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Most *Colletotrichum* species associated with tree tomato (*Solanum betaceum*) and mango (*Mangifera indica*) crops are not host-specific

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An important constraint for crop production in Colombia is the high incidence of anthracnose caused by *Colletotrichum* species. Although several studies have focused on these fungi, the relationship between the different fungal species within the genus and their hosts and whether they display any host preference or host specificity has yet to be examined. In Colombia, diseases caused by *Colletotrichum* species are particularly severe in mango (*Mangifera indica*) and tree tomato (*Solanum betaceum*) crops. In a previous investigation, the *Colletotrichum* phylogenetic species attacking these crops were identified. The present study aimed to determine whether isolates collected from tree tomato and mango showed host preference or host specificity by assessing aggressiveness, spore density, latent period, and fitness of each strain on the two hosts. In the departments of Cundinamarca and Tolima, Colombia, isolates were collected from plants that presented typical anthracnose symptoms and were identified as *C. acutatum*, *C. asianum*, *C. boninense*, *C. gloeosporioides*, *C. tamarilloi* and *C. theobromicola*. Inoculation of conidia of each isolate onto both hosts showed isolates had no host preference and only the *C. gloeosporioides* isolate showed host specificity. However, in general, isolates produced a higher spore density when inoculated on the alternate host, which may indicate a difference in the degree of adaptation to each host. Statistical analyses of the assessed parameter values revealed that isolates use different infection strategies when infecting each host. In light of these results, the implications of using quantitative estimations of fitness when studying fungal pathogens are discussed.

Keywords: *Colletotrichum*, host specificity, host–pathogen interactions, pathogenicity

Introduction

Colletotrichum species are the major causal agents of anthracnose diseases worldwide (Cannon *et al.*, 2012) and several crops of economic importance are susceptible to these pathogens (Bridge *et al.*, 2008; Prihastuti *et al.*, 2009; Nodet *et al.*, 2016). In Colombia, some of the main crops affected by this pathogen are mango (*Mangifera indica*) and tree tomato (*Solanum betaceum*; Pardo-De la Hoz *et al.*, 2016). *Colletotrichum* spp. are pre- and postharvest pathogens and thus cause high economic losses that may reach up to 50% of the total production (Ali *et al.*, 2016; Bhutia *et al.*, 2016; Peters *et al.*, 2016). Significant research efforts have focused on understanding the physiology, host–pathogen interactions, and evolution of these plant pathogenic species in order to generate accurate control strategies (Cai *et al.*, 2009; Soltani *et al.*, 2014; Rahman *et al.*, 2015).

Studying host–pathogen interactions is key to understanding the ecological aspects of a disease. For example, host specialization can be a powerful driver of divergent selection, promoting ecological speciation via host shift or host jump, depending on whether the involved hosts are genetically similar or distant (Silva *et al.*, 2012). To conduct an appropriate study of host specialization, it is important to first determine the taxonomic identity of the strains under study. In this way, the results will not be affected by presuming two different species as one. Taxonomic studies in the genus *Colletotrichum* have focused primarily on species identification and characterization of subpopulations within the species (Riccardo *et al.*, 2016; Sato, 2016; Wiesner-Hanks & Nelson, 2016). The traditional identification and characterization of *Colletotrichum* species have been mainly based on morphological differences. However, morphological criteria are not always adequate for the reliable identification of species of *Colletotrichum* because of several factors such as the environment, which influences the stability of the morphological

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features and intermediate forms coexisting in nature (Freeman *et al.*, 2000; Afanador-Kafuri *et al.*, 2003; Cai *et al.*, 2009).

Many of the current accepted *Colletotrichum* species are named after their host (Hyde *et al.*, 2009; Cannon *et al.*, 2012) and, as noted by Cai *et al.* (2009), in several cases this is an assumption of host specificity. Moreover, these fungi have been used as models to describe some of the narrowest cases of race-cultivar specificity (Barrus, 1911). However, Freeman *et al.* (1998) summarized two opposite scenarios regarding host range in *Colletotrichum* infections: (i) a single host infected by multiple species and (ii) multiple hosts infected by a single species. Several studies involving host range characterization of *Colletorichum* infections in different geographic locations concur with one of these scenarios.

In Colombia, a recent characterization of *Colletotrichum* species associated with tree tomato and mango crops used morphological, physiological, and multilocus phylogenetic approaches for accurate species identification (Pardo-De la Hoz *et al.*, 2016). The results indicated that the two scenarios mentioned above occur in these crops as (i) mango was infected by *C. asianum*, *C. gloeosporioides*, *C. siamense* and *C. theobromicola*, and tree tomato was affected by *C. kahawae*, *C. tamarilloi* and *C. siamense*, and (ii) *C. siamense* and an unidentified species belonging to the *C. gloeosporioides* species complex each infected both mango and tree tomato. However, in this study by Pardo-De la Hoz *et al.* (2016), the inoculations were performed on wounded tissue materials and thus pathogenicity of the isolates could not be evaluated. In the present study, the potential for cross infection of the isolates from the two hosts was tested to determine to what extent this set of isolates showed host specificity.

