CHITOSAN INDUCES RESISTANCE TO PITCH CANKER IN PINUS RADIATA

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ABSTRACT

Foliar application of chitosan on *Pinus radiata* D.Don seedlings resulted in the induction of resistance to wound-inoculation with *Fusarium circinatum* Nirenberg & O'Donnell, the causal agent of pitch canker. Induced resistance was expressed as the suppression of symptom development on treated seedlings compared to the controls. Chitosan treatment protected seedlings against wound inoculation with *ca* 100 spores per plant, and reduced disease incidence by 60% and deadtop development by 50%, compared to water-treated controls. However, chitosan efficacy was inoculum-dose dependent and no significant disease control was observed when treated seedlings were challenged with 500 or 8500 spores. At lower inoculum levels (10 spores/seedling) chitosan-treated seedlings remained more resistant to inoculation for at least 6 weeks after application. Furthermore, chitosan-induced resistance was shown to be systemic on 4-year-old *P. radiata* trees when chitosan application and inoculation were spatially separated. Due to the quarantine status of *F. circinatum* in New Zealand, inoculations were performed using *Sphaeropsis sapinea* (Fr.) Dyko & B.Sutton for this aspect of the study. Localised chitosan application induced an 86% reduction in average lesion length on adjacent untreated branches compared to their water-treated counterparts. These results demonstrate that chitosan can induce systemic disease resistance in *P. radiata* and there is potential for induced resistance in forest nursery disease management.

Keywords: induced resistance; chitosan; pitch canker; *Pinus radiata*; *Fusarium circinatum*; *Sphaeropsis sapinea.*

INTRODUCTION

Pitch canker disease of pines, caused by *Fusarium circinatum*, has proved to be extremely destructive to natural stands and amenity plantings of *Pinus radiata* in California (Correll *et al.* 1991; Storer *et al.* 1994, 2002), and has been devastating in pine-growing nurseries in a number of countries (Viljoen *et al.* 1994; Wingfield *et al.* 2002). It is potentially an extremely serious disease for plantation forestry in New Zealand (Dick

1998). Even in nurseries, conventional disease control strategies have not been totally effective against pitch canker (M.J.Wingfield pers. comm.). Whilst selection for resistance may be viable in the long term (Gordon *et al.*1998; Storer *et al.* 1999), it can provide no protection for trees currently growing in plantations or nurseries. Alternative strategies to reduce disease incidence and spread in the event of an incursion of *F. circinatum* into New Zealand are therefore being sought.

There is increasing interest in the practical application of elicitors for crop protection (Lyon & Newton 1999; Tally *et al.* 1999). Elicitors function by stimulating plant defences, thereby accelerating and intensifying the resistance response to subsequent infections (Hammerschmidt 1999). Various elicitors, including salicylic acid, jasmonic acid, as well as several carbohydrates, lipids, proteins, and glycoproteins (Lyon *et al*. 1995; Hahn 1996), have been identified. Furthermore, topical application of elicitors has been reported to induce resistance against fungal, bacterial, and viral pathogens in several economically important crops (Kuc 1987; Tuzun & Kloepper 1995; Oostendorp *et al.* 2001).

Oligosaccharides from fungal cell walls — such as glucan, chitin, and chitosan — are naturally released at the plant/pathogen interface during the infection process, and are amongst the most potent elicitors known (Cote & Hahn 1994). Crude fungal wall extracts have been shown to elicit defence-related mechanisms in suspension cultures of *Pinus banksiana* Lambert (Campbell & Ellis 1992 a, b) and *P. radiata* (Hotter 1997). Similarly, chitosan elicited phenylpropanoid and lignin biosynthesis in *Pinus elliottii* Engelmann (Lesney 1989; Mason & Davis 1997) and also stimulated monoterpene synthesis at wound sites in *Pinus contorta* Douglas (Miller *et al.* 1986; Croteau *et al.* 1987; Lieutier & Berryman 1988). These important defensive responses in conifers are involved with countering pathogen attack and sealing wounds. Chitosan has also been shown to be directly inhibitory to the growth of forestry root-rot pathogens, including *Fusarium oxysporum* Schlectendahl and *F. acuminatum* Ellis & Everhart (Laflamme *et al.* 1999) and has been investigated as a possible antifungal treatment for wood (Kumagai *et al.* 1990).

