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Interactions between the potato cyst nematode *Globodera rostochiensis* and diseases caused by *Rhizoctonia solani* AG3 in potatoes under field conditions

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Abstract

Findings from 2 years of field experiments investigating the relationship between *Globodera rostochiensis* and *Rhizoctonia solani* on unique field sites are reported. In 2000, a field experiment was positioned on land that had previously been used for experimental work investigating integrated potato cyst nematode (PCN) management methods. This study had produced an 'untypical' mosaic of PCN population densities ranging from 5 to 221 eggs g^{-1} soil. In 2001, the field experiment was conducted on a different field site and overlaid on a focus of *G. rostochiensis* population densities ranging from 11 to 108 eggs g^{-1} soil. In each experiment, potatoes (cv. Désirée) were grown in plots with similar population densities of *G. rostochiensis* that were either uninoculated or inoculated with *R. solani*. A series of potato plant harvests were undertaken to investigate the effects of nematode infestation on the incidence and severity of *R. solani* diseases and the associated development of plants. In both experiments, a clear relationship was found between the density of *G. rostochiensis* juveniles present in potato roots and the incidence of stolons infected by *R. solani*, 6 weeks after planting. For the first time this interaction has been determined under field conditions. The results of the study suggest that the interaction between nematode and fungus is indirect and possible mechanisms are discussed.

Introduction

The soil-borne fungus *Rhizoctonia solani* (AG3) is a widespread pathogen of potato (*Solanum tuberosum*) causing the diseases, stem and stolon canker prior to emergence of the crop and black scurf during plant senescence. The former diseases are readily identified by the presence of sunken brown necrotic lesions on both stems and stolons. When severe, such lesions may girdle the entire circumference, ultimately leading to the pruning of infected stems and stolons. Both stem and stolon canker, therefore, are of economic significance since they can result in malformation of developing daughter tubers (Scholte, 1987), a reduction in yield in some early cultivars (Hide et al., 1989) and uneven emergence that gives rise to non-target size tubers (Hide et al., 1973). Whilst the development of black sclerotia on the skins (black scurf) of harvested tubers has no effect on tuber yield, it can significantly downgrade the marketable value of seed tubers (Jeger et al., 1996) and reduce the saleability of ware to prepack markets.

Potato cyst nematodes (PCN) *Globodera rostochiensis* and *G. pallida* are sedentary endo-parasites, requiring the roots of potato to

complete their lifecycle. During PCN invasion, occupation and subsequent emergence into the rhizosphere, the roots are irreparably damaged, reducing their ability to take up water and minerals. Subsequently, infested plants senesce prematurely, are low yielding and are subject to nutrient deficiencies (Whitehead, 1998). It has been estimated that PCN may cause annual losses in the region of €300 million to potato production in the European Community (Mulholland et al., 1996). Furthermore, there are also many other additional costs associated with PCN management, such as population density monitoring, the implementation of control measures and research and development.

Nematode–fungus complexes that are synergistic in terms of disease development and yield suppression are common on a vast range of crops (Back et al., 2002). Since Morgan (1925) first described the equal abundance of *R. solani* infections and PCN cysts on the roots of potato plants sampled from fields in Lincolnshire (UK), controversy has surrounded the significance of PCN in the aetiology of *R. solani* diseases. However, much of the confusion appears to originate from the contradictory outcomes arising from the various glasshouse studies that have been undertaken over the years (Grainger and Clark, 1963; Stelter and Meinl, 1967; Mazurkiewicz-Zapalowicz and Waker-Wójciuk, 1994). Previous research has indicated that yield reductions (Grainger and Clark, 1963) and an increase in the severity of diseases caused by *R. solani* (Mazurkiewicz-Zapalowicz and Waker-Wójciuk, 1994) are dependent on the combined presence of *R. solani* and *G. rostochiensis*. However, contrasting results have shown that either no interaction exists between pest and pathogen (Stelter and Meinl, 1967) or that *R. solani* exerts antagonistic effects on *G. rostochiensis* (Janowicz et al., 1994).