Materials and methods

Colletotrichum isolates

A total of 11 isolates of *Colletotrichum*, which had been molecularly characterized by Pardo-De la Hoz *et al.* (2016), were selected from the fungal collection of the Laboratory of Mycology and Plant Pathology at Universidad de los Andes. Seven isolates were from *M. indica* 'Tommy' and four isolates from *S. betaceum* 'Común', with at least one isolate for each of the species *C. acutatum*, *C. asianum*, *C. boninense*, *C. gloeosporioides*, *C. tamarilloi* and *C. theobromicola* (Table 1). These species were the most commonly found in either of the two hosts in a previous study (Pardo-De la Hoz *et al.*, 2016).

Lyophilized strains were recovered for 7 days at 25 °C on potato dextrose agar (PDA) plates and Sabouraud dextrose yeast (SDY) broth. Subsequently, they were cultivated on fresh PDA plates for 7 days at 25 °C to obtain conidia. These conidia were then transferred onto plates containing 1.5% water agar to establish monosporic cultures.

Plant material

The most susceptible varieties for each host, Tommy for mango and Común for tree tomato, were used to test the potential for cross infection. These varieties are also the most commonly grown in Colombia. Plants were grown in a mix of top soil and rice hull (1:1), and were maintained under controlled greenhouse conditions at Universidad Militar Nueva Granada, with a photoperiod of 12 h and a temperature of 24 °C day and 18 °C night.

Table 1 List of *Colletotrichum* isolates used and their ability to infect tree tomato and mango whole plants inoculated with conidia.

Species	Isolate	Original host	Tree tomato	Mango
C. asianum	CMB4	Mango	+	+
	CMA14	Mango	+	+
	CMC3	Mango	+	+
C. theobromicola	CMF1	Mango	+	+
	CME2	Mango	+	+
C. boninense	CMC2	Mango	+	+
C. gloeosporioides	CMB1	Mango	–	+
C. acutatum	CTG3	Tree tomato	+	+
C. tamarilloi	CTJ1	Tree tomato	+	+
	CTK12	Tree tomato	+	+
	CTJ2	Tree tomato	+	+

+, Strain produced infection and/or reproductive structures; –, strain unable to infect and/or produce reproductive structures.

Pathogenicity tests

All strains were used to perform the pathogenicity assays. Isolates were grown in PDA culture medium for 8 days at 25 °C. Petri dishes were then flooded with 2 mL sterile distilled water and conidia were dislodged using a bent glass rod. This suspension was then filtered twice through sterile gauze to eliminate mycelia and then adjusted to 10^6 conidia per mL using a haemocytometer (de Freitas *et al.*, 2015).

The pathogenicity of these isolates was determined both in whole-plant inoculation as well as in detached-leaf assays. Leaves for the detached-leaf assay were collected from plants between 8 and 19 weeks old. All leaves were placed in closed Petri dishes or individual plastic boxes, depending on the size of the leaf, containing a wet paper towel. The plant material was rinsed with sterile distilled water to remove soil before inoculating. Each leaf was inoculated with four 10 μL droplets of conidial suspension (10^6 conidia mL^{-1}) on the adaxial side of the leaf lamina. For negative controls, four 10 μL droplets of sterile distilled water were used. Inoculated detached leaves were kept under controlled conditions with 80% RH, 24 °C and 12 h photoperiods in the growth chamber. Whole plants were maintained in the greenhouse as described above.

For the whole-plant assay, three leaves per plant of three whole plants were inoculated with each isolate; for the detached-leaf assay three leaves from three different plants were inoculated with each isolate. All leaves were randomly selected and corresponded to fully expanded leaves. The whole experiment was repeated three times (three biological replicates). A total of 792 leaves (198 for the detached-leaf and 594 for the whole-plant assay) were inoculated in all experiments.

A total of four parameters were measured to assess the pathogenicity of the isolates: (i) latent period (LP), calculated as the interval between inoculation and the first appearance of necrotic symptoms; (ii) the total lesion area, assessed on each host using ImageJ software (Image Processing and Analysis in Java; http://rsb.info.nih.gov/ij/) from pictures of individual leaves

taken each day from 1 to 6 days after inoculation (dai) for the detached-leaf assay and each day from 1 to 24 dai for whole-plant inoculations; aggressiveness was calculated by dividing the total lesion area (cm^2) 24 dai by the total leaf area (cm^2); the lesion growth rate (LGR; cm^2 day^{-1}) was estimated by the rate of expansion of the lesion per day, calculated by dividing the total lesion size by the duration of the experiment minus the latent period; (iii) spore density (SD), determined as the number of conidia produced on leaves divided by the total leaf area (cm^2); the total number of conidia was assessed by washing inoculated leaves with 1000 μL of sterile distilled water on the last day of the experiment, 24 dai, and determining the number of conidia using a haemocytometer; spore density was calculated by subtracting the number of conidia produced from the initial number of conidia inoculated; (iv) pathogen fitness, calculated using a single composite index of pathogen fitness (F) according to Montarry *et al.* (2010) using the following equation:

$$F = \frac{1}{1 + LP \cdot \mu} \cdot \frac{SD \cdot LGR}{\frac{LGR}{X} + \mu}$$

where X is the mean leaf size on each host, LP is latent period, SD is spore density, LGR is lesion growth rate and μ is the total duration of the experiment (in the whole-plant assay μ = 24 days; in the detached-leaf assay μ = 6).

