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Resistance to *Botrytis cinerea* in *Solanum lycopersicoides* is dominant in hybrids with tomato, and involves induced hyphal death

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Abstract

Botrytis cinerea causes gray mold disease and affects hundreds of plant species, including tomato (*Lycopersicon esculentum*). The wild nightshade, *Solanum lycopersicoides*, is cross compatible with tomato and is more resistant to *B. cinerea*, thus representing a potential source for crop improvement. Tests involving droplet inoculation of detached leaves and spray inoculation of entire seedlings demonstrated that resistance to *B. cinerea* varies among *S. lycopersicoides* accessions, with *S. lycopersicoides* LA2951 being the most resistant accession tested. Expression of resistance in the intergeneric hybrid (*L. esculentum* cv. 'VF36' × *S. lycopersicoides* LA2951) suggested that resistance is at least partially dominant in tomato. A green fluorescent protein-tagged *B. cinerea* strain was used for confocal microscopic comparison of infection in leaves of *S. lycopersicoides* and tomato. Even though *S. lycopersicoides* supported spore germination, there was evidence for hyphal lysis and death 3 days after inoculation, at a time when lesions were expanding on susceptible tomato plants. The reduced frequency of *B. cinerea* lesion spread on *S. lycopersicoides* explains why this fungus produced fewer spores in this wild nightshade than in tomato.

Abbreviations: ANOVA – analysis of variance; GFP – green fluorescent protein; PGIP – polygalacturonase inhibitor protein.

Introduction

Botrytis cinerea (teleomorph: *Botryotina fuckeldiana*) causes gray mold on more than 200 host plant species (Jarvis, 1980). As a necrotrophic pathogen, this fungus induces plant cell death to facilitate the colonization of host tissues (Govrin and Levine, 2000; Dickman et al., 2001). In addition, cell wall-degrading enzymes and toxins contribute to fungal pathogenesis (ten Have et al., 1998; Deighton et al., 2001; Colmenares et al., 2002). Because necrotrophic pathogens can exploit a variety of mechanisms to overcome host defenses, there may not be a simple gene-for-gene mechanism governing these types of interactions (Flor, 1971). On the contrary, biotrophic pathogens use different strategies to avoid incompatibility. This is determined by stereotypic genetic interactions between dominant avirulence (*Avr*) genes of the pathogen and dominant resistance (*R*) genes of the host. A mutation in an *Avr*-gene is sufficient to overcome resistance of a host containing a matching *R*-gene (Baker et al., 1997). The greater complexity of the necrotrophic plant–pathogen interaction has hampered its genetic analyses, although studies using transgenic and mutant plants have revealed several host defense components against gray mold. Expression of pear polygalacturonase inhibitor protein (PGIP) in transgenic tomato (*Lycopersicon esculentum*) plants enhanced resistance of tomato leaves and fruits to *B. cinerea*, possibly through interference with fungal pectin degradation

(Powell et al., 2000). Phytohormones also influence interactions between *B. cinerea* and tomato. While abscisic acid enhanced susceptibility (Audenaert et al., 2002), ethylene and jasmonic acid enhanced resistance to *B. cinerea* (Diaz et al., 2002). Hypersensitive responses contribute to resistance expressed toward biotrophic pathogens, but enhance susceptibility to *B. cinerea* (Hennin et al., 1999; Govrin and Levine, 2000). Synthesis of phytoalexins through heterologous expression of resveratrol synthase from grapevine rendered tobacco (Hain et al., 1993), but not tomato (Thomzik et al., 1997), more resistant to *B. cinerea*. Thus, some of the plant responses to *B. cinerea* promote susceptibility (e.g., abscisic acid and hypersensitive cell death) while others, such as ethylene and jasmonic acid, enhance resistance to gray mold (Thomma et al., 1998; 1999). The relative contributions of disease-promoting and resistance-enhancing plant defense pathways may decide whether or not disease develops.

Wild tomato species are a valuable resource for breeding. Resistance to at least 42 diseases has been identified and over half of the identified resistance factors have been introduced into cultivated tomato (Rick and Chetelat, 1995). *Solanum lycopersicoides* is a tomato-like nightshade that prefers the relatively cool and moist habitats at high elevation in its native habitat on the western slopes of the Andes (Rick, 1988). In greenhouse cultures grown for seed increase at Davis, *S. lycopersicoides* has consistently been free of *B. cinerea* symptoms, suggesting it is resistant. F_1 hybrids of *L. esculentum* × *S. lycopersicoides*, which are readily obtained by embryo culture, were resistant to *B. cinerea* following inoculation of stem cuttings (Chetelat et al., 1997). The strong male-sterility and self-incompatibility of the intergeneric hybrid prevent genetic analysis of *B. cinerea* resistance in conventional F_2 generations. However, through successive backcrosses, individual chromosome segments from *S. lycopersicoides* have been introduced into cultivated tomato (Chetelat and Meglic, 2000; Chetelat et al., 2000). As a result, mapping of *R*-genes from *S. lycopersicoides* in the background of cultivated tomato is now feasible.

