Agronomy Research **4**(Special issue), 171–175, 2006

Studies on soil application of *Stagonospora cirsii*, a candidate for biological control of *Cirsium arvense*

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Abstract. *Stagonospora cirsii*, a causal agent of Canada thistle (*Cirsium arvense*) leaf spot, is under development as a biological control agent for this perennial noxious weed. *S. cirsii* had impact as a foliar spray and it proved highly pathogenic if applied at soil surface. Since Canada thistle is a perennial weed with a very vigorous root system, the ability of fungus to survive in soil and infect roots would greatly improve its potential as a mycoherbicide. Research was conducted to study the *S. cirsii* population dynamics in sterile soil, on the possibility of infecting Canada thistle creeping roots ed with the fungus, and its ablity to survive after being incorporated into/on the soil after over-wintering. The population dynamic was studied by dilution technique. In our experiments we used 10 g and 400 g of sterile field sod-podsol soil. At two initial inoculation concentrations about 10^3 and 10^6 conidia g^{-1} of soil the CFU amount in 1 g soil gradually were stabilized at a level about 10^5 g^{-1} of soil. The population density on this level was stable for 6 months. Inoculation of the creeping roots at different stages of their development (creeping roots with buds/rosettes/young roots) in laboratory conditions showed that fungus can infect leaves only in rosettes. It seems, under natural conditions, infection of Canada thistle root system with *S. cirsii* is not possible. However, *S. cirsii* maintains viability under various conditions of over-wintering (above/under/on ground).

Key words: biological control, Canada thistle, *Cirsium arvense*, *Stagonospora cirsii*, survival in soil, mycoherbicides

INTRODUCTION

Canada thistle (*Cirsium arvense* (L.) Scop.) is an important, troublesome, perennial weed in many agricultural areas of the world. *Stagonospora cirsii* is a causal agent of brown foliar lesions on Canada thistle. *S. cirsii* had impact as a foliar spray and it proved highly pathogenic if applied at soil surface. At 48 hours dew period disease severity and plant death are close to 100% if the inoculum was used as foliar spray and close to 60% if the inoculum was used as mycelium powder at soil surface (Berestetskiy, unpublished data). *S. cirsii* kills seedlings and adversely affects (although does not immediately kill) established plants. Since Canada thistle is a perennial weed, which could grow from buds on its creeping roots, the ability of the fungus to survive in soil and to infect roots would greatly improve its potential as a mycoherbicide. This paper reports the survival and population dynamics of S. *cirsii* in sterile soil, on the possibility of infecting creeping roots of *C. arvense* with *S. cirsii,* and the survival of *S.cirsii* inoculum in/on the soil after over-wintering.

MATERIALS AND METHODS

Isolate *S. cirsii* C–163 was recovered in 2001 from diseased leaves of Canada thistle plants from Severnaya Osetia (Russia). The strain was grown on pearl-barley grains at room temperature (21˚C ± 2˚C) with 12 to 12 h of NUV light for 14 days.

The field sod-podsol soil (St.-Petersburg, Pushkin) was used for studying the population dynamics of the fungus. A water level content of 70% of complete moisture capacity was achieved, and this level of water content was maintained throughout the experiment. In all experiments the soil was sterilized twice within 1 hour at 2 atmospheres with a daily interval.

Survival of the fungus in sterile soil was studied using a modified method of membrane filters (Lagutina et al., 1992). CFU dynamics in the soil were assessed by the dilution plate technique in two volumes of the sterile soil: 10 g and 400 g. Initial concentrations of conidial suspensions were close: 10^3 and 10^6 conidia per g of soil.

Creeping roots of *C. arvense* were washed and disinfected for 10 minutes in a weak solution of potassium permanganate. The creeping roots were cut into 5 cm fragments with 1–3 buds, which were placed one by one in Petri dishes on glass slides. Their edges were wrapped up in cotton moistened with 0.004% benzimidazole solution. Before use, one part of the root fragments were stored in a refrigerator at 4–5˚C; another part was placed at 12/12 h light/darkness regime at 22 ± 2˚C to produce leaf rosettes and new roots. Small leaf rosettes (0.7–1 cm length) were developed from buds after 5–7 days of incubation. Growth of new rootlets started after 3 weeks of incubation. 1.5 mL conidial suspension of *S. cirsii* C–163 per plate, 2 x 10^6 conidia mL^{-1} was used for inoculation of 0, 1 and 4 week-old root fragments. After inoculation, the Petri dishes were maintained in darkness for 2 days, then at a 12/12 h light/darkness regime at 22 ± 2˚C. Disease was evaluated 1 and 2 weeks after inoculation. The leaves of each rosette were counted and visually rated individually for disease symptoms using a 0–6 scale (Pfirter & Defago, 1998). Disease symptoms of creeping roots, buds and rootlets were rated for area of organ surface damage (%).

