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Can we induce resistance in trees?

Tony Reglinski

Bio-Protection

Outline

1. What is Induced resistance?
2. Types of induced resistance
3. Biotic and abiotic inducers on *Pinus radiata*.
4. Conclusions

Dr Robert Hill – BioProtection Centre – Root Endophytes

Dr Beccy Ganley - Scion – Foliar Endophytes

What is Induced resistance?

"an increase in resistance to a herbivore or pathogen that results from a plant's response to a prior attack by the same or another attacker or to chemical or mechanical factors that mimic enemy attack"
(from Cipollini & Heil 2010, CAB Reviews 5, No. 005.)

What is Induced resistance?

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A natural phenomenon - first described during the late 1890s.

1933 – Chester (review) - "*the vaccination of plants results in an increased resistance of variable duration toward subsequent infection*"

Biological Induction of Resistance

Systemic Acquired Resistance (SAR)

- Triggered by local infection that causes a hypersensitive response (HR)

Localised HR

Biological Induction of Resistance

Systemic Acquired Resistance (SAR)

- Triggered by local infection that causes a hypersensitive response (HR)
- Involves systemic accumulation of salicylic acid (SA) and the expression of pathogenesis-related (PR) proteins.
- Plants exhibit SAR to subsequent attack.

Systemic accumulation of SA and PR induction

Localised HR

Biological Induction of Resistance

Induced Systemic Resistance

- ISR is phenotypically similar to SAR
- Induced by root colonising bacteria and fungi.

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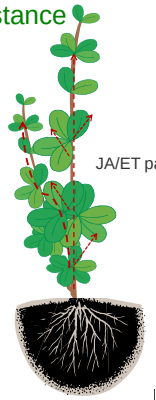
Biological Induction of Resistance

Induced Systemic Resistance

- ISR is phenotypically similar to SAR
- Induced by root colonising bacteria and fungi.
- Mediated via JA/ethylene signalling pathways
- SA-independent
- May result in 'priming' of defences



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Biotic and Abiotic Inducing Agents

Induced resistance can also be triggered by application of various biotic and abiotic agents

- Non-pathogens/endophytes - *Aureobasidium* spp., *Trichoderma* spp., *Bacillus* spp., *Pseudomonas* spp.
- Plant and microbial extracts - Glucan, chitosan, harpin proteins.
- Phytohormones – salicylates, jasmonates, brassinosteroids.
- Synthetic chemicals – acibenzolar-s-methyl, phosphite.
- UV-C

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Biological Induction in conifers

- Local inoculation of *Pinus radiata* trees with *Fusarium circinatum* induced systemic resistance to later inoculation with the same pathogen. (Bonello *et al* 2001 For. Path. 31,99-106)
- Lesion length reduced from 2.9mm to 1.04mm over two years (4 inoculations).
- Is this responsible for enhanced resistance of mature trees growing in areas with a history of the disease ? (Gordon 2006, Phytopath. Vol 96)
- Inoculation of *Pinus nigra* with *Diplodia pinea* or *D. scrobiculata* resulted in SIR to subsequent inoculation with *D. pinea. (*Blodgett et al 2007 Tree Physiology 27, 511–517)

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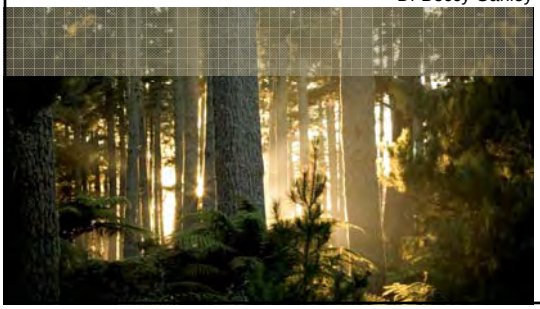
forests products innovation

Foliar endophytes

Dr Beccy Ganley



Endophyte-mediated resistance in pines

- Western white pine - white pine blister rust disease system
- Used foliar endophytes from western white pine
- Reduced mortality by 16% in seedlings pre-treated with endophytes
- Survival percentages for some treatments same as those selected for in genetic breeding trials

Ganley *et al* 2005

Ganley et al., 2008. Endophyte-mediated resistance against white pine blister rust in *Pinus monticola*. *Forest Ecology and Management* ***255, 2751-60***

