An Assessment of Chemical Treatment as Part of a Disease Management Strategy against *P. kernoviae* and *P. ramorum* Infections of Magnolia

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Summary

Phytophthora ramorum and *P. kernoviae* are newly invasive *Phytophthora* species in the UK that damage plants in nurseries, native woodlands, semi-natural- and heritage- gardens. They were discovered in Britain in 2002 and November 2003 respectively. Rhododendrons, particularly *R. ponticum*, are primary foliar hosts for both pathogens and are the main plants on which inocula are produced in outdoor situations in Europe. However, apart from rhododendron, members of the *Magnoliaceae* which are prized plant species in ornamental and heritage gardens in the UK are highly susceptible to both pathogens but particularly to *P. kernoviae*. Disease takes the form of leaf spots, and fruit and blossom blight and can be very disfiguring and even fatal in some cases. Previous research demonstrated that *P. kernoviae* overwinters in buds and leaf scars of magnolias, and sporulates on infected magnolia foliage, thus creating a self-perpetuating disease system. The same is assumed true for *P. ramorum* but still needs scientific confirmation.

Many magnolias are deciduous, so a systemic chemical treatment applied at the onset of the season was thought to be feasible as a part of a management plan to reduce disease by inactivating existing overwintering infections in buds and leaf scars, but also by protecting newly emerging blossom and foliage from external inoculum. Phosphonate applied preventatively, i.e. before arrival of inoculum, is known to enhance the plant's defence mechanisms when challenged by *Phytophthora* species, but may also inactivate existing overwintering infections. Two separate investigations were initiated in 2007, one to determine the effect of phosphonate on inactivating overwintering infections on magnolia saplings infected with *P. kernoviae*, the other to assess its effects when injected into mature magnolias diseased by *P. ramorum* although one tree was infected with *P. kernoviae*. This report describes these two investigations and their outcome.

The sapling experiment was carried out in 2007 a woodland in Cornwall. However, in October 2006 saplings were placed in a woodland in the drip line of the canopies of rhododendrons diseased with *P. kernoviae* to become infected with natural inoculum. Many of the pots were blown over during the exposure period so that the young stems of the saplings lay on a bed of infected shed rhododendron leaves. Consequently a large number of sapling stems became infected and showed symptoms of dieback. Thus the normal epidemiological pattern of disease was not realistically represented in this trial because stem dieback resulting from the inoculation process is not the usual form of disease on magnolias. *P. kernoviae* usually causes leaf spots, fruit and blossom blight on magnolias. This

prevented achievement of the aim of investigating the effect of phosphonate on the usual overwintering infections of *P. kernoviae,* but did enable an evaluation of the effect of the chemical on stem infections.

In spring 2007 prior to bud-burst the naturally infected saplings were either injected with phosphonate (as Agri-Fos 400 under experimental licenses: AEA 2005/10025PP and AEA 2005/10035PP supplied by the UK Pesticides Safety Directorate) or the chemical was applied as stem paint together with a surfactant, Pentrabark. Uninfected untreated plants were included in the experiment as indicator plants to monitor movement of inoculum.

The stem injection delivered phosphonate quickly and effectively into the plant where it was distributed to the leaves, rapidly reaching peak levels four weeks after application. By contrast phosphonate applied as a stem-paint had a lag time of 10 weeks before concentrations of the chemical peaked in foliage. The concentration of phosphonate in sapling foliage was ten times higher when applied as a stem-injection compared with the stem-painted application, but levels of phosphonate diminished throughout the season (up to November), tailing off to approximately 10% of peak levels in both application techniques.

Phosphonate applied as an injection caused severe phytotoxicity and death of 35% of the saplings due to the very high concentration delivered to plant tissues, whereas applied as a stem-paint it was not phytotoxic to plants. Application rates were calculated in accordance with those reported on producing fruit trees in the literature. However, saplings are juvenile plants and have much less dense canopies than mature trees thus lower application rates need to be tested in the future.

The untreated controls yielded the pathogen from 60% of saplings, mostly from stem infections, but in addition three plants had foliage infections. The low level of foliage infections might be attributed to low survival of the pathogen in dead shoots. A more likely explanation though, is that newly emerging susceptible spring foliage was not available in the immediate vicinity of inoculum overwintering in leaf scars, because the buds on the shoots were killed in the dieback process. The few foliage infections that occurred however, indicate that the overwintering infections in buds and leaf scars not yet killed by stem infections, reactivated in spring and caused new leaf infections. By contrast the pathogen could not be isolated from leaves, leaf scars or stems of surviving plants that had the stem injection treatment suggesting good control of the overwintering infections. Saplings with the stem-paint treatment did not fare significantly differently from the untreated controls. *P.*

kernoviae was isolated from both leaves and stems suggesting ineffectiveness of the stem-paint treatment probably due to the low levels of phosphonate penetration into plant tissue.

There was a steady increase in stem lesion extension on all infected saplings. The rate of lesion extension on the stem-injected plants was slightly faster compared with the other treatment probably due to a combination of the pathogen (initially) and after the injections, phytotoxicity. These results suggest that phosphonate was unable to stop stem infection from spreading in the stem-paint treatment. In the stem-injection treatment however, both pathogen and magnolia shoots were killed due to the high concentration of chemical in plant tissues. Phosphonate is known to retard, but not halt lesion development in tan oak (*Lithocarpus densiflorus* stem infected with *P. ramorum*) but gives effective control of foliar infections caused by *Phytophthora* species in other plants (E.g. *P. infestans* on potatoes).

Conclusions from the Sapling Experiment are as follows:

- Application of phosphonate by stem-paint does not appear to be an effective way of treating *P. kernoviae* infection.

- Application of phosphonate by injection appears to offer the best potential for treatment.

- Further investigation is needed to determine the optimum dosages for the injections and to confirm the efficacy of the treatment.

- The experiment was very useful as a pilot trial and generated much knowledge and experience.

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The second experiment was carried out on a variety of mature magnolia trees in National Trust heritage gardens, Lanhydrock and Trengwainton, in Cornwall. All of these trees were naturally infected with *P. ramorum* except one at Trengwainton that was infected with *P. kernoviae.* In spring 2007 towards the end of the flowering period these magnolias were injected with phosphonate. Levels of phosphonate in leaves were monitored over time and phytotoxic effects of the chemical noted. At least twice during the growing season foliage was tested in the laboratory for the presence of *P. ramorum* and *P. kernoviae* as appropriate.

