Project title:	Chicory: control of root rot by *Phytophthora* species
Project number:	FV 240
Project leader:	Dr K.R. Green, ADAS
Report:	Final report
Other reports:	-
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Project co-ordinator:	Mr P Cornish
Date commenced:	April 2002
Date completion due:	March 2006
Key words:	Chicory, root rot, forcing, *Phytophthora* spp, Amistar, azoxystrobin, Proplant, propamocarb hydrochloride, Novello, Bacillus laterosporus, Trianum, Trichoderma spp, Aliette, fosetyl-aluminium.

Authentication

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

Dr K.R. Green
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Signature……. Date…….

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Horticulture Research & Consultancy Manager
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Signature…….. Date……..

The results and conclusions in this report are based on a series of experiments conducted over one year. 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.

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GROWER SUMMARY

1.1 Headline

- A new species of *Phytophthora* was found to cause root rot of chicory during forcing. The pathogen is closely related, but distinct from *P. brassicae*. When not controlled, a secondary infection by *Geotrichum candidum* is frequently causing 'sour rot' symptoms.
- Azoxystrobin, fosetyl-aluminium and propamocarb hydrochloride gave almost complete control of phytophthora root rot on chicory in an artificially inoculated experiment.
- Data from this experiment supported a successful SOLA application for the use of Amistar on chicory.
- None of the biological control treatments reduced the severity of root rotting.

1.2 Background and expected deliverables

Root rot of chicory caused by *Phytophthora* spp. represents a major constraint to hydroponic chicory production in the UK. Despite attempts to manage the disease using both cultural and chemical measures at different stages of production, yield losses continue to exceed those experienced by growers in major chicory-producing countries. Although *P. cryptogea* is widely reported to be the causal organism of chicory root rot, a new species of *Phytophthora* was repeatedly isolated from rotted roots in 2000. The overall objectives of this project were to:

- Confirm the identity of the pathogen(s) causing chicory root rot in the UK.
- Develop best-practice recommendations for management of the pathogen in the field.
- Develop and evaluate practices to control *Phytophthora* during forcing and to significantly reduce the severity of root-rot.

Specific objectives were to:

- Confirm pathogen identity and determine the optimum temperature and pH range required for pathogen development.
- Develop a simple baiting and bioassay system to facilitate pathogen isolation and quantification.
- Conduct trials over two growing seasons to determine the effect of cultivation practices on root health, yield and quality.
- Determine the effect of fungicides applied at different times during storage and forcing on the incidence and severity of *Phytophthora* root rot.

- Evaluate selected alternative methods for the control of *Phytophthora* root rot during forcing.

1.3 Summary of the project and main conclusions

1.3.1 Pathogen biology

- A fungus consistently isolated from chicory roots with typical root rot symptoms was identified by molecular sequencing as a new species of *Phytophthora*. The same species was isolated from roots originating from two different farm sites. It is closely related to, but distinct from *P. brassicae*, which to date has only been isolated from brassicas.

- Artificial inoculation of healthy chicory roots with the *Phytophthora* isolate, lead to the development of typical root rot symptoms, confirming that this species of *Phytophthora* was a causal agent of root rot on chicory.

- *Phytophthora* sp. was identified as the primary cause of root rotting and was consistently isolated from the advancing edge of lesions in root tissue. However, *Geotrichum candidum* was also frequently isolated from severely rotted tissue in the same roots, as a secondary pathogen, causing 'sour rot' symptoms typical of this micro-organism.

- Optimum conditions for growth of *Phytophthora* sp. isolated from chicory roots coincided with conditions routinely used for chicon forcing. Optimum mycelial growth occurred at pH 6.0, with growth reduced at pH 5.5 and pH 7.5. The optimum temperature for mycelial growth was 15^{o}C with reduced growth rate above 19^{o}C. The optimum temperature for sporangial production was 19^{o}C.

- Laboratory studies showed that *Phytophthora* sp. from chicory roots was capable of sporangial production and zoospore release under a wide range of conditions. This demonstrated that under re-circulating forcing conditions, there is potential for tray-to-tray transmission of the pathogen by motile zoospores, in addition to localised spread of symptoms between neighbouring roots within a single tray either by zoospores or mycelial growth.

- It is probable that the new species of *Phytophthora* is not highly pathogenic to chicory, i.e. rarely causes root infection on healthy roots under field conditions. However, under favourable environmental conditions (e.g. during forcing) and particularly when root

health is poor (due to e.g. physical damage, incorrect nutrients or maturity), the pathogen is opportunistic and can cause infection and subsequent root rotting.

- *Phytophthora* is a soil-borne fungus that can survive in the soil for many years as thick-walled resting structures (oospores). During forcing, oospores in soil attached to chicory roots germinate to produce structures (sporangia), from which motile zoospores are released, capable of root infection. Once root infection has occurred, secondary disease spread from a single root can occur either between forcing trays (via motile zoospores), or between adjacent roots in the same tray (by mycelium).

1.3.2 Control

- A trial to determine the effect of root-end removal prior to forcing on phytophthora development was carried out at Jack Buck growers. The trial was inconclusive because of low infection levels in the root batch used. However, the grower subsequently modified standard practice from root-end removal to leaving roots in tact, in order to minimise risk of root infection via the cut root surface.

- A hydroponic 'minisystem' was devised at ADAS Arthur Rickwood to enable small-scale evaluation of treatments against chicory root rot to be carried out. Three pilot trials were done to optimise this system.

- The following fungicides applied to roots pre-forcing were evaluated for control of chicory phytophthora in an artificially inoculated minisystem trial: Aliette at 8 g/m^2 (industry standard), Amistar at 1 ml/m^2 (azoxystrobin) and Proplant at 10 ml/m^2 (propamocarb hydrochloride). All of the fungicides increased chicon yield in comparison with an untreated control, and eliminated root rotting compared with 100% rot incidence in the untreated control. Data from this experiment supported a successful HDC application for SOLA use of Amistar on chicory.

- An experiment was carried out to determine the effects of biological products Novello (*Bacillus laterosporus*) and Trianum (*Trichoderma* sp.) on chicory root rot development. Neither treatments reduced the severity of root rotting compared to the inoculated control treatment.

- Research in Belgium demonstrated that rhamnolipids could be effective in controlling the spread of root rot caused by *Phytophthora cryptogea* on chicory during hydroponic

forcing (Jonghe *et al*., 2005). Rhamnolipids are extracellular metabolites of the bacteria *Pseudomonas aeruginosa* which act as surfactants and can disintegrate zoospores (motile spores of *Phytophthora* species). It was found that a rhamnolipids nutrient solution (25 ug/ml) was sufficient to obtain good control of an artificial infection with a zoospore suspension of *P. cryptogea* and also under semi-commercial conditions. However, rhamnolipids did not control mycelial growth of *P. cryptogea*. This method could provide an alternative to fungicides for control of disease spread during forcing by *Phytophthora* zoospores but would not be effective for preventing short-distance spread by mycelial growth between adjacent roots in a single tray.

- A simple soil baiting system using chicory seeds/seedlings was devised that could be used to test field soil for the presence of *Phytophthora* sp. pathogenic to chicory. This method could be used as a component of a risk management system to identify fields or areas of fields from which root batches could be high risk for rot development during forcing.

1.4 Financial benefits

Phytophthora root rot represents the major constraint to chicory production in the UK, with annual losses estimated at £100,000. Proper pathogen identification together with development and evaluation of control measures, will enable the industry to make an informed decision as to what production factors can be modified to reduce production losses to an economically acceptable level.

1.5 Action points for growers

Control of phytophthora root rot on chicory is unlikely to be achieved using a single control method, however using healthy roots of excellent quality during forcing is key to disease management.

<u>Field production</u>

Based on their observations during field production and forcing, growers can develop a simple risk assessment to enable root batches that may be particularly susceptible to root rot, to be identified. Factors during field production may include:

- Susceptibility of different chicory varieties to phytophthora root rot
- Crop nutrition

- Soil type and drainage
- Cropping history – does a particular field have a history of phytophthora on other crops, indicating that soil conditions are favourable for phytophthora development?
- Conditions at the time of harvest
- Use of a simple soil baiting system (with chicory seedlings) could be used to indicate the presence of *Phytophthora* sp. pathogenic to chicory.

The new species of *Phytophthora* found to cause chicory root rot during forcing has not, to date, been isolated from other vegetable hosts so it is unlikely that specific rotations can increase disease risk. Due to the ability of *Phytophthora* oospores to survive in field soil for several years, crop rotation may reduce but not eliminate disease risk.

Cold storage prior to forcing

- Ensure that storage boxes are thoroughly cleaned before use.
- During the cold storage period, problems such as temporary failure of the refrigeration system can result in roots being stored under less than optimum conditions prior to forcing. Such root batches should be identified as higher risk for development of root rot.

Forcing

- Field soil represents the major source of inoculum for phytophthora root rot on chicory. As far as practically and economically possible, reduce disease risk by removing soil on chicory roots entering the hydroponic circulating system.
- Ensure that mechanical/physical damage to roots used for forcing is minimised. If root ends were removed at the time of field harvest, cut surfaces should be ‘cured’ prior to forcing.
- Ensure that trays are thoroughly washed and disinfected between forcing, and that re-circulating systems are flushed through, particularly if there has been an outbreak of phytopthora root rot.
- Maintain optimum nutrition during chicon forcing, according to agronomist recommendations.
- Optimise forcing conditions according to whether fresh, mid-season or late season roots are being used.
- Fungicide options for phytophthora control during forcing include the use of Aliette (fosetyl-Aluminium) or Amistar (azoxystrobin) immediately prior to forcing, following SOLA conditions of use.

- Strobilurins such as Amistar should be used with care to ensure that fungal resistance to the fungicide does not develop. In order to reduce the risk of resistance development, azoxystrobin should not be used exclusively during forcing. Due to its reported ability to reduce sporangial production and inhibit zoospore motility, Amistar could be reserved for use when root batches are particularly high risk for development of phytophthora root rot, with Aliette (for which there is lower risk of resistance development) being used more on a routine basis.

