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Forest Protection SSIF research on species other than radiata pine 2020/21

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Executive summary

Plantation species other than *Pinus radiata* (radiata pine), such as Douglas-fir, cypresses and eucalypts, continue to be of pivotal importance to ensuring New Zealand has a diversified forest estate, resilient against biosecurity threats. As part of the SWP partnership, Forest Protection (now the Ecology and Environment research group) contribute research findings from core funding (SSIF) research that is highly responsive to biosecurity threats in the diverse species areas, to ensure sustainable growth of alternative tree species. This report summarises research findings in the last financial year in the aligned projects.

Firstly, a new polyphagous ambrosia beetle known as GAB, *Xylosandrus crassiusculus*, has been introduced into New Zealand in 2019. It is native to East Asia and has been a highly successful invader worldwide. Like most invasive ambrosia beetles, *X. crassiusculus* can attack a wide range of woody plants. Adult females colonize physiologically stressed trees by excavating galleries in which they lay eggs and inoculate a symbiotic fungus, *Ambrosiella roeperi*. Both the founding female and its larval progeny feed on the fungus, not on the wood. Often the first sign of an attack is the sawdust released by the excavating adult, which takes the form of compacted “noodles” extruding from the tree trunk. Scion initiated population monitoring in November 2019 in Kumeu, Blockhouse Bay and Titirangi using panel traps baited with ethanol. Additional traps consisting of *Eucalyptus fastigata,* avocado and cherry wood bolts pre-soaked in ethanol were established at Kumeu from October 2020 to April 2021. Based on the results presented in File Note 36316895, Taiwan cherry ethanol-infused bolts were more attractive to GAB than *E. fastigata* and avocado. Wood bolts not soaked in ethanol received zero attacks. We hypothesize that *Eucalyptus* trees (among other hardwoods), but only those under stress (emitting ethanol as a stress response), will be under threat of attack from this pest, but may not be as susceptible as other species of woody trees in New Zealand. Maintaining stress-free young trees within forest nurseries will be important to avoid attacks from this new pest. We recommend FGR support Scion undertaking more research to understand this pest, how to manage it, and the relative susceptibility of alternative tree species to GAB in New Zealand.

Significant progress has also been made through the Better Border Biosecurity collaboration (b3nz.org.nz) on the safety of insect releases for biological control (through the development of a risk assessment model). Recognition of biosafety risks associated with introduced biocontrol agents (BCAs) is globally increasing, and pre-release assessments of these agents have become more rigorous in many countries, especially New Zealand. We advocate for adoption of a more comprehensive, ecologically-based, probabilistic risk assessment approach to BCA releases. An example is provided using a Bayesian network that can integrate information on probabilities and uncertainties of a BCA to spread and establish in new habitats, interact with non-target species in these habitats, and eventually negatively impact the populations of these non-target species. The new model, BAIPA (for “Biocontrol Adverse Impact Probability Assessment”), could eventually be incorporated into a structured decision-making framework that has potential to support national regulatory authorities such as the EPA (Environmental Protection Authority) in New Zealand. The authors of the File Note 36316811, along with international collaborators, have had a scientific manuscript accepted describing ecological models on biocontrol risk and proposing development of the new BAIPA model. We summarise the content, but cannot permit the entire manuscript be released publicly on the FGR website at this time, as the publication rights have been signed over to the journal.

Cypress canker disease expression is observed irregularly within New Zealand; however, it tends to be more severe in warmer areas. Because species of *Seiridium* on Cupressaceae in New Zealand have not been well characterised, there is a lack of knowledge regarding pathogenicity and distribution. Molecular research was undertaken on 63 *Seiridium* isolates in the Scion culture collection (NZFS). One gene region was sequenced, and it groups the isolates into 6 different clades, representing four described species (*Seiridium unicorne*, *S. neocupressi*, *S. kartense* and *S. carnicum*) and two possible novel species (*Seiridium* sp.) (File Note 36317015). Based on earlier pathogenicity data, it seems that *S. neocupressi*, *S. carnicum* and *Seiridium* nov. sp. 1 are the species pathogenic to *C. macrocarpa* and *Ch. lawsoniana*. This is new knowledge for New Zealand. Scion believes additional research will be required to explore the pathogenicity and identity of the possibly novel *Seiridium* species in New Zealand.

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Introduction

Plantation species other than *Pinus radiata* (radiata pine), such as Douglas-fir, cypresses and eucalypts, form an important part of a diversified forest estate. The Forest Growing Science and Innovation Strategy 2020-2035 provides a roadmap for achieving the forest growers vision for 2050. This includes a key science and innovation theme (Theme 2) "Ensuring the long-term sustainability of commercial forestry through realising value from emerging species (exotic and indigenous) and developing new models for forestry". The strategy clearly recognises the need to grow investment into science and to take emerging tree species into mainstream forestry. Doubling the planting of eucalypt species (and cypresses and redwoods) by 2035 is a medium priority focus area for Theme 2. This will be achieved by increased confidence in their resilience, achieved by a multitude of pathways, but relying heavily upon the successful biological control for suppressing the negative impacts of pests of *Eucalyptus* species. Breeding for resistance to pests and diseases and silviculture will also contribute significantly to all alternative tree species. Theme 3 covers future proofing forest growing, and another outcome is "minimising biotic risk to trees from pests and pathogens".

Research being undertaken at Scion continues to support all the aspects of the FGR strategy. *Eucalyptus* species remain important to New Zealand. The biggest threat to many *Eucalyptus* plantations has historically been from the *Eucalyptus* tortoise beetle, *Paropsis charybdis*. SWP, MPI, and large commercial growers have been supporting Scion's attempt to improve the biological control of tortoise beetle by introducing a new parasitoid. However biological control is likely to be a slow process before improved pest control and therefore crown health and growth will become measurable (Withers and Cridge, NZ Tree Grower 2021, in press). Meanwhile Scion continues to be an active partner within the Better Border Biosecurity collaboration (b3nz.org.nz) with researchers working on methods to assess and therefore reduce risk from uncertainty, and improve the safety and predictability of biological control. Recognition of biosafety risks associated with introduced biocontrol agents is globally increasing, and pre-release assessments of biological control agents (BCAs) have become a key aspect of ensuring biocontrol can be fully accepted and maintain its cultural and social licence to be a key management tool for invasive species.

In addition to eucalypts, a number of cypress species are grown in New Zealand for timber and other purposes. Cypresses have long been a favourite alternative to radiata pine for New Zealand's farm foresters, small-scale plantation owners, and some large-scale growers. The total area of cypresses in New Zealand is around 10,000 hectares. The most commonly grown species are (i) *Cupressus macrocarpa* – 'macrocarpa' and (ii) *Cupressus lusitanica* – 'lusitanica' or Mexican cypress.

The cypresses represent only a small portion of the overall SWP programme, and the priorities are to maintain the breeding populations (growth, form, canker tolerance). In recent years, controlled crossing of cypresses has been undertaken to produce new hybrids. Many species of Cupressaceae are affected by a pathogen that causes cypress canker. The distribution of cypress canker is irregular within New Zealand, however it tends to be more severe in warmer areas. Because species of *Seiridium* (Ascomycota: Amphisphaeriaceae) on Cupressaceae in New Zealand have not been well characterised, there is a lack of knowledge regarding pathogenicity and distribution. Continuing research on cypress canker-resistance will benefit from research into the genetics of the canker pathogens (*Seiridium* spp). Characterisation of Scion's *Seiridium* culture collection has been an important step towards providing understanding of what species are present in New Zealand. This knowledge will improve future research and biosecurity response. The research in this technical report has been undertaken this year from SSIF funding to Scion. The degree of completion of some of the research has been interrupted by staff changes in 2020/2021, we herein report the interim results as File Note 36317015.

