



## ORIGINAL ARTICLE



# Additive effect of the root-lesion nematode *Pratylenchus penetrans* and the fungus *Rhizoctonia solani* on potato yield and damage

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## Abstract

The significance of nematodes for disease development caused by other soil-borne pathogens has been demonstrated in many crops throughout the world and specifically prevalent are interactions between plant-parasitic nematodes and species of plant pathogenic fungi. Here, the interaction between the fungus *Rhizoctonia solani* (AG2) and the migratory endoparasitic root-lesion nematode *Pratylenchus penetrans* was investigated on potato. The hypotheses were that the combination of *R. solani* and *P. penetrans* would result in more severe canker lesions, reduced quality of the tubers and lower tuber yield, and we also expected higher nematode levels to render more nematode damage. To test this, potato plants were grown in pots in two replicate experiments and the presence and/or abundance of the two pathogens were controlled. The first three hypotheses were rejected as (1) the tuber yield decreased when the fungus and nematode occurred together but not more than the sum of their separate effects, i.e. additive, (2) there was no effect of presence of nematodes on the incidence of stem canker, and (3) the quality of tubers was actually partly improved as the presence of the nematodes reduced the likelihood of elephant hide on the tubers in one of the experiments. As expected, there were more visible nematode damages with addition of more nematodes, but beyond that the different nematode levels rendered in most cases similar responses. To have knowledge about interactions between pathogens, as the one showed here, is essential for disease control through appropriate management methods.

**Keywords** Black scurf · Disease complex · Elephant hide · *Solanum tuberosum* · Stem canker

## Introduction

The development of disease in cultivated crops has long been known to depend on complex interactions between pathogen, host and prevailing environmental conditions. Organisms occupying the same niche also interact with each other, and

plant pathogens are no exceptions. In many crops throughout the world, it has been demonstrated that nematodes play a significant role for the development of diseases caused by other soil-borne pathogens, and particularly prevalent are interactions between plant-parasitic nematodes and species of plant pathogenic fungi (Back et al. 2002). There are several possible mechanisms behind these interactions. For example, plant-parasitic nematodes may cause a wide range of lesions on the roots of the host plant that facilitate for fungal hyphae to infect (Francl and Wheeler 1993), or cause physiological changes in the host plant leading to increased secretion of root exudates, increased number of lateral roots and changes in the chemical composition of root exudates that may attract the fungi as well as increase the sensitivity for fungal infection (Back et al. 2002). Equally, fungal infection can cause a deterioration or decrease of the host resistance, which may lead to larger nematode populations (Taheri et al. 1994; Back et al. 2002). Therefore, when occurring together, fungi and nematodes may act independently and cause an additive effect on damage and yield or interact with

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each other in a synergistic or antagonistic way leading to more and less damage, respectively.

Stem canker and other symptoms caused by the plant pathogenic fungus *Rhizoctonia solani* Kühn are considered as major problems for potato growers in Sweden, especially in conventional growing systems, which often have shorter crop rotations. *Rhizoctonia solani* attacks all parts of the potato plants; the germinating stem (stem canker), the roots and stolons become brown and the tubers are often developed close beneath the soil surface and are often covered with sclerotia on the skin (black scurf), have cracks in the skin (elephant hide), have narrow holes in the tuber (dry core) or are deformed (Bandy et al. 1988; Banville 1989; Carling et al. 1989; Hausmann 2006; Muzhinji et al. 2014). The fungus can either be seed-borne, due to the sclerotia (black scurf), or soil-borne as the mycelium can survive on harvest residues. The soil-borne inoculum is considered to be the most detrimental, as there is the possibility to circumvent the seed-borne inoculum by treating the seed tubers with fungicides in conventional farming. The fungus is attracted to the plant through the root exudates released from the plant (Back et al. 2010) and therefore, there is potential for interaction with plant-parasitic nematodes.