Statistical analysis

A paired *t*-test was used to compare means recorded for each parameter and for each strain on both hosts. This was performed to determine the host preference of each strain in terms of aggressiveness, sporulation, and fitness.

A principal component analysis (PCA) and a hierarchical clustering of the principal components (HCPCA) was performed using the parameters measured during the whole-plant experiment in order to identify putative infection strategies of *Colletotrichum* strains on each host. Clusters of strains were compared in terms of the variables aggressiveness, density and fitness using an analysis of variance (ANOVA) coupled with Tukey HSD tests. All statistical analyses were performed as implemented in R v. 3.3.3 (Fox & Leanage, 2016) using the RStudio Graphic User Interface v. 0.99 (https://www.rstudio.com/products/rstudio/) and R package FactoMiner (Lê *et al.*, 2008) for the HCPCA.

Results

Host specificity of *Colletotrichum* isolates in whole-plant assays

To assess host specificity for each host and each host × isolate combination, the infection capacity of each isolate inoculated on each host was evaluated. *Colletotrichum gloeosporioides* CMB1 was the only isolate unable to infect its alternate host, tree tomato (Table 1; Fig. 1a). The other isolates were able to infect both their original as well as the alternate host (Table 1; Fig. 1a). No significant differences in lesion size between inoculations on mango and tree tomato were detected (Fig. 1a). However, it was observed that, in most cases, the isolates from mango presented higher aggressiveness values on their original host while isolates from tree tomato showed greater aggressiveness towards their alternate host (Fig. 1a).

Results showed that the majority of isolates produced a higher spore density when inoculated on their alternate host than when inoculated on the original host. In most cases the differences were statistically significant (pairwise *t*-tests, $P < 0.05$; Fig. 1b). However, the *C. acutatum* and *C. gloeosporioides* isolates produced the opposite pattern, with significantly higher spore density on their original than on their alternate host.

The estimated fitness parameter for each isolate showed a pattern similar to that observed in the spore density assessment (Fig. 1c). Eight (CTJ1, CTJ2, CTK12, CMA14, CMB4, CME2, CMF1 and CMC2) of the 11 isolates assessed had higher fitness values when infecting the alternate host and for three of these eight isolates (CTJ2, CMA14 and CMF1) this difference was found to be statistically significant (Fig. 1c).

When several isolates of a species were assessed, the overall patterns observed for the measured and estimated parameters were consistent among isolates (Fig. 1a–c). However, results from a generalized linear model showed that the variation in spore density and fitness was better explained by isolates than by species ($P < 0.05$; Table S2).

Analysis of parameters measured on different infected hosts in whole-plant assays

The HCPCA performed using all the variables assessed (latent period, total lesion area, lesion growth rate and spore density) showed that the studied isolates could be grouped into three clusters when infecting tree tomato (Fig. 2) or four when infecting mango (Fig. 3; Table S1).

The isolates from each of the three clusters identified on tree tomato were compared in terms of their aggressiveness, spore density, and fitness using ANOVA and Tukey HSD tests. No significant differences were observed in the total lesion area among the different clusters (ANOVA, $P > 0.05$; Fig. 4a), but cluster CT2 showed higher lesion growth rate than cluster CT3 (ANOVA, $P < 0.05$; Tukey HSD, $P < 0.05$; Fig. 4b). Cluster CT3 presented significantly higher spore density than clusters CT2 and CT1, and cluster CT2 presented higher spore density than cluster CT1 (ANOVA, $P < 0.05$; Tukey HSD, $P < 0.05$; Fig. 4c). An increasing trend for clusters 1, 2 and 3, respectively, was observed when comparing fitness. Cluster 3 was significantly different from clusters 1 and 2 (ANOVA, $P < 0.05$; Tukey HSD, $P < 0.05$; Fig. 4d). Each isolate × host inoculation was assigned to one of the three clusters. This assignment was always consistent in the three different replicates of each experimental inoculation on each host.

