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Preventing an increase in Verticillium wilt incidence in spinach seed production

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ABSTRACT

A semifield assay was conducted from 2009 to 2011 to distinguish between different preventive methods of reducing *Verticillium* spp. in spinach seed production. The seed treatments for controlling seed infection levels included Thiram, Signum, *Trichoderma harzianum*, *Gliocladium roseum* and Natural II, and these were tested in naturally infested and uninfested soil. Even though seed treatment by Thiram and Signum in all cases reduced the incidence of *Verticillium* spp. on the harvested seed, the soil type had a large influence on the subsequent disease pressure as a significant effect of soil was seen in 2010 and 2011 and a non-significant effect of seed treatment on *Verticillium* spp. was observed in all experimental years. As no interaction between soil and seed treatment was observed, the seed treatment had no effect on the disease pressure on the seeds subsequently harvested from the infested soil.

1. Introduction

Verticillium wilt, caused by the soilborne pathogen *Verticillum dahliae* (Kleb), is a worldwide important vascular wilt disease causing problems in many horticultural crops such as lettuce, strawberries, cauliflower and potatoes. Also more exotic crops such as watermelon and cotton are added to the list of crops affected by the disease (Atallah et al., 2011; Bilodeau et al., 2012; Davis et al., 2010; Devay et al., 1974; Xiao et al., 1997).

An introduction of infected seed in the fields may result in infection of subsequent crops susceptible to *V. dahliae* (van der Spek, 1972). Verticillium wilt is not reported to affect fresh-market or processed spinach as the symptoms of the disease develop after initiation of bolting. However, the disease is problematic in spinach seed production where it may decrease seed yield and seed quality (du Toit et al., 2005; Yang et al., 2010). Analysis of commercial seed lots from the United States, Denmark, the Netherlands and New Zealand in 2002 and 2003 revealed more than two-thirds of the lots to be infected at varying incidences from 0.3 to 84.8% (du Toit et al., 2005). The mean incidence of *Verticillium* spp. on the seed was highest for seed lots harvested in the United States (26.9%) and the Netherlands (26.3%) followed by Denmark (8.8%). The survey by du Toit et al. (2005) was groundbreaking for the Danish seed industry as Denmark is the world stronghold for hybrid spinach seed production (Deleuran, 2011). The challenge of reducing Verticillium wilt to a lower level has become one of the priorities in the Danish vegetable seed business in order to produce healthy spinach seed.

Verticillium dahliae produces spores and mycelium that systemically infect the plants. Microsclerotia serve as resting structures and can survive on the seed or in the soil for many years even in the absence of a suitable host (Keinath et al., 1991). In spinach seed production van der Spek (1972) documented how *V. dahliae* can be transmitted from artificially infested soil to harvested seed and from seed to seed. Since then, research studies have focused on seed treatments (du Toit et al., 2009b; du Toit and Hernandez-Perez, 2005), screening for resistant varieties (Mou et al., 2008) and lately detection of *V. dahliae* on the seed and in the soil by new methods, such as real-time polymerase chain reaction (PCR) (Bilodeau et al., 2012; Duressa et al., 2012).

Different seed treatments, such as hot water, steam and chlorine have proven effective in reducing the sporulation of *V. dahliae* in a freezer blotter test (du Toit et al., 2009b; du Toit and Hernandez-Perez, 2005). Thiram reduced the infection of *V. dahliae* in an experiment conducted in the United States, in which seed were evaluated in a freeze-blotter seed health assay and in a study where transmission from seed to soil was evaluated in washed sand in a

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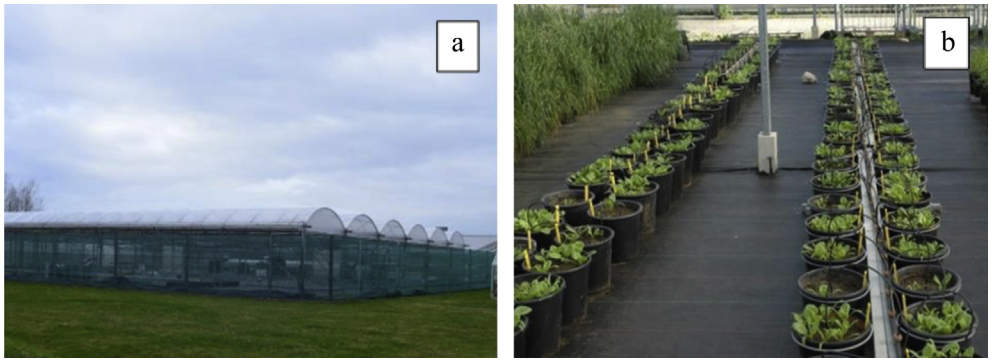

Fig. 1. Semifield assay. Semifield area (a) and germinated plants one month after sowing in 10-L pots (b).

greenhouse assay (du Toit et al., 2009a). The systemic fungicide Signum WG (a.i. boscalid and pyraclostrobin) has been tested as a spray in Danish spinach field experiments with promising results (Paaske and Olesen, 2012). From an environmental point of view alternatives to chemical treatments may also be of interest (Tinivella et al., 2009).

