Investigating Phenotypic Variability in *Colletotrichum lindemuthianum* Populations

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ABSTRACT

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Colletotrichum lindemuthianum, causal agent of anthracnose in the common bean, has wide genetic variability. Differential bean cultivars and morphological and physiological characteristics were used to analyze 74 isolates of *C. lindemuthianum* collected in two counties in the state of Minas Gerais, Brazil. Six different races were found, with a predominance of race 65 at both locations. Isolates were classified according to their sensitivities to the fungicide thiophanate-methyl, normally used in the control of common bean anthracnose. In all, $\approx 10\%$ of isolates were

resistant to the fungicide in vitro. Characteristics such as indexes of mycelia growth rate, colony diameter, sporulation capacity, and percentage of germination demonstrated the high genetic variability of *C. lindemuthianum*. We also observed variation in conidial cytology. The conidia of most isolates showed septa formation after germination, in contrast to septa absence, previously reported in the literature. Sexual and asexual reproduction were evaluated for mechanisms that may contribute in the generation of variability in *C. lindemuthianum*. Conidial anastomosis tubes were commonly found, indicating that asexual reproduction can help increase variability in this species. Information from this study confirmed high variability in *C. lindemuthianum* and will guide future studies in basic knowledge and applied technologies.

Anthracnose, caused by *Colletotrichum lindemuthianum* (Sacc. & Magnus) Briosi & Cavara, is one of the most important diseases that affects the common bean, especially during mild temperatures and moist weather conditions (17). Losses of $\leq 100\%$ in common bean yield can be observed when infected seed are used and climatic conditions favor the pathogen (33). *C. lindemuthianum* is a filamentous fungus that reproduces asexually and presents septate and ramified mycelia (36). The pathogen's sexual phase is described as *Glomerella lindemuthiana* (32) and has been identified in field isolates from Brazil (6,35). In addition to sexual recombination, other mechanisms that allow genetic material transfer have been described in the literature for this pathogen, such as a parasexual cycle (7) and asexual recombination via fusion of conidial anastomosis tubes (CATs) (13,28).

Breeding for disease resistance, which reduces production cost and environmental contamination, is the most efficient strategy for the control of anthracnose (26). However, the major limitation for the development of durable resistance in common bean cultivars is the high pathogenic and genetic variability of *C. lindemuthianum* (22). Because of this high variability, constant monitoring of the fungus in the field is essential to support breeders in the development of resistant cultivars. Synthetic fungicides are also widely used in common bean crops to control anthracnose disease (41). Despite their efficacy, the constant use of these products results in the emergence of problems, such as the appearance of resistant pathogens and the contamination of both food and the environment (38).

The variability of *C. lindemuthianum* is also demonstrated in morphological, physiological, and cultural characteristics (34).

Several characteristics of this pathogen that are essential for its development and dissemination, including mycelia growth rates, colony diameter, sporulation capacity, and germination percentage, show great variation between isolates, even within the same race (34). Another important phenotype used in the taxonomy of the genus *Colletotrichum* is conidial cytology, which measures the dimensions of nongerminated conidia and analyzes the presence of septa in germinated conidia (18). Evaluation of these characteristics in phytopathogens is also important as a morphological marker in genetic studies (34).

A population of 74 isolates of *C. lindemuthianum*, collected in two counties in the state of Minas Gerais, Brazil, was evaluated to identify the pathogenic, morphologic, and physiologic variability of this pathogen and its implications in the characterization of species, understanding of population dynamics, and common bean management. Characteristics of cell biology and fungal development and the possible mechanisms that could help increase variability in this species were analyzed. Isolates were also evaluated for their in vitro sensitivities to the fungicide thiophanate-methyl to assess the effectiveness of the active ingredient in the control of common bean anthracnose. Information presented in this study can be used to broaden the understanding of *C. lindemuthianum* biology and to facilitate common bean management.

MATERIALS AND METHODS

Fungal isolates. Isolates of *C. lindemuthianum* were obtained from bean pods, leaves, and stems of naturally infected common bean cultivars. Samples, one per plant, were collected during 2009 in two counties (Lavras and Lambari), where high anthracnose incidence in the state of Minas Gerais, Brazil was reported previously (1,33). Small pieces of infected plant tissue were surface sterilized and placed on petri dishes containing M3 culture medium (10 g of sucrose, 20 g of agar, 2 g of KH_2PO_4 , 1 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 6 g of peptone, and 1 g of yeast extract). In all,

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74 single conidia cultures were obtained and maintained in M3 medium in the dark (Supplementary Table).

Polymerase chain reaction analysis. Polymerase chain reaction (PCR) amplification was performed to confirm *C. lindemuthianum* isolates. The *MAT1-2-1* HMG domain was amplified with the specific primers HMGCLF (5'-CATGCCGCACTAAAGCAAAT-3') and HMGCLR (5'-ATCATCAGACGTTCTTGTGTG-3') for *C. lindemuthianum* (10). DNA was extracted according to the methodology developed by Raeder and Broda (24). Amplification was performed using Taq DNA Polymerase kit (Invitrogen Life Technologies, Carlsbad, CA) in a total volume of 50 μ l containing 5 μ l of 10 $\times$ PCR buffer, 1.5 μ l of $MgCl_2$ (50 mM), 0.5 μ l of Taq DNA polymerase (5 U/ μ l), 1 μ l of dNTPs (10 mM), 2.5 μ l of each primer (100 mM), and 2 μ l of DNA (10 ng/ μ l). PCR reactions were carried out in an MJ96+/MJ96G thermocycler (Biocycler Technology Co., Hangzhou, Zhejiang) using an initial denaturation of 2 min at 94°C; followed by 30 cycles of 60 s at 94°C, 30 s at 55°C, and 30 s at 72°C; and a final extension of 10 min at 72°C. Amplified products were separated by electrophoresis on 1.0% (wt/vol) agarose gels using a 100-bp DNA ladder as molecular marker with 1 $\times$ Tris-borate-EDTA buffer (0.45 M Tris-borate and 0.01 M EDTA, pH 8.0) for 3.5 h at 80 V, stained with ethidium bromide, visualized under UV light (Fotodyne Ultraviolet Trans-illuminator; Fotodyne Inc., New Berlin, WI), and photographed using a Kodak EDA-290 camera (Kodak Company, Rochester, NY).

Pathogenicity assays. Eight seeds from each of 12 common bean differential cultivars proposed by Pastor-Corrales (20) ('Michelitte', 'Michigan Dark Red Kidney', 'Perry Marrow', 'Cornell 49242', 'Widusa', 'Kaboon', 'México 222', 'PI 207262', 'TO', 'TU', 'AB 136', and 'G2333') were used for the pathogenicity test. Seed were provided by the plant breeding program of Universidade Federal de Lavras, Brazil. Each isolate was inoculated in bean pods in culture medium and incubated at 22°C for 10 to 15 days in the dark to obtain high sporulation. Ten-day-old bean seedlings with fully expanded primary leaves were sprayed with conidial suspension (1.2×10^6 conidia/ml). Inoculated plants were incubated in a moist chamber at 22°C with a 12-h photoperiod and a relative humidity of 98% for 48 h.