An underlying concern with almost all of the previous studies investigating the interaction between *G. rostochiensis* and *R. solani* is that they have been conducted under controlled conditions. In order to examine the relationship between these two organisms, it is crucial that an investigation is undertaken under natural conditions. Evans and Haydock (1993) state that the 'acid test' for determining how agriculturally significant an interaction is lies within field experimentation.

Following extensive field studies evaluating a range of integrated strategies for the management of PCN (Minnis et al., 2004), a field trial site was available at Harper Adams University College, UK that provided *G. rostochiensis* population densities of 5–221 eggs g^{-1} soil within an area of c.0.5 ha. In this study, this and a similar field site were exploited in order to study the interaction between PCN and diseases caused by *R. solani* under field conditions.

Materials and methods

Inoculum production

Isolates of *R. solani* were obtained from potato tubers infected with black scurf. Identification of the specific anastomosis group(s) (AG) responsible for the black scurf on the infected tubers was achieved using the clean slide method of Kronland and Stanghellini (1988) and staining the hyphae with safranin O. Six 5 mm mycelial plugs from the growing margin of a 7 day-old culture of *R. solani* AG3 were used to inoculate bags containing 1 kg of sand/maize meal (20 g maize meal: 980 g sand: 250 ml distilled water) (Papavizas and Davey, 1962) which had previously been autoclaved at 121 °C for 50 min. All inoculated bags were then incubated for 40 days at 25 °C.

Potato seed

The cultivar Désirée was selected for its low resistance to *R. solani* (Little et al., 1988), its susceptibility to *G. rostochiensis* infestation (Anon, 2000) and its tolerance of drought (Anon, 2000). A sample of 100 seed tubers was assessed for disease using the macroscopic and microscopic examinations of Hide et al. (1968). In both field experiments (2000, 2001), *R. solani* was detected on less than 4% of seed tubers tested.

Estimation of nematode densities in field soil

Potato cyst nematodes were sampled before and after experimentation to determine the initial population (P_i) and final population (P_f) densities. Sampling, extraction and estimation of PCN were undertaken using the methods described by Shepherd (1986).

Site selection

In both 2000 and 2001, sites at Harper Adams University College were sampled for two PCN using two different intensities of sampling (20 m and 5 m between sample points) to locate areas with a suitable range of *G. rostochiensis* population densities. In the first experiment (2000) initial population densities (P_i) of *G. rostochiensis* ranged from 5 to 221 eggs g^{-1} soil, whilst the second experiment (2001) had P_is of 11–108 eggs g^{-1} soil. The purity of the *G. rostochiensis* populations in the experimental plots was confirmed using polymerase chain reaction assays where DNA from 50 cysts was homogenised, extracted, amplified and digested by the Plant Health Clinic at Harper Adams University College, UK.

Field experiment 1 (2000)

This experiment was conducted on Four-gates field at Harper Adams University College (Ordnance Survey Grid Reference: SJ 707195), Shropshire, UK, where potatoes had previously been grown for two consecutive years. Prior to planting, the experimental area was sub-soiled, ploughed to a depth of 30 cm and a granular fertiliser applied ($N = 120$ kg, $P = 120$ kg, $K = 25$ kg; all amounts ha^{-1}). Ridges were then constructed, which were bed-tilled, de-stoned and bed-formed. Plots (four rows, 5 m in length) were marked out and sampled using an auger with a half-cylindrical blade to take 30 soil cores to a depth of 15–20 cm. These cores were bulked to produce a composite sample (300–500 g soil) for each plot and P_i was determined using standard methods (Shepherd, 1986). Using P_i data, plots with similar PCN densities were paired and randomly selected to be either *R. solani* inoculated or left uninoculated. Tubers were planted 25 cm apart, at a depth of 15 cm using a hand-held potato planter on 16 May 2000. Fifty grams of *R. solani* inoculum was applied with each seed tuber in inoculated plots whilst tubers in uninoculated plots were treated with 50 g of the silver sand/maize meal carrier.