Recommendations and conclusions

Ambrosia beetles (of which the newly invasive *Xylosandrus crassiusculus* belongs) efficiently locate and preferentially attack living, weakened plants, especially those physiologically stressed by flooding, inadequate drainage or frosting. The preliminary results presented here in File Note 36316895, suggest that although not as attractive as Taiwan cherry, nevertheless E. fastigata ethanol-infused bolts were attractive for some attack. Wood bolts not soaked in ethanol received zero attacks. This suggests Eucalyptus trees (among other hardwoods), under physiological stress will be under threat of attack from this pest. Although currently believed to be only in the Auckland region of New Zealand, with no surveillance occurring the pest could be more widely distributed, and maintaining stress-free young trees within forest nurseries will be important to avoid attacks from this new pest. We recommend FGR support Scion undertaking more research to understand this pest, how to manage it, and the relative susceptibility of alternative tree species to GAB in New Zealand.

Scion continues to be an active partner within the Better Border Biosecurity collaboration (b3nz.org.nz) with researchers working on methods to assess and therefore reduce risk from biocontrol agent introduction uncertainty, and therefore improve the safety and predictability of biological control. We believe the research underway at Scion, such as File Note 36316811, will ensure biological control can maintain its cultural and social licence to continue to be a key management tool for managing invasive pest and weed species in New Zealand.

One gene region from cypress canker isolates in the Scion culture collection was sequenced, and reveals isolates from 6 different clades, representing four described species (*Seiridium unicorne*, *S. neocupressi*, *S. kartense* and *S. carnicum*) and two possible novel species (*Seiridium* sp.) (File Note 36317015). Based on earlier pathogenicity data, it seems that *S. neocupressi*, *S. carnicum* and *Seiridium* nov. sp. 1 are the species pathogenic to *C. macrocarpa* and *Ch. lawsoniana*. Until recently, it was thought that only two species were associated with cypress canker disease in New Zealand; however, now there could be as many as six or even more, which could have implications for management and resistance breeding programmes. Research to confirm these findings will continue next year, and we recommend host pathogenicity trials are required in the future.

File Note

PAD ID: 36316895

Date: 29 June 2021

Subject: Granulate Ambrosia Beetle Trapping Research

Authors: Roanne Sutherland, Nicolas Meurisse, Toni Withers

Introduction

Ambrosia beetles are wood-boring weevils that live in a symbiotic relationship with ambrosia fungi. The beetles carry the fungus with them as they colonise new host trees and inoculate them. The fungus extracts the nutrients from the plant cells for the beetles and their offspring to feed on. Ambrosia beetles are globally successful invaders, with more than 50 species establishing outside of their native range**.** The granulate ambrosia beetle (GAB), *Xylosandrus crassiusculus* (Coleoptera: Curculionidae, Scolytinae) is native to tropical and sub-tropical East Asia and recently introduced into New Zealand.

Xylosandrus crassiusculus colonises freshly cut wood, physiologically stressed trees and occasionally seemingly healthy trees. It is one of the most polyphagous ambrosia beetle worldwide, being able to colonize over 120 broadleaf tree species in 40 families. It is also considered a major pest to nurseries overseas, attacking fruit trees such as: apple, pear, cherry, plum, peach and avocado and trees such as eucalypt and oak. Trees under physiologically stress such as frost, drought, flooding and fire produce ethanol that that acts as an attractant to the beetles. Ethanol may be crucial to the successful development of the symbiotic fungus in the galleries (Ranger, et al., 2018). The first sign of an attack are often frass noodles extruding from the trunk, branch or exposed roots. Attacks can also lead to defensive sap production and leaf wilting. There are limited management options once beetles have established in trees, the best method is to ensure tree health to reduce attractiveness (Ranger, et al., 2013).

Once a suitable host tree has been identified the adult female bore into the sapwood to create brood galleries. The foundress female does not feed on the wood but extrudes sawdust as it tunnels into the wood of new host tree, creating distinctive “toothpick noodles”. The adult female carries a symbiont fungi *Ambrosiella roeperi,* in its mycangia that she introduces to the galleries. In ambrosia beetles, both adult and offspring feed on the fungal hyphae, in contrast to bark beetles, which feed on the plant living tissue and phloem. Once the fungus has established the already mated female will lay numerous eggs. Adult, eggs, larvae and pupae can all be found together in a single brood gallery. Generation time is around 50-60 days after egg laying, during spring and summer. Adults will overwinter as adults in host tree emerging in the spring to colonize new host trees.

In New Zealand, *Xylosandrus crassiusculus* was first identified on 20th February 2019 in two pinoak (*Quercus palustris,* Fagaceae) trees in Blockhouse Bay Recreation Reserve, Auckland. The Ministry for Primary Industries (MPI) found no conclusive pathway for how it invaded New Zealand. However, it is thought to have arrived at least 18 months earlier, presumably on imported timber products. Previously, *Xylosandrus crassiusculus* represented 0.6% of Scolytinae intercepted in New Zealand during a study by Brckerhoff et al (Brockerhoff, et al., 2006).

The hosts that are of obvious concern to New Zealand include *Eucalyptus* (*camaldulensis* and *robusta,* Myrtaceae), *Grevillia robusta* and macadamia (Proteaceae), avocado (Lauraceae), prunus and plums (Rosaceae), acacia (Fabaceae), and persimmon (Ebenaceae). *Xylosandrus crassiusculus* has been found attacking at least 15 different species in West Auckland (7 native and 8 exotics as of August 2019, MPI unpublished data).

This is the first study of *X. crassiusculus* in New Zealand, the aim of the study was to 1) to test host potential using ethanol-soaked wood bolts, including *Eucalyptus* wood, 2) establish the phenology of *X. crassiusculus* to understand the number of generations it will undertake per annum in Auckland.

Methods

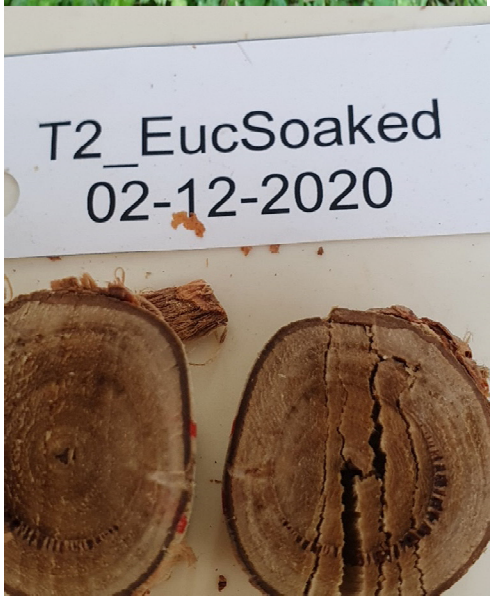
Experimental sites were chosen within the known distribution of *X. crassiusculus* in West Auckland, New Zealand on privately owned land. All sites were grass (Poaceae) pasture of turf, with established plantings of mixed native and on-native trees where *X. crassiusculus* had been found during the incursion response by MPI. Pilot study was established in October 2019 to 2020 at three sites, Kumeu, Blockhouse Bay and Titirangi, West Auckland, New Zealand to establish trapping methods and to find the most reliable site of *X. crassiusculus* population.

Kumeu was identified as the most reliable site. Experiments were set up in October 2020 as randomized complete block design with five replicates and 6 treatments plus 4 controls. Five blocks were established at least 20 m apart and the positions of treatments within blocks 10 m apart. Each block contained one treatment each of: Ethanol infused wood bolts of *Eucalyptus fastigata* (Myrtaceae), *Persea americana* Hass avocado, *Prunus campanulata* Taiwan Cherry, and control un-soaked wood bolts of each species (*E. fastigata, P. americana* and *P. campanulata*). Intercept traps baited with ethanol lures with three release rates: 1: 150 micron thick and 50 mm wide plastic lure (release rate 0.008 gr/day), 2: 150 micron thick plastic lure and 50 mm wide with a 5 mm hole punched in the top (release rate 2 gr/per day), and 3: 70 micron thick and 100 mm wide plastic lure (release rate 0.04 gr/day) and a control panel trap with no ethanol lure. Lures were produced at Scion from clear polyethene plastic tubing filled with 150 ml ethanol and stored in resealable plastic bags at -20 °C until needed. Release rates were established under field conditions during December 2019 – January 2020 by the lures being rotated and weighed daily for 14 days (Jess Kerr, unpublished data). Field traps were hung from metal standards at least 1.3 m above ground, with dry collection containers at the trap base. Traps were sprayed monthly with a long-term surface insecticide spray and lures weighed fortnightly and replaced monthly with fresh ones removed 5 hours before from the freezer.