Results of the Mature Magnolia Experiment were very promising although there was some variability. There were similarities in responses to the treatments at the two sites but there were also a number of differences in that the different species of magnolias responded variously to the uptake and transport of phosphonate to leaves. All injected trees exuded black ooze from the injection sites similar to that reported on cocoa trees which indicates a phytotoxic response to the chemical injection. Control trees were not injected with water in these tests because we were obliged to limit damage to trees in the gardens but in future work this will have to be done to confirm that the black ooze is a phytotoxic response and not a wound response. It is noteworthy that at Lanhydrock the injection wounds on the tree stems had completely healed by autumn. At both sites phosphonate levels peaked in foliage shortly after application (1–4 weeks), but it diminished fairly rapidly at Trengwainton. A visual inspection of symptoms revealed that the incidence of disease was reduced in the stem-injected magnolias at both sites

At Lanhydrock the stem-injected plants consistently tested negative for *P. ramorum* after treatment. At Trengwainton results were more variable. The untreated controls tested positive for the pathogens in most cases, especially in the spring and autumn tests, but the success rate of isolation of *P. ramorum* was lower in summer. The positive tests in spring can probably be attributed to the low levels and slow build up of the chemical in plant tissue.

Conclusions from the Mature Magnolia Experiment are as follows:

- Phosphonate applied as a stem injection was successful in reducing the incidence of disease in the treated trees.

- Further investigation is needed to determine the optimum dosages for the injections and to extend the period that active ingredient is available in the foliage.

- The effects of more frequent applications of the chemical at lower concentrations need to be tested.

Recommendations overall

- Further work on phosphonate as part of disease management should be carried out to allow treatment regimes to be formulated for ornamental trees. In particular more work is required on:

- Dose-phytotoxicity response curves should be constructed to determine non-toxic but effective levels of phosphonate in plant tissues.

- Determine the effect of low and high levels of inoculum against different concentrations of phosphonate *in vitro* and *in planta* under controlled conditions so that pathogen response to different levels of phosphonate in plants is known and preventative chemical treatments can be derived.

- The effects of appropriate levels of phosphonate on inactivating overwintering infections in leaf scars and buds also require further investigation under controlled inoculation conditions.

- The effects of frequent applications of dilute solutions of phosphonate on mature trees need investigation in an attempt to counter phytotoxicity and maintain effective plateau concentrations of phosphonate in foliage for over risk periods.

- The effects of biannual or seasonal applications of phosphonate i.e. early in spring or throughout spring and early in autumn or throughout autumn need to be tested so that host defence can be assisted at the times of the year when inoculum is usually present.

Introduction

Phytophthora ramorum and *P. kernoviae* are newly invasive *Phytophthora* species in the UK. *Phytophthora ramorum,* the cause of 'Sudden oak Death' in California, is also found in the nursery trade in Europe and the USA and is highly damaging on a wide range of plant species (Rizzo *et al*., 2005). *Phytophthora kernoviae* on the other hand was first discovered in native woodlands and semi-natural gardens in the UK in November 2003 (Brasier *et al*., 2005) and has a restricted known distribution. UK experience shows *P. kernoviae* is an extremely aggressive pathogen. Thousands of woodland rhododendrons (*Rhododendron ponticum*) have been infected and many have died, and so far about 60 beech trees (*Fagus sylvatica*) have fallen victim with lethal bleeding cankers. A number of ornamental species have also been infected including trees and shrubs which are key features in public and heritage gardens. Members of the Magnoliaceae family in particular feature prominently as highly susceptible hosts; magnolias develop leaf spots, and fruit and blossom blight and occasionally twig cankers, when infected. Recent research demonstrated that *P. kernoviae* overwinters in leaf scars, buds and bud cases (perules) of infected magnolias (Denman *et al*., 2007). Sporulation is known to occur on detached magnolia leaves (Denman *et al*., 2004) and is also thought to occur on perules, blossom and foliage of naturally infected magnolias in the field. Thus *P. kernoviae* is considered a self-perpetuating system on magnolias, with the overwintering infections in leaf scars, perules and buds infecting the newly emerging blossom and leaves in spring (Denman *et al*., 2007). It is thought that *P. ramorum* behaves in the same way but this still requires scientific confirmation.

As high value is assigned to mature established magnolias in heritage gardens, there are both cultural and economic aspects to the loss of these plants due either to compliance with statutory legislation or to disease severity. *P. kernoviae* is an aerial Phytophthora with intermittent inoculum production dependent upon favourable weather conditions, and most of the magnolias infected in the UK are deciduous, so chemical treatment might be feasible as a part of a management plan to keep plants disease free and minimise new infections. The potential efficacy of chemical treatments is assessed in the work presented here through two different experiments. In Experiment 1 the effect of phosphonate on inactivating or preventing re-activation of *P. kernoviae* overwintering on previously infected magnolia buds and shoots was assessed using magnolia saplings (*Magnolia* x *Soulangeana*). In Experiment 2, the effect of phosphonate applied as stem-injections to mature magnolias naturally-infected with either *P. kernoviae* or *P. ramorum* in heritage gardens was monitored.

Project Aims

Experiment 1

- To assess the effect of phosphonate (Agri-fos) chemical treatment in countering overwintering *P. kernoviae* infections on magnolia saplings.
- To record phosphonate levels in the leaves of the saplings.
- To document phytotoxic effects of phosphonate.

Experiment 2

- To monitor the effect of phosphonate on infections of new foliage on mature trees previously naturally infected.
- To record phosphonate levels in the leaves of mature magnolias.
- To document phytotoxic effects of phosphonate on mature magnolias.