The progress of the crop was monitored through assessments of emergence and canopy development (Burstall and Harris, 1983). At 4 and 6 weeks after planting, two rows of two adjacent plants (four plants in total) were harvested from each plot and assessed for stem canker severity by recording the number of stems and stolons infected by the disease. On each of the plants assessed for disease, the numbers of invading juvenile *G. rostochiensis* within the roots were quantified using the acid fuchsin staining methods detailed in Hooper (1986).

Following plant senescence (in September) the haulm was desiccated using diquat (Reglone®, 200 g l^{-1}) at a rate of 4 l ha^{-1}. After tuber skin set (approximately 2 weeks after desiccant application) tubers were hand-harvested from two rows of four adjacent plants in each plot and the severity of black scurf assessed using the methods of James and McKenzie (1972). Total tuber yield and tuber yield in size fractions (< 45 mm, 45–65 mm, 65–85 mm, > 85 mm) were determined using a box grader.

Field experiment 2 (2001)

In 2001, a second field experiment was undertaken at Harper Adams University College, UK (Swans Leasow, Ordnance Survey Grid Reference: SJ 715198). This experiment contained the same treatments used in the original experiment but differed in plot design. In this experiment, plots consisted of single flat beds in which a single harvest row was protected by two outer rows of discard plants. These compact plots allowed the experiment to be positioned on the PCN densities of interest. Five plants were harvested at 6 weeks after planting and at plant senescence. Measurements were taken in a similar manner to those previously described.

Statistical analysis

All statistical analyses were undertaken on Genstat Release 4.2 fifth edition © 2000, Lawes Agricultural Trust.

Results

Field experiment 1 (2000)

A clearly visible difference in emergence and canopy development was observed in field plots where seed tubers had been artificially inoculated in comparison to those left uninoculated. Four weeks after planting, a significant negative relationship

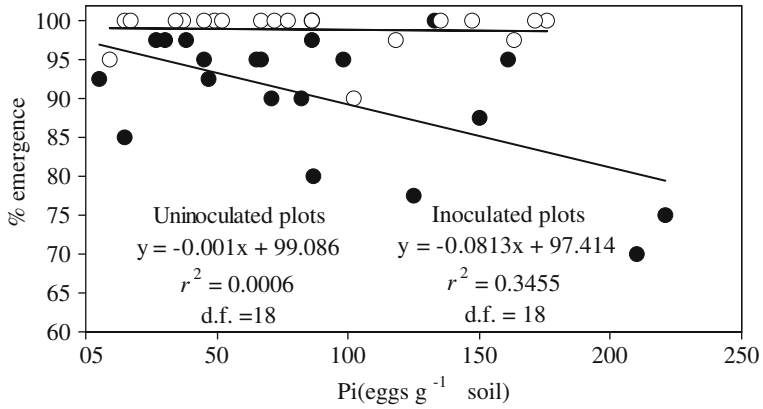

Figure 1. The relationship between initial population densities of *G. rostochiensis* and emergence of potato plants (cv. Désirée) in plots inoculated (●) and uninoculated (○) with *R. solani* during 2000.

($P < 0.01$; $r^2 = 0.346$) was found between *G. rostochiensis* P_i and % plant emergence in inoculated plots. However, no such relationship was observed where seed tubers remained uninoculated (Figure 1).

The majority of potato plants harvested and examined 4, 6 and 8 weeks after planting were found to have some degree of *R. solani* infection. It is probable that infections of potatoes from plots left uninoculated were due to naturally occurring soil-borne *R. solani*. Regression analysis undertaken on these data revealed a variety of relationships between *G. rostochiensis* densities and the incidence and severity of *R. solani* infections. However, the most consistent and strongest relationship was seen between the invasion of potato roots by *G. rostochiensis* juveniles and the percentage of stolons infected by *R. solani* (Figure 2), particularly in plants collected from the *R. solani* inoculated plots 6 weeks after planting.

Whilst significant interactions were found between *G. rostochiensis* infestations and stolon canker in the growing crop, the percentage area coverage of black scurf on tubers (square root transformed) was not found to be related to invasion of *G. rostochiensis* juveniles in inoculated plots, although a weak positive relationship was found in uninoculated plots ($P < 0.05$; $r^2 = 0.2376$).