On mango, no significant differences in aggressiveness were observed among clusters (Fig. 4e). Clusters CM1 and CM3 showed significantly higher LGR than cluster CM2 and lower LGR than cluster CM4 (ANOVA, $P < 0.05$; Tukey HSD, $P < 0.05$; Fig. 4f). Additionally, significant differences in spore density were found

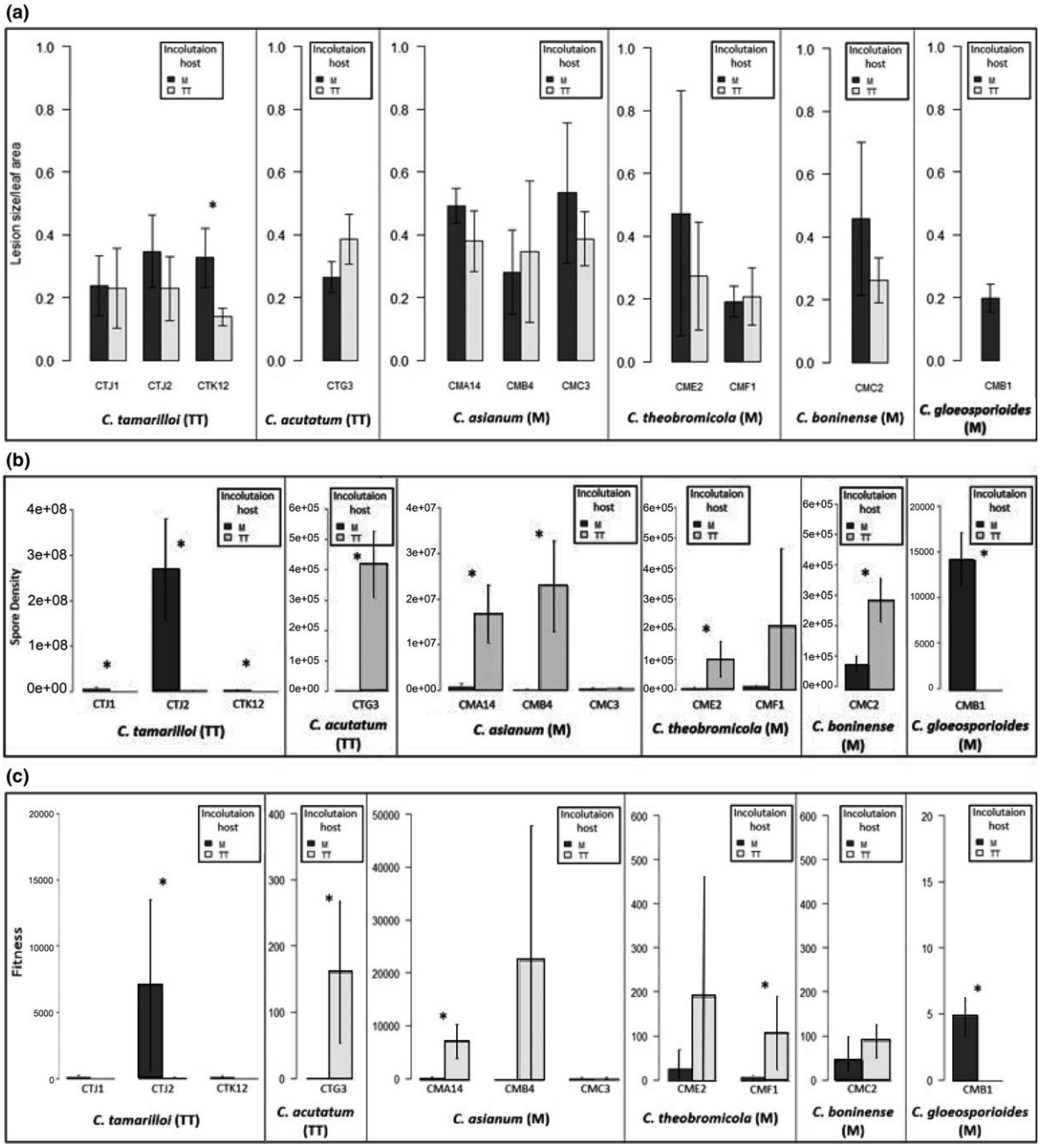

Figure 1 Differences in lesion size and spore density among isolates of *Colletotrichum* inoculated onto whole plants of mango (M) and tree tomato (TT). (a) Aggressiveness measured as the lesion size divided by the total leaf area. (b) Spore density measured as total conidial production divided by the total leaf area. (c) Fitness as defined by Montarry *et al.* (2010). Letters in parentheses indicate the host of origin of each pathogen species. Error bars represent the standard error of the mean. Asterisks indicate pairwise paired *t*-test comparisons with $P < 0.05$.

between cluster CM1 and clusters CM2, CM3, and CM4 (ANOVA, $P < 0.05$; Tukey HSD, $P < 0.05$; Fig. 4g). Only cluster CM1 showed significantly higher fitness than the remaining three clusters (Fig. 4h).

For species represented by more than two isolates, the isolates were grouped into different clusters that differed in terms of the measured disease parameters (Fig. 5a,b).

Detached-leaf assays

Most isolates produced a higher spore density when inoculated on their alternate host using the detached-leaf approach (Fig. S1a). *Colletotrichum asianum* CMA14 was an exception to the pattern as it was observed to produce higher spore density when infecting its original host (pairwise *t*-test, $P < 0.05$; Fig. S1a).

