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# A Potential Model for Pea Footrot Disease Prediction

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**Abstract:** Footrot disease due to *Nectria haematococca* (anamorph *Fusarium solani* f.sp *pisi*) is a globally, economically important disease of peas. Although the principle of inter-relationship between soil factors, inoculum density and disease potential in disease incidence, development and severity is recognised, and molecular techniques targeting a pea pathogenicity gene (*PEP3*) have recently been developed to accurately estimate the inoculum load of pathogenic forms of *N. haematococca* in soil, works incorporating soil parameters in a predictive model is yet unknown. Hence, in this research, some biotic and abiotic soil properties were assessed and the factors that influence the interaction between pathogenic *N. haematococca* and peas, culminating to footrot disease were studied. Pearson correlation results showed that pea footrot disease severity arising from the interaction between pathogenic *N. haematococca* and pea was largely dependent on the abiotic component of soil. Pea footrot disease was significantly ($p = 0.05$) related to soil pH ($r^2 = 0.52$), total oxidised nitrogen ($r^2 = 0.41$), C/N ratio ($r^2 = -0.39$), phosphate concentration ($r^2 = 0.56$) and potassium ($r^2 = 0.31$). Principal component analysis results showed that footrot disease, pea pathogenicity gene (*PEP3*), fungal richness, phosphate, potassium and magnesium were positively inter-related. A predictive disease model [DI = 1.97 + (3.48×Phosphate) + (-0.66×C/N)] ($R^2 = 0.42$) identified phosphate and C/N ratio as abiotic factors that determine footrot disease severity of peas in soil.

**Key words:** Footrot disease, peas, *Fusarium solani*, *Nectria haematococca*, pea pathogenicity gene (*PEP3*), soil DNA, soil physico-chemical properties

## INTRODUCTION

Footrot disease due to *Nectria haematococca* (anamorph *Fusarium solani* f.sp *pisi*) is a globally, economically important disease of peas (Biddle, 1984; Graham and Vance, 2003). Like many other plant diseases (Bhatti and Kraft, 1992; Navas-Cortés *et al*., 2000; Sugha *et al*., 1994), a strong and positive relationship between pathogen inoculum density and disease severity has recently been demonstrated to exist (Etebu and Osborn, 2010). However Oyarzun *et al*. (1994) showed, in an earlier study that different soils differentially affected the inoculum potential of *N. haematococca* (anamorph *F. solani* f. sp. *pisi*) in peas even though the inoculum density of the pathogen was the same in all soils. This differential influence of virulent spores of *N. haematococca* in peas was attributed to differences in the soil-environment because soil influences the interactions between a susceptible plant host and its specific pathogen in soil (Alabouvette and Steinberg, 2006).

Soil is a complex and dynamic biological system, harbouring a large number of organisms that play essential and complex roles in the conversion of organic matter and associated nutrients from one form to another, and plays vital roles in many plant-pathogen interactions that may or may not lead to plant diseases (Alabouvette and Steinberg, 2006; Lucas, 2006). Many studies have shown that soils have a capacity to suppress disease incidence or severity on susceptible host plants, in spite of the presence of a pathogen and climatic conditions favourable for disease onset and development (Baker and Cook, 1974; Schneider, 1982; Schippers, 1992; Westphal and Becker, 2001). The ability of soils to control the pathogenic activity of pathogens is dependent on inherent biotic and abiotic soil properties (Alabouvette *et al*., 1982).

Consequently, developing a pea footrot disease predictive model would require a holistic approach taking into account the biological, chemical and physical properties of soils. Although attempts have been made in the past to study biotic and abiotic factors in relation to

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pea foot (root) rot disease, inoculum densities could not be reliably assessed because existing culture-dependent assays employed in the past do not selectively quantify pathogenic forms of the fungus in natural soils (Oyarzun *et al*., 1997). Since culture-based assays do not discriminate between pathogenic and non-pathogenic forms, relationships and correlations between inoculum densities and other biotic and abiotic factors arising from such studies may not be reliable. Interestingly, Etebu and Osborn, (2010) recently developed a PCR-based molecular technique that reliably quantifies the density of pathogenic forms of *N. haematococca* in soil. The technique, however, has not been extended to experiments where biotic and abiotic soil factors were also measured concurrently.

Hence in this research, inoculum densities of 10 agricultural soils were assessed using molecular Q-PCR assays targeting a pea pathogenicity gene (*PEP3*); other biotic and abiotic soil factors were also measured concurrently, and the inter-relationship of these factors with respect to pea footrot disease in soil was studied. It is hoped that the findings of this work would offer us the opportunity to better understand the different factors that cumulatively affect pea footrot disease.

