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Short communication

Possible root infection of *Cercospora beticola* in sugar beet

Jessica Vereijssen[1], Hans J.H.M. Schneider[1] and Aad A.J. Termorshuizen[2]
[1]*IRS (Institute of Sugar Beet Research), P.O. Box 32, 4600 AA, Bergen op Zoom, The Netherlands (Phone: +31164274400; Fax: +31164250962; E-mail: vereijssen@irs.nl);* [2]*Biological Farming Systems, Wageningen University, P.O. Box 9101, 6700 HB, Wageningen, The Netherlands*

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Abstract

A potential primary infection site of the foliar pathogen *Cercospora beticola* in sugar beet is described. Sugar beet seedlings of the susceptible cv. Auris were grown in a standard soil for 14 days. A monoconidial culture of a *C. beticola* isolate was grown to produce conidia. In experiment 1, roots were immersed in a conidial suspension of isolate code IRS 00-4, or in tap water (control), for 2 days. After incubation seedlings were potted in a peat – fine river sand mixture and placed at low relative humidity (RH) (<80%) or high RH (≈100%). Twelve days after infection, seedlings at high RH showed more disease incidence (90%) than seedlings grown at low RH (disease incidence = 25%), whereas no disease symptoms developed in the control seedlings. Cercospora leaf spots (CLSs) developed on the cotyledons, leaves, petioles and stems of the seedlings. In experiment 2, roots were immersed in a conidial suspension of isolate code IRS 00-2 for 5 h. Thirty-four days after infection at high RH, 100% disease incidence was observed in the treated seedlings and one CLS in the control treatment. First indications of leaf spot development were observed as reddish purple discolouration of individual parenchymatic cells. Because splash dispersal and symptoms due to infested soil were excluded, we showed that it is possible to obtain CLS symptoms in sugar beet seedlings when their roots were immersed in conidial suspensions of *C. beticola*, thus demonstrating that roots can be a primary infection site.

Cercospora beticola causes Cercospora leaf spot (CLS) in sugar beet and is the major foliar pathogen of sugar beet world-wide (Holtschulte, 2000). In the field CLS is usually observed after row closure and in shady areas, probably due to high humidity and temperature in the crop. The fungus produces conidia that are principally splash-dispersed, and also wind-dispersed (McKay and Pool, 1918; Carlson, 1967; Meredith, 1967), but only over short distances, e.g. to adjacent fields (McKay and Pool, 1918). Leaf spots can be produced on stems, cotyledons, leaves and petioles (McKay and Pool, 1918; Nagel, 1938). In severe epidemics, the entire foliage may become necrotic and the beet starts to produce new leaves. These flushes of growth are at the expense of carbon investments of the plant into the root. After the crop is harvested, all organic debris, including the infested leaves and beet tops are ploughed under in the field. Crop debris has repeatedly been said to serve as inoculum when a new sugar beet crop is planted (McKay and Pool, 1918; Nagel, 1938; Giannopolitis, 1978). The pathogen has been said to spread from the soil to the cotyledons by splash dispersal, and later secondary infection spreads to the leaves (Nagel, 1938). However, leaf debris decomposes in soil, including the pathogen thus reducing its viability (McKay and Pool, 1918). In spring following sugar beet, the pathogen could be isolated from buried, non-decomposed leaf debris (McKay and Pool, 1918; J. Vereijssen, IRS, The Netherlands, unpubl.). Stolze (in Plotho, 1951) reported infection of beet plants in the field when infected leaf material was buried up to 25 cm deep. In spite of his results, he regarded splash dispersal as the primary infection mechanism. An alternative type of primary infection was shown by Plotho (1951),

who found mycelium and viable conidia within 3–4 month old seed balls after surface disinfection with a mixture of saponin (0.1%) and sublimat (0.1%). Nowadays, seed contamination cannot be excluded as, even after processing and pelleting the seed, conidia have been found on seeds and inexplicable CLS epidemics develop (M. Nihlgård, Syngenta, Sweden, pers. comm.). It can be concluded that conidia could be located inside the cork or close to the embryo. Its role in the CLS epidemiology, however, is not known.

During field disease assessments in the period 1998–2001, individual, growth-reduced beet plants with CLS symptoms, were frequently observed as early as late June–early July. These observations and above-mentioned results by other research scientists made us question the primary infection site. This paper reports on a potential primary infection site; *C. beticola* infects sugar beet through the root.