This study provides information about the genetic control of resistance to *B. cinerea* in *S. lycopersicoides* by comparing disease phenotypes of an intergeneric hybrid to its parents *S. lycopersicoides* and *L. esculentum*. Confocal microscopy of a green fluorescent protein (GFP)-tagged fungal strain showed that *S. lycopersicoides* inhibits the progression of gray mold infection. This work provides the basis for more detailed examinations of the contributions to tomato defense against *B. cinerea* that are provided by the *S. lycopersicoides* genome.

Materials and methods

Plant material

The parental genotypes used in this study included *L. esculentum* cv. 'VF36', a determinate, open-pollinated variety bred for canning, and the wild species *S. lycopersicoides* accession LA2951, collected at Quistagama, Tarapaca, Chile. An F_1 intergeneric hybrid (plant 90L4178) was obtained by embryo culture, using *S. lycopersicoides* as pollen parent. Hybridity was confirmed by marker analysis, as described previously (Chetelat et al., 1997). The Tomato Genetics Resource Center (TGRC), University of California at Davis (http://tgrc.ucdavis.edu/), provided seeds of the two parental species, and *in vitro*-propagated clones of the intergeneric hybrid.

In addition to the species mentioned above, we tested *L. chilense* (LA1932), *L. cheesmanii* f. *minor* (LA0317), *L. hirsutum* f. *glabratum* (LA1223), *L. peruvianum* (LA1708), *L. pimpinellifolium* (LA1589), and *S. sitiens* (LA1974 and LA2885).

Growth conditions

To break seed dormancy of *S. lycopersicoides*, seeds were germinated according to TGRC recommendations. Seeds were placed on filter paper in petri dishes, treated with 2.7% sodium hypochlorite for 30 min, washed three times with deionized water, and incubated at 25 °C in the dark. One-week-old seedlings were transferred to grower's mix A (Sungrow) and grown in the greenhouse, with regular fertilization using liquid Plantex (7-11-27) with a supplement of 0.088% (w/v) calcium nitrate. Typically, plants were 6–8 weeks of age prior to use in inoculation experiments.

A *B. cinerea* strain isolated from geranium (FP72) expressing GFP (a gift from Walt Mahaffee, USDA-ARS, 3420 NW Orchard Ave., Corvallis, OR 97331, USA) and B05.10 (derived from SAS56; Buettner et al., 1994) were maintained as glycerol stocks. Lorang et al. (2001) cloned GFP behind the strong, constitutive *ToxA* promoter using the plasmid pCT74. Fluorescence was optimized using the S65T substitution and plant-optimized codon usage

(Haseloff et al., 1997). Malt extract or potato dextrose agar was used for fungal growth and sporulation. Conidia were harvested according to published procedures (Benito et al., 1998).

Plant inoculations

Droplet inoculations of detached leaves followed standard procedures (Benito et al., 1998). Essentially, conidia (10^6 ml^{-1}) were incubated in Gamborg's B5 medium containing 10 mM sucrose and 10 mM potassium phosphate (pH 6) for 2–3 h at room temperature prior to inoculation. A total of 9 droplets (1 µl per droplet) were applied to the adaxial surface of leaves of the same chronological age. Four inoculated leaves were inserted in moist florist foam contained in a petri plate and incubated in a clear plastic box under saturating humidity. Infected leaves were kept under a 12 h light/12 h dark regime at a light intensity of 34 µmol m^{-2} s^{-1} using fluorescent white lights. Under these conditions, primary lesions generally developed within 24 h. Lesions expanded after 3 days and disease progression was scored on a daily basis for up to 7 days. Lesions were scored as spreading once they had expanded more than 1 mm beyond the primary 24 h lesion. Measuring lesions on consecutive days increased the reliability of the data. A minimum of eight leaves from at least three plants was used to detect expanding lesions.

Seedlings were spray-inoculated with a conidial suspension (Mahaffee and DiLeone, 1998). Conidia were suspended at a concentration of 10^5 ml^{-1} in sterile water. Plants were sprayed with the conidial suspension until runoff and dusted with dried ground tomato leaves to provide a nutrient-rich substrate for infection. Treated plants were incubated in mist chambers at a relative humidity greater than 85% for 4–7 days. Light conditions were 12 h light/12 h dark at a light intensity of 34 µmol m^{-2} s^{-1}. Disease severity was rated on a scale of 0–3, where 0 = no visible lesions, 1 = discrete lesions, 2 = less than $\frac{2}{3}$ of leaf with lesions, and 3 = greater than $\frac{2}{3}$ of leaf with lesions.

Spore counts

The fresh weight of infected leaves was determined. Conidia were recovered by gently rubbing the leaf surface with 0.05% (v/v) Tween 80. Filtration through glass wool was used to separate spores from mycelia. Conidia were concentrated by centrifugation for 8 min at 120 × g. Resuspended spores were counted using a hemocytometer under a compound microscope.