## MATERIALS AND METHODS

**Soil sampling and assessment of footrot disease on peas:** Ten agricultural fields (G-P) distributed across a region spanning ~20 km in East Anglia, U.K were sampled in 3 replicates on 19th May, 2006 as described by Etebu and Osborn (2009). Three viable pea seeds were planted the next day in about 2 kg of soil from every field in three pots (one seed per pot/replicate), and were watered at intervals of 2-3 days for 11 weeks. At the end of 11 weeks, the pea plants were carefully uprooted; the roots were gently washed in water to remove soil, and assessed for footrot disease as described by Etebu and Osborn (2009).

**Molecular analyses of agricultural soils:** DNA extraction: Nucleic acids were separately extracted from 0.25 g of soil samples obtained from all fields using the PowerSoil DNA kit (MoBio, Carlsbad, CA) as according to the manufacturer's protocol. DNA was eluted into a final volume of 100 ml.

**Quantitative PCR:** A pair of primers *PEP3*F1 (5'-cag aac ctc agt cat gct tca aca c-3') and *PEP3*R1 (5'-gct tga gaa gcc att ttg ggt ct-3') recently designed and demonstrated to target the *PEP3* gene of pea pathogenic *N. haematococca* (Etebu and Osborn, 2009) were used to quantify the inoculum load of the pathogen in soil as described by Etebu and Osborn (2010).

**Terminal restriction fragment length polymorphism:** The fungal community of agricultural soils were investigated using terminal restriction fragment length polymorphism analysis. PCR was carried out on soil-DNA extracted from the agricultural soils using a labelled FAM-ITS1F [[6-fam]5'-ctt ggt cat tta gag gaa gta a-3'] and ITS4 (5'-tcc tcc gct tat tga tat gc-3' ) primers targeting the Internal Transcribed Spacer (ITS) region. The PCR mixture contained 1 μL template DNA; 5 μL of 10x PCR buffer (Bioline); 1.25 μL of 50 mM $MgCl_2$; 200 μM of dNTP; 20 pmol of each primer; 0.4% glycerol; 1U of Taq polymerase (Bioline) and made up to a final volume of 50 μL with sterile nuclease free water. The thermal cycling conditions were 94ºC for 5 min, followed by 30 cycles of 94ºC for 60 s, 55ºC for 60 s and72ºC for 60 s, and a final extension at 72ºC for 7 min. Amplicons were purified using a PCR purification kit (Qiagen, Crawley, U.K.). Thereafter, a restriction enzyme digest was performed on the purified PCR products using an *Alu1* restriction endonuclease. Restriction digest mix was composed of 2 μL of *Alu1* enzyme, 1.5 μL of buffer (Applied Biosystems), 5 μL of sample DNA and made up to 15 μL with sterile distilled water. The digest was done in a 0.5 mL tube incubated in a water bath at 37 for 3 h. At the end of the digest, the digested PCR products were stored at -20 until needed. 2.5 μL of a ROX standard (Applied Biosystems) was mixed with 500μl Hi Di formamide to form a master mix. 9 μL aliquots of this master mix was added to 1 μL of digested PCR products, and then denatured at 94 for 4 min. The resulting terminal fragments were thereafter sized in an ABI 3730 automated DNA capillary sequencer (Applied Biosystems, California, USA) at an injection rate of 10 s. Sequences were analysed using GeneMapper computer software version 3.5 (Applied Biosystems, California, USA).

Terminal restriction fragments thus generated were considered as a measure of fungal richness in soil. Also, Simpson's Diversity Index (SDI) used as measure of the diversity of fungi (TRFs) was calculated from the formula

$$SDI = 1 - \frac{\sum n(n-1)}{N(N-1)}$$

Where SDI = Simpson's diversity index, n = relative abundance of the different TRF, and N = total number of TRF (Fowler *et al*., 2005).

**Analyses of physico-chemical properties of agricultural soils:**

**Soil types:** Moist soil samples from fields G-P were air dried, and weighed after gently grinding to dislodge soil particles. Thereafter, the different mineral contents of soil were assessed by passing the soils through various sieve

sizes. Soil particles that passed through the different sieves were weighed and the percentages of the various soil mineral contents were determined. Soil particles ranging from 0.5 to 2 mm were considered as sand, those ranging from 0.2 to 0.5 mm were silt, and those <0.2 mm were clay.

**pH:** Five grams of soil was suspended in 20 mL of distilled water. The suspension was allowed to stand for 15 min, stirred and shaken at intervals. pH was thereafter determined using a pH meter (model Hi 9025; Scientific Laboratories Supplies, Nottingham, UK).

