			Conidial size[u]			Growth on PDA at 28 C[v]	Ascigerous state[w]	Species (imperfect stage)
		Cyl	Int	Acu	Width (μm)	Length (μm)	Ratio			
9r2	France	90	10	0 a[x]	4.6 c[y]	12.9 bc[y]	2.84 b[y]	33.39 cd[z]	+	*C. gloeosporioides*
9n1	France	87	13	0 a	4.8 c	14.0 ef	3.02 bc	28.81 d	+	*C. gloeosporioides*
Ark P1	U.S.A. (B. Smith)	84	16	0 a	5.3 b	11.6 a	2.26 a	35.62 bc	+	*C. gloeosporioides*
Cf1	U.S.A. (B. Smith)	63	37	0 b	5.0 bc	19.5 i	4.17 g–j	43.48 a	−	*C. fragariae*
La1	U.S.A. (B. Smith)	62	38	0 b	4.5 c	12.9 bc	3.21 cd	40.42 a	−	*C. fragariae*
Cf7	U.S.A. (R. Milholland)	58	42	0 b	6.7 a	20.9 j	3.19 cd	36.69 bc	−	*C. fragariae*
Cf4	U.S.A. (R. Milholland)	34	66	0 c	3.8 de	14.7 fg	3.96 fgh	39.55 ab	−	*C. fragariae*
1079a	France	15	9	76 d	3.6 def	13.7 de	4.00 fgh	12.46 e	−	*C. acutatum*
494a	France	10	19	71 de	3.7 def	12.5 b	3.49 de	14.97 e	−	*C. acutatum*
1341a	France	10	10	80 def	3.3 ef	14.7 fg	4.62 k	13.46 e	−	*C. acutatum*
F1a	France	7	13	80 d–g	3.7 def	13.3 cd	3.89 fg	14.34 e	−	*C. acutatum*
Goff	U.S.A. (B. Smith)	4	15	81 d–h	3.5 ef	15.0 g	4.56 k	8.10 f	−	*C. acutatum*
F5a	France	4	10	86 d–i	3.7 def	14.4 e–g	4.08 ghi	15.12 e	−	*C. acutatum*
1267b	France	2	12	86 e–i	3.3 ef	16.1 h	5.22 l	16.57 e	−	*C. acutatum*
162a	France	3	11	86 e–i	3.3 ef	13.9 de	4.52 jk	13.90 e	−	*C. acutatum*
688b	France	5	6	89 e–i	3.8 de	14.6 fg	3.98 fgh	13.34 e	−	*C. acutatum*
F3a	France	5	5	90 f–i	3.8 de	14.1 ef	3.89 fg	12.78 e	−	*C. acutatum*
F2b	France	2	8	90 f–i	3.9 de	14.8 fg	3.95 fgh	11.88 e	−	*C. acutatum*
1159.5c	France	2	7	91 f–i	3.6 def	14.7 fg	4.31 h–k	14.45 e	−	*C. acutatum*
1159.2b	France	3	2	95 ghi	4.1 d	14.4 e–g	3.65 ef	12.75 e	−	*C. acutatum*
528a	France	0	8	92 hi	3.4 ef	14.2 ef	4.38 ijk	12.60 e	−	*C. acutatum*
F7a	France	0	7	93 i	3.2 f	13.9 de	4.50 jk	18.21 e	−	*C. acutatum*

[s] U.S. reference isolates are italicized.
[t] Conidial shape: Cyl, cylindrical form at both ends; Int, intermediate form acute-angled at one end and cylindrical form at the other end; Acu, acute with acute-angled at each end. One hundred conidia were examined for each isolate.
[u] Mean of 100 conidia.
[v] Colony area (cm^2) after 5 days incubation at 28 C on potato-dextrose agar (PDA).
[w] Presence (+) or absence (−) of perithecia.
[x] Values in this column with same letters do not differ significantly according to 2î test ($P \leq 0.05$).
[y] Values in these columns with same letters do not differ significantly according to Newman and Keuls test ($P \leq 0.05$).
[z] Values in this column with same letters do not differ significantly according to t test with a risk $\alpha = 0.001$.

= almost all leaves wilted; 4.5 = all leaves wilted but slightly green; 5.0 = dead plant. Inoculated plants were evaluated for disease response over a 6-wk period.