To study inoculum viability after over-wintering, pearl barley grains with *S. cirsii* C–163 pycnidia were placed in synthetic bags which were incorporated into the soil at 0, 3, 5 cm depth or fixed 5 cm above ground in the middle of November 2004. Maturity of fruit bodies and re-isolation of pathogen from grains was assessed in the end of May 2005.

All experiments consisted of 3-4 replicates per treatment and were repeated 2 times. Population densities of *S. cirsii* C–163 were transformed to its $\log_{10}$. The data were subjected to analysis of variance (ANOVA). Treatments were compared using Fisher's least significant difference test at $P = 0.05$.

RESULTS AND DISCUSSION

Applying as criterion of *S. cirsii* survival rate in the sterile soil, the percent of colonies developed from membrane filter pieces, it is possible to conclude that the survival rate of the fungus decreased gradually, and in soil, at 3 cm depth, faster than on soil surface (Fig. 1).

Two weeks after the beginning of the experiment, 50% of the colonies had grown from pieces of filters, which were placed on soil surface, and 17% of those

were incorporated into the soil. In 3 weeks these data were 42% and 0% respectively, and in 35 dpi colonies had not grown from filters, regardless of the depth of its incorporating.

In 2 dpi on the surface of membrane filters the developed mycelium was observed, cells of mycelium were dyed using an effective stain resulting in intensive rose color. On filters incorporated into soil, the mycelium was advanced more intensively than on filters located on a soil surface. At germination, spores increased in size, frequently becoming 2-3 cellular. A small amount of non- germinated spores was present on both filters; the cells of these spores were somewhat swollen. In 3–4 weeks, the dyed mycelium cells wsere markedly pale in color, the contents of mycelium cells were granular, and part of the mycelium cells had collapsed.

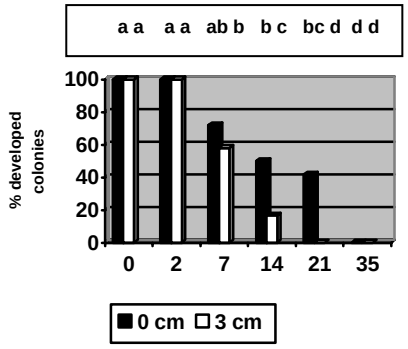

Fig. 1. Dynamics of *S. cirsii* survival on membrane filters in sterile soil. 0 cm and 3 cm depths the membrane filters were disposed in the soil. Bars marked with the same letter are not significantly different.

Possibly the conditions on membrane filters in soil (insufficient humidity, unsuitable structure of substrate) do not promote the life and growth of *S. cirsii.*.

Although on membrane filters the fungus lost viability within a month, nevertheless in the sterile soil the fungus may keep viability for as long as 6 months. In both experiences (both with 10 g, and with 400 g of soil) the tendency of alignment of CFU amount in 1 g of soil was marked at two different initial inoculation concentrations. That is, at initial inoculation concentration of about 10^3 conidia g^{-1} of soil, the CFU amount in 1 g soil was increased, and at initial inoculation concentration about 10^6 conidia g^{-1} of soil the CFU amount in 1 g of soil decreased, until the concentration in both cases was stabilized at a level of approximately 10^5 in 1 g of soil.

In the first case (10 g of soil), alignment of concentration was marked for 15 days, in the second (400 g of soil), for 7 days (Figs 2, 3). Population density was stabilized on this level until the end of the experiment (up to 6 months).

It is probable that the fungus lives in soil as live, low active mycelium, since all processes are slowed down in sterile soil where there are very few nutrients.. As a result of spore germination and mycelium growth, the conditions of the environment have been changed. Processes such as an exhaustion of nutrients, accumulation of metabolites, acidity changes, change of oxygen balance, and limitation of space obviously affect the stabilization of mycelium concentration at this level.