**Total Organic carbon (TOC):** Loss on ignition was used to measure the total organic carbon content of soil. Soil was put into crucibles and dried at 100ºC in an oven to arrive at the dry weight. Having noted the dry weight the soil sample was subjected to 500ºC in a furnace overnight. The crucibles were thereafter cooled in a desiccator and re-weighed. The soil organic content was expressed as a percentage of the dry weight of the soil (Rowell, 1994) from the equation below:

$$\text{TOC (\%)} = \frac{\text{Weight loss of soil (g)}}{\text{Weight of oven dried soil (g)}} \times 100$$

**Determination of total elements in soil:** The Kjedahl method was employed in the determination of total nitrogen, phosphorus, potassium, sodium, calcium and magnessium because of its robust suitability for the determination of total contents of a range of elements in plants, soil and water. Approximately 0.05 g of catalyst (Lithium sulphate : Copper sulphate in a 10:1 ratio) and 1ml of digest reagent (33 g of Salicylic acid, 1 L of concentrated Sulphuric acid) was added to 0.2 g of soil in a digestion tube, and then heated at 370ºC in a digestion block for 6 hours or until the solution goes clear. The digested soil sample was allowed to cool, and then cautiously diluted with about 10ml of water followed by filtration through Whatman No.44 filter paper. The filtrate was further diluted to 50 mL in a volumetric flask, and the concentrations of the individual elements were determined through flame atomic absorption spectrometry according to Allen (1989).

**Determination of organic nitrogen in soil:** Organic nitrogen (nitrate and ammonium) was extracted from 5 g of air dried soil with 50 mL 2M Potassium chloride solution for 30 min, and filtered through Whatman paper No. 42. The samples were later analysed colorimetrically for nitrate and ammonium using a continuous flow injection analyser (FIAflow2, Burkard Scientific, Uxbridge, UK) (Hofer, 2003; Knepel, 2003). Prior to subjection of extract to flow injection analysis, the filtrate was frozen at -20ºC.

**Water holding capacity:** The soil water holding capacity was adapted from the method described by Alef and Nannipieri (1995). About 20 g sub-sample of field moist soil was placed in a 10cm diameter funnel, which had the spout blocked with Whatman paper no. 42. The funnel having the soil was dipped into water contained in a beaker and left to stand for about 2 h to allow the soil to be completely saturated by water through capillary action; the top of the funnel was covered to prevent loss by evaporation, and the slurry was allowed to equilibrate for 24 h. The soil was removed, weighed and oven dried at 80ºC for 5 days. At the end of 5 days the soil samples were reweighed and the Water Holding Capacity (WHC) calculated from the following equation:

$$\text{WHC (\%)} = \frac{\text{(Weight of saturated soil – Weight of oven dried soil)}}{\text{Weight of original soil}} \times 100$$

**Data analysis:** Data was subjected to a variety of analyses to compare between various treatments depending on the data. Nucleic acid (DNA) sequence data were managed using computer softwares such as Sequence analysis version 5.1 (Applied Biosystems), and Genemapper version 3.7 (Applied Biosystems).

Numerical data were managed in Microsoft Excel computer software and statistical analyses were performed using Statistical Package for Social Science (SPSS), version 12.0. This computer programme was used to perform analysis of variance (ANOVA) using the Generalised linear model (GLM). Analysis of variance was carried out after testing for normal distribution (Gomez and Gomez, 1984) to determine whether or not a set of data requires transformation prior to ANOVA. The relationship between various agricultural soil properties and footrot disease of peas were determined through Pearson correlation, principal component, and stepwise forward regression analyses using the SPSS computer software.

## RESULTS AND DISCUSSION

A number of abiotic and biotic factors shared significant ($p = 0.05$) correlations to both *F. solani* inoculum density and pea footrot disease. Many factors (pH, TON, S-$NH_4$, C/N, $PO_4$, and K) showed significant ($p = 0.05$) simple correlations to pea footrot disease (Table 1), indicating that an increase in these factors in soil would lead to increase in footrot disease in peas.

