Project title:	*Brassica* and lettuce Propagation: Identifying means of killing clubroot and *Olpidium* resting spores in trays to avoid infection of transplants
Project number:	FV337
Project leader:	Dr John A. Walsh, Warwick HRI
Report:	Final report, May 2010
Previous report:	Annual Report April 2009
Key staff:	Dr John A. Walsh, Warwick HRI Jenny Kevitt-Jack, Warwick HRI Judith Bambridge, Warwick HRI Dr John Holah, Campden BRI Debra Smith, Campden BRI Craig Leadley, Campden BRI Joy Gaze, Campden BRI
Location of project:	Warwick HRI, Campden BRI
Project coordinator:	James Bean, Crystal Heart Salad Company
Date project commenced:	17 May 2008
Date project completed (or expected completion date):	16 May 2010
Key words:	Clubroot, big-vein, *Olpidium*, resting spores, heat, steam, microwaves, pulsed light, ozone, disinfectants.

Signed on behalf of : Warwick HRI

Name: …… **Date:** ……..
Professor Brian Thomas (Acting Director)

The results and conclusions in this report are based on an investigation conducted over a two 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.

CONTENTS

Grower Summary

Headlines

- Heat treatment, pulsed light and disinfectants appear to be effective in controlling *Olpidium brassicae* resting spores. Heat treatment and disinfectants were effective against Clubroot spores which were more difficult to control than the *Olpidium* spores.
- Ozone was ineffective and the scaling up of microwaves for commercial use looks challenging.

Background and expected deliverables

Overall aim of the project

The overall aim of the project was to evaluate new chemical and non-chemical means of controlling clubroot and *Olpidium* resting spores and to measure their efficacy against a known standard treatment.

Expected deliverables

1. A validated system for measuring the efficacy of different treatments in controling clubroot and *Olpidium* resting spores.
2. Knowledge of the efficacy of microwaves in controlling clubroot and *Olpidium* resting spores.
3. Knowledge on the efficacy of pulsed light in controlling resting spores.
4. Knowledge on the efficacy of ozone in controlling resting spores.
5. The thermal death point of clubroot and *Olpidium* resting spores.
6. Knowledge on the efficacy of conventional heat (steam) in controlling resting spores.
7. Knowledge on efficacy of new disinfectants / sterilants (chlorine dioxide, Aquaform) relative to Jet 5 in controlling resting spores.

Summary of the project and main conclusions

- Robust, reliable and reproducible bioassays for assessing the efficacy of physical and chemical treatments in controlling clubroot and *Olpidium brassicae* resting spores were developed and successfully deployed in the project.

- Microwaving under certain conditions controlled *Olpidium* resting spores, however, none of the microwave treatments killed all of the clubroot spores. Scaling up for commercial use does appear to be rather expensive.

- Pulsed light was very effective in controlling *Olpidium* resting spores, but was not effective in killing clubroot resting spores under the conditions tested.

- Ozone appears to have little potential for controlling *Olpidium* or clubroot resting spores.

- The thermal death point of *Olpidium* resting spores appears to be quite low, indicating that thermal treatments may have potential. The thermal death point of clubroot resting spores was rather high indicating that very high temperatures, or extended periods of exposure will be needed to control it.

- At the concentrations tested, Jet 5 was more effective than Difficil-S and Aquaform in controlling *Olpidium* resting spores. When tested at concentrations of roughly equivalent cost, Aquaform gave better control of clubroot resting spores than Jet 5 and Difficil-S.

Financial benefits

- The systematic and rigorous evaluation of the most promising means of controlling clubroot and *Olpidium* resting spores will allow the industry, Warwick HRI and Campden BRI to go forward with testing these under a range of intensities and durations in order to provide different options for commercial propagation systems.
- Elimination of ineffective treatments will avoid wasting time and money, and the testing of further treatments that do not control clubroot and *Olpidium* resting spores.

Action points for growers

- Microwaves, pulsed light and ozone do not appear to have great potential, or are not commercially viable for controlling clubroot, or *Olpidium* resting spores at the present time.
- Control of *Olpidium* resting spores is achieved through heating at 65^{O}C for 5 minutes. Heating at 60^{O}C is not effective.

- Treating *Olpidium* resting spores with steam for two and a half minutes also gave control.
- Clubroot resting spores had to be heated to 110^{O}C for 5 minutes in order to obtain control; heating at 105^{O}C for 5 minutes did not kill all resting spores.
- Jet 5 at 20% gave control of *Olpidium* resting spores when treated for 5 minutes;, not all spores were killed at 4%.
- Aquaform and Difficil-S at much lower concentrations (1.25% and 0.03% respectively) did not kill all *Olpidium* resting spores when treated for 5 minutes, but results suggest these would be effective if used at higher concentrations.
- Using Aquaform (80%) for 5 minutes was the only treatment that controlled clubroot resting spores when all treatments were tested at roughly equivalent rates based on cost.
- Neither Jet 5 at 40%, nor Difficil-S at 0.09% (chlorine dioxide) for 5 minutes killed all clubroot resting spores.
- Further work on a range of temperatures and a range of concentrations of disinfectants over a range of exposure times is now required in order to provide growers with a variety of options for controlling *Olpidium* and / or clubroot resting spores.

Science Section

Introduction

The project aims were

- to validate a system for measuring the efficacy of different treatments in controlling clubroot and *Olpidium* resting spores and use this to identify new systems and treatments that will control clubroot and *Olpidium* on vegetable propagation trays.

Vegetable Brassica crops in the UK were worth approximately £134 million in 2002 and the lettuce crop was worth approximately £82 million.

Clubroot, caused by the protist ***Plasmodiophora brassicae*** (Woronin) is one of the most important plant pathogenic problems in vegetable Brassica crops in the UK. The pathogen forms characteristic galls or clubs on the roots of the plant, reducing its yield potential. Up to 20% root damage, resulting in slowed growth and delayed harvesting have been reported in mild infections. Severe infections result in plant wilting and total crop failure. Since *P. brassicae* can remain dormant in contaminated soil as resting spores for as long as 18 years, affected land can be removed from Brassica production for considerable periods of time, or require disease control treatments which are expensive and have associated environmental concerns. Clubroot has become prevalent in all major areas of vegetable Brassica production.

It is essential that *P. brassicae* is not introduced in to clean soils *via* infected transplants. Production of healthy transplants is an essential component of an integrated clubroot control strategy. The sudden appearance of clubroot in a previously disease-free soil may not be due directly to incoming inoculum from infected transplants. Soils which appear to be uncontaminated (i.e. produce healthy plants) may be harboring *P. brassicae* spores at levels which do not appear to affect production. If effective and validated procedures for controlling clubroot resting spores on trays can be established, then any risk of disease transfer from contaminated to uncontaminated land via the propagation process can be minimised.

Although we demonstrated earlier (HDC Factsheet for Project FV 178) the efficacy of a number of disinfectants including Jet 5 in controlling clubroot resting spores, this involved a contact time of 20 minutes. There is no data on the efficacy of current tray washing systems in controlling clubroot resting spores.

Lettuce big-vein disease is caused by a virus that is transmitted by the soil-borne fungus *Olpidium brassicae* and is prevalent in the main lettuce producing areas of the UK. Symptoms are not well recognised and affected plants are often marketed. It may only be a matter of time before buyers begin to recognise the symptoms and reject affected consignments. The resting spores of *O. brassicae* can remain dormant in soil for >20 years and retain the ability to transmit the lettuce big-vein disease for >17 years.

