

Identification of Resistance to Target Spot of Tomato Caused by *Corynespora cassiicola* in Wild Tomato Accessions

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ABSTRACT. Tomato (*Solanum lycopersicum*) is an important vegetable crop and a valuable source of nutrients for the human diet. The southeast is the main fresh market tomato producer of the United States, with much of the production concentrated in Florida. However, production in this region is threatened by plant diseases such as target spot of tomato (TS) caused by *Corynespora cassiicola*, a multitrophic fungus widely distributed in tropical and subtropical areas. TS can infect foliage and fruit, often resulting in significant yield losses in conducive environments. There are no known TS-resistant cultivars, and control relies entirely on fungicidal sprays. However, several studies have demonstrated that the fungus is developing resistance to commonly used fungicides which further complicates disease management. The objective of this work was to identify sources of resistance to TS from wild *Solanum* accessions. Initial screens of 83 accessions informed the selection of 24 accessions for a more robust screening in which six diverse *C. cassiicola* isolates were used for single-isolate inoculation experiments. The results from a broad-sense mixed-model analysis including data from all six experiments demonstrated that all 24 accessions had significantly lower disease severities compared with the susceptible controls, suggesting that all accessions potentially harbor resistance quantitative trait loci (QTLs). *Solanum cheesmaniae* accession LA0524, *S. galapagense* accessions LA0483 and LA0532, and *S. pimpinellifolium* accession LA2093 were among the most resistant accessions tested and may be particularly useful for introgression of resistance into cultivated germplasm and for mapping of TS resistance QTLs.

Tomato (*Solanum lycopersicum*) originated in South America and was fully domesticated in Mexico (Paran and van der Knaap 2007). Tomato is a great source of lycopene, other carotenoids, vitamins, and minerals and is component of several popular processed and raw food sources, such as ketchup, sauces, and salads. It is also a model plant species subject to extensive phenotypic and genetic studies. The tomato genome has been sequenced and is composed of more than 780 million bps of DNA and more than 34,000 protein-coding genes (Genome version SL4.0, Sol genomics Network) (Hosmani et al. 2019).

Tomato is valued at more than \$92 billion annually and is one of the most important vegetable crops in the world (Food and Agriculture Organization 2018). In the United States, fresh-market tomato is valued at more than \$1.2 billion, and Florida and California contribute more than 60% of the production, with Florida alone accounting for more than half of this; much of the remaining production is spread across southeastern states (US Department of Agriculture-National Agriculture Statistics Service 2017). The Southeast, and especially Florida, have a productive advantage due to the tropical and subtropical weather in this region, which allows for production from fall through spring each year. However, the humid and warm weather is also conducive to the

development of numerous plant diseases, including target spot of tomato (TS), caused by *Corynespora cassiicola*. In recent years, TS has reemerged as a significant threat to tomato production in Florida and the southeastern United States (MacKenzie et al. 2018; Pernezny et al. 2002; Schlub et al. 2009). A large reason for the increased concern is its ability to infect both foliage and fruits, resulting in dramatic yield losses under disease-favorable conditions (Schlub et al. 2009).

Corynespora cassiicola was first reported in Florida in 1967 (Blazquez 1972). *C. cassiicola* is a necrotrophic fungus ubiquitous in the tropics and subtropics (Dixon et al. 2009; Farr and Rossman 2017). The pathogen infects more than 530 species of monocots dicots including important crops such as tomato, rubber tree (*Hevea brasiliensis*), cucumber (*Cucumis sativus*), cotton (*Gossypium* spp.), tobacco (*Nicotiana tabacum*), and soybean (*Glycine max*) (Smith 2008; Sumabat et al. 2018b). Although *C. cassiicola* is only known in its anamorph stage, there is evidence of high genetic diversity within the species (Schlub et al. 2009; Smith 2008). Cassiicolin, a diffused toxin produced by the fungus during infection, is thought to be a determinant of pathogenicity and virulence (Barthe et al. 2007; Breton et al. 2000; Kurt 2004; Onesirosan et al. 1975). However, a recent study did not detect cassiicolin isoforms from *C. cassiicola* tomato isolates, indicating that other pathogenicity factors may be involved in the infection process in this crop (Dacones et al. 2022).