Wood bolts were cut from main stems of trees, length 30 cm, and diameter between 2.5- 5 cm from two locations. *Eucalyptus fastigata* and *P. campanulata* from Pyes Pa, Tauranga, *P. americana* from Welcome Bay, Tauranga. *Persea americana* wood bolts were cut from trees that were not treated with phosphite although *Phytophthora cinnamomi* is present on the property. Each bolt was soaked in 20% ethanol bath for at least 24 hours, infused bolts were then removed, and a 30 x 3 mm eye screw was screwed into the top of the bolt then sealed in a resealable plastic bag and stored overnight at 4°C to use the following day. Bolts were hung from metal standards at least 0.7 m above the ground and replaced fortnightly.

Fortnightly site visits undertaken to: empty collection containers from the flight intercept traps (contents stored in 70% ethanol specimen vials), lures weighed, and wood bolts replaced. Wood bolts were sealed in plastic bags in a container and frozen at -20 °C for two weeks at AsureQuality, Blockhouse Bay, Auckland, before transporting to Rotorua to ensure no live organisms were moved outside of the infected zone (thereby meeting the requirements of the Hazardous Substances and New Organisms (HSNO) Act). The focus was to primary identify the presence of *X. crassiusculus* when sorting specimens and dissecting wood bolts. Hand pruners were used to split the bolts to identify the Scolytinae species using a dissecting microscope, recording the number of adults, eggs, larvae, pupae, fungal gardens, size of gallery and entrance hole.

Table 1: Images of granulate ambrosia beetle attacks on ethanol-infused wood bolts (Scion) showing the placement of the wood bolt in the field (top left), and the frass noodles produced (bottom right).



Results

Seasonal flight activity (Figure 1) was recorded over the two seasons of monitoring from October 2019 to 2021, using flight intercept traps. The results were very consistent between years, with a peak of over-wintering adults flying (emerging from host trees) caught each October and the resulting offspring (next generation) adult flight recorded each December.

Unsoaked (control) wood bolts of each species of tree, received no attacks at all, from *X. crassiusculus* or other species. Ethanol-soaked wood bolts were an effective method of attracting gallery excavation and egg laying by female *X. crassiusculus.* All adults located within galleries were identified to species. This revealed that in addition to the GAB the ethanol-infused bolts also attracted some non-target Scolytinae species, two other species established galleries in the bolts, namely (*Microperus eucalypiticus* and *Xyleborinus saxesenii)*. The entry holes are generally 0.5-0.6mm in diameter, whereas *X. crassiusculus* entry holes are 1.0mm in diameter.

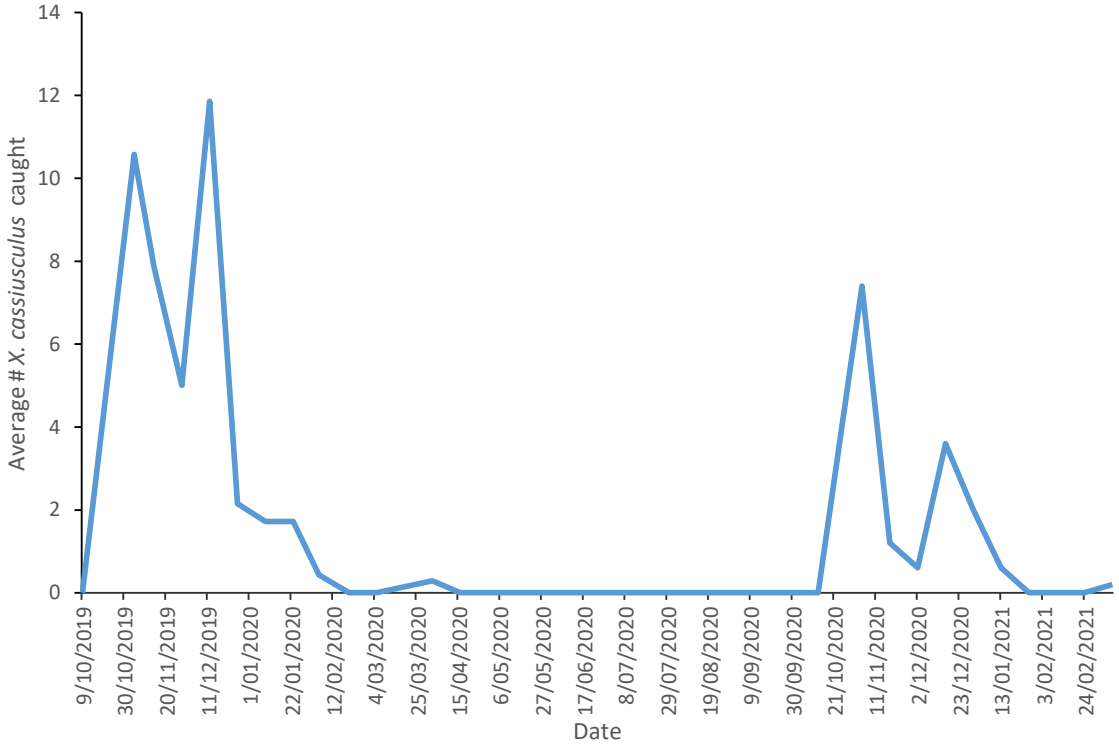


Figure 1. *Seasonal flight activity of adult* Xylosandrus crassiusculus *in Kumeu, Auckland between October 2019 and March 2021, using flight intercept traps baited with ethanol lures.*

Ethanol-infused bolt attack rates in the Kumeu field trial were variable, with the highest number of holes in December. The number of attacks per bolt ranged between 0 to 21 holes, made within a mean exposure period of 14 days. The mean number of attacks each infused wood bolt received from ***X. crassiusculus*** was highest on the Taiwan cherry *(P. campanulata*), followed by the avocado and *E. fastigata* (Figure 2).

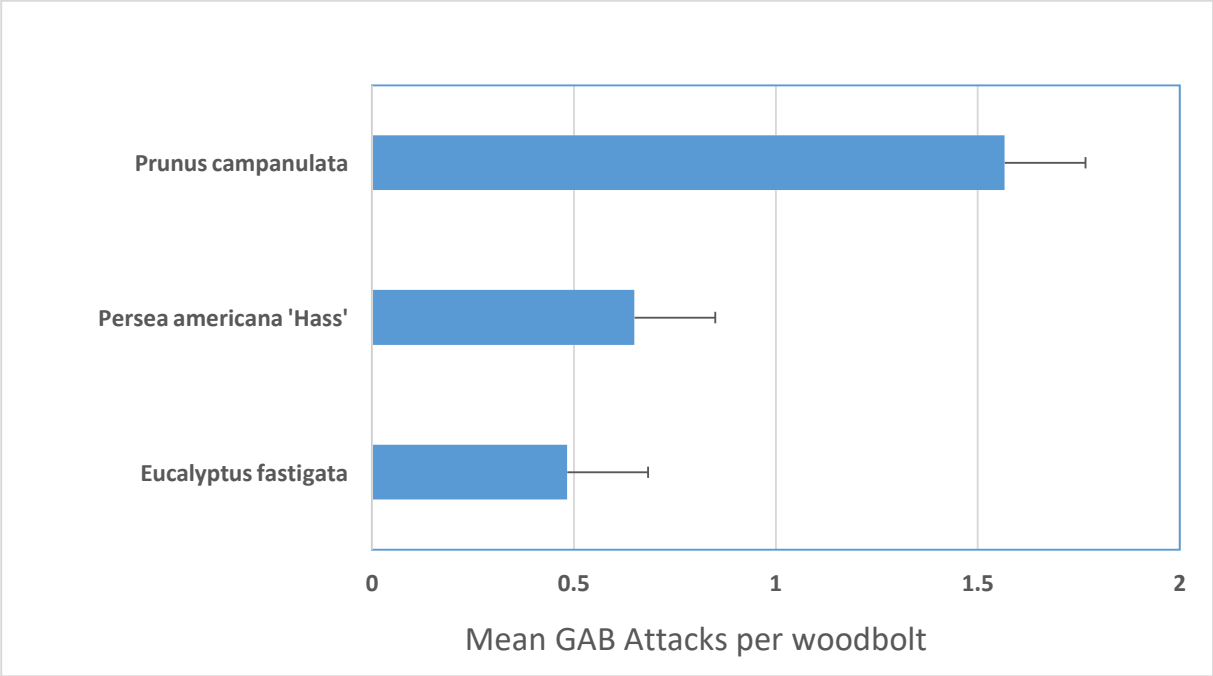


Figure 2. *Mean (+SE) number of attacks by* Xylosandrus crassiusculus *in Kumeu, Auckland per ethanol-infused bolt lure left in the field for 14 days on average. Unsoaked control bolts received zero attacks (not included in graph).*