Simple linear regression analysis revealed significant negative linear relationships between yield of tubers (sampled 18 weeks after planting) and mean infection of stolons by *R. solani* (inoculated plots: $P > 0.001$; $r^2 = 0.6665$, uninoculated plots: $P > 0.001$; $r^2 = 0.5172$) or mean *G. rostochiensis* juvenile invasion of potato roots (inoculated plots: $P > 0.001$; $r^2 = 0.4707$, uninoculated plots: $P > 0.001$; $r^2 = 0.6157$). Multiple regression analysis did not show any significant effects from interacting explanatory variates. Although measurements of tuber size fractions were taken, no significant relationships were found with either measurements of *R. solani* disease incidence or *G. rostochiensis* infestations.

Analysis was also conducted to assess whether plants infected with *R. solani* had any detrimental effect on *G. rostochiensis*. To assess this, *G. rostochiensis* multiplication rates (final population densities (P_f)/initial population densities (P_i) = multiplication rate (P_f/P_i)) were regressed against mean stolon infection measurements (from 4, 6 and 8 weeks harvests). Mean stolon infection showed a moderate negative correlation with square root transformed P_f/P_i in inoculated ($r = -0.596$; $P < 0.006$) and uninoculated ($r = -0.695$; $P < 0.001$) plots.

Field experiment 2 (2001)

As in field experiment 1 (2000), *G. rostochiensis* infestations and stolons infected by *R. solani* were positively related at 6 weeks post-planting. This relationship was only observed within plots inoculated with *R. solani* and was significant using measurements of both *G. rostochiensis* soil densities ($P < 0.001$; $r^2 = 0.488$) and the density of invading juveniles within potato root tissue ($P < 0.01$; $r^2 = 0.328$). Plotting data sets of