At 10 days postinoculation, symptoms of the disease were scored according to the descriptive scale recommended by Rava et al. (25). Plants with disease reaction scores of 1 to 3 were considered resistant, whereas plants that were scored 4 to 9 were considered susceptible. The identified races were assigned a value based on the binary nomenclature system proposed by Habgood (12). The differential cultivars were arranged in a fixed order and receive a fixed value defined by the equation 2^{n-1} , where 2 was the number of reaction classes (resistant or susceptible) and n is the order number of the cultivar. Incompatible reaction has a value of zero and nullifies the cultivar value when multiplied by it. The compatible reaction has a value 1 and gives the same cultivar value when multiplied by it. Therefore, the total sum of the values from the compatible reaction gives the number that designates the race.

Fungal isolate resistance to thiophanate-methyl. Agar blocks (6 mm in diameter) containing mycelia from each *C. lindemuthianum* isolate were cut from the edge of colonies and placed in the middle of plates containing M3 medium with thiophanate-methyl (Cercobin 700 WP; Iharabras S/A Indústrias Químicas, Sorocaba, SP, Brazil). Thiophanate-methyl (0.7 mg/ml) was added to M3 medium after autoclaving. This fungicide presents broad-action spectrum and is used on a large scale in this concentration, according to Abreu (1), to control common bean anthracnose in the field (4). The controls of this assay consisted of the growth of the same isolates in M3 medium without thiophanate-methyl.

Radial growth values were measured in two perpendicular directions after incubation at 22°C for 15 days, and the means of

two replicates were calculated (23). Mean percentages of growth of each isolate on M3 medium + thiophanate-methyl were compared with control growth. Sensitive, intermediate, or resistant isolates were defined as exhibiting <10, 10 to 60, and >60% growth, respectively, compared with the controls (31).

Indexes of mycelial growth rate and colony diameter. Experiments were carried out in a completely randomized design (CRD) with four replicates. Each plot consisted of one petri dish. Mycelial plugs (6 mm in diameter) from the second subculture were placed in the middle of M3 plates, which were incubated at 22°C in the dark. Indexes of mycelial growth rate (IMGRs) were evaluated by taking average colony measurements (in centimeters) in two perpendicular directions every 24 h over 15 days and were estimated according to the expression $IMGR = \Sigma(Dc - Dp)/N$, where Dc is average of current colony diameter, Dp is average of previous colony diameter, and N is number of days after inoculation (19). Colony diameter was assessed by measuring the perpendicular diameter average of each colony (in centimeters) after 15 days.

Sporulation capacity and percentage of germination. Sporulation capacity was analyzed using a CRD with three replicates. Each of the 74 *C. lindemuthianum* isolates was inoculated on sterilized green common bean pods that were then incubated at 22°C for 15 days in the dark. Each plot consisted of one bean pod, to which 3 ml of water was added to obtain conidial suspensions. Concentration was determined by analysis of 50 μ l of each conidial suspension according to Dias (9), using a metallized hemacytometer (Hausser Scientific Company, Horsham, PA).

For the percentage of germination assays, conidial suspensions (1.2×10^6 conidia/ml) were used for each isolate. Assays were performed using split plots in time in a CRD with two replicates. Conidial suspensions (500 μ l total) were sprayed out on petri dishes containing agar-water medium. After 24 and 48 h at 22°C in darkness, 50 conidia from each replicate were evaluated under an optical microscope Olympus CX41 (Olympus Deutschland GmbH, Hamburg, Germany). Conidial germination was defined as when the germ tubes presented size larger or equal to the smallest conidial diameter.

Conidial cytology and formation of CATs. Conidial suspensions (1.2×10^6 conidia/ml) from 18-day-old cultures were prepared as described above. Next, 200- μ l drops of each suspension were placed in an eight-well slide culture chamber (Nalge Nunc International, Rochester, NY), and the suspensions were then incubated at 22°C in darkness for 24 h. Calcofluor White (0.12 M; Sigma-Aldrich, St. Louis) was added to each well, and the samples were examined after 3 to 4 min at room temperature using an inverted epifluorescence microscope (Zeiss Axio Observer Z1; Carl Zeiss Inc., Jena, Germany). Fluorescence was detected at 420/70 nm using a $\times 40$ objective lens. Images from the epifluorescence microscope were captured using Zeiss Axiovision software and were processed further in ImageJ 1.41 software (U.S. National Institutes of Health, Bethesda, MD).

For cytological measurements, 30 nongerminated conidia from each isolate were analyzed using CRD. The width and length of conidia (in micrometers) were measured using Image Tool 3.0 software (University of Texas Health Science Center, San Antonio). After 24 h, germinated conidia from each isolate were classified with or without septa, and the percentage of septa formation was estimated. CAT fusion was quantified as the percentage of conidia or conidia germings involved in fusion, using data from three replicates, with 200 conidia/replicate after 24 h of incubation (16).

Mycelial and sexual compatibility. Mycelial discs of *C. lindemuthianum* isolates were paired in all combinations on M3 medium plates using two replicates according to the CRD. After 15 days at 22°C in the dark, the pairings were evaluated. Sexual compatibility was indicated by the formation of a dark line of

perithecia at the junction of the colonies. Mycelial compatibility was characterized by the mycelial mixture in the contact zone between two colonies.

Statistical analysis. Data were submitted to analysis of variance (ANOVA) and means were compared by the Scott-Knott test ($P = 0.05$) using the statistical program MSTAT-C 1.0 (Michigan State University, East Lansing).

RESULTS

PCR analysis. All isolates were collected from common bean cultivars, of which the etiologic agent of anthracnose is *C. lindemuthianum*. However, because of great interstrain morphological variability, these additional PCR assays were performed to confirm species. All evaluated isolates amplified a 200-bp fragment for the *MAT1-2-I* HMG domain (10), confirming they were isolates of the *C. lindemuthianum* (Fig. 1E).

Pathogenicity assays. Isolates used in this study showed different virulence patterns when inoculated in the 12 differential cultivars (Table 1). Six races were identified from the 74 isolates. Races 65 and 81 were more prevalent, accounting for 58.11 and 18.92% of the isolates, respectively. In Lavras-MG, race 65 (82.15%) was predominant, and only two other races were identified (races 1 and 81). However, in Lambari-MG, although race 65 (42.2%) was more prevalent, five other races were found, accounting for the county's higher pathogenic variability.

Thiophanate-methyl resistance. Of the isolates used in this study, 68.9% were sensitive to thiophanate-methyl, 21.6% were classified as intermediate sensitivity, and only 9.5% showed resistance to thiophanate-methyl in vitro (Table 1). Plates used as controls in this experiment exhibited radial growth, which was compatible with the colony diameter assay results for these isolates (data not shown).