Although pH was observed to be positively related to pea footrot disease (Table 1), findings by earlier workers on various fungal plant diseases were inconsistent. Whilst some workers observed a lack of relationship between pH and various plant diseases (Mallett and Maynard, 1998),

Table 1: Correlation matrix between biotic and abiotic factors of agricultural soils, and footrot disease of peas planted for 11 weeks in soils obtained from agricultural fields with history of pea cultivation

| | PEP3 | TRF | SDI | pH | WHC | TON | S-$NH_4$ | C | $NH_4$ | C/N | $PO_4$ | K | Na | Ca | Mg | DI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PEP3 | 1.00 | | | | | | | | | | | | | | | |
| TRF | - 0.38 | 1.00 | | | | | | | | | | | | | | |
| SDI | 0.33 | 0.08 | 1.00 | | | | | | | | | | | | | |
| pH | 0.16 | - 0.13 | 0.13 | 1.00 | | | | | | | | | | | | |
| WHC | 0.04 | - 0.17 | 0.01 | 0.01 | 1.00 | | | | | | | | | | | |
| TON | 0.41 | 0.01 | - 0.11 | 0.09 | 0.14 | 1.00 | | | | | | | | | | |
| S-$NH_4$ | - 0.04 | 0.06 | - 0.15 | 0.18 | 0.36 | 0.30 | 1.00 | | | | | | | | | |
| C | 0.30 | - 0.26 | 0.10 | - 0.35 | 0.19 | - 0.04 | 0.03 | 1.00 | | | | | | | | |
| NH4 | 0.26 | - 0.17 | 0.34 | - 0.15 | 0.38 | - 0.04 | - 0.004 | 0.61 | 1.00 | | | | | | | |
| C/N | 0.01 | - 0.07 | - 0.38 | - 0.25 | - 0.34 | - 0.02 | - 0.05 | 0.23 | - 0.58 | 1.00 | | | | | | |
| $PO_4$ | 0.28 | - 0.27 | 0.02 | 0.49 | 0.15 | 0.25 | 0.37 | 0.31 | 0.25 | - 0.11 | 1.00 | | | | | |
| K | 0.44 | - 0.37 | 0.12 | 0.13 | 0.17 | 0.22 | 0.13 | 0.79 | 0.43 | 0.21 | 0.56 | 1.0 | | | | |
| Na | - 0.11 | 0.05 | - 0.25 | - 0.37 | - 0.16 | 0.13 | - 0.07 | 0.23 | - 0.09 | 0.39 | - 0.42 | 0.26 | 1.00 | | | |
| Ca | 0.09 | - 0.19 | - 0.33 | 0.65 | - 0.10 | 0.19 | 0.23 | - 0.21 | - 0.30 | 0.19 | 0.53 | 0.15 | - 0.21 | 1.00 | | |
| Mg | 0.19 | - 0.18 | - 0.38 | 0.52 | - 0.05 | 0.35 | 0.21 | - 0.04 | - 0.34 | 0.40 | 0.49 | 0.31 | - 0.01 | 0.91 | 1.00 | |
| DI | 0.09 | - 0.30 | 0.07 | 0.52 | 0.19 | 0.41 | 0.26 | - 0.08 | 0.13 | - 0.39 | 0.56 | 0.31 | - 0.18 | 0.25 | 0.19 | 1.00 |

*PEP3* represent pea pathogenicity gene responsible for footrot disease of peas due to *N. haematococca*; TRF = Terminal restriction fragments (measure of fungal richness); SDI = Diversity/evenness (Simpson's Diversity index); WHC = Water holding capacity (arcsine transformed); TON (Total oxidised nitrogen, $NO_3^-$-N + $NO_2^-$-N); S-$NH_4$ = soluble Ammonium-nitrogen; C = Carbon (percentage loss of ignition); $NH_4$= total Ammonium-Nitrogen; C/N (Carbon/ total nitrogen ratio); $PO_4$ = Phosphate; K= Potassium; Na = Sodium; Ca = Calcium; Mg = Magnesium; DI = Disease index (0 = Healthy; 5 = ≥ 80%); $^x$ = Regression coefficient ($r^2$) in red coloured letters denotes significance at ($p \leq 0.05$)

Table 2: Principal component factor analysis (unrotated) of pea footrot disease with pea pathogenicity genes, soil fungal richness and soil physico-chemical properties[a]

| Plant-Soil Property | Component 1 | Component 2 | Component 3 |
|---|---|---|---|
| Footrot disease | 0.60 | NS | 0.67 |
| PEP3 | 0.60 | NS | NS |
| Fungal richness | -0.53 | NS | NS |
| Phosphate | 0.81 | NS | NS |
| Potassium | 0.83 | NS | NS |
| Magnesium | 0.50 | 0.57 | NS |
| Carbon | 0.51 | -0.78 | NS |
| Total ammonium-nitrogen | NS | -0.69 | NS |
| pH | NS | 0.69 | NS |
| Total oxidised nitrogen (TON) | NS | NS | 0.63 |
| Variance | 35.25 | 19.24 | 10.87 |
| Cummulative variance (%) | 35.25 | 54.49 | 65.36 |