Our earlier work identified the best disinfectants and minimum effective rates for controlling resting spores of *O. brassicae* during lettuce propagation to ensure healthy transplants (HDC Factsheet FV/PC 62, December 1994). We also demonstrated efficacy of the new formulation of Bavistin (DF) in giving some control of big-vein in the field (Final Report on HDC project no FV/PV 62) and obtained a SOLA for Bavistin DF on lettuce for control of big-vein.

Although we have demonstrated the efficacy of a number of disinfectants including Jet 5 in controlling *Olpidium* resting spores, this involved a contact time of 20 minutes. There is no data on the efficacy of current tray washing systems in controlling *Olpidium* resting spores.

Materials and methods

Techniques are available at Warwick HRI for purifying and quantifying clubroot resting spores and techniques have been developed for purifying and quantifying *Olpidium* resting spores. These were used to produce quantified batches of resting spores which were then subjected to a number of physical and chemical treatments to determine the efficacy of the treatments in controlling the spores.

Bioassays have also been developed at Warwick HRI to determine the viability of clubroot and *Olpidium* resting spores following physical and chemical treatments of resting spores.

Campden BRI have expertise and practical facilities to undertake research in established thermal sterilisation treatments (steam, microwaves) and novel decontamination systems (pulsed light, ozone). Campden BRI also has staff qualified to undertake efficacy testing of disinfectants and the associated test facilities.

A slight modification of the existing bioassay for determining whether *Olpidium* resting spores were controlled was developed. The spores were dried on to polypropylene discs or part modules and then their viability checked using the existing bioassay. Polypropylene discs with the resting spores dried on were supplied to Campden BRI where the treatments outlined below were applied. The discs were then returned to Warwick HRI where the viability of the spores was then assessed using the bioassay.

A range of concentrations of clubroot resting spores were used to infect Brassica

(broccoli) seedlings growing in sand. Appropriate numbers were then dried on to polypropylene discs as for *Olpidium* and their viability checked using the existing bioassay with Brassica seedlings growing in sand or compost. Finally a range of clubroot spore suspensions were used to infect Brassica seedlings growing only in compost.

Microwaves were tested under three regimes for their ability to control *Olpidium* resting spores dried on to polypropylene plastic discs in a fully replicated experiment, including untreated *Olpidium* controls and uncontaminated controls. For clubroot control, microwaves were tested under two regimes in solutions in small plastic petri dishes in a fully replicated experiment including untreated clubroot controls and controls with no clubroot or *Olpidium* spores.

Pulsed light was tested at two different intervals for its ability to control *Olpidium* resting spores that had been dried down on to polypropylene plastic discs or clubroot resting spores in solutions in small plastic petri dishes, in fully replicated experiments, including untreated *Olpidium* and clubroot controls and controls with no clubroot or *Olpidium* spores.

Ozone was tested at 2 different concentrations for its ability to control *Olpidium* resting spores (dried on to the polypropylene plastic discs or rewetted on the polypropylene discs) and clubroot resting spores (in solution in small plastic petri dishes) in fully replicated experiments, including untreated *Olpidium* and clubroot controls and controls with no clubroot or *Olpidium* spores.

As a prerequisite to conventional heat treatments, replicated thermal death experiments were be carried out on *Olpidium* and clubroot resting spores. Quantified aliquots of *Olpidium* and clubroot resting spores were provided to Campden BRI by Warwick HRI. These were transferred to capillary tubes, treated at a range of temperatures and for different periods of time and then returned to Warwick HRI, where bioassays were carried out to determine the viability of the treated resting spores. Again appropriate untreated and controls with no clubroot or *Olpidium* spores were included.

Conventional wet heat (steam) for three different durations was tested for its ability to control *Olpidium* resting spores dried on to the polypropylene plastic discs in fully replicated experiments, including untreated controls and controls with no clubroot or *Olpidium* spores.

A system for measuring the efficacy of different chemical disinfectants (Difficil-S, Aquaform and Jet 5) in controlling *Olpidium* resting spores on modular trays was established. Plastic discs were contaminated with known quantities of *Olpidium* resting spores which were dried on to the discs, for clubroot known quantities of spores were aliquoted in to Falcon tubes. These were supplied to Campden BRI who treated sets of discs and spores in tubes with the chemical disinfectants. Following disinfectant treatment, residues were chemically neutralised and diluted. One set of contaminated discs and one set of tubes were left untreated as controls and there were uncontaminated controls. The discs and spores in tubes were returned to Warwick HRI, where they were tested using the

bioassays to determine the efficacy of the disinfectants in controlling the resting spores. Appropriate untreated and uncontaminated controls were included.

Objective 1. Validation of a system for measuring the efficacy of different treatments in killing* Olpidium brassicae *resting spores

In a fully replicated experiment with appropriate controls, circular discs of plastic, 29mm diameter, of the same material used in the manufacture of 216 modular trays (polypropylene) (Figure 1) and parts of 216 modular trays (Figure 2) were contaminated with 200, 600, or 1000 *Olpidium brassicae* resting spores per disc / part modular tray. The discs were then placed into single cells of clean P40 modular trays pre-planted with lettuce in sand (J. Arthur Bowers, sharp sand) (Figure 3). Similarly, young susceptible lettuce plants were placed in the contaminated 216 part trays. After 3 weeks, the transplanted lettuce plants were removed from the modules, the sand washed from their roots and assessed for *Olpidium* infection by microscopical examination of root systems. By this means, the optimum concentration of spores on the plastic discs and part modules for future experiments to evaluate different physical and chemical treatments was determined.

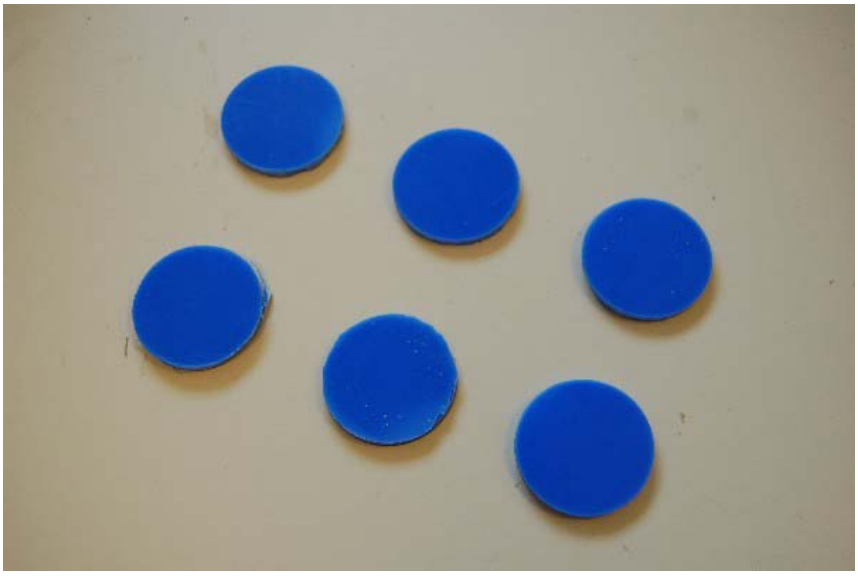

Figure 1: Polypropylene discs that spores were dried on to for development of the bioassay for determining the effect of various treatments on *Olpidium brassicae* resting spores.

