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Weather and inoculum factors associated with kiwifruit bud rot

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Pseudomonas syringae pv. actinidiae biovar 3 (Psa) causes kiwifruit bacterial canker and also bud rot, which destroys developing flower buds and can become a severe problem, particularly in green-fleshed cultivars. The effects of weather and inoculum factors on bud-rot development were investigated. Experiments were conducted on two green kiwifruit cultivars: Actinidia chinensis var. deliciosa ‘Hayward’ and A. chinensis var. chinensis × A. chinensis var. deliciosa ‘Zesh004’ (known as Green14), at four sites for two consecutive years. Temperature and rainfall were recorded from bud burst to flowering and bud-rot incidence was monitored from approximately two weeks after flower bud appearance until flowering. Correlations between weather parameters and final bud-rot incidence, and between initial bud-rot and final bud-rot incidence were investigated. There was no significant association between temperature and final bud-rot incidence, but total rainfall and number of days of rain were positively correlated with final bud-rot incidence. Initial bud-rot incidence showed the strongest correlation with final bud-rot incidence and appeared to be the main factor that contributed to bud-rot.

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Propidium monoazide combined with qPCR to differentiate live and dead conidia of Neofabraea actinidiae

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Neofabraea actinidiae can occasionally cause post-harvest rot in kiwifruit. Quantitative polymerase chain reaction (qPCR) analysis represents a feasible and accurate option for identifying and quantifying this rot but is limited because qPCR results do not differentiate live and dead conidia. Propidium monoazide (PMA) is a photoreactive dye that penetrates into the damaged cell-wall membranes of dead conidia binding to the DNA and thus suppressing its amplification by qPCR. A commercial kit containing PMA was trialled for differentiating between live and dead N. actinidiae conidia. The most suitable conditions were 1 μM PMA with 10 min light emitting diode (LED) exposure, and could clearly distinguish high concentrations of live from similar concentrations of dead conidia when tested separately and as a mixture. Low concentrations of live N. actinidiae conidia could be distinguished from dead ones when tested separately, but not as a mixture. Additional work is needed to optimise the effectiveness of the PMA binding and apply this concept in the orchard.
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**Velvetleaf regenerates and reproduces after cropping**

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Velvetleaf (Abutilon theophrasti) was accidentally introduced into New Zealand in 2016 and intensive efforts are being implemented to mitigate the risk of its naturalisation. This study investigated the possibility of velvetleaf plants surviving in maize fields following silage harvest in late summer and early autumn. Velvetleaf plants were grown to maturity in a glasshouse during the summer of 2017–2018. They were left to seed and senesce naturally and, on 28 February 2018, five senesced plants were “harvested” at each height (0, 30, 50, 70 and 150 mm) with varying numbers of leaf nodes remaining (0, 0, 1, 2 and 3 pairs respectively). Velvetleaf was capable of regeneration 2–4 weeks after “harvest” from these leaf nodes. Forty percent regeneration occurred with 1 pair of nodes; 80% (2 pairs); and 100% (3 pairs). All regenerated plants subsequently produced mature seed pods with viable seed (95% germination) 6–10 weeks following “harvest”. Maize silage crops are normally cut ~100 mm above ground level. In this study, velvetleaf was able to regenerate and reproduce following simulated maize silage harvest at, below and above, normal cutting height. Growers are advised to continue management strategies for velvetleaf to prevent seed set following maize silage harvest and before sowing of the next crop or pasture.

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**Local adaptation in a New Zealand invader, Mimulus guttatus?**

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The New Zealand flora comprises proportionately more alien species than anywhere else on Earth. Many of these species are ‘sleeper’ species, currently not invasive but with the potential to become so. Understanding what traits lead to sleepers becoming invasive is a key question in invasion biology. One hypothesis is local adaptation – that is, selection pressures in an alien habitat select for certain genetic traits favouring species spread. In New Zealand, the semi-aquatic herb Mimulus guttatus, ‘monkey flower’, is already showing signs of becoming invasive and is widespread across the South Island, blocking waterways and ditches. A common garden experiment was used to test for local adaptation in 37 populations of monkey flower from 8 regions across the North and South Islands of New Zealand. Possible adaptations in plant physiology (including, fresh vs dry weight, flower size, and photosynthetic rate) were examined. Observable differences include significant differences in biomass and leaf morphology. Results to date indicate significant genetic differences among New Zealand monkey flower populations. This is indicative of invasive potential.
Differences in insect anatomy may affect tolerance to the fumigant ethanedinitrile