Conclusion

The granulate ambrosia beetle, *X. crassiusculus*, has been a highly successful invader worldwide. The beetles tiny size, the increasing global trade of timber and other woody material, and the ability for a single foundress female to initiate a new colony are traits that have undoubtedly assisted it to invade New Zealand. Like most invasive ambrosia beetles, *X. crassiusculus* can attack a wide range of woody plants across diverse habitats and they prefer physiologically stressed trees. They will excavate galleries in which they lay eggs and inoculate a symbiotic fungus, *Ambrosiella roeperi* on which the beetles feed. We do not currently know the beetles full plant host range in NZ and it could already have established outside of the known range of West Auckland. Currently no formal delimitation surveys are taking place and the population is likely to be dispersing. Often the first sign of an attack is the sawdust released by the excavating adult, which takes the form of compacted “noodles” extruding from the tree trunk, branch or exposed roots. This could be one useful symptom that can be reported from surveillance, to help us track the spread of this pest. Monitoring in nurseries will be important during the peak flight periods in late October and December, during this time it will be critical to ensure optimal tree health to reduce likelihood of attacks and establishment. Once adults have established galleries in the heartwood there are limited control options. Insecticides could possibly be applied as a deterrent to the invading female, but the best action to prevent establishments is promoting good tree health.

Scion’s ethanol-infused bolt traps (the ethanol mimics the “stress signal” given off by a stressed tree) revealed Taiwan cherry was preferred over eucalypts and avocado, but all tree species were subject to invasion. Due to the nature of the beetle, there are concerns it could in the future become a pest within forestry nurseries attempting to raise young tree stock for planting. While *Pinus* species are immune from this particular ambrosia beetle, there are concerns for future impacts on many other hardwood species that form an important part of a diversified New Zealand forest industry.

Next year Scion will analyse the data from the current study, and plan future research to examine the host range and potential impact of *X. crassiusculus* on New Zealand native trees, shrubs, as well as key horticultural and forestry species.

Acknowledgments

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File Note

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Update on the diversity of *Seiridium* species associated with Cypress Canker Disease (CCD) in New Zealand

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Abstract

Cypress canker or cypress canker disease (CCD) is a serious disease of the Cupressaceae. It is caused by a number of different *Seiridium* species, including *S. cardinale*, *S. cupressi* and *S. unicorne*. This disease severely limits the expansion of New Zealand's ~10,000 ha of planted cypress.
Recently, there has been a taxonomic overhaul of the *Seiridium* genus, including the description and redescription of species. Given the taxonomic changes within the genus, a review of the *Seiridium* species identified and kept in Scion's National Forest Culture Collection (NZFS) was needed. Sixty-three isolates of the ~450 NZFS *Seiridium* isolates from Cupressaceae and Pinaceae were selected for a preliminary analysis. All the isolates were sequenced using a partial region of the β-tubulin gene region (*BTUB*) and a Maximum Likelihood (ML) phylogenetic tree constructed. Based on the results of the ML analysis, the 63 *Seiridium* isolates grouped into 6 different clades, representing at least four known species (*Seiridium unicorne*, *S. neocupressi*, *S. kartense* and *S. cancrinum*) and two clades identified as *Seiridium* sp. *Seiridium cupressi* was not detected in the sampled isolates. Based on earlier pathogenicity data, it seems that *S. neocupressi*, *S. cancrinum* and a *Seiridium* sp. isolate are pathogenic to *Cupressus macrocarpa* and *Chamaecyparis lawsoniana*. Characterizing these isolates further with additional gene regions, along with more NZFS *Seiridium* isolates, is needed to fully explore the diversity hidden in the collection and New Zealand. In addition, more pathogenicity data is needed to determine the threat any of these isolates play to commercial forestry in New Zealand.

Introduction

Cypress canker or cypress canker disease (CCD) is a serious disease of the Cupressaceae (Danti & Della Rocca 2017; Graniti 1998). Until recently, it was thought to be caused by three fungal pathogens, *Seiridium cardinale* (Wagner 1939), *Seiridium cupressi* (Boesewinkel 1983; Fuller & Newhook 1954) and *Seiridium unicorne* (Boesewinkel 1983). All three species have a wide geographical distribution, of which *S. cardinale* has the widest. After *S. cardinale* was discovered in California in 1928, the pathogen has since spread to New Zealand, Europe, the entire Mediterranean basin, Africa and Australia (Danti and Delta Rocca 2017).

Within the Cupressaceae, *S. cardinale* has been associated with disease on *Cupressus*, *Chamaecyparis*, *Juniperus*, *Thuja* and x*Cupressocyparis* (Danti et al. 2013, 2014; Graniti 1998). Although the pathogen can establish and infect a number of different hosts; epidemics are favoured by suitable climatic conditions, as seen in the Mediterranean, South Africa and New Zealand, and the density and continuity of susceptible hosts (Graniti 1998). The CCD pathogens typically infect through wounds, created by wind, frost, pruning or insects, upon which cankers appear (Hood et al. 2001; Danti and Della Rocca 2017). Once a susceptible host is infected, tissue necrosis begins, spreading steadily inward to the cambium, which eventually causes the plant to die (Graniti 1998).

Due to the severity of *S. cardinale* compared to other species of *Seiridium*, more is known and published about this species. This is partly because of the confusion around the taxonomy and identification of the pathogens causing CCD. Early identifications were based on the morphology of conidial appendages (Barnes et al. 2001; Bonthond et al. 2018); *S. cardinale* lacked appendages, while the appendages of *S. cupressi* followed the curve of the conidia and those of S*. unicorne* were perpendicular to the conidia. These morphological characters can vary between isolates, and species were often misidentified.

Molecular tools, such as DNA sequencing and the construction of phylogenetic trees to infer relatedness, are useful for delineating species. Protein coding gene regions offer more resolution when compared to ribosomal gene regions for this genus (Barnes et al. 2001; Graniti 1998; Swart 1973; Viljoen et al. 1993). Bonthond et al. (2018) showed that with the combination of four protein coding gene regions, namely *ITS*, *TEF, BTUB* and *RPB2* they could describe and redescribe many different *Seiridium* species associated with cypress, including *S. cardinale*, *S. unicorne*, *S. cancrinum*, *S. cupressi* and *S. kenyanum*. These scientists also introduced *S. neocupressi,* which was represented as *S. cupressi* in earlier studies (Barnes et al. 2001; Cunnington et al. 2007; Tsopelas et al. 2007) and originally collected from Australia and New Zealand. It appears that *S. neocupressi* and not *S. cupressi* could be the most important causal agent of CCD in these countries (Bonthond et al. 2018).

In this study, we explored the diversity of *Seiridium* isolates currently housed in Scion's National Forest Culture Collection (NZFS), that represented a range of host species and geographic locations. Many of the isolates were originally identified as either *S. cupressi* or *S. unicorne*, based on morphology (Table 1). Given that the genus has seen a taxonomic overhaul, it is important from a diagnostics and biosecurity point of view to understand which species are present in New Zealand and which cause the greatest damage to New Zealand's commercial forestry industry.