IMGRs and colony diameter. The 74 isolates were grouped in four different groups according to IMGRs and colony diameters (Table 2). Isolates with lower mycelial growth rates exhibited a lower radial growth, except for isolate LV 157, which was classified in a distinct group in both analyses. For both analyses, isolates showed great variability, and average growth (IMGR) varied from 1.76 cm/day (LV 187) to 4.5 cm/day (LV 186); colony diameter varied from 3.2 cm (LV 187) to 7.3 cm (LV 186) after 15 days.

Sporulation capacity and percentage of germination. Sporulation capacity data did not meet the assumptions of the ANOVA.

Transformation of data was required to fit the homogeneity of variances and normality of errors. A logarithmic transformation was used as suggested by Box and Cox (5). Mean tests classified the 74 isolates into five groups (Table 2). Sporulation data were variable, and the numbers of conidia varied from 0.13×10^6 conidia/ml (LV 155) to 18.87×10^6 conidia/ml (LV 122).

The statistical analyses performed for conidial germination rates showed that all factors were significantly different: strain ($P < 0.0001$), incubation time ($P < 0.0001$), and the strain-time interaction ($P < 0.0001$). Significant interactions indicated that percentages of germinating isolates were not coincident at both times of incubation assessed (24 and 48 h). For example, isolate LV 121 was classified in the low germination percentage group at 24 h; however, this isolate exhibited an intermediate germination percentage at 48 h. Thus, mean tests were performed independently for each evaluation time. Different groups were formed according to germination percentages (Table 3). Isolate LV 190 exhibited higher germination percentages at 24 h ($88.5 \pm 12.02\%$) and 48 h ($94.5 \pm 4.76\%$), whereas some strains (LV 122, LV 137, LV 142, and LV187) still had $<30\%$ germinated conidia at 48 h (Table 3).

Conidial cytology and formation of CATs. Significant statistical variations ($P < 0.05$) were observed for the width and length of conidia. Width varied from $4.09 \mu\text{m}$ (LV 158) to $6.19 \mu\text{m}$ (LV 159), forming three groups of statistically different isolates (Table 3). Measurement of conidia length yielded four different groups, with measurements varying from $14.46 \mu\text{m}$ (LV 136) to $18.98 \mu\text{m}$ (LV 143) (Table 3).

After 24 h of incubation in water, CAT fusion was not observed in four isolates (LV 157, LV 178, LV 184, and LV 201); thus, they were not included in the statistical analysis. From the 70 isolates analyzed, five groups were formed (Table 3). LV 121 exhibited the highest percentage of CAT fusion ($\approx 60\%$) (Fig. 1B and C), with long chains of conidia visualized (Fig. 1A). Evaluation of germings indicated that only 11 isolates (LV 122, LV 143, LV 155, LV 171, LV 172, LV 182, LV 187, LV 195, LV 199, LV 200, and LV 203) did not show septa formation after germination (Fig. 1D). The percentage of conidia with septa after germination varied among the other 63 isolates evaluated, with values of conidia with septa ranging from 6% (LV 170) to 88% (LV 139) (Fig. 2).

Mycelial and sexual compatibility. Isolate pairing resulted in 48 combinations (1.78% of the total) that had sexual compati-

Fig. 1. Conidial anastomosis tube (CAT) fusion in different *Colletotrichum lindemuthianum* isolates stained with Calcofluor White (0.12 M) and polymerase chain reaction (PCR) amplification products from five representative isolates and (M) molecular marker (100-bp ladder). **A**, Chain of fused conidia from isolate LV 146; **B**, conidia fusion from isolate LV 135; **C**, conidia fusion from isolate LV 138; and **D**, conidia fusion from isolate LV 174. **E**, PCR amplification products obtained using primers HMGCLF and HMGCLR for indicated isolates. Bar: $10 \mu\text{m}$.

bility, marked by the presence of a dark contact line between pairings. However, none of the 48 combinations showed viable and fertile perithecia in the contact line after 15 days of incubation. Microscopic analysis revealed only the formation of proto-perithecia, without production of asci and ascospores (data not shown). Isolates LV 195 and LV 141 were compatible in a higher number of combinations, 12.33 and 10.96%, respectively (Table 1). A group of 30 isolates was incompatible in all combinations. Mycelial compatibility was not observed in any pairings.

DISCUSSION

All traits evaluated in this study demonstrated high variability in *C. lindemuthianum* populations. Pathogenicity tests revealed six virulence patterns in the 74 isolates of *C. lindemuthianum* studied (Table 1). These results show that *C. lindemuthianum* is a highly variable plant pathogen, especially considering that sampling was performed in only two counties, Lavras and Lambari, both in the state of Minas Gerais, Brazil, in 2009. Race 65 was most prevalent (62.16%) in both counties and was identified in 82.14% of the isolates collected in Lavras. Although the high prevalence of race 65 in Brazil has been confirmed in previous studies (15,37), the infected material was collected from a single host (BRS Majestoso cultivar) in Lavras (Supplementary Table 1). This fact may have contributed to the high prevalence of the race in our report.

Five other races of *C. lindemuthianum* were identified in this study: race 81 (18.92%), race 73 (9.46%), race 66 (2.70%), race 64 (8.11%), and race 1 (2.70%) (Table 1). Race 66 has not been previously reported in Minas Gerais (33,37). Despite its low prevalence, this race must not be ignored, because an emergence of new virulence alleles may promote the breakdown of genetic resistance in the improved cultivars that are currently used. Therefore, monitoring races in producing areas of the common bean is important for plant breeding programs because this monitoring allows for the identification of newly emerging pathogenic races and new sources of resistance.

In addition to genetic resistance, the use of fungicide is a strategy often employed to control pathogens. Chemical control of anthracnose is achieved with the fungicide Cercobin 700, which has thiophanate-methyl as its active ingredient (1). The effect of this fungicide in reducing in vitro mycelial growth of *C. lindemuthianum* isolates was evaluated. The results showed that 9.5% of the isolates studied were resistant (Table 1). In Brazil, cultivation of common bean occurs three seasons a year, meaning that inoculum is constantly present in the field. Therefore, the fungus is exposed to the fungicide continuously, thus providing opportunity for the selection of resistant individuals in the pathogen population. In addition, thiophanate-methyl is a systemic fungicide that has a specific mode of action at a site of the pathogen's metabolism, facilitating the emergence of resistant individuals in the population (4). The results suggest that studies are needed to determine the best strategies for the efficient use of fungicides in addition to genetic resistance.

