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Evaluation of the causes of legume yield depression syndrome using an improved diagnostic tool

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ABSTRACT

The aim of the study was to establish a diagnostic tool to narrow down the causes for pea yield depressions. A differential two-level diagnostic test system was established under controlled conditions using peas (*Pisum sativum* L.) as test plants. Soils from 22 organically managed sites with unexplained moderate to high pea yield losses were tested in level 1 diagnostics (γ-irradiation to eliminate potentially harmful organisms, nutrient additions to compensate for potential nutrient deficiencies or activated charcoal amendment to bind and thereby to immobilize potentially phytotoxic compounds). Results showed that organisms harmful to the test plant were the primary cause of limited germination and growth in most of the sampled soils, whereas a positive effect of nutrient addition was rarely found and toxins were not involved. Level 2 diagnostics (pesticides targeting ascomycetes, oomycetes, Rhizoctonia spp., nematodes) further narrowed down the organisms involved in yield depressions. Oomycetes were identified as the primary reason for limited germination rates, and, in some soils, also for limited growth of established seedlings. In other soils, a multitude rather than a single group of pathogens was involved in limited growth. Plant-pathogenic nematodes were never found to be limiting for crop growth parameters. Harmful effects of pesticides were found in several soils, hinting at an important role of beneficial soil organisms in the suppression of pathogens causing yield depression in legumes. The bioassay used in the present study was robust and could thus serve as a low-cost tool for agricultural advisors and farmers to predict the risk of yield losses in legumes and to narrow down causes, helping them to develop appropriate strategies.

1. Introduction

Due to their ability to fix atmospheric nitrogen in symbiosis with rhizobacteria, legumes are of outstanding importance in organic agriculture, and are often included in crop rotations. In stockless farming systems lacking manure input, soil fertility has to be built using N_2 fixing cover crops in the off-season and marketable grain legumes; under central European conditions these are peas (*Pisum sativum* L.) and fava beans (*Vicia faba* L.) (Finckh et al., 2014). Despite their importance for soil fertility, the use of peas and fava beans has been declining in organic agriculture in Germany and elsewhere for many years (Rahmann et al., 2004). This is mainly because productivity is much below expectations based on pedoclimatic conditions. A multitude of factors and their interactions may contribute to such yield depressions, including abiotic factors such as impaired soil structure (Allmaras et al., 2003; Tu, 1992, 1994), lack of nutrients (Kraft and Pfleger, 2001) and presence of toxic compounds (Narwal, 2000). Factors may also include biotic limitations such as soil infestation with plant-pathogenic nematodes and soil- and seed-borne pathogens (Kraft and Pfleger, 2001). In grain legumes, particularly in peas, seed rot, seedling damping-off, foot rot as well as foliar diseases caused by various soil- and seed-borne pathogens such as *Pythium* spp., *Rhizoctonia solani*, *Fusarium solani*,

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Aphanomyces euteiches and the Ascochyta complex (*Ascochyta pisi*, *Mycosphaerella pinodes* and *Phoma medicaginis*) have a high yield depression potential. Pathogenic nematodes such as *Ditylenchus capsici* and *Meloidogyne hapla* can also cause high losses (Kraft and Pfleger, 2001). Often, complex interactions of various pathogens occur, and synergistic effects have also been reported (Kerr, 1963). Furthermore, impaired microbial activity and diversity (Chen et al., 1988; Garbeva et al., 2004; Nitta, 1991) and/or the absence of specific antagonists such as *Pseudomonas fluorescens* or *Trichoderma* sp. (Stutz et al., 1986; Wiseman et al., 1996) may result in enhanced development of plant pathogens. The lack of rhizobia or suboptimal rhizobia colonization may also play a role in yield depressions of leguminous crops (Bardin et al., 2004; Chakraborty and Chakraborty, 1989; Dileep Kumar et al., 2001; Hemissi et al., 2013; Sayeed Akhtar and Siddiqui, 2008).

Yet, often the source of yield depressions cannot be identified, particularly in the case of complex interactions. The term 'soil fatigue' was coined for unexplained yield depressions after repeated cultivation or in perennial cultures (Schreiner and Sullivan, 1909). Soil fatigue has been studied in many crops, including sugar beet, cereals, many vegetables and fruit production (Anon., 1982).

