Project title:	Towards the development of a laboratory based assay for the detection of Common Root Rot (*Aphanomyces euteiches*) in vining peas.
Project number:	FV 429
Project leader:	Dr Lea Wiesel, PGRO
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Key staff:	Lea Wiesel, Carole Smith, Rebecca Ward (PGRO) Neil Murray (Bruce Farms)
Location of project:	Processors and Growers Research Organisation, The Research Station, Great North Road, Thornhaugh, Peterborough, PE8 6HJ.
Industry Representative:	Neil Murray, Bruce Farms, Balmyle, Meigle, Perthshire PH12 8QU
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AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

[Name]

[Position]

[Organisation]

Signature ..... Date .....

[Name]

[Position]

[Organisation]

Signature ..... Date .....

Report authorised by:

[Name]

[Position]

[Organisation]

Signature ..... Date .....

[Name]

[Position]

[Organisation]

Signature ..... Date .....

CONTENTS

GROWER SUMMARY

Headline

A quick and reliable plate test to determine levels of common root rot (*Aphanomyces euteiches*) in soil samples has been developed. This test is now available at PGRO as a service to growers and can be used to assess risk levels of disease development prior to pea planting.

Background

Vining peas for the frozen pea market are grown in eastern parts of the UK due to climatic conditions, and in close proximity to processing factories to comply with 150 minutes from field to frozen. These two factors put huge pressures on land and pea yields have been declining over the last 10 years not only in the UK but worldwide, in pea cropping areas. Foot rot diseases are a major reason for yield losses and are caused by a complex of soil-borne pathogens including *Fusarium solani* f.sp *pisi*, *Fusarium oxysporum* f.sp *pisi*, *Didymella pinodella* and *Aphanomyces euteiches*. Disease symptoms usually appear when the plant begins to flower or earlier when plants are stressed due to waterlogging or other environmental factors. Strong disease development can lead to complete crop losses and in less severe incidences uneven maturity of the crop and associated reduction in product quality. All of the pathogens produce long-lasting resting spores leading to increased pathogen levels in soils over pea cropping cycles. In France and the Great Lakes Regions of the USA, inoculum levels of *A. euteiches* in soil have become so high that pea production and the processing factories have been relocated to less infected areas. *Aphanomyces* levels in the UK seem to be on the rise and if we do not find ways to accurately determine pathogen levels in soil we could be at risk of not being able to grow peas in the future.

Chemical treatment against foot rot diseases is not available and once the disease has developed there is very little a grower can do to save the crop. Disease development is weather and soil structure dependent, and is favoured by high soil moisture and often seen in soils where there has been a history of soil compaction and water-logging. Another factor is drilling time and peas sown in cold wet soils appear to be more susceptible than those grown later in the season. Mitigation is limited to crop rotation strategies.

One strategy for pea growers to reduce risks of yield losses is to assess pathogen levels in fields before planting pea crops. Prediction of pathogen levels in soil will give an indication about likelihood of disease development when conditions are favourable for the disease. At PGRO, a soil test is offered to test for abundance of *F. solani* f.sp *pisi* and *D. pinodella*. However, no such test exists for *A. euteiches* and therefore this project aimed to develop a

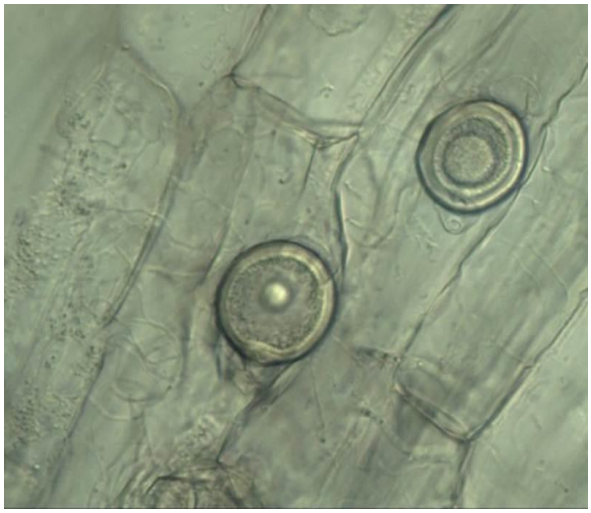
quick and reliable laboratory test to measure abundance of *A. euteiches* in soils to be able to assess risk levels of disease development prior to pea planting.

Summary

One mitigation strategy to avoid yield losses due to common root rot disease in peas is to assess *A. euteiches* levels in soils prior to pea cropping and to choose fields with low levels where disease development is unlikely to occur. A soil bait method is available to assess *A. euteiches* levels in soils but requires up to six weeks to deliver results which is too long for growers who need to use it for rotational planning.

To overcome this limitation, a quick laboratory test to assess levels of *A. euteiches* in soils has been developed. Pea seedlings are grown for eleven days in a dish whilst being exposed to the test soil (Picture 1). After the incubation period the roots of the seedlings are assessed for infection by *A. euteiches*. Roots of infected seedlings are honey coloured and softer than healthy roots (Picture 1). The infection is scored on a scale from 0-5 based on the percentage of the root tissue showing disease symptoms. To validate that the disease symptoms are caused by *A. euteiches* the roots are microscopically examined for the presence of thick walled oospores (Picture 2).

Picture 1: Plate test using soil. Plates on the left were inoculated with *A. euteiches* infected test soil and seedlings show disease symptoms (honey discolouration). Plates on the right were inoculated with sterilised soil as a negative control and seedlings are completely healthy.



Picture 2: Thick walled *Aphanomyces euteiches* oospores in roots of pea seedling that had been growing in infected test soil.

The plate method using soil was developed alongside a second method that uses organic matter extracted from test soils instead of the test soils themselves. *Aphanomyces euteiches* oospores are concentrated in the organic matter fraction of soils and it was proposed that using organic matter instead of soil might give better infection. However, all three methods, the traditionally used soil bait method (pot), the plate method using soil (soil) and the plate method using organic matter (OM) gave similar results on all test soils (Figures 1 and 2). It is known that *A. euteiches* occurs in Scotland and therefore, Scottish fields were selected for soil sampling. In 2014, eight soil samples and in 2015, nine soil samples were collected from fields near Perth, Scotland. Results obtained using the three different methods significantly correlated in both years, demonstrating that all three methods give the same consistency for assessing risk levels of *A. euteiches* in soils.

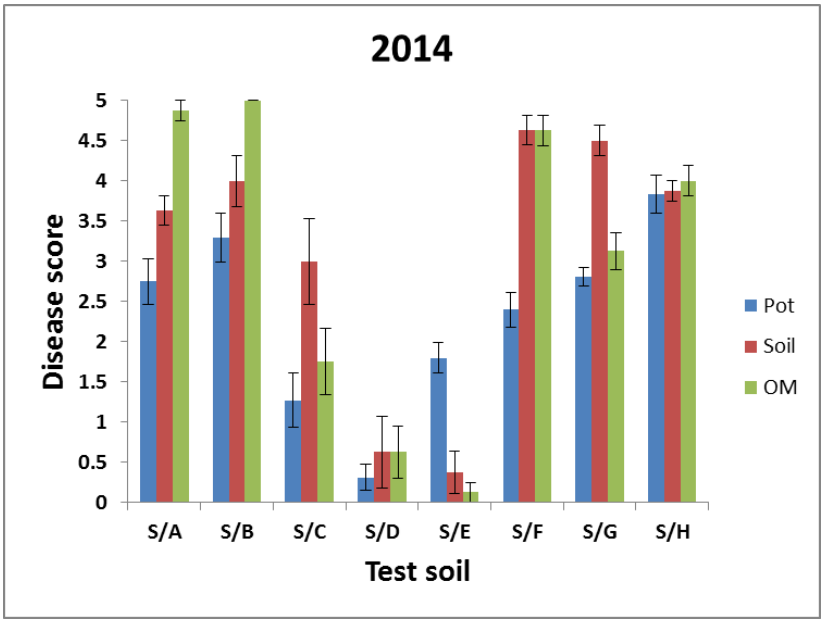


Figure 1: Disease scores (scale 0 to 5) for levels of *A. euteiches* infection of pea roots assessed using the soil bait method (pot), the plate method using soil (soil) and the plate method using organic matter (OM). Test soils had been collected from Scottish fields in 2014. Data show mean values and standard error (n>8).