Background

Cypress trees belong to the family Cupressaceae, which contains more than 30 genera and over 140 species. Seventeen of those species, including *Callitris, Cryptomeria*, *Cupressus and Thuja*, have been planted across New Zealand since the mid-to-late 19th century. For commercial forestry, *Chamaecyparis*, *Cupressus*, *Juniperus*, *Sequoia* and *Thuja* are the more important genera. The planted area of cypress spans over 10,000 ha and has remained relatively the same since 2010 (Bulman and Hood, 2018).

In New Zealand, cypress canker or CCD is a major limiting factor restricting the wider planting of cypress as a commercial crop. Cankers may form on stems or branches and cause shoot or tip dieback and the disease can kill a tree of any age (Bulman & Hodd 2018). Cypress canker was first described in New Zealand as "gummosis" disease on *Cupressus macrocarpa* (macrocarpa) and *Chamaecyparis lawsoniana* (Lawson's cypress) and attributed to *S. cardinale* (Birch, 1933), who found the disease widespread in the North Island, mainly in plantations. Later, Fuller and Newhook (1954) reported cypress cankers on *Ch. lawsoniana* shelterbelts in the Waikato district. The causal agent according to the authors was *S. unicorne* and not *S. cardinale*. Their study focused on shelterbelts and not plantations. Since the mid-twentieth century, *C. macrocarpa* and *Ch. lawsoniana* have generally been avoided for planting because of the threat of disease (Weston 1957, 1971; Newhook 1962; Gilmour 1966). *Cupressus lusitanica* (lusitanica) was then favoured as an alternative species as it was considered less susceptible to cypress canker (Fuller & Newhook, 1954; Bannister & Orman 1960; Newhook 1962).

In 1981 and 1982, a study was performed by van der Werff (1985) to assess the potential and status of *C. macrocarpa*, *Ch, lawsoniana* and *C. lusitanica,* considering a number of factors, including their susceptibility to cypress canker. It was reported that cypress canker was widespread in both the North and South Islands; however, disease levels were low compared to previous reports, especially amongst older trees. Shelterbelts seemed harder hit by the disease when compared to plantations. The widespread distribution of the CCD pathogens is thought to be the result of New Zealand's favourable temperate climate with no seasonal temperature extremes and constant rainfall throughout the year (van der Werff 1985). Interestingly, cypress canker was the only organism found threatening the health of these trees from van der Werff's study.

Since the early 1900s, it seemed as if there was a shift in the populations of *S. cardinale* and *S. unicorne* in New Zealand. *Seiridium unicorne* was being identified more often compared to *S. cardinale*, which seemed to have become a 'residual population' (van der Werff, 1988b). It was hypothesised that a reduction in virulence of *S. cardinale* was to blame; however, a study by Chou et al. (1990) proved, with

pathogenicity trials on *C. macrocarpa* and *C. lusitanica*, that *S. cardinale* was still the more virulent pathogen. Host preference could explain the observed differences in populations, as *S. cardinale* was readily isolated from *T. plicata*, where *S. unicorne* was not. *Seiridium unicorne* was more readily isolated from *Ch. lawsoniana*, where *S. cardinale* was not (Wagerner 1939; Strouts 1973).

Some of the observations around population density, pathogenicity and host differences between the different CCD pathogens could be explained by taxonomic confusion. While *S. cardinale* was correctly identified (in most cases) in New Zealand, many of the *S. unicorne* isolates were misidentified and were later identified as *S. cupressi* (van der Werff 1988a). Until recently, CCD in New Zealand was thought to be caused by *S. cardinale* and *S. cupressi*, both of which are distributed throughout the country on a number of cypress species (Bulman & Hood 2018). However, a recent phylogenetic analysis of a global collection of *Seiridium* isolates, which included a limited number of New Zealand isolates, showed that *S. cardinale*, *S. neocupressi* and *S. unicorne* were present in NZ (Bonthold et al. 2018). Following this finding, updated diagnostics procedures in the Scion Forest Health Reference Laboratory confirmed that *S. cancrinum* was also present in a number of sites in the Bay of Plenty on *C. macrocarpa* (Smallman et al. unpublished)

While the CCD pathogens have historically been associated with the Cupressaceae, canker symptoms were identified on a *Pinus pinaster* during routine Forest Biosecurity Surveillance in the Bay of Plenty in 2018. A species of *Seiridium* was isolated from the canker. This was the first report of *Seiridium* from *Pinus* anywhere. The finding was immediately reported to MPI. Since then a few more isolates of *Seiridium* have been isolated from cankers on or needles of *Pinus radiata* in New Zealand.

Knowing the diversity of *Seiridium* spp. associated with New Zealand's Cupressaceae and *Pinus* is critical to ensure we know what species are present (and which are absent) and represent a biosecurity risk. Also, understanding the role they play in canker/disease development. Having the correct identities is also necessary for any breeding programmes. It is well understood that resistance to CCD varies among the cypresses (Beresford and Mulholland 1982) and work is underway to develop more canker-resistant *C. macrocarpa* genotypes (Aimers-Halliday et al. 2002; Gea & Low 1997) and hybrids; however, we need to understand what *Seiridium* species are present in New Zealand and most important in causing da image. This will enable the use of the most appropriate isolates in future resistance screening.

Methods

Selected *Seiridium* isolates from Scion's NZFS culture collection, representing a range of cypress and pine host species and geographic range, were cultured on top of cellophane sheets on the surface of malt extract agar (2% MEA) plates. DNA was extracted from fungal mycelia with the Promega Genomic DNA purification kit (Fitchburg, Wisconsin, USA) according to the manufacturer's protocol. Region 1 of the β-tubulin gene region (BTUB) was amplified with the primers T1 and bt2b (5'-AACATGCGTGAGATTGTAAGT-3' and 5'-ACCCTCAGTGTAGTGACCCTTGGC-3') (Glass and Donaldson 1995; O'Donnell and Cigelnik 1997). The PCR programme was 7 min and 30 s of initial denaturation at 95 °C, 35 cycles of 30 s denaturation at 95 °C, 30 s annealing at 53°, and 90 s elongation at 72 °C with a final elongation step of 7 min 30 s at 72 °C. The amplicons were sequenced with both forward and reverse primers, using an ABI Prism 3730XL Sequencer (Applied Biosystems). Sequences were quality checked and assembled using DNASTAR Lasergene SeqMan Pro v. 8.1.3 software.

All of the BTUB sequences generated from this study were combined with the BTUB dataset compiled by Bonthond et al. (2018) and aligned using MAFFT v. 7. Alignments were checked, trimmed and edited in BioEdit v. 7.2.5. A Maximum-likelihood (ML) analysis of the BTUB dataset were performed with PhyML v. 3.1 using an HKY+I+G substitution model, determined by jModelTest 2.1.10, with 1000 bootstrap iterations. The constructed ML tree was viewed and edited in MEGA X.

