Chapter 7

# Relations of *Cercospora beticola* with Host Plants and Fungal Antagonists

**Robert T. Lartey, Soumitra Ghoshroy, TheCan Caesar-TonThat, Andrew W. Lenssen, and Robert G. Evans**

Cercospora leaf spot (CLS) caused by *Cercospora beticola* Sacc., is still considered to be the most important foliar disease of sugar beet. The disease has been reported wherever sugar beet is grown (Bleiholder and Weltzien 1972). Since the disease was first identified, management of CLS of sugar beet has been an ongoing mission of plant pathologists. Today, several strategies are available and applied either singly or in combination to manage the disease. These management strategies, which were summarized by Windels et al. (1998), include cultural practices such as deep tillage, rotation with non-host crops and identification and elimination of secondary weed hosts. Others include breeding and use of resistant cultivars and application of fungicide. The use of resistant sugar beet cultivars has long been an integral part of CLS management; however, problems associated with selection of resistant cultivars against CLS are well documented and were recently reviewed by Weiland and Koch (2004). According to the authors, resistance to CLS in sugar beet has been described as quantitatively inherited and rate limiting with respect to disease development (Smith and Gaskill 1970, Rossi et al. 1999). Although resistant cultivars have proven effective in both North America and Europe, they nonetheless exhibit low heritability (Smith and Ruppel 1974), and cultivars bred for *Cercospora* resistance can still exhibit leaf spots if climatic conditions favorable for the disease occur.

Early attempts at chemical control of CLS focused on the application of Bordeaux mixture, a mixture of copper sulfate ($CuSO_4$) and hydrated lime. Currently, different classes of fungicides applied to manage CLS include benzimidazoles [thiophanate methyl (Topsin M)], sterol biosynthesis inhibitors [tetraconazole (Eminent)], protectant fungicides such as [TPTH (SuperTin, AgriTin), mancozeb (Dithane M45, Penncozeb)], or stobilurin fungicides [trifloxystrobin (Gem) and azoxystrobin (Quadris)]. While applications of fungicides have proven effective for the management of CLS, rapid development of resistance by *C. beticola* against several fungicides has reinforced the need to develop and implement other management strategies. More recently, models have been developed to increase efficiency in chemical control and manage development of resistance against fungicides (Windels et al. 1998, Wolf et al. 2004).

There have also been some attempts in recent years to control CLS with biological agents (Bargabus et al. 2002, Jacobsen et al. 2004). However, these promising endeavors are still in their early stages. Reacting to frustrations with the lack of success in using biological control for plant diseases sixty years after the first attempt by Hartley in 1921, Katan (1981) stressed the need to reevaluate the whole concept of biological control. Baker (1987) suggested the need to acquire more information on mechanisms to achieve efficient

biological control. A crucial requirement for developing a comprehensive management system is understanding the interactions between the host, the pathogen and the environment that includes potential antagonists (Lartey and Conway 2004).

In this chapter, we present results of ongoing work on interactions between host plants and *C. beticola* as a step toward developing a new comprehensive management system for CLS. This new management system is based on: 1) Rapid detection, identification and subsequent elimination of alternate hosts; 2) Critical examination of interactions between the pathogen and host plants to understand the basis for resistance against *C. beticola* and 3) Examination and understanding of the basis for antagonism by potential biological agents to develop direct control or elimination of inoculum from target sources.

## Interaction Between *Cercospora beticola* and Host Plants

### Host range and survival of *Cercospora beticola*

In addition to sugar beet, *C. beticola* causes leaf spot lesions on most *Beta* spp. such as red garden beets, Swiss Chard and Mangel-wurzel (McKay and Pool 1918). Other previously reported hosts are species of *Atriplex*, *Cycloloma* and *Chenopodium* (Chenopodiaceae) and *Amaranthus* (Amaranthaceae). Specifically, several weeds were described by Vestal (1931) as susceptible to *C. beticola*. These include *Chenopodium album* L., *Amaranthus retroflexus* L., *Malva rotundifolia* L., *Plantago major* L., *Arctium lappa* L. and *Lactuca sativa* L. In recent years other common weeds such as bindweed (*Convolvulus arvensis* L.) (Windels et al. 1998), winged pigweed *Cycloloma atriplicifolium* (Spreng) Coult, wild buckwheat *Polygonum convolvulus* L, and common unicorn plant *Proboscidea louisianica* (P. Mill.) Thellung (Jacobsen et al. 2000) have been named as hosts of C. *beticola*.

Nagel (1938) documented that *C. beticola* could survive in soil for 20 months. More recently, Khan et al. (2008) provided additional evidence that *C. beticola* could survive in soil for up to 22 months. The natural inoculum of *C. beticola* in a sugar beet field begins as stromata in infected beet residue debris where the pathogen overwinters (Windels et al. 1998). According to the authors, the severity of CLS varies from year to year, depending upon weather condition and effectiveness of disease control. Under favorable conditions which are characterized by relatively high humidity or heavy dew, conidiophores and conidia are produced on the stromata and are carried to host leaves by wind or water to initiate infection (Ruppel 1986). As stated earlier, management of CLS thus focuses on timely application of different classes of fungicides in addition to the use of resistant cultivars, cultural practices in rotation with non-host crops, elimination of weed hosts and deep plowing of beet residues after harvest.

Secondary hosts and surviving propagules in the soil thus play a major role as sources of inoculum and occurrence of CLS under favorable environmental conditions. Understanding variations in interactions of the pathogen with secondary hosts and sugar beet may improve understanding of and serve as a basis for developing resistant cultivars against CLS. In addition, interactions with antagonists could form the basis for biological control strategies to manage inoculum of *C. beticola* in the survival stage.