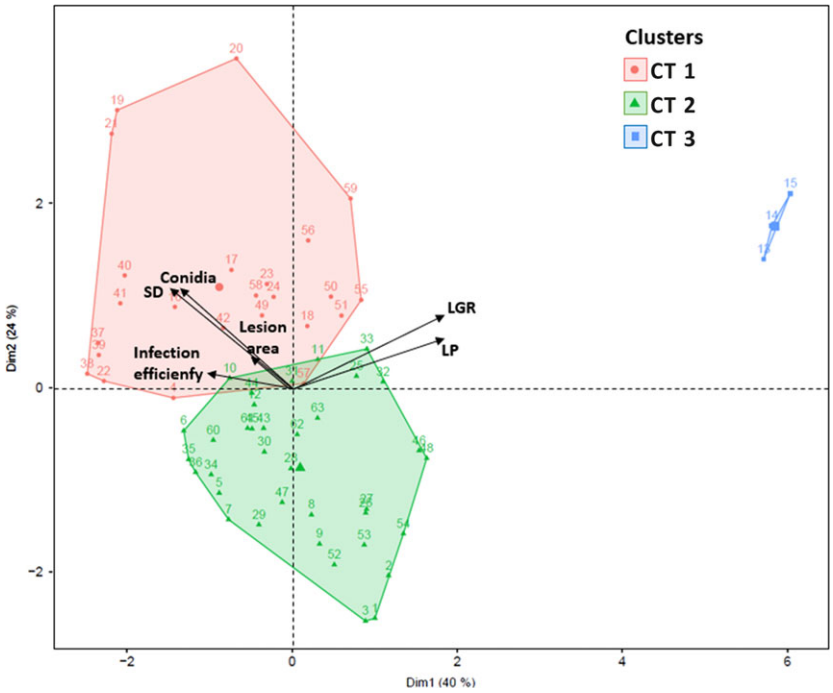

Figure 2 Clustering of *Colletotrichum* isolates infecting tree tomato based on hierarchical clustering of the principal components analysis (HCPCA). Axes represent principal components explaining more than 60% of the variance. CT, cluster tree tomato; LP, latent period, calculated as the interval between inoculation and the first appearance of necrotic symptoms; lesion area, the total area of infected leaf tissue by the end of the experiment; LGR, lesion growth rate, estimated as the total lesion size divided by the duration of the experiment minus the latent period; infection efficiency, defined as the total lesion area divided by the total leaf area; conidia, total conidia production, calculated as the total conidial count at the end of the experiment subtracting the initial inoculum; SD, spore density, estimated as total conidial production divided by the total leaf area. [Colour figure can be viewed at wileyonlinelibrary.com]

As in the whole-plant assay, the detached-leaf assay also showed *C. gloeosporioides* CMB1 to be specific to its original host, mango, as it was not capable of infecting tree tomato (Fig. S1b). However, unlike the results of the whole-plant experiments (Fig. 1a), *C. theobromicola* CME2 was also specific to mango and was unable to infect tree tomato (Fig. S1b). The remaining isolates were pathogenic to both their original and alternate host (Fig. S1b).

Fewer significant differences were found in the fitness estimation from the detached-leaf experiments data. However, for fitness the same trend as in the spore density data was observed. However, CMA14 behaved in the opposite way compared to the results obtained in the whole-plant assay; its fitness was higher on mango, the host from which it was isolated (Fig. S1c). In general, results were consistent between the whole-plant and the detached-leaf assays regarding host preference. However, correlation could not be statistically determined because the experiment was neither performed under the same conditions nor with the same number of replicates.

Discussion

This is the first study to investigate host specificity among *Colletotrichum* isolates from mango and tree tomato in Colombia using whole-plant inoculations. Most isolates showed neither host preference nor specificity. Aggressiveness was not significantly different when isolates were inoculated on mango or tree tomato. The isolates seemed to show host preference in the field as they were only obtained from one of the two crops, but this was not reproduced in the detached-leaf or whole-plant assays. It was also shown that the *Colletotrichum* species maintain a diversity of infection strategies in natural conditions as suggested by the grouping of isolates into clusters with different aggressiveness (ability to produce lesions) and transmission abilities (ability to produce conidia).

