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 Kerry Maguire, PGRO
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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

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.

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CONTENTS

GROWER SUMMARY

Headline

Root rot is a major problem for growers of peas in the UK. A soil diagnostic test is brought a step closer with the first year of a two year project looking at different methods to evaluate *A. euteiches* in soil.

Background

Peas are valuable crops for the environment, crop rotation, diet and human health. Production is threatened by increasing levels of soil-borne diseases as peas are grown in restricted areas in the UK due to climate and location of processing facilities. There are increasing incidences of pea root rot. Symptoms usually appear as the plants begin to flower or earlier if the plants are stressed by waterlogging or other factors. This can result in complete crop loss or in less severe incidences uneven maturity of the crop. Chemical control of soil borne pathogens is unavailable. Once the symptoms have been observed there is very little the grower can do to save the crop.

There are three main fungal species which cause the problem *Fusarium solani* pv *pisi*, *Phoma medicaginis* var *pinodella* and *Aphanomyces euteiches*. Infection is dependent on weather and soil conditions. Disease levels are favoured by high soil moisture and are often seen where there has been a history of soil compaction and water logging although this is not always the case. Another factor is drilling time; peas sown in cold wet soils appear to be more susceptible than those grown later in the season. There is a test available to identify the risk of root rot for *P. medicaginis* and *F. solani*. The test is used by growers to identify fields with a high disease risk and either lengthen the rotation or plant later in the season. The incidence of *A. euteiches* root rot is on the increase. Recent trials at PGRO have identified low levels of infection without obvious above ground symptoms. This could reduce yield without the appearance of diseased plants and also allow the fungal levels to increase in the soil undetected.

A. euteiches is a very resilient fungus and is able to survive in the soil as thick walled oospores which form in abundance in the decaying pea roots. The area of infection spreads out across the field between pea crops. Field infection cannot be predicted or tested for and identification is based on the symptoms and the oospores in the crop.

This project aims to evaluate methods used in research projects to identify *A. euteiches* in soil samples and to identify a time and resource efficient method for identifying *A. euteiches* from field samples.

Summary

Soils with confirmed *A. euteiches* infection were used in eight methods of isolating the fungus from soil samples. The initial test involved soil baiting. Peas were grown in soil and after approximately five weeks the plants were assessed for disease presence. This method is inefficient in terms of time, space and resources but allows the testing of large volumes of soil. Alternative methods used peas grown in boiling tubes, rolled paper towels and in petri dishes. These all had the benefit of more efficient use of time, space and resources. Although infection did occur, it was lower than that seen in the soil bait test.

An agar plate method was also tested using selective medias. This was inconclusive in the initial experiments. There is a huge diversity of fungi in soil and attempting to select one is complex.

Financial Benefits

Recommendations not available yet.

Action Points

Not identified as yet.

SCIENCE SECTION

Introduction

Growers are increasingly concerned about the problems of root rots when growing vining peas especially in land that has had peas in the rotation for a number of years. Peas are especially susceptible if the soil is wet or consolidated or if there has been a long history of growing vining peas. These rots can result in complete crop loss or, in less severe incidences, uneven maturity of the crop. Both of these cause major problems for the grower. In the past year approximately one third of samples into the PGRO crop clinic were diagnosed with root rots. This is the largest single category of crop disorders seen and is a frequent topic for discussion with growers and agronomists. It can be a devastating disease as once it is identified, it is generally too late to save the crop and complete loss is likely (Biddle & Cattlin, 2007).

In the UK, root rots are caused by different soil dwelling fungi. Each has its own symptoms but shares the common outcome of death of the below ground parts. *Fusarium spp*. and *Phoma medicaginis* often occur together whereas *Aphanomyces euteiches* is more likely to occur on its own and has become an increasing problem especially for growers in colder areas or those who drill early. There are soil tests available for *Fusarium spp*. and *P. medicaginis* which identifies the disease levels in the soil and provides a risk rating which can be used to influence crops grown and drilling times (PGRO Technical Update 34). However there is not a test for *A. euteiches* and very little is known about its distribution in the UK.