Target spot earns its name from the ringed appearance that is frequently observed in lesions (Pernezny and Simone 1999). Foliar symptoms begin as small, dark-brown necrotic lesions with darkened margins. As lesions develop further, patterns of concentric rings appear within them, providing a target-like appearance. The lesions are typically surrounded by yellow margins (Kurt 2004; Pernezny and Simone 1999). TS symptoms

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may be confused with other aerial diseases including bacterial spot and early blight (Pernezny and Simone 1999). Foliar infections often result in defoliation, which can become extensive and lead to the death of infected plants (de Lamotte et al. 2007; Fulmer et al. 2012; MacKenzie et al. 2018). Fruit lesions typically begin as small, brown, sunken, necrotic flecks with darkened margins; lesions can expand rapidly as fruits ripen, becoming large and sunken with concentric rings and often with noticeable dark gray to black fungal growth in the center (Pernezny and Simone 1999). Fruit lesions can be extremely problematic because even fruit with small lesions must be culled or these lesions will develop during ripening and transit and cause significant postharvest losses (Fajola 1979; MacKenzie et al. 2018).

Breeding for resistance against TS was not of primary interest in the past (Pernezny and Simone 1999), and consequently, no resistant tomato cultivars currently exist. There is no evidence of host resistance within cultivated germplasm, and the only report of such dates to 1973 when Bliss et al. (1973) identified resistance in *S. pimpinellifolium* accession (PI 112215) and a derived breeding line. However, screenings conducted in 2011 did not demonstrate resistance in this material to recently collected isolates (G. Vallad, personal communication). Due to the lack of resistant cultivars available and low efficacy of cultural methods, chemical control is the primary management approach. However, recent studies have warned of the increasing insensitivity of *C. cassicola* to commonly used fungicides, such as quinone outside inhibitors and succinate dehydrogenase inhibitors (Mackenzie and Vallad 2017; MacKenzie et al. 2020). In light of this, the identification of sources of TS resistance for the development of TS

resistant cultivars is needed. The objective of this work was to identify resistance to TS from wild *Solanum* accessions for subsequent mapping and introgression into cultivated tomato. The results of several screenings of resistance to TS in a broad set of wild tomato accessions are presented in this section.

Materials and Methods

GROWING CONDITIONS AND PLANT MATERIALS. Seeds from accessions used in all screens were obtained from the Tomato Genetics Resource Center (Davis, CA, USA). Seedling establishment methods were similar for all assays. Seeds from accessions and controls were directly sown in 128-well plastic transplant trays. Seedlings were maintained in an open-sided greenhouse, watered as needed, and fertilized weekly using a 20N-8.7P-16.6K water-soluble fertilizer at 1000 mg/L of nitrogen.

Three experiments were conducted: an initial germplasm survey, a confirmation experiment, and then more comprehensive experiments using single isolate inoculations. In the initial germplasm survey, the inoculation was done directly in the transplant trays (see discussion of inoculation methods later in the article). For the confirmation experiment and the individual isolate assays, individual seedlings were transferred into 296-mL plastic pots ~4 weeks after sowing and inoculated 3 weeks later.

INITIAL GERmplasm SURVEY AND CONFIRMATION EXPERIMENT. The initial germplasm survey was conducted in 2017 and included 83 *Solanum* accessions and two commercial hybrids ('Florida 47' and 'Sanibel') as susceptible controls (Table 1). On the basis of results from that survey, accessions with potential resistance were

Table 1. Resistance response of the accessions tested in the initial germplasm survey.