Although all morphological and physiological characteristics evaluated in this study showed high variability in *C. lindemuthianum*, no grouping was observed in relation to collection local or pathogenic race. In analyzing race 65 variability, the most prevalent race in the study, we observed variation for all traits. Physiological traits showed significant differences ($P < 0.05$) in mean diameter and IMGR between *C. lindemuthianum* isolates. IMGR values for all isolates were >1.70 cm/day, whereas isolates evaluated by Souza et al. (34) grew <1.11 cm/day. The differences in IMGR values found by these authors may be related to genetic factors and also the number of subcultures and the storage times prior to evaluation of the isolates. In this study, we used fresh isolates, which were evaluated a few weeks after collection of the infected samples in the field.

TABLE 1. Race classification, percentage of colony growth inhibition by fungicide thiophanate methyl, and percentage of compatible sexual crosses for 74 *Colletotrichum lindemuthianum* isolates

Isolate	Race	Inhibition (%) ^y	Compatible crosses (%) ^z
LV 121	64	100.00 S	nc
LV 122	64	7.63 R	nc
LV 130	81	4.29 R	nc
LV 131	65	100.00 S	nc
LV 132	81	86.60 S	nc
LV 134	65	100.00 S	1.37
LV 135	81	75.44 S	1.37
LV 136	65	52.78 I	1.37
LV 137	81	15.56 I	1.37
LV 138	64	88.19 S	6.85
LV 139	65	64.43 S	5.48
LV 140	65	25.00 I	nc
LV 141	66	78.13 S	10.96
LV 142	81	9.65 R	nc
LV 143	64	100.00 S	nc
LV 144	1	5.26 R	2.74
LV 145	65	82.15 S	4.11
LV 146	64	11.97 I	2.74
LV 147	65	6.90 R	1.37
LV 148	65	12.28 I	1.37
LV 149	81	20.37 I	1.37
LV 152	64	12.50 I	1.37
LV 153	81	26.47 I	1.37
LV 154	1	70.76 S	1.37
LV 155	81	100.00 S	nc
LV 156	73	23.00 I	nc
LV 157	73	10.78 I	1.37
LV 158	73	81.04 S	1.37
LV 159	73	71.31 S	nc
LV 160	65	84.69 S	nc
LV 161	65	80.77 S	5.48
LV 162	65	72.22 S	2.74
LV 163	65	51.39 I	nc
LV 164	65	66.96 S	nc
LV 165	81	13.79 I	2.74
LV 166	65	100.00 S	nc
LV 167	65	76.67 S	nc
LV 168	65	76.36 S	1.37
LV 169	65	85.92 S	2.74
LV 170	81	70.42 S	nc
LV 171	66	100.00 S	1.37
LV 172	65	81.14 S	nc
LV 173	65	100.00 S	nc
LV 174	65	91.83 S	nc
LV 175	65	69.79 S	5.48
LV 176	65	78.00 S	1.37
LV 177	65	86.57 S	1.37
LV 178	65	100.00 S	1.37
LV 179	65	80.21 S	1.37
LV 180	65	77.28 S	nc
LV 181	65	76.32 S	nc
LV 182	81	5.88 R	1.37
LV 183	73	100.00 S	1.37
LV 184	65	56.86 I	1.37
LV 185	65	91.93 S	2.74
LV 186	65	21.24 I	2.74
LV 187	81	89.07 S	nc
LV 188	65	60.16 S	nc
LV 189	65	92.56 S	nc
LV 190	65	89.58 S	nc
LV 191	65	100.00 S	nc
LV 192	65	85.46 S	5.48
LV 193	65	61.36 S	2.74
LV 194	65	100.00 S	4.11
LV 195	65	100.00 S	12.33
LV 196	81	91.00 S	2.74
LV 197	65	100.00 S	nc
LV 198	65	94.35 S	6.85
LV 199	65	100.00 S	6.85
LV 200	65	21.16 I	nc
LV 201	81	2.68 R	5.48
LV 202	73	76.92 S	nc
LV 203	65	100.00 S	1.37
LV 204	73	31.45 I	1.37

^y Inhibition by fungicide. Resistance to thiophanate-methyl: S = sensitive, R = resistant, and I = intermediate.

^z Abbreviation: nc = compatible sexual crosses were not observed for this isolate.

Physiological traits can also help directly classify isolates that have greater capacity for spread in the field and that can easily establish themselves on host surfaces. The rate of sporulation of a pathogen, for example, influences the determination of their capacity for dispersal, affecting the spatial distribution of the species (21). Furthermore, *C. lindemuthianum* has been reported to have limited dispersal capacity, which occurs preferentially between different parts of the plant and occasionally between adjacent plants (35). Therefore, studying the capacities for sporulation of different *C. lindemuthianum* isolates may assist in inferring which isolates have greater chances for spread in the field and high spore production, such as isolate LV 122. Initial establishment and development of plant pathogens in their hosts requires germination to hyphal form and, subsequently, production of infective structures (40). In this study, high levels of diversity were detected when we analyzed the germination of conidia from different strains, including between the two evaluated periods (24 and 48 h). Knowledge of the process of spore germination is important for attaining a broader understanding of the events before and after host infection by a pathogen (14).

Moreover, the analysis of morphological traits is useful to verify species variability, in genetic analysis (e.g., when isolates identified differed in a given trait), and in the differentiation of *Colletotrichum* spp. (18,34). Cytological analysis in this study measured the lengths and widths of ungerminated conidia and the presence of septa in germinated conidia. The ranges of variation in conidial length (14.46 to 18.98 μm) and width (4.09 to 6.19

μm) are in agreement with those reported for the species (27,29). However, the presence of septa in germinated conidia contradicts what has been reported in the literature (18). In that study, the authors state that *C. lindemuthianum* spores remain nonseptate after germination. On the basis of our results, this species' germinated conidia may be inferred to have varying percentages of septa formation; in our analyses, isolates exhibited percentages of conidia with septa of 6 to 89% (Fig. 2). This contradiction may be attributed to the fact that O'Connell et al. (18) evaluated only two *C. lindemuthianum* isolates, which did not cover the entire variability for this trait. Therefore, our data suggests that this trait, the absence of septa in germinated conidia, cannot be used for species classification, as has been previously proposed (18). *C. lindemuthianum* isolates may or may not produce septate conidia after germination when using a large population. PCR reactions using primers specific for the *C. lindemuthianum* MAT1-2-1 HMG domain (10) confirmed that all strains isolated were from this species.