A. 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 tan discolouration and the outer root cells disintegrate. The fungus is very resilient and is able to survive in the soil as thick walled oospores which form in abundance in the decaying pea roots and are usually found in the organic matter of the soil after the crop (Fig 1).

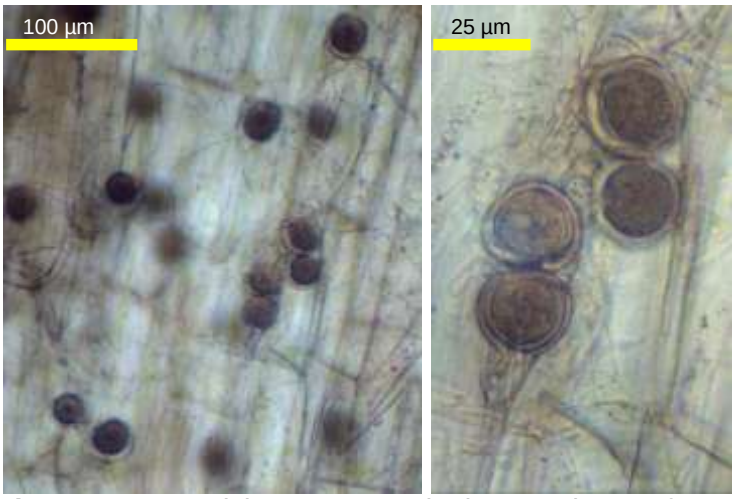

Figure 1. *A. euteiches* oospores in the root tissue of pea.

An infected area in a field tends to spread with time. Field infection cannot be predicted or tested for and identification is based on plant symptoms and the presence of oospores in infected material. The only method of control is to extend the rotation to at least ten years to allow the oospore population to decline. *A. euteiches* has a range of hosts including clover, vetch, barley, oats and some weed species (CMI description No 600). Recent trials at PGRO have identified low levels of infection without obvious above ground symptoms. This could reduce yield without the appearance of diseased plants and also allow the fungal levels to increase in the soil undetected.

The aim of this project is to evaluate known methods used to isolate *A. euteiches* using UK soils and start the development of a time efficient assay for testing soils for disease risk. This project investigates published techniques to isolate *A. euteiches* from soils and to culture the fungus on agar plates. Although these methods work well for research purposes, and some are used to establish disease levels, they do not appear to be time or resource efficient and have not been tested on UK soils. This project proposes to evaluate these methods for suitability to develop an assay to identify *A. euteiches* in field soils and to look at ways a soil test could be developed.

The first year of this project aimed to look at some of these methods. In selecting which methods to evaluate, it was important that the method was able to use a realistic amount of soil and could be reliable carried out on a routine basis. The method needed to be reproducible as well as time and resource efficient.

Soil Bait method. This is a widely used method to identify the presence of soil pathogens. Peas are grown in pots of the test soil in the glasshouse and are kept in conditions to favour disease development. After 38 d the peas are assessed for root discolouration on a scale of 1 – 5 (Malvick *et al.*, 1994). Infection is confirmed by checking for oospores in the root

tissue using microscopy. This method evaluates the presence of viable fungal spores and includes any potential suppression from other soil microbes. However it is a long test (over four weeks) and is labour and space intensive.

Separation of organic matter from soils. The oospores of *A. euteiches* are found in infected plant debris as it decomposes and can be found in the organic matter of soils (Kraft *et al.*, 1990). To obtain cultures of *A. euteiches,* soil samples (a few grams) are washed to concentrate the organic matter. This is carried out using beakers and sieves to obtain the floating organic matter. A potential alternative method would be the use of the Modified Fenwick Can. This device is used to separate cyst nematodes found in the organic soil fraction from large (hundreds of grams) soil samples. The concentrated organic material can be then be used in tests for *A. euteiches*.

Rolled Paper towel infection assay and the modified assay. This method is used to obtain infected plant material from which the fungus can be isolated and has the potential to be used as a test method for the presence of *A. euteiches*. The original method was developed by Mitchell *et al.*,(1969) and further developed by Kraft *et al.*, (1990) and Malvick *et al.* (1994). The soil is laid 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 the infection is confirmed using microscopy. The addition of pentachloronitrobenzene (PCNB) resulted in less contaminants and peas with less secondary disease at the end of the assay (Williams-Woodward *et al*., 1998).