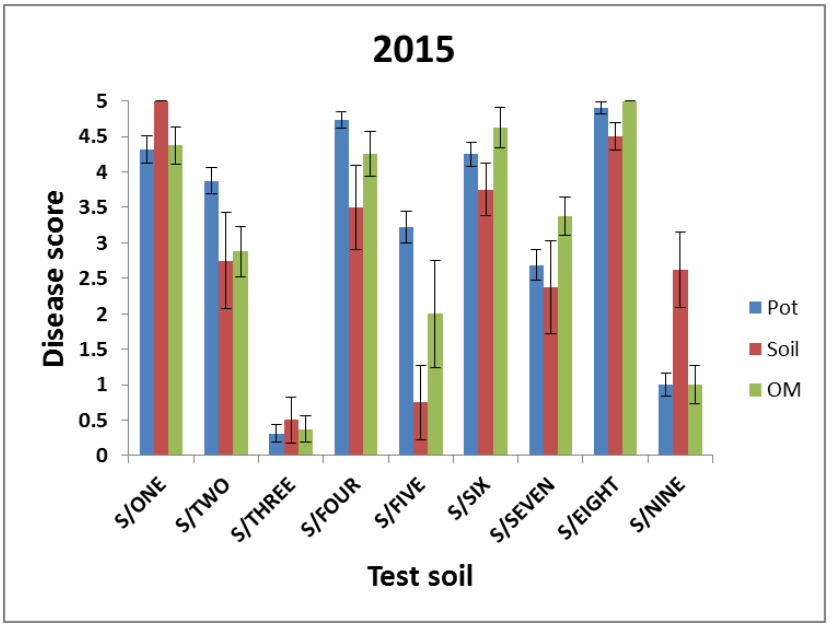


Figure 2: Disease scores (scale 0 to 5) for levels of *A. euteiches* infection of pea roots assessed using the soil bait method (pot), the plate method using soil (soil) and the plate method using organic matter (OM). Test soils had been collected from Scottish fields in 2015. Data show mean values and standard error (n>8).

The fields from which the tests soils had been collected were visually rated for disease development. When foot rot levels in the pea crop were high the soils scored a high *A. euteiches* rating (Table 1). This shows that *A. euteiches* levels in soils can be used to predict disease development in the field when conditions are favourable for disease development.

Table 1: Visually rated disease status of pea crops and average *A. euteiches* risk score determined using the soil bait method, the plate method using soil and the plate method using organic matter.

Sample	Year	Status of crop	*A. euteiches* risk score
S/A	2014	Slightly sick crop	3.75
S/B	2014	Healthy crop	4.10
S/C	2014	Sick crop	2.01
S/D	2014	Healthy crop	0.52
S/E	2014	Very healthy crop	0.77
S/F	2014	Sick crop	3.88
S/G	2014	Very sick crop	3.48
S/H	2014	Very sick crop	3.90
S/One	2015	Very sick crop	4.56
S/Two	2015	Very sick crop	3.17
S/Three	2015	Healthy crop	0.40
S/Four	2015	Very sick crop	4.16
S/Five	2015	Healthy crop	1.99
S/Six	2015	Healthy crop	4.21
S/Seven	2015	Very sick crop	2.81
S/Eight	2015	Slightly sick crop	4.80
S/Nine	2015	Slightly sick crop	1.54

The test using soil instead of organic matter is quicker because it does not require the extra time for extraction of organic matter from soil. The soil test is therefore cheaper to run and has been chosen to be offered as a service to pea growers by PGRO.

Financial Benefits

Presently, it is not possible to link levels of *A. euteiches* in soils prior to pea planting with potential yield losses. However, work undertaken in Wisconsin, USA in the 1980s suggested that yield losses range from 42% to 86% in susceptible pea varieties. In extreme cases total crop loss can occur. Furthermore, PGRO is already working on linking *A. euteiches* levels in soils with potential yield losses. In addition to yield losses, foot rot diseases also lead to reduction in product quality and in most cases when clear foot rot symptoms are visible in the field the crop is not commercially viable. Since the disease cannot be controlled chemically, assessing pathogen levels in soils holds great potential to minimise impacts of the disease. If *A. euteiches* levels in soils are high (scores >3) it seems very likely that disease will develop especially in wet years or in fields with soil compaction or waterlogging issues. Furthermore,

levels of *A. euteiches* in some areas of France and of the USA have become so high that pea cropping had to be abandoned in these areas. Testing soil samples for the presence of *A. euteiches* will also help to monitor pathogen distribution across the UK. PGRO recommends testing soils for levels of *A. euteiches* prior to pea planting to help control disease development and spread especially in the northern regions of the UK where high levels of *A. euteiches* are already present in soils.

Action Points

To measure *A. euteiches* levels in soils, a soil sample of around 2 kg needs to be collected by growers using a W shape across the field. The soil samples need to be sent to PGRO where the developed plate test will be used to assess risk levels in soils at a price of £149 per sample. Within two weeks, growers will be informed of risk levels in their fields which can be used to inform decisions on whether to plant a pea crop or not. To avoid potentially high yield losses due to common root rot fields with *A. euteiches* levels of greater than 3 should not be used for pea cropping.

SCIENCE SECTION

Introduction

Vining peas for the frozen pea market are grown in eastern parts of the UK due to climatic conditions and close proximity to processing factories, to comply with 150 minutes from field to frozen. These two factors put huge pressures on land and pea yields have been declining over the last 10 years not only in the UK but worldwide in pea cropping areas (Bennett *et al*. 2012). Pathogenic fungi and oomycetes have been identified as the major causes for pea yield depression (Fuchs *et al*. 2014) and foot rot diseases are caused by a complex of soil-borne pathogens including *Fusarium solani* f.sp. *pisi*, *Fusarium oxysporum* f.sp. *pisi*, *Didymella pinodella* and *Aphanomyces euteiches* (Bødker and Leroul 1993). Disease symptoms usually appear when the plant begins to flower or earlier when plants are stressed due to waterlogging or other environmental factors. High disease development can lead to complete crop losses and in less severe incidences uneven maturity of the crop. All of the pathogens produce long-lasting resting spores leading to increased pathogen levels in soils over pea cropping cycles. In France and the Great Lakes Regions of the USA, inoculum levels of *A. euteiches* in soil have become so high that pea production and the processing factories had to be relocated to less infected areas (BASF AgSolutions, Canada). *Aphanomyces* levels in the UK seem to be on the rise and if we do not find ways to accurately determine pathogen levels in soil we could be at risk of not being able to grow peas in the future.