The interactions between *R. solani* and the potato cyst nematodes *Globodera rostochiensis* (Wollenweber) Behrens (Back et al. 2006, 2010) and *Globodera pallida* (Stone) Behrens (Bhattarai et al. 2009, 2010) have been quite well studied. These nematodes are sedentary endoparasites that establish a permanent feeding site inside the root. It was found that juveniles of *G. rostochiensis* attacking potato roots were associated with higher abundance of stolons infected by *R. solani* (Back et al. 2006). The suggested explanation was that the juveniles modify the discharge of exudates during the beginning of the invasion of the root (Back et al. 2010). The disease development was also greater with *G. pallida* than with *G. rostochiensis*, which may be due to the longer juvenile stage for *G. pallida* (Robinson et al. 1987; Bhattarai et al. 2009). A long juvenile stage with low hatching rate and high survival rate due to better utilization of the fat reserves will prolong the interaction period, as for *G. pallida* (Robinson et al. 1987).

Interactions between *R. solani* and plant-parasitic nematodes with less specialized feeding behaviour are less well studied. In potato, studies on the interaction between *R. solani* and root-lesion nematodes, which are migratory endoparasites, have yielded contradictory results. Scholte and s'Jacob (1989) did not find any interaction between *Pratylenchus neglectus* (Rensch) Filipjev & Schuurmans-Stekhoven and *R. solani*, while Kotcon et al. (1985) found lower tuber yield in plots infested with both *R. solani* and *Pratylenchus penetrans* (Cobb) Filipjev & Schuurmans-Stekhoven. In addition, interactions between *P. penetrans* and other fungal pathogens have been found in several other

agricultural crops. For example, strawberry plants inoculated with *P. penetrans* and *Rhizoctonia fragariae* Husain & McKeen on the same half of a split-root strawberry crown had greater levels of black root rot than plants either inoculated with *P. penetrans* and *R. fragariae* on different halves of the root system, or inoculated with *P. penetrans* or *R. fragariae* separately (LaMondia 2003). In potato, the disease complex of *Verticillium dahliae* Kleb. and *P. penetrans* is well known and has been found to result in greater yield loss than caused by *V. dahliae* alone (Rowe et al. 1985).

The aim of the present study was to make a first step in unravelling the interactions between the plant pathogenic fungus *R. solani* and the root-lesion nematode *P. penetrans*, by controlling the presence and/or abundance of the pathogens in pot experiments. The hypotheses were that the combination of *R. solani* and *P. penetrans* would result in (1) more severe stem canker, (2) reduced quality of the tubers due to higher incidence of black scurf and other tuber symptoms, and (3) concomitant lower tuber yield. It was also hypothesized that adding a high level of nematodes would result in (4) more nematode damages.

## Materials and methods

This study consisted of two experiments with some minor differences in the experimental setup concerning the nematode treatments. In addition, the grading of damages in experiment 1 was insufficient, indicating a need for a second experiment to be able to address the hypotheses properly. The second experiment was part of a larger experiment (Edin et al. 2019).

### Fungus

An isolate of *Rhizoctonia solani*, anastomosis group 2 (AG2-1), originating from Vara, Sweden, was kindly provided by Dr. S. Ahlström at the Department of Forest Mycology and Plant Pathology, SLU, Uppsala, Sweden, which was the same isolate used in the experiments previously published (Edin and Viketoft 2017; Viketoft et al. 2017; Edin et al. 2019). The methodology was also the same as in those experiments, i.e. the fungus was grown on potato dextrose broth (PDB) dishes for a week and then the filamentous mycelium was harvested and washed with phosphate-buffered saline (PBS). The mycelium was mixed to small pieces and diluted in tap water to correspond to 0.1 g mycelium pot<sup>-1</sup> within the same volume as for the inoculation of the nematodes.

### Nematodes

Second stage juveniles of the root-lesion nematode, *Pratylenchus penetrans*, were purchased from Plant

Research International, Wageningen, the Netherlands, and kept in culture on maize. The nematodes were extracted from the maize roots using trays (Whitehead and Hemming 1965), and kept in a cold storage room (4 °C) in a 3L glass beaker that was aerated with an aquarium air pump (Super-Fish Air-Flow mini) until inoculation. At inoculation, the concentration of nematodes in the solution was determined by counting the nematodes in five replicates of 2 ml samples in a counting disc.