The Dish and Towel Method. This is a cross between the rolled towel method and the soil bait method. The peas are distributed in a sterile dish on damp towelling. The soil is placed over the roots and the plants assessed for disease symptoms (Oyarzun and Van Loon, 1989).

Boiling tube infection assay. This assay is used to assess the pathogenicity of *Fusarium solani* pv *pisi* (Gravanis, 1986). A germinated pea is placed on an agar slope inside a boiling tube with a soil sample. The plants are assayed for disease symptoms. Although this assay is used for root infecting *Fusarium spp* it has the potential to also be successful for *A. euteiches* assay.

Agar plates *A. euteiches* can be grown *in vitro* on agar plates. The agar media provides the nutrients the fungus requires to grow without the presence of the plant. These can be used to induce the formation of spores or mycelial growth depending on the media used to aid the identification of the fungus. Additional additives such as fungicidal compounds and antibiotics suppress the growth of other organisms. This is particularly important when using soil samples as there are a lot of cultural soil microbes which could mask the sparse,

arachnoid mycelial growth of *A. euteiches*. *A. euteiches* can be grown on Tap Water Agar (TWA), Potato Dextrose Agar (PDA) or Corn Meal Agar (CMA) with the addition of Metaxyl M, Benomyl and Streptomycin sulphate (Pfender *et al.*, 2001) to suppress or prevent the growth of other microbes.

Agar plate sandwich technique. Ideally a test would be based on a selective media and plated out directly from the soil. An agar plate with actively growing cultures is overlaid with another media which has different selection compounds. These can either be antibiotics or a media which stimulates spore formation for identification of the fungus.

Grass overlay. A blade of sterile grass placed over a fungal culture encourages the growth of *A. euteiches* into the grass where it forms oospores, sporangia and zoospores (Pfender, 1984). These can be visualised under the microscope and used as a method to identify a potential culture on agar.

Disease assessment. The quickest method of disease assessment would be the visual assessment of roots on a scale similar to that used in the soil bait technique. However soil contains many microbes which have the potential to discolour the roots. Therefore the assessment method needs to distinguish between miscellaneous rots and *A. euteiches*. A characteristic symptom is the disintegration of the root. This can be quickly identified and assessed but does take time to develop. Alternatively the presence of oospores in the plant tissue can be used to identify the fungus. This requires a trained eye to identify the correct morphological features of the oospores and is time consuming to evaluate each root samples. However it can be used to verify the scoring system.

Materials and methods

Soil samples were received from growers and selected for use based on their likely disease incidence. Soil samples from the east coast of Scotland (Perth) were selected based on the number of pea crops grown and if there was a history of disease. All had peas in them at the time of sampling. Methods were taken from published literature where *A. euteiches* has been studied. In some instances this was adapted to the requirements of the project.

Three soils were chosen to test the different methods of disease. Some soils were mixed to reduce the disease level as in early experiments the plants were dying prematurely. Each assay included a steam sterilised control soil and infection was confirmed by the presence of oospores where appropriate.

Protocol 1) Soil Bait method

Soil samples were mixed in open trays before being placed in 4 x 14 cm diameter pots. All pots and trays were washed with a 10% Sodium hypochlorite solution (bleach) prior to use. Five pea seeds, cv. Kelvedon Wonder, were sown per pot. After 10 d the soil was saturated and a high water content maintained for 14 d. Soil moisture was reduced and the plants left to grow for a further 14 d before assessment.

The plants were removed from the pots and the roots gently washed. The roots were assessed using a 1-5 scale.

