European Journal of Soil Biology 69 (2015) 41–51

Contents lists available at ScienceDirect

European Journal of Soil Biology

journal homepage: http://www.elsevier.com/locate/ejsobi

Original article

Fusarium-suppressive effects of green manure of turnip rape

Ling Zou [a, *], Antti Tuulos [a], Anu Mikkonen [b, d], Frederick L. Stoddard [a], Kristina Lindström [c], Merja H. Kontro [d], Hilkka Koponen [a], Pirjo S.A. Mäkelä [a]

[a] Department of Agricultural Sciences, PO Box 27, Latokartanonkaari 5, FIN-00014, University of Helsinki, Finland
[b] Department of Food and Environmental Sciences, PO Box 27, Latokartanonkaari 11, FIN-00014, University of Helsinki, Finland
[c] Department of Environmental Sciences, PO Box 65, Viikinkaari 2a, 00014, University of Helsinki, Finland
[d] Department of Environmental Sciences, University of Helsinki, Niemenkatu 73, 15140 Lahti, Finland

ARTICLE INFO

Article history:
Received 11 February 2015
Received in revised form
4 May 2015
Accepted 12 May 2015
Available online 17 May 2015
Handling editor: B.S. Griffiths

Keywords:
Allelochemicals
Botrytis cinerea
Cereal monoculture
Fusarium spp.
Rhizoctonia solani
Sclerotinia sclerotiorum
Turnip rape

ABSTRACT

Allelochemicals of *Brassica* can inhibit a wide range of soil-borne pathogenic fungi. In the present study, the effects of different turnip rape (*Brassica rapa*) cultivation treatments on the soil fungal community were investigated in two experiments in 2009 2010 and 2010 2011. The treatments used turnip rape as a green manure, as a mixed-crop with barley, or as a rotational crop after barley, with continuous barley as the reference. The composition of the soil fungal community was monitored with capillary-based length heterogeneity PCR using primers ITS1F/ITS4. The operational taxonomic units (OTUs) identified as the pathogens *Botrytis cinerea*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum* did not respond to different treatments. Fungal diversity was the lowest and the relative abundance of *Fusarium* spp. the highest in barley monoculture. Incorporation of turnip rape plants into the soil as green manure was associated with a low relative abundance of *Fusarium* spp. Turnip rape as a mixed crop or rotational crop did not substantially decrease the relative abundance of *Fusarium* spp. Aqueous and non-polar extracts of allelochemicals from turnip rape had very little effect on the growth of *Fusarium culmorum in vitro*. We conclude that high fungal diversity, resulting from either application of fertilizer or incorporation of organic matter of turnip rape, was more important than allelochemicals in affecting the growth of *Fusarium* spp. in field soils.

1. Introduction

Winter turnip rape [*Brassica rapa* L. ssp. *oleifera* (DC.) Metzg.] is used for oil, seed and forage production. Although usually grown as a single crop, it can be cultivated in a mixture with barley [1], which led to a substantial reduction in N leaching in experiments in Finland [2]. Glucosinolate-derived isothiocyanates from *Brassica* species inhibited the growth of a wide range of soil-borne pathogenic fungi, including *Rhizoctonia solani* J.G. Kühn, *Gaeumannomyces graminis* (Sacc.) Arx and D.L. Olivier, and *Fusarium* spp. [3,4]. Thus, including turnip rape in a crop mixture with barley or as a rotational crop after barley could be complementary to conventional methods relying on synthetic fungicides for management of soil-borne pathogens.

Green manuring, the incorporation of actively growing plant materials to capture their nutrients, organic matter and allelochemicals, of some species of *Brassica* was suppressive to some soil-borne pathogenic fungi, such as *R. solani* that causes scab disease of potato [5], and species of the genera *Cylindrocarpon*, *Phytophthora*, *Pythium*, and *Rhizoctonia* that cause apple replant disease [6]. Glucosinolates can be transported through the phloem from the leaves to the roots of *Arabidopsis thaliana* [7], leading to an impact on microbes in the rhizosphere [8]. Thus, root exudates from plants in the Brassicaceae may suppress the growth of soil-borne pathogens. Green manure and root exudates not only release potentially fungicidal glucosinolate-derived isothiocyanates, but also can be sources of nutrients that promote growth of microbes in the rhizosphere [9]. The exudate could create a suppressive environment for pathogens because of competition for nutrients and the presence of antagonistic microbes; thus high microbial biomass and diversity are considered important indicators of suppressive soils [10].