### Protocol for rapid detection of *Cercospora beticola* in plant hosts

A PCR protocol was developed for the rapid detection and identification of *C. beticola* in infected sugar beet, other crops and weed hosts (Lartey et al. 2003). Templates are prepared using a modification of N-Amp Plant PCR Kits (Sigma Chemical Co. St. Louis, MO) protocol. For total DNA extraction, leaf disks from lesions of infected plants are homogenized in extraction solution, incubated, and extracted DNA is subjected to PCR for amplification.

The PCR reaction is carried out in Extract-N-Amp PCR mix containing buffer, salts, dNTPs, Taq polymerase and TaqStart antibody. The reaction is primed respectively with forward and reverse primers consisting of CBACTIN959L (5′ AGCACAGTATCATGATTGGTATGG 3′) and CBACTIN959R (5′ CACTGATCCAGACGGAGTACTTG 3′), designed to amplify about 959 bp fragment of *C. beticola* actin gene sequence. Furthermore, the primers ITS1 5′ TCCGTAGGTGAACCTGCGG 3′ and ITS4 5′ TCCTCCGCTTATTGATATGC 3′ (Weiland and Sundsbak 2000) also are used to amplify an additional segment of the actin gene as an added confirmation. Amplifications are carried out using MasterCycler gradient thermocycler (Eppendorf Scientific Inc., Westbury, NY). The amplified PCR products are resolved by electrophoresis in appropriate gels.

## Safflower: A new host of *Cercospora beticola*

Until recently, safflower *Carthamus tinctorius* L., an annual, broadleaf oilseed and forage crop adapted to the small-grain production areas of the Lower Yellowstone River Valley (LYRV), was not considered a host of *C. beticola*. The only previously identified *Cercospora* species that infected safflower was *Cercospora carthami* Sund and Ramak. CLS of safflower, caused by *C. carthami*, was first reported in India in 1924 and has since been reported in other old world countries such as Israel, Iran and Pakistan (Ashri 1971). Appearance of unusual spots on safflower in the LYRV prompted us to examine safflower as a potential host of *C. beticola*.

Using the previously described PCR protocol, Lartey et al. (2005a,b) documented and identified safflower as a host of *C. beticola* causing leaf spot of safflower. Isolates C1 and C2 of *C. beticola* (Whitney and Lewellen 1976) and Sid1 and Sid2 which were isolated from infected sugar beet at Sidney, MT by Anthony J. Caesar (ARS/NPARL, Sidney, MT) were used for the study. Inocula from each of the tested isolates were used to spray inoculate safflower plants at the 4–6 leaf stage. Inoculated plants were first incubated under 90% minimum relative humidity (RH) and 8 h photoperiod at 32°C for 3–4 days. Plants were then transferred to a growth chamber and maintained at a 60% minimum RH, 8 h photoperiod and 26°C. Inoculated plants were observed daily for symptoms and subsequently assayed for *C. beticola* infection by PCR as previously described.

Between 2–3 weeks after inoculation with each of the four *C. beticola* isolates, the first leaf spot symptoms were observed on safflower plants (Fig. 1Aa). Symptoms appeared as round to irregular light brown to black spots without splits openings, frequently with dark brown to black borders. With the aid of a dissecting scope or microscope at a low magnification (10×), dark brown to black fructifications of the pathogen could be observed in some of the leaf spot lesions. Uninoculated control safflower plants did not develop leaf spot symptoms (Fig. 1Ab).

Figure 1B illustrates the results of PCR detection of *C. beticola* in infected safflower tissues. Both blank control (lane 2) and uninfected safflower (lane 3) did not reveal any amplification by the actin primers. An ITS amplicon from the control uninfected plant was detectable in lane 4. Using *C. beticola* actin and ITS primers, two fragments were detected in the control *C. beticola* culture. They are presented in lane 5 from the actin fragment and lane 6 for ITS primers amplicon. From infected safflower lesions, expected fragments of all tested *C. beticola* isolates (C1, C2, Sid1, and Sid2) were amplified using *C. beticola* actin primers CBACTIN959L and CBABTIN959R (lanes 7, 9, 11, and 13 respectively). The amplicons of about 1 Kbp correspond to the control amplicon in lane 5. Two sets of ITS primers-based amplicons were also amplified and are presented in lanes 8, 10, 12, and 14, respectively. The upper fragments correspond to the control uninfected plant ITS in lane 4, and the lower to the ITS amplicon from the *C. beticola* control culture in lane 6.

