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Effect of successive cauliflower plantings and *Rhizoctonia solani* AG 2-1 inoculations on disease suppressiveness of a suppressive and a conducive soil

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ABSTRACT

Disease suppressiveness against *Rhizoctonia solani* AG 2-1 in cauliflower was studied in two marine clay soils with a sandy loam texture. The soils had a different cropping history. One soil had a long-term (40 years) cauliflower history and was suppressive, the other soil was conducive and came from a pear orchard not having a cauliflower crop for at least 40 years. These two soils were subjected to five successive cropping cycles with cauliflower or remaining fallow in a greenhouse experiment. Soils were inoculated with *R. solani* AG 2-1 only once or before every crop. Disease decline occurred in all treatments cropped with cauliflower, either because of a decreased pathogen population or increased suppressiveness of the soil. Disease suppressiveness tests indicated that the conducive soil became suppressive after five subsequent cauliflower crops inoculated each cycle with *R. solani* AG 2-1. Suppressiveness of all treatments was measured in a seed germination test (pre-emergence damping-off) as well as by measuring the spread of *R. solani* symptoms in young plants (post-emergence damping-off). Results showed that suppressiveness was significantly stimulated by the successive *R. solani* inoculations; presence of the cauliflower crop had less effect. Suppressiveness was of biological origin, since it disappeared after sterilization of the soil. Moreover, suppressiveness could be translocated by adding 10% suppressive soil into sterilized soil. The suppressive soil contained higher numbers of culturable filamentous actinomycetes than the conducive soil, but treatments enhancing suppressiveness did not show an increased actinomycetes population. The suppressiveness of the soil samples did also not correlate with the number of pseudomonads. Moreover, no correlation was found with the presence of different mycoparasitic fungi, i.e. *Volutella* spp., *Gliocladium roseum*, *Verticillium biguttatum* and *Trichoderma* spp. The suppressive soil contained a high percentage of bacteria with a strong *in vitro* inhibition of *R. solani*. These bacteria were identified as *Lysobacter* (56%), *Streptomyces* (23%) and *Pseudomonas* (21%) spp. A potential role of *Lysobacter* in soil suppressiveness was confirmed by quantitative PCR detection (TaqMan), since a larger *Lysobacter* population was present in suppressive cauliflower soil than in conducive pear orchard soil. Our experiments showed that successive cauliflower plantings can cause a decline of the damage caused by *R. solani* AG 2-1, and that natural disease suppressiveness was most pronounced after subsequent inoculations with the pathogen. The mode of action of the decline is not yet understood, but antagonistic *Lysobacter* spp. are potential key organisms.

1. Introduction

Rhizoctonia solani Kühn (teleomorph *Thanatephorus cucumeris* (Frank) Donk) is a soil-borne fungal pathogen, which causes world wide serious losses in many different agricultural crops (Domsch et al., 2007). Disease incidence and severity, and consequent economic losses due to *R. solani* are unpredictable and fluctuate from season to season. Damage by *R. solani* is also dependent on soil texture (Lewis, 1979), soil moisture content (Höper and Alabouvette, 1996), management practices including minimal tillage (Rovira, 1986), and crop rotation (Rovira, 1986; Larkin and Honeycut, 2006). The occurrence of disease suppressive soils, i.e. soils where pathogens are limited in their ability to establish or to produce disease symptoms, have been described for *R. solani* in different crops for at least three decades ago (Henis et al., 1978; Jager et al., 1979; Lewis, 1979). Disease suppression of *R. solani* was found to be stimulated by for example the addition of some types of compost (Tuitert et al., 1998; Pérez-Piqueres et al., 2006; Termorshuizen et al., 2006) and cellulose-containing products (Kundu and Nandi, 1985; Croteau and Zibilske, 1998). Crop rotation

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also influences disease suppressiveness of the soil (Garbeva et al., 2006; Postma et al., 2008). Although increased disease suppressiveness has been described, experimental results are often difficult to repeat and the occurrence of disease symptoms by *R. solani* is still rather unpredictable.

Besides stimulation of disease suppressiveness by adding organic compounds or other management practices, decline of disease due to monoculture of a crop has been described. One would expect heavy disease development after continuously growing a host crop in the same soil due to build up of the pathogen population. However, examples of the opposite exist. The best described example of disease decline is take-all (*Gaeumannomyces graminis* var. *tritici*) decline in barley and wheat (Gerlagh, 1968; Sarniguet and Lucas, 1992; Raaijmakers and Weller, 1998). For *R. solani* a decline in disease has also been reported in both field and pot experiments for wheat (Lucas et al., 1993; Roget, 1995; Wiseman et al., 1996; Mazzola and Gu, 2002), sugar beet (Hyakumachi et al., 1990; Sayama et al., 2001), radish (Henis et al., 1978; Chet and Baker, 1980; Chern and Ko, 1989), potato (Velvis et al., 1989; Jager and Velvis, 1995) and cauliflower (Davik and Sundheim, 1984). However, knowledge on the mode of action of *Rhizoctonia* disease decline is lacking. In most pathogen-crop combinations, it is unknown if the host crop or the pathogen itself are needed for the development of disease decline. In few cases it was described that virulent *R. solani* was required to induce *Rhizoctonia* disease decline (Lucas et al., 1993; Sayama et al., 2001). Furthermore, knowledge about the antagonistic organisms inhibiting the disease development would enable better prediction of the occurrence of disease decline. Different species or microbial groups have been suggested to be involved in the mechanism of *R. solani* decline, i.e. *Trichoderma* spp. (Henis et al., 1978; Chet and Baker, 1980; Wiseman et al., 1996), *Verticillium biguttatum* or other mycoparasitic fungi (Jager et al., 1979; Velvis et al., 1989), an increased *R. solani* population of other AGs (anastomosis groups) (Jager and Velvis, 1995), part of the *Pseudomonas* population (Mazzola and Gu, 2002), and a combination of *Pantoea*, *Exiguobacterium* and Microbacteria (Barnett et al., 2006). However, none of these organisms have really been proven to be a key factor in *Rhizoctonia* disease decline.