Some of the mechanisms studied that could help the generation of the observed variability are sexual recombination (6,35), the parasexual cycle (7), and asexual recombination via CAT fusion (16,28). For sexual recombination, only 1.78% of the combinations evidenced sexual compatibility. However, these pairings did not produce asci or ascospores. Studies of the sexual cycle of the *Colletotrichum* genus indicate that genetic control of reproduction in this pathogen is not equivalent to a simple mating system; more than one locus in the genome is likely involved in the control (2,39). The production of protoperithecia between

TABLE 2. Mean values and standard errors for indexes of mycelia growth rate (IMGR), colony diameter, and sporulation rate from 74 *Colletotrichum lindemuthianum* isolates investigated

Isolate	IMGR (cm/day) ^z	Colony diameter (cm) ^z	Sporulation rate ($\times 10^6$ conidia/ml) ^z	Isolate	IMGR (cm/day) ^z	Colony diameter (cm) ^z	Sporulation rate ($\times 10^6$ conidia/ml) ^z
LV 121	2.00 $\pm$ 0.62 a	3.50 $\pm$ 9.42 a	16.33 $\pm$ 8.07 c	LV 168	3.35 $\pm$ 0.46 c	5.53 $\pm$ 6.95 c	12.52 $\pm$ 4.01 c
LV 122	3.55 $\pm$ 0.53 c	5.83 $\pm$ 7.93 c	18.87 $\pm$ 9.74 c	LV 169	4.40 $\pm$ 0.25 d	7.13 $\pm$ 4.11 d	5.23 $\pm$ 3.75 b
LV 130	4.33 $\pm$ 0.24 d	7.00 $\pm$ 3.56 d	10.43 $\pm$ 3.30 b	LV 170	4.40 $\pm$ 0.30 d	7.10 $\pm$ 4.55 d	3.57 $\pm$ 1.17 a
LV 131	4.03 $\pm$ 0.61 d	6.65 $\pm$ 9.15 d	1.28 $\pm$ 0.98 a	LV 171	3.78 $\pm$ 0.23 c	6.20 $\pm$ 3.83 c	7.45 $\pm$ 3.28 b
LV 132	3.38 $\pm$ 0.62 c	5.58 $\pm$ 9.32 c	1.93 $\pm$ 0.53 a	LV 172	3.18 $\pm$ 0.41 b	5.28 $\pm$ 6.13 b	1.20 $\pm$ 0.45 a
LV 134	3.27 $\pm$ 0.27 b	5.40 $\pm$ 4.08 b	1.48 $\pm$ 0.41 a	LV 173	4.30 $\pm$ 0.30 d	6.95 $\pm$ 4.51 d	1.10 $\pm$ 1.09 a
LV 135	3.45 $\pm$ 0.29 c	5.68 $\pm$ 4.43 c	1.57 $\pm$ 0.44 a	LV 174	2.95 $\pm$ 0.87 b	4.93 $\pm$ 13.1 b	1.22 $\pm$ 1.11 a
LV 136	4.43 $\pm$ 0.43 d	7.15 $\pm$ 6.45 d	3.07 $\pm$ 1.91 a	LV 175	2.87 $\pm$ 0.53 b	4.80 $\pm$ 8.04 b	1.77 $\pm$ 0.26 a
LV 137	2.65 $\pm$ 0.29 b	4.48 $\pm$ 4.27 b	12.23 $\pm$ 6.98 c	LV 176	2.97 $\pm$ 0.47 b	4.95 $\pm$ 7.05 b	2.18 $\pm$ 1.31 a
LV 138	4.63 $\pm$ 0.35 d	7.20 $\pm$ 9.09 d	0.98 $\pm$ 0.20 a	LV 177	4.12 $\pm$ 0.55 d	6.68 $\pm$ 8.26 d	2.52 $\pm$ 0.83 a
LV 139	3.07 $\pm$ 0.66 b	5.15 $\pm$ 9.29 b	1.90 $\pm$ 1.50 a	LV 178	3.35 $\pm$ 0.50 c	5.53 $\pm$ 7.50 c	1.10 $\pm$ 0.26 a
LV 140	2.75 $\pm$ 0.27 b	4.63 $\pm$ 4.03 b	13.25 $\pm$ 3.49 c	LV 179	2.85 $\pm$ 0.31 b	4.78 $\pm$ 4.57 b	1.40 $\pm$ 0.83 a
LV 141	3.90 $\pm$ 0.23 d	6.35 $\pm$ 3.42 d	7.72 $\pm$ 5.09 b	LV 180	4.07 $\pm$ 0.66 d	6.60 $\pm$ 9.83 d	2.77 $\pm$ 2.14 a
LV 142	3.43 $\pm$ 0.65 c	5.65 $\pm$ 9.75 c	9.90 $\pm$ 2.98 b	LV 181	2.17 $\pm$ 0.29 a	3.75 $\pm$ 4.36 a	2.70 $\pm$ 1.28 a