Accessions	<i>Solanum</i> species	Response
LA0522, LA0749, LA0932, LA1447	<i>S. cheesmaniae</i>	R
LA2099	<i>S. habrochaites</i>	R
LA2094	<i>S. lycopersicum</i>	R
LA2093	<i>S. pimpinellifolium</i>	R
LA0421, LA0422, LA0521, LA0524, LA0528B, LA0746, LA1037, LA1040, LA1042, LA1043, LA1138, LA1402, LA1404, LA1414, LA1427	<i>S. cheesmaniae</i>	MR
LA0526	<i>S. galapagense</i>	MR
LA1721	<i>S. habrochaites</i>	MR
LA1322, LA2190	<i>S. neorickii</i>	MR
LA1617, LA2102, LA2173	<i>S. pimpinellifolium</i>	MR
LA0434, LA0437, LA0529, LA1035, LA1036, LA1139	<i>S. cheesmaniae</i>	MS
LA1932, LA1958	<i>S. chilense</i>	MS
LA1318, LA2663	<i>S. chmielewskii</i>	MS
LA1753	<i>S. habrochaites</i>	MS
LA1299, LA1657, LA1674	<i>S. pennellii</i>	MS
LA1474	<i>S. peruvianum</i>	MS
LA3124	<i>S. cheesmaniae</i>	S
LA1930, LA1972	<i>S. chilense</i>	S
LA1927, LA2651	<i>S. habrochaites</i>	S
LA1226	<i>S. lycopersicum</i>	S
LA1515, LA1522, LA1649, LA1656, LA1724, LA1732, LA1733, LA1735, LA1809, LA1911, LA1912, LA1920, LA1940, LA1941, LA1942, LA1943, LA2560, LA2580, LA0716, LA0751, LA1273, LA1277, LA1282, LA1297, LA1302, LA1303, LA1340, LA1356, LA1367	<i>S. pennellii</i>	S
LA1336, LA1954	<i>S. peruvianum</i>	S
LA1375, LA1992	<i>S. pimpinellifolium</i>	S
'Florida 47'	<i>S. lycopersicum</i>	S
'Sanibel'	<i>S. lycopersicum</i>	S

R = resistant (0% to 25% disease); MR = moderately resistant (26% to 50% disease); MS = moderately susceptible (51% to 75% disease); S = susceptible (76% to 100% disease). Scoring is based on at least two replicates of eight seedlings per accession.