Methods

Experiment 1

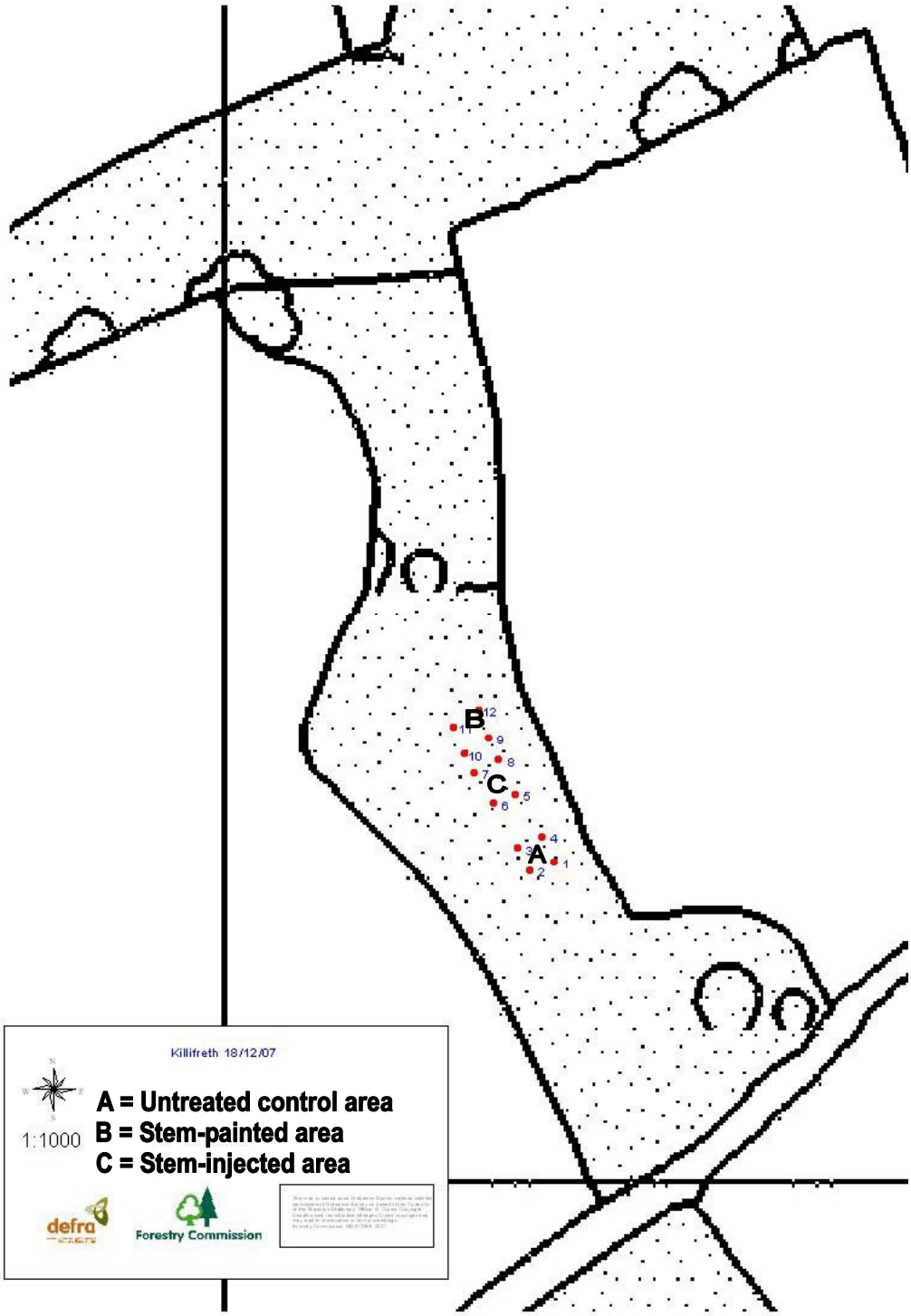
Since artificial inoculation of plants in outdoor environments with either of the quarantine pathogens *P. kernoviae* and *P. ramorum* is not permitted in the UK, experiments were designed to use natural inoculation. All field work was carried out in naturally infected woodlands or those that have recently been cleared of naturally infected rhododendrons on the same site. Every precaution was taken to prevent transmission of the pathogen in accordance with the procedures stipulated in the Plant Health and Seed Inspectors Handbook. In October 2006 sixty 5-yr-old *Magnolia* x *Soulangeana* saplings were placed in the drip line of the canopy of woodland *Rhododendron ponticum* plants heavily infected with *P. kernoviae*, which served as an inoculum source for the saplings. By November 2006 natural infection of the saplings had been confirmed with LFD tests (an ELISA test kit developed by Pocket Diagnostic, Central Science Laboratory (CSL), York, UK, Lane *et al.,* 2007). Once the leaves had fallen off the saplings and plants were moved to the experiment site which is immediately adjacent (20m) and separated only by a farm track to the woodland where natural infection took place. The bases of the saplings sustained rabbit damage during the process of natural infection. Most pots containing the saplings were also blown over during the infection period causing young stems and branches of the saplings to lie on a bed of infected rhododendron leaves which resulted in severe *P. kernoviae*-induced shoot dieback. The end point of the dieback was marked on five stems of each plant with a cable tie prior to application of the treatments in spring 2007.

The experiment was laid out in a randomised design in a woodland at Killifreth Farm, Cornwall, that previously had *P. kernoviae*-infected *R. ponticum* which were removed 6-mo prior to setting out the experiment. Sixty naturally infected plants were randomly allocated to the three treatments (20 plants per treatment) consisting of: untreated plants (which meant that no chemicals were applied and no wounding took place - stems were not injected with water), phosphonate applied as a stem-injection, phosphonate and Pentrabark applied as a stem-paint. Chemicals were applied under the experimental licenses: AEA 2005/10025PP and AEA 2005/10035PP supplied by the UK Pesticides Safety Directorate. As there was a risk that inoculum production may occur from naturally infected untreated plants and disseminate to nearby plants surrounding them, these plants had to be grouped together to avoid confounding investigation of the effects of phosphonate in inactivating the pathogen with protection from new infections. Therefore, same-treated plants as well as uninfected control plants were randomly positioned within a designated experiment area on the experimental site (see Figure 3). There were thus, three experiment areas (one area per treatment), approximately 10 m apart and these areas were as uniform as possible minimising the possibility of cross infection (Figure 1). The phosphonate formulation used was Agri-fos 400 (supplied by Agrichem Manufacturing Industries Pty. Ltd., Loganholme, Queensland, Australia). Chemical treatments were applied in spring (01 March 2007) prior to bud burst. The average stem diameter of the saplings was approximately 30 mm, thus stem-injections were applied by boring 5mm into the stem and inserting into each sapling a 50 mL spring loaded-syringe (Chemjet® Trading Pty. Ltd., Bongaree, Queensland, Australia) containing 10 ml of chemical. The dosage of the phosphonate was 20% a.i., 15 ml per m canopy diameter as recommended for avocado and cocoa trees (Whiley *et al*., 1995; Opoku *et al*., 1998. Where phosphonate was applied as a stem-paint the surfactant Pentrabark (Agrichem) was mixed with the phosphonate at 2.5% a.i. Since the canopy diameter of the saplings was less than 1m only 10 ml of the solution was applied to each sapling for both the injections and the stem-paints; it took a few hours for the syringes to empty in most cases but some took as much as a day. A 20 mm commercial paint brush was used to apply the phosphonate stem-paint solution to the base of the saplings 10 cm above ground level. Twenty uninfected, untreated plants, 5 per treatment area and 5 on the experiment site beyond the boundaries of the treatment area, were placed as indicator plants to detect inoculum movement. A further 5 uninfected plants per chemical treatment application (i.e. stem-paint or stem-injection) were added to the plants in the appropriate treatment areas as indicators of phytotoxicity.