## Experimental setup

The experiments were designed as pot experiments with six and four treatments, respectively (Table 1), with eight replicates. Pots (12 × 12 × 25 cm, drainage holes covered with filter paper) were filled with 1600 g of washed, sterilized sand (0–3 mm Ø; 200 °C for 6 h) and 200 ml of tap water was added. One pre-germinated mini-tuber of cultivar King Edward VII (produced from meristem cultures, cultivated at The Finnish Seed Potato Centre Ltd (SPK), Tyrnävä, Finland) was placed in each pot with the most germinated sprouts facing upwards. For experiment 1 the mini-tubers were of size class 25–30 mm and for experiment 2 they were of size class 20–25 mm. Another 900 g sand were added on top of the tuber and 100 ml tap water was applied. In experiment 2 the inoculations were performed the day after the planting but in experiment 1 they were made 4 days after planting; the pots were covered with aluminium foil and received 200 ml of tap water during that period. During the inoculation, the fungus, the nematodes, and water were added in different combinations to the pots: control (tap water), fungus (0.1 g fresh mycelium per pot), low level of nematodes (ca 2500 nematodes per pot), medium level of nematodes (ca 5000 nematodes per pot) and high level of nematodes (ca 7500 nematodes per pot). Both fungus and

nematodes were added to the pots as a water solution spread over the soil surface with a 0.25 L metal cup. The actual inoculum volume differed between the experiments due to variations in nematode density, but the volumes added for each treatment were always the same within each experiment. For example, in experiment 1 the volume of 60 ml of liquid solution contained either 15 ml (low level) or 45 ml (high level) of nematode solution, 15 ml fungal solution and/or tap water according to the treatments in Table 1.

The experiments were carried out in a climate chamber set to 12 °C with a day/night cycle of 16/8 h and artificial light (152 LUX on average). This temperature was chosen to mimic the conditions of spring in areas suitable for growing potatoes; the 30-year average temperature for May is 6–11 °C and 12–15 °C for June (SMHI 2017). The pots were placed on trolleys in a randomized complete block design. The trolleys were rotated once a week to provide uniform exposure to light. The pots were watered twice a week with tap water to ensure normal moisture and during the last weeks, depending on the growth level, the plants were watered thrice a week. In addition, all pots were fertilized at four occasions with a complete fertilizer (Blomstra NPK: 100:18:86 and micronutrients, Orkla Care, Solna, Sweden). In total, each plant received 290 mg N in experiment 1 and 200 mg N in experiment 2.

## Harvest

The potato plants were harvested 10 or 11 weeks after inoculation (Table 1). The potato plant was taken out of the pot, washed carefully with tap water, dried with a paper towel and divided into stems, roots, stolons and tubers. The tubers were divided into small (0.5–2 cm in diameter) and large (> 2 cm in diameter), and tubers smaller than 0.5 cm were regarded as stolons. In experiment 1, the plant parts were

**Table 1** Details on inoculations and harvest in the two experiments with the pathogenic fungus *Rhizoctonia solani* and the root-lesion nematode *Pratylenchus penetrans*. For details on the grading of plant parts see Supplementary Table S1

		Exp. 1	Exp. 2	
Inoculations	1. Control (C)	X	X	
	2. Fungus (F)	X	X	
	3. Nematode	3(a) Low level (NL): 1 nematode g <sup>-1</sup> sand	X	
		3(b) Medium level (N): 2 nematodes g <sup>-1</sup> sand		X
		3(c) High level (NH): 3 nematodes g <sup>-1</sup> sand	X	
	4. Combination	4(a) F + NL	X	
		4(b) F + N		X
		4(c) F + NH	X	
	Harvest		11 weeks	10 weeks
		Canker (stem, stolons)	Graded	Graded
Sclerotia (stem, stolons, roots, tubers)		Graded	Counted	
Elephant hide (tubers)		Graded	Graded	
Nematode lesions (stem, stolons, roots, tubers)		Graded	Counted	

graded regarding nematode damage (number of lesions), stem canker, sclerotia and elephant hide (Supplemental Table S1). The severity of stem canker and elephant hide were graded in the same way in experiment 2, while the actual number of sclerotia and nematode lesions were instead counted on each plant part. The mother tuber was measured in length and diameter and graded regarding nematode damage and sclerotia (black scurf).