The lack of host specificity of *Colletotrichum* isolates from mango and tree tomato can favour the migration of populations from one host to another. This could be a threat for these crops because they are grown in geographically overlapping regions of Colombia. *Colletotrichum* species have long been considered broad-range pathogens (Freeman *et al.*, 1998). However, due to the complexity of *Colletotrichum* systematics, studies prior to the major phylogenetic revision of the genus by Cannon *et al.* (2012) may have underestimated the species richness, thus limiting the scope of conclusions regarding host range. Giblin *et al.* (2010) found that putative *C. gloeosporioides* strains isolated from mango and avocado were able to cross-infect both the alternate and original host in experimental conditions. However,

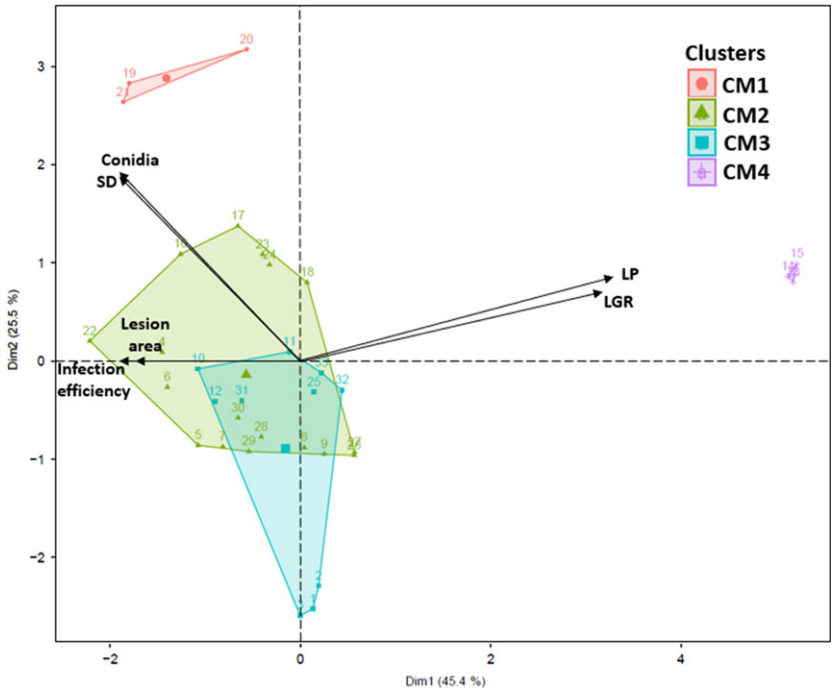

Figure 3 Clustering of *Colletotrichum* isolates infecting mango, based on hierarchical clustering of the principal components analysis (HCPCA). Axes represent principal components explaining more than 60% of the variance. CM, cluster mango; LP, latent period, calculated as the interval between inoculation and the first appearance of necrotic symptoms. Lesion area, the total area of infected leaf tissue by the end of the experiment; LGR, lesion growth rate, estimated as the total lesion size divided by the duration of the experiment minus the latent period; infection efficiency, defined as the total lesion area divided by the total leaf area; conidia, total conidia production, calculated as the total conidial count at the end of the experiment subtracting the initial inoculum; SD, spore density, estimated as total conidial production divided by the total leaf area. [Colour figure can be viewed at wileyonlinelibrary.com]

several species from the *C. gloeosporioides* species complex can be responsible for anthracnose in these crops. Likewise, Freeman (2008) discussed the host range of several *Colletotrichum* species that are now known to comprise more than one, sometimes even dozens, of evolutionary different lineages. Despite this, recent studies have reported that strains belonging to the same *Colletotrichum* species are able to infect different hosts. For example, Souza *et al.* (2013) reported that *C. gloeosporioides* and *C. acutatum* isolated from either mango or citrus could cause anthracnose symptoms on leaves of mango cultivars Palmen and Tommy Atkins and blossom blight symptoms in citrus flowers. These results indicate a lack of host specificity of *Colletotrichum* species.

The HCPCA performed using all assessed variables allowed further identification of putative strategies that this pathogen uses when infecting mango and tree tomato. The analysis grouped the isolates into clusters on each host based on the values obtained for the assessed parameters. Isolates from three of the species able to infect both hosts, *C. acutatum*, *C. asianum* and *C. tamarilloi*, were consistently grouped into clusters with opposite behaviour in terms of LGR and spore density when infecting the original and the alternate host. For example, *C. tamarilloi* isolates were grouped into clusters CT1 and CT2 (relatively low spore density and high LGR) when infecting tree tomato and into clusters CM1 and CM2 (relatively high spore density and lower LGR) when infecting mango.

In this study, fitness of isolates was used as a proxy to determine host preference. Similar to spore density, fitness was higher on the alternate rather than original host. As can be seen in the fitness equation, spore density is the variable that most influences fitness estimation. Interpretation of this fitness estimation might be misleading as this result indicates that, in general, isolates from different species tend to have a higher fitness when infecting their alternate host. Fitness is defined as a measure of the reproductive success of an allele, individual or group of individuals (Wu *et al.*, 2013) and so reproductive parameters are essential predictors for its estimation. However, in organisms with trophic and reproductive lifestyles as diverse as fungi, it has been historically difficult to establish common metrics to assess fitness (Pringle & Taylor, 2002; Gilchrist *et al.*, 2006). Spore production and viability have long been used as proxy to determine the fitness of saprophytic fungi, which entails both reproductive success and dispersal capacity (Gilchrist *et al.*, 2006). This has also been the case for asexual plant pathogens, where fitness estimated using mainly sporulation parameters has been used to inform the selection of resistant cultivars (Gallet *et al.*, 2013). Indeed, spore production is a key factor when determining the fitness of a pathogen in a given host.