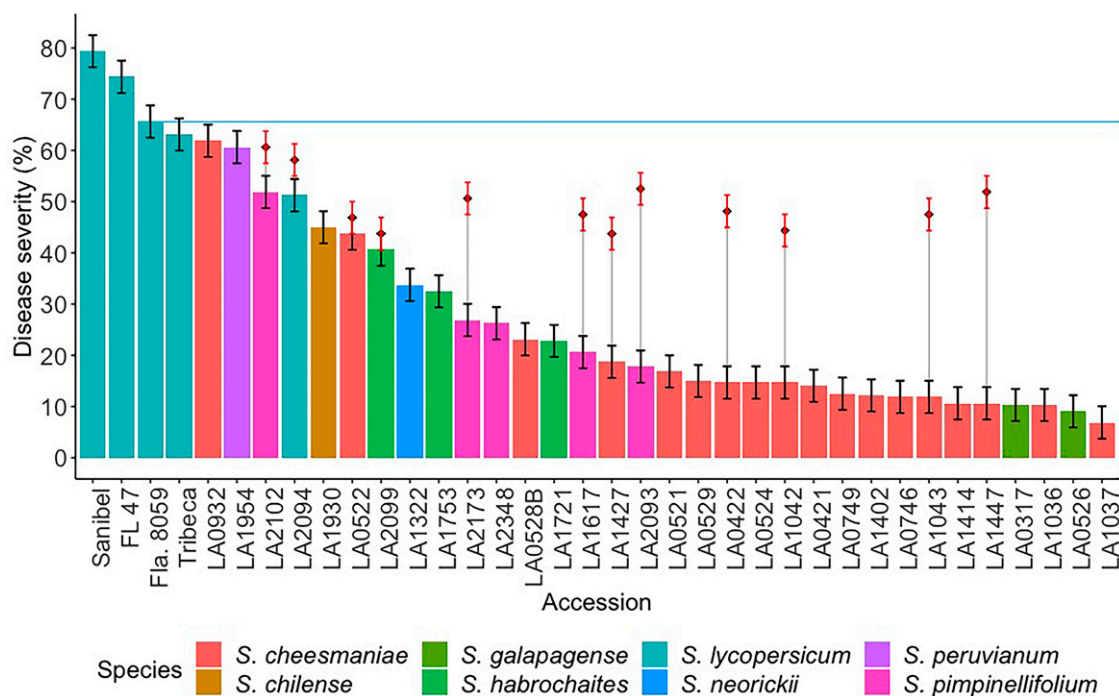


Fig. 1. Disease severity estimates (y-axis) of the accessions and controls (bars) and select F₁s between Fla 8059 and indicated accession (red diamond) tested in the second screen. Values are based on a 0 to 10 rating scale corresponding to percent disease (0% to 100%). Bars represent the standard error of the mean as calculated by the mixed model. Different colors group different *Solanum* species. The horizontal blue line represents the rating of Fla. 8059 to use as a reference for the hybrids' disease ratings.

selected for replicated testing in a confirmation experiment. This experiment was conducted in Spring 2019 and included 32 accessions from *S. cheesmaniae* (18), *S. chilense* (1), *S. galapagense* (2), *S. habrochaites* (3), *S. lycopersicum* (1), *S. neorickii* (1), *S. peruvianum* (1), and *S. pimpinellifolium* (5), along with four cultivated tomato controls (hybrid cultivars Sanibel, FL 47, and Tribeca; and inbred Fla. 8059) (Fig. 1). Additionally, 12 interspecific hybrids from the crosses of select accessions to Fla. 8059 were included to investigate gene action of resistance (Fig. 1). The breeding line Fla. 8059 was set as female in all crosses.

INDIVIDUAL ISOLATE ASSAYS. On the basis of results from the confirmation experiment, 18 accessions from three species (*S. cheesmaniae*, *S. galapagense*, and *S. pimpinellifolium*) were selected for assays conducted during 2019 (Fig. 2). These assays also included six *S. galapagense* accessions that were not previously tested due to the unavailability of seed. Each assay included the hybrids 'Tribeca' and 'HM1823', and the breeding line Fla. 8059 as susceptible controls.

INOCULUM PREPARATION AND INOCULATION METHODS. For the initial germplasm survey and the confirmation experiment, a mixture of three *C. cassicola* isolates from Gary E. Vallad (GEV) isolate collection from Gulf Coast Research and Education Center, University of Florida, Wimauma, were used for the inoculation (GEV3125, GEV3183, and GEV3223). For the individual isolate assays, the set of accessions was inoculated with six isolates in six separate experiments. The isolates used were GEV3127, GEV3131, GEV3140, GEV3142, GEV3153, and GEV3240, chosen to represent the genetic diversity within a large collection of 123 isolates collected across Florida between 2015 and 2017 (MacKenzie et al. 2020; Xavier et al. 2020).

Isolates stored in filter paper were reactivated by transferring a piece of filter paper to full-strength potato dextrose agar (PDA) (39 g of PDA/L) and incubating for 5 to 7 d in 25 ± 2 °C and a

12:12 photoperiod under fluorescent light. Isolates were plated in PDA media using four mycelial plugs per plate. After a week of mycelial growth, the inoculum was prepared by blending seven plates of each isolate culture per liter of water to obtain a suspension of spores and hyphae at a concentration of ~10⁴ spores or hyphae/mL. The plants were spray-inoculated until runoff either with the cocktail of isolates (initial germplasm survey or confirmation experiment) or with individual *C. cassicola* isolates for each of the six single-isolate experiments (individual isolate assays).

ASSAY CONDITIONS AND RATING OF SYMPTOMS. After inoculation, plants were placed in clear plastic tubs (81.6 cm length × 48.6 cm width × 34.9 cm height), and ~500 mL of water was added to the base of each tub to increase relative humidity. The tubs were covered with a clear lid and incubated in a growth room with controlled temperature and light (27/21 °C day/night temperature, 13 h of light) for 4 d.

After incubation, plants were individually rated for foliar disease severity. For the initial germplasm survey, plants were rated as resistant, moderately resistant, and susceptible based on foliar symptoms. For the confirmation experiment and individual isolate assays, a 0 to 10 rating scale corresponding to percent disease (0% to 100%) was used to assess disease severity (DS) among accessions tested. The rating of each plant from the confirmation experiment and individual isolate assays was used in the statistical analysis.