In experiment 1, conducted in October 1999, sugar beet seedlings of the susceptible cv. Auris were sown in the greenhouse in seedling trays with a standard soil (fine river sand, 0–1 mm; pH = 6–6.5; 0% organic matter) and grown for 14 days. A monoconidial culture of *C. beticola* isolate code IRS 00-4, originally isolated from a field at Toldijk in the province of Gelderland (The Netherlands), was grown to produce conidia according to a method modified from L.W. Panella (USDA-ARS, USA, pers. comm.). Briefly, *C. beticola* was grown on potato dextrose agar (PDA) for 7 days and flooded with sterile water. Mycelium was scraped off, and the mycelial suspension was poured onto sugar beet leaf agar (SBLA; 50 g of fresh cut leaves in 450 ml of demi water and 10 g PDA was autoclaved at 121 °C for 35 min) still containing pieces of leaves, and left for 4 h. Excessive water was decanted and the SBLA-plates were incubated at 23 °C for 7 days. The culture was flooded with 5 ml of tap water and the conidia were gently brushed off the culture plate using a hairbrush and collected in a jar. A conidial suspension was prepared and divided over several small glass jars. Seedlings were gently washed out of the standard soil. Ten seedlings were tied together with Parafilm (American National Can, USA) and the roots were immersed in the conidial suspension for 2 days at ambient temperature (18–22 °C) and 16 : 8 light : dark. During the incubation period, air was gently blown through with a pipette to agitate the suspension twice a day. Control treatment seedlings (40 plants) were immersed with their roots in tap water for 2 days under similar conditions. After 2 days, each seedling was transferred to a pot (180 ml) containing a peat (30% dry matter; 27% organic matter; pH zone 5–6.5) – fine river sand mixture (1 : 9). Control seedlings were transplanted first. Incubated seedlings were retrieved only at the cotyledons and stems and care was taken not to touch the upper soil with the incubated roots by making large transplanting-holes in the peat–sand mixture, to prevent future splash dispersal. To assess the influence of relative humidity (RH) on disease expression seedlings were put at either ambient greenhouse RH (<80%; low) (40 plants) or at high (≈100%) RH (40 plants) in the greenhouse for 1 week. Ambient 100% RH was achieved by placing a plastic cover over the plants. After 1 week, RH was set back to ambient greenhouse RH. Temperature in the greenhouse was set at 22±1 °C. Control and treated seedlings were separated to prevent cross-contamination.

In experiment 2, conducted in October 2001, we modified the experimental conditions compared to experiment 1 in order to improve the consistency of the disease expression. Sugar beet seedlings cv. Auris were grown for 14 days as described in experiment 1. *C. beticola* monoconidial isolate code IRS 00-2, originally isolated from a field at Veendam, province of Groningen (The Netherlands), was cultured as in experiment 1 and conidial suspensions containing 10^5 conidia/ml were prepared in small photographic film cans. A new isolate was used because in three succeeding experiments with isolate code IRS 00-4 less symptoms developed, and the consistency of aggressiveness after repeated culturing was questioned. Roots were immersed for 5 h in the conidial suspension, or in water for the control treatment. Seedlings were potted and placed in a greenhouse similar to experiment 1. The seedlings were allowed to grow for 2 days at low RH and were then placed at high RH (≈100%) for 1 week for induction of leaf spots and conidial formation. After 1 week, RH was set back to ambient greenhouse RH. Here, only high RH is used as experiment 1 showed that high RH favours leaf spot expression. Temperature in the greenhouse was set at 22 ± 1 °C during the experiment. Twenty seedlings for both treatment and control were used, and placed apart to prevent cross-contamination. Infected roots, stems and petioles were sterilised with 1% hypochlorite (1 : 4 Glorix : water) for 1 min and washed with sterile water twice for 1 min and plated on both PDA (Merck, Germany) and water

agar (Oxford, England) with oxytetracycline (50 ppm) and chlortetracycline (50 ppm).