The following measurements were recorded:

- Laboratory assays quantified the level of phosphonate in a sample of leaves bulked over plants per treatment every fortnight.
- Phytotoxic effects were assessed by visual inspection based on overall appearance of the plant.
- Stem lesion extension was measured monthly.
- At the end of the experiment both leaves and stems were tested for the presence of the pathogen. Isolations were made from the dead-live junctions and leaf scars of the five-tagged stems that had been measured monthly. The remaining stems were cut out of the plants at the end of the experiment and the dead-live junctions were bulked and baited. At least 10 leaves per plant (where available) were also brought to the laboratory for testing. Isolations were made from the three leaves per plant that had been observed monthly; those remaining were surface sterilised and baited. Elisa (LFD) tests were carried out on a bulked sample per plant of stems that had dieback which largely served to indicate that the pathogen had been present there previously.
- Observations on the general condition of plants were recorded at the end of the trial (June 2007).
- Statistical treatments of the re-isolation and stem lesion data were carried out by fitting binomial generalised linear models with logit link.

Figure 1 Phosphonate sapling experiment site layout in Killifreth woodland, Cornwall. Numbers 1–12 indicate the four poles marking the corners of the areas A, B and C.



Experiment 2

Magnolia trees naturally infected with *P. ramorum* in two National Trust heritage gardens, Lanhydrock and Trengwainton, were identified. One tree at Trengwainton was infected with *P. kernoviae*. Trees were selected based on what was available at sites that were amenable to participating in the work. Magnolia infections at these sites were largely caused by *P. ramorum*. Six trees were used at Lanhydrock and three *Magnolia salicifolia* trees at Trengwainton (Table 1). Original natural infections were confirmed either by PHSI sampling (CSL testing) or by Forest Research and although trees were re-tested prior to application of the chemical not all trees tested positive on this occasion (see results section). Phosphonate was applied as a stem-injection at the rate of 20%a.i., 15 mL per m canopy diameter as indicated above on 17 and 18 April 2007. Holes evenly spaced around the perimeter of the tree trunk were drilled with a hand drill to a depth of 30 mm. Spring-loaded syringes containing the appropriate volume of phosphonate were pushed into the holes and the spring catch released so that the chemical solution could be taken up by the xylem of the trees.

Table 1 Location of mature Magnolia species and treatments applied in Experiment 2.

Area	Species	Treatment
Lanhydrock	Magnolia x *loebneri* 'Leonard Messel'	Stem-injected
Lanhydrock	Magnolia x *loebneri* 'Star Bright'	Untreated
Lanhydrock	Magnolia x *loebneri* 'Leonard Messel'	Stem-injected
Lanhydrock	*Magnolia proctoriana*	Stem-injected
Lanhydrock	*Magnolia quinquepeta*	Stem-injected
Lanhydrock	*Magnolia stellata* 'Rosea'	Untreated
Trengwainton	*Magnolia salicifolia*	Stem-injected
Trengwainton	*Magnolia salicifolia**	Stem-injected
Trengwainton	*Magnolia salicifolia*	Untreated

* This tree was infected with *P. kernoviae* while the rest were infected with *P. ramorum*

The following measurements were recorded:

- Levels of phosphonate in foliage were monitored fortnightly for more than two months. Foliage was tested for infection at least twice in the season using isolations from symptomatic material.

- The experiment was evaluated on a binomial basis using visual inspection. The parameter recorded was pathogen absent or present (confirmed through isolation) at the end of the trial (June 2007).

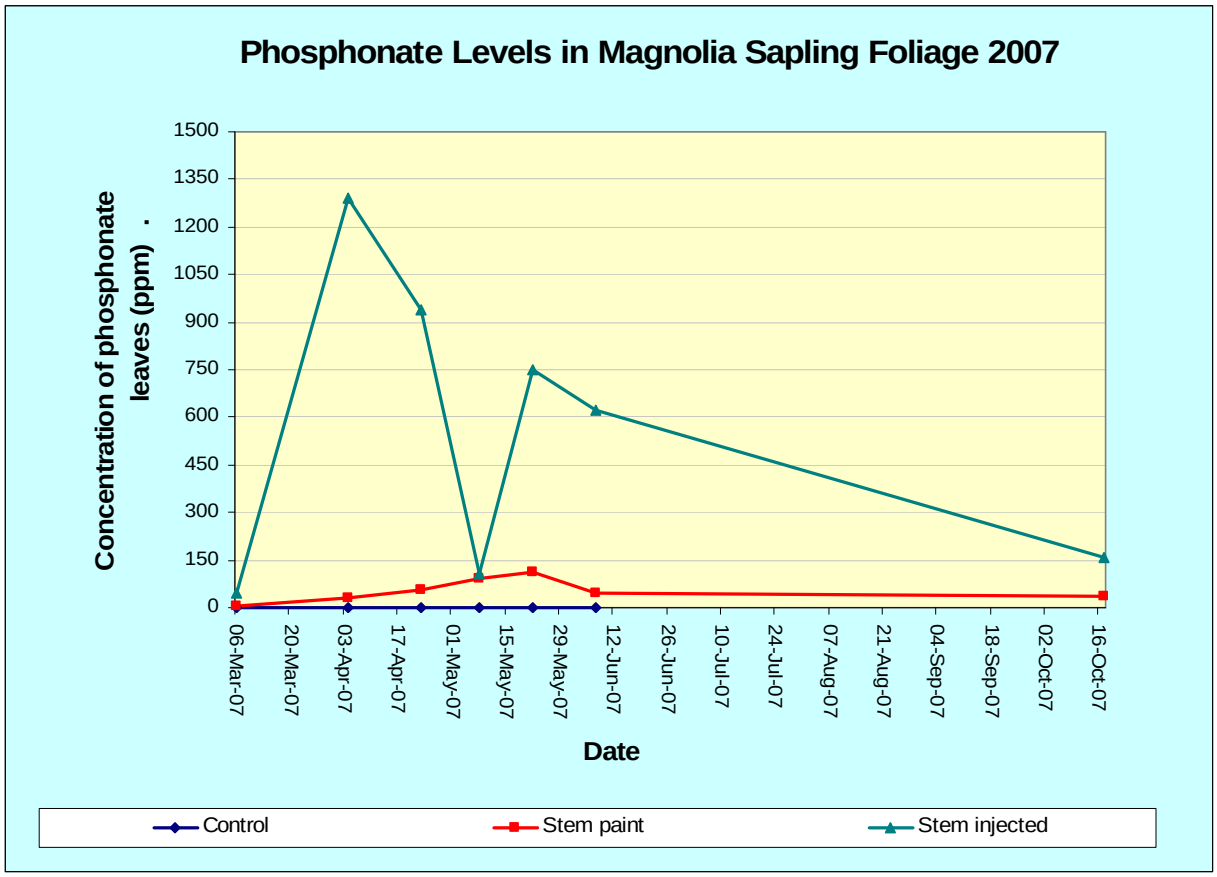
Results

Experiment 1 (Sapling trial)

Phosphonate levels in foliage of saplings

Figure 2 summarises the lab results indicating phosphonate levels in foliage (ppm). Delivering the chemical to the saplings via injection showed a quicker uptake and transportation to the leaves with peak levels being evident 4 weeks after application, compared with stem-painted plants that took 10 weeks for levels to peak. There was a ten-fold increase in the concentration of phosphonate in the foliage of stem-injected leaves compared with the stem-painted plants. The concentration of phosphonate in foliage diminished over time reaching levels of approximately 10% of the peak concentration. Untreated controls had negligible levels of phosphonate levels throughout the test period.