Results

Table 1: Isolates of *Seiridium* used in the construction of the *BTUB* Maximum likelihood analysis

Isolate number:	Other number:	Host:	Location[1]:	Isolation Date:	Initial ID2:	Putative ID[3]:	Pathogenicity data[4]:
NZFS 4188	L.87.2	*C. macrocarpa*	BP	07/2000	*S. cupressi*	*S. cancrinum*	n/a
NZFS 4177	L.101.2	*C. macrocarpa*	BP	10/2000	*S. cupressi*	*S. cancrinum*	n/a
NZFS 4178	L.101.4	*C. macrocarpa*	BP	10/2000	*S. cupressi*	*S. cancrinum*	Yes (2)
NZFS 2586	n/a	*C. macrocarpa*	BP	03/1996	*S. cupressi*	*S. cancrinum*	n/a
NZFS 4176	L.85.1	*C. macrocarpa*	BP	07/2000	*S. cupressi*	*S. cancrinum*	n/a
1810035-1	n/a	*Pinus pinaster*	BP	03/2018	*Seiridium* sp.	*Seiridium* sp.**	n/a
L114.03	6383	*Sequoiadendron giganteum*	NC	06/1999	*S. cupressi*	*S. neocupressi*	n/a
NZFS 5025/b	1909081-1	*Chamaecyparis sp.*	New Zealand	09/2019	*Seiridium* sp.	*S. neocupressi*	n/a
NZFS 4183	L195.3	*C. lusitanica*	NC	05/2003	*S. cupressi*	*S. neocupressi*	n/a
NZFS 4239	L156.2.2	*C. macrocarpa*	New Zealand	09/2002	*S. cupressi*	*S. neocupressi*	Yes (3)
NZFS 4233	L.195.2	*C. lusitanica*	NC	05/2003	*S. cupressi*	*S. neocupressi*	n/a
NZFS 4218	L.195.1	*C. lusitanica*	NC	05/2003	*S. cupressi*	*S. neocupressi*	n/a
2007075-1	n/a	*C. macrocarpa*	SL	07/2000	*Seiridium* sp.	*S. neocupressi*	n/a

NZFS 4245	L.156.2.1	*C. macrocarpa*	WI	02/2002	*S. cupressi*	*S. neocupressi*	n/a
NZFS 4249	n/a	*n/a*	New Zealand	n/a	*S. cupressi*	*S. neocupressi*	n/a
NZFS 4219	L.195.4	*C. macrocarpa*	NC	05/2003	*S. cupressi*	*S. neocupressi*	n/a
NZFS 4227	L.156.2.3	*C. macrocarpa*	WI	02/2002	*S. cupressi*	*S. neocupressi*	n/a
NZFS 114.02	6383/1	*Sequoia sempervirens*	NC	06/1999	*S. cupressi*	*S. neocupressi*	n/a
NZFS 114	n/a	*C. macrocarpa*	MC	10/1983	*S. cupressi*	*S. neocupressi*	n/a
NZFS 4213	L.188.2	*Ch. Lawsoniana*	WO	09/2002	*S. cupressi*	*S. neocupressi*	n/a
NZFS 4190	L.180.2	*S. giganteum*	NN	06/2002	*S. cupressi*	*S. neocupressi*	n/a
NZFS 4225	L.180.1	*S. giganteum*	NN	06/2002	*S. cupressi*	*S. neocupressi*	n/a
NZFS 2591	n/a	*C. macrocarpa*	TK	05/1983	*S. cupressi*	*S. neocupressi*	n/a
NZFS 4204	L.175.2	*Platycladus orientalis*	SC	04/2002	*S. cupressi*	*S. neocupressi*	Yes (3)
NZFS 4206	n/a	*Callitris rhomboidea*	TK	06/2000	*S. cupressi*	*S. neocupressi*	n/a
NZFS 4202	L.155.5.1	*C. macrocarpa*	HB	02/2002	*S. cupressi*	*S. neocupressi*	Yes (2)
NZFS 4181	L.233.5	*Cupressocyparis ovensii*	AK	07/2004	*S. cupressi*	*S. neocupressi*	n/a
NZFS 4193	L.233.2	*C. ovensii*	AK	07/2004	*S. cupressi*	*S. neocupressi*	n/a
NZFS 4184	L.188.7	*Ch. lawsoniana*	WO	09/2002	*S. cupressi*	*S. neocupressi*	n/a

NZFS 4226	L.188.6	*Ch. lawsoniana*	WO	09/2002	*S. cupressi*	*S. neocupressi*	n/a
NZFS 4238	L188.4	*Ch. lawsoniana*	WO	02/2002	*S. cupressi*	*S. neocupressi*	n/a
2006042-1	n/a	*Ch. lawsoniana*	AK	06/2000	*S. unicorne**	*S. unicorne*	n/a
2006055-3	n/a	*Ch. lawsoniana*	AK	06/2000	*S. unicorne**	*S. unicorne*	n/a
NZFS 2587	n/a	*C. macrocarpa*	BP	03/1994	*S. cupressi*	*Seiridium* sp.	n/a
NZFS 4246	L.217.2	*Ch. lawsoniana*	ND	10/2003	*S. cupressi*	*Seiridium* sp.	n/a
NZFS 4230	L.155.6.1	*C. macrocarpa*	HB	02/2002	*S. cupressi*	*Seiridium* sp.	n/a
NZFS 4236	L155.4.1	*C. macrocarpa*	HB	02/2002	*S. cupressi*	*Seiridium* sp.	n/a
NZFS 4237	L156.6.2	*C. macrocarpa*	WI	02/2002	*S. cupressi*	Seiridium sp.	n/a
NZFS 4241	L217.1	*Ch. lawsoniana*	ND	10/2003	*S. cupressi*	*Seiridium* sp.	n/a
NZFS 4182	L.155.7.1	*C. macrocarpa*	HB	02/2002	*S. cupressi*	*Seiridium* sp.	n/a
NZFS 4194	L.233.4	*Cupressocyparis ovensii*	AK	07/2004	*S. cupressi*	*Seiridium* sp.	n/a
NZFS 4229	L.156.6.3	*C. macrocarpa*	WI	02/2002	*S. cupressi*	*Seiridium* sp.	n/a
NZFS 4192	L.217.3	*Ch. lawsoniana*	ND	10/2003	*S. cupressi*	*Seiridium* sp.	n/a
NZFS 4235	L155.3.1	*C. macrocarpa*	HB	02/2002	*S. cupressi*	*Seiridium* sp.	Yes (1)
NZFS 4265	L176	*Cupressocyparis leylandii*	AK	04/2002	*S. cupressi*	*Seiridium* sp.	n/a

NZFS 4191	L.190.7	*Ch. lawsoniana*	AK	09/2002	*S. cupressi*	*Seiridium* sp.	n/a
2005017-4	n/a	*Ch. lawsoniana*	AK	05/2000	*Seiridium* sp.	*Seiridium* sp.	n/a
NZFS 2016	4617	*C. macrocarpa*	AK	05/1996	*S. cupressi*	*Seiridium* sp.	n/a
NZFS 2590	n/a	*n/a*	BP	10/1994	*S. cupressi*	*Seiridium* sp.	n/a
NZFS 2589	n/a	*n/a*	BP	10/1994	*S. cupressi*	*Seiridium* sp.	n/a
NZFS 4217	L.190.2	*Ch. lawsoniana*	AK	09/2002	*S. cupressi*	*Seiridium* sp.	n/a
NZFS 4196	L.206.1	*C. macrocarpa*	AK	08/2003	*S. cupressi*	*Seiridium* sp.	n/a
NZFS 4244	L204.6	*C. macrocarpa*	WO	08/2003	*S. cupressi*	*Seiridium* sp.	n/a
NZFS 4185	L.192.5	*Ch. lawsoniana*	New Zealand	03/2003	*S. cupressi*	*Seiridium* sp.	n/a
NZFS 4216	L.190.1	*Ch. lawsoniana*	AK	09/2002	*S. cupressi*	*Seiridium* sp.	n/a
NZFS 4199	L.204.3	*C. macrocarpa*	WO	08/2003	*S. cupressi*	*Seiridium* sp.	n/a
NZFS 4240	L206.2	*C. macrocarpa*	AK	08/2003	*S. cupressi*	*Seiridium* sp.	n/a
NZFS 4243	L.204.1	*C. macrocarpa*	WO	08/2003	*S. cupressi*	*Seiridium* sp.	n/a
2003045	n/a	*Thuja plicata*	WI	03/2000	*Seiridium sp.*	*Seiridium* sp.	n/a
NZFS 4186	L.192.1	*Ch. lawsoniana*	New Zealand	03/2003	*S. cupressi*	*Seiridium* sp.	n/a
NZFS 4220	L.205.1	*C. macrocarpa*	BR	07/2003	*S. cupressi*	*Seiridium* sp.	n/a

NZFS 4231	L.192.7	*Ch. lawsoniana*	New Zealand	03/2003	*S. cupressi*	*Seiridium* sp.	n/a
NZFS 4242	L.215.2	*Ch. lawsoniana*	AK	09/2003	*S. cupressi*	*Seiridium* sp.	n/a