Fusarium sp. can infect a wide range of cereal hosts, causing significant yield loss and degraded quality. In 2001 and 2002 in Finland, up to 44% of the harvested grain was contaminated by

* Corresponding author.
E-mail address: ling.zou@helsinki.fi (L. Zou).

http://dx.doi.org/10.1016/j.ejsobi.2015.05.002
1164-5563/

Fusarium spp. [11]. Mycotoxins produced by this group of fungi have been associated with long-term weakening of immune system of humans, along with symptoms of acute poisoning such as nausea, vomiting and stomach pain [12].

Hence, in the present study we aimed to investigate effects of turnip rape as sources of allelochemicals and nutrients on the soil fungal community. We set out to determine whether turnip rape used as a green manure incorporated into the soil either in the autumn or in the early spring, or grown as a rotational crop after barley, or as a mixed crop with barley, affected soil fungal community structure. The effects of the different treatments were evaluated by capillary-electrophoresis-based length heterogeneity PCR (LH-PCR), a method that is widely used for this purpose due to its high reproducibility and technical simplicity [13–15]. Primers ITS1F and ITS4 were used because of their specificity to fungi and their ability to amplify more species than other ITS primers [16,17]. Soil chemical properties were investigated as factors potentially affecting microbial growth, with focus on moisture content, pH, and mineral N concentration because of the intensive competition for N between plant roots and microorganisms [18]. Finally, we sought to test whether the effects of turnip rape could be attributed to either polar or non-polar allelochemicals by using an *in vitro* test of fungal growth.

2. Materials and methods

2.1. Evaluation of LH-PCR to investigate soil fungal diversity

The feasibility of using primers (ITS1F: 5′-CTTGGTCATTTAGAGGAAGTAA-3′, ITS4: 5′-TCCTCCGCTTATTGATATGC-3′) and capillary-electrophoresis-based LH-PCR to distinguish soil fungal diversity was tested by determining the differences in the length of amplicons of reference strains of *Fusarium* sp., *Botrytis cinerea*, *R. solani* and *Sclerotinia sclerotiorum* that were isolated in the experimental sites (Table A.1). Genomic DNA from about 0.2 g freeze-dried fungal tissue was extracted using a commercial kit (E.Z.N.A.® Fungal DNA kit, OMEGA bio-tek, Norcross, NC, USA) and subjected to PCR, and the products were stored at 4 °C overnight before capillary electrophoresis (Appendices). The range of each run was limited between 150 bp and 1000 bp length standards by GeneScan version 3.7 (Applied Biosystems®). The runs were normalized using size standards, and peaks of each run were converted to bands of different sizes and intensities in a lane of an artificial gel. The size of each band was estimated by BioNumerics 5.0 (Applied Maths NV, Sint-Martens-Latem, Belgium). PCR and capillary electrophoresis for extracted genomic DNA of each fungal reference strain was replicated three times.

To test the reproducibility of recovery of fungal DNA from soil, inocula of the four reference strains (underlined in Table A.1) were prepared by mixing sterile sand with fungal pure culture. Fungi were grown on potato dextrose powder (BD Difco™, Franklin Lakes, NJ, USA) including 9% gelatin (BD Difco™) and 0.4% agar (Agar for Microbiology, Sigma–Aldrich, Co. St. Louis, MO, USA). Inocula were prepared by melting the solid medium at about 40 °C (non-lethal to fungi) and homogenizing 60 mL of it with 240 g of sterile sand. One part of the prepared inoculum was mixed with 5 parts of air-dried soil from the experimental farm in four replicate 1-L pots for each strain. Four pots of soil moisturized with water were included as negative controls. Pots were covered by plastic film that was perforated with small holes and kept at 22 °C for three days. Soil genomic DNA was extracted using a commercial kit (E.Z.N.A.® Soil DNA Isolation kit) from 0.8 g of soil (Appendices). In Bionumerics analysis, optimization was set to 0.37% (approx. 1 bp shift allowed) and the peak detection threshold was lowered to a surface area of 1% of the total peak area.