Figure 2 Phosphonate levels in leaves of treated magnolia saplings in Experiment 1.



Phytotoxicity

Phytotoxic effects were observed in the stem-injected plants only. The injected plants were extremely slow to leaf out and the spring flush was very poor. Leaves were small, yellowish in colour and appeared weak at first. The summer flush leaves (which probably appeared late June to July) were much stronger and healthier, appearing normal when appraised in October 2007. Naturally infected, stem-injected plants fared worse than the uninfected, injected plants. Seven out of 20 (35%) infected, injected plants died (Table 2). Typically the infected, injected plants also showed very severe and rapidly progressing shoot dieback (Table 3). Certainly there was up to 800 mm dieback on the shoots of injected saplings so that the summer growth of 2006 and some spring 2006 growth had no leaf flushing at all. A large amount of the dieback was initially pathogen induced as confirmed by the positive LFD tests. However, this dieback seems to have been compounded by phosphonate phytotoxicity since four of five uninfected stem-injected plants also suffered shoot dieback although it was much less severe than the infected, injected plants (Table 3). Exposure to severe weather may also have advanced dieback. Phytotoxic effects were not evident on the stem-painted plants.

Table 2 Survival and re-isolation of *P. kernoviae* at the end of Experiment 1 in June 2007.

Area	Infected	Treatment	Total saplings	Final *P. kernoviae* positive saplings	Final saplings dead
A	Infected	Untreated	20	12	0
A	Uninfected	Untreated	5	0	0
B	Infected	Stem-painted	20	15	0
B	Uninfected	Stem-painted	5	3	0
B	Uninfected	Untreated	5	3	0
C	Infected	Stem-injected	20	0	7
C	Uninfected	Stem-injected	5	1	0
C	Uninfected	Untreated	5	1	0

Isolation results

Stem injections significantly reduced re-isolation of the pathogen ($P<0.001$). None of the naturally infected, stem-injected plants tested positive for the pathogen at the end of the trial (Table 2). Although most plants showed LFD positive tests from the dead stem tissue indicating initial infection with *P. kernoviae*, neither leaf nor stem isolations or baiting techniques yielded the pathogen. A single originally uninfected, injected sapling yielded *P. kernoviae* from isolation of symptomatic foliage at the end of the experiment. Additionally a single uninfected untreated indicator plant also tested positive for *P. kernoviae* in this area (see Figure 3, Area C, for distribution of infected plants).

Table 3 Mean stem lesion length and extension over time on magnolia saplings treated with phosphonate.

Recording period	Untreated (cm)	SE [c]	Stem-injected (cm)	SE	Stem-painted (cm)	SE
February[a]	37.90	3.03	16.74	1.45	24.98	2.13
March [b]	2.50	0.40	2.69	0.41	2.89	0.36
April	9.25	1.03	20.18	1.80	8.70	0.56
May	15.33	1.33	73.68	7.05	17.02	0.73
June	19.66	1.43	81.92	4.58	21.74	0.73

[a] Original lesions formed during natural infection were recorded in February at the end of winter

[b] Lesion extensions were recorded additively on a monthly basis from March through June

[c] SE = Standard Error

In the stem painted plants 75% (15/20 plants) yielded *P. kernoviae* upon isolation (Table 2); 13/20 plant stems tested positive and 7/20 plants had leaves that tested positive (some plants had both leaves and stems that were positive). These figures are not significantly different from the untreated controls. On the stems the pathogen was isolated from leaf scars and the dead-live junctions on stems, as well as being baited from a number of plant stems. Two of the five uninfected, stem-painted plants yielded *P. kernoviae* from symptomatic foliage that was isolated at the end of the trial in June 2007 (see Figure 3, Area B, for distribution of infected plants).

In the naturally infected, untreated control plants the pathogen could be isolated from 60% (12/20) of saplings (Table 2). All most all the positive isolations were from stems, but 3 plants yielded *P. kernoviae* from foliage (see Figure 3, Area A, for distribution of infected plants).

None of the indicator plants (uninfected, untreated saplings) were positive in the untreated experiment area (Area A), but three were positive in the stem painted area (Area B) one in the stem-injected area (Area C) of the experiment (see Figure 3 for distribution of infected plants). A number of the indicator plants that became infected were in close proximity to plants that tested positive for foliage infections (see Figure 3 for distribution of infected plants).