1.6 Reference

De Jonghe, K., De Dobbelaere, I. Sarrazyn, R. & Höfte, M. 2005. Control of *Phytophthora cryptogea* in the hydroponic forcing of witloof chicory with the rhamnolipid-based biosurfactant formulation PRO1. *Plant Pathology* **54**: 219-226.

2 SCIENCE SECTION

2.1 Introduction

Phytophthora root rot of chicory (*Cichorium intybus*) has become a major constraint to hydroponic chicon production in the UK. Current yield losses are estimated at 20%, compared with 5-10% yield losses reported by growers in major chicory-producing countries such as France, Italy, The Netherlands and Belgium. In addition to yield reduction (stunted chicon development), phytophthora root rot also affects chicon quality, because leaves from infected roots develop tip burn, which is unacceptable to retailers. In Holland, Belgium, France and USA, chicory root rot is caused by *Phytophthora cryptogea.* Prior to this project the causal agent of chicory root rot in the UK had not been determined.

Symptoms of root rot are usually first observed 12 days after the start of forcing. Since *Phytophthora* sp. has not been detected in water entering the hydroponic system (B. Read, pers. comm.), it is probable that soil is the main source of inoculum for the pathogen. This hypothesis was supported by observations from Jack Buck Growers that the incidence of root rot during forcing was greatly reduced in washed roots compared with unwashed roots with soil attached (normal growers' practice). Once resting spores have germinated, infection of chicory roots can occur either through wounds or via root hairs. This process could commence either in the field to give a symptomless root infection, during cold storage or during forcing. Irrespective of the time at which root infection occurs, once forcing commences, the hydroponic system provides an ideal environment for the rapid development and spread of *Phytophthora* sp., due to the continual presence of water and temperatures of approximately 20^{o}C. Low oxygen levels in water at the corners of forcing trays (older design) where water circulation is poor, appear to further promote the development of phytophthora.

In other European countries, phytophthora root rot is maintained at economically acceptable levels, largely through the use of fungicides during forcing. At the start of this project, however, Aliette (fosetyl-aluminium) was the only fungicide approved (off-label) specifically for control of phytophthora rot during forcing in the UK. Cultural practices can also form an important component of disease management and there is increasing interest in the use of alternatives to conventional fungicides for control of chicory root rot.

This report describes studies undertaken to confirm the identity of the pathogen causing chicory root rot in the UK and to develop recommendations for integrated management of the disease.

2.2 Review of potential management strategies for chicory root rot

In the UK, chicory growers have adopted techniques and disease management practices comparable to those used in other European countries. However, not all practices are possible or relevant for UK growers, due largely to differences in soil type and fungicide approvals.

2.2.1 Field cultivation practices

Extensive research has been done on the field production of chicory as it is recognised that successful forcing depends on the quality of the root. For example, a detailed growers manual produced by PPO Lelystad (Kruistum & Buishand, 1986) provides production recommendations based on research conducted throughout The Netherlands, Belgium and France. Root rot severity has been observed to vary with variety, the field used for root production and root maturity. Growers and agronomists contacted in the major-producing countries stressed that the most important measure for preventing root rot development during forcing was field selection, taking account of soil type, rotation and field drainage. UK growers are aware of these cultivation requirements and their importance for management of phytophthora rot. They practice a wide range of crop rotations, although until the causal pathogen for chicory root rot is confirmed the suitability of these rotations cannot be evaluated. They have experimented with the use of ridging as an alternative to beds to improve soil drainage but this was not wholly effective. The use of potassium phosphite fertiliser as a crop health promoter and to stimulate plant growth has been shown to incidentally reduce the severity of *Phytophthora capsici* on peppers and other *Phytophthora* species (Smillie *et al*., 1989; Förster *et al*., 1998). Use of the fertiliser in chicory production did not seem to influence the subsequent health of roots during forcing (B. Read, pers. comm.).

2.2.2 Hydroponic forcing

The hydroponic system used for chicon forcing in the UK is largely comparable to the techniques used in the major chicory-producing countries. For example, investments in the UK include a new design of forcing trays (as used by producers in France), that minimise conditions conducive for development of phytophthora rot by facilitating more thorough tray washing and improved water circulation. UK chicory production is done on heavier soils than in The Netherlands, so more vigorous root cleaning equipment has been installed to reduce the quantity of soil (primary inoculum source for *Phytophthora* sp.) entering the hydroponic circulation system.

The major difference in production method lies in the routine use of a range of fungicide products for the control of phytophthora root rot by the major European producers. While some research has been done on alternative management strategies, commercial practice is still highly dependent on fungicides (G. van Kruistum, PPO Lelystad, pers. comm.). In France, use of the fungicide active ingredients mancozeb, azoxystrobin, and propamocarb hydrochloride are permitted for phytophthora rot control during forcing as well as fosetyl-aluminium. In The Netherlands, dimethomorph (which is a component of the fungicide Invader used for potato blight control in the UK) is approved for use during forcing and gives effective disease control. *In vitro* studies indicate that dimethomorph is most effective against the growth, sporulation and zoospore cyst germination of *Phytophthora* species when compared with azoxystrobin, fluazinam, fosetyl-aluminium and metalaxyl (Matheron, 2000). When comparing, azoxystrobin and fosetyl-aluminium (routinely used for control of chicory root rot) it was found that azoxystrobin was more effective for inhibition of zoospore motility while fosetyl-aluminium gave better inhibition of mycelial growth (Matheron, 2000). Research has also been done on the use of mefenoxam (metalaxyl-M) for control of phytophthora rot on chicory (Benigni & Bompeix, 2006). The product was found to be effective. However, it was found that one isolate of *P. cryptogea* out of six tested was resistant to mefenoxam *in vitro*, highlighting the need for resistance management if this active ingredient were to be used commercially.

A range of alternative management practices have been considered both for phytophthora control during chicory forcing but also for control of oomycete fungi in other nursery re-circulating systems:

- **Nutrition:** Producers recognise the importance of correct nutrition within the chicory forcing system, particularly to ensure the development of healthy and vigorous root hair systems. Observations suggest that incorrect nutrition during forcing can result in a batch of roots being more susceptible to phytophthora rot.
- **Slow sand filters:** Although effective for disease management in certain nursery hydroponic situations (HDC, 1998, 2000), the extensive filter surface area and water reservoir required for treating water circulating at a continually high flow rate within the chicory forcing system, would be impractical (T. Pettitt, pers. comm).
- **Ozone**: Ozone can be used as a water disinfectant within chicory forcing systems. Disadvantages of ozone use include possible deleterious effects on fungicides, added nutrients and beneficial micro-organisms.
- **Surfactants**: In Belgium, laboratory studies showed promising results using a range of surfactants versus *P. cryptogea* from chicory roots. Certain surfactants such as Agral 90 inhibited zoospore release (Demeulenaere & Höfte, 2000). Further to this work, Jonghe *et al.*

(2005) investigated the use of rhamnolipids, which are extracellular metabolites of *Pseudomonas aeruginosa* with surfactant properties, against phytophthora rot in chicory. Such surfactants can exhibit a lytic activity against zoospores. Experiments using a mini-hydroponic system and under semi-commercial conditions showed that rhamnolipids have good potential to limit the spread of *P. cryptogea* in a hydroponic chicory forcing system, and can be used as a preventative measure against root rot.

- **Ultraviolet light (UV):** Good results have been obtained using UV versus *Phytophthora cryptogea* and *Fusarium oxysporum* f.sp. *lycopersici* in tomatoes (HDC, 1996). The method is, however, not successful in situations where water is cloudy, turbulent or muddy (HDC, 1992), as occurs routinely during chicory forcing.
- **Micro-filtration**: Micro-filtration has been used effectively in cucumber production to prevent the spread of *Pythium aphanidermatum* and to reduce yield loss (HDC, 1996). A variety of equipment is available depending on the requirements of the system. An advantage of micro-filtration compared with 'active' disinfection methods (such as ozone, chemicals and UV), is that a proportion of potentially beneficial micro-organisms that may help to suppress pathogen activity, remain in the system (HDC, 1996). However, as with UV treatment, the high soil content in the water could limit the practicality of this technique.
- **Sonication**: Laboratory results from Canada suggest that with sonication, a selective elimination of *Pythium* zoospores is possible while potentially beneficial bacteria such as *Pseudomonas* bacteria remain in tact. Work is ongoing to test the incorporation of an in-line continuous sonicator within recirculating greenhouse systems (Tu & Zhang, 2000).
- **Chemical disinfectants:** Substances such as Jet 5, hydrogen peroxide and chlorine dioxide have been used with varying success for disinfection in commercial horticulture (HDC, 1992).

2.3 Identification of the causal pathogen

2.3.1 Introduction

In Holland, Belgium, France and USA, phytophthora root rot on chicory is most commonly caused by *P. cryptogea* (Forlot *et al*., 1966; Stanghellini & Kronland, 1982; B.M. Schober, pers. comm.). In 1990, *P. cryptogea* was also implicated but not confirmed as the cause of root rot on chicory in the UK (T. O'Neill, pers. comm.). Prior to project commencement, however, the species to be isolated from rotted chicory roots in the UK (originating from Jack Buck Growers) were *P. syringae*, *P. megasperma* and *P. parasitica* but not *P. cryptogea* (C. Brewster, J. Scrace, pers. comm.). In addition, one species of *Phytophthora* that was consistently isolated from rotted roots from Jack Buck Growers was diagnosed by PCR-testing as a previously unidentified species of *Phytophthora* (D. Cooke, J. Scrace, pers. comm.). Further work was carried out to identify the causal pathogen of chicory root rot in the UK.