1 = no necrosis of roots and hypocotyls
2 = slight necrosis of roots and hypocotyls
3 = necrosis of roots and lower hypocotyls, slight chlorosis of cotyledons and moderate stunting of stem
4 = extensive necrosis of roots, hypocotyls and cotyledons and severe stunting of stems
5 = dead seedling

Protocol 2) Organic matter separation

a) Sieve and Beaker. Soil samples were mixed well and 100 g of soil was passed through a 2 mm sieve, clods were broken down and stones removed. This was mixed for 3 min in sterile distilled water. The organic and mineral fraction was collected on a 75 µm mesh sieve and washed in running water, the retained sample was suspended in water and the heavier fraction was allowed to settle for 15 seconds. The suspended organic material was collected and stored at 3^{o}C.

b) Modified Fenwick Can. Four hundred grams of soil were dried slightly and mixed well before washing through a 2 mm sieve into a Modified Fenwick Can (Fig 2). The organic matter floated in the column of water and was collected using a 75 µm sieve. This material was stored at 3^{o}C.

Figure 2 The Modified Fenwick Can used to separate out the organic matter content of the soil.

Protocol 3) Rolled towelling method

This followed the method of Kraft *et al*. (1990). Pea seeds, cv. Kelvedon Wonder, Ambassador and Avola were surface sterilised (10% bleach for 15 min and washed 6 times in SDW) and germinated on 1.2% Tap Water Agar (TWA) for 5 d. Seedlings were placed on sterile paper towels moistened with sterile distilled water (SDW) and approx. 10 mm^3 of soil/organic matter was placed on each root. The plants were wrapped in tissue and kept at 20^oC constant with 11.5 h light for 21 d. Each rep consisted of 5 plants and there were 6 reps per soil per variety. Plants were assessed for stem browning and the presence of oospores in the root tissue.

Protocol 4) The modified rolled towel method

This followed the modifications detailed in Williams-Woodward *et al.* (1998). In summary the rolled towel method was followed with the exceptions of peas pre-germinated in sterile vermiculate for 5 d at 22^oC and the addition of pentachlorobenzene (PCNB) [0.1 g l^{-1}] to moisten the towels. These were rolled between two layers of plastic film and then treated the same as protocol 3).

Protocol 5) Dish and Towel method

This followed the method of Oyarzun and Van Loon, 1989. In brief, three sterilised and pre-germinated seeds (in sterile vermiculite) of cv Ambassador were placed on damp filter papers in 9 cm petri dishes. The seedling roots were covered in soil. These were placed at 20^{o}C for 7 d. Plants were assessed for root discolouration and the presence of oospores.

Protocol 6) Boiling tube infection assay

This assay is based on the pathogenicity assay for *F. solani* pv *pisi* (Gravanis, 1986). A surface sterilised pre- germinated pea (cv Ambassador or Kelvedon Wonder) was placed on a 1.5% TWA agar slope in a boiling tube with a cotton wool bung and aluminium foil cover. One gram soil sample or organic matter was suspended in 10 ml SDW. One millilitre of this solution was placed on each seedling. Plants were incubated at 20^{o}C 16 h light for 20d. The plants are assayed for disease symptoms using the 1-5 scale.

Protocol 7) Agar Plates

Three media types were tested (Table 1). Organic matter or soil from the soil bait test was diluted 1 g in 10 ml SDW and spread onto the agar plate. After 7 d at 20^{o}C the cultures were examined for the appearance of arachnoid fungal growth. Plates were then examined at weekly intervals for oospore formation.

Table 1. The ingredients for the three medias used. All made up to 1 litre.

Media type	Dried peas (g)	Glucose (g)	Potato Dextrose Agar (g)	Cornmeal Agar (g)	Technical Agar (g)	Benomyl (ppm)	Streptomycin sulphate (ppm)	Metalaxyl M (ppm)
Corn Meal Agar (CMA)	0	0	0	10	10	5	200	30
Potato Dextrose Agar (PDA)	0	0	39	0	0	5	200	30
Pea Decoction Agar	180	7	0	0	20	5	200	30

Protocol 8) Plate sandwich

The plates were set up as for the agar media tests above. The base media was CMA. The plates were incubated at 20^{o}C for 4 d before a layer of either PDA or CMA (both without

benomyl, Metalaxyl M and Streptomycin sulphate) was layered over the culture. The plates were returned to the incubator and assessed weekly for *A. euteiches* cultures.