Fluorescent microscopy

Three different fluorescent microscopes were used for visualizing GFP-tagged *B. cinerea* in infected plant tissues. (1) A Leica DMS DAS (Leica, Mannheim, Germany) equipped with filter blocks with spectral properties matching those of GFP; excitation at 488 nm and emission at 580 nm. In order to improve the resolution of germinating and adhering spores, fine forceps were used to remove epidermal peels from the adaxial side of tomato leaves. (2) A Leica DMRB fluorescent compound microscope with an Endow GFP filter cube; exciter HQ470/40, emitter HQ520/50, and beam splitter Q495LP. (3) A Leica TCS 4D laser scanning confocal microscope equipped with an Omnichrome Ar/Kr laser (Leica, Wetzlar, Germany), excitation at 488/568 nm excitation and emission at 580 nm through a barrier filter, and an INNOVA Enterprise Ion Laser (Coherrent, Santa Clara, CA). Optical sections were digitally processed using Image Pro (Carlsbad, CA) or Photoshop 5.0 (Adobe Corp., Mountain View, CA).

Statistical analysis

Statistical tests included analysis of variance (ANOVA) using the SPSS program package. Differences in gray mold resistance among *Lycopersicon* and *Solanum* species were analyzed using the repeated measures procedure of a general linear model (GLM). Plants were grown in a randomized complete block design. The genotypes were assigned random positions in two flats with 50 wells each. Plants were of the same age and grown under identical environmental conditions in the greenhouse. Eight leaves from a minimum of three plants per accessions were used to compare resistance to *B. cinerea*. Leaves of a similar developmental stage (fourth leaf from the apex) from nine genotypes were assigned random positions in nine clear plastic boxes per experiment. The experiment was repeated. The percentage of lesion expansion on three consecutive days was treated as within-subjects variables; genotypes were treated as between-subjects factors. Whereas the effect of genotype was highly significant ($F_{8,54} = 5.25$; $P < 0.001$), there was no significant effect of blocks ($F_{8,54} = 3.90$; $P = 0.053$). Tukey's test was used for separation of means.

Results

Resistance to B. cinerea

Reproducible comparisons of gray mold resistance in *S. lycopersicoides* and tomato were made possible by devising assays that have revealed contributions of PGIP and phytohormones to this plant–pathogen interaction (Powell et al., 2000; Audenaert et al., 2002; Diaz et al., 2002). Droplet inoculations of excised tomato leaves with *B. cinerea* are known to produce primary lesions within the first day (Benito et al., 1998). A subset of these lesions will enlarge after a quiescent period of ~2 days. The proportion of lesions that expand after 3 days provides a discrete measure of disease susceptibility.

A survey of six *Lycopersicon* and two *Solanum* species revealed substantial variation for resistance to *B. cinerea* (strain B05.10) among wild relatives of cultivated tomato (Figure 1). Based on the accessions tested, *S. lycopersicoides* and *L. esculentum* ranked as the most and least resistant genotypes, respectively. In addition to *S. lycopersicoides*, *L. chilense*, and *L. cheesmanii* were significantly more resistant than tomato, suggesting that additional wild relatives could be exploited to improve resistance of tomato to *B. cinerea*.

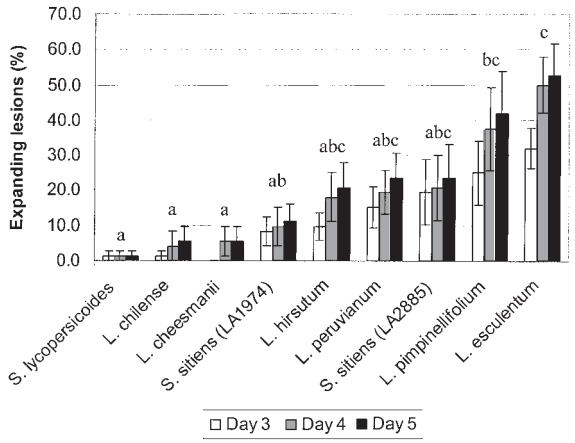


Figure 1. Resistance of different *Solanum* and *Lycopersicon* species to *B. cinerea*. Detached leaves were droplet-inoculated and expanding lesions were scored at the times indicated. Percentages represent the proportion of primary lesions that supported pathogen spread. Accession numbers are indicated for *S. sitiens*. The other accession numbers are *S. lycopersicoides* (LA2951), *L. chilense* (LA1932), *L. cheesmanii* f. *minor* (LA0317), *L. hirsutum* f. *glabratum* (LA1223), *L. peruvianum* (LA1708), *L. pimpinellifolium* (LA1589), and tomato cv. 'VF36'. Data are means and standard errors. Repeated measures procedure of a GLM was used in conjunction with Tukey's test to separate means.

Besides variation between species, there was within species variation for resistance to *B. cinerea* (Figure 2). Three different accessions of *S. lycopersicoides* were compared to cultivated tomato cv. 'VF36'. Whereas accession LA2951 was considerably more resistant than tomato, accessions LA1964 and LA2408 were intermediate in phenotype. In a statistical analysis of the data using the repeated measures procedure of a GLM, the effect of genotype on gray mold infection was highly significant ($F_{3,44} = 8.01$, $P < 0.001$). Spray inoculations of intact seedlings with *B. cinerea* (B05.10) essentially confirmed the results obtained with droplet inoculation of excised leaves. Means and standard errors of disease scores 5 days after spray inoculation of tomato (VF36) and *S. lycopersicoides* (LA2408, LA1964, and LA2951) were 2.87 ± 0.13, 2.11 ± 0.11, 1.56 ± 0.11, and 1.20 ± 0.17, respectively. The same trends were observed in a separate experiment of spray-inoculated seedlings.