[a]: Loading values greater than or equal to the absolute value of 0.5 indicates significant inter-relatedness with a component; Loading values less than 0.5 were considered not significant

others observed significant (p = 0.05) relationships between pH and disease which were either negative or positive. The role of pH in soil suppressiveness (less disease) seems to depend on the host plant and pathogen involved. Oyarzun *et al*. (1998) while studying factors associated with soil receptivity with respect to three root rot pathogens (*Thielaviopsis basicola*, *Aphanomyces euteiches* and *Fusarium solani* f. sp. *pisi*) of peas observed that pH was positively related to black root rot caused by *T. basicola* but not with footrot disease caused by *F. solani* f. sp. *pisi* or soft root rot caused by *A. euteiches*. Soil pH depends on the chemical factors introduced into soil, partly due to agricultural management practice and the biotic components inherent in soil. As a result, *T. basicola* proved more pathogenic on peas in soils with a relatively high content of elements indicative of alkalinity such as total calcium and nitrate, while its pathogenicity was less severe on peas in soils with increasing carbon and high magnesium and phosphorus content (Oyarzun *et al*., 1998)

Rimé *et al*. (2003), however, observed a relationship similar to the findings of this work, between pH and plant disease due to soil ectoparasitic nematodes. Similarly, a positive relationship between pH and disease (i.e., the more acidic the soil, the less severe the disease) was reported by Lacey and Wilson (2001) with respect to potato scab caused by *Streptomyces scabies*, and by Duffy *et al*. (1997) with respect to take-all disease of wheat caused by *Gaeumannomyces graminis*. However, Höper *et al*. (1995) observed a dissimilar (an inverse) relation between pH and *Fusarium* wilt disease (i.e. the more acidic the soil, the more severe the disease). They observed a positive correlation between pH and soil suppressiveness with respect to *Fusarium* wilt. The findings from this work therefore seem to suggest that acidic soils would generally lead to more suppressive soils (less disease) with respect to footrot disease due to *F. solani* f. sp. *pisi*.

Apart from pH, total phosphate concentration was also significantly (p = 0.05) correlated to pea footrot disease. Like pH, it was positively related to pea footrot disease (Table 1 and 2). Duffy *et al*. (1997) in their study of take-all disease of wheat caused by *Gaeumannomyces graminis* also observed a significant positive relationship

between phosphorus and disease. Similarly, Oyarzun *et al*. (1998) also found a positive relationship between soluble phosphorus and footrot disease in peas due to *F. solani* f. sp. *pisi* but not with soft rot disease due to *A. euteiches*. The lack of a relationship between phosphorus and pea soft rot disease due to *A. euteiches*, again suggests that the role of abiotic factors on soil suppressiveness or conduciveness would depend on the pathogen in question. A number of other workers also did not observe significant ($p = 0.05$) relationships between phosphorus and various diseases. Some of these includes Armillaria root disease of forest pines (Mallett and Maynard, 1998); Potato scab disease of potatoes (Lacey and Wilson, 2001), and black root rot of tobacco (Ramette *et al*., 2003).

Although, phosphate inputs have been reported to have no effect on pea yields (McKenzie *et al*., 2001), the significant ($p = 0.05$) positive correlation observed between phosphate and pea footrot disease makes it a potential indicator in assessing the likelihood of pea footrot disease in agricultural fields prior to cultivation.

Total Oxidized Nitrogen (TON) had a significant ($p = 0.05$) positive correlation to pea footrot disease. Oyarzun *et al*. (1998) also observed the same relationship between soluble nitrogen in soil and pea footrot disease. Soil factors influence plant diseases by affecting the pathogen, host plant or the interaction between plant and pathogen (Alabouvette and Steinberg, 2006). In particular, plant and microbial growth are both limited by nitrogen availability in many ecosystems (Kaye and Hart, 1997). Although in the majority of agricultural management practices, nitrogenous fertilizers are often applied to the soil, peas are relatively unresponsive to fertilizers, particularly nitrogen, except when nodulation is poor or fails completely (Muehlbauer *et al*., 1983). Peas, in association with *Rhizobium*, are capable of fixing atmospheric nitrogen which meets their requirement for high yield (Crozat *et al*., 1994). This capacity to fix nitrogen probably explains its unresponsiveness to fertilizer application, particularly nitrogen. As a result, excessive nitrogen in soil, not utilized by pea plant may lead to an increase of footrot disease as observed in this work (Table 1) due to any or all of the following reasons. (i) Nitrogen, in its nitrate form, is indicative of alkalinity (Oyarzun *et al*., 1998), and as such would lead to an increase in soil pH which has been demonstrated to show a significant positive correlation with plant disease in this work and by some earlier works (Workneh *et al*., 1993; Oyarzun *et al*., 1998). (ii) Since peas are capable of meeting their nitrogen requirements through atmospheric nitrogen fixation, excess TON not utilized by pea plants in soil infested with pathogenic *F. solani* f. sp. *pisi* may become available to the pathogen and other microbes for growth and reproduction, thereby increasing the chances of inoculum proliferation in soil. The significant ($p = 0.05$) positive relationship between TON and pea footrot disease is probably as a result of a relative increase in fungal pathogen numbers. The fact that competition for nitrogen by biocontrol agents in soils suppresses the growth of soil-borne plant pathogens (Scher *et al*., 1984), coupled with the significant ($p = 0.005$) positive relationship observed between TON and *PEP3* gene in this present work (Table 1) further supports this assumption. It is important also to note that total ammonium-nitrogen ($NH_4$-N) had no significant ($p = 0.05$) relationship with pea footrot disease. The form of nitrogen ($NO_3$ or $NH_4$) has been noted as an important factor when it comes to its role in disease suppression in soil (Janvier *et al*., 2007).