The submerged part of the main stem (from the mother tuber up to the surface level, 5–8 cm), the largest tuber, ten roots and 20–25 g of the potting medium were weighed separately and put in plastic bags for cold storage (4 °C) for later extraction of nematodes. The remaining plant parts were weighed and dried for dry weight measurements (75 °C for 48 h) and 100 g of the potting medium were weighed and dried (105 °C for 24 h) for determination of water content.

### Nematode extraction

For nematode extraction, the plant material and potting medium were placed in mesh net sieves covered with vliesseline filters. The main stem part and roots were cut into 1 cm pieces and the tubers were divided into four pieces and placed skin side down. The sieves were placed in Baermann funnels, and the nematodes were extracted for 24 h, heat-killed and fixated in formalin (Viketoft et al. 2005). As the focus of the study is the difference among the treatments, we believe that an extraction time of 24 h from the plant parts give valuable information although we will not recover all nematodes. The number of nematodes was estimated in the suspensions from each extraction under low magnification (50×) and expressed as number of nematodes per gram dry weight of each plant part or per gram dry potting sand.

### Statistical analyses

In experiment 1, the effects of additions of fungus and nematodes on dry weight of stems, tubers, roots and stolons as well as the number of nematodes in the different plant parts and in the potting medium were analysed with randomized complete block design ANOVA followed by Tukey adjusted multiple comparisons using R version 3.1.1 (R Core Team 2013). To account for heteroscedasticity, heteroscedastic consistent covariance estimation, packages multcomp (Hothorn et al. 2008) and sandwich (Zeileis 2004, 2006), was used. The number of tubers and stems were analysed with Poisson regression using R, with the control treatment used as baseline, followed by Tukey adjusted multiple comparisons to investigate differences in numbers among the treatments. The proportion of small tubers were analysed with binomial regression using SAS for Windows 9.3 (SAS Institute Inc., Cary, NC, USA). To analyse the effect on fungal and nematode damage we used a dichotomous

variable—damage (some gradings per plant > 0) or no damage (all gradings per plant 0). In addition, due to too few observations (Agresti 2013), only damage on stems and tubers could be analysed. The data was analysed with binomial regression using R.

For experiment 2, randomized complete block design ANOVA (estimated using R version 3.5.2 (R Core Team 2018) and the lme4 package (Bates et al. 2015) with block as random factor, was applied to stem, root and stolon biomass, number of stems and tubers, proportion of small tubers, severity of sclerotia, stem canker and nematode damage on stem, and abundance of plant-feeding nematodes in growing medium, tubers, stem and roots. The log-transform was used for number of tubers and the log-plus-one transform was used for severity of stem canker and nematode damage on stem, due to better results in tests for normality and equal variance for the residuals (diagnostics test performed using the car package (Fox and Weisberg 2011)). Significance of added nematodes, added fungus and the interaction of nematodes and fungus was tested using Type II Wald Chi-square tests and post hoc comparisons between factor levels were done by the Tukey adjusted multiple comparisons, using the emmeans package (Lenth 2018). All statistical tests were performed with a significance level of 5% ( $p \leq 0.05$ ).

## Results

There was no obvious difference in emergence time between the different treatments, and all potato plants developed well. The potato plants in pots inoculated with *R. solani* were infected by stem canker and black scurf. The nematode *P. penetrans* were present in all plant parts and potting medium samples where nematodes had been added. Bacteriophagous nematodes were also present, especially in the potting medium samples, but were not considered important for the experiment.

### Impact on stems

At harvest, the dry weight of stems differed among the treatments in experiment 1. The treatment with *R. solani* and high level of nematodes had more aboveground biomass than the control ( $p = 0.002$ ) and the two nematode treatments ( $p < 0.001$  and  $p = 0.002$ ) (Table 2). In addition, the dry weight of stems was also greater for the fungal treatment compared to the two nematode treatments ( $p < 0.001$  and  $p = 0.002$ ). The treatment with *R. solani* and low level of nematodes did not differ in dry weight from the other treatments, but these plants had more stems compared to the fungus treatment ( $p = 0.005$ ) and low nematode treatment ( $p = 0.002$ ) (Table 2). In