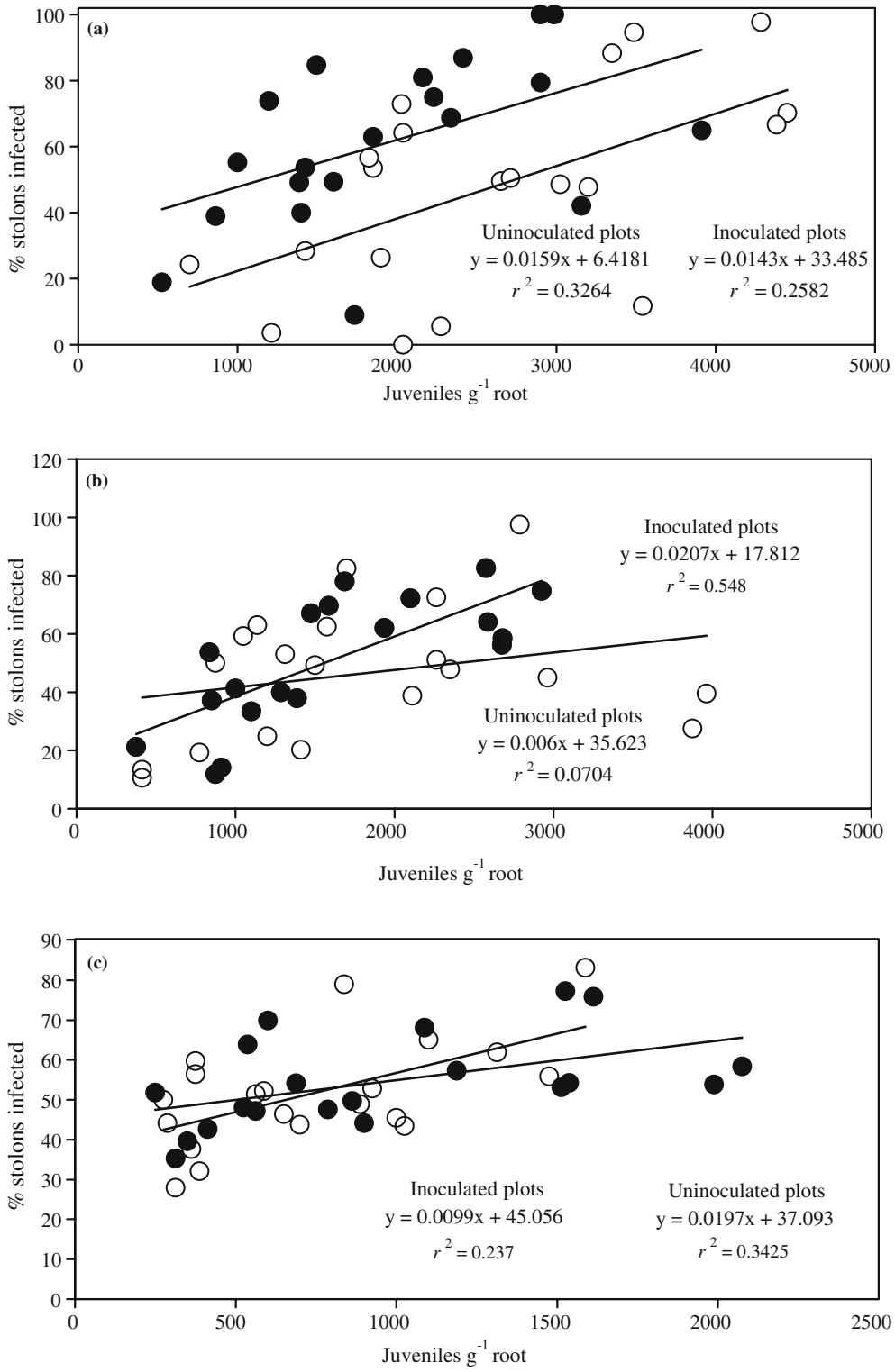

Figure 2. The relationship between *G. rostochiensis* juvenile invasion of potato roots (cv. Désirée) and the percentage of stolons infected by *R. solani* in plots inoculated (●) and uninoculated (○) with *R. solani* at 4 (a), 6 (b) and 8 (c) weeks after planting field experiment 1 (2000).

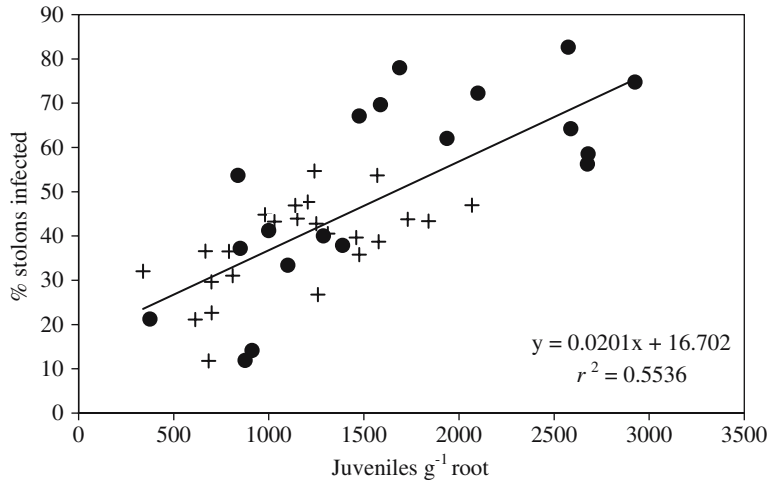

Figure 3. The relationship between invasion of potato (cv. Désirée) roots by *G. rostochiensis* juveniles and infection of stolons by *R. solani* in inoculated plots, 6 weeks after planting field experiments 2000 (●) and 2001 (+).

nematode invasion and stolon canker from both field experiments (Figure 3) made clear that the measurements from the 2 years followed similar trends. Linear regression analysis of the two data sets did not indicate any significant difference between the regression lines. A single line of best fit was therefore used to describe the relationship found over the 2 years of field studies (Figure 3). A significant and relatively strong relationship ($r^2 = 0.5536$) was observed between the population density of *G. rostochiensis* within potato roots (measured as the number of juveniles gram^{-1} of root tissue) and stolon infection by *R. solani* during the two field studies.