Identification of the underlying causes of yield depressions is essential to develop appropriate remediation strategies such as fertilization with deficient nutrients, the use of suppressive composts (Darby et al., 2006; Fuchs, 2002; Noble and Coventry, 2005; Schüler et al., 1989), introduction of microbial antagonists (Haas and Défago, 2005; Wang et al., 2003), or solarization or biofumigation (Deadman et al., 2006; Handiseni et al., 2012; Matthiessen and Kirkegaard, 2006). Thus, the aim of the present study was to establish a method to narrow down the causes for unexplained pea yield depressions. For that purpose, a differential two-level diagnostic test system first described by Bouhot and Bonnel (1979) was modified. The aim of a first level was to assess the impact of soil nutrients, toxins and harmful organisms as putative limiting factors. The aim of a second level was to further narrow down the underlying causes based on the results of the first level, e.g. in the case of harmful organisms, to identify groups of organisms involved in soil fatigue. In the present study, soil treatments suggested by Bouhot and Bonnel (1979) to assess the impact of different factors were modified. Namely, repeated autoclaving was replaced by γ-irradiation, which is known to be much less destructive to soil structure and chemistry (McNamara et al., 2003), nutrient solutions containing macro- and all instead of only six micro-elements were used, and pesticides used for level 2 diagnostics were selected according to current knowledge and availability. Furthermore, experimental and growth conditions as well as disease assessment were optimized for peas instead of celery. The suitability of the modified test system was assessed using 22 soils from sites showing different levels of soil fatigue.

2. Materials and methods

2.1. Sites and soil samples

Representative soil samples were taken in autumn (September–October) 2008–2010 from a total of 22 sites in Germany (Table 1). These sites are a subset of a large-scale survey with the overall aim to increase the added value of organically grown legumes by optimization of soil fertility management (www.bodenfruchtbarkeit.org). At all selected sites, farmers and advisors have reported legume yields remaining moderately to strongly below expectations in one or several years, without finding obvious explanations such as impaired soil structure, pathogen damage or lack of nutrients in the field. At all sites, peas as part of the crop rotation were the last crop before taking soil samples. 180 l of soil were taken from the top 20 cm with a spade from at least 12 sites per field, sieved to 1 cm, homogenized and stored at 3 °C in Styrofoam boxes in darkness under aerobic conditions until further use.

2.2. Physical and chemical soil properties

Particle size distribution was analyzed according to DIN ISO 11277 (2002) by the Bavarian State Research Centre for Agriculture (Institute for Organic Farming, Soil and Resource Management) and soil types were classified according to DIN 4220 (DIN ISO, 2008) (Table 1). Chemical properties were analyzed by the Institute Koldingen GmbH, AGROLAB Group (Sarstedt, Germany). N_{tot} and organic matter were measured by dry combustion according to DIN ISO 13878 (ISO, 1998) or DIN ISO 10694 (ISO, 1995) respectively, pH and calcium supply according to Schachtschabel (VDL 1A5.2.1 in VDLUFA Methodenbuch (1991)), phosphorus and potassium in a CAL-extract (VDL 1A6.2.1.1), Mn, Cu, B and Zn in a CAT extract (VDL 1A6.4.1), and Mg in a calcium chloride extract (VDL 1A6.2.4.1).

2.3. Biological parameters and activity of soils

Soil microbial biomass (C_{mic}, N_{mic}) was determined by a chloroform fumigation extraction assay (Brookes et al., 1985; Vance et al., 1987). Ergosterol content (a measure of fungal biomass) was determined according to the method described by Zelles et al. (1987). As indicators of general soil microbial activity, soil basal respiration (Isermeyer, 1952) and fluorescein diacetate hydrolization rate (FDA) (Schnürer and Rosswall, 1982) were analyzed. Activity of enzymes involved in N- (protease activity) and P-cycling (alkaline phosphatase activity) were determined with methods described by Ladd and Butler (1972) and Margesin (1993) respectively. Measured values were compared to minimal comparative values as described by the Swiss work group 'Implementation of Soil Biology' (2009).

2.4. PCR multiscan for selected plant pathogens and scan for plant-pathogenic nematodes

In all soil samples, the presence of potential pathogenic microorganisms was determined by DNA Multiscan by the company Relab denHaan (Waterlingen, Netherlands). The scan is semiquantitative (7 infestation levels, 0 no, 1 starting, 2 light, 3 moderate, 4 intermediate, 5 heavy, 6 very heavy infestation). It included 42 fungi, 18 oomycetes, and 12 bacteria (Table 2). In addition, the presence and abundance of 22 nematodes potentially detrimental or beneficial to plants was determined by the same company by washing 100 ml of soils as described by Oostenbrink (1954) and observation in light microscopy. Damage expected by nematodes depends on plant species, nematode species and stress-level of the plant. Yet, as a rough guideline for expected damage in susceptible plants with medium stress level, infestation levels were attributed to 3 classes, with (i) 0–59 no damage to plants expected, (ii) 60–159 damage expected and (iii) >160 severe damage expected.

2.5. Bioassays

2.5.1. Differential diagnostics, level 1

A controlled conditions bioassay to quantify different levels of soil fatigue and to identify causal relationships between soil fatigue levels and nutrient imbalance, toxic compounds, and/or noxious organisms was established according to Bouhot and Bonnel (1979). Subsamples of the soil were either left untreated or (i) fertilized weekly with a 3-fold diluted nutrient solution (Knop's solution