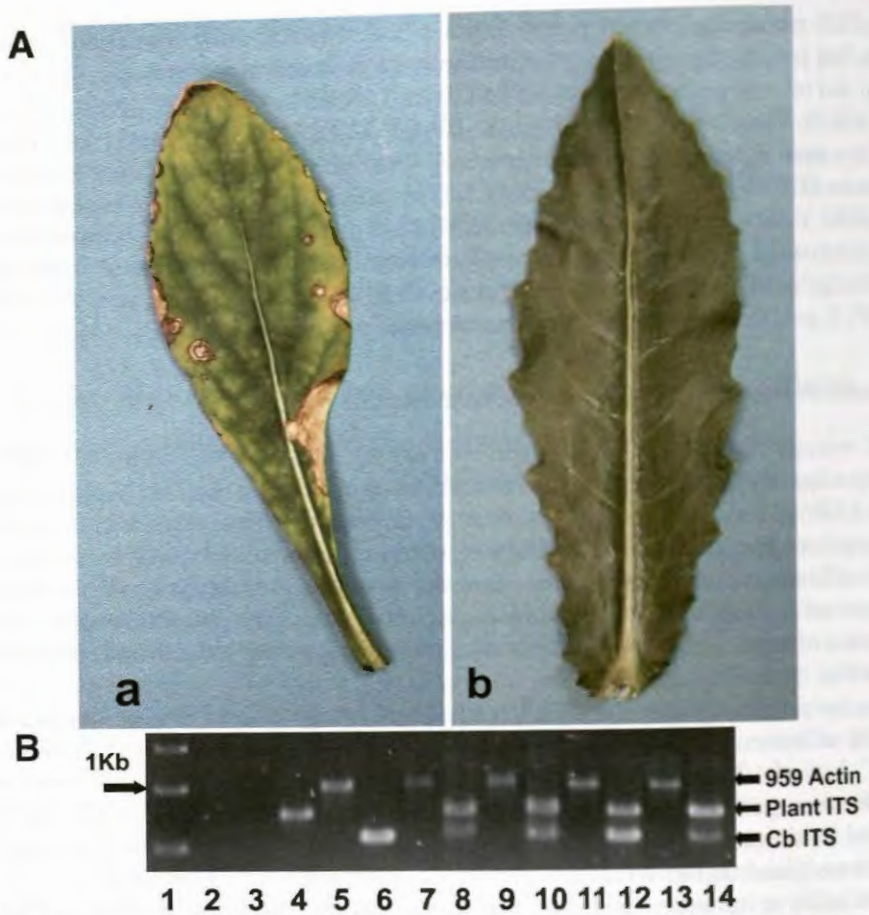


**Fig. 1.** (Aa) Diseased safflower leaf after infection with *Cercospora beticola* (C2). Lesions are characterized by light brown round to irregular spots. The dark brown to black border is noticeable around some of the disease lesions which were positively shown to contain *C. beticola*. (Ab) Healthy safflower leaf. (B) Detection of *Cercospora beticola* in infected safflower by PCR. Direct detection by amplification of *C. beticola* segments from lesion leaf tissues with actin specific (lanes: 3, 5, 7, 9, 11, and 13) and ITS (lanes: 4, 6, 8, 10, 12, and 14) primers. 1 = 1KB Ladder; 2 = Blank control; 3 and 4 = Uninfected safflower control; 5 and 6 = Sid1 control culture; 7 and 8 = C1 from infected leaf lesion; 9 and 10 = Sid1 from infected leaf lesion; 11 and 12 = C2 from infected leaf lesion; 13 and 14 = Sid2 from infected leaf lesion. (Reproduced with permission from Lartey et al. 2005a)

## Isolation of *C. beticola* from safflower and infection assay in sugar beet

Single spores of each of the four *C. beticola* isolates were isolated from infected safflower and cultured on PDA plates as previously described. Inocula were produced and were then used to infect sugar beet plants. The plants were first maintained under high humidity and then transferred to a chamber as described for safflower. After development of disease symptoms, infected sugar beet leaves were examined with a low power microscope for pseudostromata development and the lesions were assayed for *C. beticola* by PCR as previously described.

As observed with safflower, symptoms of CLS were observed between 2–3 weeks after inoculation of sugar beet. All four isolates of *C. beticola* caused symptoms which appeared as round to irregular light brown spots with dark brown to black borders, frequently with

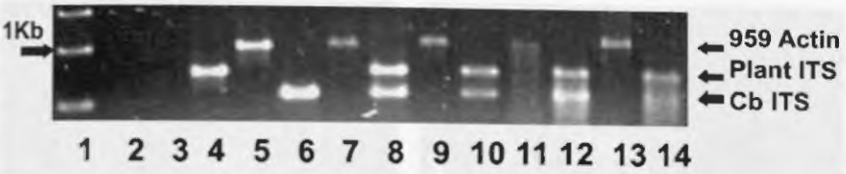


**Fig. 2.** Detection of *Cercospora beticola* in infected sugar beet by PCR with actin specific (lanes: 3, 5, 7, 9, and 11) and ITS (lanes: 4, 6, 8, 10, and 12) primers. 1 = 1KB Ladder; 2 = Blank control; 3 and 4 = Uninfected sugar beet control; 5 and 6 = Sid1 control culture; 7 and 8 = C1 from infected leaf lesion; 9 and 10 = Sid1 from infected leaf lesion; 11 and 12 = C2 from infected leaf lesion; 13 and 14 = Sid2 from infected leaf lesion. (Reproduced with permission from Lartey et al. 2005a)

splits of various sizes in the lesions. Pseudostromata occasionally were evident in the lesions. The uninoculated sugar beet plants did not develop the leaf spot symptoms.

Results of the PCR-based confirmation of *C. beticola* infection of sugar beet are presented in Figure 2. As with safflower, blank control and uninoculated sugar beet in lanes 2 and 3, respectively, did not reveal amplification with the actin primers. From uninfected sugar beet, however, a single ITS fragment was amplified and detected in lane 4. Amplicons also were amplified from the control *C. beticola* culture by *C. beticola* actin primers CBACTIN959L and CBABTIN959R and by the ITS primers in lane 5 and 6, respectively. As with safflower, samples from sugar beet lesions infected by all isolates, C1, C2, Sid1, and Sid2, produced positive fragments from the actin primers in lanes 7, 9, 11, and 13, respectively. The 1 Kbp amplicon corresponded to the control fragment in lane 5. Additionally, two positive ITS amplicons were also amplified in lanes 8, 10, 12, and 14, respectively. The upper fragment corresponds to the uninoculated sugar beet ITS in lane 4 and the lower to the ITS amplicon from the *C. beticola* control culture in lane 6.