There are very few reports demonstrating the use of elicitors to protect conifers against subsequent pathogenic infection. Foliar application of 5-chlorosalicylic acid (5CSA) caused a reduction in susceptibility of *P. radiata* seedlings to infection with *Sphaeropsis sapinea* (Reglinski *et al.* 1998). Similarly, exposure of Norway spruce (*Picea abies* (L.) Karsten) seedlings to methyl jasmonate for 3 days resulted in a reduction in the incidence of damping-off after inoculation with *Pythium ultimum* Trow (Kozlowski *et al.* 1999). In both examples the reduction in disease incidence was attributed to the induction of host defence mechanisms.

We investigated the potential for using chitosan to induce resistance in *P. radiata* seedlings against fungal infection. The two specific objectives were to determine the effectiveness of chitosan in protecting *P. radiata* seedlings against *F. circinatum*, and to establish whether the induced response was local or systemic.

METHODS

Trial Location and Plant Material

Experiments were conducted in the Forest Research Quarantine Facility or a shade house at Forest Research, Rotorua, New Zealand. *Pinus radiata* seed obtained from the

Forest Research nursery was stratified in water for 24 hours before being sown. For Experiment One, seedlings were grown in pots (170 × 170 mm) with 9–12 plants per pot. For Experiment Two, seedlings were grown in HIKO V93 trays (40 plant cells/tray). In each experiment the plants were reared in a glasshouse and transferred to the Quarantine Facility before inoculation. The trees used for Experiment Three were 4-year-old *P. radiata* shoot cuttings with a physiological age of approximately 7 years. These trees were too large for the Quarantine Facility and so, for biosecurity reasons, inoculations were made using *S. sapinea*.

Chitosan Preparation

Chitosan (crab shell, minimum 85% deacetylated, Sigma Chemical Co., St Louis, Mo. USA) was ground to a powder and then 10 g were solubilised by stirring for 4 h in 500 ml of 1 M acetic acid. Insoluble material was removed by filtration through three layers of cheesecloth. The filtrate was then precipitated by neutralisation with 2 M NaOH and the chitosan recovered by centrifugation at 10 000 rpm for 20 minutes. The chitosan pellets were thoroughly washed with milliQ water to remove salts and then freeze dried. The freeze-dried powder was dissolved in 0.025 M acetic acid and the pH adjusted to 6 with 1 M NaOH to obtain a 1 mg/ml stock solution. The stock was diluted in water to obtain a chitosan concentration of 0.1 mg/ml and a pH of 5.5 for all experiments.

Inoculum Preparation

Fusarium circinatum (NZFS 308B ex *P. radiata* Santa Cruz, California) was maintained on 3% w/v malt extract agar (MEA). Fresh cultures, incubated for 5 days at 25°C in the dark, were used for inoculum preparation. Spores were harvested by pouring 2 ml of sterile distilled water on to the culture, agitating the surface of the mycelium with a sterile spatula, and then collecting the resultant suspension. The concentration of spores was estimated using a haemocytometer and adjusted to the desired concentration (Tables 1 and 2) with sterile deionised water. Spore viability was routinely tested by carrying out a serial dilution, pipetting a standard amount from each dilution to 3% (w/v) MEA petri plates (three replicates per dilution), and counting the resultant colonies. *Sphaeropsis sapinea* (NZFS 897) was cultured on 3% w/v MEA. Mycelial plugs taken from 1-week-old *S. sapinea* cultures were used as inoculum.