The research described in the current paper started with the observation that a field with continuous cauliflower cropping maintained extremely low levels of *R. solani* root rot for several years, while *R. solani* AG 2-1 was detected in the soil and even inoculated in previous field trials. It was questioned if the low level of *R. solani* disease was the result of a high soil suppressiveness to this disease. A second question was whether a conducive soil can become suppressive and whether successive pathogen or host plant (or the combination) introduction is essential for suppressiveness development. Finally, we wanted to know if any microbial group correlated with the occurrence of suppressiveness. A better understanding of disease decline in cauliflower as well as in other crops, and the underlying aspects of soil suppressiveness, may lead to the development of improved disease management methods, which will reduce fungicide use and increase yield.

In the present study soil suppressiveness against *R. solani* AG 2-1 was evaluated under standardized conditions. Suppressiveness of the cauliflower soil which was supposed to be suppressive, as well as soil of a nearby pear orchard without any cauliflower crop for many years, were compared. Subsequently, both soils were used to study the nature of disease decline. Soils were treated with i) successive plantings or fallow and ii) one initial *R. solani* inoculation or inoculation before each planting. The composition of the microflora in the different treatments was assessed and correlated with the soil suppressiveness data. For this purpose, mycoparasitic fungi as well as commonly reported antagonistic bacteria (i.e. *Pseudomonas* spp. and *Streptomyces* spp.) were assessed. Additionally, the presence of a recently described bacterial genus correlating with *Rhizoctonia* suppression (*Lysobacter* spp.) (Postma et al., 2008) was tested in part of the soil samples.

2. Materials and methods

2.1. Soils

Two marine clay soils with sandy loam texture having similar physical and chemical characteristics (Table 1) were collected from Zwaagdijk, province Noord-Holland in the Netherlands. The major differences between the soils resulted from their cropping history. One soil was collected from a field in which cauliflower had been grown for more than 40 years. The other soil was collected within the rows of a nearby 40-year-old pear orchard with grass between the rows. The soils were collected in winter, after a period of frost. They were air-dried at 9 °C up to field capacity, sieved (15 mm pore size) and kept in darkness in a cycle of 12 h at 14 °C and 12 h at 18 °C.

The presence of the cauliflower pathogen *R. solani* AG 2-1 was assessed in both soils after a wet sieving procedure. Particles >0.4 mm were concentrated on a sieve by washing 100 g of soil with excessive amounts of water. Then, 0.5 g of this sieve fraction was disrupted by bead beating and DNA was extracted with an Ultra Clean™ Soil DNA isolation kit (MoBio Laboratories, BIOzymTC, Landgraaf, the Netherlands). After DNA purification with a PVPP column (polyvinyl polypyrrolidone; Sigma), PCR with AG 2-1 specific ITS-primers AG2TF9 5′-GCACACCTTCCTCTTTCATC-3′ and AG2TR7 5′-GATTGATAAAGGTGTTGTCC-3′ (Van den Boogert et al., 2000) was performed. Results showed that *R. solani* AG 2-1 was present in the cauliflower soil, but not in the pear orchard soil. From both soils *R. solani* could be isolated using *Juncus effusus* baiting (Doornik, 1981).

The soil to be used as a sterilized control was collected from grassland close to the cauliflower field. After air drying to field capacity and sieving, the soil was treated with gamma radiation (6 Mrad), and stored in double plastic bags for up to 6 months at 9 °C. A sample of the cauliflower soil was sterilized by gamma radiation to test the biological component of disease suppression.

Disease suppressiveness of these three soils was determined at the start of the experiment and after storage in darkness in a cycle of 12 h at 14 °C and 12 h at 18 °C for 3–5 months. The methods are described below.

Table 1
Physical and chemical properties of the two sandy loam soils.

Soil	pH-KCl	% Sand[a] (>50 μm)	% Silt[a] (2–50 μm)	% Clay[a] (<2 μm)	C (g/kg)	N total (g/kg)	NO_3 (mg/kg)	Wetness[b] at WHC (%)	Wetness[b] at pF 1.7 (%)
Cauliflower	7.1	52	20	12	26.3	2.1	57	40.8	25.6
Pear orchard	7.2	44	28	16	33.4	2.8	46	48.9	32.5

[a] The percentages sand, silt and clay were calculated by dividing the weight of the dried fraction (105 °C) by the weight of the air-dried (40 °C) original soil. The percentages do not add up to 100%; the remaining fraction consists of organic matter, lime and water.
[b] Wetness was calculated as the percentage of water per dry soil; WHC = water-holding capacity.