Responses of an F_1 intergeneric hybrid and its female *L. esculentum* cv. 'VF36' and male *S. lycopersicoides* (LA2951) parents to gray mold were compared to test the genetic expression (dominance) of resistance. Excised leaves were droplet-inoculated and the

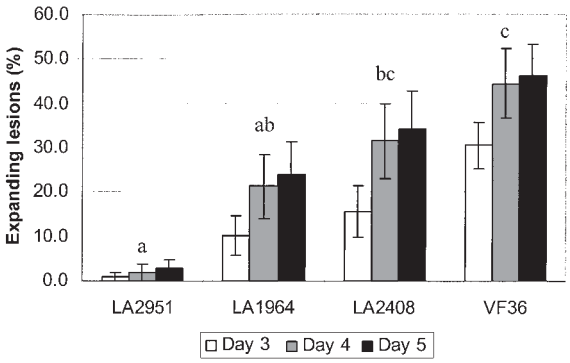


Figure 2. Resistance to *B. cinerea* (B05.10) of detached leaves from *L. esculentum* cv. 'VF36' and three different *S. lycopersicoides* accessions. Percentages represent the proportions of primary lesions that supported pathogen spread. Means and standard errors of 12 leaves (4 leaves times 3 plants) from each accession are shown. Plants were of the same age, and leaves from a comparable developmental stage. Repeated measures procedure of a GLM was used in conjunction with Tukey's test to separate means.

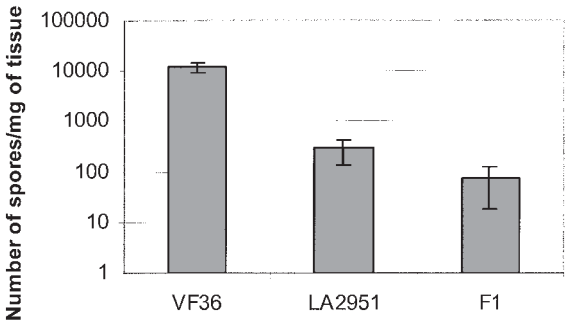


Figure 3. Expression of resistance to *B. cinerea* in an intergeneric hybrid of *L. esculentum* cv. 'VF36' × *S. lycopersicoides* LA2951. Excised leaves were droplet-inoculated and incubated for 1 week, after which spores from each leaf were harvested. Spore counts were determined and means and standard errors of 12 leaves from each parent and the F_1 hybrid are shown. Unlike the parents (3 plants times 4 leaves), hybrid plants were clonally propagated and do not represent genetic variation. Note that the data are logarithmically transformed.

number of spores that were produced on inoculated leaves was counted. *B. cinerea* produced ∼40 times more spores on tomato than on *S. lycopersicoides* (Figure 3). The difference in spore production between *S. lycopersicoides* and the intergeneric hybrid was not significant. In addition, the proportion of lesion expansion was determined 4 days after inoculation. Means and standard errors of lesion expansion in tomato, *S. lycopersicoides*, and the hybrid were 56% ± 7%, 7% ± 3%, and 2% ± 2%, respectively. Both sets of observations suggest that the gray mold resistance of *S. lycopersicoides* is dominant in hybrids with tomato.

Microscopy of B. cinerea *infections*

The morphology of primary lesions differed between *S. lycopersicoides* and tomato. Tomato leaves produced concentric primary lesions (Figure 4a), some of which developed a water-soaked appearance and supported lesion spread (Figure 4b). Conversely, primary lesions of *S. lycopersicoides* appeared dry and dispersed, indicating that *B. cinerea* had problems establishing itself on this wild tomato relative (Figure 4c).

Microscopic analysis of a GFP-tagged *B. cinerea* strain revealed several aspects of infection processes in droplet-inoculated tomato leaves. First indications of fungal germination were observed 12 h after inoculation (Figure 5a and b). Single conidia supported between 1 and 5 sites of germination. Germinating hyphae were firmly attached to leaf surfaces after ∼24 h (Figure 5c). Penetration of plant tissues occurred after about 32 h. Two main routes of fungal penetration were found, either between cells (Figure 5d) or via appressorium formation (Figure 5e). There was evidence for plant cell death in response to penetration (data not shown). In addition, *B. cinerea* infrequently grew through stomatal openings. Hyphal growth occurred on the leaf surface (Figure 5f) as well as within leaf tissues (Figure 6d). *Botrytis cinerea* was able to colonize intercellular spaces and vascular tissues of *L. esculentum*. One week after infection, conidiophores started to form on the leaf surfaces (Figure 5g).