Experimental Design and Statistical Analysis

CONFIRMATION EXPERIMENT. The confirmation experiment included two assays using a randomized complete block design (RCBD), each with eight replicates and with single plants as

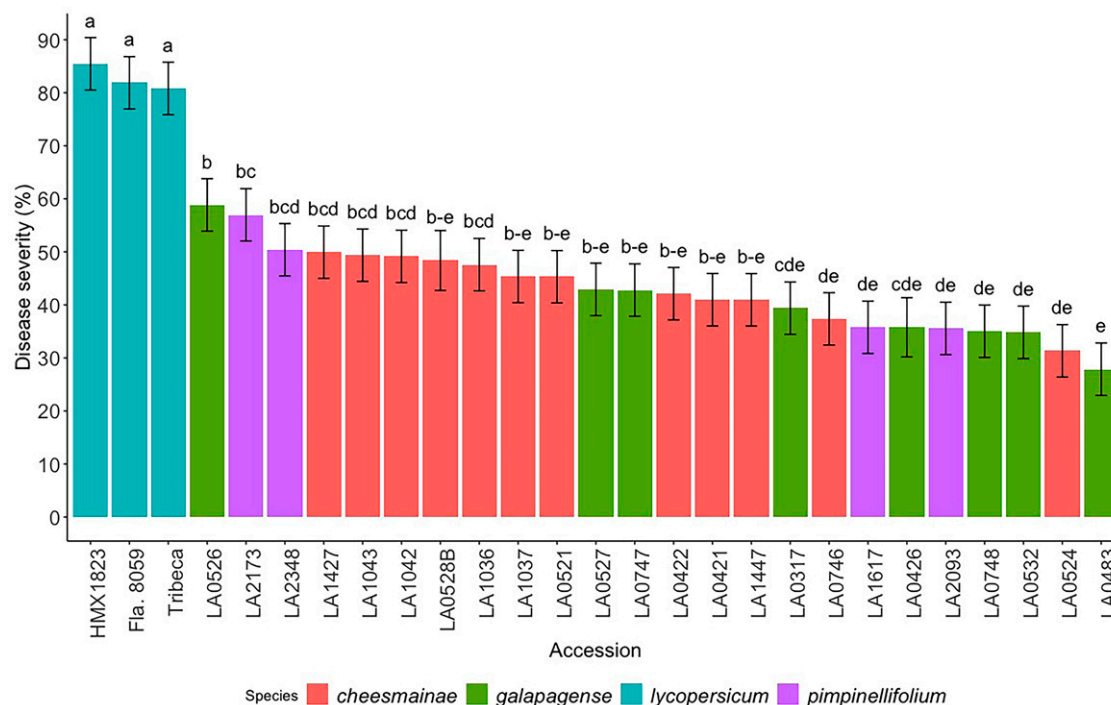


Fig. 2. Combined performance of 24 *Solanum* accessions and three cultivated susceptible controls against six individual *Corynespora cassiicola* isolates based on least square (LS) mean disease severity. The y-axis shows the LS mean disease severity and the x-axis the line tested. Disease severity is based on performance across six separate experiments, each testing a distinct *C. cassiicola* isolate. Experiments were randomized complete block designs with 16 blocks, and data were analyzed using a linear mixed model analysis. Error bars represent the standard error of the mean. Different letters separate significantly different means based on the Tukey's honestly significant difference pairwise comparison method for an alpha of 0.05.

experimental units. Accession was set as independent variable in the model and included 48 levels; rating was the dependent variable. Block was nested within experiment and both variables were set as random. The model was specified as follows:

$$\text{Rating} = \text{Accession} + \text{Block}(\text{Experiment}) + \text{Experiment}$$

INDIVIDUAL ISOLATE ASSAYS. The final set of 24 accessions was tested individually against six *C. cassiicola* isolates in six separate experiments. Each experiment tested a different *C. cassiicola* isolate, and thus this factor was not replicated. For this reason, *C. cassiicola* isolate differences are confounded with experiment. Each of the six experiments was designed as an RCBD with 16 replicates and with single plants as experimental units. The single treatment factor included 27 levels (24 accessions plus three susceptible controls). Eight plastic tubs were divided using a polystyrene foam barrier such that each tub contained two blocks.