In experiment 1, we obtained 90% disease incidence 12 days after incubation at high RH, 25% disease incidence at low RH and no disease incidence for the control seedlings at either RH. Leaf spots were observed 5 days earlier at high RH than at low RH, concluding that high RH favours disease expression. Spots developed not only on the leaves, but also on the stems, cotyledons and petioles. First CLS symptoms appeared on the stem followed by petiole and cotyledon/leaf symptoms. Spots on the leaves and cotyledons quite often formed at the leaf margins or leaf tip. Leaf spot symptoms on the stems were elliptical and located 1.5–2 cm above the soil surface. Leaf spots obtained under greenhouse conditions are dissimilar to those in the field. Conidiophores and conidia of *C. beticola* were microscopically visible on leaf spots on all plant parts. From each leaf spot conidia were collected and examined under the microscope for identification. In experiment 2, we obtained 100% disease incidence (1–20 leaf spots per plant) in the incubated seedlings and one leaf spot on one plant in the control treatment, 34 days after root infection. Germinated spores were present in the conidial suspension from which the seedlings were taken. In this experiment more attention was paid to the development of primary CLS symptoms. These symptoms can be seen as reddish-purple discolouration of individual parenchymatic cells and were interpreted as the initial stage of cell death. The pathogen was not observed on the PDA and water agar plates for all plant parts. Because *C. beticola* is a slow growing pathogen on artificial media, the plates were soon overgrown with fast growing fungi. Of each leaf spot, conidia were collected and examined under the microscope for identification.

We have demonstrated that leaf symptoms arose as a result of root infection as splashing of conidia from the soil and symptom development due to already infested soil (during potting) were excluded. One possible mode of primary infection might be that conidia germinate at the root surface and mycelia enter the root and vascular system and systemically colonise the plant. Alternatively, mycelia may grow epiphytically along with the plant on the epidermis, with infection occurring at the stem base. Lesions have not been observed at the stem base. Root infection has been shown for other foliar fungi showing leaf blight, including *Trichometasphaeria turcica*, on sorghum (El-Shafie and Webster, 1979) and on citrus by *Fusarium solani* (Nemec et al., 1980), scorched leaves on soybean by *F. solani* f.sp. *glycines* (Njiti et al., 2000), mildew on faba bean by *Peronospora viciae* f.sp. *fabae* (van der Gaag and Frinking, 1997) and sunflowers by *Plasmopora halstedii* (Gunawardena and Hawes, 2002). Of these pathogens, fungal hyphae of downy mildew were shown to be present in xylem vessels in buckwheat (Zimmer et al., 1990) and sorghum (Balasubramanian, 1973), all plant tissue except xylem in radish (Ohguchi and Asada, 1981), cortex and pith in sorghum (Balasubramanian, 1973), petioles, flowers, stems, leaves and seeds in buckwheat (Zimmer et al., 1990) and epicotyl in faba bean (van der Gaag and Frinking, 1997). Most of the above-mentioned pathogens germinate at the root surface and subsequently the fungal hyphae enter the root and thus colonise the plant, which confirms the above-mentioned first possible mode of primary infection. The second possible mode of primary infection, epiphytical growth of *C. beticola*, is less plausible as one would expect abundant lesions on the stem. Lesions on the stem were observed, but not as abundant as expected with epiphytical growth. The above-mentioned studies on foliar pathogens indicate that although a fungus is a foliar pathogen, it can have its site of primary infection at the roots.

The leaf spot observed in the control treatment of experiment 2 can be the result of cross-contamination or is seed-borne, which is less plausible. In a greenhouse, CLS can be wind-dispersed (Carlson, 1967; Meredith, 1967) by blowers for temperature regulation or dispersed by insects (McKay and Pool, 1918; Plotho, 1951; Meredith, 1967). Although disease expression varied according to the experimental conditions, CLS symptoms were recorded in both experiments. Difference in aggressiveness and colonisation rate of the two *C. beticola* isolates used may account for some of the differences. Other variables such as day length, temperature and RH during night time and light intensity may also play a role in CLS symptom development. Therefore, we hypothesise that *C. beticola* can be present in a sugar beet seedling or plant without showing symptoms, not only in a greenhouse but also in the field. Beet plants with and without symptoms need to be screened for *C. beticola* using molecular techniques, since plating-out infested plant parts failed to show the pathogen.

In conclusion, we have demonstrated that it is possible to obtain CLS symptoms in sugar beet seedlings when they are infected with a conidial suspension

via the root. Knowing infection occurs via the roots offers novel breeding strategies and novel strategies for controlling the disease.

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