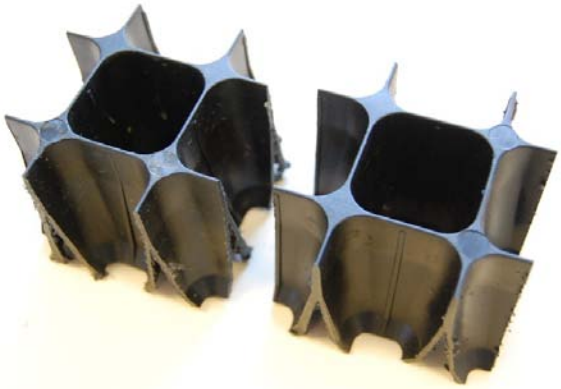

Figure 2: Parts of 216 modular trays that spores were dried on to for developing the bioassay for determining the effect of various treatments on *Olpidium brassicae* resting spores

Figure 3: A young lettuce seedling growing in sand in a cell from a P40 tray with an *Olpidium brassicae* contaminated disc inserted

Objective 1. Validation of a system for measuring the efficacy of different treatments in killing clubroot resting spores

A range of quantities ($5x10^3$, $5x10^5$, or $5x10^7$) of clubroot resting spores were used to infect Brassica seedlings growing in sand in single cells of P40 modular trays. The root systems of the Brassica seedlings were then examined by microscopy for infection. A further experiment was then carried out to infect Brassica seedlings growing in sand, or compost (steam sterilised Levingtons M2) in single P40 cells with a range of quantities of clubroot resting spores ($5x10^6$, $5x10^7$, or $5x10^8$) dried on to the circular plastic discs, with appropriate controls. After 7 weeks, the root systems were examined for clubroot symptoms and where

there were no symptoms, roots were examined for the presence of clubroot spores by light microscopy. Finally, a range of quantities ($5x10^6$, $5x10^7$, or $5x10^8$) of clubroot resting spores in water were used to infect Brassica seedlings growing in M2 compost (Figure 4), with appropriate controls. After 7 weeks, the root systems were examined for clubroot symptoms and where there were no symptoms, roots were examined by light microscopy for the presence of clubroot spores.

Figure 4: A young Brassica seedling growing in sand in a cell from a P40 modular tray after inoculation with clubroot resting spores

Objectives 2-4. Determining the efficacy of microwaves, pulsed light and ozone in killing* Olpidium *and clubroot resting spores

The small plastic discs were contaminated with *Olpidium* resting spores at Warwick HRI as determined in Objective 1 and supplied to Campden BRI. Clubroot resting spores were supplied in bulk solution and aliquots pipetted in to small petri dishes immediately prior to treatment. Fully replicated experiments with appropriate contaminated untreated and uncontaminated control discs (for *Olpidium*) or extracts of infected and healthy Brassica roots in petri dishes (for clubroot), were carried out at Warwick HRI and Campden BRI to determine the efficacy of microwaves, pulsed light and ozone in controlling clubroot and *Olpidium* resting spores as follows:

Microwaves were tested in three regimes for their ability to control *Olpidium* resting

spores on the plastic discs. Discs with 600 resting spores on were tested using the best conditions that were practically achievable from those established during thermal imaging of discs treated with microwaves (Figure 5). Thirty discs were tested dry, thirty after the discs have been wetted and 30 more after soil had been added and wetted. Microwaves were tested in two regimes for their ability to control clubroot resting spores ($5x10^7$) in small petri dishes. Thirty petri dishes were tested with spore solution in and 30 with soil added to the spore solution. The microwave heating time was 45 seconds. Heating for longer was likely to result in overheating of the microwave oven components because there was insufficient material in the cavity to absorb sufficient microwave energy. Thermal imaging of treated discs was carried out in order to determine what temperatures were achieved on the discs following microwave treatment (Figure 5).

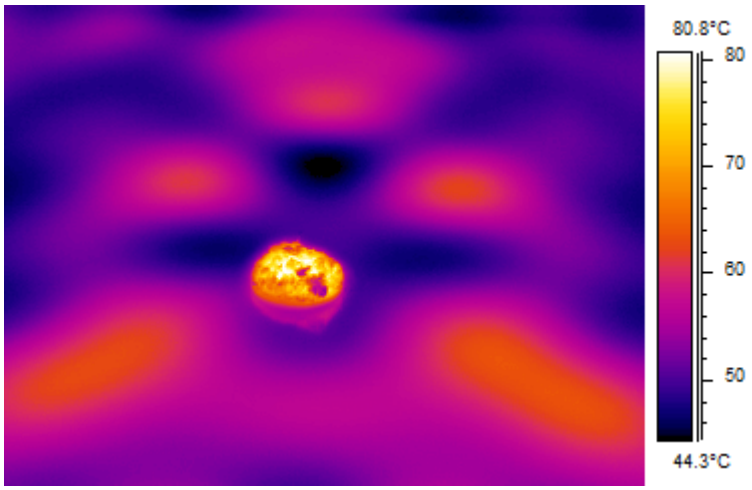

Figure 5: Thermal imaging of a polypropylene disc after treatment in the microwave oven

Pulsed light was tested at two different rates for its ability to control *Olpidium* resting spores that had been dried down on to the plastic discs (600 resting spores / disc) or clubroot spores in small petri dishes ($5x10^7$ resting spores / petri dish). Thirty discs (*Olpidium*) and 30 petri dishes (clubroot) were subjected to 1 flash (at 3000 volts, 1.3 $J.cm^{-2}$) at maximum pulsed light intensity in a commercial pulsed light unit (Clarinor, France) (Figure 6) and 30 discs and 30 petri dishes were subjected to four flashes (at 3000 volts, 1.3 $J.cm^{-2}$) at maximum intensity.