2.3.2 Methods

2.3.2.1 Symptom types and fungal morphology

Batches of chicory roots with rot symptoms were sent from Jack Buck Growers to ADAS Arthur Rickwood. Rot symptoms from each batch were photographed and described. Root tissue sections (2 mm^3) cut from the leading edge of typical symptoms were surfaced sterilised (30 sec in 1% sodium hypochlorite, 1 min rinse in sterile distilled water (SDW)) and plated onto either potato dextrose agar amended with streptomycin (PDA+S) or a selective agar medium (P_{10}ARP; Appendix 1). *Phytophthora* sp. was very slow growing in culture and easily became overgrown by other organisms, hence the need for a selective medium. Tissue from the leading edge of root lesions was also examined microscopically following incubation in float culture using either SDW, Petri's solution (Appendix 1) or soil water.

The morphology of isolates of *Phytophthora* sp. obtained from chicory roots, on agar and in float culture was described.

2.3.2.2 Molecular sequencing

Pieces of tissue with typical symptoms of phytophthora rot obtained from chicory roots originating from two different farms (codes JB and PCT) were placed in 90% ethanol and sent to D. Cooke, Scottish Crops Research Institute for genetic sequencing by PCR. Isolates of *Phytophthora* sp. from these root batches were maintained at ADAS Arthur Rickwood. The

genetic sequences were compared with an isolate of *Phytophthora* sp. obtained from chicory roots by J. Scrace (ADAS) prior to the project (isolate code: JS).

2.3.2.3 Kochs postulates

Three studies were done to determine the pathogenicity of isolates of *Phytophthora* sp. obtained from chicory.

Experiment 1

Isolates of *Phytophthora* sp. obtained from chicory roots originating from two different farms were used (isolate codes: JB and PCT). Soil was scrubbed from the surface of visibly healthy chicory roots and then roots wiped with 90% ethanol. Roots were inoculated by two different methods. Firstly, a sterile cork borer was used to remove 5 mm diameter plugs of root tissue (two per root). A disc of mycelium cut from actively growing, pure cultures of *Phytophthora* sp (on PDA+S agar) was placed within each hole (3 roots per isolate). The plug of root tissue was replaced and sealed with petroleum jelly. Three uninoculated control roots were also set up, using discs of PDA+S agar only. The roots were placed on damp paper towel in plastic boxes with lids. Secondly, the bases of roots were removed with a clean knife to expose root tissue. Roots were placed in a 1 L beaker of distilled water (three roots per beaker) containing two 5 mm discs of mycelium cut from actively growing, pure cultures of *Phytophthora* sp (on PDA+S agar). Discs of PDA+S agar only were used in the uninoculated control treatment. Boxes and beakers containing roots were incubated in the dark at 18-21°C. Symptom development in the roots was assessed after 22 days. Tissue pieces (2 mm^3) cut from the edge of developing lesions were plated onto PDA+S and P_{10}ARP agar and incubated, to confirm the causal organism.

Experiment 2

Soil was scrubbed from the surface of visibly healthy chicory roots and then roots wiped with 90% ethanol. A sterile cork borer was used to remove 5 mm diameter plugs of root tissue (two per root). A disc of mycelium cut from actively growing, pure cultures of *Phytophthora* sp (on P_{10}ARP agar) was placed within each hole. The plug of root tissue was replaced and sealed with petroleum jelly. Uninoculated control roots were also set up, using discs of P_{10}ARP agar only. Roots were wrapped in wet paper towel, placed in a plastic box with a lid (first wiped with ethanol) and incubated at 21°C. The roots were checked for rot development after 18 days. Tissue pieces (2 mm^3) cut from the edge of developing lesions were plated onto PDA+S and P_{10}ARP agar and incubated, to confirm the causal organism. Tissue pieces were also floated in sterile distilled water.

The isolates used in the experiment were as follows (two roots per isolate):

Code	**Source of *Phytophthora* sp.**
-	Uninoculated control
JS	*Ex* chicory root (Isolated by J. Scrace)
CH112	*Ex* Strawberry*
PCT	*Ex* chicory root (P.C. Tinsley farm)

*similar sequence to JB and PCT isolates (see Figure x)

Experiment 3

Visibly healthy chicory roots were scrubbed clean in tap water and wiped with 90% ethanol. Roots were placed individually in wide-necked glass flasks (250 ml volume) containing distilled water. The roots were inoculated using three different methods with *Phytophthora* sp. *ex* chicory (isolate code: PCT): i) roots inoculated with mycelial plugs (three per root) as described for Experiment 1, ii) root bases cut and mycelial plugs placed in the distilled water, and iii) root bases cut and mycelial plugs pressed on to the freshly cut root bases. The flasks containing roots were placed on a rotary shaker and incubated at ambient temperature (20^{o}C) and light. Equivalent uninoculated control roots were also set up. After one week, the roots were examined for rot development around the inoculation points. Tissue pieces (2 mm^3) cut from the edge of developing lesions were plated onto PDA+S and P_{10}ARP agar and incubated, to confirm the causal organism.

2.3.3 Results and discussion

A range of rot symptoms was observed in chicory roots. Rots were most commonly associated with the lower 4 cm of the root, corresponding with the section that had been immersed in water during forcing. In particular, rots developed from the bases of roots (where root ends had been removed according to standard grower practice) or from mechanical wound sites. *Phytophthora* sp. was most consistently isolated from the advancing edges of rots that had a light grey watersoaked appearance and that were firm in texture (Fig. 1A). Mixed infections were common, with affected root tissue often appearing light grey and water-soaked at the very leading edge of a lesion, with darker, soft watery rots behind the leading edge (Fig. 1B). *Geotrichum candidum* was consistently isolated from dark brown, watery rots and a film of white spores could sometimes be seen on the surface of severely affected roots. This type of symptom was sometimes accompanied by a sour odour. *G. candidum* is a common soil inhabitant and causes symptoms known as sour rot on crops such as carrot (during storage) and tomato, thriving under warm (greater than 20^{o}C), moist

conditions. It is probable that primary infection due to *Phytophthora* sp. and/or mechanical wounds on roots, predisposes chicory roots to secondary infection by *G. cladium*.

A B

Figure 1. Pale grey watersoaked lesion due to *Phytophthora* sp. (A) and mixed infection due to *Phytophthora* sp. (at leading edge) and *Geotrichum candidum* (dark brown rot) (B)

2.3.3.1 Fungal morphology

Phytophthora isolates PCT and JB had similar morphological characteristics in agar culture. Hyphal swellings were present and abundant. Chlamydospores were present and abundant with diameter ranging from 20-45 µm (mean 33 µm). Mycelium was correloid. No oogonia, antheridia or oospores were observed in agar culture.

For both isolates, sporangia were produced in float culture and occasionally in agar culture. Sporangia were mainly ovoid and non-papillate. There was occasional extended proliferation of sporangia. There was no evidence of caducity (detachment of sporangia from sporangiophores). Sporangial dimensions varied slightly between isolates. Sporangia from the JB isolate had mean dimensions of 40 x 31 µm with a length:breadth ratio of 1.4, compared with 55 x 42 µm for the PCT isolate, with a length:breadth ratio of 1.3.

A B C

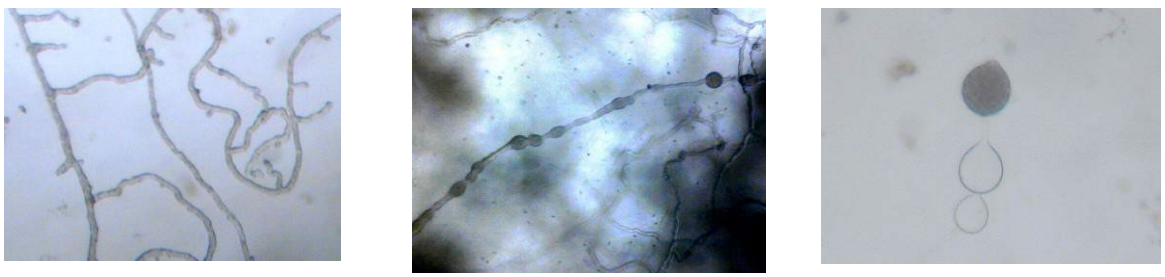


Figure x. Characteristics of *Phytophthora* sp. *ex* chicory: correloid mycelium (A); hyphal swellings and chlamydospores (B); proliferating sporangia (C)

2.3.3.2 Molecular sequencing

DNA sequencing showed that the isolate JS (obtained from chicory prior to the project) was closely related to, but distinct (10-11 base pairs different) from *Phytophthora porri* and *P. primulae*. Isolates of *Phytophthora* sp. obtained from chicory roots (sourced from two different farms) during this project (codes JB and PCT) were identical to each other but distinct from the JS isolate. They were more closely related to the newly described species *P. brassicae*, to date only isolated from brassicas, being distinct by only a few base pairs from this species (Figure x). Unexpectedly, isolates PCT and JB also closely matched an isolate of *Phytophthora* sp. causing root rot on strawberry outside the UK.

2.3.3.3 Kochs postulates

Experiment 1

There was no symptom development in the uninoculated control roots incubated in boxes or beakers. Rot development did not occur consistently on the inoculated roots, however typical symptom development occurred in association with at least one inoculation point, for each inoculation method / isolate combination. *Phytophthora* sp. was isolated from these lesions on P_{10}ARP.

Experiment 2

There was no symptom development in the uninoculated control roots or the roots inoculated with the strawberry isolate CH112. Inoculation with isolate JS led to slight rotting around the inoculation points. Extensive rotting typical of phytophthora developed in association with the inoculation points following inoculation with isolate PCT (Figure x). Fungi developing from tissue pieces on agar could not be identified due to contamination. Sporangia typical of *Phytophthora* sp. developed in float culture from tissue pieces cut from roots inoculated with isolate PCT but not from other roots.