## Interaction of *Cercospora beticola* with sugar beet and safflower

The interaction between *C. beticola* and the host plants was investigated recently by Lartey et al. (2007). Sample leaves from inoculated and uninoculated safflower and sugar beet plants were harvested every three days and examined for disease and pathogen development. The freshly harvested leaves were observed under scanning electron microscope (SEM) operated under variable pressure mode using Hitachi 3200N (Hitachi High Tech., Pleasanton, CA). Specimens were observed using a backscattered electron detector. Additionally, leaves from safflower and sugar beet were also fixed with glutaraldehyde, post fixed in osmium tetroxide, dehydrated in ethanol series, critically dried using a Ladd critical point dryer, and sputter coated with gold using a Desk II sputter coater (Denton Vacuum, Moorestown NJ) before viewing under SEM. Images were captured digitally for subsequent analysis.

Using the scanning electron microscopic, a comparison of interactions between *C. beticola* and the two different host crops showed some similarities in infection process between the two crops. Figure 3 shows *C. beticola* infection of safflower and sugar beet under SEM. Following growth on the leaf surface, hyphal strands were observed entering into stomatal openings of safflower (Fig. 3A). The hyphal penetration of safflower through the stomata is consistent with previous observations in sugar beet (Rathaiah 1977). However, the entry of *C. beticola* through stomatal opening of safflower did not appear to follow a particular pattern as some hyphal strands grew over some stomatal openings without apparent entry.

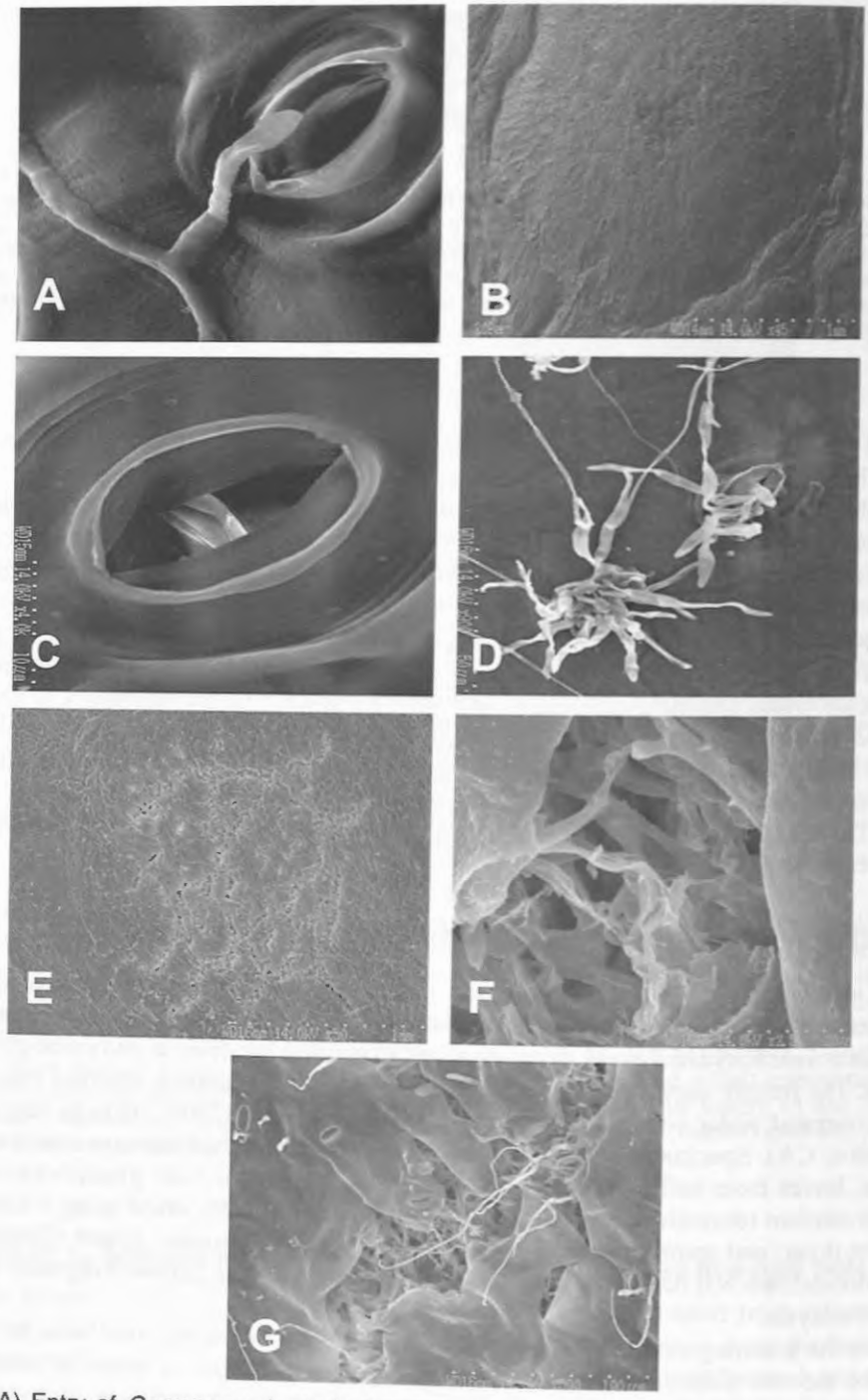


**Fig. 3.** (A) Entry of *Cercospora beticola* through stomatal opening of a safflower leaf. (B) Lesions of leaf spot Cercospora after infection of safflower with *C. beticola.* (Notice absence of splits in safflower lesion.) (C) Hypha of *C. beticola* in a stomatal opening of safflower lesions. (D) Emerged hyphal structures of *C. beticola* from stomatal openings of safflower lesion. (E) Lesions of Cercospora leaf spot of sugar beet. (Notice the presence of splits in sugar beet lesion.) (F) Hyphal strands of *C. beticola* in splits within the sugar beet lesions. (G) Emerged hyphal strands of *C. beticola* from split openings of sugar beet lesion. (Reproduced with permission from Larley et al. 2007)