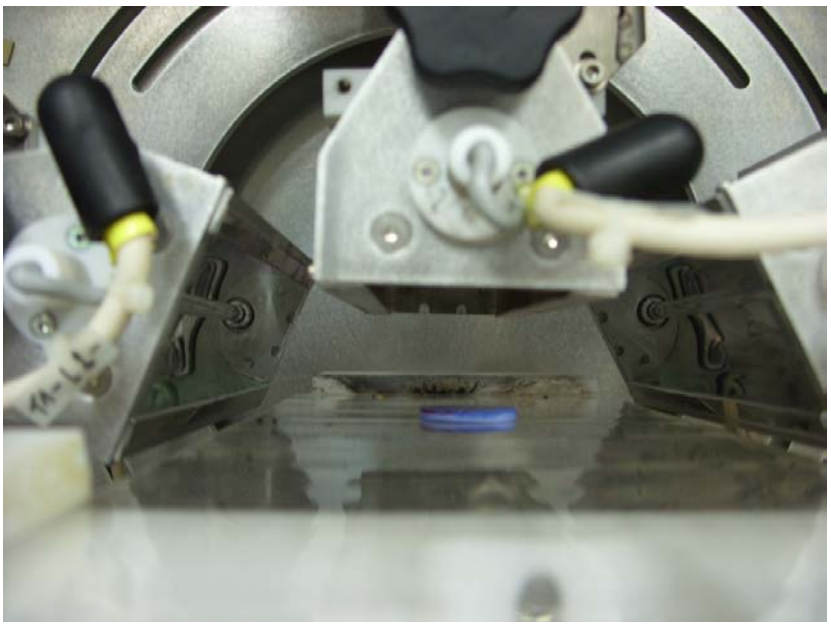

Figure 6: A polypropylene disc in the pulsed light unit

Ozone was tested at 2 different concentrations (8ppm and 25ppm) and under one regime for its ability to control clubroot resting spores and two regimes for *Olpidium* resting spores. The two regimes for *Olpidium*, spores that had been dried onto the plastic discs and spores that had been dried on to discs and then rewetted, whereas clubroot spores were treated in solution in the small petri dishes. Both ozone concentrations were in excess of the HSE exposure limit. The exposure time was 30 minutes and 30 discs (1000 *Olpidium* resting spores / disc) and 30 petri dishes ($5x10^7$ clubroot resting spores / dish) were treated at each of the two ozone rates. The ozone levels were monitored throughout the experiment and this showed that the target levels (8ppm and 20ppm) were achieved and maintained for the exposure time of 30 minutes. The experiment also included 30 dry discs and 30 rewetted discs with *Olpidium* spores and 30 petri dishes with clubroot spores in that remained untreated as controls that accompanied the discs that were treated. The control discs were kept and stored in the same place and treated in exactly the same way as the treated discs, except for the actual experimental treatments. Additionally, there were 30 discs without spores on and 30 petri dishes with extracts from healthy roots in for the 'healthy' controls.

The discs and petri dishes were returned to Warwick HRI where they were assayed as described in Objective 1 to determine the viability of the spores.

Objective 5. Determining the thermal death point of* Olpidium *and clubroot resting spores

As a prerequisite to conventional heat treatments, replicated thermal death experiments were carried out on *Olpidium* and clubroot resting spores. Quantified aliquots of *Olpidium* and clubroot resting spores were provided to Campden BRI by Warwick HRI. These were

then transferred to capillary tubes (5,000 *Olpidium*, or $5x10^7$ clubroot spores / capillary) (Figure 7). These were then treated at a range of temperatures in an oil bath (Figure 8) for different durations (30 seconds-15 minutes) and then returned to Warwick HRI, where bioassays were carried out to determine the viability of the treated resting spores. Untreated capillaries and capillaries containing distilled water or extracts from healthy roots were also tested in the bioassays.

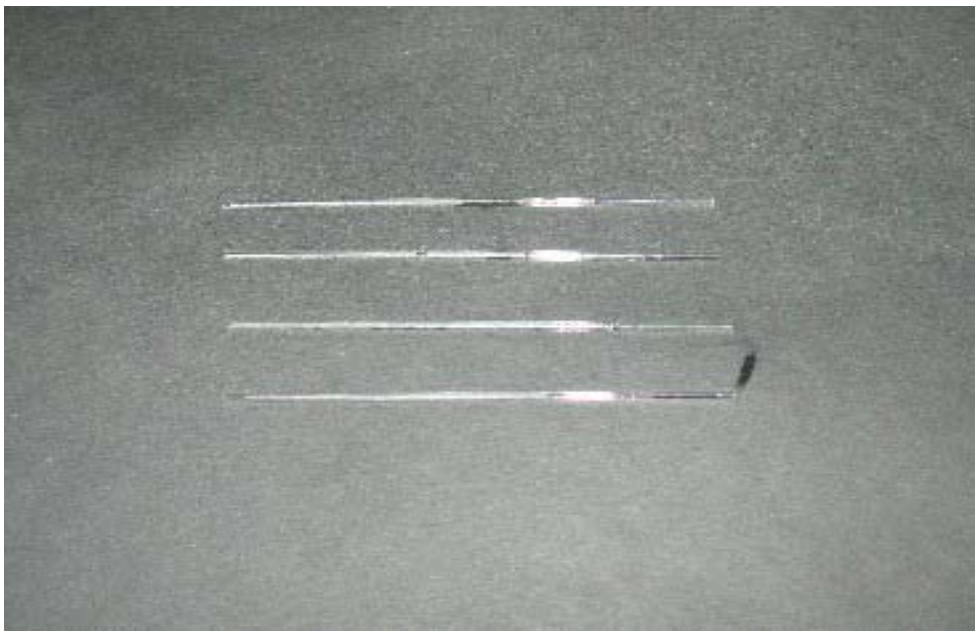

Figure 7: Capillary tubes used for heat treatments of *Olpidium brassicae* resting spores

Figure 8: Oil bath in which the *Olpidium brassicae* resting spore filled capillaries were heat-treated

Two formats of experiments were carried out to determine kinetic parameters analogous to 'd' and 'z' values for *Olpidium* resting spores. D values are the decimal log reduction time for the numbers of viable organisms at any given temperature. For example, a d value of 1 min at 80^{O}C means that the viable population of the organism is reduced by 90% for every minute of heating at this temperature. Z values are the degrees of temperature over which a 90% change in the d value is observed. For example, a z value of 8^{O}C means that for every change of 8^{O}C, there is a 10 fold change in the d value.

The first experiment was to establish a temperature range in which 90% of plants that are used to test spores from the capillaries did not become infected following the heat treatments on the spores. This was investigated by heating 30 capillary tubes containing *Olpidium* resting spores at each of three temperatures in the range 110-130^{O}C for 5, 10, or 15 minutes. Thirty capillaries containing untreated *Olpidium* and 30 capillaries with just water in, were also assessed in the bioassay as controls.

In a second experiment to establish a kinetic parameter analogous to a z value, 30 capillary tubes containing *Olpidium* resting spores were heated at each of 6 temperatures (60-110^{O}C) for three different times (1, 2.5, 5, or 10 mins). Thirty capillaries containing untreated *Olpidium* and 30 uncontaminated tubes with just water in, were also assessed in the bioassay as controls.

For clubroot, temperatures in the range 65-85^{O}C didn't kill all clubroot spores, so a second experiment in the range 90-100^{O}C was carried out which also didn't kill all clubroot spores. Finally a third experiment was carried out in the range 105-120^{O}C.

Objective 6. Testing the efficacy of conventional heat in killing the resting spores

Three different durations of steam treatment (2.5, 5 and 7 minutes) were tested for their ability to control *Olpidium* resting spores dried on to the polypropylene plastic discs (1000 spores / disc) in fully replicated experiments, including untreated *Olpidium* controls and uncontaminated controls. Temperatures in the steam tunnel were monitored.

Objective 7. Testing the efficacy of new disinfectants / sterilants (Difficil-S, Aquaform) relative to Jet 5 in killing the resting spores

Polypropylene discs were contaminated with 1000 *Olpidium* resting spores / disc at Warwick HRI and clubroot resting spores were aliquoted in to Falcon tubes (5x10^{7} / tube) and supplied to Campden BRI. Fully replicated treatments with Difficil-S (chlorine dioxide), Aquaform and Jet 5 were applied to the *Olpidium* contaminated discs and clubroot spores in tubes at Campden BRI. Appropriate contaminated, untreated and uncontaminated controls were included in the experiment and accompanied the *Olpidium* contaminated discs and tubes of clubroot spores that were to be treated at Campden BRI; they were kept and stored in the same way and place as the treated discs in every way except for the treatments. The

discs and tubes were then returned to Warwick HRI where they were tested in the bioassays to determine the efficacy of Difficil-S and Aquaform relative to Jet 5, in controlling *Olpidium* and clubroot resting spores.