Statistical analysis. The likelihood ratio chi-squared 2î test was performed for conidial shape distribution (1). Pairwise comparisons of growth means were performed using the Student's t test with a level of significance (α) equal to 0.001. Analysis of variance (SAS Institute, Inc., Cary, NC) was performed for conidial size followed by a Newman and Keuls test. Since the scale of disease symptoms comprises 11 steps, disease response data were considered as quantitative and were subjected to a variance analysis performed with the general linear model procedure (SAS Institute, Inc., Cary, NC). Conformity of data to the main assumptions (normality of distribution of the error terms, adequacy of the model, and homogeneity of residual variances) of the analysis of variance was checked. A Newman and Keuls test was used to separate means. Analyses were performed on disease response data obtained on the 38th day after inoculation when differences between cultivars and between isolates were more apparent under our experimental conditions.

Data are presented as means for each treatment.

RESULTS

Morphological and cultural characteristics.

Conidial shape. Since the shape distribution of the 50 conidia per isolate was similar in both experiments, the 2î test was performed on the combined total of 100 conidia. Data presented in Table 1 show that isolates without acute conidia were easily separated from those with acute conidia; the former could be assigned to two groups depending on the proportion of cylindrical and intermediate conidia. In the first group, cylindrical conidia predominated in ArkP1 (*C. gloeosporioides*), and in 9r2 and 9n1. The second group (*C. fragariae*), comprising the Cf1, La1, Cf7, and Cf4 isolates, showed more than 30% of intermediate conidia. The remaining 15 isolates, which included the Goff isolate (*C. acutatum*), showed a predominance of acute conidia.

Conidial size. Analysis of conidial size was performed according to length, width, and length/width ratio. Data for length/width ratio and width showed greater differences between isolates than conidial length, the latter showing large variations within isolates (Table 1). Three isolates—ArkP1 (*C. gloeosporioides*), 9r2, and 9n1—could be distinguished from the others by their smaller length/width ratio (< 3.1). Moreover, they fitted well into the classification based on a predominantly cylindrical conidia. However, according to the Newman and Keuls test, the separation of the remaining 19 isolates based on length/width ratio was different from that based on conidial width.

Temperature reaction on PDA. Isolate growth of the three species showed considerable variation with temperature, though the magnitude of this variation was similar for each isolate, as reported previously (24,26). Thus, growth was slow at 10 C (areas from 0.53 cm^2 to 8.41 cm^2) and increased with temperature. The optimal growth temperature was isolate-dependent, occurring between 25 and 30 C. Statistical Student's t tests performed for growth at different temperatures showed the greatest discrimination between isolates at 28 C (data not shown), in agreement with Smith (26). Therefore, only the results obtained at 28 C were presented in Table 1. Isolates were clearly separated into two groups based on growth areas at 28 C: a fast-growing group (area > 28.8 cm^2), and a slow-growing group (area < 18.2 cm). The fast-growing group overlapped with the group without acute conidia while the slow-growing group overlapped with the group showing a predominance of acute conidia (Table 1). However, isolates belonging to the same group were assigned to different classes using the t test because there was growth variation occurring within group (Table 1).

Sexual state. Three isolates (9r2, 9n1, and ArkP1) produced perithecia with asci and ascospores typical of *G. cingulata* (teleomorph of *C. gloeosporioides*). These isolates were those belonging to the fast-growing isolates that produced cylindrical conidia (Table 1). All other isolates examined failed to produce perithecia.

Colletotrichum spp. in France. Data obtained for conidial shape, conidial size, temperature reaction and sexual state allowed identification of the French *Colletotrichum* isolates. Two isolates, 9r2 and 9n1, appeared to belong to the same species as ArkP1, an isolate of *C. gloeosporioides* (teleomorph, *G. cingulata*). Isolates were characterized by small cylindrical conidia, a high growth rate on PDA at 28 C, and perithecia. Colonies on PDA were very dark due to the presence of perithecia, and showed aerial mycelia for ArkP1 and flat for 9n1 and 9r2. The remaining 14 French isolates were similar to Goff and were identified as *C. acutatum*. They were characterized by the production of acute conidia and slow growth on PDA at 28 C. On PDA, they had dense white aerial mycelia, and the conidial masses were well developed with a salmon-orange or salmon-pink coloration, except for the 494a isolate, which presented a chromogenic pink color. All the *C. fragariae* isolates studied originated from the United States. The conidia of each *C. fragariae* isolate were distributed between cylindrical and intermediate shape, and isolate growth was rapid on PDA at 28 C. The mycelia were gray and aerial, sparser than the isolates mentioned above. Orange spore masses were observed infrequently.