Chemical treatment against foot rot diseases is not available. Disease development is weather and soil structure dependent and is favoured by high soil moisture. It is often seen in soils where there has been a history of soil compaction and waterlogging. Another factor is drilling time, and peas sown in cold wet soils appear to be more susceptible than those grown later in the season. Mitigation is limited to crop rotation strategies; one strategy for pea growers to reduce risks of yield losses is to assess pathogen levels in fields before planting pea crops. Prediction of pathogen levels in soil will give an indication of the likelihood of disease development when conditions are favourable for the disease. At PGRO, a soil test is offered to test for abundance of *F. solani* f.sp. *pisi* and *D. pinodella*. Soil dilutions are spread on selective media, and colony numbers of the pathogenic fungi are counted (Biddle 1993). However, no quick laboratory test exists for *A. euteiches*.

Aphanomyces euteiches infection begins with the germination of oospores in response to pea root exudates. The released zoospores swim in soil water towards the seedling and infect the root tissue. The roots develop a honey discolouration and the outer root cells disintegrate. The oomycete develops thick walled oospores which form in abundance in the decaying pea roots. *Aphanomyces euteiches* is a very resilient fungus and the oospores can survive in soils

for more than ten years. The currently available test to determine *A. euteiches* levels in soils is a soil bait test performed in a glasshouse which takes about six weeks to deliver results. Although the bait test gives reliable results it takes too long to be used by growers for crop rotational planning. This project aimed to develop a quick and reliable laboratory test to measure abundance of *A. euteiches* in soils to be able to assess risk levels of disease development prior to pea planting.

Materials and methods

Methods tested during the first year of the project

During the first year of the project, six different methods were tested to decide whether they hold the potential to be used as a laboratory test to assess *A. euteiches* levels in soils. The soil bait method was used as a standard test. For all methods (except for the agar plate methods) infected pea roots were assessed on a scale of 1 – 5 (Figure 3).

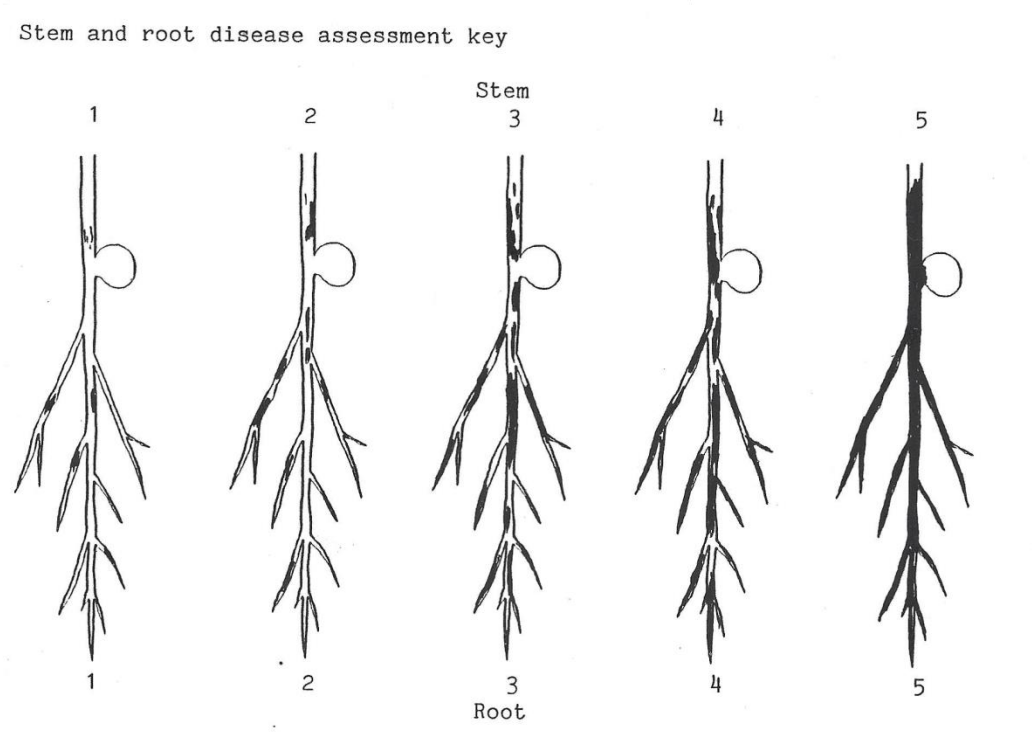


Figure 3: Scoring sheet to assess root and stem infection levels caused by *A. euteiches*.

Generally, infection was confirmed by checking for oospores in the root tissue using microscopy. *Aphanomyces* oospores are concentrated in the organic matter part of the soil. Organic matter was extracted from a few soil samples to be used instead of straight soil in some of the methods. The methods are described in detail in the FV 429 Annual Report 2015 and brief descriptions are provided here.

1) **Soil bait method:** Peas are grown in pots containing the test soils in a glasshouse for up to six weeks. The pots are kept under waterlogged conditions at 26°C for two weeks after seedling establishment to provide ideal conditions for disease development. The disease is allowed to develop for a further three to four weeks before pea roots are assessed for root discolouration.

2) **Rolled towel method:** Soil is placed next to pre-germinated peas on damp tissue and rolled up. These are kept in controlled environmental conditions until the roots begin to discolour and infection levels are assessed.

3) **Modified rolled towel method:** The method works as described in 2) with the addition of pentachlorobenzene (PCNB) [0.1 g l^{-1}] to moisten the towels and cling film is wrapped around the towel.

4) **Dish and towel method:** Pre-germinated pea seedlings are placed on a Petri dish with moist tissue. Soil is added to cover the roots and the plates are kept in controlled environmental conditions until disease development occurs and infection levels are assessed.

5) **Boiling tube infection assay:** A pre-germinated pea seedling is placed on an agar slope in a boiling tube and a soil suspension is added to the tube. Tubes are kept in controlled environmental conditions until disease development occurs and infection levels are assessed.

6) **Agar plates:** Soil solutions are spread on plates with three different kinds of solid media. After 7 days at 20^{o}C the cultures are examined for the appearance of arachnoid fungal growth. Plates are then examined at weekly intervals for oospore formation.

7) **Sandwich plates:** The plates contain a base layer of CMA medium and a soil suspension is spread over the medium. After 4 d of incubation a layer of either PDA or CMA is layered over the culture. The plates are returned to the incubator and assessed weekly for *A. euteiches* cultures.

Methods used in final laboratory tests

In the second year of the project, the dish and towel method was optimised, using both soil and organic matter as inoculum, and developed into a reliable laboratory tests for assessing *A. euteiches* levels in soils. Infection of seedlings by *A. euteiches* is confirmed using microscopy to check for presence of oospores in the root sample.

1) Organic matter extraction from soil

a. Weigh out 2,000 g of the test soil to extract organic matter using a modified Fenwick Can Cyst washer (Picture 3).