Experiment 3

Typical symptoms of phytophthora rot developed in all of the roots inoculated with *Phytophthora* sp. (isolate code: PCT) in association with the inoculation points, either plugs cut in the root, or cut root bases. For one root, mycelium and sporangia typical of *Phytophthora* sp. were visible by microscopic examination, emerging from one of the root inoculation points. *Phytophthora* sp. was isolated consistently from tissue pieces cut from the leading edge of root rots and plated on to P_{10}ARP. There was no symptom development on the uninoculated control roots.

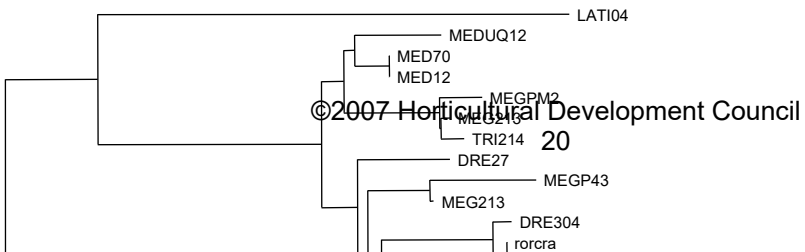
Visibly healthy scrubbed roots were subsequently placed on the rotary shaker in flasks containing either fluid that had contained the inoculated roots or fluid that had contained uninoculated roots. After 8 days, rot development had occurred in roots placed in the inoculated fluid and not in the uninoculated flasks.

The experiments confirmed that two isolates of *Phytophthora* sp. (codes JB and PCT) obtained from chicory roots grown at two different farms, were pathogenic to chicory roots. Another isolate of *Phytophthora* sp. obtained from chicory roots (isolate JS) was slightly pathogenic. An isolate of *Phytophthora* sp. *ex* strawberry (genetically similar to isolates JB and PCT), was not pathogenic.

Fig x. Symptoms of rot developing in a chicory root after inoculation with *Phytophthora* sp. *ex* chicory

To summarise, two distinct new species of *Phytophthora* were isolated from chicory. Isolate JS was closest taxonomically to *P. porri*, previously reported from Allium and a few other hosts (carrot, carnation, lily, and tulip). This species was extremely slow growing in culture and was slightly pathogenic on chicory roots. The other species of *Phytophthora* which was more consistently isolated (from roots originating from two different farms), is closely related to a species found only on brassicas (*P. brassicae*). This species was found to be highly pathogenic on chicory roots. It is likely that these newly discovered species are opportunistic pathogens of a broad host range. Despite their close taxonomic relationship to *P. porri* and *P. brassicae* respectively, it can not be assumed that these species will have the same biological characteristics as the related species in terms of host range or temperature/pH optima.

Figure x. Dendrogram showing relationship of *Phytophthora* isolates from chicory to other *Phytophthora* species



2.4 Effect of pH and temperature on the growth of *Phytophthora* sp. isolated from chicory roots

2.4.1 Methods

2.4.1.1 Effect of pH on mycelial growth

Batches of potato dextrose agar (PDA) were amended to pH values 5.5, 6.0, 6.5, 7.0 and 7.5 (representing the pH range used during chicon forcing) using sodium hydroxide, and poured into 9 cm-diameter Petri dishes. Discs (5 mm diameter) were cut from just within the leading edge of an actively growing isolate of *Phytophthora* sp. *ex* chicory (isolate code: PCT). For each pH treatment, one agar disc was placed (mycelial side down) on the centre of each of 12 PDA plates (four replicate blocks of three plates). The plates were incubated at 16°C in the dark. Two perpendicular lines were drawn on the base of each plate to enable colony diameters to be measured (two per plate, to give a mean value). Colony diameters were measured every 7 days for 4 weeks.

2.4.1.2 Effect of temperature on mycelial growth

Discs (5 mm diameter) were cut from just within the leading edge of an actively growing isolate of *Phytophthora* sp. *ex* chicory (isolate code: PCT). For each of five temperature treatments, agar discs were placed mycelial side down on the centre of each of ten PDA plates. The plates were incubated at the test temperatures (12, 15, 18, 21, 24°C, representing the temperature range used during chicon forcing in different seasons) in the dark. Colony diameters were measured twice weekly for 2 weeks.

2.4.1.3 Effect of temperature on sporangial production

For each of five temperature treatments, eight discs (5 mm diameter) were cut from just within the leading edge of an actively growing isolate of *Phytophthora* sp. *ex* chicory (isolate code: PCT). The discs were immersed in Petri's solution (Appendix 1) and incubated in the dark at the test temperatures (12, 15, 18, 21 and 24°C) for 72 h. For each pH treatment, a 1 mm^3 section from each of four discs was mounted on a microscope slide and the number of sporangia visible in four random fields was counted (Falloon & Grogan, 1991).

Negligible sporangial development had occurred for any treatments when this experiment was assessed. The experiment was therefore repeated, instead immersing mycelial discs in recirculation fluid obtained from the uninoculated treatment of one of the minisystem experiments (Section 2.6), instead of Petri's solution. Data were analysed by analysis of variance (ANOVA) in Genstat.

2.4.2 Results and discussion

Phytophthora sp. *ex* chicory grew *in vitro* at all the pH values tested, although there was a significant effect of pH on colony diameter (Table x) and mycelial growth rate over 21 days (P<0.05) (Figure x). Growth was slower at pH 5.5 and 7.5 compared with optimum growth at pH 6.0. During chicory forcing, pH is adjusted to 6.8 on day 1 and then reduced to pH 6.2 from day 3 onwards with limited scope for modifying these conditions (B. Read, pers. comm). These results indicate that growth of *Phytophthora* sp. would not be limited under these conditions.

Phytophthora sp. *ex* chicory grew *in vitro* at all of the temperatures tested. However, there was a significant effect (P<0.001) of temperature on the growth rate of *Phytophthora* sp. from chicory on PDA (Figure x), with an optimum at 15°C and a reduced growth rate at 22°C and above. Significant results (P<0.001) were obtained for temperature effects on sporangial production, with highest numbers of sporangia developing at 19°C and reduced sporangial production at temperatures of 22°C or above (Figure x). The temperature range used for chicon forcing varies with season as follows (B. Read, pers. comm.): early forcing (when roots are newly harvested, from October to January) at 22-24°C; mid-season forcing (from February to May) at 18-20°C; and late season forcing (from June to September) at 12-16°C. It has been observed that batches of old roots that have been kept in cold storage for several months and that are used for late forcing can be particularly susceptible to outbreaks of phytophthora root rot. The results from this experiment show that the temperatures used for forcing during this period are also particularly conducive for pathogen development.

Preliminary laboratory studies (data not presented) showed that *Phytophthora* sp. from chicory roots was capable of sporangial production in different liquid media, including sterile distilled water, distilled water and soil water (8 g soil in 800 ml distilled water, left to settle, then filtered). In particular, abundant sporangia formed when mycelial discs were placed in the re-circulated fluid collected from chicory forcing trays. Zoospore release was also observed under these conditions. This demonstrated that under re-circulating forcing conditions, there is potential for tray-to-tray transmission of the pathogen by motile zoospores, in addition to localised spread of symptoms between neighbouring roots within a single tray.

Table x. Effect of pH on colony diameter of *Phytophthora* sp. *ex* chicory

pH	Colony diameter (mm)

	7 days	14 days	21 days
5.5	25.0	46.3	72.3
6.0	29.4	56.8	79.6
6.5	26.3	52.6	76.9
7.0	28.4	55.1	77.5
7.5	26.1	51.5	75.3
F. probability	0.003	0.004	0.008
S.e.d. (12 d.f.)	0.937	2.149	1.589

Figure x. Effect of pH on the growth rate of *Phytophthora* sp. *ex* chicory

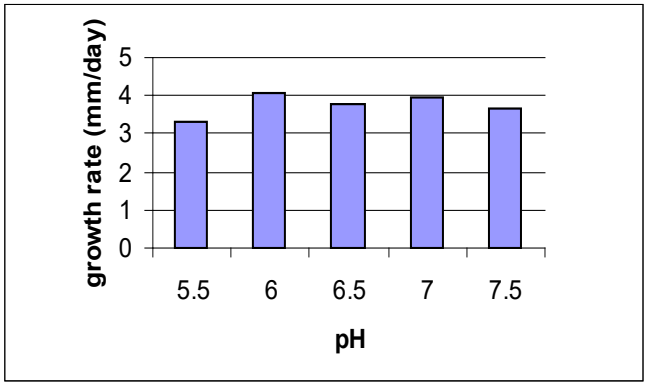


Figure x. Effect of temperature on the mycelial growth rate of *Phytophthora* sp. *ex* chicory

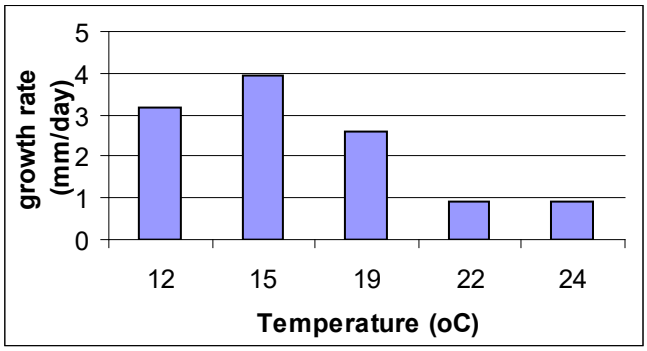
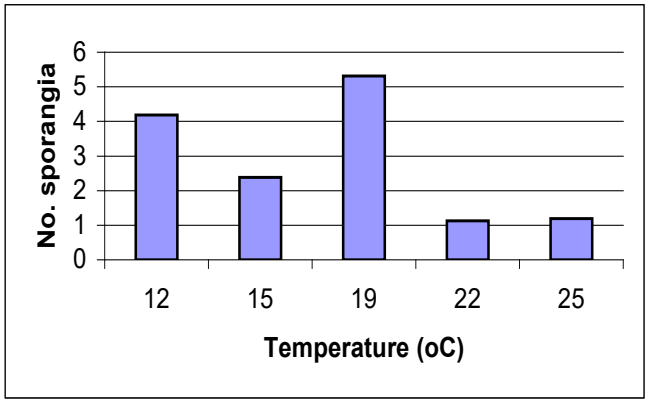


Figure x. Effect of temperature on sporangial production of *Phytophthora* sp. *ex* chicory



2.5 Effect of chicory root-end removal on the incidence and severity of phytophthora root rot and chicon yield and quality

2.5.1 Introduction

Standard grower practice was previously to remove the end of chicory roots (approximately 3 cm) immediately prior to forcing, in order to standardise root length to allow more uniform root stacking within forcing trays. It was hypothesised that root-end removal could make chicory roots more susceptible to phytophthora infection during the forcing process by creating a fresh 'wound site'.