The three disinfectants were tested on *Olpidium* at both their manufacturers' recommended concentration and at five times this concentration. Following the results of the experiment on *Olpidium*, higher rates of the disinfectants, based on equivalent costs were tested on clubroot resting spores. The discs and tube contents were treated for 5 minutes and then placed in a neutralising solution (a universal neutralisation medium based on BS EN 1672, the Food Hygiene bactericidal suspension test [Lecithin, 3g/l, Polysorbate 80, 30g/l (V/V); Sodium thiosulphate, 5g/l; L-histidine, 1g/l; Saponin, 30g/l; made up in diluent consisting of. Tryptone, 1.0g/l and NaCl, 8.5g/l]). One set of 30 *Olpidium* contaminated discs and 30 tubes of clubroot spores were left untreated as controls and another set of 30 contaminated discs and 30 tubes were treated with the neutralising solution as a further control. Following treatment of all discs in the neutralising solution, the solution was filtered through a membrane to recover any spores that were detached from the discs. There was also a set of 30 uncontaminated, untreated discs and tubes as controls. The discs, membranes and tubes were returned to Warwick HRI following the treatments, where they were placed in the individual P40 modules containing lettuce or Brassica seedlings to determine the viability of the spores. In this way the efficacy of the disinfectants in controlling the resting spores was determined. In the clubroot experiment to verify the ability of the neutralising solution to neutralise the disinfectants, it was mixed with the highest concentration of each disinfectant (10 reps each) and left to stand for 5 minutes, after which clubroot resting spores were added and then tested for viability in the normal bioassay.

Results

Objective 1. Validation of a system for measuring the efficacy of different treatments in killing Olpidium *resting spores*

All three rates of resting spores dried on to the polypropylene discs and part 216 modules gave rise to infection in the bioassay (Table 1). The 200 spores / disc gave rise to infection in 26 out of the 30 lettuce bioassay plants and the 200 spores / part 216 module gave rise to infection in 28 out of 30 lettuce bioassay plants, whereas 600 and 1000 spores / disc and part 216 module gave rise to infection in all 30 bioassay plants. No infection was seen in bioassay plants from the control treatments involving discs or part modules with no spores on, indicating there was no cross contamination between treatments. Rates of 600 and 1000 spores / disc were used in subsequent bioassay experiments.

Table 1: Validation of the bioassay for determining the efficacy of various physical and chemical treatments in controlling *Olpidium brassicae* resting spores

Number of spores per polypropylene disc or part 216 module	**Number of lettuce plants infected with *Olpidium* / number of plants tested in the bioassay**
0 spores / disc	0 / 30
200 spores / disc	26 / 30
600 spores / disc	30 / 30
1000 spores / disc	30 / 30
0 spores / module	0 / 30
200 spores / module	28 / 30
600 spores / module	30 / 30
1000 spores / module	30 / 30

Objective 1. Validation of a system for measuring the efficacy of different treatments in killing clubroot resting spores

None of the rates of resting spores used to inoculate Brassica seedlings growing in sand gave rise to visibly clubbed roots after 3, 4, 5, or 7 weeks, although microscopy of roots did reveal infection in some cases. Drying spores on to the plastic discs and attempting to infect Brassica seedlings growing in sand didn't give rise to any clubbed roots, but did give rise to clubbed roots of some, but not all plants growing in compost. Adding spore suspensions to Brassica seedlings growing in compost gave rise to clubbed roots of all plants at all spore concentrations (Table 2). No infection was seen in bioassay plants from the control treatment where no spores were added, indicating there was no cross contamination between treatments. Rates of $5x10^7$ spores / replicate were used in subsequent bioassay experiments.

Table 2: Validation of the bioassay for determining the efficacy of various physical and chemical treatments in controlling clubroot resting spores

Number of spores in solutions added to modules with Brassica seedlings in compost	Number of Brassica plants with visible clubroot / number of plants inoculated in the bioassay
0 spores	0 / 15
$5x10^6$	15 / 15
$5x10^7$	15 / 15
$5x10^8$	5 / 5

Objectives 2-4. Determining the efficacy of microwaves, pulsed light and ozone in killing* Olpidium *and clubroot resting spores

Treating *Olpidium* resting spores dry on plastic discs with microwaves failed to kill all resting spores, 26 out of the 30 bioassay plants were infected by *Olpidium* (Table 3). Treating spores that had been rewetted or with soil added and wetted, with microwaves appeared to control all *Olpidium* resting spores. The untreated controls gave 100% infection and the no spore control gave no infection, indicating that there was no cross contamination between treatments.

Table 3: The effect of microwaves on *Olpidium brassicae* resting spores

Treatment (duration)	Number of bioassay plants infected with *Olpidium* / number tested
Dry treated (45secs)	26 / 30
Wet treated (45 secs)	0 / 30
Soil wet treated (45 secs)	0 / 30
Dry untreated	30 / 30
Wet untreated	30 / 30
Soil wet untreated	30 / 30
No spores control discs	0 / 30

Treating clubroot resting spores in small petri dishes with microwaves failed to kill all resting spores in the absence and in the presence of soil. All 30 bioassay plants had clubbed roots in both cases (Table 4). The untreated controls gave 100% infection and the no spore control gave no infection, indicating that there was no cross contamination between treatments.

Table 4: The effect of microwaves on clubroot resting spores

Treatment (duration)	Number of Brassica plants with visible clubroot / number of plants inoculated in the bioassay
Spore suspension (45 secs)	30 / 30
Spore suspension + soil (45 secs)	30 / 30
Spore suspension untreated	30 / 30
Spore suspension + soil untreated	30 / 30
Healthy root preparation control	0 / 30

Treating *Olpidium* resting spores dry on plastic discs with pulsed light appeared to control all the resting spores. Both the single flash and four flash treatments appeared to control all spores (Table 5). All plants were infected in the positive control treatment, indicating the inoculum was viable and the results from treatments real. Once again, the no spore control gave no infection of lettuce seedlings.

Table 5: The effect of pulsed light on *Olpidium brassicae* resting spores

Pulsed light treatment	**Number of bioassay plants infected with *Olpidium* / number tested**
1 flash	0 / 30
4 flashes	0 / 30
Spores, untreated control	30 / 30
No spores control discs	0 / 30

Treating clubroot resting spores in petri dishes with pulsed light appeared had little effect on the resting spores. The single flash had little or no effect and although the four flash treatment appeared to control some spores, it didn't give complete control (Table 6). All plants were infected in the positive control treatment, indicating the inoculum was viable and the results from treatments real. Once again, the no spore control gave no infection of Brassica seedlings.

Table 6: The effect of pulsed light on clubroot resting spores

Pulsed light treatment	**Number of Brassica plants with visible clubroot / number of plants inoculated in the bioassay**
1 flash	30 / 30
4 flashes	21 / 26
Spores, untreated control	30 / 30
Healthy root preparation control	0 / 30

At the rates tested ozone appeared to be relatively ineffective in killing the *Olpidium* resting spores on plastic discs under dry conditions. At 8ppm and 25ppm almost all of the treated discs still had live resting spores on (30/30 and 29/30 bioassay plants infected respectively) (Table 7). Treating discs that had been wetted prior to treatment was also ineffective (28/30 bioassay plants infected after treatment at 8ppm and 30/30 infected at 25ppm) (Table 7). All plants were infected in the wet and dry positive control treatments. The no spore control gave no infection of lettuce seedlings indicating there was no cross-contamination between treatments.