Infection processes were compared between *S. lycopersicoides* (LA2951) and *L. esculentum* cv. 'VF36' to obtain more information about the resistance mechanisms of the wild species. *Botrytis cinerea* germinated at essentially the same rate in both species. Within the first 2 days, 95% and 90% of the conidia germinated on tomato and *S. lycopersicoides*, respectively. There was no difference in hyphal growth during this time period (Figure 6a and b). However, hyphal death and lysis occurred on the third day after *B. cinerea* inoculation of *S. lycopersicoides* (Figure 6c). At the same time, *B. cinerea* continued to grow on and within tomato leaves. Colonization of vascular tissue of tomato leaves may accelerate fungal spread and symptom formation (Figure 6d). Large differences in fungal colonization of each plant species were evident 5 days after inoculation. Whereas only a few surviving hyphae were present on *S. lycopersicoides* leaves (Figure 6e), there was profuse hyphal growth on the tomato host (Figure 6f). As a consequence, tomato leaves supported abundant sporulation 16 days after inoculation (mean and standard error, 5809 ± 1428 spores per mg tissue, $n = 3$) but *S. lycopersicoides* did not (0 spores observed, $n = 3$). The geranium strain used for GPF-tagging was less virulent than *B. cinerea* (B05.10), causing a lower frequency and slower development of infection (data not shown). We detected a total of seven spreading lesions on tomato leaves but only one expanding lesion on *S. lycopersicoides* leaves ($\chi^2 = 4.5$, $P < 0.05$), thus replicating differences in resistance between both species that were established with the B05.10 strain.

Discussion

Wild relatives of tomato are potential resources for introducing resistance against *B. cinerea* into cultivated varieties. A screen using excised leaves of eight wild