LV 143	3.90 $\pm$ 0.18 d	6.35 $\pm$ 2.65 d	4.05 $\pm$ 2.77 b	LV 182	4.17 $\pm$ 0.24 d	6.75 $\pm$ 3.70 d	8.18 $\pm$ 1.95 b
LV 144	2.22 $\pm$ 0.24 a	3.83 $\pm$ 3.59 a	13.07 $\pm$ 3.10 c	LV 183	2.38 $\pm$ 0.38 a	4.08 $\pm$ 5.68 a	3.90 $\pm$ 1.34 a
LV 145	3.37 $\pm$ 0.74 c	5.56 $\pm$ 11.12 c	9.22 $\pm$ 4.96 b	LV 184	3.07 $\pm$ 0.47 b	5.10 $\pm$ 6.98 b	0.97 $\pm$ 1.11 a
LV 146	4.41 $\pm$ 0.31 d	7.13 $\pm$ 4.65 d	6.98 $\pm$ 2.14 b	LV 185	3.77 $\pm$ 0.53 c	6.15 $\pm$ 7.94 c	0.53 $\pm$ 0.18 a
LV 147	3.50 $\pm$ 0.20 c	5.75 $\pm$ 3.00 c	5.03 $\pm$ 1.43 b	LV 186	4.50 $\pm$ 0.27 d	7.25 $\pm$ 4.04 d	1.60 $\pm$ 1.19 a
LV 148	3.45 $\pm$ 0.73 c	5.68 $\pm$ 10.97 c	0.93 $\pm$ 1.06 a	LV 187	1.76 $\pm$ 0.37 a	3.15 $\pm$ 5.51 a	4.05 $\pm$ 2.37 b
LV 149	3.48 $\pm$ 0.39 c	5.35 $\pm$ 1.73 b	12.18 $\pm$ 6.18 c	LV 188	4.08 $\pm$ 0.37 d	6.63 $\pm$ 5.56 d	4.65 $\pm$ 2.36 b
LV 152	2.83 $\pm$ 0.31 b	4.75 $\pm$ 4.65 b	10.15 $\pm$ 2.90 b	LV 189	2.77 $\pm$ 0.42 b	4.65 $\pm$ 6.35 b	6.83 $\pm$ 3.31 b
LV 153	3.03 $\pm$ 0.25 b	5.08 $\pm$ 3.77 b	9.00 $\pm$ 5.81 b	LV 190	2.83 $\pm$ 0.45 b	4.75 $\pm$ 11.21 b	1.23 $\pm$ 0.73 a
LV 154	3.18 $\pm$ 0.48 b	5.28 $\pm$ 7.18 b	0.65 $\pm$ 0.28 a	LV 191	4.32 $\pm$ 0.39 d	6.98 $\pm$ 5.91 d	2.13 $\pm$ 1.37 a
LV 155	2.41 $\pm$ 0.27 a	4.13 $\pm$ 4.03 a	0.13 $\pm$ 0.08 a	LV 192	3.35 $\pm$ 0.75 c	5.53 $\pm$ 11.24 c	10.92 $\pm$ 7.30 b
LV 156	2.95 $\pm$ 0.55 b	4.93 $\pm$ 8.22 b	9.98 $\pm$ 3.06 b	LV 193	2.59 $\pm$ 0.85 b	4.38 $\pm$ 12.71 b	0.47 $\pm$ 0.25 a
LV 157	3.06 $\pm$ 0.32 b	5.10 $\pm$ 4.76 b	11.12 $\pm$ 6.44 b	LV 194	3.03 $\pm$ 0.39 b	5.05 $\pm$ 5.80 b	3.27 $\pm$ 2.97 a
LV 158	3.53 $\pm$ 0.40 c	5.80 $\pm$ 5.94 c	2.08 $\pm$ 1.40 a	LV 195	3.53 $\pm$ 0.63 c	5.80 $\pm$ 9.49 c	1.25 $\pm$ 0.62 a
LV 159	3.73 $\pm$ 0.16 c	6.10 $\pm$ 2.45 c	3.02 $\pm$ 3.67 a	LV 196	2.95 $\pm$ 0.25 b	4.93 $\pm$ 3.77 b	3.92 $\pm$ 3.99 a
LV 160	2.90 $\pm$ 0.34 b	4.85 $\pm$ 5.07 b	0.68 $\pm$ 0.42 a	LV 197	2.60 $\pm$ 0.36 b	4.40 $\pm$ 5.35 b	2.70 $\pm$ 1.69 a
LV 161	3.10 $\pm$ 0.42 b	5.15 $\pm$ 6.24 b	3.83 $\pm$ 0.80 b	LV 198	3.77 $\pm$ 0.25 c	6.15 $\pm$ 3.70 c	9.53 $\pm$ 5.00 b
LV 162	3.23 $\pm$ 0.24 b	5.35 $\pm$ 3.70 b	1.47 $\pm$ 0.85 a	LV 199	4.15 $\pm$ 0.26 d	6.75 $\pm$ 4.12 d	7.08 $\pm$ 2.92 b
LV 163	2.03 $\pm$ 0.28 a	3.55 $\pm$ 4.20 a	4.43 $\pm$ 2.54 b	LV 200	3.12 $\pm$ 0.43 b	5.18 $\pm$ 6.40 b	15.37 $\pm$ 3.82 c
LV 164	3.37 $\pm$ 0.52 c	5.55 $\pm$ 7.85 c	1.57 $\pm$ 0.94 a	LV 201	3.37 $\pm$ 0.40 c	5.55 $\pm$ 5.92 c	6.80 $\pm$ 2.95 b
LV 165	3.52 $\pm$ 0.23 c	5.78 $\pm$ 3.50 c	9.70 $\pm$ 1.47 b	LV 202	3.12 $\pm$ 0.31 b	5.18 $\pm$ 4.72 c	3.28 $\pm$ 2.61 a
LV 166	3.37 $\pm$ 0.27 c	5.55 $\pm$ 4.04 c	2.45 $\pm$ 1.42 a	LV 203	3.60 $\pm$ 0.52 c	5.90 $\pm$ 7.79 c	1.90 $\pm$ 1.17 a
LV 167	2.68 $\pm$ 0.36 b	4.53 $\pm$ 5.38 b	2.08 $\pm$ 2.18 a	LV 204	3.82 $\pm$ 0.69 c	6.23 $\pm$ 10.34 c	10.18 $\pm$ 1.55 b