The aim of the experiment was to determine the effect of chicory root-end removal on the incidence and severity of phytophthora root rot, chicon yield (gross and marketable) and the incidence of tip burn on chicons.

2.5.2 Methods

The experiment was done at Jack Buck Growers, Holbeach, Lincs using a batch of chicory roots var. Totem (ref: PC Tinsley, batch L) for which outbreaks of phytophthora root rot had been observed by the grower in previous weeks.

There were two treatments:

1. Chicory root-end removal prior to forcing (standard grower practice)
2. Chicory roots left in tact

Each treatment was replicated eight times, with a single forcing tray representing a replicate (16 trays in total). The trays were arranged in a randomised block design within the usual stacks of trays in the forcing room (2 stacks of 6 boxes high, 11 boxes long). For practical reasons, the trays used for the experiment were positioned close to one end of the stacks for easy access, but were not placed in either the top or bottom row, or the end column, in order to avoid edge effects (see experimental layout in Appendix 2).

Eight trays were set with roots that had not had the ends removed. These trays were labelled and positioned in the forcing room, together with eight labelled trays containing roots cut in the usual manner.

Aliette (fosetyl aluminium) was applied (8 g/m^2) to all of the experimental trays according to normal grower practice (overhead spray onto roots after trays had been set and 1 day prior to commencement of forcing). Forcing procedures followed standard grower practice. Ozone was not used on the system during forcing.

At the time of harvest (21 days after the commencement of forcing), fifty roots per tray (selected at random) were collected for root rot assessment. Each root was cut longitudinally and the percentage internal surface area affected with typical symptoms of phytophthora rot estimated. Twenty chicons per tray (selected at random) were weighed to get their gross and marketable yield.

2.5.3 Results and discussion

Roots from three treatment 1 trays and three treatment 2 trays were weighed and cut open (a total of 300 roots). No phytophthora root rot was observed in any of the roots. It was concluded that the level of phytophthora infection in the trial was too low to be able to discern treatment differences, and assessments were discontinued. Planned post-harvest assessments to determine the incidence of chicon tip burn, were not carried out because of negligible disease development.

It was concluded that I) subsequent experiments would need to be artificially inoculated to ensure development of phytophthora root rot and to enable treatment comparison, and ii) that a small scale experimental system was needed to avoid the practical difficulties of operating on a commercial scale. For this purpose, a hydroponic forcing mini-system was designed at ADAS Arthur Rickwood.

Subsequent to this experiment, standard grower practice was modified such that root end removal was no longer done immediately prior to harvest. Instead, roots harvested from the field were dug to a fixed depth. This produced roots of a standard length and the opportunity for ‘curing’ of cut ends to occur well in advance of the forcing process.

2.6 Development of a hydroponic system for studying the biology and control of phytophthora rot of chicory roots

2.6.1 Objectives

- To design and set-up a small-scale hydroponic system for forcing chicory roots based on standard UK production methods
- To develop a reliable method for producing symptoms of root rot on chicory roots by inoculation of the hydroponic system with *Phytophthora* sp.

2.6.2 Methods

2.6.2.1 Run 1

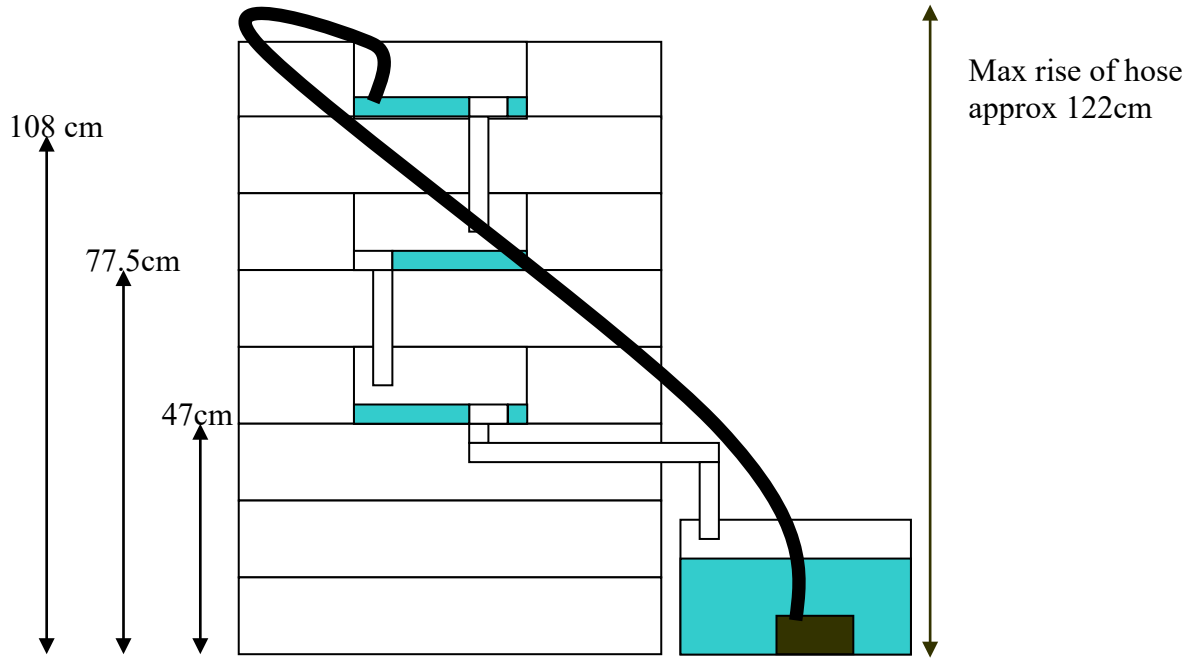
Chicory roots were collected from Jack Buck Growers on the day of experimental set-up and transported to ADAS Arthur Rickwood in polythene sacks contained in insulated boxes (to keep them cool in transit). Prior to collection, roots were treated with calcium chloride and root ends removed, according to standard grower practice.

A hydroponic 'mini-system' was set-up in a controlled temperature store at ADAS Arthur Rickwood. Figure 1 shows the design of the mini-system which was set up using two stacks of three 'trays'. Trays were custom made using plastic washing-up bowls (approximately 33 x 30 x 14 cm) with a drainage hole (4 cm diameter) cut in the base. The trays were separated using plastic crates with holes cut in the bases to allow piping carrying recirculating water to pass between trays. Sections of PVC pipe (4 cm long) were glued vertically over the drainage hole in each tray to enable a water level of 4 cm to be maintained in the trays during chicon forcing (comparable to grower practice).

One stack of three trays was artificially inoculated with *Phytophthora* sp. from chicory roots (isolate code: PCT), prepared as follows. Actively growing cultures of the fungus growing on PDA+S were cut into pieces and placed in three muslin bags. A bag containing phytophthora inoculum was placed inside a Universal tube with several holes (5 mm diameter) pierced in the side of the tube. One tube was placed in the centre of each tray. The second stack of trays remained uninoculated.

The trays were maintained in the dark for the whole period of the trial in a controlled temperature store at ADAS Arthur Rickwood, aiming for the following conditions to reflect grower practice:

Figure x. Design of hydroponic 'mini-system' for forcing chicory



pH: Day 1 = 6.8; Day 3 onwards = 6.2

EC:

Day 1 = 1.0

Day 7 = 1.6

Day 10 = 2.0

Day 20 = 1.0

Nutrient feed consisted of :

Trace element feed 6 kg/100 L (provided by grower)

Calcium nitrate 2.4 kg/100 L

Pottassium nitrate 3.8 kg/100 L

Acid for pH regulation consisted of:

60% nitic acid diluted to 5.6 L of acid in 100 L of water

Water temperature (using an electric water heater):

Week 1 24°C

Week 2 23°C
Week 3 22°C
(Air temperature to be 2°C lower)
The aim was to maintain the chicory root pith temperature at 20°C for days 1-7, 19°C for days 8-14 and 18°C for days 15-21 (monitored using a temperature probe).

Water Flow rate:
1 L/minute reducing to 0.6 L/minute by the end of the cycle
(equivalent to 15 L/minute then 10 L/minute per stack of six 1.2 m^2 boxes, by grower)

Water depth in tray = 4 cm

A daily check was done to ensure that the pumps were running, and that the air and water heaters were operating. EC, pH, temperature and water depth were checked twice-weekly.

After 21 days, for each tray, chicons were cut, counted and weighed (total for the tray). Each root was cut longitudinally and the incidence of root rots recorded.

After harvesting was complete, roots and chicons were discarded. The system was flushed through with sodium hypochlorite solution then clean water. The trays were washed with Jet 5 then clean water.

2.6.2.2 Run 2

In Run 2, the set-up method, forcing conditions and method of inoculation were the same as for run 1 apart from the following modifications:

- To try and encourage development of phytophthora rot, no nutrients were added to the recirulating fluid. In addition, a plastic pipe was placed around the inoculum and four roots in each inoculated tray, to create an area of reduced water movement (similar to restricted water movement at the corners of older style forcing trays).
- Water heaters were not used during this run, because of difficulties in controlling water temperature in Run 1, leading to temperatures that were too high in comparison with commercial conditions. Instead, air temperatures were maintained at a higher level, using heaters.