With inoculation of creeping roots, the symptoms of infection were expressed as vast necrosis at 1 week after inoculation, and were detected only in leaf rosettes of creeping root fragments. No symptoms were observed on creeping root fragments, young roots and buds. The fungus, *S. cirsii* was re-isolated only from leaves of rosettes.

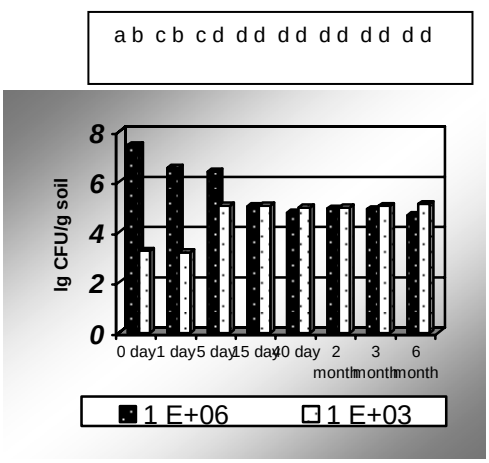

Fig. 2. Population dynamics of *S. cirsii* in sterile soil at initial concentrations of close to 1 E + 03 and 1 E + 06 conidia g^{-1} of soil (in 10 g of soil). Bars marked with the same letter are not significantly different.

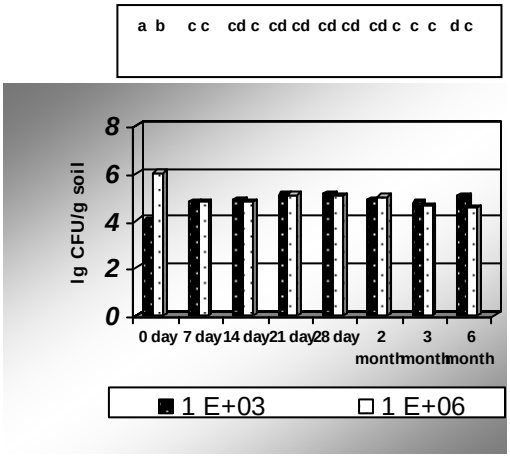

Fig. 3. Population dynamics of *S. cirsii* in sterile soil at initial concentrations of close to 1 E + 03 and 1 E + 06 conidia g^{-1} of soil (in 400 g of soil). Bars marked with the same letter are not significantly different.

After over-wintering, the surface of the grains from 3 and 5 cm depths were covered with stroma with numerous empty fungal fruit bodies. In the variant with inoculum storage at a height of 5 cm above ground level, grains were covered with dark-brown stroma with numerous fungal fruit bodies, whose content was represented by hyaline cells, not differentiated on spores. In the case of over-wintering on soil surface, the fruit bodies were pseudothecia with asci. Irrespective of over-wintering conditions (above/under/on ground) the fungus was re-isolated from infected pearl barley grains.

CONCLUSIONS

S. cirsii can survive in sterile soil for a long time. The use of the dilution plate technique has shown stable population density of *S. cirsii* (near 10^5 CFU g^{-1} of soil) for 6 months irrespective of the initial concentration. No significant difference was observed between inoculation concentrations of near 10^3 and 10^6 conidia g^{-1} of soil from the 15th (10 g of soil) or 7th (400 g of soil) day of measurement until the end of incubation.

Inoculation of the creeping roots at different stages of their development (creeping roots with buds/rosettes/young roots) in laboratory conditions showed that fungus can infect only leaves in rosettes. It seems, under natural conditions, infection of Canada thistle root system with *S. cirsii* is not possible. However, *S. cirsii* remains viable on organic substrate under various conditions of over-wintering (above/under/on ground).

Since Canada thistle is a perennial weed with a very vigorous root system, the ability of fungus to survive in soil and infect roots would greatly improve its potential

as a mycoherbicide. *S. cirsii* may survive in sterile soil. To determine its survival in natural soil, it is necessary to receive a marked strain (for example, resistance to fungicide) and study its relations with soil fungi.

ACKNOWLEDGEMENTS. The authors thank EU project Food–CT–2003–001687 (2E–BCA in Crops) for financial support of this work, and C. Alabouvette for teaching methods of soil fungi studies.

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