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Ethanedinitrile (EDN) is a potential alternative fumigant to methyl bromide for the phytosanitary treatment of timber and logs. Previously, adult golden-haired bark beetles (*Hylurgus ligniperda*) have shown high rates of tolerance to EDN, while all life stages of burnt-pine longhorn beetles (*Anthopinus ferus*) were highly susceptible. These results suggest that the fumigant mode of action might be species-specific and more complex than previously thought. Therefore, the anatomy of these beetles was examined to investigate potential differences in EDN toxicity related to these insect traits. The mesothoracic and fourth abdominal cuticles of 20 individuals were measured and compared across the two species. Spiracle sizes of 20 adult insects were also measured and compared across and within species. Of the two species, *A. ferus* had the thicker dorsal thoracic and abdominal cuticle. Adults of *A. ferus* had also the larger total spiracle area. These results provide important information to help explain the observed differences in EDN tolerance across the two species. Further work will test the main route of entry of EDN into the bodies of target insects and additional effects on EDN on insect behaviour.

Comparing the toxicity of two fumigants to insects from the field vs laboratory – does insect origin matter?

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The golden-haired bark beetle, *Hylurgus ligniperda* (F.), is a common forest insect which may be associated with pine (*Pinus radiata* D.Don) logs exported from New Zealand. We tested the dose-mortality responses of *H. ligniperda* adults, from two different origins (field vs laboratory), to methyl bromide (MB), the most widely used fumigant worldwide; and to ethanedinitrile (EDN), a potential alternative to MB. Naked insects were fumigated with either MB or EDN at 10°C for 4 and 3 hours, respectively. Laboratory adults had been reared on artificial diet under controlled conditions for >10 generations. Field adults, by contrast, had been recently collected from Lindgren funnel traps with lures of alpha-pinene and ethanol. Tolerance to the two fumigants tested was significantly different, according to the origin of the insects, with field-collected adults being less tolerant to MB and EDN than laboratory-reared ones. The implications of our results for the development of disinfection schedules for New Zealand export logs will be discussed.
Susceptibility of kale cultivars to the wheat bug, *Nysius huttoni* (Hemiptera: Lygaeidae) in New Zealand

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Seedlings of kale cultivars are highly susceptible to direct feeding by the wheat bug *Nysius huttoni* (Hemiptera: Lygaeidae), an endemic New Zealand insect pest. Damage from this pest leads to reduced plant establishment so is currently managed by prophylactic use of pesticides. Encouraging farmers to use less susceptible kale cultivars can reduce pesticide costs and improve the environment. A series of choice and no-choice tests was conducted in a controlled-temperature room to evaluate the relative susceptibility of seedlings of the six most widely grown kale cultivars in New Zealand (Gruner, Kestrel, Regal, Colear, Corka, and Sovereign). Bugs settled most readily on Kestrel in both sets of tests, although mean settlement times did not differ significantly among cultivars. However, feeding damage on Kestrel occurred significantly earlier than on Corka or Gruner. These results indicate that Kestrel is the cultivar most susceptible to wheat bugs and that pesticide use could be reduced if less susceptible cultivars were more widely grown in New Zealand. These results also provide important information for developing integrated-management protocols for brassica pests.