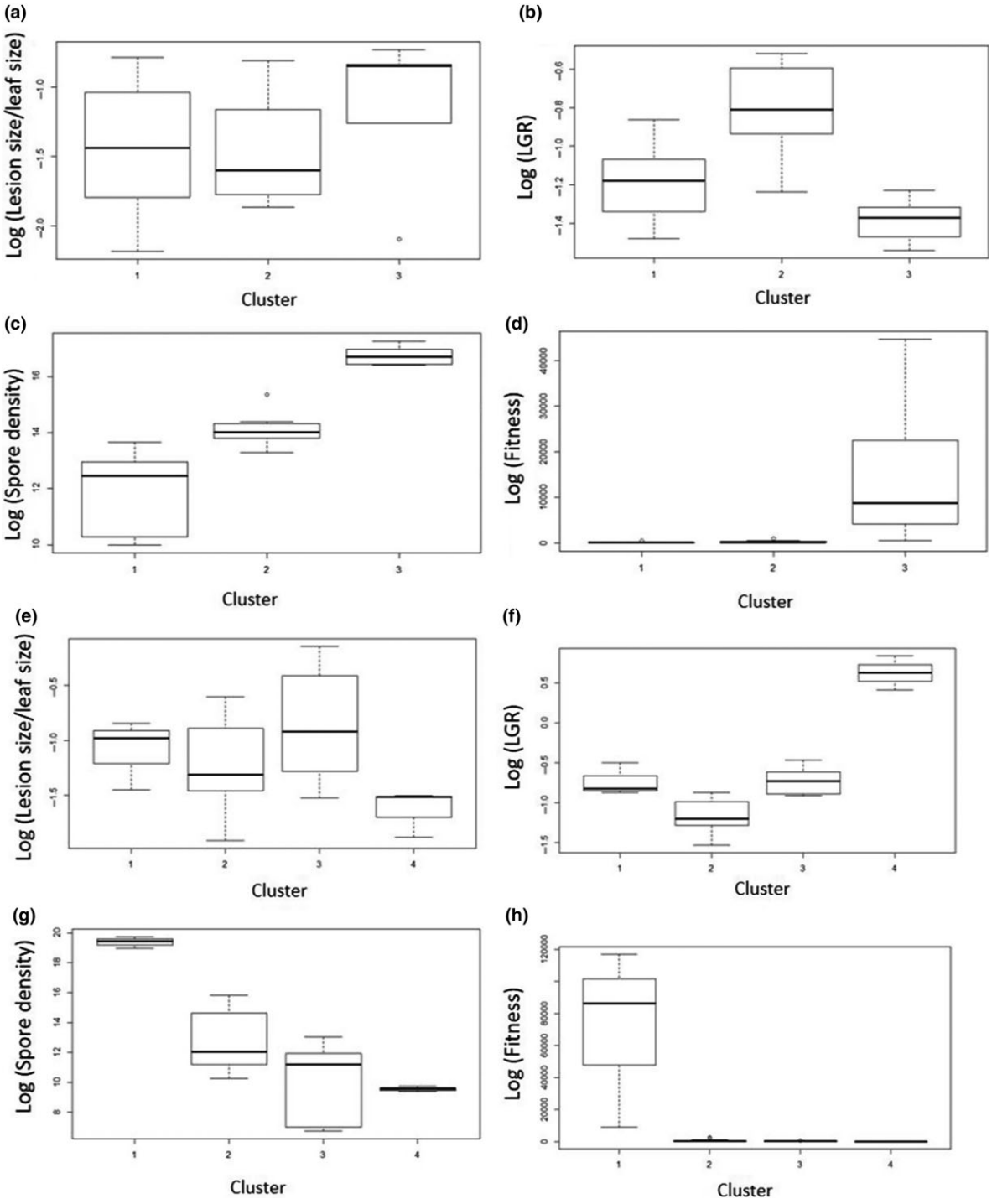

Figure 4 Differences among clusters of *Colletotrichum* isolates inferred with the hierarchical clustering of the principal components analysis (HCPCA) for each host, tree tomato (a, b, c, d) and mango (e, f, g, h). (a, e) Total lesion area, measured as lesion size divided by the total leaf area; (b, f) lesion growth rate (LGR), estimated as the total lesion size divided by the duration of the experiment minus the latent period; (c, g) spore density, measured as the total conidial production per leaf divided by the total leaf area; and (d, h) fitness, as defined by Montarry *et al.* (2010) for each isolate.