2.6.2.3 Run 3

- Forcing conditions (including nutrition) were the same as for run 1 except that no water heaters were used.
- Chicory roots artificially inoculated with phytophthora isolate (isolate code PCT) and showing typical symptoms of phytophthora root, were used as inoculum. Three infected roots were placed in the corner of each tray of the inoculated stack, close to the water input pipe.

2.6.3 Results and discussion

Conditions for the duration of each minisystem run are provided in Appendix 3.

2.6.3.1 Run 1

Good chicon development occurred over the 21 day forcing period. The mean chicon weight in each tray was 0.22 kg and there was excellent root hair development on the roots. There was no significant effect of inoculum or tray position on tray weight. There was no root rot development in any of the trays (inoculated or uninoculated).

Water temperatures were too high in comparison to commercial conditions and water heaters were subsequently not used.

2.6.3.2 Run 2

Chicon development was poor with a mean yield of 0.18 kg in the uninoculated trays and 0.12 kg in the inoculated trays. Poor chicon development was due largely to nil addition of nutrients (planned). In addition, there was a high incidence of sclerotinia rot (*Sclerotinia sclerotiorum*) in the roots. Rot incidence ranged from 31 – 95% in the inoculated trays. Two out of three of the uninoculated trays were unaffected by sclerotinia rot, while one tray had an incidence of 68%. It is probable that inoculum of *S. sclerotiorum* was present in soil attached to chicory roots and that the disease was able to establish due to the poor nutrient status of the roots. Root rots due to *Phytophthora* sp. were not observed.

2.6.3.3 Run 3

The inoculated trays were assessed after only 16 days into the 21-day forcing period because of severe root rotting and poor chicon development. Approximately 50% of roots in the inoculated trays had no chicon development. Chicons that had developed were shrivelled and discoloured. The maximum weight of an individual chicon was 0.44 kg. There was very

little root hair development and roots were shrivelled. When the roots from the inoculated trays were cut longitudinally, there was 100% rot incidence in each of the three trays (containing an average of 47 roots per tray). Rots extended upwards from the base of the root, often three-quarters of the way up the root. The leading edge of the rot in each root was water-soaked and grey. Mycelium typical of *Phytophthora* sp. (aseptate and correloid) was visible under the microscope, in root tissue cut from the leading edge of the lesion. Sporangia typical of *Phytophthora* sp. developed from tissue sections floated in sterile distilled water for 2-3 days. Behind the leading edge of root lesions, rots were darker and softer. A strong smell had developed in the recirculating water from 10 days into the forcing period, suggesting the development of sour rot. *Geotrichum candidum* (cause of sour rot) was consistently isolated from rotted tissue that was darker in colour.

The uninoculated trays were harvested at the end of the 21-day forcing period. Chicon development was generally good and mean chicon yield was 0.2 kg. Typical symptoms of phytophthora rot were observed in each of the three trays suggesting the presence of natural inoculum, however, the incidence and severity of rots was not as severe as in the artificially inoculated trays. An average of 47% of roots per tray had rot symptoms. Lesions developed from the root base up to the water surface, and were mainly present in the surface 2 mm of the root tissue. Mycelium typical of *Phytophthora* sp. (aseptate and correloid) was visible under the microscope, in root tissue cut from the leading edge of the lesion. Sporangia typical of *Phytophthora* sp. developed from tissue sections floated in sterile distilled water for 2-3 days. There was very little secondary infection in the roots.

While environmental conditions recorded during the three runs were not always optimum, the development of good quality chicons in the uninoculated trays during Run 1 and Run 3 (uninoculated trays), demonstrated that the minisystem reflected grower production sufficiently to provide a useful system for comparison of treatments to control phytophthora rot. The design using three trays per stack was satisfactory, with similar yields and disease levels obtained for trays within the same stack. The use of chicory roots articificially inoculated with *Phytophthora* sp, was identified as the most effective method for ensuring high inoculum pressure and ensuring disease development in untreated roots.

Effect of fungicides applied prior to chicory forcing on the development of phytophthora root rot and chicon yield

2.6.4 Objective

The aim of the experiment was to determine the effect of fungicides applied to chicory roots immediately prior to forcing on:

- The incidence and severity of phytophthora root rot
- Chicon yield
- Chicon quality

Fungicides to be tested were selected based on reports of efficacy in other chicory-producing countries and potential for approval in the UK. Dimethomorph was not included, based on advice that this was unlikely to get UK approval (V. Powell, HDC, pers. comm.).

2.6.5 Methods

Fungicide treatments were as shown in Table x.

Table x. Fungicides applied to chicory roots prior to forcing

	Treatment	Rate of ai / m^2	Rate of product / m^2*
1.	Control (no fungicide)	-	-
2.	Amistar (azoxystrobin)	0.25 g	1 ml
3.	Aliette (fosetyl-aluminium)	6.4 g	8 g
4.	Proplant (propamocarb hydrochloride)	7.2 g	10 ml

*In 5 L water

The experiment made use of the mini-system previously developed (see Section 2.6 for details). The experiment comprised four stacks of three trays containing chicory roots (approximately 40 per tray). Within each stack, water was re-circulated through the three trays (see Figure x) for the duration of the experiment, to simulate hydroponic forcing of chicory roots as carried out by a commercial UK producer. Each fungicide treatment was applied to one stack, with the three trays representing replicate plots. Each stack had a separate water circulation system. All of the trays were artificially inoculated with a *Phytophthora* species from chicory roots.

Artificial inoculum of *Phytophthora* sp. *ex* chicory was prepared as follows: Pieces of root tissue (2 mm^3) were cut aseptically from the leading edge of typical symptoms of

phytophthora root rot in chicory roots. The tissue pieces were placed in sterile distilled water and incubated for approximately 16 h. After this period, actively growing mycelium typical of phytophthora was visible by microscopic examination around the tissue pieces. 48 visibly healthy roots of chicory var. Atlas were scrubbed to remove mud and dried with paper towels. Each root was wiped thoroughly with 90% ethanol. Using aseptic technique, a sterile cork borer (6 mm diameter) was used to cut 3 plugs (approximately 5 mm depth) along the length of each root. One piece of infected root tissue was placed in each 'hole' cut in the chicory roots. The plug of healthy root tissue was replaced and sealed with petroleum jelly. The inoculated roots were labelled using plastic plant labels tied with elastic bands around the top of the root.

Chicory roots (var. Atlas) were collected from Jack Buck Growers, Lincolnshire on the day of experimental set-up. The grower was requested to treat the roots with calcium chloride before collection (standard grower practice) but to ensure that roots had not been treated with Aliette (fosetyl-aluminium). Sufficient roots were collected to fill twelve 'trays' (washing-up bowls) used in the mini-system (approximately 550 roots). The roots were transported in polythene sacks contained in large plastic insulated boxes (to keep roots cool while in transit).

Twelve trays (adapted washing-up bowls, see Section 2.6) were filled with healthy chicory roots, closely packed. Four inoculated roots (labelled) were placed within each tray (evenly positioned). For each treatment, the roots in each of three trays were sprayed at the appropriate rates (Table x), using an Oxford precision sprayer with single nozzle.

The mini-system was set-up as described in Section 2.6. The experiment was located in a controlled temperature store at ADAS Arthur Rickwood and maintained in the dark for 21 days. The aim was to maintain the following conditions, to reflect grower practice:

pH: Day 1 = 6.8; Day 3 onwards = 6.2

EC:
Day 1 = 1.0
Day 7 = 1.6
Day 10 = 2.0
Day 20 = 1.0

Nutrient feed consisted of:
Trace element feed 6 kg/100 L
Calcium nitrate 2.4 kg/100 L
Potassium nitrate 3.8 kg/100 L

Acid used for pH regulation consisted of:
60% nitic acid diluted to 5.6 L of acid in 100 L water

Chicory root pith temperatures (measured with a temperature probe):
20^{o}C (days 1-7)
19^{o}C days 8-14)
18^{o}C (days 15-21)
(achieved by maintaining air temperature approximately 3 degrees C higher than water temperature)

Water Flow rate: 750 ml/minute (equivalent to 12 L/minute per stack of six 1.2 m^2 boxes, by grower)

Water depth in tray: 4cm

The experiment was checked daily to ensure that the pumps were running and that the air heating was operating. PH, EC, temperature and water depth were monitored twice-weekly and adjusted if necessary. The water temperature in each of the four water reservoirs and air temperature was logged for the duration of the trial using a Delta-T logger.

After 21 days, all of the chicons were cut away from the roots. The number of chicons per tray and total chicon weight per tray was recorded. The marketability of each chicon was scored where 1 = marketable, 2 = marketable, but chicon slightly loose, and 3 = unmarketable (chicon open or too small). Each root was cut longitudinally and the presence or absence of root rot recorded. Root rot severity was assessed using the scale of Benigni & Bompeix (2004) where: 0 = healthy root, 1 = slight necrosis, 2 = moderate necrosis (<25 % root height), 3 = severe necrosis (>25 % root height). Root symptoms observed were plated out in the laboratory to check for causal organisms.

The mini-system was flushed through with sodium hypochlorite solution then clean water. Trays were washed with Jet 5 then clean water.

Mean chicon weights were analysed by ANOVA. Chicon quality scores (non-parametric data) were analysed using Friedman's statistic.

2.6.6 Results and discussion

Conditions (pH, EC and pith temperature) for the duration of the trial are shown in Appendix 3.

There was a significant effect of fungicide treatment on mean chicon weight (Table x), with a mean weight of 173 g for chicons in the untreated control, compared to mean weights of over 200 g for the fungicide-treated chicons. Chicon weight did not vary significantly between the fungicide treatments. The effect of fungicides on chicon quality was not statistically significant, although there was a trend for lower quality chicons in the trays treated with Proplant (propamocarb hydrochloride).