Table 7: The effect of ozone on *Olpidium brassicae* resting spores

Treatment conditions, ozone concentration and duration	**Number of bioassay plants infected with *Olpidium* / number tested**
Dry, 8ppm, 30mins	30 / 30
Dry, 25ppm, 30mins	29 / 30
Wet, 8ppm, 30mins	28 / 30
Wet, 25ppm, 30mins	30 / 30
Dry, spores untreated control	30 / 30
Wet, spores untreated control	30 / 30
No spores untreated control	0 / 30

At the rates tested, ozone appeared to be ineffective in killing the clubroot resting spores in solution in the small petri dishes. After treatment with ozone at 8ppm and 25ppm, all of the replicates still had live resting spores (30/30 bioassay plants infected at both concentrations; Table 8). All plants were infected in the positive control treatment. The no spore control gave no infection of Brassica seedlings indicating there was no cross-contamination between treatments.

Table 8: The effect of ozone on clubroot resting spores

Ozone concentration and duration of treatment	**Number of Brassica plants with visible clubroot / number of plants inoculated in the bioassay**
8ppm, 30mins	30 / 30
25ppm, 30mins	30 / 30
Spores untreated control	30 / 30
No spores untreated control	0 / 30

Objective 5. Determining the thermal death point of* Olpidium *and clubroot resting spores

In the first experiment, virtually all the *Olpidium* resting spores were controlled in the temperature range 110-130^{O}C after treating for 5, 10, or 15 minutes (Table 9). Spores in untreated capillaries gave rise to infection in all 30 bioassay plants, indicating that the spores were viable and the results from treatments real. The no spore control gave no infection of lettuce seedlings indicating there was no cross-contamination between treatments.

Table 9: The effect of heat treatment on *Olpidium brassicae* resting spores

Heat treatment of resting spores in capillaries (duration)	**Number of bioassay plants infected with *Olpidium* / number tested**
110ºC (5 mins)	1 / 30
110ºC (10 mins)	0 / 30
110ºC (15 mins)	0 / 30
120ºC (5 mins)	0 / 30
120ºC (10 mins)	0 / 30
120ºC (15 mins)	0 / 30
130ºC (5 mins)	0 / 30
130ºC (10 mins)	0 / 30
130ºC (15 mins)	0 / 30
Spores in untreated capillary	30 / 30
No spores untreated control	0 / 30

In the second experiment again, virtually all the *Olpidium* resting spores were controlled in the temperature range 60 - 110^{O}C after treating for 1, 2.5, 5, or 10 minutes (Table 10). Again spores in untreated capillaries gave rise to infection in all 30 bioassay plants indicating that the spores were viable and the results from treatments real. The no spore control gave no infection of lettuce seedlings indicating there was no cross-contamination between treatments.

Table 10: The effect of heat treatment on *Olpidium brassicae* resting spores

Heat treatment of spores in capillaries (duration)	**Number of bioassay plants infected with *Olpidium* / number tested**
60ºC (1 min)	0 / 15
60ºC (5 mins)	0 / 15
60ºC (10 mins)	0 / 15
70ºC (1 min)	0 / 15
70ºC (5 mins)	0 / 15
70ºC (10 mins)	0 / 15
80ºC (1 min)	0 / 15
80ºC (5 mins)	0 / 15
80ºC (10 mins)	0 / 15
90ºC (1 min)	0 / 15
90ºC (5 mins)	0 / 15
90ºC (10 mins)	1 / 15
100ºC (1 min)	0 / 15
100ºC (5 mins)	0 / 15
100ºC (10 mins)	0 / 15
110ºC (1 min)	0 / 15
110ºC (2.5 mins)	0 / 15
110ºC (5 mins)	0 / 15
Spores untreated	30 / 30
No spores untreated control	0 / 30

As these large experiments were not in the temperature range suitable for determining the thermal death point of *Olpidium* resting spores, a further small experiment was carried out to get a better idea of the temperatures the spores could resist. This showed that spores could

resist 45^{O}C and confirmed that some were killed at 60^{O}C after treatment for 5 minutes (Table 11). The positive control treatment confirmed the viability of the spores and the negative control indicated there was no cross-contamination of treatments.

Table 11: The effect of heat treatment on *Olpidium brassicae* resting spores

Heat treatment of spores in capillaries (duration)	**Number of bioassay plants infected with *Olpidium* / number tested**
30ºC (5 mins)	5 / 5
45ºC (5 mins)	5 / 5
60ºC (5 mins)	2 / 5
Spores untreated	5 / 5
No spores untreated control	0 / 5

This made it possible to carry out a further experiment at more temperatures in a similar range, in order to calculate the thermal death point of *Olpidium* resting spores. This experiment showed that heating spores to 65ºC for 5 minutes gave control (Table 12).

Table 12: The effect of heat treatment on *Olpidium brassicae* resting spores

Heat treatment of spores in capillaries (duration)	**Number of bioassay plants infected with *Olpidium* / number examined**
50ºC (5 mins)	14 / 24
55ºC (5 mins)	1 / 27
60ºC (5 mins)	1 / 26
65ºC (5 mins)	0 / 25
70ºC (5 mins)	0 / 24
Spores untreated	27 / 28
Healthy root preparation untreated	0 / 14
Healthy root preparation, 50ºC (5 mins)	0 / 13

To determine the 'z' value for *Olpidium*, we can extrapolate that the increase in temperature required to reduce infection from 100% to 0% (i.e. 100% reduction) in the plants was 20ºC. Using these data we can predict that it would take at least 50 minutes at 45ºC to achieve the same level of destruction as at 65ºC for 5 minutes.

The first experiment on clubroot revealed that some, but not all resting spores were controlled in the range 65-85ºC after treatment for 5 minutes (Table 13). Spores in untreated capillaries gave rise to infection in all 30 bioassay plants, indicating that the spores were viable and the results from treatments real. The healthy root preparation controls gave no infection of Brassica seedlings, indicating there was no cross-contamination between treatments.

Table 13: The effect of heat treatment on clubroot resting spores

Heat treatment of spores in capillaries (duration)	Number of Brassica plants with visible clubroot / number of plants inoculated in the bioassay
65ºC (5 mins)	25 / 30
70ºC (5 mins)	17 / 30
75ºC (5 mins)	12 / 30
80ºC (5 mins)	4 / 30
85ºC (5 mins)	4 / 30
Spores untreated	30 / 30
Healthy root preparation untreated	0 / 30
Healthy root preparation, 85ºC (5 mins)	0 / 30

A further experiment carried out at higher temperatures in the range 90-100ºC also failed to give complete control of clubroot spores (Table 14).

Table 14: The effect of heat treatment on clubroot resting spores

Heat treatment of spores in capillaries (duration)	Number of Brassica plants with visible clubroot / number of plants inoculated in the bioassay
90ºC (5 mins)	4 / 30
95ºC (30 secs)	1 / 30
95ºC (2.5 mins)	7 / 30
95ºC (5 mins)	10 / 30
100ºC (5 mins)	13 / 30
Spores untreated	30 / 30
Healthy root preparation untreated	0 / 30

A final experiment showed that although some spores were able to survive treatment at 105ºC, no infection was detected from spores that were treated for 5 minutes at 110ºC and higher (Table 15).