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Reduced mortality

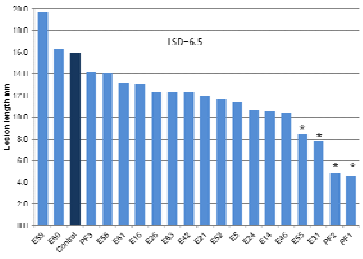
- Variation between endophyte treatments – some worked better than others
- No indication of any induced susceptibility
- Effective over time
- First demonstration of endophyte-mediated resistance in a pine species

Endophyte-mediated resistance in other tree species

- Many examples of endophytes that prevent herbivory
  - Endophytes produce metabolites that deter herbivores
- Fewer examples of resistance against pathogens*
  - Reduced leaf necrosis and mortality in Cacao seedlings against *Phytophthora* pathogen
  - Reduced severity of rust disease in coffee trees
  - Foliar endophytes reported to contribute to quantitative resistance to rust in Poplar (Raghavendra & Newcombe 2013 New Phytol 197, 909-918.
- More examples in agricultural crops and grass systems

Testing endophytes against *Diplodia pinea*

- Endophytes applied as foliar spray 10 weeks before stem inoculation with *D. pinea.*
- A second experiment recorded no significant differences



Pinus radiata studies

Methyl Jasmonate

UV-C

Trichoderma spp., root endophytes (Robert Hill)

1. Disease Resistance
   - Diplodia dieback
   - Terminal crook
2. Enzyme activation
3. Terpene analysis

Defence mechanisms against *Diplodia pinea*

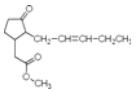
- Diplodia can gain entry via wounds.
- Resin 'bleeds' from fresh wounds.
  - Toxic terpenes and phenolics.
  - Volatiles – warning signal, recruitment.
- Wound site – lignification, synthesis of terpenoids and phenolics.
  - Peroxidase.
  - Polyphenol oxidase
  - Phenylalanine ammonia lyase
- Anatomical modifications
  - Polyphenolic parenchyma cells
  - Traumatic resin ducts

1. Chemical elicitor - Methyl Jasmonate

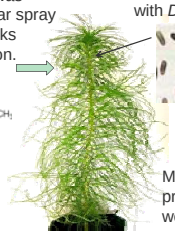
- Jasmonates play regulatory role in defence.
- Jasmonic acid is converted to methyl jasmonate (MeJA) in tissues damaged by wounding or herbivory.
- MeJA operates as a systemic signal for defence activation (also an interplant signal).
- In conifers, MeJA has been used as a 'tool' to 'mimic' wounding and herbivory.
- MeJA shown to induce the formation of traumatic resin ducts.

MeJA–Induced resistance in *Pinus radiata*

MeJA (4.5mM) was applied as a foliar spray 1, 2, 3, or 5 weeks before inoculation.



Wound inoculation with *Diplodia pinea*

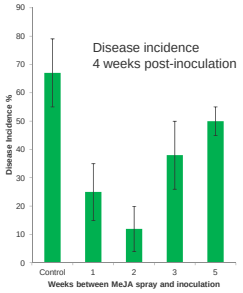


Pinus radiata seedling <1yr

Monitor disease progress over 4 weeks

MeJA–Induced resistance in *Pinus radiata*

- Resistance peaked between 1 and 3 weeks after MeJA treatment.
- Resistance was transient and was not significant after 5 weeks.
- Induced resistance is accompanied by growth retardation



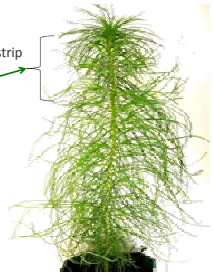
From Gould N, Reglinski T, Spiers M & Taylor JT (2008). *Can. J. For. 38, 677-674*

Induced defence responses in *Pinus radiata* stems

What is induced by MeJA?

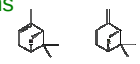
- Treatments
  - Spray whole seedling with 1mM MeJA
  - Wound – strip needles.
- Stems tissue was harvested at 0, 1, 4, 7 and 14 days after treatment.

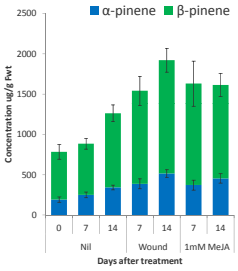
3cm strip



Terpene levels in *P. radiata* stems

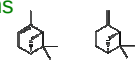
- MeJA and wounding stimulated accumulation of α- and β-pinene.