Picture 3: Modified Fenwick Can Cyst washer used to separate the organic matter fraction of soils.

b. Bleach and thoroughly rinse all equipment after every soil sample to avoid cross contamination. It is important to remove all bleach residues to avoid killing any oospores during the subsequent washing process. Place the coarse sieve in the top of the Fenwick Can Cyst washer. Place the 75 µm sieve under the spout of the washer on top of the base.

c. Attach the hose to a tap and place the bung in the bottom of the can. Fill the cylinder with water. Calibrate to a very slow rate of cold running water (approximately 250 ml min^{-1}). Of the 2,000 g of soil, wash approximately 500 g at a time; this makes it easier to wash. Trickle water over the soil in the coarse sieve at the top of the Fenwick Can Cyst washer. Break up any clods to assist in washing. Trickle the water over the soil until all soil is washed through.

d. As the soil is washed at this very slow rate, the soil sediment goes to the bottom of the cylinder and the organic matter floats to the top and flows over onto the spout, collecting on the fine sieve. When each fraction of 500 g soil is washed, remove the coarse sieve. From this, place any remaining organic matter, stones, roots, etc. into a clean 2,000 ml beaker. Add a large amount of water, swirl, allow settling and then pour into the 75 µm sieve to collect any

floating organic matter. Do this several times until there is very little organic matter to collect. Scrape the organic matter off the fine sieve, place into a small universal and store at 4°C. Approximately 10-25 g of organic matter can be harvested from 2,000 g of soil.

2) Preparation of pea seedlings

   a. Use a pea variety susceptible to *A. euteiches* infection (for example Ambassador). Peas are pre-germinated approximately one week before inoculation. Use a healthy seed lot to avoid seed-borne disease. Do not use treated seed.
   b. Make up tap water agar (TWA) plates. Surface-sterilise seeds with 20% bleach solution for 20 minutes. Under a flow hood, rinse six times with sterilised distilled water. Place eight seeds onto each TWA plate. Wrap stacks of plates with foil to keep out the light and leave to germinate at 20°C for one week.

3) Laboratory plate test using soil

   a. Prepare four replicates per test soil.
   b. Line Petri dishes (9 cm diameter) with autoclaved, moist double-layered disk of paper towelling. Smooth out bumps, leaving a relatively even surface. As the zoospores will be swimming over this surface to find the roots, a smooth surface if essential. Do not tear.
   c. Place two pea seedlings into each dish. Take care to use seedlings with even root length. Replace lid immediately after pea placement to keep towelling from drying out.
   d. Carefully place 15 g of air dried test soil onto the roots of the two pea plants, covering the main and side roots as completely as possible. The soil should not go over the seed or epicotyl.
   e. Very slowly, drop by drop, allowing the water to permeate into the soil, distribute 9 ml of sterile distilled water over the soil in each dish. Depending on the soil type, the soil will be at saturation to over-saturation.
   f. Incubate plates in the dark at 26°C for 11 days. To alleviate peas pushing off the lids as they grow and the contents drying out, affecting infection and disease development, place a suitable flat weight on top of the stacks of petri dishes. If plates dry out slightly add 1-2 ml of sterile distilled water at day 8 or 9.

4) Laboratory plate test using organic matter

    a. Prepare four replicates per test soil.

    b. Line Petri dishes (9 cm diameter) with autoclaved, moist double-layered disk of paper towelling. Smooth out bumps, leaving a relatively even surface. As the zoospores will be swimming over this surface to find the roots, a smooth surface if essential. Do not tear.

    c. Place two pea seedlings into each dish. Take care to use seedlings with even root length. Replace lid immediately after pea placement to keep towelling from drying out.

    d. Carefully place 0.5 g of test organic matter between the roots of the two pea plants. Do not place the organic matter onto the roots as it will to too virulent too quickly.

    e. Disperse 5 ml of sterilised distilled water over the organic matter, allowing for maximum dispersal and submersion of the organic matter. The organic matter should be relatively dispersed and flattened out in the water solution, proximal, but not on the roots.

    f. Incubate plates in the dark at 26°C for 11 days. To alleviate peas pushing off the lids as they grow and the contents drying out, affecting infection and disease development, place a suitable flat weight on top of the stacks of petri dishes. If plates dry out slightly add 1-2 ml of sterile distilled water at day 8 or 9.

5) Assessment for *A. euteiches* infection

    a. Samples where soil has been used as inoculum need to be soaked in cool water for approximately 15-30 minutes to soften soil for easier removal from the roots. After soaking gently wash roots under running tap water to remove all soil residues.

    b. Organic matter residues wash off very easily from seedling roots and soaking is not necessary.

    c. Each seedling is rated for infection on a 1-5 scale (Figure 3).

        i. 0 = no discolouration, healthy seedling

        ii. 1 = up to 20% of the roots discoloured

        iii. 2 = 20% - 40% of the roots discoloured

iv. 3 = 40% - 60% of the roots/and or stem base discoloured

v. 4 = 60% - 80% of the roots/and or stem base discoloured

vi. 5 = all tissue discoloured, dead seedling

d. Do not consider or rate other infections which do not adhere to the specifications of colour and description for *A. euteiches.* This fungus exhibits a water-soaked, honey-coloured root upon infection. Any other coloured infections are caused by other soil borne pathogens and will give a false reading.

6) Verification of *A. euteiches* infection using microscopy

a. Using a clean, alcohol flamed scalpel for each treatment, cut a 1-2 cm section of the main root from each seedling, where likely *A. euteiches* infection has been noted. If no infection is noted, cut anywhere on upper/mid main root. Also cut several side roots, approximately 5 mm per section; do this for three to five side roots per plant, where there is suspected *A. euteiches* infection. Place root pieces on a labelled microscopy slide, add a drop of sterile water and lay cover slip over root sections, avoiding air bubbles. Gently flatten roots sections for easier viewing with the microscope.

b. Using a microscope at 10x magnification, scan the root sections in a thorough manner for presence of thick-walled oospores. *Aphanomyces euteiches* oospores can be seen in a reasonably prepared root section at 10 x magnification but for confirmation a 40 x magnification should be used.

c. The standard used for a positive confirmation of *A. euteiches* infection is the presence of two thick-walled oospores per seedling. Oospores should measure 18-25 µm in diameter, with a uniformly thickened wall of 1-5 µm width (CMI Descriptions of Pathogenic Fungi and Bacteria No. 600). The outside of the oospores are smooth without jagged or spiked edges and oospore centres are often granulated in appearance. Swimming zoospores are rarely present, but are not considered diagnostic for this test.

d. If the root visual assessment has scored positively for *A. euteiches* and no oospores are found on the root sections, another sample of sections of roots should be taken in the same manner from the same seedling. Often, a different section will confirm the presence of oospores, but not always. If a second sample is needed and no oospores are found, it can be assumed that the affirmative visual score was a false positive.

A few modifications to the methods have been tested to arrive at the final tests. Amounts of soil placed on the pea roots were increased from 9 g of soil to 15 g of soil to increase infection efficiency. Incubation times from eight to twelve days were tested. Generally, infection in organic matter inoculated plates is slightly faster than in soil samples. Eleven days incubation time was chosen to allow enough time for infections from soils with low levels of *A. euteiches* to develop but any incubation time longer than that carries the risk of masking differences between soils with medium and high levels of *A. euteiches*. To start with, organic matter was blended in a food processor to achieve better distribution between the pea seedlings in the dish. Blending was very time-consuming and it was difficult to maintain accuracy due to the amount of water needed to rinse the cylinder. Unblended organic matter gave consistent infection levels so the use of blending the organic matter was abandoned. During one test, the organic matter was placed directly on the roots. This made the infection too rapid and virulent. Placement of organic matter on the paper towelling between the pea roots was therefore chosen. To avoid secondary infection of pea seedlings due to seed-borne contamination, bleach concentration and timing was increased from 10% bleach for 15 minutes to 20% bleach for 20 minutes. During method development, negative controls using autoclaved soil were included to make sure that no cross contamination was introduced at any point during the set up. Negative controls were abandoned for the final test using soils from Yorkshire with the aim to reduce costs of the service. The addition of positive controls had to be stopped after initial attempts during the first year of the project because a definite *A. euteiches* culture could not be obtained at PGRO.