Table 15: The effect of heat treatment on clubroot resting spores

Heat treatment of spores in capillaries (duration)	Number of Brassica plants with visible clubroot / number of plants inoculated in the bioassay
105ºC (5 mins)	3 / 30
110ºC (5 mins)	0 / 30
115ºC (5 mins)	0 / 30
120ºC (5 mins)	0 / 30
Spores untreated	15 / 15
Healthy root preparation untreated	0 / 30

To determine the 'z' value for clubroot, we can extrapolate from 83% of plants with symptoms after treatment at 65ºC for 5 minutes, to 100% of plants with no symptoms after treatment at 110ºC for 5 minutes. Similarly we can extrapolate from 0% of plants with symptoms after treatment at 110ºC for 5 minutes to 17% of plants with no symptoms after treatment at 65ºC for 5 minutes. Using these data, we can predict that to obtain a shift from 100% symptoms to 0% symptoms in the plants (i.e. 100% reduction) would require an

increase in temperature of greater than 45ºC. From this, we can predict that it would take at least 50 minutes at 65ºC to achieve the same level of control as achieved at 110ºC for 5 minutes.

Objective 6. Testing the efficacy of conventional heat in killing* Olpidium *resting spores

Steam treatment of *Olpidium* resting spores dried on to the polypropylene discs gave good control, with no infection of lettuce seedlings detected after spores had been treated for 2.5, 5, or 7 minutes (Table 16). Control treatments confirmed that the spores tested were viable and that there was no cross contamination of treatments.

Table 16: The effect of steam treatment on *Olpidium brassicae* resting spores

Duration of steam treatment of spores on discs	Number of bioassay plants infected with *Olpidium* / number tested
2.5 mins	0 / 30
5 mins	0 / 30
7 mins	0 / 30
Untreated	30 / 30
Healthy root preparation untreated	0 / 30
No spores untreated control	0 / 30

Monitoring the temperatures achieved in the steam tunnel over a four hour period showed that they ranged from 90-96.5ºC. As temperatures of 110ºC for 5 minutes were needed to control clubroot (Table 15), no tests were carried out on spores in the steam tunnel.

Objective 7. Testing the efficacy of new disinfectants / sterilants (Difficil-S, Aquaform) relative to Jet 5 in killing* Olpidium *and clubroot resting spores

Neither the Difficil-S, nor the Aquaform at the recommended rates, or five times the recommended rates were able to control all the spores on the discs (Table 17). At the recommended rates, Difficil-S was better than Aquaform, but not as good as Jet 5. At five times the recommended rate, only Jet 5 controlled all *Olpidium* resting spores.

Table 17: The effect of disinfectants on *Olpidium brassicae* resting spores

Disinfectant, rate (duration of treatment)	Number of bioassay plants infected with *Olpidium* / number tested
Jet 5, 40ml/l (5 mins)	1 / 30
Jet 5, 200ml/l (5 mins)	0 / 30
Difficil-S, 60ppm* (5 mins)	4 / 30
Difficil-S, 300ppm* (5 mins)	2 / 30
Aquaform, 0.25% (5 mins)	16 / 30
Aquaform, 1.25% (5 mins)	3 / 30
Spore filter positive control	30 / 30
Neutraliser only control	21 / 30
No spore untreated control	0 / 30

*Concentration of available chlorine dioxide.

In the absence of disinfectants, the neutraliser appeared to have an adverse effect on the *Olpidium* resting spores, this could possibly have slightly exaggerated the effect of the disinfectants.

At much higher concentrations than those used in the *Olpidium* experiment, neither the Jet 5, nor the Difficil-S were able to control all the clubroot spores (Table 18). However, at a concentration equivalent (on a cost basis) to the higher concentrations of Jet 5 and Difficil-S, Aquaform controlled the clubroot spores (Table 18).

Table 18: The effect of disinfectants on clubroot resting spores

Disinfectant, rate (duration of treatment)	**Number of Brassica plants with visible clubroot / number of plants inoculated in the bioassay**
Jet 5, 80ml/l (5 mins)	9 / 30
Jet 5, 400ml/l (5 mins)	1 / 30
Difficil-S, 180ppm* (5 mins)	26 / 30
Difficil-S, 900ppm* (5 mins)	9 / 30
Aquaform, 18% (5 mins)	19 / 30
Aquaform, 80% (5 mins)	0 / 30
Spore positive control	15 / 15
Spore neutraliser control	30 / 30
Jet 5 + neutraliser control**	0 / 10
Difficil-S + neutraliser control**	10 / 10
Aquaform + neutraliser control**	0 / 10
Healthy root preparation control	0 / 15

*Concentration of available chlorine dioxide.
**The neutraliser was added to the highest rate of each disinfectant, left for 5 minutes and then clubroot spores were added, prior to testing the viability of the spores in the bioassay.

In the absence of the disinfectants, the neutraliser had no noticeable effect on the clubroot resting spores. The results obtained from adding the neutraliser to the disinfectants prior to treating the clubroot resting spores suggested that at the higher concentrations, the neutraliser wasn't effective against Jet 5 and Aquaform. This would have resulted in the contact time for these two disinfectants, albeit at reduced rates, being greater than the 5 minutes. In practical situations, disinfectants might not be neutralised (though they might be diluted or washed off by rinsing), so the performance of Difficil-S relative to Jet 5 and Aquaform, might be better than seen in this experiment

Discussion

The results from the experiments carried out to validate a system for measuring the efficacy of different treatments in controlling *Olpidium* and clubroot resting spores resulted in the development of robust, reproducible and reliable bioassays that were successfully deployed in subsequent experiments. Both 600 and 1000 *Olpidium* spores dried on to discs or part 216 modules resulted in 100% infection of lettuce seedlings in the bioassays. Clubroot

spores didn't give reliable infection when dried on to surfaces so all experiments were carried out on spore suspensions and $5x10^7$ spores gave reproducible development of clubbed roots.

Provisional experiments looking at the amount of heat absorbed by dry polypropylene discs when microwave heated indicated that under dry conditions, discs would not be heated to particularly high temperatures, hence it was decided to add water and soil plus water to discs in subsequent experiments in order to increase the heating of spores on the discs. By doing this it was possible to dramatically improve the efficacy of microwaves in controlling the *Olpidium* resting spores (from very little control to 100% control). Although under wet conditions 100% control was achieved, there are reservations about the practicality of scaling up microwaving for treatment of whole trays (see conclusions). Microwaving is also a potentially complex treatment. The results from work in objective 5 would suggest that *Olpidium* is not particularly heat resistant and even hot water washing may be sufficient for inactivation. Unfortunately none of the microwave treatments appeared to have any effect on clubroot resting spores, indicating that they are more resilient than *Olpidium* resting spores.

The pulsed light treatments gave 100% control of the *Olpidium* resting spores on discs. If resting spores adhering to propagation trays are covered in soil in commercial situations, this may reduce the efficacy of this treatment. Also, it might be difficult to ensure exposure of all parts of propagation trays to pulsed light in commercial situations. Under ideal conditions, the process is clearly effective against *Olpidium*, but a great deal more development work would be required to determine whether it would be a feasible technology in a commercial setting. Pulsed light did have a slight effect on clubroot, but the lack of control made it clear that even if exposure could be increased, the expense and practical considerations would dictate that it would not provide a viable means of controlling clubroot. The results provided further evidence that clubroot spores are more robust and more difficult to control than *Olpidium* spores.