Stomatal entry was followed by development of leaf spot lesions in the infected leaves. No sign of the pathogen was observed in the early stages of lesion development which first appeared about two weeks post inoculation. The SEM examination did not show presence of splits in lesions of safflower tissues (Fig. 3B). Additional examination showed hyphae of *C. beticola* in stomatal openings of safflower lesions (Fig. 3C). Finally, the pathogen reemerged only through the stomatal openings within the safflower leaf lesions (Fig. 3D).

In sugar beet, lesions also appeared about two week after inoculation. However, the SEM examination of the surface of the lesions in sugar beet exhibited deep splits (Fig. 3E). Further examination of the splits within the lesions of sugar beet revealed dense hyphal mass (Fig. 3F). Similar to safflower, emerging hyphal structures of *C. beticola* were observed from lesions of sugar beet. However, emerging young hyphae were also observed piercing tissues near and away from the stomatal openings within the sugar beet lesions in addition to stomatal openings, (Fig. 3G). Eventually, the emerged pathogen appeared as hyphal mass in both safflower and sugar beet lesions.

## Interaction Between *Cercospora beticola* and Fungal Antagonists

Research on biological control of *C. beticola* to manage CLS of sugar beet has been rare. However, in one study, Jacobsen et al. (2004) integrated *Bacillus mycoides* Flügge isolate Bac J as a foliar antagonist with fungicide and successfully controlled *C. beticola* and CLS. The application of a foliar antagonist to manage *C. beticola* is consistent with recognition of CLS as a foliar disease. The following fungal antagonists were also examined for their potential to suppress *C. beticola* and CLS. The selected agents include three species of *Trichoderma*, several of which have previously been studied and applied for management of other diseases. The fourth agent, *Laetisaria arvalis* Burdsall, was first isolated by Boosalis from sugar beet fields in Nebraska in 1960 and has since been shown to control several plant pathogens. Recently, Lartey (2006) suggested the need to understand the interaction between the potential antagonist and the target pathogen as the basis for developing and using biological agents to manage the pathogen. Thus, interactions between *C. beticola* and these potential agents could serve as a basis for biological control of CLS.

### Interaction with *Trichoderma* species

For decades, *Trichoderma* species have been studied for biological control of various plant pathogens, particularly soil borne pathogens (Weindling 1932, Cook 1993, Harman and Kubicek 1998). While *Trichoderma harzianum* Rifai, remains the most widely studied, the potential of other *Trichoderma* species have increasingly received significant attention.

Three *Trichoderma* species, *T. harzianum*, *Trichoderma virens* (Miller et al.) and *Trichoderma aureoviride* Rifai were evaluated as potential biological agents as an integral part of developing a comprehensive management system against *C. beticola*. In the preliminary standard antibiosis test to evaluate inhibition of *C. beticola*, four *C. beticola* isolates C1, C2, Sid1, and Sid2 were challenged with the three *Trichoderma* species on Potato Dextrose Agar (PDA) at 25°C (Johnson and Curl 1972). Mycelial discs of *C. beticola* and each of the three *Trichoderma* species were positioned simultaneously, approximately 7 cm apart on PDA plates. Controls consisted of unchallenged *C. beticola* mycelial discs that were positioned simultaneously at the center of the plates. Contact between isolates of the pathogen and the *Trichoderma* spp were established between four and five days after transfer of the antagonists to the plates. Figure 4a shows that the colony size of the challenged *C. beticola* (isolate C2) in presence of *T. harzianum* was notably smaller than the