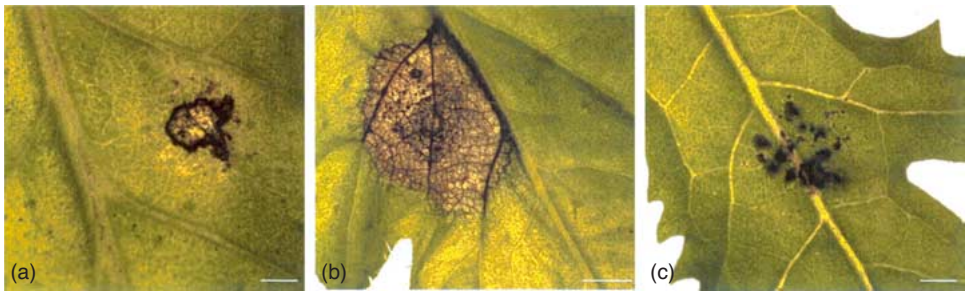


Figure 4

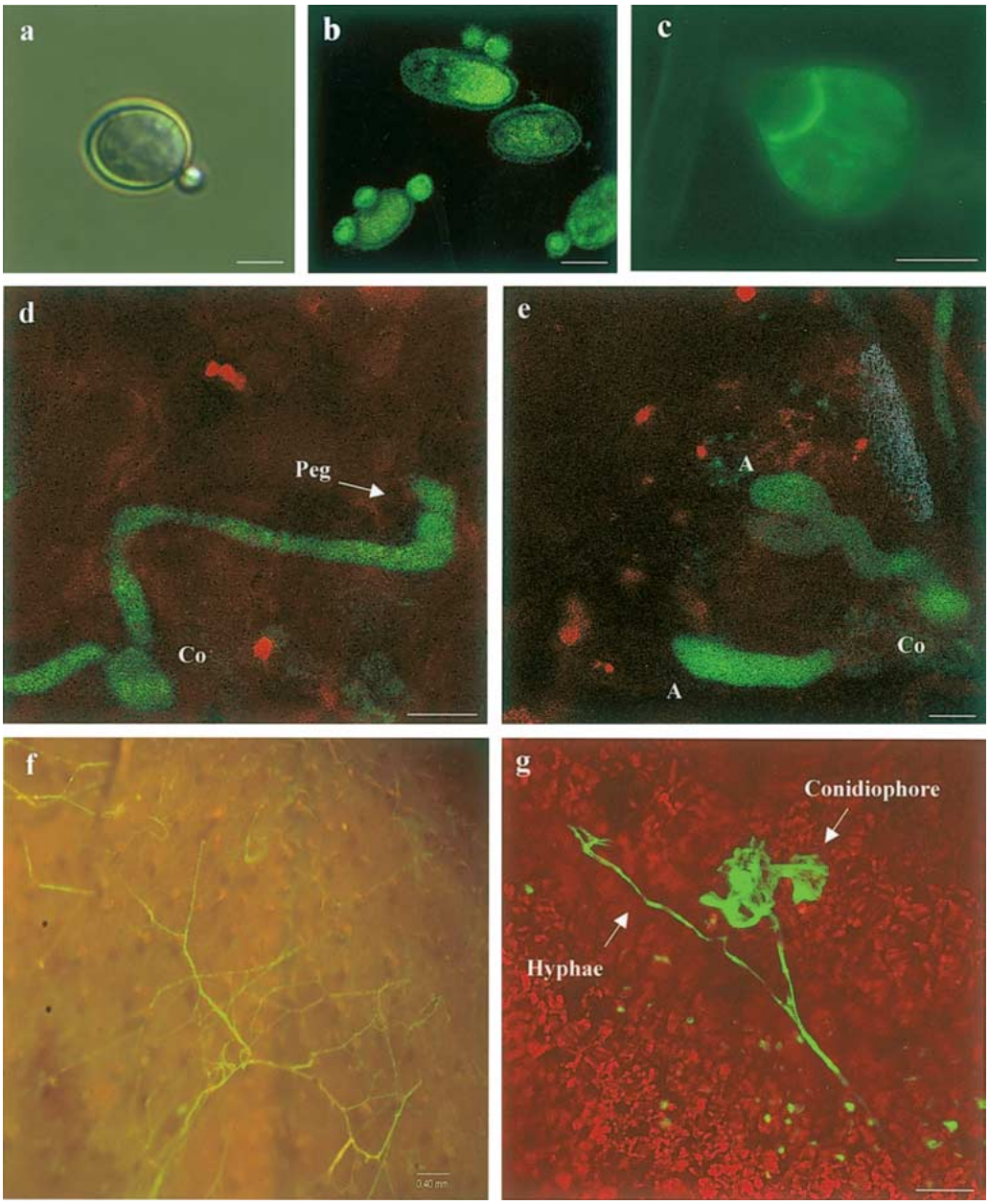


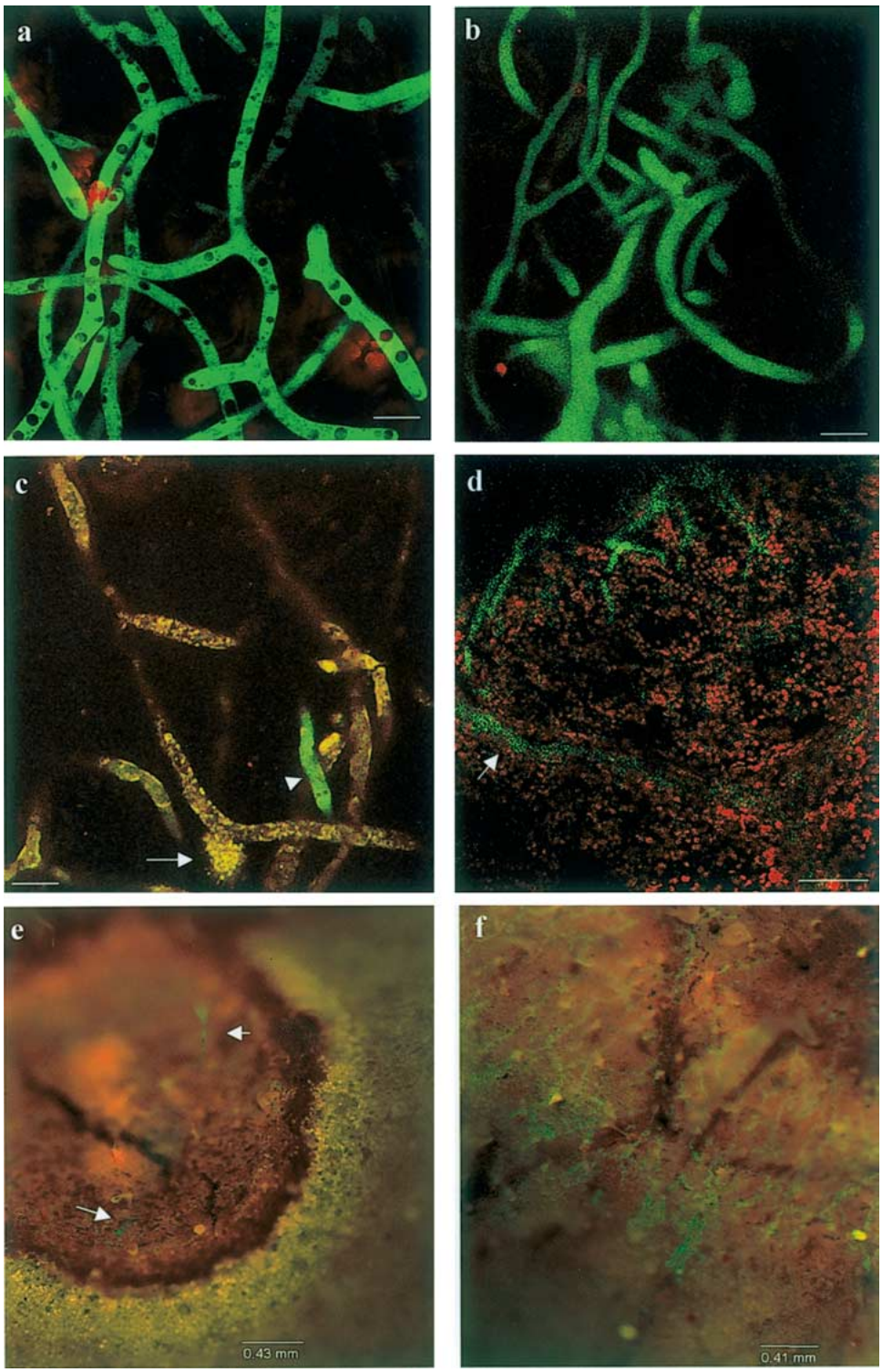
Figure 5

tomato accessions revealed that *S. lycopersicoides*, *L. chilense*, and *L. cheesmanii* are significantly more resistant to *B. cinerea* than cultivated tomato. Significant variation in resistance was also observed among different accessions of *S. lycopersicoides*; LA2951 was the most resistant accession in both excised leaf and whole plant assays. In this context it may be significant that LA2951 was collected at lower latitude, which represents the southernmost distribution of the species, and lower elevation than the other accessions (Chetelat et al., 1997). Environmental conditions, such as temperature, humidity, and slope (north- vs. south-facing) undoubtedly differ between collection sites, and may favor development of *Botrytis* to varying degrees, thus resulting in different selection pressures. In addition, browsing by herbivores may be a significant environmental variable, as the wound sites would facilitate penetration by *B. cinerea* hyphae. Grazing of animals such as llamas, alpacas, goats, and sheep, is common in the highlands of N. Chile and S. Peru and threatens the survival of local populations of wild tomatoes, including *S. lycopersicoides*. With respect to genetic diversity, all three accessions are self-incompatible and relatively variable. Using a set of 28 isozyme markers, these *S. lycopersicoides* accessions were polymorphic at an average of ~43% of loci, and contained an average of ~1.68 alleles per locus (Chetelat, unpublished data). This level of within population polymorphism is similar to that reported for allogamous (self-incompatible) populations of other tomato species, such as *L. pennellii* and *L. hirsutum* (Rick et al., 1979; Rick and Tanksley, 1981). Therefore, variation in the level of resistance to *B. cinerea* within, as well as between, populations of *S. lycopersicoides* can be expected.

Previous publications indicated variation in gray mold resistance among *Lycopersicon* and *Solanum* species (Urbasch, 1986; Egashira et al., 2000; Nicot et al., 2002). *Lycopersicon hirsutum* and *L. peruvianum* were more resistant than tomato after inoculation of wounded petioles (Nicot et al., 2002). Leaf colonization of spores released from infected stems was also evaluated, and *L. chilense* (LA1969) and *L. chmielewskii* performed better than tomato. The susceptibility of leaves and stems was not significantly correlated, suggesting that gray mold infection depends on the host tissue (Egashira et al., 2000; Nicot et al., 2002). In addition, plant development, environmental conditions, and fungal strains influence interactions between *B. cinerea* and its hosts. Despite the contribution of various factors on gray mold infections, several wild tomato relatives are significantly more resistant than commercial tomatoes and a potential source for crop improvement. *Solanum lycopersicoides* is a promising genetic resource because its resistance to *B. cinerea* is an order of magnitude higher than tomato.