Grass overlay. Young blades of grass free of obvious disease or damage were collected from the lawn at PGRO. The grass was cut into 3.5 cm lengths and boiled for 5 min in distilled water. These were transferred to the top of potential cultures and left for 5 d. The grass was transferred to sterile tap water and the blade visualised under the microscope after 24 h for signs of infection, oospores or sporangia.

Results

Protocol 1) Soil Bait method

The plants in the sterile soil were disease free and those grown in the test soils had the characteristic discolouration of the roots and sloughing off of the outer parts of the root (Table 2). This infection was confirmed by microscopy. The germination rate of the peas in the test soil was lower than the sterile soil. When these seeds were removed from the soil they were heavily infested with fungal and bacterial infections.

Table 2. Results of the Soil Bait method. The test soils (A-I) all had high *A. euteiches* levels. The sterile soil did not have infected plants.

Soil sample	No of germinated seeds (max 5)	*A. euteiches* root score
A	2.50	4.13
B	1.50	4.88
C	3.25	4.10
D	3.00	3.88
E	2.00	4.25
F	3.00	3.92
G	3.50	4.16
H	3.25	4.29
I	3.75	4.54
Sterile Soil	4.50	0.00

Protocol 2) Organic matter separation

Both methods were effective at separating the organic matter fraction from the bulk soil. The organic matter was analysed under the microscope and oospores were present in the decaying material.

Protocol 3) The Rolled Towel method

This method used the organic matter from the soil bait test with confirmed *A. euteiches.* The method was also tested without soil and with a sterile soil. Two pea cultivars were compared with Kelvedon Wonder to test their susceptibility to *A. euteiches*. Both Avola and

Ambassador were infected with *A. euteiches* with a maximum root score of 4. However infection levels were low across all the reps (Table 3). Plants without soil and those inoculated with clean soil did not have root discoloration. The Kelvedon Wonder seed had 93% other fungal and bacterial contamination coming from the seed.

Table 3. The results from the rolled towel method.

	Mean infection score		
Variety	test material	Without soil	Sterile soil
Ambassador	0.17	0	0
Avola	0.37	0	0
Kelvedon Wonder	0.6	0	0

Protocol 4) The Modified Rolled Towel method

In the first experiment all the plants died as the towelling was too wet and the plants were smothered in the towelling. The second experiment resulted in some very healthy plants (Fig 3). Some plants had a general discolouration. To ascertain if this was the result of *A. euteiches* infection root segments were studied under the microscope for the presence of oospores (Table 4).

Figure 3 Pea plants unrolled at the end of the Modified Rolled Towel method. The plants were looking healthy with some root discolouration.

Table 4. The percentage of discoloured roots and those with oospores in the roots.

Treatment	Discoloured roots (%)	Presence of oospores (%)
Test soil (a)	33	0
Test soil (b)	25	12.5
Test soil (c)	57	25
Test soil (d)	75	50
Sterile soil	0	0

Protocol 5) Dish and Towel method

This method was quick to set up and used very little space (Fig 4). Soil samples were used rather than organic matter. The plants had root discoloration but it was not clear from looking at the roots if this was *A. euteiches* or other fungi especially as this discolouration was also found on the plants under the sterile soil. The main root from each plant was divided into three sections and studied for the presence of oospores. Oospores were not identified in the plants grown in the sterile soil. Twenty nine of the 35 plants with the test soil had oospores in the roots (Table 5).

Figure 4. Pea plants in the Dish and Towel method with soil samples placed on top of the plants.

Table 5. The number of plants with discoloured roots and the number of plants with *A. euteiches* infection confirmed by the presence of oospores in the root tissue.

Treatment	No of discoloured roots	Confirmed *A. euteiches* infection	Oospore presence		
			Upper root section	Mid root section	Lower root section
Test soil	35	29	18	19	23
Sterile soil	2	0	0	0	0

Protocol 6) Boiling tube method

Two pea cultivars were tested. The Kelvedon wonder seeds developed secondary infections and could not be assessed. The Ambassador plants inoculated with the sterile soil solution germinated well, the peas inoculated with the soil suspension quickly succumbed to infection (Fig 5).