**Table 2** Dry weights [g; mean (SE)] and numbers of different parts of potato plants subjected to different combinations of the plant pathogenic fungus *Rhizoctonia solani* and the root-lesion nematode *Pratylenchus penetrans* ( $n=8$ )

Treatment	No. of stems	Stem biomass	Stolon biomass	Root biomass	No. of tubers	Proportion small tubers <sup>a</sup>
<i>Experiment 1</i>						
Control (C)	3.3 (0.3) ab	5.85 (0.22) bc	0.26 (0.03) a	1.08 (0.08)	8.8 (1.1) ab	0.41 (0.08) b
Fungus (F)	2.3 (0.4) b	7.46 (0.40) ab	0.04 (0.01) b	1.13 (0.08)	8.3 (1.2) ab	0.52 (0.05) ab
Nematodes low level (NL)	2.0 (0.3) b	5.49 (0.15) c	0.29 (0.04) a	0.98 (0.06)	10.0 (0.9) ab	0.51 (0.05) ab
Nematodes high level (NH)	3.6 (0.8) ab	5.71 (0.24) c	0.22 (0.02) a	1.04 (0.04)	11.8 (1.6) a	0.56 (0.05) ab
F + NL	6.0 (1.3) a	6.55 (0.77) abc	0.12 (0.03) b	0.95 (0.08)	8.6 (1.8) ab	0.75 (0.07) a
F + NH	4.6 (0.9) ab	8.34 (0.72) a	0.05 (0.01) b	1.23 (0.10)	6.6 (1.1) b	0.56 (0.06) ab
<i>Experiment 2</i>						
C	1.6 (0.2)	4.52 (0.3)	0.21 (0.03) a	0.84 (0.09) a	6.50 (0.6) ab	0.37 (0.04) a
F	1.5 (0.3)	3.95 (0.2)	0.09 (0.02) b	0.78 (0.08) ab	4.63 (0.6) b	0.32 (0.08) a
N	2.1 (0.3)	4.00 (0.2)	0.16 (0.03) ab	0.65 (0.06) ab	7.25 (1.1) ab	0.50 (0.05) a
F + N	2.0 (0.3)	4.13 (0.4)	0.10 (0.03) b	0.57 (0.05) b	8.50 (1.4) a	0.47 (0.08) a

<sup>a</sup>In experiment 2, the ANOVA test shows a significant effect of added nematodes, but the Tukey test does not show any significant separation when all four treatment levels are used

Treatments with the same letter within a column and experiment are not significantly different at level  $p \leq 0.05$

experiment 2, presence of *R. solani* or nematodes did not significantly affect either dry weight of stems or number of stems (Table 2).

There was no effect of treatment on either fungal damage (stem canker, sclerotia) or nematode damage on the stems in any of the experiments (Table 3). There was a significant effect of treatment on the number of nematodes in stems in experiment 1 ( $p = 0.02$ ), but not in experiment 2. However, the multiple comparisons could not distinguish among the treatments, but the presence of the fungus seemed to increase the number of nematodes when the level was high (Table 4).

### Impact on stolons

In both experiments, the dry weights of stolons were reduced in all treatments containing *R. solani* ( $p < 0.001$ ) (Table 2). The stolons were both few and very short due to the fungus.

### Impact on tubers

In experiment 1, neither the addition of fungus nor nematodes had an effect on the number of tubers compared to the control, but when high level of nematodes were present, the combination with *R. solani* resulted in fewer tubers than if the nematodes occurred alone ( $p = 0.011$ ) (Table 2). In experiment 2, inoculation of nematodes

**Table 3** Percentage of stems and tubers with fungal and nematode damages from potato plants subjected to different combinations of the plant pathogenic fungus *Rhizoctonia solani* (F) and the root-lesion nematode *Pratylenchus penetrans* (N)—low (L) and high level (L) in experiment 1

Treatment	Stems			Tubers		
	Stem canker <sup>a</sup>	Sclerotia	Nematode lesions	Black scurf	Elephant hide	Nematode lesions
<i>Experiment 1</i>						
Fungus (F)	100	50	–	39	59 a	–
Nematodes low level (NL)	–	–	100	–	–	11
Nematodes high level (NH)	–	–	100	–	–	43
F + NL	83	27	77	20	22 b	26
F + NH	84	24	65	25	32 b	53
<i>Experiment 2</i>						
F	77	77	77	–	54	51
N	–	–	–	100	–	–
F + N	75	81	88	94	37	62