[1]Locations based on the Crosby regions (Crosby et al 1998). Where the regions is unknown New Zealand is stated.
[2]Identification based on morphology at time of diagnosis. *Based on BLAST of DNA sequence
[3]Putative identification based on Maximum likelihood tree based on *TUB* alignment
[4]Pathogenicity data according to Hood et al. (2009): 1-More virulent; 2- Similar virulence; 3-Less virulent than L101.4 (Hood et al. 2004). L101.4 (NZFS 4178) was here identified as *S. cancrinum*.
**Based on further DNA work with MPI, this isolate is closely related to but not identical to *S. kartense*

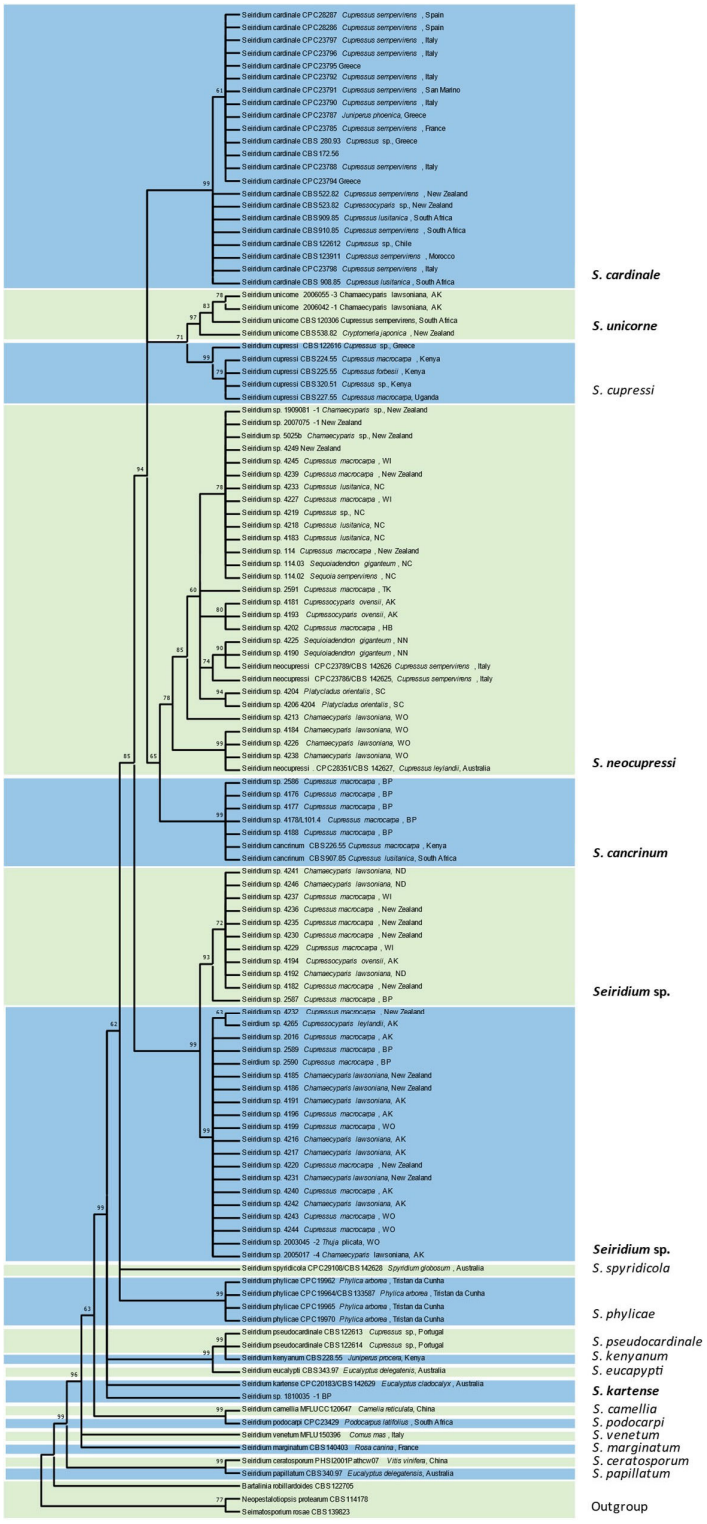

Figure 1: The best Maximum Likelihood tree from *BTUB*. Nodes are labelled with bootstrap values from PhyML. Nodes below 60 bootstrap values are not labelled. Clade colours and labels indicate monophyletic lineages, while names in bold represent groups present in New Zealand.

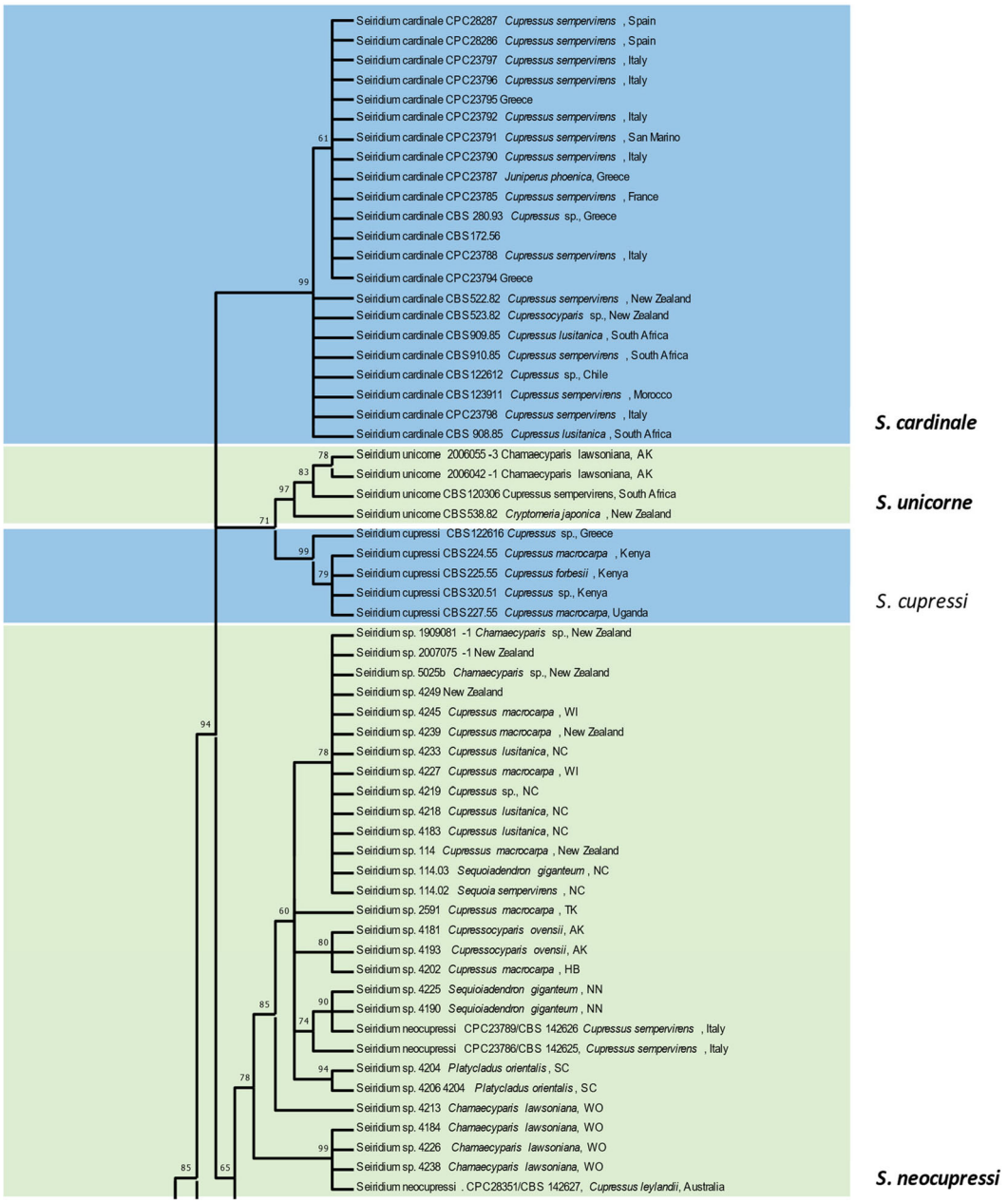

Figure 2: The top half of the best Maximum Likelihood tree from *BTUB* cut at *Seiridium neocupressi*. Nodes are labelled with bootstrap values from PhyML. Nodes below 60 bootstrap values are not labelled. Clade colours and labels indicate monophyletic lineages, while names in bold represent groups present in New Zealand.