However, when studying interactions between a host and a pathogen with a varying degree of host preference, these measures may not be the most accurate because variations in spore production can be the result of factors other than the fitness of the pathogen on a given host. For example, molecular and biochemical evidence

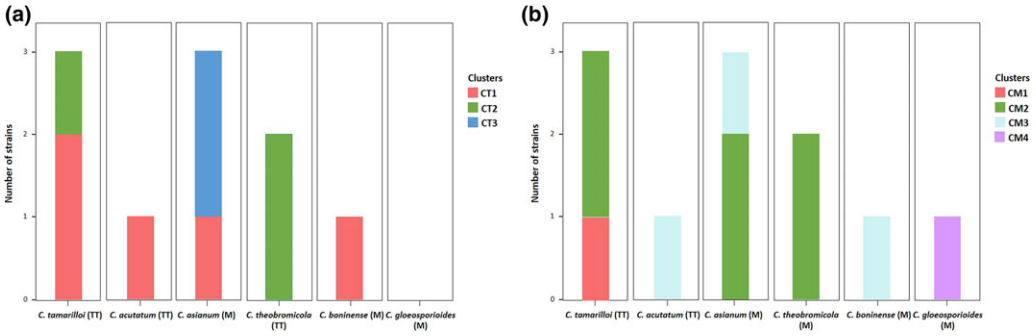

Figure 5 Distribution of cluster assignment by hierarchical clustering of the principal components analysis (HCPCA) for isolates of *Colletotrichum* species infecting tree tomato (a) and mango (b). CT, clusters of tree tomato isolates; CM, clusters of mango isolates; TT, isolates from tree tomato; M, isolates from mango. [Colour figure can be viewed at wileyonlinelibrary.com]

has shown that increased spore production in asexually reproducing fungi can be the result of exposure to stress triggering sporulation as a dispersal mechanism to seek better environmental conditions (Han *et al.*, 2004; Vargas-Perez *et al.*, 2007). Additionally, the characteristics of conidia on the original and the alternate host can shed light on the fitness of conidia produced in different conditions, but these have not been determined. Moreover, studies in several pathosystems have shown that increased virulence, defined as the capacity of a pathogen to colonize the host, can be a better selection predictor and a more accurate description of the degree of preference of a pathogen for a given host (Bolker *et al.*, 2010; Berngruber *et al.*, 2013). A study of *Phytophthora infestans* showed how the hemibiotrophic lifestyle results in evolutionary trade-offs that lead to the different use of reproductive strategies, thus affecting our interpretation of evolutionary metrics such as the fitness (Andrivon *et al.*, 2013).

In this study, high LGR and relatively low sporulation can be seen as a signature of host preference. This is consistent with the trend observed when isolates were inoculated on their original host, although aggressiveness was not statistically significant. This argument assumes that isolates from different species would show preference towards their host of isolation, especially because most were consistently isolated from only one of the two hosts studied (Table 1 in Pardo-De la Hoz *et al.*, 2016). However, most isolates were able to infect both hosts in the detached-leaf and whole-plant experiments.

To search for evidence of host specificity in isolates of *Colletotrichum* species in mango and tree tomato, the latent period, aggressiveness, conidial production, and fitness were assessed through two different inoculation models: detached-leaf and whole-plants. The detached-leaf assay showed similarity with the whole-plant assay. Although the different parameters tested on detached leaves and whole plants showed roughly similar results, the recommendation is to use whole-plant assays given that the conditions are nearest to natural conditions and greater significant differences were found in the fitness parameter. Interestingly, a few contradictory results were obtained between the detached-leaf and the whole-plant assays. For example, *C. acutatum* and *C. asianum* differed in their production of conidia between the two assays, *C. theobromicola* in its aggressiveness, and *C. tamarilloi* and *C. asianum* in their fitness. Another study using *Colletotrichum* as a model also found differences in the parameters assessed in detached-leaf and whole-plant assays (Liu *et al.*, 2007); they found no response to the pathogen in the detached-leaf assay and some of the symptoms observed were probably associated with senescence and dehydration. Thus, it is recommended that whole-plant rather than detached-leaf assays are used to evaluate *Colletotrichum* pathogenicity.

Overall, the results of this study showed that most *Colletotrichum* species causing anthracnose in mango and tree tomato are not host-specific and do not show host preference in a laboratory setting. This is consistent with the long-standing view of *Colletotrichum* as a generalist pathogen. Nonetheless, field isolation frequencies as well as examination of several disease parameters showed that, although most species are capable of causing disease in both hosts, they might not be equally adapted nor use the same pathogenic strategies on each of them. Furthermore, these differences can be found even at the isolate level within some species; indeed, one isolate was specific to its original host in this investigation. These results should inform the development of anthracnose management strategies for these two crops and highlight the host range of *Colletotrichum* species as far more complex than simply generalist or specialist.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Figure S1. Parameters assessed in whole-plant assays of mango (M) and tree tomato (TT) inoculated with isolates of *Colletotrichum*. (a) Total lesion area measured as lesion size divided by the total leaf area. (b) Spore density measured as total conidial production per leaf divided by the total leaf area. (c) Fitness, calculated using equation of Montarry et al. (2010) for each isolate.

Table S1. Distribution into clusters of *Colletotrichum* isolates on tree tomato and mango after hierarchical clustering of the principal components analysis (HCPCA). Isolate, species, or complex and host and specified on the left. The cluster distribution of three replicates is shown.