<sup>a</sup> Separated into severity grade 1 and severity grade 2–3 for experiment 2

**Table 4** Abundances of the root-lesion nematode *Pratylenchus penetrans* (no. g<sup>-1</sup> dry weight; mean (SE)) in different parts of potato plants subjected to the plant pathogenic fungus *Rhizoctonia solani* (F) and nematodes (N)—low (L) and high (H) level in experiment 1

Treatment	Stems	Tubers	Roots	Potting medium
<i>Experiment 1</i>				
Nematodes low level (NL)	196 (41)	0.7 (0.6) b	1569 (497)	0.55 (0.21) b
Nematodes high level (NH)	389 (98)	0.7 (0.2) b	1591 (402)	0.93 (0.28) ab
Fungus (F) + NL	177 (69)	3.8 (2.1) ab	950 (404)	0.55 (0.21) b
F + NH	686 (384)	5.1 (2.3) a	1200 (324)	2.01 (0.82) a
<i>Experiment 2</i>				
N	122 (35)	1.6 (0.7)	743 (113)	0.25 (0.12)
F + N	130 (36)	3.9 (1.1)	1391 (425)	0.13 (0.04)

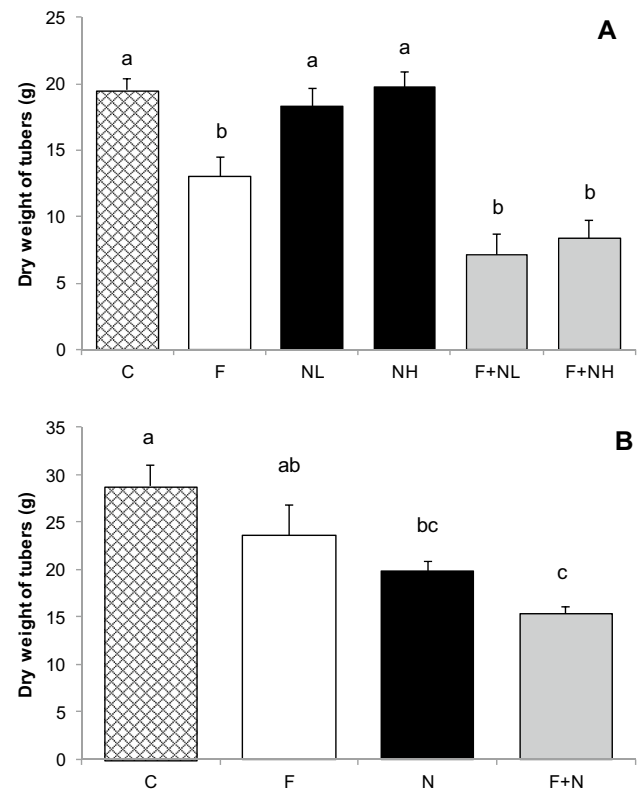
Treatments with the same letter within a column and experiment are not significantly different at level  $p \leq 0.05$

increased the number of tubers ( $p = 0.026$ ). In addition, in experiment 1 the combination of low level of nematodes and fungus resulted in a greater proportion of small tubers compared to the control ( $p = 0.003$ ), while in experiment 2 addition of nematodes in general increased the proportion of small tubers ( $p = 0.026$ ), although the multiple comparisons could not distinguish among the treatments (Table 2).

In experiment 1, the dry weight of tubers differed among the treatments ( $p < 0.001$ ). Plants in all treatments containing the fungus had reduced dry weight of tubers compared to the control and the two nematode treatments (Fig. 1a). In experiment 2, there was an individual effect of both fungus and nematodes, both leading to lower dry weight of tubers, but no interaction effect (Fig. 1b).