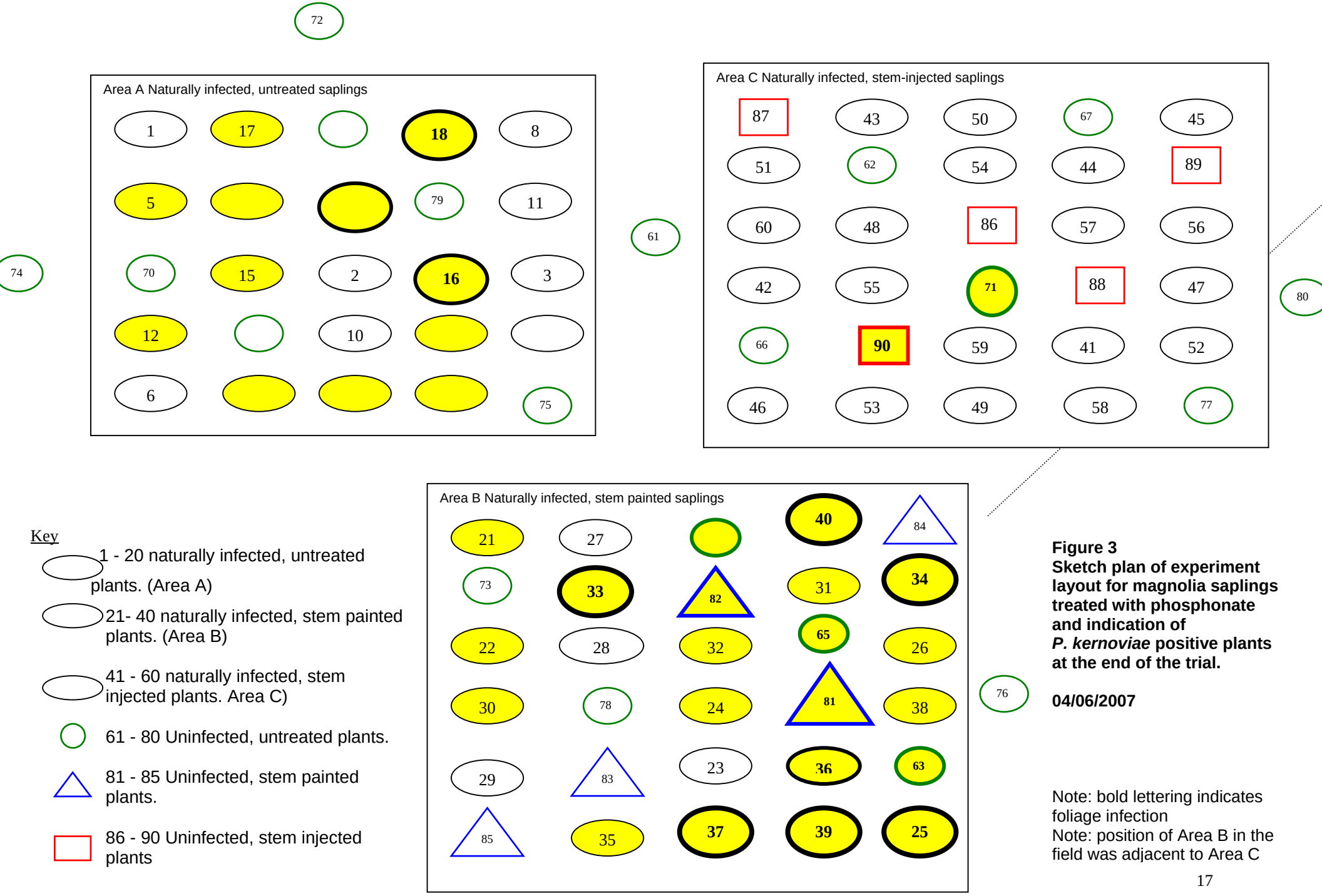


Figure 3
Sketch plan of experiment layout for magnolia saplings treated with phosphonate and indication of *P. kernoviae* positive plants at the end of the trial.

04/06/2007

Note: bold lettering indicates foliage infection
Note: position of Area B in the field was adjacent to Area C

Stem lesion extension

The stem lesion length showed a steady increase in size of lesions over the four-month observation period (Table 3). The overall increases were larger for the stem-injected treatment but differences between untreated controls and stem-painted plants were not significant.

Experiment 2 (Mature magnolia Experiment)

Phytotoxicity

Black ooze exuded from all injection wounds (Figure 4). A similar response has been reported from cocoa trees injected with phosphonate and was described as a phytotoxicity, therefore based on this information I interpret the response of the magnolias to be a phytotoxicity reponse but in future trials it will be necessary to prove that this is not a wound reponse. At Trengwainton by the end of the growing season there was still active bleeding and peeling away the bark revealed a restricted, zone of inner bark death down to the wood (25 cm long x 3 cm wide and 4 mm deep) (Figures 5 and 6). However, trees at the two sites responded differently over the longer term. At Lanhydrock by autumn the injection wounds had healed over with callus tissue and there was very little evidence of a previous bleeding response (Figure 7), consequently the outer bark was not peeled away on trees at this site. There were no other phytotoxic effects evident, i.e. foliage was normal, at both sites.

Phosphonate levels in foliage of injected trees

The levels of phosphonate in foliage are indicated in Figures 8 and 9. At Trengwainton although the same magnolia species was treated the trees responded differently in up take of phosphonate. The tree in the walled garden (TTN SD 17) which was a slightly younger tree (22 years-old) than the other reached peak levels (92 ppm) within a fortnight of being injected and then levels fluctuated before diminishing. On the other hand the other magnolia (TTN SD 14) which was 27 years-old took longer to reach peak level (one month), the highest level attained was more than three times the concentration of the other (330 ppm). This tree was older and the canopy was significantly denser than the former. See discussion for possible explanations about the differences in up take.

At Lanhydrock most of the injected trees reached peak levels shortly after application, except *M. quinquepeta* and *M. proctoriana*. The concentration of phosphonate in leaves of the different trees also varied quite a lot (Fig 7) ranging from approximately 100 ppm to 600ppm. Generally the level diminished consistently once the peak had been reached.

Figure 4 Phytotoxic response of *Magnolia salicifolia* stem-injected with phosphonate (20% a.i.; 15mL per m canopy diameter).

Figure 5 Outer bark peeled away to show shallow phototoxic response around injection hole on *M. salicifolia* lesion (25 cm x 3 cm).

Figure 6 Depth of phytotoxic response of *M. salicifolia* to phosphonate injected into the stem.

Figure 7 Callus response of *M. proctoriana* at Lanhydrock October 2007, following phosphonate injected into the stem in spring 2007.