Seedling Treatment and Wound Inoculation

Experiment One

Six-month-old *P. radiata* seedlings were sprayed to run-off with either chitosan (0.1 mg/ml) or sterile water. Pulse® (a.i. 1.02 g/litre polyether modified polysiloxane, Monsanto, Wellington, New Zealand) at 0.05% (v/v) was included as a wetting agent/ penetrant in each treatment. Treatments were applied using a pressurised sprayer with eight replicate pots containing 9–12 seedlings per pot for each treatment. Chitosan was applied on two occasions, 14 days and 7 days before inoculation. Water was applied 7 days before inoculation. When dry, the seedlings were transferred to the Quarantine Facility where the ambient temperature was 19–20°C with 16:8 h light:dark. Seedlings were inoculated by removing one needle fascicle, 2–5 cm from the apex of each seedling, and placing a 5-µl

droplet of *F. circinatum* spore suspension containing 2×10^4, 1×10^5, or 1.7×10^6 spores/ml on the wound site. This equated to approximately 100, 500, or 8500 spores per inoculation respectively. Plants were examined twice weekly for symptom development. The first indication of infection was a purplish oily lesion at the inoculation point. Infection was scored on each occasion in the following categories: (a) lesion length, (b) dead top — wilting and death of the crown, and (c) mortality — death of the seedling. Isolations were carried out from representative plants exhibiting various levels of dieback. Plants were harvested, surface sterilised in 10% hypochlorite, rinsed in sterile water, and then cut into segments and plated on to potato dextrose agar (PDA). Petri dishes were incubated at 22°C and examined after 7 days for fungal colonies.

Experiment Two

Three-month-old *P. radiata* seedlings were sprayed to run-off with either chitosan (0.1 mg/ml), thiabendazole at 30 g a.i./litre, or sterile water. Pulse at 0.05% was added to each treatment. There were 27 replicate plants per treatment. Chitosan was applied at either 6 weeks or 1 week prior to inoculation. Water and thiabendazole were each applied 1 week before inoculation. Seedlings were inoculated with *F. circinatum* suspensions containing 2×10^3 spores/ml (*ca* 10 spores per inoculation site) using the same techniques as described in Experiment One. Symptom development was recorded at regular intervals up to 50 days post-inoculation. Isolations from inoculated plants were carried out as described above.

Experiment Three

Experiment Three was carried out on 4-year-old *P. radiata* cuttings (physiological age of approximately 7 years) in the shade house. Chitosan (0.1 mg/ml) in 0.05% Pulse was applied with a hand sprayer to one single branch per tree on two occasions, 8 days prior to inoculation and again 1 day before inoculation. Control plants were similarly treated with 0.05% Pulse in water. There were 25 replicates per treatment. Inoculations were carried out on adjacent untreated branches of each tree by cutting a shallow wound (12–15 × 5–8 mm × 1 mm deep) on the upper side of the branches and then placing a 5-mm-diameter plug of *S. sapinea* mycelium on to the fresh wound. The plug was wrapped with moist cotton wool and held in place by a strip of plastic film. The inoculated branches were harvested 4 weeks later and the lengths of visible lesions were measured.

Statistical Analysis

Treatment effect on disease incidence and lesion size were determined by analysis of variance (ANOVA), with each seedling treated as an individual replicate. Differences between treatment means were separated by the Least Significant Difference (LSD) at $p>0.05$, $p>0.01$, and $p>0.001$. All statistical analyses were carried out using GENSTAT 5.

RESULTS

There was a significant reduction ($p<0.05$) in disease incidence on *P. radiata* seedlings that were treated with chitosan before wound inoculation with 100 *F. circinatum* spores per wound site (Table 1). At 21 days post inoculation, only 25% of chitosan-treated plants had developed disease symptoms compared to 63% of the water-treated control plants.

TABLE 1–Disease symptoms on *P. radiata* seedlings treated with chitosan and then inoculated with *F. circinatum* at 2×10^4 (low), 1×10^5(medium), and 1.7×10^6 (high) spores/ml (100, 500, and 8500 spores per wound site respectively). Data were recorded 21 days post inoculation.