Information on test soils

It is known that *A. euteiches* occurs in Scotland and therefore Scottish fields were selected for soil sampling. In 2014, eight soil samples from fields near Perth, Scotland were used for method development (Table 2). In 2015, nine soil samples from fields near Perth were used for method validation (Table 2). All 17 soil samples were tested using the soil bait method and both laboratory plate tests using soil and organic matter as inoculum. The distribution of *A. euteiches* in the UK is not known. In order to test whether the developed plate test can be used on unknown soil samples to detect presence of *A. euteiches*, eight soil samples from fields near Hull, Yorkshire were tested in 2015 using the laboratory test with soil as inoculum (Table 2).

Table 2: Location of fields from which test soils were sampled, year of sampling and status of pea crop at harvest.

Sample	GPS Co-Ordinates Grid reference	Year	Status of crop
S/A	56.488424 -3.275263	2014	Medium foot rot - Slightly sick crop
S/B	56.52033 -3.36463	2014	Healthy crop
S/C	56.55843 -3.34923	2014	Medium / High Footrot - Sick crop
S/D	56.602962 -3.150115	2014	Healthy crop
S/E	56.60137 -3.094690	2014	Very healthy crop
S/F	56.64212 -3.11720	2014	Medium / High Footrot - Sick crop
S/G	56.696555 -2.857973	2014	High Footrot - Very sick crop
S/H	56.65013 -2.90396	2014	High Footrot - Very sick crop
S/One	56.61678 -3.17283	2015	High Footrot - Very sick crop
S/Two	56.56716 -315840	2015	High Footrot - Very sick crop
S/Three	56.61336 -3.05970	2015	Virgin field - Healthy crop
S/Four	56.61215 -3.0254	2015	High Footrot - Very sick crop
S/Five	56.60200 -3.10884	2015	Virgin field - Healthy crop
S/Six	56.61636 -3.11796	2015	Healthy crop
S/Seven	56.55843 -3.34923	2015	High Footrot - Very sick crop
S/Eight	56.52753 -3.28556	2015	Medium foot rot - Slightly sick crop
S/Nine	56.59786 -3.2658	2015	Medium foot rot - Slightly sick crop
BE1	SE969430	2015	High Footrot - Very sick crop
BE2	SE971430	2015	High Footrot - Very sick crop
BE3	TA022413	2015	High Footrot - Very sick crop
BE4	TA025413	2015	High Footrot - Very sick crop
BE5	SE970427	2015	High Footrot - Very sick crop
BE6	SE984379	2015	High Footrot - Very sick crop
BE7	SE873303	2015	High Footrot - Very sick crop
BE8	SE806299	2015	High Footrot - Very sick crop

Plate tests to assess levels of Fusarium solani f.sp pisi and Didymella pinodella

All soil samples were also tested for levels of *Fusarium solani* f.sp *pisi* and *Didymella pinodella*. These tests are offered as a service by PGRO to growers and methods are described in Biddle (1993). Briefly, soil dilutions are spread on selective media, and colony numbers of the pathogenic fungi are counted. A risk index is calculated on a scale of 0.2 - 5 and risk scores are linked to potential yield loss. An increase of 1 in the risk scores is linked to potential yield loss of 0.85 t/ha in wet years favouring disease development. A score lower than 0.5 indicates very low foot rot risk and levels are unlikely to cause any serious yield loss. A score between 0.5 and 1.2 indicates low risk in most seasons, but yield loss of up to 20% can occur in unfavourable conditions. A score between 1.2 and 2 indicates a medium risk of

foot rot development especially in wet seasons. A score greater than 2 indicates a high risk of serious yield loss in most seasons.

Results

Overview of tested methods in first year of the project

During the first year of the project, six different methods were tested with the aim of finding a method that reliably quantifies levels of *A. euteiches* in soils and is quicker than the traditionally used soil bait method.

The rolled towel method and the modified rolled towel method both showed root infection but despite being quicker than the soil bait technique are complex to perform. The dish and towel method was identified as a method with great potential to be investigated further because infection occurred on seedling roots and the method was quick and relatively easy to perform. The boiling tube method, although showing infected roots, was more difficult to rate for infection levels and was prone to secondary infection. The use of agar plates would have been the quickest and potentially easiest method but soil samples contain many different fungi most of which will grow on the growth media selected. This resulted in growth of other fungal mycelium on the agar plates and reliable quantification of *A. euteiches* mycelium was not possible. The modified Fenwick Can Cyst washer method to extract organic matter from the test soils was identified to hold potential for further use in the method development and it was decided to test selected methods with both, straight soil and extracted organic matter.

Three methods to assess A. euteiches levels in soils

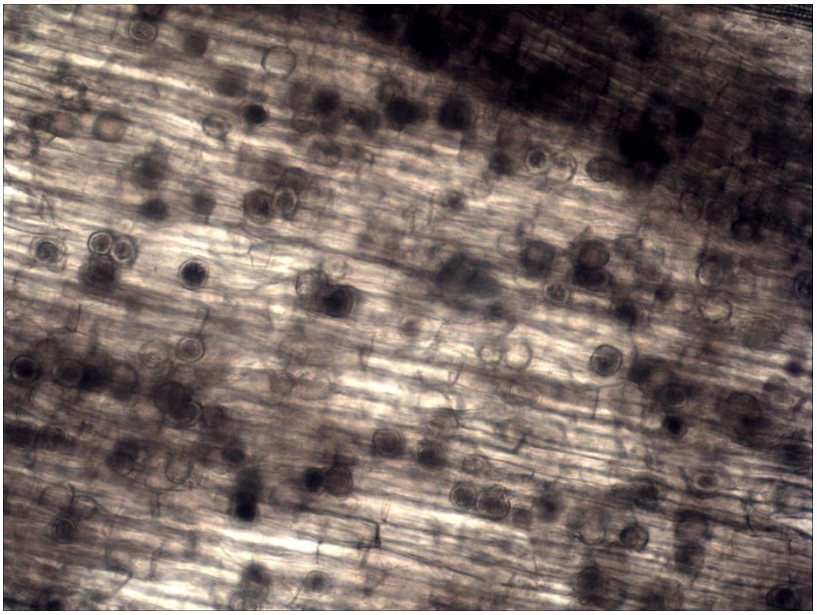
For the second year of the project it was decided to try to optimise the dish and towel method and to assess its potential to be used as a screening tool to assess risk levels of *A. euteiches* in soils. The aim was to develop a fast and reliable method that can be offered as a service to pea growers.