A high percentage of roots (97%) in the untreated control treatment had root rot symptoms (Table x), indicating high inoculum pressure due to the articially inoculated roots placed in each tray. Symptoms were typical of phytophthora root rot, and the development of sporangia on infected root tissue floated in sterile distilled water for 2 days, confirmed *Phytophthora* sp. as the causal organism. In contrast, the incidence of root rot in the fungicide-treated trays was reduced to 1% or less, irrespective of treatment. The severity of symptoms in these affected roots was low. In the fungicide-treated trays, even roots adjacent to the inoculated roots (completely rotten by the end of the experiment), remained healthy.

The efficacy of Amistar (azoxystobin) for controlling phytophthora root rot on chicory was also demonstrated by Benigni & Bompeix (2004) in France. They showed that the protection supplied by azoxystrobin at 0.25 g/m^2 (same rate as used in this experiment) was either equivalent (4 trials) or better (1 trial) than that obtained with fosetyl-aluminium (12 g/m^2). The use of azoxystrobin at 0.25 g/m^2 against *P. cryptogea* on chicory roots before the forcing period was officially authorised in France in March 2002.

Table x. Effect of fungicide treatments applied to chicory roots pre-forcing on chicon weight and quality

Fungicide treatment	Tray position in stack	Mean individual chicon weight (g)	Mean chicon quality score (1-3)*
Untreated control	Top	172.6	1.80
	Middle	189.7	1.68
	Bottom	156.8	1.91
	Mean	173.0	1.80
Amistar	Top	189.4	1.66
	Middle	208.4	1.79
	Bottom	226.1	1.71
	Mean	207.9	1.72
Aliette	Top	209.1	1.82
	Middle	224.1	1.60
	Bottom	205.6	1.81
	Mean	212.9	1.74
Proplant	Top	202.5	2.00
	Middle	211.3	1.97
	Bottom	208.0	2.00
	Mean	207.3	1.99
	d.f	6	3
	F. probability (block)	0.319	-
	SED (block)	9.1	-
	F. probability (treatment)	0.029	-
	SED (treatment)	10.5	-
	P (Friedman's test)	-	0.164

*chicon quality score: 1 = marketable, 2 = marketable, but chicon slightly loose, and 3 = unmarketable (chicon open or too small)

Table x. Effect of fungicide treatments applied to chicory roots pre-forcing on the incidence and severity of phytophthora root rot

Fungicide treatment	Mean % incidence of root rot	Mean root rot severity (0-3 score)*
Untreated control	97	2.10
Amistar	0	0.00
Aliette	1	0.03
Proplant	1	0.03

*root rot severity score: 0 = healthy root, 1 = slight necrosis, 2 = moderate necrosis (<25 % root height), 3 = severe necrosis (>25 % root height)

2.7 Effect of two biological products on the development of chicory root rot caused by *Phytophthora* sp.

2.7.1 Introduction

The aim of the experiment was to determine the effect of two biological products (that could be used as alternatives to conventional fungicides during forcing) on the development of chicory root rot, caused by *Phytophthora* sp. in a laboratory experiment.

Trianum (produced by Koppert) contains the beneficial fungus *Trichoderma harzianum*. It is reported to protect the plant by four mechanisms; competition for space, competition for nutrients, mycoparasitism and by strengthening the plant (promoting a healthier root system) (see www.koppert.nl/e005.shtml). Product literature indicates that it can offer protection against a range of soil-borne diseases.

Novello Liquid (produced by Cotswolds Hydroponics Ltd) contains *Bacillus laterosporus* and can be used in both hydroponic systems and soil. Product literature indicates that the product can promote root development and can have a significant inhibitive effect on a range of pathogens including *Phytophthora* species.

The experiment was conducted as a small-scale laboratory trial.

2.7.2 Methods

Treatments were as follows:

	Inoculation with *Phytophthora* sp.	Root soak solution	Microorganism	Product rate
1	No	Distilled water	-	-
2	Yes	Distilled water	-	-
3	Yes	Distilled water + Novello	*Bacillus laterosporus*	2 ml per L
4	Yes	Distilled water + Trianum G	*Trichoderma harzianum*	0.75 g per L

Each treatment was applied separately to seven chicory roots, each with three inoculation points.

The roots were artificially inoculated as follows: Pieces of root tissue (2 mm^3) were cut aseptically from the leading edge of typical symptoms of phytophthora root rot in chicory roots. The tissue pieces were placed in sterile distilled water and incubated for approximately 16 h. After this period, actively growing mycelium typical of phytophthora was visible by microscopic examination around the tissue pieces. Twenty eight visibly healthy roots of chicory were scrubbed to remove mud and blotted dry with paper towels. Each root was wiped thoroughly with 90% ethanol. Using aseptic technique, a sterile cork borer (5 mm diameter) was used to cut 3 plugs (approximately 5 mm depth) in the lower 5 cm of each root. For 21 roots, one piece of infected root tissue was placed in each 'hole' cut in the chicory roots. The plug of healthy root tissue was replaced and sealed with petroleum jelly. For the uninoculated control treatment (7 roots), the same procedure was followed but infected root pieces were not added.

The roots were placed in 500 ml glass conical flasks (one root per flask), in the appropriate suspension (Table x). The flasks were placed on a rotary shaker for 7 d at approximately 20^{o}C. After this period, each root was cut longitudinally and the diameter of lesion around each inoculation point measured.

2.7.3 Results and discussion

Lesion development was not extensive in any of the roots. Lesion diameters were significantly higher for the inoculated control treatment compared to the uninoculated control (Table x). The two biological products did not result in a significant reduction in lesion size compared to the inoculated control.

Further testing of these products and others available could be carried out by the grower under commercial production conditions.

Table x. Effect of biological products on the development of phytophthora root rot in chicory roots

Treatment	Mean lesion diameter (mm)
Uninoculated control	7.0
Inoculated control	9.8
Trianum	8.7
Novello	8.6
D.f.	24
P	0.047
SED	0.92

2.8 Development of a soil-baiting system for *Phytophthora* sp. pathogenic to chicory

2.8.1 Introduction

The aim of the experiment was to develop a technique for baiting *Phytophthora* sp. pathogenic to chicory roots, from soil samples, based on a published method (Falloon, 1982).

2.8.2 Methods

Chicory seeds (var. Platine) were surface sterilised in 3% sodium hypochlorite for 3 seconds and rinsed three times in sterile distilled water. The seeds were sown at a depth of 10-20 mm in moist sterilised sand in seed trays (four trays of 20 seed). The trays were placed in large polythene bags to prevent dehydration and were incubated in the dark at 20°C for 8 days until seedlings had emerged.

Two litres of soil (collected from ADAS Arthur Rickwood) was thoroughly mixed and divided into two samples. Chicory roots with confirmed symptoms of phytophthora root rot were chopped into pieces (approximately 1 cm^3) and blended with sufficient distilled water in a Waring blender to give a slurry of root inoculum. 100 ml of the inoculum was added to 1 L of soil and incorporated thoroughly throughout the soil. The second soil sample was left uninoculated. The soil samples were stored at ambient temperature in sealed bags until required for seedling baiting tests.

Chicory seedlings were gently removed from the sand and washed in distilled water. The seedlings were prepared for baiting by removing any seed remains and by lightly crushing the plumule and radicle with forceps approximately 10 mm above and below the point of seed attachment, to encourage infection.

For each of the inoculated and uninoculated treatments, soil was placed in each of four plastic boxes to a depth of 5 cm. Each box was flooded with distilled water to a depth of 1 cm above the soil surface. Five of the prepared seedlings was floated on the water in each box. The boxes were covered to prevent them from drying out and incubated at 20°C. Five prepared seedlings were also floated in sterile distilled water (SDW) in a Petri dish as a check.

The roots were observed after 4 days for symptoms of phytophthora infection (seedlings water-soaked and flaccid). Seedlings suspected to be infected were observed using an inverted microscope to check for sporangial development.

2.8.3 Results and discussion

Seedlings floated on SDW remained healthy in appearance. Seedlings floated on water over infested soil all showed symptoms of phytophthora infection (water-soaked and flaccid). When viewed microscopically, sporangia typical of *Phytophthora* sp. *ex* chicory could be seen developing from all of the seedlings. Seedlings floated on uninfested soil deteriorated slightly but not to the same extent as those floated over the infested soil, and no sporangial development was observed.

A similar baiting method was recently used in another HDC project (FV 246a) to determine the presence of *Phytophthora asparagi* in asparagus fields. The technique has the advantage that if confirms or otherwise the presence of a *Phytophthora* species specifically pathogenic to the host crop (in this case chicory). This method if further refined could potentially be used as a component of a risk management system to identify fields or areas of fields from which root batches could be at high risk of phytophthora rot development during forcing.

2.9 Overall conclusions

2.9.1 Disease biology

- A fungus consistently isolated from chicory roots with typical root rot symptoms was identified by molecular sequencing as a new species of *Phytophthora*. The same species was isolated from roots originating from two different farm sites. It is closely related to, but distinct from *P. brassicae*, which to date has only been isolated from brassicas.

- Artificial inoculation of healthy chicory roots with the *Phytophthora* isolate, lead to the development of typical root rot symptoms, confirming that this species of *Phytophthora* was a causal agent of root rot.

- *Phytophthora* sp. was identified as the primary cause of root rotting and was consistently isolated from the advancing edge of lesions in root tissue. However, *Geotrichum candidum* was also frequently isolated from severely rotted tissue in the same roots, as a secondary pathogen, causing 'sour rot' symptoms typical of this micro-organism.

- Optimum conditions for growth of *Phytophthora* sp. isolated from chicory roots coincided with conditions routinely used for chicon forcing. Optimum mycelial growth occurred at pH 6.0, with growth reduced at pH 5.5 and pH 7.5. The optimum temperature for mycelial growth was 15^{o}C with reduced growth rate above 19^{o}C. The optimum temperature for sporangial production was 19^{o}C.

- Laboratory studies showed that *Phytophthora* sp. from chicory roots was capable of sporangial production and zoospore release under a wide range of conditions. This demonstrated that under re-circulating forcing conditions, there is potential for tray-to-tray transmission of the pathogen by motile zoospores, in addition to localised spread of symptoms between neighbouring roots within a single tray.