Pathogenicity tests. All inoculations were successful: a high percentage of conidial germination (70–95%) was obtained with each inoculation.

Since the inoculation of the F5a isolate was not repeated, it was not included in the analyses of variance. Results of the analyses of variance on disease severity from studying the effects of isolate, cultivar, [isolate × cultivar] interaction, and experimental effects are presented in Table 2. No significant experimental effects were found.

Variation in C. gloeosporioides. The effect of isolate was not significant while a significant cultivar effect was observed (Table 2). Isolates showed either no virulence or only a weak virulence. Cultivars Valeta and Elsanta were slightly more susceptible than Addie, Sequoia, and Dover, with disease responses of 0.44, 0.43, 0.15, 0.03, and 0.04, respectively.

Variation in C. acutatum. The nonenvironmental variation of the disease severity can be explained by isolate, cultivar, and [isolate × cultivar] interaction effects.

Since the effect of [isolate × cultivar] interaction was significant, we carried out an analysis of variance for each isolate, followed by a Newman and Keuls test in order to rank cultivars according to their susceptibility. Results are shown in Table 3. Isolates were classified into two groups according to their pathogenicity on the five cultivars. When group 1 isolates were inoculated, Addie

TABLE 2. Analyses of variance in disease response of five strawberry cultivars, 38 days after inoculation with *Colletotrichum gloeosporioides* (three strains) and *C. acutatum* (14 strains)

Species	Source of variation	df	MS[y]	$P > F$[z]
C. gloeosporioides	Experiment	1	0.018	0.6889 NS
	Isolate	2	0.233	0.1249 NS
	Cultivar	4	1.502	0.0001**
	Experiment × isolate	2	0.163	0.2315 NS
	Experiment × cultivar	4	0.074	0.6122 NS
	Isolate × cultivar	8	0.119	0.3828 NS
	Experiment × isolate × cultivar	8	0.141	0.2603 NS
	Error	148	0.110	
	Total	177		
C. acutatum	Experiment	1	1.902	0.2079 NS
	Isolate	13	45.020	0.0001**
	Cultivar	4	188.622	0.0001**
	Experiment × isolate	13	1.223	0.4279 NS
	Experiment × cultivar	4	2.159	0.1262 NS
	Isolate × cultivar	52	8.341	0.0001**
	Experiment × isolate × cultivar	52	1.056	0.7075 NS
	Error	669	1.197	
	Total	808		

[y] Mean squares.

[z] NS = nonsignificant at $P = 0.05$; ** = significant at $P = 0.01$.

had a similar susceptibility to Sequoia and Dover. In this group, F7a and F1a had a very low virulence. When group 2 isolates were inoculated, Addie was susceptible but Sequoia and Dover were resistant and did not shown any lesion.

A statistical approach confirmed the different behavior of the two groups. We calculated the contribution of each isolate to the [isolate × cultivar] interaction. This is given as follows:

$$\text{interaction component} = \frac{\text{(variance of the interaction due to an isolate)}}{\text{(total variance of the interaction)}}$$

The greater the contribution the more the isolate takes part in the interaction effect. As shown in Table 3, the interaction effect was mainly attributed to the four isolates of group 2 (688b, 1341a, 1159.5c, and 1267b) that showed an interaction component greater than 10%. These four isolates represented 61.3% of the [isolate × cultivar] interaction. In addition, results of the analysis of variance carried out within each group of isolates showed that nonenvironmental variation was explained by isolate and cultivar effects but not by [isolate × cultivar] interaction effects that were either nonexistent or only weakly significant (Table 4). Within each group, cultivar ranking was the same for the isolates that differed in their ability to cause more or fewer symptoms.

Cultivars Elsanta and Valeta were susceptible to most isolates, while Addie varied from susceptible to intermediate according to the isolates used, and Sequoia and Dover were either intermediate or resistant.

Figure 1 shows the effects of isolate, cultivar, and [isolate × cultivar] interaction. Isolate curves belonging to the same group (Goff, 494a, and F1a for group 1; 1341a and 1267b for group 2) developed in a similar way as the isolates have the same pattern of pathogenicity. However, between the two groups the curves do not have the same development as Sequoia and Dover, which show a resistance specific to group 2. Furthermore, within group 1, Goff causes more damage than 494a and 494a more damage than F1a.