	Incidence (%)	Average lesion length (mm)	Deadtop (%)	Mortality (%)
Water – 100 spores	63	37	42	2
Chitosan – 100 spores	25*	44	21*	0
Water – 500 spores	52	47	38	4
Chitosan – 500 spores	55	47	42	4
Water – 8500 spores	71	38	48	13
Chitosan – 8500 spores	65	44	46	8
LSD	22	13	17	11

* Significantly ($p<0.05$) better than respective water control

Furthermore, disease symptoms were less severe in treated plants than control plants, with dead tops occurring in 21% of chitosan-treated seedlings compared to 42% of water-treated seedlings. No significant treatment effect was observed on seedlings that were inoculated with 500 or 8500 spores per wound site.

In Experiment Two, chitosan treatment slowed down the onset and severity of disease whether applied 1 week or 6 weeks before inoculation (Table 2). Average lesion length was significantly smaller ($p<0.05$) on chitosan-treated seedlings than on untreated controls for the duration of the experiment. By 32 days post-inoculation, 82% of water-treated controls had developed dead top compared to only 44% of the seedlings treated with chitosan 6 weeks earlier ($p<0.05$). Furthermore, by this phase of the trial 11% of water-treated controls had died compared to 0% mortality amongst the fungicide- and chitosan-treated seedlings. By 50 days post-inoculation, the mortality level had risen to 78% for water-treated controls compared with only 29–37% for chitosan-treated seedlings ($p<0.01$). There was 52% mortality amongst fungicide-treated seedlings but this was not significantly different to the control. In the *F. circinatum* studies the pathogen was isolated from plants expressing symptoms and confirmed as the causal agent; no other pathogenic fungi were isolated.

Localised chitosan application induced systemic resistance to wound inoculation with *S. sapinea* in 4-year-old *P. radiata* cuttings (Table 3). Average lesion lengths on branches that were untreated, but were adjacent to chitosan-treated branches on the same tree, were significantly ($p<0.001$) smaller (19.6 mm) than lesions on respective water-treated controls (49.6 mm). Isolations from infected plant material confirmed *S. sapinea* as the causal agent.

DISCUSSION

There is increasing interest in the practical implementation of induced resistance for disease management (Lyon & Newton 1999; Oostendorp *et al.* 2001; Tuzun & Kloepper 1995). In this study we demonstrated that foliar application of chitosan can induce resistance in *P. radiata,* and that the induced resistance response is systemic. Chitosan is a potent elicitor of defence mechanisms in conifers (Miller *et al.* 1986; Lieutier & Berryman

TABLE 2–Disease incidence and severity on *P. radiata* seedlings 15, 32, and 50 days after wound inoculation with *F. circinatum* at 10 spores/wound site.

	Incidence (%)			Average lesion length (mm)			Dead top (%)			Mortality (%)		
	15 days	*32 days*	*50 days*	*15 days*	*32 days*	*50 days*	*15 days*	*32 days*	*50 days*	*15 days*	*32 days*	*50 days*
Water	81	96	97	10	35	36	7	82	0	0	11	78
Fungicide	48*	78*	82	7*	28*	16*	15	66	0	0	0	52
Chitosan 6 weeks pre-inoculation	52*	85	85	6*	25*	23*	15	44*	0	0	0	30*
Chitosan 1week pre-inoculation	63	89	92	6*	21*	27*	18	74	0	0	0	37*
LSD	26	17	16	1.3	8	8	19	26	0	0	10	27

* Significantly (p<0.05) better than untreated control,

TABLE 3–Systemic induction of resistance to *S. sapinea* on 4-year-old *P. radiata* trees.

Treatment	Average lesion length (mm)
Water control	49.6
Chitosan	19.6*
LSD	7.5

* Significantly (p<0.001) better than untreated control

1988; Lesney 1989; Mason & Davis 1997) but, to our knowledge, it has not previously been shown to elicit systemic resistance to pathogens in coniferous species.