The fungi *Trichoderma* spp. and *Gliocladium* spp. are known to be capable of producing antimicrobial compounds that can suppress disease by diverse mechanisms (Howell et al., 1993). *Gliocladium roseum* was demonstrated as a potential biological control agent of *V. dahliae* in soil (Keinath et al., 1991), and *Trichoderma* spp. was mentioned as an antagonist in studies conducted by van der Spek (1973). Natural II with actiomycete as active ingredient is another biological seed treatment, which has reduced *Verticillium* spp. In a study by Cummings and du Toit (2009) with spinach seed samples, a reduction in the percentage of seeds infected by *Verticillium* spp. was reduced from 49.75% in non-treated to 4.75% in seed samples treated with Natural II after 14 days in a freeze-blotter test.

Despite numerous studies on *V. dahliae* in agriculture and several suggestions on how to reduce the incidence of *V. dahliae* on harvested seed, the disease still poses significant challenges before a strategic integrated management plan can be suggested in spinach seed production.

With support from the Danish seed business, the aim was to allow testing of naturally infested soil and seed in an assay that could be adopted by the growers and in that way support a sustainable Danish spinach seed production. A semifield assay was established where spinach for seed set is grown in pots. This assay allows for a completely randomised design of two different soil types 1: Soil infested by *Verticillium* spp. 2: Uninfested control soil. Differing from the research by du Toit (2005, 2009a, b) and van der Spek (1972, 1973), the first objective of our research was to imitate a field situation with a natural microflora and develop a reproducible semifield assay. Second, the purpose of this study was to evaluate how different seed treatments, such as mentioned above, may reduce the level of infection on the subsequent harvested seed, when treated seed is sown in infested soil versus uninfested soil.

2. Materials and methods

Experiments were conducted as a semifield assay (Fig. 1) at Research Centre Flakkebjerg, Aarhus University, Denmark in the growing seasons of 2009, 2010 and 2011.

2.1. Semifield assay

In 2009, 2010 and 2011, 10-L pots were filled with soil 2 (described in paragraph 2.2.) to a total of 3/4 of the pot volume; the upper layer was filled with 1 L of soil 1 or soil 2. At the beginning of May, 20 seeds of *Spinacia oleracea* L. were sown in each pot. The pots were placed on fibre cloth on top of the ground (Fig. 1b) and protected against rain with a plastic roof (Fig. 1a). Thus, irrigation was the only controlled parameter and each pot was irrigated three times a day with an automatic drip irrigation system.

2.2. Soil and seed material

Two different soils were tested. Soil 1, infested soil, was obtained from a field with a high level of Verticillium wilt in 2008. An uninfested control soil, soil 2, which contained 17.5% clay (<2 μm), 25.2% silt (2–63 μm), 55% sand (>63 μm) and 2.3% organic matter, was obtained from Research Centre Flakkebjerg and mixed with sand and sphagnum (Kekkilä Garden white 420 W). In earlier experiments, soil 2 did not show any sign of infestation by *Verticillium* spp. or other soilborne pathogens.

Soil 1 was collected from the same field each experimental year (2009, 2010 and 2011) in March and mixed 1:1 with soil 2 in order to make the soil sample uniform. The soil was kept in a cold storage until sowing.

Seed was obtained from commercial seed companies and each year a new seed lot with a different level of *Verticillium* spp. was obtained and employed in the experiment. In 2009, lot A with no infection; in 2010, lot B with high infection (49%) and in 2011, lot C with intermediate infection level of *Verticillium* spp. (14%). After receiving the seed lots, a seed health test of 4 × 100 seeds was performed to verify the infection level of *Verticillium* spp. and other seedborne fungi (Table 1).

2.3. Seed treatments

Each sample consisting of 10 g of seed was mixed with the treatments shown in Table 2.

Table 1
Percent infected seed based on a seed health test of 4 × 100 seeds from seed lots A, B, and C. Seed was tested before sowing. Fungi classified as others include fungi such as *Acremonium*, *Epicoccum*, *Ulocladium*, *Botrytis* and *Bipolaris spp*.

Fungi	Lot A 2009	Lot B 2010	Lot C 2011
Verticillium spp.	0	49	14
Cladosporium spp.	1	1	12
Stemphylium botryosum	5	40	14
Colletotrichum spp.	0	0	0
Fusarium spp.	14	0	0
Alternaria alternata	19	61	82
Others	7	2	7