In 2014, eight soil samples and in 2015, nine soil samples were tested for *A. euteiches* levels using the soil bait method and the plate method (dish and towel) with both soil and organic matter as inoculum. All three methods reliably identified *A. euteiches* presence and all of the methods can be used to score infection levels to advice risk levels of *A. euteiches* in soils. Picture 4 shows an overview of the soil bait test performed in 2015 and Picture 5 shows plants that had been growing in test soil S/Eight (see Table 2). Pictures 6 (10 times magnification) and Picture 7 (40 times magnification) show *A. euteiches* oospores in roots of seedlings that had been growing in test soil S/Eight (see Table 2). Picture 8 shows on overview of the plate method using soil. Seedlings were inoculated with test soil S/B (see Table 2) or with sterilised

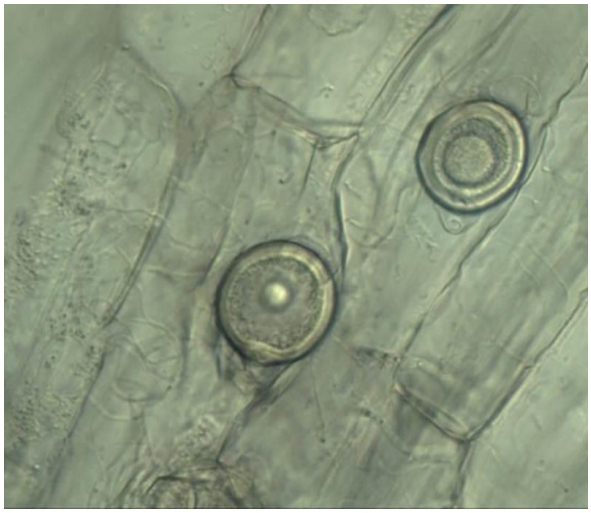
soil as negative control. Picture 9 shows a close up of a sick seedling inoculated with soil extracted from test soil S/B. Picture 10 shows on overview of the plate method using organic matter. Seedlings were inoculated with organic matter extracted from test soil S/B (see Table 2) or with sterilised test organic matter as negative control. Picture 11 shows a close up of a sick seedling inoculated with organic matter extracted from test soil S/B.

Picture 4: Overview of the soil bait test performed with soils collected in 2015.

Picture 5: Sick pea plants that had been growing in pots filled with test soil S/Eight during the soil bait tests in 2015.



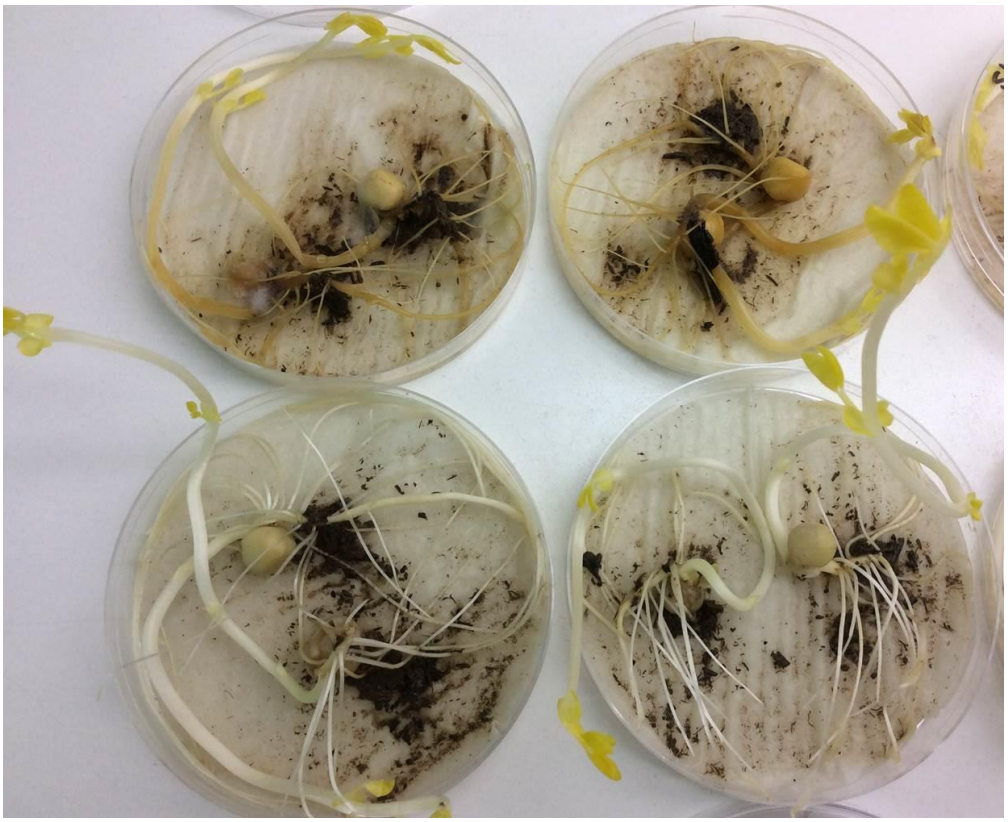
Picture 6: *Aphanomyces euteiches* oospores in roots of pea seedlings that had been growing in test soil S/Eight (10 times magnification).



Picture 7: *Aphanomyces euteiches* oospores in roots of pea seedlings that had been growing in test soil S/Eight (40 times magnification).

Picture 8: Plate test using soil. Plates on the left were inoculated with test soil S/B and seedlings show honey discolouration due to *A. euteiches* infection. Plates on the right were inoculated with sterilised soil and seedlings are completely healthy.

Picture 9: Plate test using soil. Seedlings were inoculated with test soil S/B and show honey discolouration due to *A. euteiches* infection.



Picture 10: Plate test using organic matter. Plates on the top were inoculated with organic matter extracted from test soil S/B and seedlings show honey discolouration due to *A. euteiches* infection. Plates on the bottom were inoculated with sterilised organic matter and seedlings are completely white and healthy.

Picture 11: Plate test using organic matter. Seedlings were inoculated with organic matter extracted from test soil S/B and show honey discolouration due to *A. euteiches* infection.

The test soils collected in Scotland in 2014 showed varied levels of *A. euteiches* as determined using the soil bait method, the plate method using soil and the plate method using organic matter (Figure 4). All plant roots were assessed for presence of *A. euteiches* oospores using microscopy (Table 3).

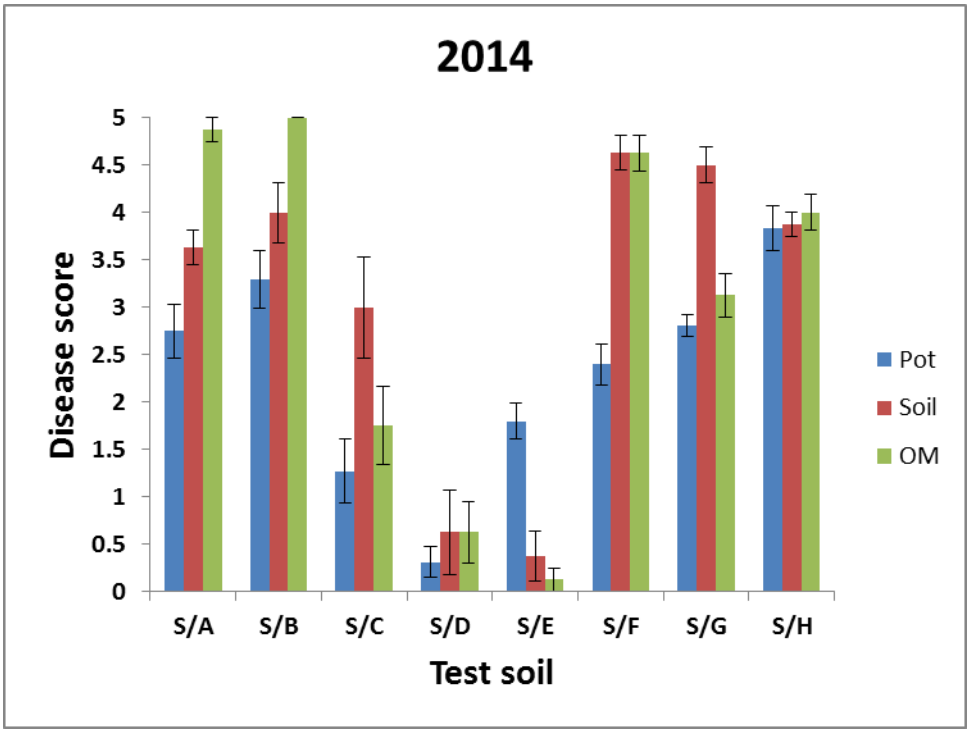


Figure 4: Disease scores (scale 0 to 5) for levels of *A. euteiches* infection of pea roots assessed using the soil bait method (pot), the plate method using soil (soil) and the plate method using organic matter (OM). Test soils had been collected from Scottish fields in 2014. Data show mean values and standard error (n>8).

