Applied Soil Ecology 107 (2016) 191–204

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Traditional and new soil amendments reduce survival and reproduction of potato cyst nematodes, except for biochar

Negin Ebrahimi[a,b,*], Nicole Viaene[a,c], Bart Vandecasteele[a], Tommy D'Hose[a], Jane Debode[a], Pieter Cremelie[a], Caroline A. De Tender[a], Maurice Moens[a,b]

[a] Institute for Agricultural and Fisheries Research (ILVO), Burg. Van Gansberghelaan 96, 9820 Merelbeke, Belgium
[b] Department of Crop Protection, Ghent University, Coupure links 653, 9000 Ghent, Belgium
[c] Department of Biology, Ghent University, K.L. Ledeganckstraat 35, 9000 Ghent, Belgium

ARTICLE INFO

Article history:
Received 16 March 2016
Received in revised form 5 June 2016
Accepted 13 June 2016
Available online xxx

Keywords:
Viability
Pig slurry
Cattle slurry
Compost
Biochar
Hatching

ABSTRACT

Potato cyst nematodes (PCN), *Globodera rostochiensis* and *G. pallida*, are major constraints to potato crop production. We studied the effects of several soil amendments on PCN survival and reproduction in pot experiments. Pig slurry, cattle slurry, mineral nitrogen fertilizer (NH_4NO_3), crab shell compost and wood chip compost at 170 kg N ha^{-1} reduced the number of viable eggs in cysts of both PCN species in the absence of potato. This resulted in fewer second-stage juveniles (J2) hatching from these cysts and penetrating potato roots than from cysts of non-amended soils. When potato was planted, the same amendments resulted in less reproduction than in non-amended soil. Most reduction of reproduction was achieved in soils amended with pig slurry (87%) and wood chip compost (82%). Adding biochar at 0.3 and 1% did not reduce the survival or the reproduction of any of PCN species; moreover, it inhibited the suppressing effect of wood chip compost and pig slurry on PCN reproduction when added together with these amendments. The release of ammonium and changes in soil microbial community, determined by phospholipid fatty acid (PLFA) analysis, are involved in nematode suppression in soil amended with pig and cattle slurries. However, the suppressing effect of wood chip and crab shell compost can only be explained by the changes in soil microbiota, while the effect of mineral nitrogen fertilizer can only be related with the production of ammonium. Ammonium and microorganisms most probably have affected PCN directly by killing the eggs and juveniles or indirectly by changing the physiology of the root as mentioned amendments reduced hatch and movement of J2, penetration of the roots and females' fecundity.

1. Introduction

Potato cyst nematodes (PCN), *Globodera rostochiensis* and *G. pallida*, are managed by integrating different control options, *e.g.*, nematicides, resistance and crop rotation (Anonymous, 2007). However, application of nematicides has become very restricted and only a limited number of potato cultivars are (partially) resistant to *G. pallida* (Anonymous, 2007). Crop rotation with non-hosts, i.e. any crop but potato for most field situations, allows for natural decline in viability of the resilient cysts of PCN. Studies showed that the rate of this decline not only depends on the length of the period without host crop, but also varies with climatic conditions, soil type and other unknown soil factors (Turner and Subbotin, 2013; Schomaker and Been, 2013).

Annually, a large amount of agricultural and agro-industrial by-products, all considered waste, is generated. Many of these products can be recycled for agricultural use, *e.g.*, as fertilizer or carbon (C)-rich soil amendment. Many organic amendments reduce disease incidence caused by plant pathogens and nematodes (Abawi and Widmer, 2000; Lazarovits et al., 2001; Renčo et al., 2007; Oka, 2010). Nematode suppression by soil amendments can be caused by different mechanisms including *i*) improvement of the physical and chemical properties of the soil, which may have an adverse influence on hatching, mobility and survival, *ii*) the release