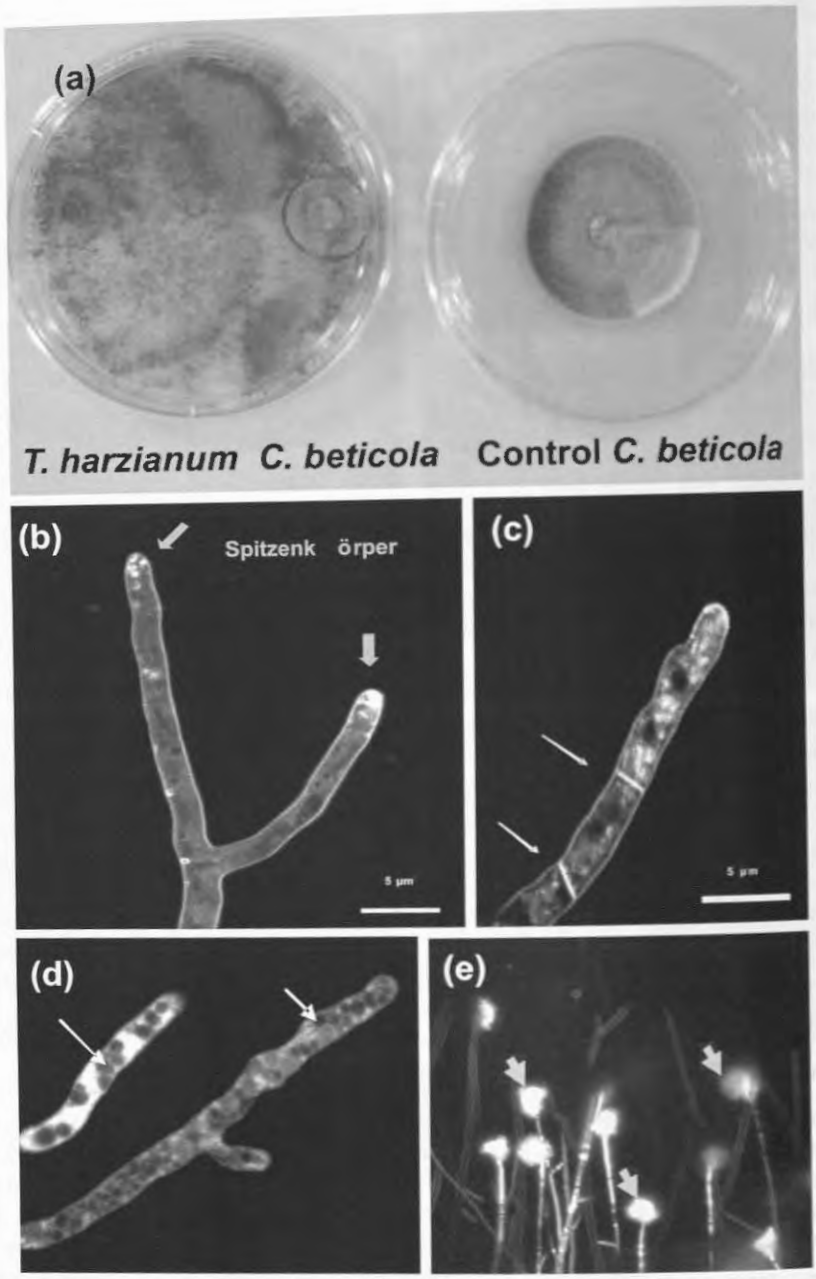


**Fig. 4.** (a) Inhibition of *Cercospora beticola* by *Trichoderma harzianum*. Colony plugs from fungal cultures were transferred simultaneously to potato dextrose agar (PDA) and were incubated for 15 days. Growth of *C. beticola* was severely inhibited as *T. harzianum* overwhelmed *C. beticola* completely growing over the colony. Control *C. beticola* colony was significantly larger than the inhibited colony. Confocal laser scanning microscopy (CLSM) revealed an interactive mechanism between *C. beticola* and each tested antagonism. (b) The presence of apical vesicle cluster (Spitzenkörper) (arrows) at the apical tip of *C. beticola* after staining with FM4-64 staining indicates a healthy and unchallenged growth pattern using CLSM. (c) Challenge of *C. beticola* with *T. harzianum* provokes morphological changes at the hyphal growing tip; disruption of the Spitzenkörper and appearance of septa (arrows) at the subapical region and (d) Intense vacuolization dead *C. beticola* hyphae were indiced by *T. virens*. (e) *T. aureoviride* induced release of cytoplasmic materials from burst hyphal tips, resulting in cell death. No conclusive changes were detected with *T. harzianum*.

unchallenged control after 10 days incubation. *T. aureoviride* and *T. virens* also significantly inhibited growth of all tested *C. beticola* isolates (data not shown).

Microscopy was used to study morphological changes of *C. beticola* (isolate C2) caused by *T. harzianum*, *T. virens* and *T. aureoviride* (Fig. 4b–d). A two-week culture of *C. beticola* grown on slides coated with a film of potato dextrose agar medium was challenged with three species of *Trichoderma* by placing an inoculum plug 2.3 cm away from the growing tips of *C. beticola*. Plates were incubated for an additional 24 h until hyphal tips of the challenging fungi came in contact with *C. beticola*. Agar with the zone of contact was cut out, stained with FM4-64 (Molecular Probes, Eugene, OR), a membrane-selective fluorescent dye used as general cytological stain and endocytosis markers for living hyphae (Fisher-Parton et al. 2000). Samples were observed with a confocal laser scanning microscope (Zeiss LSM 410). Observations were made with a C-Apochromat 63 × 1.2 N water immersion objective. The unchallenged control *C. beticola* showed normal elongation tip with intact Spitzenkörper (Fig. 4b). Contact with *T. harzianum* (Fig. 4c) and *T. virens* (Fig. 4d) induced disruption of the apical vesicles at the hyphal tips of *C. beticola* (Fig. 4c) and high vacuolization indicated by the black spots (Fig. 4d, arrows); formation of septa were frequently seen when *C. beticola* was in contact with *T. harzianum* (Fig. 4c, arrows). The hyphal tips of *C. beticola* in contact with *T. aureoviride* revealed disruption and release of cytoplasmic materials from extensive bursting of hyphal tips (Fig. 4e).

### Interaction with *Laetisaria arvalis*

*Laetisaria arvalis*, a basidiomycete, was initially referred to as *Corticium sensu lato* (Hoch and Fuller 1977, Odvody et al. 1977, Burdsall et al. 1980). It was later placed in the genus *Laetisaria* by Burdsall et al. (1980). The fungus has since been shown to control a variety of soil-borne pathogens in several crops (Lartey et al. 1991, 1994) and has biological control activity over a wide range of soil water potentials (Hoch and Abawi 1979). Allen et al. (1982) observed control of *Rhizoctonia solani* Kühn by *L. arvalis* on sugar beet. In sugar beet field plots naturally infested with *R. solani*, application of sclerotia of *L. arvalis* in fall and spring significantly depressed populations of *R. solani* (Larsen et al. 1985). Martin et al. (1984) applied *L. arvalis* to seeds of table beet, or as a soil amendment in soil naturally infested with *R. solani* and *Phoma betae* Frank, and respectively observed reductions of pre- and post-emergence damping-off and wire-stem symptoms.