Plots inoculated with *R. solani* produced significantly lower tuber yields than those left uninoculated ($P < 0.001$). In addition, weak negative relationships were found between *G. rostochiensis* juvenile invasion of the roots of 6 week-old plants and tuber yield in both *R. solani* inoculated and uninoculated plots ($P < 0.05$). Multiple regression analysis revealed that interactions between *G. rostochiensis* root invasion and stolon infection by *R. solani* had a significant effect on final tuber yield in both inoculated ($P < 0.024$) and uninoculated plots ($P < 0.017$). The proportions of tubers belonging to 10 mm size brackets up to 70 mm were not found to be related to either *R. solani* disease intensity or densities of *G. rostochiensis*.

When *G. rostochiensis* initial population densities (P_i) are plotted against the multiplication rate (final population density/initial population density, P_f/P_i), a negative double exponential curve was obtained (Evans and Stone, 1977). Observations from this experiment indicated that the multiplication rate was additionally affected by inoculation treatments of *R. solani* (Figure 4). Comparison of the two lines for the two inoculation treatments revealed a significant difference ($P < 0.005$).

Discussion

This is the first time field experiments have successfully demonstrated a clear interaction between the nematode *G. rostochiensis* and the fungus *R. solani*. Positive relationships were consistently found between *G. rostochiensis* densities and *R. solani* infection of various potato parts, but particularly between the invasion of potato roots by *G. rostochiensis* juveniles and the incidence of stolons infected by *R. solani*. This relationship is the key finding of this work and has not been reported elsewhere. The closest record outside this work is that of Mazurkiewicz-Zapalowicz and Waker-Wójciuk (1994), where necrosis-type symptoms were reported to increase during combined infestations of *G. rostochiensis* and *R. solani* in comparison to treatments of either organism alone.

Synergistic interactions between plant parasitic nematodes and fungal pathogens are triggered by a

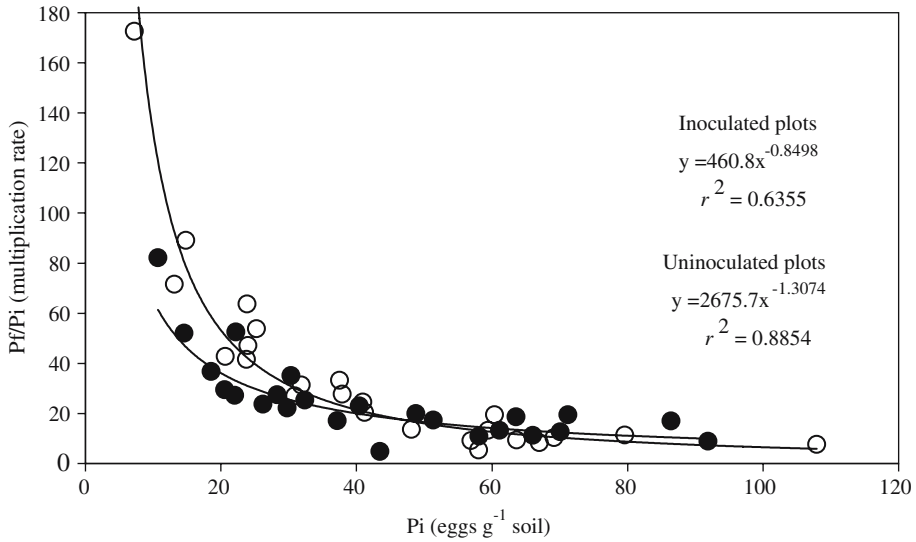

Figure 4. The relationship between initial population densities (P_i) and multiplication rate of *G. rostochiensis* in field plots either inoculated with *R. solani* (●) or left uninoculated (○) during 2001.

variety of specific mechanisms (Back et al., 2002). In view of the current findings, it seems likely that the nature of the interaction between *G. rostochiensis* and *R. solani* is indirect, since pest and pathogen infect different regions of the plant. Given that the roots and stolons of potatoes are within close proximity to one another, there may be an association between root damage caused by invading *G. rostochiensis* juveniles and the release of root metabolites attractive to *R. solani*.