Rating was set as the dependent variable and accession, isolate, tub, and block were set as independent variables. All independent variables were set as random factors except for accession to assess the accession resistance across all possible levels of isolates. The Isolate \times Accession interaction was also set as random to make a broad-sense inference on the resistance of each accession across all possible levels of isolates (i.e., a population of isolates) as suggested by Dixon et al. (2018).

The model was specified as follows:

$$\text{Rating} = \text{Accession} + \text{Isolate} + \text{Tub}(\text{Isolate}) + \text{Block}(\text{Isolate} * \text{Tub}) + \text{Isolate} * \text{Accession}$$

In the confirmation experiment and the individual isolate assays, a linear mixed model was fitted using the PROC GLIMMIX

procedure of SAS version 9.4 (SAS Institute, Cary, NC, USA). The Student panel was inspected to ensure compliance with the model assumptions. Estimation of fixed effects was done with the *lsmeans* statement, and the multiple mean comparison was done using the Tukey's honestly significant difference method with an alpha of 0.05. Graphics were done using the ggplot2 package in R (R Core Team 2019; Wickham 2016).

Results

INITIAL GERMLASM SURVEY. An initial germplasm survey showed several accessions with resistance or moderate resistance to TS (Table 1). This initial screening served as an early exploration of resistance and as a basis for the two subsequent experiments with a more systematic and robust screening. It also helped avoid selecting susceptible accessions for subsequent experiments.

CONFIRMATION EXPERIMENT. In addition to accessions with resistance or moderate resistance from the initial germplasm survey, new entries were included in the confirmation experiment. DS ranged from less than 10% for accession LA1037, to 79% for the susceptible cultivar Sanibel (Fig. 1). The susceptible controls 'Sanibel', 'Florida 47', Fla. 8059, and 'Tribeca' showed the highest DS ranging from 63% to 79%. All accessions had significantly lower DS relative to the four susceptible controls ($P < 0.05$, Supplemental Table 1), with exception of *S. cheesmaniae* LA0932 (only significantly different from 'Sanibel'), *S. peruvianum* LA1954 (only significantly different from 'Sanibel' and 'Florida 47'), *S. pimpinellifolium* LA2102, and *S. lycopersicum* LA2094 (not significantly different from 'Tribeca'). Interestingly, all *S. cheesmaniae* accessions, except for LA0932 and LA0522, exhibited low DS relative to the other accessions. Among the

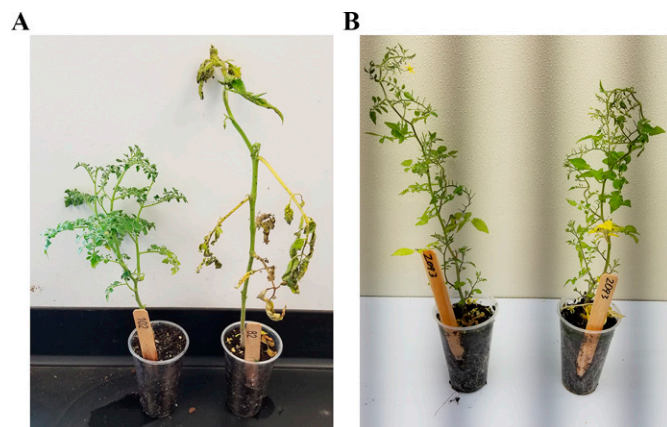


Fig. 3. Resistant and susceptible phenotypes against target spot of tomato caused by *Corynespora cassiicola* in the seedling disease assay. Pictured are (A) *Solanum galapagense* accession LA0483 (left) and the susceptible control 'HM1823' (right) and (B) two plants of *S. pimpinellifolium* accession LA2093. Photos courtesy of authors.

S. pimpinellifolium accessions tested, DS ranged from 52% for LA2102 to as low as 18% for LA2093. Finally, both *S. galapagense* accessions (LA0317 and LA0526) had among the lowest DS of the accessions tested (albeit not significantly different from most accessions tested).