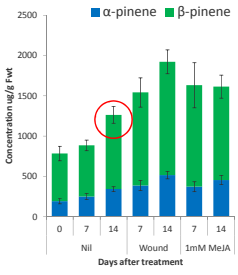




Terpene levels in *P. radiata* stems

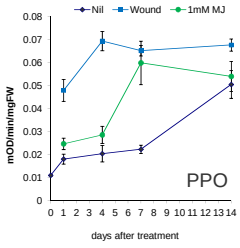
- MeJA and wounding stimulated accumulation of α- and β-pinene.
- Terpene accumulation was observed in 'Nils' after 14 days.
- Possibly induced by volatiles released from treated plants?

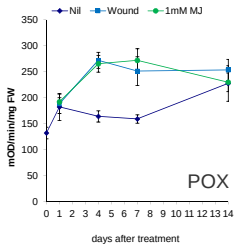




Enzyme activity in *Pinus radiata* stems

•Polyphenol oxidase (PPO) and peroxidase (POX).

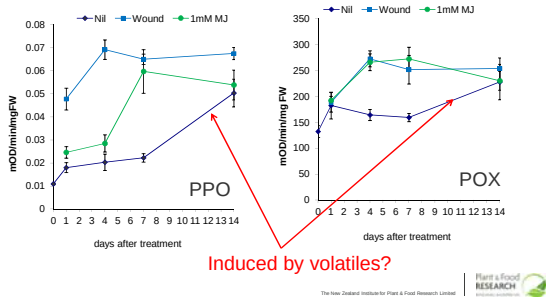




Enzyme activity in *Pinus radiata* stems

•Polyphenol oxidase (PPO) and peroxidase (POX).

• PPO and POX also increased in the 'Nils' between 7-14 days



Induced by volatiles?

Bio-Protection

HEALTHY PLANTS NEED FU

***Trichoderma* root endophytes enhance plan health and vigour**

Dr Robert Hill

Bio-Protection Research Centre, PO Box incoln University, Lincoln 7647, New Zealand

Trichoderma root endophytes

- Opportunistic symbionts
- Penetrate root cortex
- Promote plant growth
- Enhance plant health
  - Antagonism
  - Induced resistance/priming

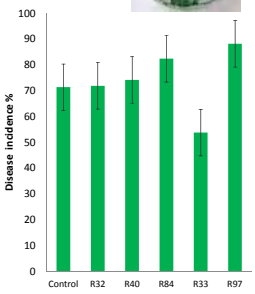
(*T. harzianum* T22, *T. hamatum* 382, *T. atroviride* P1)

Shoresh et al., Ann. Rev. Phytopath. 48,21-43, 2010

Trichoderma Root Endophytes

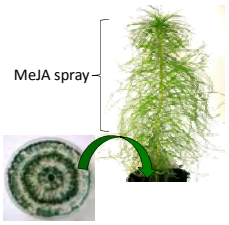
Biological induction - Trichoderma

- *Trichoderma spp.* root drench (10^7 spores/plant)
- 2-weeks before inoculation with *D. pinea.*
  - *T. atroviride* – R32, R33, R40, R84
  - *T. asperellum* – R97
- R33 induced a slight reduction in disease
- No effect on terpenes, POX or PPO.



Combination of Trichoderma and MeJA

- Is there an additive benefit of combining R33 and MeJA?
- Treatments (applied 2 weeks before inoculation)
  1. Nil
  2. MeJA (1mM) foliar spray
  3. R33 root drench
  4. R33 root drench plus MeJA spray.

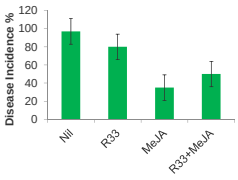


R33 root drench (10^7 spores)

Combination of Trichoderma and MeJA

- R33 reduced disease incidence by ~18% (not significant).
- MeJA reduced disease incidence by ~60%.
- There was no additive effect.
- No additive effect on terpenes or phenolics.

But.