The tubes were assessed 20 days post inoculation using the 1-5 scale used for the soil baiting technique. It was observed that the plants were either clean or black so the data was presented as number infected plants with a maximum of five plants assessed (Table 6). *A. euteiches* was confirmed by the presence of oospores.

Figure 5. Peas grown in boiling tubes in the presence of the test soil. Tube a) has been inoculated with sterile soil, tube b) with the test soil.

Table 6. The results of the plants grown in the boiling tubes

Treatment	Variety	Number discoloured roots	Confirmed *A. euteiches* infection
Test soil (1g in 10ml SDW)	Kelvedon Wonder	2	3
Test soil (1g in 20ml SDW)	Kelvedon Wonder	3	3
Test soil (1g in 30ml SDW)	Kelvedon Wonder	0	0
Test soil (1g in 10ml SDW)	Ambassador	3	3
Test soil (1g in 20ml SDW)	Ambassador	1	1
Test soil (1g in 30ml SDW)	Ambassador	0	0
Sterile soil (1g in 10ml SDW)	Kelvedon Wonder	0	0
Sterile soil (1g in 10ml SDW)	Ambassador	0	0

Protocol 7) Plate test

The plates were inoculated with organic matter suspended in SDW. There was a lot of fungal growth on the plates especially on the PDA and pea decoction plates. These were overrun with mycelium. Both media supported the fast growth of a wide range of fungi. There were cultures which looked like *A. euteiches* on the CMA plates and this was confirmed by the presence of oospores in the media.

Protocol 8) Plate Sandwich and the grass over lay

There was a lot of fungal growth on the base layer and this was reduced when it was overlaid with the second agar. However the media used were too nutrient rich for this assay and too many fungi grew (Table 7). Oospores were not identified in the agar suggesting *A. euteiches* was either not present or not producing oospores. *A. euteiches* mycelium was not

identified within the extensive hyphal growth on the plates. The grass overlay was not colonised by *A. euteiches*.

Table 7. The results of the plate sandwich method.

Soil type	Media bottom	Media Top	Outcome
Organic matter (1 g in 10 ml water)	CMA (plus additives)	PDA	Rampant fungal growth no oospores
Organic matter (1 g in 20 ml water)	CMA (plus additives)	PDA	Rampant fungal growth no oospores
Sterile soil (1 g in 10 ml water)	CMA (plus additives)	PDA	No growth
Organic matter (1 g in 10 ml water)	CMA (plus additives)	CMA	Rampant fungal growth no oospores
Organic matter (1 g in 20 ml water)	CMA (plus additives)	CMA	Rampant fungal growth no oospores
Sterile soil (1 g in 10 ml water)	CMA (plus additives)	CMA	No growth

Discussion

The current method used for identifying *A. euteiches* is the soil bait technique. This method is quick to set up and reliably identifies infected soils. Peas are not tolerant of water logging, which is a requirement of this test, and become stressed as a result. All the peas grew poorly even in uninfected soil. The infected plants had the characteristic honey colouration and the disintegration of the roots. The test is not specific to *A. euteiches* and other root rot infecting fungi were also identified. However from a growers perspective this would not be a problem as they are interested in the total root rot risk (*A. euteiches*, Fusarium and *P. medicaginis*). The test takes 38 days which is too long when a grower needs to make a decision in season. This illustrated the need to look at alternative methods.

One of the problems with soil assays is the amount of soil used for testing. The sample needs to be representative of the field but also of a realistic size to transport to the laboratory. *A. euteiches* oospores are found in the organic matter therefore a method of washing out the organic debris from the soil would enable a large volume of soil to be

analysed whilst the test remains small. The beaker and sieve method of extracting the organic debris from the soil is intricate and not suited to samples larger than a few grams. The Modified Fenwick Can is designed to handle much larger samples and the resulting sample was of a similar quality to the beaker and sieve method. The Modified Fenwick Can has the potential to be included as part of future developments. All of the tests are reliant on field sampling. *A. euteiches* can be a patchy disease and soil sampling would need to use the accepted 'W' shape sampling. In addition samples would need to be taken from areas of concern such as those with signs of waterlogging or previous premature senescence of the crop.