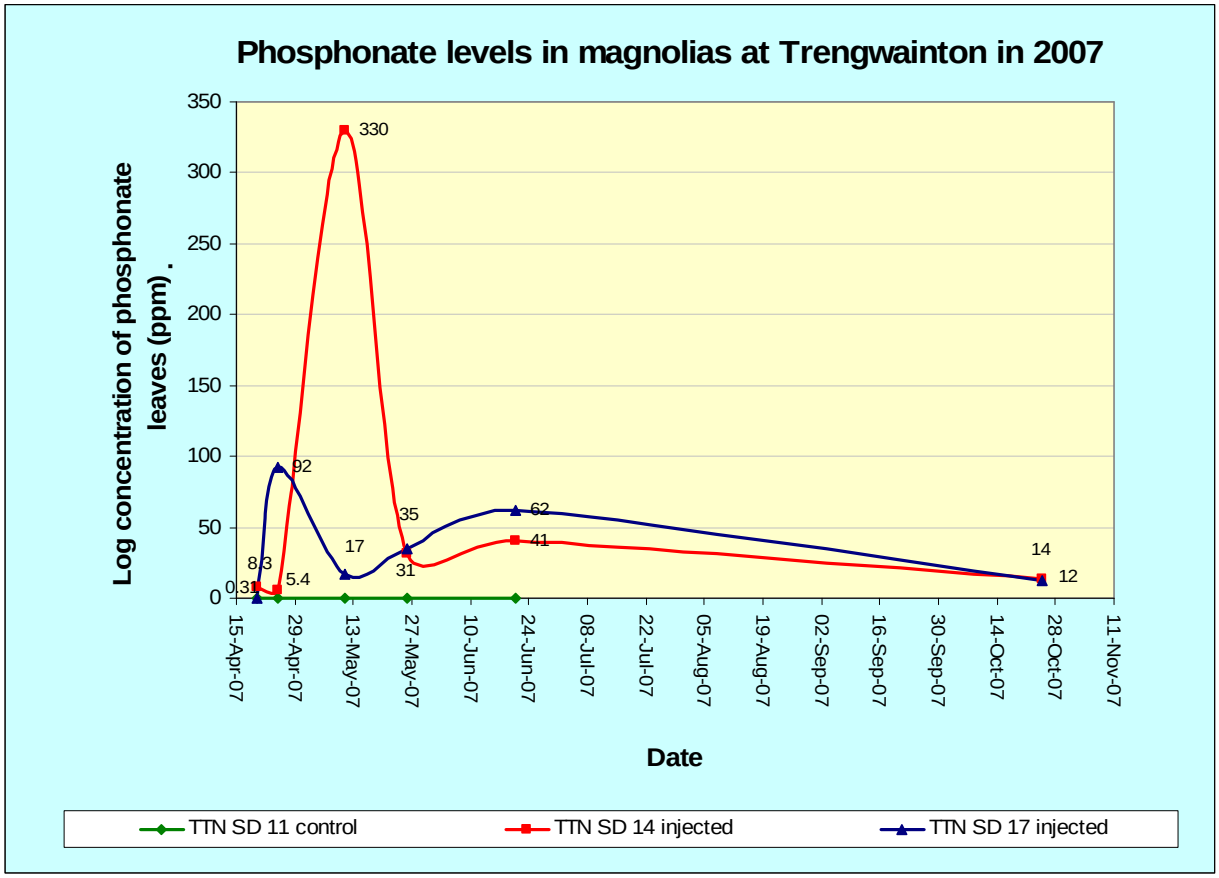


Figure 8 Phosphonate levels in magnolia foliage at Trengwainton in Experiment 2.

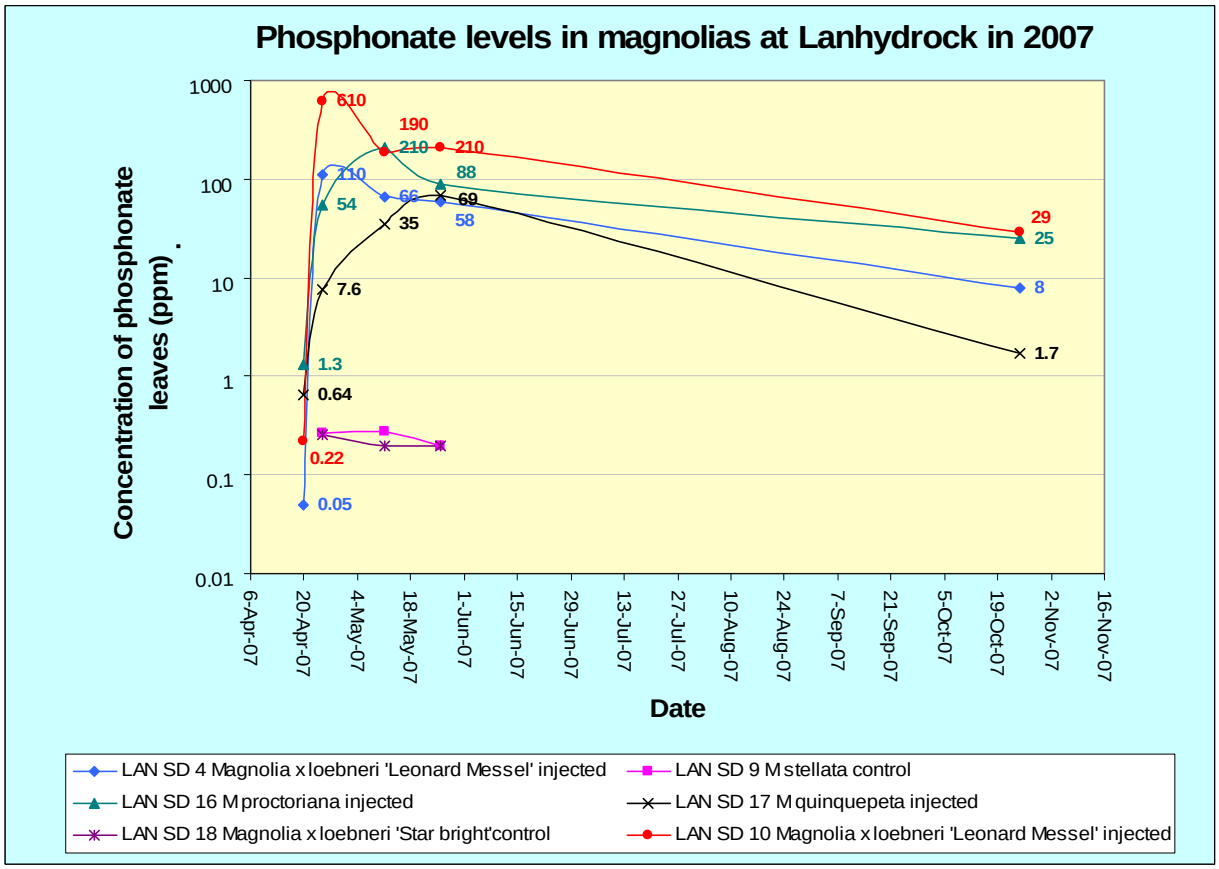


Figure 9 Phosphonate levels in magnolia foliage at Lanhydrock in Experiment 2.

Isolation results

These are summarised in Table 4. Foliage was tested at least three times a year (in most cases) once during the spring (April through June), once in high summer (August) and finally in autumn (October). Two of the three control plants tested positive for *P. ramorum* in spring and autumn but only one of the three tested positive in summer. In the spring tests on the controls one of the positive plants was at Lanhydrock and one was at Trengwainton whereas in autumn both controls were positive at Lanhydrock but the one at Trengwainton was not. The summer positive was found at Lanhydrock.

By contrast at Lanhydrock the isolations from the stem injected plants all gave negative results, with the exception of the highly susceptible M x *loebneri* 'Leonard Messel', which only became positive in autumn when phosphonate levels were low. The positive in autumn is like to be caused by external inoculum that could infect the leaves because the levels of phosphonate were too low to protect the plant by this time. Alternatively, the autumn infection may also be caused by inoculum that survived the spring dose of phosphonate and lay dormant until levels of the chemical were too low to have an effect against infection. At Trengwainton on the other hand, one of the injected trees was positive in spring while the other was positive in summer; neither was positive in autumn. The positive in spring is probably a consequence of levels of phosphonate being too low and rising too slowly during the critical period. The absence of infection is autumn is difficult to explain but could be down to human error (poor sampling or failure to isolate it).

Observations

Disease incidence on control plants at Lanhydrock was much higher than on injected trees. Disease was also present in both the inner and outer canopy of controls, but on the injected plants disease was often not visible on the outer canopy and leaves with lesions had to be sought from the inner canopy. In some cases lesions were present on leaves but the pathogen was not isolated. It is thus uncertain what the cause of the symptom was. This was the case at Trengwainton in the October isolations.