What’s buzzing? A snapshot analysis of pest notifications

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The Ministry for Primary Industries’ PHELs receive many notifications each year of pests suspected of being new to New Zealand. Most notifications are received through the Ministry’s free Pest and Disease Hotline and are an important source of surveillance data. During the period January 2015–February 2018, PHELs received ~9400 notifications, with each March the busiest (1,160) period. Fifty-one percent of notifications came from Auckland, Waikato or the Bay of Plenty. The brown soldier bug, *Cermatulus nasalis*, was the most frequently reported (483) invertebrate. Publicity campaigns targeting specific pests (e.g. brown marmorated stink bug), past incursion-response publicity (e.g. fruit flies, termites) and ‘strange-looking’ invertebrate notifications are all reflected in the organisms reported. Excluding targeted campaigns and incursion responses, guava moth, *Coscinoptycha improbana* (181; ~2%), was the most frequently reported, followed by the three-lined hover fly, *Helophilus seelandicus* (97; ~1%). Spiders, as a group, were reported in considerable numbers (14.4%). Ant samples were frequently submitted (9.1%). These data indicate the number of notifications geographically is proportional to human-population levels. These results will assist PHEL in planning and engaging in future surveillance initiatives.
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**Eucalyptus variegated beetle: genetic diversity of New Zealand and Australian collections**

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Paropsisterna variicollis, the eucalyptus variegated beetle, was first detected in New Zealand in 2016. It threatens a growing eucalyptus forestry sector through larvae and adults causing significant defoliation to important plantation species. This work aimed to clarify the identification and origin of the New Zealand incursion to inform selection of suitable biological control agent(s). Australian and New Zealand specimens from the Paropsisterna obovata-varicollis-cloelia species complex were analysed by PCR and sequencing of two genetic loci, cytochrome c subunit I (COI) and cytochrome b (Cyt b). Molecular analysis of both genetic regions showed three major clusters of diversity. Cluster 1, proposed as Paropsisterna variicollis, had maximum 1.3% genetic variation and was collected from New Zealand, Western Australia and from geographically diverse locations in eastern Australia. Taxonomic results identified distinctive phenotypes of other closely related beetle species, assisting in proposing Cluster 2 as Paropsisterna near decorolata and Cluster 3 as Paropsisterna agricola. Molecular results were compared to morphological structures on adult beetles.

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**Parasitoids associated with codling moth (Cydia pomonella) in apple-growing regions in New Zealand**

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Codling moth (Cydia pomonella) (CM) is a major pest of apples in New Zealand. Several biocontrol agents introduced in the past to control CM have only been partially successful at reducing CM populations, so a parasitoid wasp, Mastrus ridens (Hymenoptera: Ichneumonidae), was recently released into apple-growing regions. This study sought evidence of the establishment of CM parasitoids. Corrugated cardboard bands (2-cm wide bands with sentinel CM larvae and 10-cm wide empty bands to trap wild CM larvae) were used to assess the presence of M. ridens and other CM parasitoids in Hawke's Bay, Nelson, Central Otago and Waikato regions. Five CM parasitoid species, including M. ridens, were recovered from sentinel and wide bands. Liotryphon caudatus (Hymenoptera: Ichneumonidae, released to control CM in 1906) was found in Hawke’s Bay and Waikato. Ascogaster quadridentata (Hymenoptera: Braconidae, released to control CM in the 1930s), Glabridorsum stokesii (Hymenoptera: Ichneumonidae, released in 1967 to control light brown apple moth), Dibrachys microgastri (Hymenoptera: Chalcidoidea: Pteromalidae, an accidental arrival) and M. ridens were found in all regions. The interspecific competition between M. ridens and other parasitoid species remains to be investigated.
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**Developing apple leafcurling midge rearing techniques**

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The study of *Neonectria ditissima* causing European canker in apple trees is often dependent on controlled inoculation of tree wounds and development of canker lesions. This makes the success of the initial inoculation crucial for time-efficient research. The effect of morning vs. evening inoculation on the successful development of canker lesions was investigated. Ninety-six ‘Royal Gala’ trees were inoculated on six different days (February–March 2017), which covered several different weather conditions. On each inoculation day, 16 trees were inoculated on four rasp and four picking wounds, at 10:00 and 19:00 hours. These were then assessed over the following 8 months for presence of disease symptoms. Irrespective of rain (0–4.5 mm rainfall at the day of inoculation), temperature (average daily temperature of 14.8–21.3°C), and humidity (average daily relative humidity of 59.8–94.2% rH), neither the day nor the hour of inoculation affected symptom development. However, more inoculated wounds developed symptoms in rasp wounds (91%) than in picking wounds (63%). Under the environmental conditions tested, inoculation timing (date and hour) had little effect on *N. ditissima* symptom expression.
Effect of heat or chemical disinfection on the viability of ‘Fuji’ apple graft wood