Overall, higher levels of resistance were observed among *S. galapagense*, *S. cheesmaniae*, and *S. pimpinellifolium* accessions as shown by their lower DS. For this reason, accessions from these three species were selected for further testing using the individual isolate assays, and the remaining species were not tested further.

The DS of each of 12 interspecific hybrids included in this experiment was intermediate to the responses of Fla. 8059 and the corresponding accession but generally closer to that of Fla. 8059 (such as the hybrids from LA2173, LA1617, LA2093, LA0422, LA1042, LA1043, and LA1447). This suggests that resistance from those accessions is likely recessive or incompletely recessive (Fig. 1). However, further studies would be needed to determine gene action because this was not consistently clear.

INDIVIDUAL ISOLATE ASSAYS. On the basis of results from the confirmation experiment, 18 accessions were selected for further evaluation against single *C. cassiicola* isolates. These included 12 accessions from *S. cheesmaniae* and four accessions from *S. pimpinellifolium*. Because of the low DS observed for the two *S. galapagense* accessions tested in the confirmation experiment, six additional accessions from this species were included for a total of 24 accessions. Significant differences in DS were observed between accessions and the susceptible controls 'HM1823', Fla. 8059, and 'Tribeca' (Figs. 2–4, Supplemental Table 1).

DS from these assays ranged from 10% to 90% for the accessions, and from 40% to 100% for the susceptible controls 'HM1823', Fla. 8059, and 'Tribeca' (Fig. 4). The experiment inoculated with isolate GEV3153 produced DS close to 100% for the controls, indicating that this isolate was one of the most aggressive, followed by the experiments inoculated with isolates GEV3140 and GEV3142 (Fig. 4). In contrast, experiments inoculated with isolates GEV3127 and GEV3240 resulted in lower DS, suggesting that these isolates were less aggressive. In general, experiments using isolates GEV3131 and GEV3153 had the highest DS for the accessions with fewer accessions showing lower DS. Although the interaction isolate \times accession was treated as a random

effect in the combined analysis, the distribution of the raw data showed similar accession rankings among the isolates tested suggesting that the isolate \times accession interaction was low (Fig. 4). Pearson's correlation tests of DS of accessions among isolates were significant ($P < 0.05$), which further supports this (Supplemental Table 2).

In the combined mixed model analysis, all 24 accessions exhibited lower DS than the susceptible controls 'HM1823', Fla. 8059, and 'Tribeca' ($P < 0.05$, Fig. 2). Although accessions from *S. galapagense* tended to have the lowest DS, most of the accessions were not significantly different from one another, and all three *Solanum* species were represented by the six accessions with the lowest DS.