Similar to the *Trichoderma* spp, the two *L. arvalis* isolates LA-60 and LA-67 were evaluated as potential agents for control of the *C. beticola*. The two *Laetisaria* isolates were first tested against *C. beticola* isolates C1, C2, Sid1 and Sid2 in a standard antibiosis test on PDA in a petri dish. Mycelial discs of each *C. beticola* isolate were paired against the antagonists by placing them approximately 6 cm apart on PDA plates. Controls consisted of unchallenged *C. beticola* cultures, placed at the center of separate PDA plates concurrently with transfer of the *Laetisaria* isolates to the challenged cultures. The cultures which were incubated under 12 h photoperiod at 22°C for fifteen days were examined daily for growth, changes in growth patterns of the fungi, and evidence for contact inhibition of the pathogen by *Laetisaria*.

After two days, first contact was established between *L. arvalis* and the *Cercospora* colonies. Growth of all isolates of *C. beticola* ceased after the contact with *L. arvalis* isolates, which continued to grow and overwhelmed *C. beticola* within one week. Figure 5a shows a colony of *C. beticola* (isolate C2) challenged with *L. arvalis* (LA 60) that is much smaller than the unchallenged control.

Examination of the interaction between *L. arvalis* (LA 60) and *C. beticola* (C2) is presented in Figure 5b–e. To establish hyphal contact between *L. arvalis* and *C. beticola*, mycelial discs of *C. beticola* were deposited on slides and the pathogen was challenged by

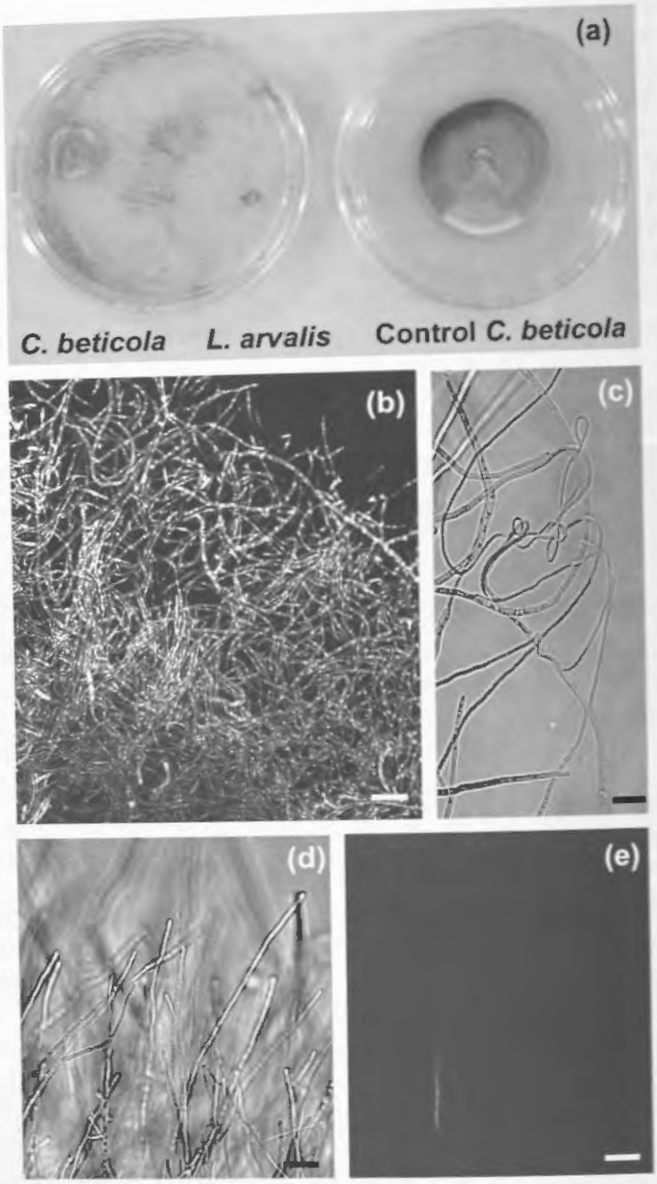


**Fig. 5.** (a) Antagonistic inhibition of *Cercospora beticola* by *Laetisaria arvalis*. Colony plugs from fungal cultures were transferred simultaneously to PDA and were incubated for 15 days. Growth of *C. beticola* was severely inhibited as *L. arvalis* overwhelmed *C. beticola* completely growing over the colony. Control *C. beticola* colony was significantly larger than the inhibited colony. (b,c,d,e) Microscopic observation of interaction between *L. arvalis* and *C. beticola*. Difference interference contrast (DIC) microscopy and confocal laser scanning microscopy (CLSM) of propidium iodide-stained three week-old-*Cercospora beticola* culture (isolate C2) challenged or not challenged with *L. arvalis* (isolate LA-60) culture for 24h. (b) View by CLSM of the edge of C2 colony that had been challenged with LA-60 without hyphal contact. Nuclei are fluorescent in dead cells after propidium iodide staining. (c) View by DIC microscopy of C2 colony edge challenged with LA-60 without hyphal. Hyphae display an abnormal growth pattern with twisted and contorted hyphae. (d) DIC microscopy view of the edge of a control C2 colony without challenging with LA-60. Hyphae exhibit elongated shape with no contortion. (e) View by CLSM of the edge of C2 control colony without challenging with LA-60 after staining with propidium iodide. Few fluorescent nuclei are visible: bar, 30 µm for b; bar, 10 µm for c, d, and e.