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Bundles of dormant wood were submerged in water (45°C for 45 min or 50°C for 15 min), or were wrapped in moist cloth, placed inside zip-locked bags and submerged for 3–6 h or treated with one of three GRAS chemicals or untreated (two bundles per treatment). Scion wood was grafted onto ‘M9’ rootstocks then planted in a randomised order, with growth assessed after 16 weeks. Two of the GRAS treatments and submersion at 45°C for either 45 min or 3 h in a bag did not significantly affect viability compared with the untreated control (83–95% viable). Submersion at 50°C for 15 min reduced viability significantly (70%). Scions did not survive exposure to 50°C for 3–5 h in bags (P<0.001). No significant differences in mean scion shoot length were observed between the untreated wood (107 cm) and that submerged in 45°C water for 45 min, 3 h in a bag, or GRAS-treated. Wood treated at 45°C for 5–6 h in a bag or at 50°C for 15 min had significantly shorter shoots (54–75 cm, P<0.001). Dormant ‘Fuji’ wood remained viable after treatments known to eliminate pathogens from wood.

Organic and inorganic nitrogen effects on spore production and mycelial growth of Neonectria ditissima in vitro

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Nitrogen (N) is known to influence the growth of Neonectria ditissima (N.d.), the causal organism of European canker. In vitro, inorganic N inhibits conidia germination at N concentrations above 0.2 mol/L while, in planta, foliar urea application increased disease expression of leaf scar wounds up to nine-fold. The influence of organic and inorganic N sources on mycelial growth and spore production of N.d. in vitro was investigated. Four organic and six inorganic N sources were tested on agar at concentrations of N between 0 and 0.2 mol/L, with 3 different N.d. isolates. Spore production was generally increased by the addition of low concentrations of N, with varying results at higher concentrations dependent on the N source; however, this also differed among N.d. isolates. Spore production was generally incompletely inhibited at the higher N concentrations tested. However, germination from the resulting conidia decreased, possibly due to morphological changes to the spores. Mycelial growth generally decreased with the addition of N. Understanding the N effect in planta will be further complicated by the physiological plant-N and plant-pathogen interaction processes.
Validating outsourced high throughput automated qPCR for increased research outputs from forest pathology trials

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Needle diseases of Pinus radiata caused by Phytophthora pluvialis and Phytophthora kernoviae have been increasingly recognised since the discovery of red needle cast in 2008. There is a need for rapid diagnostic screening of numerous samples, but sample processing time, equipment and staff availability limit the throughput and utilisation of diagnostic qPCR analysis in the research environment. Automated and high-throughput capable DNA extraction and real-time PCR provides the opportunity to expand the capacity of research trial analysis and a potential alternative to laborious isolation and plating but must be thoroughly validated before results can be used with confidence. The use of a high-throughput format for qPCR assays targeting Phytophthora pluvialis and Phytophthora kernoviae was validated on a robotic platform, proving to be consistently more sensitive than isolation, achieving qPCR detection down to 1% diluted inoculated material for Phytophthora kernoviae and 10% for Phytophthora pluvialis. Plating results yielded a 60% detection rate of Phytophthora pluvialis in inoculated needle fragments, whereas qPCR yielded a 100% detection on the same material. High throughput automated qPCR can, therefore, be utilised with confidence in forest pathology research trial analyses in future.