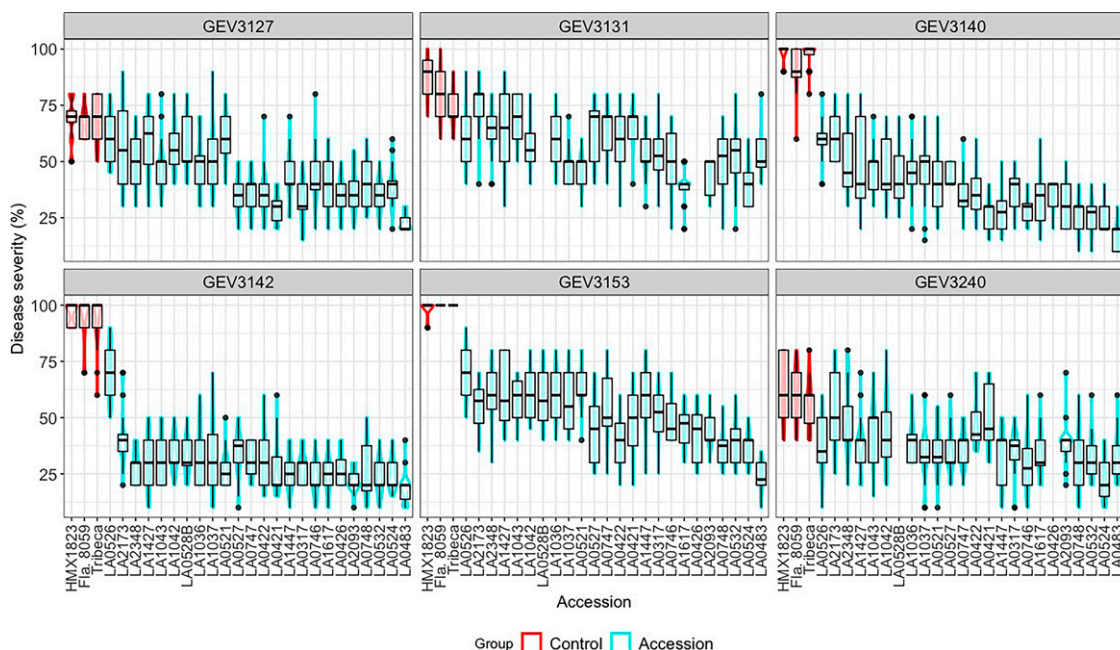
Discussion

This study used successive TS disease screenings to identify wild tomato accessions that may serve as useful sources to introgress resistance into cultivated tomato. A preliminary germplasm survey, which focused largely on those *Solanum* species that easily hybridize with cultivated tomato (Grandillo et al. 2011; Strickler et al. 2015), included 83 accessions from *S. cheesmaniae*, *S. chilense*, *S. chmielewskii*, *S. habrochaites*, *S. neorickii*, *S. galapagense*, *S. pennellii*, *S. peruvianum*, and *S. pimpinellifolium*. Results of this initial survey identified 32 accessions for further testing and final characterization of 24 accessions from *S. pimpinellifolium*, *S. galapagense*, and *S. cheesmaniae*, for their responses to six individual *C. cassiicola* isolates.

The mixed model analysis of data from experiments using six individual *C. cassiicola* isolates showed that all accessions tested were significantly more resistant than susceptible controls. Most accessions were not significantly different from each other, suggesting the possibility of common genetics for resistance in this germplasm set. On the other hand, four of the six accessions with the lowest DS were *S. galapagense*, further supporting the potential utility of this species as a donor of resistance.

S. pimpinellifolium accession LA2093 showed the lowest DS among all *S. pimpinellifolium* accessions in the confirmation experiment and the individual isolate assays. However, the difference was not significant among most *S. pimpinellifolium* accessions tested within each experiment. DS varied slightly among the accessions within this species in both screens, with values of 18% to 52% in the confirmation experiment and from 36% to 57% in the overall analysis from the individual isolate assays. Bliss et al. (1973) identified *S. pimpinellifolium* accessions with full or intermediate resistance in their study which is in line with our results showing varying levels of resistance among the accessions within this species. A previous study remarked the high genetic diversity within *S. pimpinellifolium* (Blanca et al. 2015). The variation observed among the *S. pimpinellifolium* accessions tested could thus be caused by diverse resistance alleles among these accessions.

Plants typically defend against pathogens through two well-studied pathways: pathogen-associated molecular patterns (PAMP)-triggered immunity (PTI) is activated via pattern recognition receptors located at the membrane that recognize PAMPs; the second, called effector-triggered immunity (ETI), initiates when receptors located mostly in the cytoplasm and encoded by R genes sense pathogen elicitors (Chisholm et al. 2006; Jones and Dangl 2006). Because both pathways require only one functional receptor allele; resistance through either pathway typically behaves as dominant (Gomez-Gomez and Boller 2000; Joosten and de Wit 1999).



for significant isolate \times accession interactions, only slight changes in rankings were observed among the accessions for each individual isolate experiment. Additionally, the correlation of DS of accessions among experiments was significant. This suggests that responses were generally consistent from isolate to isolate. However, further work is needed to examine in depth the potential isolate \times accession interaction in advanced lines and ensure that broad resistance is advanced into those lines.

For breeding purposes, plant materials with lower foliar disease are sought that can potentially provide durable and reliable genetic resistance to control TS. Those accessions with the lowest overall DS may therefore prove the most useful for the introgression of resistance into cultivated tomato.

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