2.9.2 Control

- A trial to determine the effect of root-end removal prior to forcing on phytophthora development was carried out at Jack Buck growers. The trial was inconclusive because of low infection levels in the root batch used. However, the grower subsequently modified standard practice from root-end removal to leaving roots in tact, in order to minimise risk of root infection via the cut root surface.

- A hydroponic 'minisystem' was devised at ADAS Arthur Rickwood to enable small-scale evaluation of treatments against chicory root rot to be carried out. Three pilot trials were done to optimise this system.

- The following fungicides applied to roots pre-forcing were evaluated for control of chicory phytophthora in an artificially inoculated minisystem trial: Aliette (industry standard), Amistar (azoxystrobin) and Proplant (propamocarb hydrochloride). All of the fungicides increased chicon yield in comparison with an untreated control, and eliminated root rotting compared with 100% rot incidence in the untreated control. Data from this experiment supported a successful HDC application for SOLA use of Amistar on chicory.

- An experiment was carried out to determine the effects of biological products Novello (*Bacillus laterosporus*) and Trianum (*Trichoderma* sp.) on chicory root rot development. Neither treatments reduced the severity of root rotting compared to the inoculated control treatment.

- A simple soil baiting system using chicory seeds/seedlings was devised that could be used to test field soil for the presence of *Phytophthora* sp. pathogenic to chicory. This method could be used as a component of a risk management system to identify fields or areas of fields from which root batches could be high risk for rot development during forcing.

2.10 References

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2.11 Technology transfer

2.11.1 Meetings and presentations

- Project planning visits to Jack Buck Growers (K. Green) in May 2002, January 2003 and June 2003.
- Visit with to Jack Buck Growers with Tim Pettitt, September 2003
- Visit to T. Pettitt at Warwick HRI by K. Green for training in laboratory techniques relevant to the project, March 2004
- Project summary provided for chicory growers group, January 2003
- Project review meeting, June 2003, Jack Buck Growers
- Project review meeting, March 2004, ADAS Arthur Rickwood
- 'Biology and management of Phytophthora diseases on vegetables': FV 240 project results presented in a talk given by K. Green at the HDC Field Vegetable research roadshow, Kirton, March 2005.
- Project results and references provided to Vivian Powell (HDC) as the basis for a SOLA application for Amistar (July 2005).

2.11.2 Articles

Green, K. 2003. Chicory root rot. *HDC News*, December 2003, p 13.

Green, K. 2006. Reducing root rot risk. *HDC News*, July/August 2006. **125**:21.

2.12 Acknowledgements

We thank Robin Buck of Jack Buck Growers for contributions to project funding. Technical input from Tim Pettitt was appreciated. The technical assistance of Brian Read (Jack Buck Growers), Amanda Shepherd, Tareka Ratcliffe and Alan Green (ADAS) is gratefully acknowledged.

3 APPENDICES

Appendix 1.

Petri's solution (for stimulation of sporangial production)

Calcium nitrate	0.4 g
Magnesium sulphate	0.15 g
Potassium acid phosphate	0.15 g
Potassium chloride	0.06 g
Distilled water	1 L

P_{10}ARP (selective media for *Phytophthora* spp.)

To make 1 litre:

- Autoclave 17 g corn meal agar in 1 L distilled water
- Cool to 45oC
- Add 5 mg/L pimaricin, 250 mg/L ampicillin, 10 mg/L rifampicin and 100 mg/L PCNB

Pimaricin:
100 mg pimaricin in 20 ml water = 5 mg/ml
Add 1 ml to 1 L

Ampicillin
560 mg in 11.2 ml water = 50 mg/ml
Add 5 ml to 1 L

Rifampicin
100 mg rifampicin in 20 ml 90% ethanol = 5 mg/ml
Add 2 ml to 1 L

PCNB
2 g of quintozene (if 50:50 w/w) in 20 ml = 100 mg/ml
Add 1 ml to 1 L

Appendix 2. Site plan for root-end removal experiment

Plan (side view)

	Stack 1	Stack 2	Stack 3	Stack 4	Stack 5	Stack 6	Stack 7	Stack 8	Stack 9	Stack 10	Stack 11
Box 1		Top									
Box 2		**P1 T1**	**P2 T2**								
Box 3		**P3 T2**	**P4 T1**								
Box 4		**P5 T2**	**P6 T1**								
Box 5		**P7 T1**	**P8 T2**								
Box 6		Bottom									

Central channel

Box 1		Top									
Box 2		**P9 T2**	**P10 T1**								
Box 3		**P11 T1**	**P12 T2**								
Box 4		**P13 T2**	**P14 T1**								
Box 5		**P15 T1**	**P16 T2**								
Box 6		Bottom									

Sliding door - entrance

Treatments

T1 Cut root at base

T2 No cut

Appendix 3 Forcing conditions during minisystem develoment trials

Run 1

Flow rates:

Treatment	Start flow rate (L/min)	Finish flow rate (L/min)
Uninoculated (Trt1)	0.98	0.62
Inoculated (Trt 2)	0.89	0.60

Data Recording:

		Temperature (Degrees C)				pH		EC		Pith Temp	
Date	Time	Unt Water	Trt Water	Unt Air	Trt Air	Unt	Trt	Unt	Trt	Unt	Trt
03/12/03	0800	19.6	21.1	19.1	19.4	7.0	6.8	1.3	1.4	21.1	21.0
05/12/03	0836	24.4	23.1	19.2	19.3	6.5	6.3	1.2	1.3	20.6	19.7
08/12/03	0745	18.3	27.2	17.7	18.1	6.8	6.8	0.7	0.6	19.1	19.0
10/12/03	0745	18.6	27.9	18.3	18.9	6.8	6.5	0.9	0.7	21.0	21.1
12/12/03	0712	21.2	19.3	17.8	18.0	7.2	7.3	0.6	0.9	18.8	18.1

Datalogger information:
Water temperatures ranged from 13.1°C to 29.0°C; air temperature ranged from 13.8°C to 21.3°C .

Run 2

Flow rates:

Treatment	Start flow rate (l/min)	Finish flow rate (l/min)
Uninoculated (Trt1)	0.610	0.607
Inoculated (Trt 2)	1.090	0.816

Data Recording:

		Temp degrees C				pH		EC		Pith Temp	
Date	Time	Unt Water	Trt Water	Unt Air	Trt Air	Unt	Trt	Unt	Trt	Unt	Trt
14/01/04	1600	-	-	-	-	7.4	7.4	0.7	0.7	8.4	8.4
16/01/04	0825	21.7	22.2	23.4	21.5	7.6	7.5	0.9	0.8	20.1	20.5
20/01/04	0808	24.7	25.0	25.8	24.3	7.5	7.4	0.8	0.9	24.2	23.8
23/01/04	0810	25.3	25.6	26.6	25.1	7.6	7.2	0.7	0.8	24.7	24.2

28/01/04	0815	20.6	20.8	17.2	16.2	7.5	7.2	0.7	0.8	20.5	20.3
30/01/04	0930	20.0	20.7	21.0	19.6	8.0	6.9	0.6	0.8	19.3	19.5
03/02/04	0715	23.2	22.7	21.0	21.9	7.3	8.3	0.5	1.0	22.1	21.8

Datalogger information:
Water temperatures ranged from 9.55 Deg C to 25.6 Deg C, Air temperature ranged from 11.83 Deg C to 26.45 Deg C.

Run 3

Data recording:

		Temp degrees C				pH		EC		Pith Temp	
Date	Time	Unt Water	Trt Water	Unt Air	Trt Air	Unt	Trt	Unt	Trt	Unt	Trt
16.02.04						7.6	7.3	0.7	0.7		
17.02.04		19.0	22.5	23.0	18.4	7.8	7.6	0.7	0.7		
20.02.04	0810	24.0	26.6	26.8	23.7	7.9	7.8	0.7	0.7	22.9	23.6
20.02.04						6.2	6.1	1.1	1.1		
24.02.04		22.2	24.8	25.2	19.2	6.2	6.2	1.1	1.1	22.4	22.2
02.03.04	0825	21.4	24.5	24.8	21.6	6.2	6.0	1.5	2.2	22.4	23.7
05.03.04	0815	19.2	22.8	19.8	21.6	6.2	-	2.0	-	22.6	

Appendix 4. Measurements for duration of fungicide treatment trial

Date	Day of forcing	Unit	pH	EC	Pith temperature (°C)
12.11.04	2	1	6.2	1.0	15.9
12.11.04		2	6.2	1.0	16.1
12.11.04		3	6.2	1.0	15.8
12.11.04		4	6.1	1.0	15.9
16.11.04	6	1	6.4	1.0	17.5
16.11.04		2	6.3	1.0	17.4
16.11.04		3	6.2	1.0	17.4
16.11.04		4	6.2	1.0	16.9
19.11.04	9	1	6.2	1.6	17.0
19.11.04		2	6.2	1.6	17.0
19.11.04		3	6.2	1.6	16.9
19.11.04		4	6.2	1.6	17.3
22.11.04	12	1	6.7	2.1	25.8
22.11.04		2	7.1	1.7	25.9
22.11.04		3	6.5	1.6	25.6
22.11.04		4	6.3	1.9	25.1
24.11.04	14	1	6.4	2.0	22.8
24.11.04		2	6.3	2.0	22.5
24.11.04		3	6.7	1.9	21.8
24.11.04		4	6.5	1.9	21.5
29.11.04	19	1	7.6	1.9	19.1
29.11.04		2	6.7	1.6	19.2
29.11.04		3	6.8	1.5	18.8
29.11.04		4	6.9	1.4	18.5
01.12.04	21	1	6.9	1.2	18.9
01.12.04		2	6.8	1.2	18.5
01.12.04		3	6.8	1.1	18.7
01.12.04		4	6.8	1.2	18.2