Potassium was observed to show a significant ($p = 0.05$) positive correlation to footrot disease ($r^2 = 0.31$) (Table 1). Although Mckenzie *et al*. (2001) had shown that it was generally not a major factor in pea yields, potassium seemed to influence footrot disease.

Total sodium was not significantly ($p = 0.05$) correlated to pea footrot disease, but it is the only abiotic factor that showed a significant ($p = 0.05$) negative correlation to other abiotic factors that themselves showed significant positive correlation to pea footrot disease (Table 1). In particular, it was negatively related to pH and phosphate which are themselves positively related to pea footrot disease. Whilst the role of phosphate in the association between pea plant and *F. solani* f. sp. *pisi*, if any, is not yet known, sodium may remotely influence its impact.

Total magnesium and calcium are the two most significantly ($p = 0.05$), strongly correlated ($r^2 = 0.91$) abiotic factors assessed in this work. Results showed that magnesium was significantly ($p = 0.05$) positively related to pH ($r^2 = 0.52$), Total Oxidised Nitrogen (TON) ($r^2 = 0.35$), C/N ratio ($r^2 = 0.40$), phosphate ($r^2 = 0.49$), and potassium ($r^2 = 0.31$) (Table 1), and significantly ($p = 0.05$) negatively related to total ammonium-nitrogen ($NH_4$-N) ($r^2 = -0.34$) and fungal diversity ($r^2 = -0.38$) (Table 1). The capacity of soils to suppress soil-borne plant diseases has, in part, been attributed to diversity of soil microbial communities (Abawi and Widmer, 2000; Garbeva *et al*., 2004; Kennedy and Smith, 1995; Nitta, 1991; Sullivan, 2004; van Elsas *et al*., 2002). These results suggest that increase in magnesium may result in a less diverse fungal population, while favouring the relative proliferation of pathogenic *F. solani* and pea footrot disease severity. Several works have shown that *F. solani* f. sp *pisi* isolates that lack the *PDA* and/or *PEP3* gene were not highly virulent even when other *PEP* genes are present (Etebu and Osborn, 2009; Temporini and VanEtten, 2002). This underscores the potential relevance of Mg in pea footrot disease, being significantly ($p = 0.05$) positively related to the *PEP3* gene in soil.

Principal Component Analysis (PCA) was used to determine the relationship between the biotic and abiotic components of soils that cumulatively interacts with pea

Table 3: Summary of coefficients of predictors in a pea footrot disease predictive model obtained from Stepwise multiple regressions analysis

| | Unstandardized coefficient | | | | | Collinearity Statistics | |
|---|---|---|---|---|---|---|---|
| Model | β | Std.Error | Standardized Coefficients β | t | Sig | Tolerance | VIF |
| 1 (Constant) | - 1.00 | 1.07 | | - 0.94 | 0.36 | | |
| P | 3.73 | 1.04 | 0.56 | 3.58 | 0.00 | 1.00 | 1.00 |
| 2 (Constant) | 1.97 | 1.68 | 1.17 | 0.25 | | | |
| P | 3.48 | 0.98 | 0.52 | 3.54 | 0.00 | 0.99 | 1.01 |
| C/N | 0.66 | 0.30 | - 0.33 | - 2.20 | 0.04 | 0.99 | 1.01 |

$R^2$ Model 1 (0.31); Model 2 (0.42); Dependent variable: DI; Disease model: DI = 1.97 + (3.48*Phosphate) + (-0.66*C/N)

plants to influence pea footrot disease. Additionally, all variables except Ca being very strongly correlated to Magnesium were subjected to Stepwise regression analysis to identify a potential pea footrot disease predictive model (Field, 2006).