Damages of both nematodes and fungus, respectively, were found on the tubers in the pots where they had been inoculated in both experiments. For fungal damage in the form of elephant hide, the likelihood was lower when the fungus was added in combination with nematodes in experiment 1, both at low ( $p < 0.001$ ) and high level ( $p = 0.009$ ). This is indicated by the lower percentage of tubers with elephant hide in the combined treatments (Table 3). However, this was not found in experiment 2 where nematodes did not affect the likelihood of elephant hide. The occurrence of black scurf on the tubers was not affected by the presence of nematodes in either of the experiments (Table 3).

For occurrence of visible nematode damages, addition of the higher level of nematodes in experiment 1 resulted in higher probability than the low level ( $p < 0.001$ ) (Table 3), but presence of the fungus had no effect on the probability of nematode damages in either of the experiments. In addition, the number of nematodes in the tubers was affected by treatment in experiment 1 ( $p = 0.02$ ), where the combination of *R. solani* and high level of nematodes increased the prevalence of nematodes in tubers compared to addition of the nematodes alone (Table 4).



**Fig. 1** Dry weight of tubers [g; mean (SE)] of potato plants subjected to different combinations of the plant pathogenic fungus *Rhizoctonia solani* (F) and the root-lesion nematode *Pratylenchus penetrans* (N). In experiment 1 (a) there were two levels of nematode addition, low (NL) and high (NH), while in experiment 2 (b) there was only one (intermediate). Treatments with the same letter above the bar are not significantly different at level  $p \leq 0.05$

### Impact on roots

Addition of fungus and nematodes did not significantly affect the dry weight of roots in experiment 1, but in experiment 2 inoculation of nematodes reduced the root biomass (Table 2). The roots had brown lesions or sections regardless of treatment and were always covered with sclerotia if *R.*

*solani* had been added. There was no significant difference among the treatments in the number of nematodes in roots in both experiments (Table 4).

### Potting medium

There was a significant effect of treatment on the number of nematodes in the potting medium in experiment 1 ( $p=0.02$ ), but not in experiment 2. In experiment 1, more nematodes were found in the potting sand with addition of fungus and high level of nematodes compared to low nematodes only ( $p=0.01$ ), and fungus and low nematodes ( $p=0.01$ ) (Table 4).

### Discussion

Contrary to our first hypothesis, we found no effect of presence of nematodes on the incidence of stem canker. The second hypothesis was not supported either as we found no effect of nematodes on the severity of black scurf, and the likelihood for elephant hide was even reduced in presence of nematodes in one of the experiments. Also, the hypothesis about lower tuber yield when both pathogens occurred together was rejected, as the decrease in yield did not exceed the sum of the individual effects of the two organisms. As hypothesized, a high level of nematodes resulted in more visible nematode damages, but beyond that the different nematode levels rendered in most cases similar responses. Interestingly, there was no effect of presence of *R. solani* on visible nematode damages, but the fungus increased the number of nematodes in the tubers.

All the plants emerged well in both experiments, so the conditions seemed to have been optimal for growth of the potato plants enabling them to emerge before being heavily attacked by the fungus. However, there are differences in plant growth between the experiments, with more stem biomass and stems in experiment 1, possibly related to the larger size of the seed tubers in combination with longer growth period and greater total *N* addition. It is usually the seed infection of *R. solani* that reduces the emergence and not the soil-borne inoculum (Carling et al. 1989). Therefore, it was not surprising that there were no large differences among the treatments in stem dry weight at harvest either. The number of stems has previously been found to be affected by both *R. solani* and potato cyst nematodes (PCN) (Kiani et al. 2013), but the effect was cultivar specific. However, it is interesting that in the present study it is only the combination of *R. solani* and low level of nematodes that had more stems.