As a first step toward exploring resistant genes from wild accessions, we compared the gray mold resistance of an intergeneric hybrid to its *L. esculentum* and *S. lycopersicoides* parents. This particular hybrid (plant 90L4178) has been used to transfer the genome of *S. lycopersicoides* into the background of cultivated tomato through a series of introgression lines (ILs), which contain chromosome segments of the nightshade in *L. esculentum* (Chetelat and Meglic, 2000; Chetelat et al., 2000). Evaluation of the ILs for response to *B. cinerea* should provide information on the number of genes controlling resistance, and their gene action (dominance) in a tomato background. Because *S. lycopersicoides* is a self-incompatible, outcrossing species, it is genetically heterogeneous, and variation within populations for traits such as disease resistance can be expected. Therefore, the F_1 hybrid we tested (clones of a single individual) may not be representative of all other such hybrids. However, all tested individuals of *S. lycopersicoides* were consistently more resistant than tomato, suggesting that resistance to *B. cinerea* exists within this nightshade

Figures 4 and 5. (4). Primary lesion phenotype in response to *B. cinerea* infections. *L. esculentum* supports (a) concentric necroses that can result in (b) lesion expansion. (c) In contrast, *S. lycopersicoides* produces dispersed lesions that frequently confine *B. cinerea* without further development of disease symptoms. Pictures were taken 3 days post inoculation. Scale bars represent 2 mm. *(5)*. Growth and development of GFP-tagged *B. cinerea* on tomato leaves. (a) DIC image of a conidium 12 h after inoculation. (b) Confocal micrograph of germinating conidia 12 h after inoculation. (c) Fluorescent micrograph of a conidium attached to an epidermal peel 24 h after inoculation. (d) Confocal micrograph of germ tube penetrating between adjacent cells 32 h after inoculation. This image is a composite of 3 Z-sections each of them 10 μm apart. Co: conidia, arrow indicates penetration peg. (e) Confocal micrograph of a germ tube forming appressoria, 32 h after inoculation; A: appressorium, Co: conidia. (f) Fluorescent micrograph of hyphae growing on the surface of tomato leaves 5 days after inoculation. (g) Confocal micrograph of hyphae forming a conidiophore 7 days after inoculation. This image is a composite of 3 Z-sections each of them 10 μm apart. Fluorescent micrographs of conidiophores have been published previously (Lorang et al., 2001). Scale bars represent 5 μm (a, b, and c), 10 μm (d and e), 0.4 mm (f), and 100 μm (g).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species. Moreover, a sesquidiploid F_1 hybrid, consisting of two genomes of *L. esculentum* cv. 'UC82B' and one genome of *S. lycopersicoides* (LA1964), also displayed enhanced resistance to *B. cinerea* (two isolates) in vegetative tissues (Chetelat et al., 1997). These observations provide further evidence that resistance to *B. cinerea* is widespread in *S. lycopersicoides*, and that its expression is dominant in hybrids with tomato.