Results from 2000 indicate that the interaction between *G. rostochiensis* densities within potato roots and the percentage of stolons infected was strongest at 6 weeks after planting. At this time, higher proportions of sedentary *G. rostochiensis* juvenile stages (J3–J5) (data not included here) were recovered from potato roots, which would indicate that a larger density of nematode feeding cells (syncytia) were present.

In 2000, a relationship was found between densities of *G. rostochiensis* within the soil (P_i) and percentage emergence of plants in plots inoculated with *R. solani*. Whilst *R. solani* is well known to delay emergence (Secor and Gudmestad, 1999) the combined presence of these organisms may further extend the period taken for plants to emerge as indicated by Figure 1. A further delay in emergence may be critical since previous research has shown that stem canker symptoms increase very little (Hide et al., 1989) or even decrease (Simons and Gilligan, 1997) after plant emergence. Van Emden (1965) hypothesised that potato stems gained resistance to stem canker following exposure to light, which causes a switch to autotrophic nutrition.

It is well established that infestations of potato cyst nematodes *G. rostochiensis* and *G. pallida* adversely affect the yield of potato (Haydock and Evans, 1998). In contrast, 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. It is difficult to determine whether a combination of *G. rostochiensis* and *R. solani* have an effect on yield. Multiple regression analysis revealed that the yield was significantly affected by the combined presence of *G. rostochiensis* and *R. solani* in 2001 but not in 2000. This result is surprising given the reasonable relationship between *G. rostochiensis* densities and *R. solani* stolon infections.

In both field experiments, there were indications that the development of *G. rostochiensis* is disrupted in plants that are co-infected with *R. solani* diseases. In 2000, a negative linear relationship was found between mean stolon infections caused by *R. solani* and the multiplication rate of *G. rostochiensis*, suggesting that fewer female nematodes mature into cysts on potato plants heavily infected with

R. solani. Furthermore, results of multiple regression analyses demonstrated both that densities of *G. rostochiensis* juveniles found in potato roots and the incidence of stolon infections caused by *R. solani* were related to the multiplication rate of *G. rostochiensis* in plots inoculated with *R. solani*. These results were further supported by results from 2001, which indicated a lower multiplication rate in plots inoculated with *R. solani* compared to plots left uninoculated. The development of individual *G. rostochiensis* juveniles into females may be affected by a number of factors. Previous reports have demonstrated a reduction in the ratio of *G. rostochiensis* juveniles developing into females during heavy infestations of *G. rostochiensis* (Trudgill, 1967) or when the host plant is co-infected with pathogens such as *R. solani* (Ketudat, 1969). In the case of high nematode densities it is likely that sex is determined by the lower nutrient supply to individual nematodes (Trudgill, 1967). However, plant pathogens may reduce multiplication directly by colonising and damaging nematode feeding sites. This is illustrated in the work of Fattah and Webster (1983), who used transmission electron microscopy to compare the feeding sites (giant cells) of *M. incognita* during normal development and in the presence of *Fusarium oxysporum* f.sp. *lycopersici*. In this study, nematode feeding sites from tomato plants co-infected with *F. oxysporum* f.sp. *lycopersici* were seen to be much smaller with fragmented and swollen nuclei.

Previous studies (Grainger and Clark, 1963; Stelter and Meinl, 1967; Mazurkiewicz-Zapalowicz and Waker-Wójciuk, 1994) investigating the interaction between *G. rostochiensis* and *R. solani* have been undertaken under controlled conditions. Such studies allow the comparison of independent and combined effects of nematode and fungus on disease development and plant growth. However, field experiments allow interactions between nematodes and fungi to be assessed in the natural soil environment where biotic and abiotic factors are not excluded. Back et al. (2002) highlight characteristics of the soil environment such as temperature, moisture, biota and soil type that may affect the interaction between nematode and fungus.

It is clear from this work that relationships between nematodes and fungi are often multifaceted, whereby differential outcomes occur for host, nematode and pathogen. The present study has demonstrated that *G. rostochiensis* has an important role in the development of *R. solani* diseases, particularly stolon canker, whilst such infections may hinder nematode development.

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