Principal components 1 and 2 cumulatively accounted for 54.49% (λ1= 35.25%; λ2= 19.24%) of the total variance between variables. Although no simple correlation was established between the pea pathogencity gene and footrot disease, principal component factor analysis showed that the *PEP3* pathogenicity genes was related to footrot disease (Table 2). The goal of principal component analysis is to identify the combination of variables that explains the largest amount of variation in a multivariate data set (Fowler *et al*., 2005). This suggests that inoculum density, measured as *PEP3* gene copy numbers may have in some ways influenced pea footrot disease. Results showed that inoculum density measured as pathogenicity genes (*PEP3*), fungal richness, phosphate, potassium, magnesium and pea footrot disease were inter-related (Table 2). Whilst $PO_4$, K and Mg produced significant positive relationships (p = 0.05) with footrot disease as given by Principal Component Analysis (PCA), Oyarzun *et al*., (1998) observed a negative relationship between these same soil factors with increasing suppressiveness to *F. solani* f. sp. *pisi* (i.e., an increased P, K and Mg in soil was related to a correspondingly lower level of footrot disease of peas grown in such soils). Conflicting reports on the relationship between soil factors and plant disease abound in literature. For example, Duffy *et al.* (1997) working on take-all disease of wheat observed a relationship between ammonium-nitrogen and disease, using PCA, similar to the findings of Oyarzun *et al*. (1998). Additionally, others have also reported that ammonium-nitrogen could reduce the take-all disease of wheat (Colbach *et al*., 1997). In contrast, Huber and Wilhelm (1988) observed that the decrease in rhizosphere pH associated with ammonium-nitrogen increases the availability of manganese to the wheat plant, which in turn is known to influence take-all disease (Huber and McCay-Buis, 1993; Huber and Wilhelm, 1988). These apparent conflicting results observed by different workers underlines the complex nature of soil borne plant disease incidence, development and severity. Höper and Alabouvette (1996) in a review on soil suppressiveness concluded that the importance of soil physicochemical factors towards plant diseases is yet to be understood, owing to the complexity of the interactions between these factors. Oyarzun *et al*. (1995), observed that soil receptivity to root rot of grain legumes is essentially a product of the biotic environment.

Two predictive models were identified to significantly (p = 0.001) account for pea footrot disease variability in agricultural soils (Table 3). The first model accounted for 31% of the variability, and involved phosphate as the only predictor (Table 3). The second on the other hand accounted for 42% of pea footrot disease variability in agricultural soils (Table 3). This second model had phosphate and C/N ratio as potential pea footrot disease predictors. Both potential predictors contributed significantly (p < 0.05) to the variability in pea footrot disease observed among the different agricultural soils. Whilst phosphate contributed 31% of the variation in pea footrot disease, C/N ratio accounted for an additional 11% (Table 3).

The identified potential pea footrot predictive model resulting from stepwise forward regression analysis was [DI = 1.97 + (3.48*Phosphate) + (-0.66*C/N)]. Where DI represents disease index; phosphate measured in mg/g soil; N represents total ammonium nitrogen, also measured in mg/g soil and C represented soil organic carbon measured as percentage Loss of Ignition (LOI). The identified model showed that phosphate is significantly (p = 0.05) positively related to pea footrot disease, while as part of the same model, C/N ratio is negatively related to disease. Stepwise regression analysis did not reveal any direct relationship between footrot disease and any of the *PEP3* gene copy numbers (Table 3).

Although both predictors significantly (p<0.05) account for pea footrot disease, phosphate was shown to be a more important contributor to pea footrot disease than C/N ratio (Table 3) All correlations between the individual variables and the outcome (pea footrot disease) showed that phosphate was more highly related to the disease than C/N ratio (Table 3). This was the case for Zero-order correlation (Pearson correlation) which measures the relationship between each predictor and the outcome (Field, 2006). Also, Phosphate was shown to correlate more strongly to pea footrot disease than C/N ratio with partial correlation. Partial correlation measures the relationship between a predictor and the outcome, while controlling for all other predictors in a model. Furthermore, part correlation which is described to

represent the relationship between each predictor and the part of the outcome (footrot disease) that is not explained by the other predictors in a model (Field, 2006), also showed, by their regression coefficients, that phosphate was more strongly correlated to pea footrot disease than C/N ratio.