The rolled towelling method was tested on three pea cultivars. The Kelvedon Wonder seed (from two sources) had a high level of seed borne disease and decayed in the damp conditions in the towelling. Ambassador and Avola were both susceptible to *A. euteiches* and the seed was less susceptible to secondary rots than Kelvedon Wonder. Both towelling methods were quicker than the soil bait technique and have the potential to be used as a soil test. However they are complex to carry out. A less intricate and quicker approach was the Dish and Towel method and this will be investigated further to encourage better disease development and to use organic matter rather than soil in the assay. The boiling tube method was very effective at identifying the soils with *A. euteiches* present. This assay has potential to be developed further.

The use of the agar plates would be the quickest and possibly the easiest method to develop. However soil samples contain many different fungi many of which will grow on the media tested. This can result in growth of other fungi. *A. euteiches* mycelium grows in a relatively sparse arachnoid fashion which can easily be lost within other fungal mycelium. Alternative media will be tested.

With all the methods, plant or agar plates were checked for the presence of oospores to confirm the presence of *A. euteiches*. This pathogen discolours the roots. However the honey colouration is very slight especially in the towel and dish assay method. This method terminates before the roots start to disintegrate. Therefore the best way to confirm disease presence is to check for *A. euteiches* oospores. It is envisaged that this would only need to be done for a couple of roots in a test rather than every sample.

All of the methods ascertain the presence of viable fungal propagules. They do not take into account the soil conditions, weather conditions or general crop health all of which play an important part in the development of disease.

Conclusions

Eight methods of isolating *A. euteiches* have been tested. All the assays involving plants identified the pathogen although there was over estimation of the disease levels. This project enters its second and final year and the assays will be refined. These will then be used to test soils from different areas and compare *A. euteiches* levels with the number of pea crops grown.

Knowledge and Technology Transfer

PGRO Open day 2014 (Oral and Poster presentation)

Cereals 2014 (Poster presentation)

6th International Food and Legume Research Conference and the 7th International Conference on Legume Genetics and Genomics Canada 7-11 July 2014 (Poster presentation)

British Society of Plant Pathology Presidential meeting 2014 (Poster presentation)

VAA Meeting November 2014 (Oral presentation)

Holbeach Marsh Pea Growers Technical Meeting 2014 (Oral presentation)

Warwick Crop Centre Seminar November 2014 (Oral presentation)

The Pulse Magazine Spring 2014 (Article)

PGRO Staff Away day 2014 (Oral presentation)

Bruce Farms Technical meeting 2014 (Oral presentation)

References

Biddle A. and Cattlin N. 2007 Pests, Diseases and Disorders of Peas and Beans, A Colour Handbook. Academic Press.

CMI Description of Pathogenic Fungi and Bacteria No 600 *Aphanomyces euteiches*. 1978 Commonwealth Agricultural Bureaux.

Gravanis F. (1986) A study if the *Fusarium* foot and root rot of peas and an evaluation of certain chemicals for its control. PhD Thesis University of Manchester.

Kraft, J., Marcinkowska, J. and Muehlbauer F. (1990) Detection of *Aphanomyces euteiches* in field soil from northern Idaho by a wet sieving/baiting technique. Plant Disase 74:716-718.

Malvick D., Percich J., Pfelger, F., Givens, J. and Williams J. (1994) Evaluation of methods for estimating inoculum potential of *Aphanomyces euteiches* in soil. Plant Disease 78:361-365

Mitchell, J., Bhalla, H. and Yang, G (1969) An approach to the study of the population dynamics of *Aphanomyces euteiches* in soil. Phytopathology 59: 206-212

Pfender, W. (1984) Chapter 25 Aphanomyces Root Rot. In Compendium of Pea Diseases Ed Hagedorn D. The American Phytopathology Society.

Pfender, W., Malvick, D., Pfleger F. and Grau C. (2001) Chapter 9 Aphanomyces Root Rot. In Compendium of Pea Diseases 2nd Edition. Ed Hagedorn D. The American Phytopathology Society.

Oyarzun, P. and Van Loon, J. (1989) *Aphanomyces euteiches* as a component of the complex of foot and root pathogens of peas in Dutch soils. Netherlands Journal if Plant Pathology 95:259-264