Table 3: Percentage congruency of positively scored *A. euteiches* disease presence on roots of seedlings in plate tests using soil (soil) and plate tests using organic matter (OM) with presence of thick-walled *A. euteiches* oospores in root samples from 2014 test soils.

Sample	**Soil %**	**OM %**
S/A	87	100
S/B	100	67
S/C	100	63
S/D	86	63
S/E	50	63
S/F	87	60
S/G	100	100
S/H	100	86
Average	88.75	75.25

The test soils collected in Scotland in 2015 also showed varied levels of *A. euteiches* as determined using the soil bait method, the plate method using soil and the plate method using organic matter (Figure 5). All plant roots were assessed for presence of *A. euteiches* oospores using microscopy (Table 4).

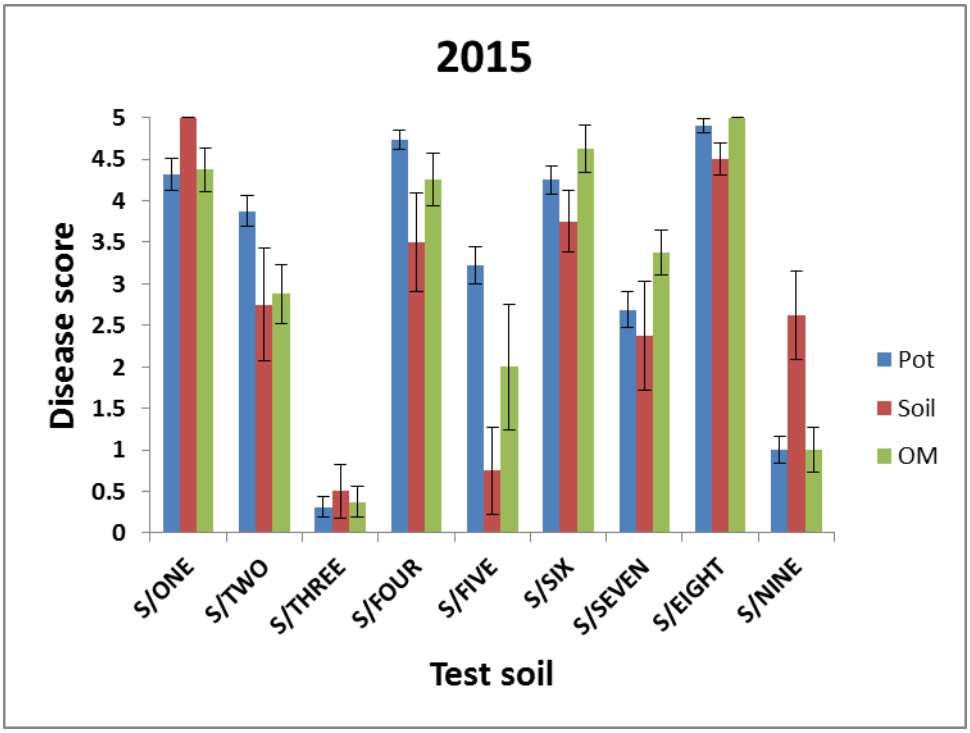


Figure 5: Disease scores (scale 0 to 5) for levels of *A. euteiches* infection of pea roots assessed using the soil bait method (pot), the plate method using soil (soil) and the plate method using organic matter (OM). Test soils had been collected from Scottish fields in 2015. Data show mean values and standard error (n>8).

Table 4: Percentage congruency of positively scored *A. euteiches* disease presence on roots of seedlings in plate tests using soil (soil) and plate tests using organic matter (OM) with presence of thick-walled *A. euteiches* oospores in root samples from 2015 test soils.

Sample	Soil %	OM %
S/ONE	87	75
S/TWO	87	37
S/THREE	75	62
S/FOUR	87	62
S/FIVE	87	62
S/SIX	100	85
S/SEVEN	87	62
S/EIGHT	100	100
S/NINE	50	50
Average	84.44	66.11

Results obtained using the three different methods significantly correlated in both years demonstrating that all three methods give the same consistency for assessing risk levels of *A. euteiches* in soils. In 2014, results from the soil bait method significantly correlated with results from the plate method using soil and with results from the plate method using organic matter (Table 5). Results from the plate method using soil also correlated with results from the plate method using organic matter (Table 5). In 2015, results from the soil bait method significantly correlated with results from the plate method using soil and with results from the plate method using organic matter (Table 6). Results from the plate method using soil also correlated with results from the plate method using organic matter (Table 6).

Table 5: Pearson correlation of *A. euteiches* levels obtained from eight fields in 2014. Pathogen levels were determined using the soil bait method (pot), the plate method using soil (soil) and the plate method using organic matter (OM). Results for the three methods are significantly correlated ($p<0.05$).

Correlations 2014

		OM	Soil	Pot
OM	Pearson Correlation	1	.879**	.774*
	Sig. (2-tailed)		.004	.024
	N	8	8	8
Soil	Pearson Correlation	.879**	1	.719*
	Sig. (2-tailed)	.004		.044
	N	8	8	8
Pot	Pearson Correlation	.774*	.719*	1
	Sig. (2-tailed)	.024	.044	
	N	8	8	8

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table 6: Pearson correlation of *A. euteiches* levels obtained from nine fields in 2015. Pathogen levels were determined using the soil bait method (pot), the plate method using soil (soil) and the plate method using organic matter (OM). Results for the three methods are significantly correlated ($p<0.05$).

Correlations 2015

		OM	Soil	Pot
OM	Pearson Correlation	1	.839**	.928**
	Sig. (2-tailed)		.005	.000
	N	9	9	9
Soil	Pearson Correlation	.839**	1	.725*
	Sig. (2-tailed)	.005		.027
	N	9	9	9
Pot	Pearson Correlation	.928**	.725*	1
	Sig. (2-tailed)	.000	.027	
	N	9	9	9

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

All soils were also tested for levels of *F. solani* f.sp. *pisi* and *D. pinodella* (Table 7) but levels were generally low.

Table 7: Foot rot index based on presence of *F. solani* f.sp. *pisi* and *D. pinodella* in test soils (scale 0.2 - 5). Test soils had been collected in Scotland during 2014 and 2015.

Sample	Foot rot index
S/A	0.20
S/B	0.20
S/C	0.20
S/D	0.20
S/E	0.20
S/F	0.20
S/G	0.20
S/H	0.20
S/ONE	1.39
S/TWO	1.56
S/THREE	0.32
S/FOUR	1.62
S/FIVE	0.20
S/SIX	0.20
S/SEVEN	1.42
S/EIGHT	0.20
S/NINE	1.89

Prediction of disease development in the field

The fields from which the tests soils had been collected were visually rated for disease development. When foot rot levels in the pea crop were high the soils had been given a high *A. euteiches* scoring (Table 8). This shows that *A. euteiches* levels in soils can be used to predict disease development in the field.