Ozone showed little potential to control *Olpidium* or clubroot resting spores even at levels in excess of HSE occupational exposure limits (note: these are occupational exposure limits for people and are not related to the effects on spores) after 30 minutes of treatment, suggesting it has little potential as a commercial treatment for controlling *Olpidium* or clubroot resting spores. Wetting discs prior to treatment failed to improve the performance of ozone.

The thermal death point of *Olpidium* resting spores appears to be 65^{O}C. The 'z' value indicates that it would be possible to control *Olpidium* at the lower temperature of 45ºC, but it would require 50 minutes. The thermal death point of clubroot spores was much higher (110ºC), confirming earlier results that clubroot spores are much more difficult to control than

Olpidium spores. The 'z' value for clubroot spores indicates that treating them at 65ºC for 50 minutes might give control.

The disinfectants that had not previously been tested for their efficacy in controlling *Olpidium* resting spores (Difficil-S and Aquaform) didn't give 100% control at the recommended, or five times the recommended rates, whereas at the recommended rate (4%) Jet 5 controlled most of the resting spores and at five times the recommended rate (20%), it controlled all the resting spores. However, it should be noted that Jet 5 was used at much higher concentrations than both Difficil-S and Aquaform and if the latter two had been tested at the same higher concentration of Jet 5 (20%), our results suggest they may have controlled all the resting spores. In our previous tests on Jet 5 (HDC Fact Sheet on Project No. FV/PC 62), it controlled all *Olpidium* resting spores at a concentration of 2%, however, this involved a much longer contact time (20 minutes) than used in current tests (5 minutes with disinfectant before neutralising solution was added). In tests described here, even at 40%, Jet 5 (5 minutes with disinfectant before neutralising solution was added) didn't quite control all clubroot resting spores. Our previous work (HDC Fact Sheet 42/97 on Project No. FV 178) showed that 5% was the minimum concentration needed to control resting spores (with a contact time of 20 minutes) in the absence of soil. In the presence of soil, 8% Jet 5 was needed to control clubroot resting spores again with a contact time of 20 minutes. On a cost basis, Aquaform appears to be a good alternative to Jet 5, being the only disinfectant that controlled clubroot with the short contact time of 5 minutes before the neutralising solution was added.

Conclusions

Robust, reliable and reproducible bioassays for assessing the efficacy of physical and chemical treatments in controlling *Olpidium brassicae* and clubroot resting spores have been developed and successfully deployed in the project. The results from all experiments showed clearly that clubroot resting spores are much more difficult to control than *Olpidium* resting spores.

Under certain (wet) conditions the use of microwave energy appeared to be capable of controlling *Olpidium* resting spores but not clubroot spores. The results indicated that the microwave energy itself had little effect on the *Olpidium* resting spores directly, as death only occurred when the spores were heated in a microwave energy absorbing media (e.g. wet soil). Hence controlling of the *Olpidium* resting spores using microwave energy was attributed to thermal effects rather than to any direct effect of the microwave field.

Problems are likely with scaling up the use of microwave energy. The trials were performed using a single (known) location in the microwave cavity and a short heating time. Due to the wide range in microwave energy distribution (microwave field pattern) inside a microwave oven and between different microwave ovens, the amount of heating is likely to

vary depending on the exact position inside the microwave cavity. This could result in some areas (of a tray for example) overheating and some areas not heating too well (possibly leaving viable *Olpidium* spores). However, the biggest problem is likely to be problems with microwave failure due to the overheating of components. Microwave ovens generate powerful electric and magnetic fields. The energy within these fields needs to be absorbed by materials with the necessary characteristics (water is a good absorber of microwave energy, plastic is generally a poor absorber). If there is insufficient material to absorb the energy the oven components will overheat. Using a domestic microwave oven empty (or with a very small load e.g. a dry plastic tray) can result in irreversible failure within a few minutes. Using an oven under these conditions can also constitute a fire risk.

Large scale processing using pulsed light is perfectly feasible and the technology is in commercial use for the decontamination of packaging components at a rate of 40,000 units per hour. Under the ideal conditions used in these experiments, pulsed light showed great potential as a treatment against *Olpidium* spores but little potential for clubroot decontamination. However, as has previously been discussed, the efficacy of a pulsed light treatment could be reduced in the presence of contaminants e.g. soil contamination. Considerable further development work would therefore be required to establish the feasibility of commercialising pulsed light for propagation tray decontamination including testing of heavily soiled trays.

Ozone appears to have little potential for controlling *Olpidium* or clubroot resting spores on propagation trays commercially.

The thermal death point of *Olpidium* resting spores appears to be 65^{O}C when exposed for 5 minutes and that of clubroot 110^{O}C. The potential of conventional wet heat (steam, achieving temperatures of >90^{O}C) in controlling *Olpidium* resting spores seems good with control being achieved after 2.5 minutes treatment. However, the potential for clubroot control looks less promising, unless extended exposure periods were possible.

It is possible for the disinfectants to control *Olpidium* and clubroot resting spores with the short contact time of 5 minutes, but very high concentrations are needed. The ability of Jet 5 to control *Olpidium* resting spores was confirmed. Difficil-S and Aquaform didn't give 100% control at the recommended, or five times the recommended rates. A cost comparison of Aquaform and Difficil-S with Jet 5 indicated that it would be possible to use them at much higher rates without increasing costs. Neither Jet 5, nor Difficil-S were able to control all clubroot resting spores even at very high concentrations with a contact time of 5 minutes before the neutralising solution was added, however, Aquaform did control clubroot spores after 5 minutes exposure before the neutralising solution was added, albeit at a high concentration.

Pulsed light, heat treatment and disinfectants appear to be the treatments most promising for *Olpidium* and heat treatment and disinfectants appear to be best for clubroot.

Microwaving under certain conditions controlled *Olpidium* resting spores, however scaling up for commercial use appears to be very expensive and challenging.

Having identified heat treatment and disinfectants as the best options for controlling *Olpidium* and clubroot contamination of propagation trays, further work on a range of temperatures and a range of concentrations of disinfectants for different exposure times is now required in order to provide growers with a variety of options for their propagation operations.

Technology transfer

John Walsh gave a presentation on progress of the project at the AGM of Plant Propagators Ltd., in Stamford, on 2 October, 2008, entitled, '*Brassica* and Lettuce Propagation: Identifying means of killing clubroot and *Olpidium* resting spores in trays to avoid infection of transplants'**.**

John Walsh gave a presentation on progress of the project at the AGM of Plant Propagators Ltd., in Huntingdon, on 7 October, 2009, entitled, '*Brassica* and Lettuce Propagation: Identifying means of killing clubroot and *Olpidium* resting spores in trays to avoid infection of transplants'**.**

John Walsh gave an informal presentation on progress of the project to the technical committee of Plant Propagators Ltd., at Warwick HRI on 19 November, 2009. Debra Smith, John Holah and Craig Leadley were also present and contributed to the meeting.

The project was featured in HDC News 'Field Vegetables Review 2010' on page 13.

References

Walsh, J.A. & Bambridge, J.M. (1994). Disinfection for the control of lettuce big-vein. HDC Fact Sheet on Project No. FV/PC 62. 4pp.

Walsh, J.A. & Bambridge, J.M. (1997). Disinfection for clubroot. HDC Fact Sheet 42/97 on Project No. FV 178. 2pp