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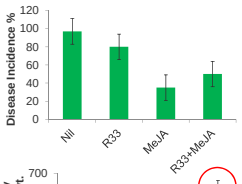
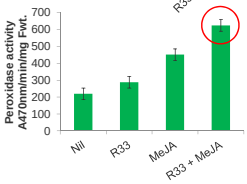
- There was no additive effect.
- No additive effect on terpenes or phenolics.

But.

- R33+MeJA induced peroxidase activity more than either component alone.
- Does Trichoderma R33 operate by 'priming'?



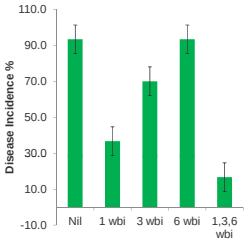


Ultraviolet light

- Ultraviolet radiation (UV) can alter leaf chemistry.
- UV absorbing components accumulate in conifer needles exposed to UV-B (280-315nm) e.g. at higher altitude.
- UV-C (100-280nm) often used as a postharvest fruit treatment.
  - Directly antimicrobial.
  - Induces phenols and antioxidant enzymes in plant tissue.

UV-C induced resistance to diplodia

- UV-C (1.2mW/cm^{-2} x 30 min) up to 6 weeks before inoculation (wbi).
- Disease incidence and severity (lesion size) was significantly reduced ($p<0.05$)



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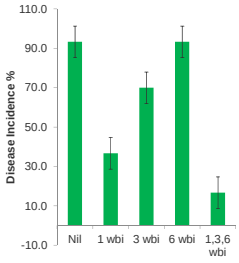
- UV-C (1.2mW/cm^{-2} x 30 min) up to 6 weeks before inoculation (wbi).
- Disease incidence and severity (lesion size) was significantly reduced ($p<0.05$)
- UV-C did not affect levels of α & β-pinene or total phenolics.
- PPO and POX were elevated by 70 and 200%, respectively.



Brief Update on Recent Studies

Glasshouse/Lab studies/Field

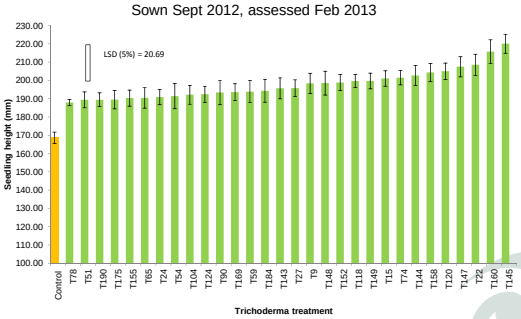
- New Trichoderma isolates
- Diplodia
- Terminal crook

Enhancing *Pinus radiata* health and vigour using selected *Trichoderma* isolates: PF Olsen Nursery

www.bioprotection.org.nz

PF Olsen Nursery 2013

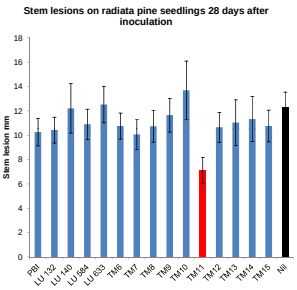
Sown Sept 2012, assessed Feb 2013



www.bioprotection.org.nz

New Trichoderma Mixtures

Diplodia stem infection



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Terminal Crook

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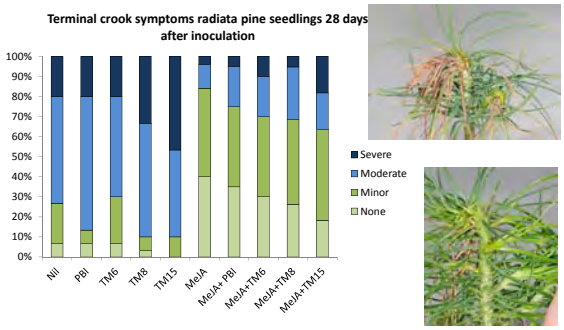
MeJA/Trichoderma Combinations



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Can We Induce Resistance?

Chemical inducers – transient effect
Endophytes – duration?
Combinations – synergy?
Implementation – nursery ok, Plantation?

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Knowledge Gaps

Environmental factors affecting induced resistance – soil, water, temp.
Ecological interactions – mycorrhizae, insects…
Endophytes – duration, population successions (nursery to plantation).
Genetics – are some genotypes more responsive?

Redwoods Photos by Michael Nichols natgeo.com

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Beccy Ganley (Scion)
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