placing a mycelial disc from the leading edge of a *L. arvalis* colony about 2 cm away from the growing tips of *C. beticola*. After 24 h, staining with propidium iodide to label nucleic acids of membrane-compromised fungal hyphae (Dengler et al. 1995; Steinkamp et al. 1999) revealed an extensive number of dead cells at the edge of the *C. beticola* colonies before physical contact, as demonstrated by the presence of fluorescent nuclei or by intense fluorescence in compromised hyphae (Fig. 5b). Interference contrast microscopy revealed marked morphological changes in the growth pattern of *Cercospora* hyphae challenged with LA-60 before hyphal contact of the two fungi; contorted hyphae, sometimes twisted, were observed at the edge of the colony reflecting a change in the polarized growth direction (Fig. 5c). In contrast, the control *C. beticola* without LA-60 showed elongated hyphae with tips oriented approximately in the same direction (Fig. 5d). Hyphae of the control *C. beticola* without LA-60 showed very few dead cells after staining with propidium iodide (Fig. 5e). The data suggest a likely antibiosis based induced death of *C. beticola* by *L. arvalis*, as death cells of *C. beticola* were observed prior to physical contact with *L. arvalis*.

## Potential Implication of Pathogen Interaction with Plant Hosts and Fungal Antagonists

### Crop interaction

According to Bosemark (2006), currently available resistant sugar beet cultivars were developed from materials produced by Munerati from 1919 to 1920. He called attention to some setbacks associated with the early resistant cultivars such as significant yield loss under high disease pressure. More recently developed resistant cultivars, while showing higher degrees of resistance, do not exhibit good combining ability. He therefore called additional attention to the need for broadening the Cercospora resistance gene pool by introducing new sources of Cercospora resistance.

While other *Beta* and weed species have been identified as hosts of *C. beticola*, no comparative studies have been carried out to identify a potential difference on interaction with these hosts. Such a study may help in identifying the basis for resistance in some hosts, and could serve as the basis for developing resistant sugar beet cultivars. Current molecular techniques could help in understanding and adapting these resistance mechanisms to sugar beet.

The PCR technique for rapid detection of *C. beticola* could help to quickly screen a broad spectrum of crops and weeds for potential hosts. The results should aid identification of crops for rotation with sugar beet. Additionally, potential weed hosts could be identified and eliminated as an integral part of developing a comprehensive management system.

### Fungal antagonists

In the standard antibiosis tests, all isolates of *C. beticola* were inhibited by all tested fungal agents, each of which exhibited a different effect on *C. beticola*. Noteworthy is the observation of *L. arvalis* which unlike previous observations (Lartey et al. 1994, Gupta et al. 1999), did not exhibit mycoparasitism as a mode of action.

The relatively few attempts at biological control of Cercospora leaf spot have followed the conventional biocontrol dogma: foliar antagonists to control foliar pathogens and soil applied antagonists to control soil borne pathogens. Thus, the successful combined foliar applications of *Bacillus mycoides* isolate (Bac-J) with fungicide by Jacobsen et al. (1998, 2000) and Bargabus et al. (2002) reduced incidence of *C. beticola* and also prevented the development of resistance to the fungicide. The greater part of the life cycle of *C. beticola*

is spent as overwintering propagules, such as stromata, on infected sugar beet residue in the soil. Under optimal conditions during the following growing season, the propagules germinate to produce conidiophores and conidia on the stromata. These are dispersed as primary inoculae that initiates infection. Even with extensive rotation of sugar beet with non-host crops, incidence of Cercospora leaf spot can be severe under optimal environmental conditions, suggesting long-term persistence and an increased role of overwintering propagules from infected sugar beet and yet to be identified secondary host residues in the soil. Longevity studies (Nagel 1938) showed that propagules of *C. beticola* could remain viable for over 27 months in sterile soil and 20 months in field soil. Indeed, recent studies indicate a potential for *C. beticola* to infect sugar beet through the root (Vereijssen et al. 2004, 2005). Thus, a comprehensive management system against *C. beticola* should also consider and target these sources of soil-borne inoculum and potential sources of infection. Species within the genus *Trichoderma* have a proven history of control of a wide range of fungal pathogens. *L. arvalis* is a remarkable colonizer of organic matter in the soil, moving horizontally in the thatch layer and vertically within the upper 2 cm of soil (Conway et al. 2000). The tested fungal agents show good potential for application as biological agents for management of CLS of sugar beet.

Our work lays the foundation for rapid detection and identification of alternate crop and weed hosts and thus can be used for selection of appropriate crops in rotation with sugar beet as well as elimination of potential alternate hosts. In spite of continuous efforts over a century, breeders have not been able to produce sustainable resistant cultivars. A critical examination of biological interactions between *C. beticola* and various hosts such as safflower and sugar beet may provide clues toward breeding for resistance. Finally, a novel approach to combat CLS would be to decrease or even eliminate primary inoculum with microbial antagonists. The tested biological control agents have provided evidence to warrant additional research on soil application to reduce inoculum of *C. beticola* in the soil. For this purpose a recently developed ELISA technique for direct detection of *C. beticola* in field soil (Caesar et al. 2007), could play a major role in evaluating efficacy of soil applied biological control agents on *C. beticola* and inoculum in field soils.

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Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information to benefit the readers and does not imply any recommendation or endorsement by the USDA or the University of South Carolina.

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