* Corresponding author at: Burgemeester Van Gansberghelaan 96, 9820 Merelbeke, Belgium.
E-mail addresses: negin.ebrahimi@ilvo.vlaanderen.be, neginebrahimi55@gmail.com (N. Ebrahimi), nicole.viaene@ilvo.vlaanderen.be (N. Viaene), bart.vandecasteele@ilvo.vlaanderen.be (B. Vandecasteele), tommy.dhose@ilvo.vlaanderen.be (T. D'Hose), jane.debode@ilvo.vlaanderen.be (J. Debode), pieter.cremelie@ilvo.vlaanderen.be (P. Cremelie), caroline.detender@ilvo.vlaanderen.be (C.A. De Tender), maurice.moens@ilvo.vlaanderen.be (M. Moens).

http://dx.doi.org/10.1016/j.apsoil.2016.06.006
0929-1393/

of nematicidal compounds by the organic material, *e.g.*, organic acids, phenolic compounds and ammonium, *iii*) improvement of plant growth, and *iv*) the production of allelochemicals, *e.g.*, antibiotics or chitinases by the soil microflora (Widmer et al., 2002; Oka, 2010). It is known that organic amendments affect the microbial community of the soil, including bacteria, fungi and free-living nematodes (Oka, 2010). Many of these organisms, or their metabolites, are beneficial for plant growth or antagonistic towards plant pathogens and nematodes (Viaene et al., 2013). Organic materials also appear to induce plant systemic resistance to nematodes by introduction of specific microorganisms such as bacteria, fungi and mycorrhiza (Reitz et al., 2000; Dababat and Sikora, 2007; Vos et al., 2012).

Animal waste products and several kinds of compost and plant extracts have been studied for their potential to manage plant-parasitic nematodes. Tian et al. (2000) found that soil amended with chitin improves the development of nematode antagonistic soil fungi and bacteria, leading to suppression of the soybean cyst nematode, *Heterodera glycines*. It was reported that swine manure inhibited hatch and movement of second-stage juveniles (J2) of *H. glycines* towards the host root (Reynolds et al., 1999). Xiao et al. (2008) showed that volatile fatty acids (VFA)-enriched swine manure also inhibited *H. glycines* hatch and root penetration. Swine manure contains a large amount of nitrogen that can be converted to VFA and ammonium (NH_4^+), both having nematicidal activities (Xiao et al., 2008). Anaerobically digested cattle slurry reduced the densities of *Meloidogyne incognita* in tomato (Jothi et al., 2003). Renčo et al. (2007) examined the effects of five composts of different origins (plants, animals and fungi) on *G. rostochiensis* on potato in pot experiments. All tested composts reduced the number of eggs and J2. Many plant extracts have nematicidal effects (Chitwood, 2002; Valdes et al., 2011). For example, extracts from *Allium* spp. have nematicidal activities (Auger and Thibout, 2005) and some were shown to be as toxic as methyl bromide for nematodes and insects (Arnault et al., 2004).

Few studies have investigated the effects of the addition of biochar on soil inhabiting nematodes. Biochar is produced by thermal treatment of biomass at oxygen deficiency, *e.g.*, by pyrolysis or gasification. It can be used as a soil amendment (Lehmann and Joseph, 2009) and is put forward as a way to sequester carbon (C) to mitigate climate change (Lehmann et al., 2006). Biochar has a high specific surface area (400–800 $m^2 g^{-1}$) providing a habitat for soil microorganisms. It has also been shown to change the composition and abundance of the soil biological community (Fischer and Glaser, 2012). However, Nelissen et al. (2015) and De Tender et al. (2016) reported no significant influence of the addition of biochar to the soil on the total amount of microbial biomass.