Previous studies have shown an effect of PCN on the incidence and damage severity of stem canker (Bhattarai et al. 2009; Kiani et al. 2013). *Pratylenchus penetrans* used in the

present study is less specialized and does not modify the metabolism of the plants as PCN do (Hussey and Grundler 1998). Instead, *P. penetrans* is not only restricted to attack the roots, and therefore a more direct interaction between *R. solani* and *P. penetrans* could be expected than between *R. solani* and PCN. However, we could not find any effect of the nematode on the incidence and damage severity of stem canker, which could be a result of the fact that *P. penetrans* prefer the roots over the stem (Table 3). It is even possible that the nematodes first attack the roots, and then later attack the stem when the fungus is already present there (Nordmeyer and Sikora 1983). The fact that we could not detect any effect of nematodes on the incidence and severity of stem canker could also be related to the statistical method. As severity of stem canker is a graded measure, the normality assumption of the ANOVA is not met and the use of binomial regression on the dichotomized variable may have resulted in lower statistical power. However, it is difficult to compare the actual amount of *R. solani* inoculated in those studies with the amount inoculated in the present study, as the inoculation methods differed. It is possible that the inoculum level used in the present study was higher leading to severe stem canker even in the absence of nematodes. With a lower fungal inoculum level, it is likely that wounding by the nematodes is of greater importance.

The most pronounced effect of *R. solani* was found on the tubers. Potatoes infected with stem canker (*R. solani*) do not necessarily suffer an overall yield loss but instead a reduction in the number of marketable tubers (Weinhold et al. 1982). However, we did find a yield loss even in the presence of the fungus alone in one of the experiments. That may depend on the amount of added fungal inoculum being greater than the levels usually present in agricultural fields (Budge et al. 2009). Interestingly, the presence of nematode reduced the likelihood of elephant hide on the tubers in experiment 1. It was also noted that black scurf and elephant hide only rarely occurred together on a tuber, which also have been observed by others (J. Woodhall, FERA, personal communication). However, the reasons for that are still unknown. It is suggested that elephant hide originates from a stolon lesion caused by *R. solani* on a pre-tuber which then grows out to form a tuber (J. Woodhall, FERA, personal communication), while black scurf is formed from mycelia growing on the tuber itself (Dijst 1988). These symptoms are therefore likely to be differently affected by nematodes, but the mechanisms behind the reduction in incidence of elephant hide are difficult to discern from this study.

The root-lesion nematode *P. penetrans* may alone cause considerable yield loss, by 30–70%, in the potato crop (e.g. Bernard and Laughlin 1976; Holgado et al. 2009; Morgan et al. 2002; Olthof 1989; Philis 1995). However, in the present study a lower tuber yield due to nematodes compared to the control was only found in experiment 2 (Fig. 1).

The economic threshold of *P. penetrans* on potato crops is reported to be of 100–250 specimens per 250 g soil (Brodie et al. 1993; Holgado et al. 2009), and this would infer that even our low level (1 nematode g<sup>-1</sup> potting sand) could potentially be economically damaging. In experiment 2, the nematodes were retrieved from locally kept maize cultures and added to the pots as soon as there was enough inoculum, while for experiment 1 the nematodes were retrieved from the maize roots in the Netherlands and then transported to us. Therefore, due to the extended extraction and transportation the nematodes were kept around 1 week in water before being added to the pots in experiment 1 and their vigour may have been reduced at inoculation. In addition, the seed tubers just barely had sprouts at inoculation, which means there was also a limited food supply for the nematodes at first. The fact that the potato plants were harvested prematurely in these pot experiments could also account for some of the difference to estimated yield losses due to *P. penetrans* in the field.

The amount of visible nematode damages on the tubers was consistently related to the number of inoculated nematodes across the experiments, as the percentage of tubers with nematode damage in experiment 2 nicely fits in between the percentages found in experiment 1. However, the number of nematodes in the different plant parts increased with the higher level of nematodes but not corresponding to the difference in inoculum level. This suggests that the carrying capacity had been reached and the nematodes were constrained by competition. The greater amount of nematodes in the potting sand in the high level nematode treatment, especially in combination with *R. solani*, also points towards the fact that the nematodes were looking for new fresh roots to attack.

## Conclusion

We have shown that there seems to be an additive effect of the plant pathogenic fungus *R. solani* and the root-lesion nematode *P. penetrans* on yield loss in potato. In addition, the nematode levels in this experiment did not affect the incidence of stem canker and in some cases the nematodes could even reduce tuber symptoms in form of elephant hide. Our results suggest that the fungus and nematodes affect each other in different ways in the different parts of the potato plant, but the exact mechanisms still need to be elucidated. Knowledge about interactions between pathogens, as the one showed here, is essential for choosing appropriate management methods for disease control.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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