^z Mean values in columns followed by the same letter are not significantly different, according to the Scott-Knott test ($P = 0.05$).

compatible isolates indicates that genetic information to complete the sexual cycle is not available in these individuals, although the initial phase of reproduction has been demonstrated. Another hypothesis that could explain these results is the absence of any environmental stimuli, such as nutrients, temperature, or lighting, to induce the pathogen's complete sexual cycle.

The fusion of vegetative hyphae to form heterokaryons is a prerequisite for the occurrence of the parasexual cycle (8,11). Our results indicate that mycelium compatibility was not observed between tested isolates, indicating that the isolates belong to different vegetative compatibility groups (VCGs). The occurrence of a large number of VCGs in *C. lindemuthianum* has been de-

Fig. 2. Percentage of conidia that presents septa formation after germination for 74 isolates of *Colletotrichum lindemuthianum* evaluated after 24 h of incubation in water and stained with Calcofluor White (0.12 M). Bars represent standard deviation from mean values of two replicates.

TABLE 3. Mean values and standard errors for conidial germination, conidial anastomosis tube (CATs) fusion, and conidia measurements from 74 *Colletotrichum lindemuthianum* isolates investigated

Isolate	Germination (%) ^y		CATs (%) ^{y,z}	Conidia measurements ^y	
	24 h	48 h		Width	Length
LV 121	14.0 ± 4.24 a	50.5 ± 10.61 d	59.75 ± 15.20 e	5.32 ± 0.58 c	17.12 ± 1.39 c
LV 122	1.5 ± 0.71 a	14.5 ± 3.54 a	1.30 ± 1.06 a	5.09 ± 0.61 b	18.04 ± 1.68 d
LV 130	4.0 ± 1.41 a	30.5 ± 4.95 b	3.00 ± 1.41 a	5.49 ± 0.64 c	17.73 ± 1.71 d
LV 131	48.5 ± 4.95 d	79.0 ± 4.24 f	45.75 ± 13.08 d	5.06 ± 0.68 b	16.78 ± 2.54 b
LV 132	64.0 ± 1.41 e	88.5 ± 7.78 g	6.75 ± 5.30 a	4.98 ± 0.80 b	16.94 ± 1.82 b
LV 134	34.5 ± 4.95 c	75.0 ± 2.83 f	16.75 ± 13.08 a	4.78 ± 0.81 b	15.24 ± 2.87 a
LV 135	37.5 ± 9.19 c	76.0 ± 2.83 f	14.25 ± 8.13 a	5.45 ± 0.74 c	17.87 ± 1.82 d
LV 136	37.0 ± 19.7 c	66.5 ± 20.5 e	7.75 ± 0.35 a	5.04 ± 0.67 b	14.46 ± 2.88 a
LV 137	6.0 ± 1.41 a	22.0 ± 4.24 a	2.75 ± 2.47 a	5.24 ± 0.71 c	18.26 ± 1.91 d
LV 138	22.5 ± 2.12 b	81.0 ± 2.83 f	10.25 ± 7.42 a	5.27 ± 0.58 c	16.20 ± 2.52 b
LV 139	4.5 ± 0.71 a	54.0 ± 7.07 d	9.25 ± 7.42 a	5.39 ± 0.76 c	17.11 ± 1.71 c
LV 140	13.5 ± 6.36 a	42.0 ± 5.66 c	1.50 ± 0.71 a	5.21 ± 0.60 c	18.40 ± 1.73 d
LV 141	2.5 ± 0.71 a	26.0 ± 8.49 b	5.50 ± 5.66 a	5.20 ± 0.61 c	18.53 ± 2.01 d
LV 142	5.5 ± 0.71 a	15.0 ± 4.24 a	3.50 ± 2.12 a	5.07 ± 0.76 b	18.05 ± 2.31 d
LV 143	1.0 ± 1.41 a	29.5 ± 2.12 b	2.25 ± 1.77 a	4.59 ± 0.40 a	18.78 ± 1.99 d
LV 144	55.5 ± 9.19 e	76.0 ± 4.24 f	6.50 ± 1.41 a	5.44 ± 0.48 c	16.44 ± 2.64 b
LV 145	2.0 ± 1.41 a	37.5 ± 2.12 c	5.75 ± 6.72 a	4.94 ± 0.50 b	17.43 ± 2.15 c
LV 146	29.5 ± 13.33 b	78.0 ± 16.97 f	24.25 ± 10.25 b	5.14 ± 0.54 b	16.51 ± 1.89 b
LV 147	58.0 ± 5.66 e	86.0 ± 4.24 g	7.50 ± 1.41 a	5.47 ± 0.71 c	17.73 ± 2.32 d
LV 148	25.5 ± 7.78 b	94.5 ± 2.92 g	9.50 ± 4.96 a	4.88 ± 0.70 b	16.48 ± 1.70 b
LV 149	50.0 ± 2.83 d	81.5 ± 0.71 f	8.00 ± 4.96 a	4.50 ± 0.59 a	16.31 ± 1.74 b
LV 152	64.0 ± 11.31 e	89.5 ± 9.19 g	9.00 ± 0.94 a	5.84 ± 0.85 c	17.25 ± 1.64 c
LV 153	44.0 ± 5.66 d	75.5 ± 3.54 f	2.50 ± 2.12 a	5.13 ± 0.59 b	16.51 ± 1.70 b
LV 154	58.5 ± 10.61 e	86.5 ± 4.95 g	11.00 ± 0.71 a	5.71 ± 1.05 c	17.04 ± 1.99 c
LV 155	25.5 ± 6.36 b	40.5 ± 2.32 c	0.75 ± 0.35 a	5.41 ± 0.47 c	17.39 ± 1.52 c
LV 156	47.5 ± 4.95 d	77.0 ± 1.44 f	2.00 ± 0.00 a	5.09 ± 0.42 b	17.78 ± 1.56 d
LV 157	47.5 ± 2.12 d	83.0 ± 5.66 g	nf	5.01 ± 0.46 b	18.41 ± 1.89 d
LV 158	67.0 ± 4.24 e	89.5 ± 4.95 g	4.50 ± 2.12 a	4.09 ± 0.58 a	17.34 ± 1.57 c
LV 159	43.5 ± 3.54 d	85.0 ± 5.66 g	1.50 ± 0.71 a	6.19 ± 2.17 c	16.34 ± 2.21 b
LV 160	61.5 ± 2.12 e	90.5 ± 6.12 g	2.30 ± 1.77 a	5.56 ± 0.78 c	16.63 ± 1.68 b
LV 161	69.0 ± 8.49 e	86.5 ± 12.02 g	6.00 ± 4.24 a	4.64 ± 0.55 a	15.56 ± 1.62 a
LV 162	77.5 ± 0.71 f	94.5 ± 5.00 g	3.75 ± 1.77 a	5.25 ± 0.81 c	17.72 ± 2.27 d
LV 163	59.0 ± 5.66 e	85.5 ± 6.36 g	2.00 ± 1.41 a	5.40 ± 0.68 c	18.31 ± 2.54 d
LV 164	72.0 ± 11.31 f	92.0 ± 5.96 g	4.75 ± 3.89 a	5.32 ± 0.92 c	17.00 ± 2.52 c
LV 165	61.5 ± 7.36 e	86.5 ± 4.95 g	5.75 ± 3.89 a	5.12 ± 0.41 b	18.02 ± 1.69 d

(Continued on following page)

^y Mean values in columns followed by the same letter are not significantly different, according to the Scott-Knott test ($P = 0.05$).

^z Abbreviation: nf = CATs fusion were not observed for this isolate.

TABLE 3. (continued from preceding page)