In agreement with previous work, single conidia of the GFP-labeled strain formed 1–5 germ tubes (Coley-Smith et al., 1980; Salinas and Verhoeff, 1995). Penetration occurred most commonly through anticlinal wall junctions between adjacent epidermal cells or by penetration of epidermal cells. Penetration pegs and appressoria were frequently observed. Penetration via stomatal openings was less frequent. These modes of germination and penetration are similar to those of *Botrytis* following inoculation in the presence of aqueous glucose (Cole et al., 1996). These environmental conditions favor the formation of elongated germ tubes and appressoria as illustrated in the review by Prins et al. (2000). Low spore concentrations result in preferential penetration of guard cells or stomatal openings when lily leaves are infected with *B. elliptica* (Doss et al., 1988). In contrast, inoculation by dusting with dry conidia leads to the formation of short germ tubes without specialized penetration structures (Cole et al., 1996). Thus, inoculation conditions appear to have a greater influence on germination and penetration structures than either *Botrytis* or host species (Cole et al., 1996).

Microscopic studies of a GFP-tagged *B. cinerea* strain in infected *L. esculentum* and *S. lycopersicoides* leaves revealed no differences in spore germination. *Botrytis cinerea* penetrated both plant species and caused host cell death. The major difference in growth on tomato and *S. lycopersicoides* was an induction of extensive hyphal death 3 days after inoculation of the wild nightshade. Penetration of *S. lycopersicoides* by *B. cinerea* resulted in fewer, smaller and more dispersed necrotic lesions than in tomato with little or no survival of hyphae. In contrast, tomato supported penetrations that resulted in large necrotic lesions that coalesced into a single large primary lesion. Approximately half of these primary lesions developed a water-soaked appearance and subsequently spread. Primary lesions, which did not develop into spreading lesions, showed some extent of hyphal degradation but there were more surviving hyphae than in *S. lycopersicoides*. The development of B05.10 and GFP-tagged *B. cinerea* strains on tomato slowed after initial lesion formation, perhaps reflected by the slight decrease in fungal actin expression after 3 days reported by Benito et al. (1998). They reported, however, that there is a rapid increase in fungal actin mRNA and biomass 4 days after inoculation, indicating resumption of rapid fungal growth we observed in this study. In contrast, fungal growth and reproduction were severely reduced in *S. lycopersicoides*, resulting in less than 3% of the spore production observed on tomato.

The nature of the resistance in *S. lycopersicoides* is not known but hyphal death and lysis point towards the potential involvement of induced antifungal compounds. Possible mediators of resistance include chitinases, $\beta(1 \rightarrow 3)$ glucanases, osmotins, antimicrobial peptides, phytoalexins, and glycoalkaloids. Chitinases and $\beta(1 \rightarrow 3)$ glucanases, in particular, might be important because these enzymes degrade fungal cell walls and some of them induce hyphal lysis *in vitro* (Sela-Buurlage et al., 1993; Ji and Kuc, 1996). Moreover, a basic chitinase and an osmotin from ripening grape fruits (Salzman et al., 1998) as well as 5 kDa peptides from bean seeds specifically inhibit *B. cinerea* mycelial growth *in vitro* (Ye and Ng, 2001). Evolutionary data provide additional evidence for a role of these types of proteins in defense because class I chitinases of Solanaceae are under intense positive selection pressure, perhaps a reflection of 'arms-races' with pathogens (Bishop et al., 2000).

Solanum lycopersicoides and tomato differ in primary lesion development. These differences in regulation of plant cell death are reminiscent of lesion mimics (Dangl et al., 1996). The *S. lycopersicoides* phenotype may be analogous to initiation class mutants because lesions are scattered and determinate in size. Conversely, the tomato phenotype may be related to

Figure 6. Infection of (a, c, and e) resistant *S. lycopersicoides* (LA2951) and (b, d, and f) susceptible *L. esculentum* cv. 'VF36' with a GFP-tagged *B. cinerea* strain. Confocal images were taken 48 (a and b) and 72 h (c and d) after inoculation. Fluorescent micrographs were taken 5 days (e and f) after inoculation. Both plant species supported hyphal growth during the first 2 days. *Solanum lycopersicoides* limited hyphal growth beyond day 3 after inoculation. (c) Yellow fluorescence indicates hyphal death. The arrow indicates hyphal lysis. The arrowhead indicates a surviving hypha that fluoresces green. (d) Arrow indicates that *B. cinerea* infects vascular traces. (e) Arrows indicate surviving hyphae. Scale bars represent 10 μm (a, b, and c), 100 μm (d), and 0.4 mm (e and f).

propagation class mutants because lesion expansion is less constrained than in the case of *S. lycopersicoides*. *Botrytis cinerea* probably profits from a single spreading lesion because, being a necrotroph, it thrives on dead tissue. Moreover, hypersensitive cell death and reactive oxygen species make hosts more vulnerable to gray mold attack (Govrin and Levine, 2000). In conclusion, multiple and small lesions may generate a larger area of plant defenses than a single large lesion in addition to providing less dead substrate for survival of *B. cinerea*. We do not yet know the nature of resistance in *S. lycopersicoides* but we cannot exclude the possibility of an *R*-gene-like mechanism. Inactivation of a fungal toxin is another plausible mechanism of resistance. Alternatively, resistance to *B. cinerea* may be based on an entirely new mechanism.

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