Induced resistance is generally characterised by a reduction in the size and incidence of lesions that develop on elicitor-treated plants after inoculation (Hammerschmidt 1999). Foliar application of chitosan reduced disease incidence on *P. radiata* seedlings inoculated with *ca*100 *F. circinatum* spores/wound, but did not elicit effective resistance in seedlings challenged with the higher inoculum levels (500 and 8500 spores/wound). This inoculum dose dependence may indicate the limitations of the induced host response. However, it is estimated that some twig beetle vectors (*Pityopthorus* spp.) carry less than 10 spores per individual (Gordon *et al.* 1998), and so this result may not necessarily reflect the potential for induced resistance in natural conditions. *Fusarium circinatum* had a devastating effect on container-grown pine seedlings in South Africa (Viljoen *et al*. 1994), and it was reported (M.J.Wingfield pers. comm.) that fungal mycelia spread rapidly across the soil to infect tender young plants. Chitosan efficacy in these circumstances, where the relevance of inoculum concentration is not known, remains to be determined.

Having established the ability of chitosan to induce resistance in *P. radiata* seedlings, our next goal was to investigate the duration of the induced response. For this study *ca* 10 *F. circinatum* spores were applied per wound site in order to reflect more natural inoculum levels, as discussed above. Previous studies on *P. radiata* indicated that treatment with 5-chlorosalicylic acid induced resistance to *S. sapinea* inoculation for up to 32 days after application (Reglinski *et al.* 1998). In this investigation, chitosan treatment enhanced resistance in *P. radiata* seedlings to *F. circinatum* inoculation for at least 42 days. Induced resistance was expressed as a reduction in disease severity rather than a reduction in disease incidence. Indeed, by 50 days post-inoculation there was no difference in disease incidence between treatments. However, average lesion length and seedling mortality were significantly lower in chitosan-treated seedlings than in water-treated controls.

The next objective of this study was to determine whether the chitosan-induced resistance response in *P. radiata* was systemic. The quarantine status of *F. circinatum* in New Zealand dictated that all experiments involving this pathogen should be carried out in a containment facility. However, since the 4-year-old trees selected for systemic studies could not be relocated to this facility we elected to challenge the treated trees with *S. sapinea* which shares similarities with *F. circinatum* in that it can infect through wounds in shoots and stems, and dieback may follow. The finding that chitosan application induced systemic resistance to *S. sapinea* in 4-year-old *P. radiata* trees is arguably the most interesting and exciting result in our investigation. To our knowledge this is the first report of foliar elicitor application resulting in the systemic induction of disease resistance in

coniferous trees. Induction of resistance to *S. sapinea* has previously been reported in 5CSA-treated *P. radiata* (Reglinski *et al.* 1998), and to *Pythium ultimum* in methyl jasmonate-treated Norway spruce (Kozlowski *et al.* 1999). However, in each of these studies small seedlings were used and the whole plant was exposed to the elicitor, so negating the opportunity to investigate a systemic response.

Our data indicate that induced resistance may have potential as a disease control method in *Pinus radiata* seedlings and young trees. Furthermore, the finding that chitosan treatment elevated resistance to both *F. circinatum* and *S. sapinea* suggests that the induced response is not pathogen specific and therefore may offer broad-spectrum control. This may be a particularly attractive option for forest nurseries where elicitor application could be easily integrated with existing management practices. Further investigations are required to determine more accurately the onset and duration of the chitosan-induced response in *P. radiata.*

From an ecological perspective, induced resistance is arguably a more complex disease control option than the use of fungicides. This is because elicitor efficacy is reliant upon a physiological and biochemical response by the plant, and therefore is affected by climatic and agronomic factors that influence general plant health. For example, light is known to affect plant defence signalling (Karpinski *et al.* 2003) and so the induced resistance response of seedlings under glass or under artificial light may be different from that of those in the field. Furthermore, glasshouse-reared plants are generally more tender and more susceptible to infection than their field-grown counterparts. This may also affect elicitor-competence and partly explain the failure of chitosan to elicit an effective resistance response against high inoculum levels in these seedlings. A final note of caution is warranted since plant resistance against pathogens and insects is regulated by several interconnecting, and sometimes antagonistic, pathways (Feys & Parker 2000; Thaler *et al.* 2002). Pathogen-induced resistance has been shown to compromise subsequent resistance to insects and vice versa (Bostock *et al.* 2001) and so elicitation of resistance to fungi may predispose *P. radiata* to insect attack. Further experiments are required to confirm such issues before proceeding with implementation of elicitors on a large scale.