Although the pea pathogenicity gene (*PEP3*) was not identified in the model, it nonetheless would have played an important role in disease incidence and severity. As earlier stated, all the agricultural fields assessed in this work seem to possess the threshold amount of the *PEP3* pea pathogenicity gene (100 per g soil), capable of inflicting maximum pea footrot disease (Etebu and Osborn, 2010). For agricultural fields having a density of $\geq$ 100 *PEP3* gene copy numbers per gram of soil the variables that would determine disease severity as identified in the model are phosphate and the C/N ratio present in soil prior to pea cultivation.

Similarly, although the role of C/N ratio in pea footrot disease could not easily be ascertained as it showed no significant ($p = 0.05$) correlation to the pea pathogenicity genes assessed in this work, it was the only abiotic factor that showed a significant ($p = 0.05$), inverse correlation ($r^2 = -0.39$) to pea footrot disease (Table 1). This relationship was again observed in the pea footrot disease model (Table 3). An increase in the amount of nitrogen in soil with no corresponding increase in organic carbon would result in a low C/N ratio value, and that would in turn result in increased pea footrot disease as shown in the identified pea footrot model (Table 3). Some workers have shown that a high supply of nitrogen results in severe disease conditions in plants (Graham, 1983). Oyarzun (1993) showed that foot/root rot disease reduced pea yield by over 50% even when more than 200 kg N/ha was applied. High plant nitrogen is thought to remove carbon from metabolic pathways that lead to the synthesis of defence substances, such as phytoalexins, alkaloids and phenolics (Horsfall and Cowling, 1980). This may explain why a high amount of nitrogen with no corresponding amount of carbon in soil renders pea plants vulnerable to footrot disease.

Alternatively, a high amount of carbon in soil, without a corresponding amount of total ammonium-nitrogen would result in a high C/N ratio. Using the pea footrot disease model identified in this work, the higher the C/N ratio value, the lower the footrot disease severity. The expression of the pea pathogenicity genes required in footrot disease has been shown to be suppressed by glucose and amino acids (Khan and Straney, 1999). Soil carbon and nitrogen in carbohydrate and amino acids may therefore suppress the expression of the *PDA* gene, needed to initiate footrot disease in peas. Cues have been identified to regulate different pathogenesis-associated genes in several plant-fungal relationships (Khan and Straney, 1999). These include changes in nutrient levels (Talbot *et al*., 1993; Van den Ackerveken *et al*., 1994), and plant-specific compounds such as pectin or cutin fragments (Gonzalez-Candelas and Kolattukudy, 1992; Lin and Kolattukudy, 1978).

As predicted in this model and proposed herein, the other variable that would determine the incidence or severity of pea footrot disease in soils, provided such soils do have the threshold number of *F. solani* f. sp. *pisi* required for disease, is the concentration of phosphate. Although phosphate was found to occur at relatively low concentrations compared to other abiotic soil chemical properties, it nevertheless was significantly related to many of the variables including the pea pathogenicity gene (*PEP3*) (Table 1). The *PEP3* gene is the only pea-pathogenicity gene present exclusively in highly virulent strains of *N. haematococca* (Han *et al*., 2001, Temporini and VanEtten, 2002). There are contrasting views on the role of phosphorus on peas. Whilst some observed phosphorus as being desirable for pea growth (Hadavizadeh and George, 1988), others such as, McKenzie, *et al*. (2001) reported that adding phosphate into soil has no effect on pea yields. Findings from this work, however, show that high amounts of $PO_4$ would lead to severe pea footrot disease, and by extension would reduce yield (Table 3). It probably enhances disease by conferring a comparative advantage on *N. haematococca* (*F. solani* f. sp. *pisi*) during pea infection. There is a clear need to further study its role in the pea plant-pathogen interaction.

Annual pea production in the UK is valued at about £55M in 1984 (Biddle, 1984). Footrot disease causes yield loss of peas between 35-57% (Kraft, 1984, 2001; Oyarzun, 1993). Footrot disease could therefore be responsible for a loss of as much as £19.25-£31.35M (being 35- 57% of £55M) annually in the agricultural sector of the UK economy. The predictive model identified herein accounts for 42% of the variability of pea footrot disease in agricultural soils, meaning that the use of this model would potentially explain 42% of pea yield loss due to *N. haematococca* that could cost the UK economy as much as £19.25-£31.35M annually.

However, as with every model identified through statistical analysis, this model needs validation (Ramette, 2007) through experimentation across agricultural soils with diverse cropping histories. Despite the need for further work to confirm the applicability of this model, as far as we know, for the first time a potential predictive model is proposed with respect to pea footrot disease.

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