Table 8: Visually rated disease status of pea crops and average *A. euteiches* risk score determined using the soil bait method, the plate method using soil and the plate method using organic matter.

Sample	**Year**	**Status of crop**	***A. euteiches* risk score**
S/A	2014	Slightly sick crop	3.75
S/B	2014	Healthy crop	4.10
S/C	2014	Sick crop	2.01
S/D	2014	Healthy crop	0.52
S/E	2014	Very healthy crop	0.77
S/F	2014	Sick crop	3.88
S/G	2014	Very sick crop	3.48
S/H	2014	Very sick crop	3.90
S/One	2015	Very sick crop	4.56
S/Two	2015	Very sick crop	3.17
S/Three	2015	Healthy crop	0.40
S/Four	2015	Very sick crop	4.16
S/Five	2015	Healthy crop	1.99
S/Six	2015	Healthy crop	4.21
S/Seven	2015	Very sick crop	2.81
S/Eight	2015	Slightly sick crop	4.80
S/Nine	2015	Slightly sick crop	1.54

Eight fields in Yorkshire with severe foot rot disease were tested for levels of *A. euteiches*, *F. solani* f.sp. *pisi* and *D. pinodella* in 2015 (Table 9). The aim was to test which pathogens were responsible for the disease development.

Table 9: Levels of *A. euteiches*, percentage congruency of positively scored *A. euteiches* disease presence on roots of seedling with presence of thick-walled *A. euteiches* oospores in root samples and foot root index based on presence of *F. solani* f.sp *pisi* and *D. pinodella* in test soils. Soils had been collected in Yorkshire in 2015.

Sample	*A. euteiches* score	% congruency oospores	Foot rot index
BE1	0.5	75	2.72
BE2	0	100	1.96
BE3	0	87	2.49
BE4	0	100	1.35
BE5	1	12	1.11
BE6	0.5	100	2.36
BE7	1.125	100	2.53
BE8	0.125	87	2.17

Discussion

Two quick and reliable laboratory based methods have been developed to assess levels of *A. euteiches* in soil samples. The first method uses soil as inoculum whereas the second method uses organic matter extracted from soil as inoculum. Infection scores from both methods not only correlate significantly with each other but also with scores obtained from the traditionally used soil bait test. This demonstrates that the laboratory methods can replace the much more time consuming soil bait test to assess levels of *A. euteiches* in soils. PGRO will offer the plate method using soil as an inoculum as a service to growers. *Aphanomyces euteiches* oospores are concentrated in the organic matter fraction of soils and it had been proposed that using organic matter instead of soil might give better infection. However, this did not turn out to be the case and infection levels were as good using soil as inoculum as they were using organic matter. The soil method is quicker to perform because the step of extracting the organic matter does not have to be performed. The soil test is therefore cheaper to the growers.

Levels of *A. euteiches* in soils have also been compared to occurrences of disease symptoms in the fields. Generally, when levels of *A. euteiches* in soils were high, crops were sick whereas in fields with low levels of *A. euteiches* the crops were healthy. However, in two occasions (soil S/B and soil S/Six) levels of *A. euteiches* in the soil samples were high but crops remained looking healthy. Trials performed at PGRO have previously identified low levels of *A. euteiches* infection without obvious above ground symptoms. This could lead to reduced yield without the appearance of diseased plants and also allow the pathogen levels to increase in the soil undetected. As mentioned above, inoculum levels of *A. euteiches* in soils in some areas in France and the USA have become so high that pea production and the

processing factories had to be relocated to less infected areas (BASF AgSolutions, Canada). This highlights the importance of monitoring *A. euteiches* distribution in the UK. So far, presence of *A. euteiches* had only been confirmed to occur widely in soils from Scotland. The tests of the soils from Yorkshire showed that *A. euteiches* does not only occur in Scotland but also further south in the UK. Levels of *A. euteiches* in Yorkshire soils were low but the pathogen occurred together with two of the other foot rot causing pathogens *F. solani* f.sp *pisi* and *D. pinodella* leading to very sick crops.

To get reliable results for *A. euteiches* infection of soils, a soil sample of around 2 kg needs to be collected by growers using a W shape across the field. *Aphanomyces euteiches* can be patchy in occurrence and it is therefore important to cover a greater area of the field. In addition, samples need to be taken from areas of concern such as those with signs of waterlogging or previous premature senescence of the crop. The soil samples should be sent to PGRO where the developed plate test will be used to assess levels of *A. euteiches* in soils. Results will be reported within two weeks. The price per soil sample is £149 because a time consuming microscopy step is needed to assure presence of *A. euteiches* oospores in infected root samples. This is the only way of guaranteeing that the disease symptoms on the seedling roots are caused by *A. euteiches*.

Currently, levels of *A. euteiches* in soils cannot be related to potential yield losses. Estimation of yield losses due to *A. euteiches* under field conditions can be difficult to establish because of the presence of other foot rot causing pathogens (Gaulin et al. 2007) but, work undertaken in Wisconsin, USA in the 1980s suggested that yield losses due to *A. euteiches* ranged from 42% to 86% in susceptible pea varieties (Pfender and Hagedorn 1983). Since the disease cannot be controlled chemically assessing pathogen levels in soils holds great potential to minimise crop losses. Using the developed plate test, growers will be informed of risk levels in their fields which can be used to inform decisions on whether to plant a pea crop or not. If *A. euteiches* levels in soils are high it seems very likely that disease will develop especially in wet years or in fields with soil compaction or waterlogging issues. PGRO therefore recommends that fields with *A. euteiches* levels of greater than 3 should not be used for pea cropping to avoid potentially high yield losses. PGRO will use the developed plate method to perform further research on *A. euteiches*. Aims are to relate levels of *A. euteiches* in soils to yield losses, to assess distribution of *A. euteiches* across the UK and to find mitigation strategies to avoid further spread of the pathogen and to keep levels low, especially in intensively cropped vining pea areas.

Conclusions

Two laboratory based methods to assess risk levels of common root rot (*Aphanomyces euteiches*) have been developed. One method uses soil as inoculum whereas the other method using organic matter extracted from soils as inoculum. Both plate tests reliably indicate *A. euteiches* levels in soils and results are obtained within two to three weeks of receiving a soil sample. Results do not only correlate between the two plate tests but also to the more time consuming, traditionally used soil bait method. The plate test using soil is quicker than the test using organic matter and was therefore chosen to be offered as a service to growers by PGRO. The price per soil sample is £149. This project provides growers with a risk assessment tool to assess levels of *A. euteiches* in soils prior to pea planting and is crucial to avoid high crop losses due to *A. euteiches* infection.

Knowledge and Technology Transfer

Agronomy training CCC Agronomy (February 2015)

Agronomy training Saffron Waldon (February 2015)

Cereals 2015 (June 2015)

Dengie Crops grower meeting (February 2015)

Legume panel meetings (February and November 2015)

PGRO and Syngenta Roadshows (6 meetings in January and February 2015)

PGRO Crop Protection course (February 2015, February 2016)

PGRO Pulse Open Day Stubton (July 2015)

PGRO Vining pea Open Day Nocton (June 2015)

TerresInovia and PGRO meeting (June 2015)

The Vegetable Magazine (December 2015)

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