Evaluation of different methods for isolating Phytophthora spp. from a Canterbury waterway

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Phytophthora spp. pose a risk to New Zealand’s managed and natural ecosystems. As Phytophthora spp. are well adapted to aquatic environments, water surveillance can be used to identify their distribution. Seven bait species (Rhododendron arborescens, Pittosporum undulatum, Banksia attenuata, Camellia japonica, Pittosporum eugenioides, Pinus radiata, and Cedrus deodara) were evaluated for Phytophthora spp. isolation. Water was collected from 2 sites in the Suckling river (Tai Tapu) and half was membrane-filtered (3-μm pore size) to capture spores. Leaf baits were floated directly on unfiltered water at room temperature in the laboratory for 7 days. Baits were also placed in nylon-mesh bags and floated in the Suckling river sites (in situ) for 7 days. Leaf lesions and membrane filters were cultured on Phytophthora spp. selective media. Eighty-six Phytophthora spp. isolates representing 5 colony morphotypes were recovered, 6 (3 morphotypes) from membrane filters, 25 (4 morphotypes) from baits on collected river water, and 55 (5 morphotypes) from in situ baits. The highest numbers of isolates were recovered from R. arborescens (50.6%; 4 morphotypes), Pinus radiata (17.2%; 3 morphotypes) and Pittosporum undulatum (12.6%; 2 morphotypes). In situ baiting using Rhododendron arborescens and Pinus radiata was the most effective method of isolating Phytophthora species.
Compiling myrtle rust surveillance data for the 2017-18 New Zealand incursion

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As the observed distribution of myrtle rust (\textit{Austropuccinia psidii}) changes within New Zealand, many stakeholders are interested in the spread of this disease, resulting in a number of organisations carrying out various levels of monitoring. There is a need to compile these monitoring data from multiple sources and provide easy access to basic disease distribution information, for the benefit of all interested parties. This should include not only locations of positive myrtle-rust detections, but also the rate of monitoring in areas where it has not yet been detected (confirmed absence). This project provided visual information in the form of maps that are easy to interpret by general and/or specific users. Resolution was useful and informative while maintaining privacy of landowners. A series of maps is presented, showing the advance in sampling effort and the disease distribution across New Zealand, as measured from surveillance effort from Ministry for Primary Industries, Department of Conservation, botanical gardens, Plant and Food Research and the Myrtle Rust Reporter app (NatureWatch NZ). Further analysis of such data will inform ongoing management and research.

Location of overseas botanic gardens with New Zealand Myrtaceae in relation to myrtle rust occurrence

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New Zealand’s productive systems and natural ecosystems depend on a robust biosecurity framework to exclude invasive pests, diseases and weeds. Risk assessment is an important component of this biosecurity framework identifying potential threats posed by invasive organisms to specific plants and plant systems before they arrive in New Zealand. A major challenge in risk assessment is determining the potential impact of a pest or disease when it is not present in New Zealand. The International Plant Sentinel Network (IPSN), a network of botanic gardens and arboreta, aims to provide early warning of new and emerging tree and plant pests and diseases. The utility of the IPSN in providing information on the potential impact of myrtle rust for New Zealand’s indigenous Myrtaceae. Botanic gardens were identified around the world with New Zealand Myrtaceae and where myrtle rust is present. This is a first step in an ex-post study on the use of sentinel or expatriate plants as a biosecurity risk-assessment tool. The approach could be extended to other plant pathogens or pests to explore their impact on New Zealand indigenous or cultivated plant species overseas.
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Biosecurity partnership and collaboration: key similarities and differences between the Australian and New Zealand plant-biosecurity government–industry agreements

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New Zealand and Australia have each established legal partnerships between government and industry to improve biosecurity outcomes. Both partnerships take a collaborative approach to build capability and capacity to manage biosecurity risk across the biosecurity system. Key differences between the New Zealand and Australian systems include: the scope of responsibilities and accountability for management of biosecurity pests; governance arrangements; and cost sharing. Key similarities include: a shared role in response decision making; shared costs for pest eradication; advanced agreement on the potential liabilities surrounding pest eradication; provision for compensation to growers for direct costs incurred during an eradication response; minimum commitments to maintain capability and capacity; and an established basis for a transparent, consistent and equitable partnership. The approach taken by both countries encompasses the view that biosecurity outcomes will be improved by building genuine industry–government partnerships. In New Zealand, successes include development of operational agreements, strategies for fruit fly and brown marmorated stink bug, and completion of readiness activities for future incursions of these pests.