2.2. Genbank database analysis

To evaluate if the length of amplified ITS regions of different fungal species listed in Table 1 differed significantly in Genbank (National Center for Biotechnology Information), corresponding sequences of each species were obtained in the Nucleotide database in Genbank by searching "18S ribosomal RNA gene, partial sequence, internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence, and 28S ribosomal RNA gene, partial sequence". Sequences of non-target species were left out by using the Taxonomy group filter in the website, and a few sequences longer than 1000 bp or shorter than 300 bp were considered as outliers and removed. Sequence sizes of different species were recorded and the means and confidence intervals ($P = 0.05$) were calculated and rounded to integers.

2.3. Study site, sampling and analysis

Two similar 2-year field experiments in 2009–2010 and 2010–2011 were conducted in different parts of the Viikki experimental farm (60°13′ N, 25°10′ E), University of Helsinki, Finland. The soil is a silty clay loam with organic matter content 3.8%, pH 6.3 in 0.01 M $CaCl_2$, tentatively classified as Vertic Stagnosols [19]. The preceding crops were common wheat (*Triticum aestivum* L. emend Thell.) and barley (*Hordeum vulgare* L.). The experiment comprised four replicates of five treatments (Table 2 and Fig. 1) in a randomized complete block design and each plot was 12.5 m^2 (2.5 m × 5 m). Turnip rape (cv 'Largo'), containing 3–20 μmol glucosinolates per gram of leaf dry matter in our growing conditions, was used [1]. Treatment MC (mixed culture) was a mixture of barley and turnip rape sown on 13 May 2009 (first experiment) and 27 May 2010 (second experiment), with 80 kg ha^{-1} of fertilizer N (N–P–K: 20-2-12, Pellon Y4, Yara, Espoo, Finland) applied to the seed bed at sowing; the barley was harvested in the middle of August when the turnip rape was at the rosette stage. Treatment TAB (turnip rape after barley) consisted of barley sown on the same dates with the same fertilizer, followed by turnip rape sown in late July with 30 kg ha^{-1} of fertilizer N. Treatment ETI (early turnip rape incorporation) was the same as MC, but turnip rape was incorporated into the soil on 5 October 2009 and 12 October 2010. In treatment LTI (late turnip rape incorporation), the turnip rape overwintered, 80 kg ha^{-1} of fertilizer N was applied as top dressing using a precision fertilizer spreader on 4 May in both years, and the plant green manure and fertilizer were incorporated on 1 June 2010 and 31 May 2011. In treatment BM (barley monoculture), barley was sown in the middle of May with 80 kg ha^{-1} of fertilizer N, harvested in early August, and its stubble was left on the soil. In the second year of each experiment, barley was sown into the plots of treatment ETI, LTI and BM and treated with 80 kg ha^{-1} of fertilizer N, while in treatments MC and TAB, 80 kg ha^{-1} of fertilizer N was applied as a top dressing to the overwintered turnip rape plants using a precision fertilizer spreader on 4 May. Soil samples were taken six times during each experiment (Table 3). During the growing seasons, rhizosphere soil samples were taken in the following manner, adapted from Ref. [20]. In the first year, six barley plants from each plot, and in the second year, six plants of turnip rape and six of barley from each plot of treatments ETI and LTI were chosen. The plants were pulled up and shaken gently to remove loose soil, then the tightly adhering rhizosphere soil was collected by vigorously shaking the roots. Between growing seasons, samples of bulk soil (15–20 cm deep) were taken by an auger and sieved through 2-mm mesh to homogenize the soil and remove plant residues. Genomic DNA was extracted from 0.8 g of each composite sample of soil using the E.Z.N.A. kit and subjected to PCR, as described above. Capillary electrophoresis using the products