Most research on soil amendments has focused on their influence on PCN during the potato crop; little effort has been placed on their effect on the survival and viability of PCN during crop rotation with non-hosts. However, the latter type of research may yield extra information on the factors affecting decline rates of PCN. Schomaker and Been (2013) reported a mortality rate of 69% for *G. rostochiensis* in the absence of the host in the first year after a potato crop; in subsequent years they observed 20–30% mortality rate. In view of this, we evaluated in pot experiments the suppressive effect of different soil amendments on *G. rostochiensis* and *G. pallida* in the presence of potato as well as in its absence. Our research was focused on amendments that were locally available and economically acceptable by farmers, but also on crab shell compost and new products like biochar. The specific objectives of this study were *i*) to determine the effect of a series of soil amendments in the absence of a host on the viability (number of live eggs) of PCN and the subsequent hatching and infectivity of surviving J2, *ii*) to examine the impact of the incorporation of amendments into the soil planted with potato on the reproduction of PCN, *iii*) to understand the modes of action of these soil amendments in PCN suppression by looking at their effect on hatching, migration and host finding ability and root penetration of J2, and *iv*) to unravel mechanisms of nematode suppression by soil chemical and biological analyses.

2. Materials and methods

2.1. Nematodes

The populations of *G. rostochiensis* (Kruishoutem, Belgium) and *G. pallida* (Chavornay, Switzerland) used in this experiment were obtained from stock cultures. Cysts were maintained on potato cv. Désirée (PCN susceptible standard) under greenhouse conditions (20–25 °C, 16 h light). After sixteen weeks, newly formed cysts were extracted using a Seinhorst elutriator (Seinhorst, 1964). Cysts were stored for 4 months at 4 °C to overcome the diapause. The content of the harvested cysts of *G. rostochiensis* and *G. pallida* was 435 ± 46 and 418 ± 51 eggs, respectively (n = 50).

2.2. Amendments

The following amendments were used in the experiments: pig slurry (PS), cattle slurry (CS), crab shell compost (CC), mineral nitrogen (N) fertilizer (N) in the form of ammonium nitrate (NH_4NO_3) (Sigma Aldrich, St Louis, MO, USA) (0.068 g kg^{-1} soil (dry basis)), wood chip compost (WC) (made from 30% straw, 18% wood chip, 23% poplar bark, 14% maize straw and 14% leek residue), biochar (BC) (0.3% and 1% V/V) (produced during slow pyrolysis of hard and soft wood at 480 °C), biochar-blended wood chip compost (WCBC), biochar-blended pig slurry (PSBC) and biochar-blended crab shell compost (CCBC). The blended amendments consisted of 0.3% biochar (Table 1). The two slurry-amended soils were collected from the long-term experimental site BOPACT at the Institute for Agricultural and Fisheries Research (ILVO), Merelbeke, Belgium, immediately after the slurries were applied to the soil (57% sand, 37.7% loam, 5.3% clay and 0.67% organic carbon). The amount of pig slurry, cattle slurry, mineral N fertilizer and composts applied contained the maximum N doses (170 kg N ha^{-1}) permitted by the European legislation (91/676/EEC) (Anonymous, 1991; Anonymous, 2011).

2.3. Experiment 1: Effects of amendments on the viability of G. rostochiensis and G. pallida

Pot experiments were conducted to examine the impact of the application of amendments in the soil in the absence of potato on the viability (number of live eggs) of PCN. The subsequent hatching and infectivity of surviving J2 after amendment treatment was also determined by a hatching assay and an infectivity test.

The amendments were thoroughly mixed with soil collected from the BOPACT site at ILVO (0–15 cm) and added to 2.5 l pots (concentrations of amendments are shown in Table 1); the soil bulk density was 1.3 g cm^{-3}. Only the crab shell compost was mixed with the soil and incubated at room temperature for 8 months prior to its use, to stimulate growth of chitinolytic bacteria in the soil during the incubation period. Non-amended soil served as a control. Batches of cysts of *G. rostochiensis* or *G. pallida* were placed in retrievable nylon mesh bags and placed at a depth of 6 cm in 4 pots (replicates) of every amendment. Pots were left outside for 16 weeks during spring/summer in a completely randomized design and were exposed to prevailing weather conditions to simulate field conditions. Each treatment had 4 replicates. Every pot received 3 bags containing 14 cysts; they served to estimate the viability of the cyst content at 8, 12 and 16 weeks after soil