Table 4 Outcome of isolation tests for *P. ramorum* or *P. kernoviae* on foliage of treated or untreated on mature magnolias in heritage gardens

Site	Species	Tree Number	Treatment	Date	Test Result	Authority carrying out tests
Trengwainton	*M. salicifolia*	TTN SD 11	Prior to treatment	2006	Pr positive[a]	PHSI/CSL[b]
			Control (No treatment)	May 2007	Pr positive	FR[c]
				August 2007	Negative	FR
				October 2007	Negative	FR
Trengwainton	*M. salicifolia*	TTN SD 14	Prior to treatment	November 2006	Pr positive	FR
			Stem-injected	May 2007	Pr positive	FR
				August 2007	Negative	FR
				October 2007	Negative	FR
Trengwainton	*M. salicifolia* (infected with *P. kernoviae*)	TTN SD 17	Prior to treatment	2006	Pk positive	PHSI/CSL
				June 2007	Negative	FR
				August 2007	Pk positive	FR
				October 2007	Negative	FR
Lanhydrock	*M.* x *loebneri* 'Leonard Messel'	LAN SD 4	Prior to treatment	November 2006	Pr positive	PHSI/CSL
			Stem-injected	April 2007	Negative	FR
				August 2007	Negative	FR
				October 2007	Pr positive	FR
Lanhydrock	*M. stellata*	LAN SD 9	Prior to treatment	November 2006	Pr positive	PHSI/CSL
			Control (No treatment)	June 2007	Pr positive	FR
				August 2007	Pr positive	FR
				October 2007	Pr positive	FR
Lanhydrock	*M.* x *loebneri* 'Leonard Messel'	LAN SD 10	Prior to treatment	November 2006	Pr positive	PHSI/CSL
			Stem-injected	April 2007	Negative	FR
				August 2007	Negative	FR
				October 2007	Negative	FR
Lanhydrock	*M. proctoriana*	LAN SD 16	Prior to treatment	November 2006	Pr positive	PHSI/CSL
			Stem-injected	April 2007	Not tested	–
				August 2007	Negative	FR
				October 2007	Negative	FR
Lanhydrock	*M. quinquepeta* x *stellata* 'Jane'	LAN SD 17	Prior to treatment	November 2006	Pr positive	PHSI/CSL
			Stem-injected	April 2007	Not tested	-
				August 2007	Negative	FR
				October 2007	negative	FR
Lanhydrock	*M.* x *loebneri* 'Star Bright'	LAN SD 18	Prior to treatment	November 2006	Pr positive	PHSI/CSL
			Control (No treatment)	April 2007	Not tested	–
				August 2007	Negative	FR
				October 2007	Pr positive	FR

[a] Pr positive = foliage positive for *P. ramorum.* Pk positive = foliage positive for *P. kernoviae*.
[b] PHSI = UK Plant Health and Seed Inspectorate.
[c] CSL = Central Science Laboratory.
[d] April through June tests approximately equate to spring season. August tests equate to mid-summer tests; October tests equate to autumn tests.

Discussion and conclusions

The sapling trial laid an excellent foundation for fine tuning further detailed work on the effects of phosphonate on inactivation of the pathogen and its sporulation. It demonstrated the need for determining dose-response tests for phytotoxicity but indicated a potential ideal range of phosphonate concentrations between 300–600 ppm. At concentrations below 100 ppm there was no significant difference from untreated plants, in fact, although not statistically validated in this trial, there was a suggestion that there could even be enhancement of infection if levels of phosphonate are too low. Further research would have to validate this hypothesis.

The lack of control over inoculation of the plants significantly impeded achieving the goal of determining the efficacy of phosphonate on inactivating overwintering infections. Severe stem infection is not the normal mode of *P. kernoviae*-initiated disease on this host, while foliage infections spreading into the petioles and overwintering in leaf scars are common. The stem infections dominated, overriding leaf scar infections killing stems and buds in many cases, thereby interfering with the normal epidemiological pattern of infecting spring foliage. Thus further work on this aspect should be carried out in a controlled environment with controlled inoculation points simulating natural infections.

The sapling work enabled us to determine the most effective method of applying phosphonate to magnolias to get the chemical to the developing buds and leaves. Application of phosphonate to saplings as a stem-paint was ineffective. The stem injections were clearly the most effective method of getting the chemical systemically into the plant. Although there may be a case for using chemical sprays to deliver the phosphonate to buds and leaves the practical considerations of this method render it not feasible in garden environments. However, experience of stem-injections in both the sapling and mature tree trials indicates that some work is required on how to reduce the initial stem-tissue phytotoxicity response of the trees. Applying larger volumes of a dilute solution of phosphonate but delivering the required total amount of active ingredient could be investigated. Furthermore, further investigation is required to determine means of prolonging the presence of the active ingredient in foliage over high-risk periods. More frequent applications of phosphonate may fulfil this requirement.

The mature tree trials have yielded encouraging first results although there was some variability in the response of the trees. The differences in bleeding reactions to phosphonate

injections at the two different sites may in part be attributed to the application technique. Trengwainton was the first site on which the trees were injected. Mindful of the negative impact of potential damage to the trees we were over cautious in applying the chemical and did not drill deeply enough into the stem. Therefore quite a lot of the chemical was probably delivered in a concentrated form to the phloem tissue of the tree where it caused necrosis. A more robust application to a depth of 25–40 mm in diffuse porous trees seems to enable the trees to heal the wound site and not to cause undue damage to the inner bark. Experience at Lanhydrock where the depth of injecting was about 30 mm supports this conclusion. Since control trees were not wounded and injected with water it will be important in future to establish that the bleeding response is in fact due to phytotoxicity rather than to wounding.

Both observations and isolation tests indicated that phosphonate appeared effective in lowering disease incidence at Lanhydrock, but results were more variable at Trengwainton. This is probably in part due to the less robust method of application of the chemical at Trengwainton but may also partly be attributed to the rapid peak and decline of phosphonate levels in leaves at this site. Much remains unknown about the uptake and distribution of the chemical in mature trees. At Trengwainton the phosphonate had dropped to very low levels within about a month of application, whereas at Lanhydrock the decline curve was not as steep. At both sites however, low levels were present in autumn, when as previous research has shown, a second flush of inoculum is usually available for late season infections. The positives in October at Lanhydrock are thus probably attributable to low levels of the chemical in the leaves, host resistance responses slowing down (tree preparing for winter) and external inoculum available for infection. The autumn infections are an important means for the pathogen to overwinter and thus perpetuate new spring infections the following season. A method to create an extended spring application of phosphonate that would maintain an effective dose of the chemical in leaves needs to be developed and the efficacy of an autumn application of the chemical also needs to be investigated.

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