DISCUSSION

Among the four criteria used in the present study for the identification of *Colletotrichum* spp. pathogenic to strawberry (*C. fragariae*, *C. acutatum*, and *C. gloeosporioides*), conidial shape provided the best means of discrimination. These results are in agreement with previous data (13) and the interest in conidial shape for separating species was reported by several authors dis-

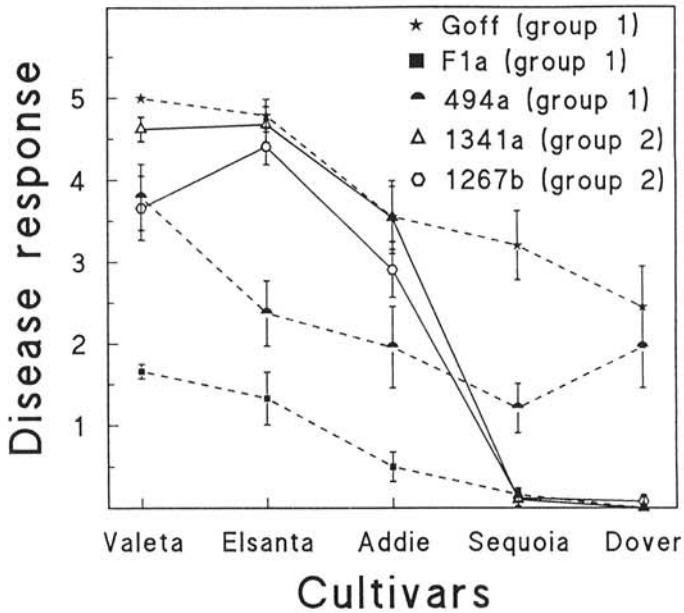

Fig. 1. Pattern of pathogenicity of five isolates belonging to groups 1 and 2 on five strawberry host cultivars. Each point is the mean of 12 disease response data recorded 38 days after inoculation. Vertical bars represent standard errors.

TABLE 3. Disease response of five strawberry cultivars, [isolate × cultivar] interaction component for 14 isolates and isolate groups of *Colletotrichum acutatum*

Source designation[x]	Cultivar disease response[x]					Pooled cultivar mean	[Isolate × cultivar] interaction component per isolate[y]	Groups classification
	Valetta	Elsanta	Addie	Sequoia	Dover			
F2b	2.0 a[z]	2.3 a	1.1 ab	0.4 b	0.2 b	1.2	0.7	1
Goff	5.0 a	4.8 a	3.6 b	3.2 b	2.5 b	3.8	1.2	1
1159.2b	2.8 a	2.6 a	1.3 b	0.8 b	1.1 b	1.7	1.3	1
F1a	1.7 a	1.3 a	0.5 b	0.2 b	0.0 b	0.7	1.5	1
162a	2.4 a	1.6 ab	1.1 b	0.7 b	0.9 b	1.3	3.3	1
494a	3.8 a	2.4 b	2.0 b	1.2 b	2.0 b	2.3	4.5	1
528a	1.8 ab	2.3 a	0.8 c	1.1 bc	0.6 c	1.3	5.0	1
1079a	2.2 a	1.7 b	0.9 b	0.5 b	1.0 b	1.2	6.0	1
F7a	0.9 a	0.5 b	0.0 c	0.0 c	0.0 c	0.3	6.1	1
F3a	2.7 a	1.7 a	2.0 a	1.2 a	2.1 a	1.9	9.1	1
1267b	3.7 b	4.4 a	2.9 c	0.1 d	0.1 d	2.2	11.0	2
1159.5c	4.3 a	4.5 a	2.3 b	0.0 c	0.0 c	2.2	13.1	2
1341a	4.6 a	4.7 a	3.5 b	0.1 c	0.0 c	2.6	18.3	2
688b	4.0 a	4.9 a	4.0 a	0.3 b	0.0 b	2.6	18.9	2
Pooled isolate mean	3.0	2.8	1.9	0.7	0.7			

[x] Disease response is based on a 0–5 disease severity scale where 0 = no symptom and 5 = dead plant. Each datum is the mean of 12 plants.
[y] Newman and Keuls test was carried out for each isolate. Values in each line with the same letter do not differ significantly ($P = 0.05$).
[z] Percentage of the total sum of square of [isolate × cultivar] interaction per isolate.