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REFERENCES

BOSTOCK, R.M.; KARBAN, R.; THALER, J.S.; WEYMAN, P.D.; GILCHRIST, D. 2001: Signal interactions in induced resistance to pathogens and insect herbivores. *European Journal of Plant Pathology 107(1)*: 103–111.

CAMPBELL, M.M.; ELLIS, B.E. 1992a: Fungal-elicitor mediated responses in pine cell cultures. 1. Induction of phenylpropanoid metabolism. *Planta 186*: 409–417.

——1992b: Fungal elicitor-mediated responses in pine cell cultures: cell wall-bound phenolics. *Phytochemistry 31(3)*: 737–742.

CORRELL, J.C.; GORDON, T.R.; McCAIN, A.H.; FOX, J.W.; KOEHLER, C.S.; WOOD, D.L.; SCHULTZ, M.E. 1991: Pitch canker disease in California: pathogenicity, distribution, and canker development on Monterey pine. *Plant Disease 75*: 676–682.

COTE, F.; HAHN, M.G. 1994: Oligosaccharins: structures and signal transduction. *Plant Molecular Biology 26(5)*: 1379–1411.

CROTEAU, R.; GURKEWITZ, S.; JOHNSON, M.A.; FISK, H.J. 1987: Biochemistry of oleoresinosis. Monoterpene and diterpene biosynthesis in lodgepole pine saplings infected with *Ceratocystis clavigera* or treated with carbohydrate elicitors. *Plant Physiology 85*: 1123–1128.

DICK, M.A. 1998: Pine pitch canker — the threat to New Zealand. *New Zealand Forestry 42*: 30–34.

FEYS, B.J.; PARKER, J.E. 2000: Interplay of signaling pathways in plant disease resistance. *Trends in Genetics 16(10)*: 449–455.

GORDON, T.R.; WIKLER, K.R.; CLARK, S.L.; OKAMOTO, D.; STORER, A.J.; BONELLO, P. 1998: Resistance to pitch canker disease, caused by *Fusarium subglutinans* f. sp. *pini,* in Monterey pine (*Pinus radiata.*) *Plant Pathology 47*: 706–711.

HAHN, M.G. 1996: Microbial elicitors and their receptors in plants. *Annual Review of Phytopathology 34*: 387–412.

HAMMERSCHMIDT, R. 1999: Induced disease resistance: how do induced plants stop pathogens. *Physiological & Molecular Plant Pathology 55(2)*: 77–84.

HOTTER, G.S. 1997: Elicitor-induced oxidative burst and phenylpropanoid metabolism in *Pinus radiata* cell suspension cultures. *Australian Journal of Plant Physiology 24*: 797–804.

KARPINSKI, S.; GABRYS, H.; MATEO, A.; KARPINSKA, B.; MULLINEAUX, P.M. 2003: Light perception in plant disease defence signalling. *Current Opinion in Plant Biology 6*: 390–396.

KOZLOWSKI, G.; BUCHALA, A.; MÉTRAUX, J.-P. 1999: Methyl jasmonate protects Norway spruce (*Picea abies* (L.) Karst.) seedlings against *Pythium ultimum* Trow. *Physiological & Molecular Plant Pathology 55*: 53–58.

KUC, J. 1987: Plant immunization and its applicability for disease control. Pp.255–274 *in* Chet, I. (Ed.) “Innovative Approaches to Plant Disease Control”. John Wiley and Sons, New York.

KUMAGAI, H.; FURUKAWA, I.; SAKUNO, T.; KISHIMOTO, J. 1990: Anti-mold activity of chitosan-treated wood. Evaluation of anti-mold activity of chitosan and chitosan/copper sulfate jointly-treated wood. *Research Bulletin of the Tottori University Forests 19*: 59–65.

LAFLAMME, P.; BENHAMOU, N.; BUSSIERES, G.; DESSUREAULT, M. 1999: Differential effect of chitosan on root rot fungal pathogens in forest nurseries. *Canadian Journal of Botany 77(10)*: 1460–1468.