Isolate	Germination (%) ^y		CATs (%) ^{y,z}	Conidia measurements ^y	
	24 h	48 h		Width	Length
LV 166	11.0 ± 1.41 a	47.0 ± 19.80 d	8.50 ± 7.78 a	5.48 ± 0.59 c	17.12 ± 2.05 c
LV 167	4.0 ± 0.00 a	82.5 ± 2.03 g	1.50 ± 0.71 a	5.38 ± 0.61 c	16.14 ± 1.99 b
LV 168	30.0 ± 11.31 b	46.5 ± 9.19 d	2.75 ± 2.47 a	5.27 ± 0.72 c	17.23 ± 2.18 c
LV 169	8.0 ± 7.07 a	66.5 ± 6.36 e	8.50 ± 3.53 a	5.12 ± 0.69 b	17.64 ± 2.17 c
LV 170	66.5 ± 14.85 e	88.0 ± 9.90 g	6.00 ± 1.41 a	5.49 ± 0.51 c	17.08 ± 1.92 c
LV 171	25.5 ± 3.36 b	53.0 ± 8.49 d	1.50 ± 1.89 a	5.30 ± 0.57 c	16.80 ± 2.59 b
LV 172	4.0 ± 1.41 a	94.5 ± 0.71 g	2.00 ± 0.71 a	4.99 ± 0.87 b	15.84 ± 1.95 a
LV 173	47.5 ± 11.92 d	85.5 ± 12.02 g	6.50 ± 5.66 a	5.20 ± 0.64 c	16.85 ± 2.03 b
LV 174	41.0 ± 1.41 c	94.0 ± 1.36 g	22.00 ± 13.43 b	5.23 ± 0.70 c	17.63 ± 2.25 c
LV 175	28.5 ± 3.54 b	53.5 ± 7.78 d	2.00 ± 1.41 a	5.31 ± 0.91 c	17.09 ± 2.24 c
LV 176	25.5 ± 2.12 b	80.0 ± 4.24 f	1.50 ± 1.82 a	5.25 ± 0.57 c	16.56 ± 1.72 b
LV 177	44.0 ± 8.49 d	90.5 ± 4.97 g	8.25 ± 5.30 a	5.21 ± 0.68 c	17.44 ± 2.18 c
LV 178	40.0 ± 4.24 c	84.0 ± 5.68 g	nf	5.24 ± 0.59 c	16.79 ± 2.06 b
LV 179	61.0 ± 18.38 e	83.0 ± 11.31 g	3.00 ± 1.82 a	5.13 ± 0.84 b	17.99 ± 2.34 d
LV 180	45.0 ± 4.24 d	77.5 ± 4.95 f	1.50 ± 0.71 a	5.11 ± 0.74 b	17.40 ± 1.38 c
LV 181	37.0 ± 1.41 c	85.0 ± 2.83 g	1.75 ± 1.14 a	4.94 ± 0.78 b	16.22 ± 2.05 b
LV 182	35.0 ± 8.49 c	58.5 ± 10.61 d	1.50 ± 1.41 a	4.96 ± 0.63 b	17.77 ± 1.13 d
LV 183	43.0 ± 9.90 d	80.0 ± 4.24 f	2.25 ± 1.77 a	5.45 ± 0.81 c	16.74 ± 2.14 b
LV 184	42.5 ± 17.48 d	77.0 ± 16.87 f	nf	5.04 ± 0.51 b	16.28 ± 2.42 b
LV 185	53.0 ± 8.49 d	89.0 ± 6.18 g	12.80 ± 8.13 a	5.39 ± 0.90 c	15.19 ± 2.15 a
LV 186	6.0 ± 4.24 a	59.0 ± 5.36 d	3.00 ± 2.83 a	5.31 ± 1.05 c	17.11 ± 1.83 c
LV 187	4.0 ± 2.83 a	26.0 ± 9.90 a	2.25 ± 1.06 a	4.91 ± 0.39 b	16.78 ± 1.93 b
LV 188	7.0 ± 2.83 a	42.0 ± 5.65 c	1.75 ± 1.06 a	5.08 ± 0.50 b	15.64 ± 2.45 a
LV 189	22.5 ± 10.6 b	47.5 ± 19.09 d	4.50 ± 3.53 a	4.95 ± 0.34 b	16.68 ± 1.14 b
LV 190	88.5 ± 12.02 g	94.5 ± 4.76 g	27.20 ± 5.30 b	5.06 ± 0.62 b	18.25 ± 1.84 d
LV 191	16.0 ± 8.49 a	42.0 ± 2.97 c	4.00 ± 3.53 a	5.10 ± 0.55 b	16.17 ± 1.75 b
LV 192	8.0 ± 4.24 a	30.5 ± 2.27 b	11.75 ± 3.18 a	5.19 ± 0.65 c	17.01 ± 2.51 c
LV 193	63.0 ± 16.97 e	90.0 ± 9.85 g	1.55 ± 6.36 a	5.05 ± 0.57 b	15.59 ± 1.78 a
LV 194	25.5 ± 3.54 b	54.5 ± 9.19 d	5.75 ± 2.47 a	5.05 ± 0.96 b	17.08 ± 2.79 c
LV 195	26.5 ± 3.54 b	50.5 ± 2.49 d	51.50 ± 0.94 d	4.44 ± 0.52 a	15.93 ± 2.11 a
LV 196	72.5 ± 3.54 f	94.0 ± 2.83 g	3.50 ± 0.71 a	5.17 ± 0.55 b	16.66 ± 2.05 b
LV 197	8.5 ± 6.36 a	38.5 ± 12.02 c	1.75 ± 1.06 a	4.76 ± 0.53 b	17.84 ± 1.59 d
LV 198	71.5 ± 4.95 e	91.5 ± 6.36 g	36.25 ± 13.08 c	5.16 ± 0.52 b	15.56 ± 2.07 a
LV 199	1.5 ± 2.12 a	26.0 ± 4.24 b	2.00 ± 0.71 a	5.76 ± 0.94 c	18.08 ± 1.35 d
LV 200	4.0 ± 1.41 a	33.0 ± 2.98 b	4.00 ± 4.24 a	4.86 ± 0.59 b	16.81 ± 2.40 b
LV 201	4.0 ± 1.41 a	28.5 ± 3.54 b	nf	5.01 ± 0.58 b	16.87 ± 2.28 b
LV 202	27.5 ± 3.54 b	65.5 ± 6.78 e	3.25 ± 1.77 a	5.06 ± 0.76 b	16.86 ± 2.10 b
LV 203	10.5 ± 3.56 a	35.5 ± 10.6 b	3.58 ± 0.94 a	5.39 ± 0.61 c	17.43 ± 1.66 c
LV 204	37.0 ± 5.66 c	73.0 ± 8.49 f	24.00 ± 5.66 b	5.37 ± 0.52 c	16.76 ± 1.71 b

scribed. Barcelos et al. (3) observed the formation of 45 VCGs: 44 isolates were incompatible and only 1 combination successfully formed heterokaryons, demonstrating the wide species variability for this trait. In addition, vegetative incompatibility is common in natural populations of filamentous fungi and could limit the occurrence of the parasexual cycle in nature (8).

An alternative method of asexual recombination between incompatible isolates, CAT fusion, has been suggested by Ishikawa (13). In this study, we observed that CAT fusion is common for most of the isolates (94.6%) analyzed. Whereas CATs have an important role in the pathogen's population dynamics, the high frequency may significantly influence the variability of pathogen populations. Previous work has shown that CAT fusion occurs only in water and that it is dependent on the culture age (e.g., it occurs more often in older colonies) and strain used (e.g., the highest percentage of CAT fusion was 25.8% for race 65 isolate LV 115) (16). One advantage for using large populations for characterization studies is the possibility of finding extreme phenotypes. In this study, five isolates had CAT percentages that were higher than previously reported (>25%) (Table 3). LV 121 showed a higher percentage of CAT fusion (≈60%), close to what is found for *Neurospora crassa* (30). These isolates could be used in future studies to elucidate the role of CATs in genetic variation, pathogenicity, and population dynamics. Furthermore, all strains that present CAT extreme values belongs to race 65, except isolate LV121 (race 64); and, interestingly, all the strains showed high susceptibility to commercial fungicide. A high percentage of CATs can be a possible strategy of these isolates to change genetic

information to seek resistance to one of the fungicides most used in the field for anthracnose.

Our main findings were as follows: (i) genetic and morphological variations occurred between race 65 isolates; (ii) *C. lindemuthianum* isolates demonstrated wide variations in morphological and physiological analyses and could not be grouped by origin or race; (iii) resistance to the fungicide thiophanate-methyl is strain dependent for *C. lindemuthianum*; (iv) CAT fusion occurred at higher frequencies in these populations, specially isolate LV 121 (60%); and (v) *C. lindemuthianum* conidia can form septa after germination, which is strain dependent. The information gathered in this study can be applied in future studies: basic knowledge (e.g., cell biology and genetics of pathogenic fungi), applied areas in phytopathology (e.g., management and control of disease), and plant breeding for resistance to *C. lindemuthianum* races are of great importance in the region.

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