TABLE 4. Analyses of variance in disease response 38 days after inoculation within two groups of *Colletotrichum acutatum* isolates

	Source of variation								
	Cultivar			Isolate			[Cultivar × isolate]		
Groups of *C. acutatum*[x]	df	MS[y]	$P > F$[z]	df	MS	$P > F$	df	MS	$P > F$
Group 1 = 10 strains	9	52.16	0.000**	4	54.01	0.000**	36	1.78	0.106 NS
Group 2 = 4 strains	3	3.00	0.013*	4	228.12	0.000**	12	1.49	0.046*

[x] Groups were described in Table 3.
[y] Mean squares.
[z] NS = not significant at $P = 0.05$; * = significant at $P = 0.05$; ** = significant at $P = 0.01$.

cussing *Colletotrichum* taxonomy (13,20,24,30). Using the temperature responses on PDA, the identification of *C. acutatum* is effective, whereas the species *C. fragariae* and *C. gloeosporioides* cannot be separated. These two species were considered as synonymous by Von Arx (31). Since variation is apparent, conidial size is of little value in distinguishing species (13,20), even though conidia of *C. gloeosporioides* may appear smaller. The presence of perithecia allows us to distinguish *C. gloeosporioides* from *C. acutatum* and *C. fragariae*, though this is not a reliable characteristic (2,20). The use of the shape of setae produced on strawberry leaf medium, as a further morphological criterion for separating these three species, has been reported recently (13). However setae formation may be influenced by environmental factors (4,12). Separation of these three species is achieved by using molecular markers such as isozymes (6) or restriction fragment length polymorphism (RFLP) of ribosomal DNA and random amplified polymorphism DNA (RAPD) (B. Denoyes, *unpublished*).

Since 1990, about 200 samples of anthracnose-infected plants or fruits have been received at the Laboratory of Plant Protection at Bordeaux (A. Baudry, *unpublished data*). Isolates obtained from this material produced conidia typical of *C. acutatum*. Thus, our observations of anthracnose isolates from strawberry have shown that, among the French isolates, *C. acutatum* is prevalent, while the occurrence of *C. gloeosporioides* is only occasional. This is the first time that a characterization of *Colletotrichum* spp. of anthracnose isolates from strawberry in France has been carried out. *C. acutatum* has been observed in other European countries, e.g., the United Kingdom (27) and Italy (9). *C. acutatum* is also the major species present in the southwestern United States (California) from which the everbearing-type strawberry has been introduced to Europe. None of the French isolates examined were identifiable as *C. fragariae*, a species that causes severe losses in the eastern United States. Several hypotheses may explain why it has not been observed either in France or more generally in Europe: *C. fragariae* has been found mainly in the southeast region of the United States, particularly in Florida (19), from which exchange of the host material has been limited.

In the present study we infected five strawberry genotypes with three isolates of *C. gloeosporioides* and 14 isolates of *C. acutatum*. In *C. gloeosporioides*, the isolates tested induced either no or only very few symptoms on the varieties used. However, other isolates belonging to this species are capable of causing severe disease (19). In *C. acutatum*, we have shown an [isolate × cultivar] interaction that explains the association of the isolates tested with two different groups. Highly pathogenic isolates from group 1, such as 494a, may cause necrosis and crown rot on cvs. Sequoia and Dover, which behave similarly to the intermediate cv. Addie. Isolates from group 2 are nonvirulent on cvs. Sequoia and Dover but virulent on Addie.

The susceptibility or resistance of cultivars to the 14 *C. acutatum* isolates, and the three isolates of *C. gloeosporioides* of low pathogenicity, did not vary with the incubation temperature used (i.e., 25 ±C 4 C and 29 C ±1 C). In contrast, variability of susceptibility was detected in cv. Sequoia when it was inoculated with *C. fragariae* under similar environmental conditions (10,11,25).

With respect to host resistance, cvs. Elsanta and Valeta are susceptible while Addie is less susceptible. Due to the noncompatible reaction of group 2 isolates with Sequoia and Dover described as resistant by various authors (10,16,25), the resistance of these cultivars to group 2 isolates seems to be specific. Resistance of these cultivars to group 1 isolates seems to be due to a partial resistance caused by the intermediate degree of compatibility of group 1 isolates. Specific resistance represents merely mono- or oligogenic resistance (21). This is in agreement with our preliminary results obtained from crosses between resistant cultivars Sequoia and Dover and susceptible cultivars such as Elsanta inoculated with 1267b (group 2). In these results, resistance to 494a (group 1) is different and seems to be polygenic. Nevertheless, these assumptions need to be confirmed by studying the inheritance of resistance to each group of isolates using genetic design. The identification of a genetic control of resistance would allow us to optimize the selection for anthracnose resistance.

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