LESNEY, M.S. 1989: Growth responses and lignin production in cell suspensions of *Pinus elliottii* ‘elicited’ by chitin, chitosan or mycelium of *Cronartium quercum* f.sp. *fusiforme. Plant Cell Tissue and Organ Culture 19*: 23–31

LIEUTIER, F.; BERRYMAN, A.A. 1988: Preliminary histological investigations of the defence reactions of three pines to *Ceratocystis clavigera* and two chemical elicitors. *Canadian Journal of Forest Research 18*: 1243–1247.

LYON, G.D.; NEWTON, A.C. 1999: Implementation of elicitor-mediated induced resistance in agriculture. *In* Agrawal, A.A.; Tuzun, S.; Bent, E. (Ed.) “Induced Plant Defences Against Pathogens and Herbivores”. APS Press, St. Paul.

LYON, G.D.; REGLINSKI, T.; NEWTON, A.C. 1995: Novel disease control compounds: The potential to ‘immunize’ plants against infection. *Plant Pathology 44*: 407–427.

MASON, M.E.; DAVIS, J.M. 1997: Defense response in slash pine: chitosan treatment alters the abundance of specific mRNAs. *Molecular Plant Microbe Interactions 10(1)*: 135–137.

MILLER, R.H.; BERRYMAN, A.A.; RYAN, C.A. 1986: Biotic elicitors of defence reactions in lodgepole pine. *Phytochemistry 25*: 611–612.

OOSTENDORP, M.; KUNZ, W.; DIETRICH, B.; STAUB, T. 2001: Induced disease resistance in plants by chemicals. *European Journal of Plant Pathology 107(1)*: 19–28.

REGLINSKI, T.; STAVELEY, F.J.L.; TAYLOR, J.T. 1998: Induction of phenylalanine ammonia lyase activity and control of *Sphaeropsis sapinea* infection in *Pinus radiata* by 5-chlorosalicylic acid. *European Journal of Forest Pathology 28*: 153–158.

STORER, A.J.; WOOD, D.L.; GORDON, T.R. 2002: The epidemiology of pitch canker of Monterey pine in California. *Forest Science 48*: 694–700.

STORER, A.J.; BONELLO, P.; GORDON T.R.; WOOD, D.L. 1999: Evidence of resistance to the pitch canker pathogen (*Fusarium circinatum*) in native stands of Monterey pine (*Pinus radiata.*) *Forest Science 45*: 500–505.

STORER, A.J.; GORDON, T.R.; DALLARA, P.L.; WOOD, D.L. 1994: Pitch canker kills pines, spreads to new species and regions. *California Agriculture 48*: 9–13.

TALLY, A.; OOSTENDORP, M.; LAWTON, K.; STAUB, T.; BASSI, B. 1999: Commercial development of elicitors of induced resistance to pathogens. *In* Agrawal, A.A.; Tuzun, S.; Bent, E. (Ed.) "Induced Plant Defences Against Pathogens and Herbivores". APS Press, St. Paul.

THALER, J.S.; KARBAN, R.; ULLMAN, D.E.; BOEGE, K.; BOSTOCK, R.M. 2002: Cross-talk between jasmonate and salicylate plant defense pathways: effects on several plant parasites. *Oecologia 131(2)*: 227–235.

TUZUN, S.; KLOEPPER, J. 1995: Practical application and implementation of induced resistance. Pp.152–168 *in* Hammerschmidt, E. *et al*. (Ed.) "Induced Resistance to Disease in Plants". Academic Publishers.

VILJOEN, A.; WINGFIELD, M.J.; MARASAS, W.F.O. 1994: First report of *Fusarium subglutinans* f. sp. *pini* on pine seedlings in South Africa. *Plant Disease 78*: 309–312.

WINGFIELD, M.J.; JACOBS, A.; COUTINHO, T.A.; AHUMADA, R.; WINGFIELD, B.D. 2002: First report of the pitch canker fungus, *Fusarium circinatum,* on pines in Chile. *New Disease Reports 4*. [Aug 2001 – Jan 2002 www.bspp.org.uk/ndr]