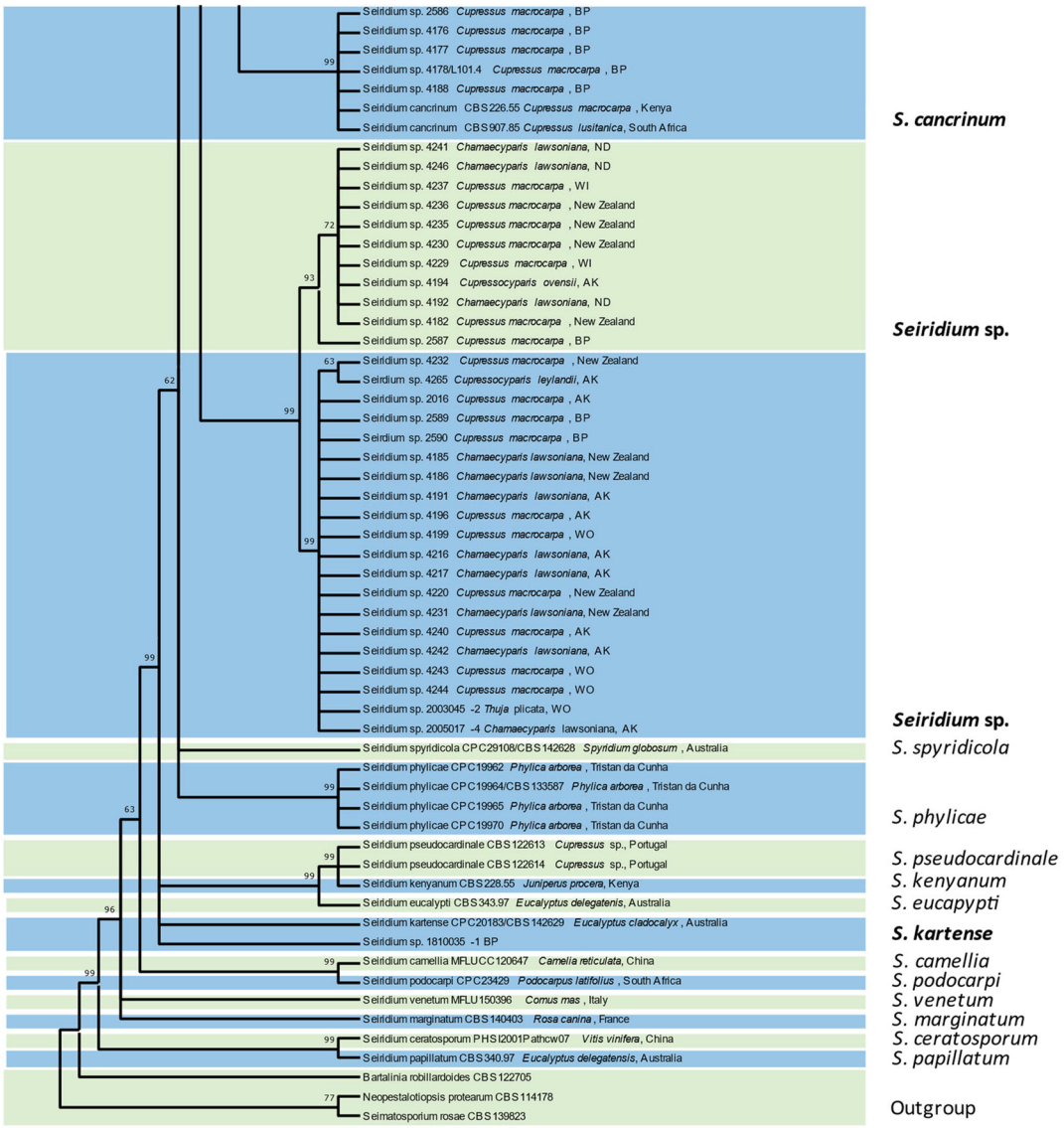

Figure 3: The bottom half of the best Maximum Likelihood tree from *BTUB* cut at *Seiridium cancrinum*. Nodes are labelled with bootstrap values from PhyML. Nodes below 60 bootstrap values are not labelled. Clade colours and labels indicate monophyletic lineages, while names in bold represent groups present in New Zealand.

Discussion

Based on the *BTUB* Maximum likelihood (ML) tree (Figures 1,2,3) that included a suite of new *Seiridium* taxa from Bonthond et al. (2018), there is wide diversity of *Seiridium* associated with the Cupressaceae in New Zealand, across the North and South Island. This diversity may yet increase upon further examination of the remaining ~370 *Seiridium* isolates still in the NZFS. It is known that *S. cardinale* and *S. unicorne* were spread to a number of countries around the world, including New Zealand, on ornamental cypress (reviewed in Graniti 1998). Currently, the origin of the wider diversity we have uncovered in New Zealand is unknown.

The 63 putatively identified *Seiridium* isolates (Table 1) located from this research clusters into six clades, namely, *S. cancrinum*, *S. kartense*, *S. neocupressi*, *S. unicorne*, and two clades with no species

identification, namely *Seiridium* sp. The isolates grouping with *S. cancrinum*, *S neocupressi* and *S. unicorne* are well represented by our *BTUB* ML tree and show similar morphological characters to the respective type strains. While isolate "1810035-1" forms a clade with *S. kartense* according to *BTUB*, the *TEF, ITS* and *RPB2* gene sequences generated by MPI suggest it is not. To correctly identify this species and the isolates identified as *Seiridium* sp., additional data will be needed from additional gene regions, such as *ITS*, *TEF* and *RPB2* (Bonthond et al. 2018).

All clades, except '*S. kartense*' and *S. unicorne* included isolates collected in New Zealand prior to 1998. Organisms that were present in New Zealand before 29 July 1998 are not considered new (HSNO Act). Bonthold (2018) included a New Zealand isolate of *S. unicorne* from *Cryptomeria japonica* with a collection date of 1981, demonstrating that this species is also not a "new to New Zealand" organism. The isolate grouping in the '*S. kartense*' clade was found after 1998 but may or may not be new depending on its identity. No isolates clustered with sequences of *S. cupressi* from overseas, making it possible that this species may not be present in the collection and in New Zealand, although the sequencing of additional cultures will make this clear. Isolates identified as *S. cardinale* in the NZFS were not included in this part of the study but will be included in the next part of the project given what we have learned about isolates previously identified as "*S. cupressi*".

The majority of the NZFS isolates included in this study had been initially identified as *S. cupressi* based on fungal morphology, demonstrating the importance of applying molecular tools available to differentiate morphologically cryptic species. These findings bring into question the identity of some of the earlier identifications of *S. unicorne* and *S. cupressi* and the observations made from these species. The isolates used in the pathogenicity trials by Hood et al. (2004) and (2009) were all believed to be either *S. cupressi* or *S. cardinale*. Some of the "*S. cupressi*" isolates, according the *BTUB* phylogenetic tree, are more likely something else (*S. neocupressi*, and *S. cancrinum*).

Based on these tentative identities, the results of Hood et al. (2004; 2009) show that *S. neocupressi*, *S. carnicum* and an isolate of the uncharacterized *Seiridium* sp. clade are pathogenic to *C. macrocarpa* and *C lusitanica*. Isolates of *S. neocupressi* and *Seiridium* sp. are also as, or more, virulent than the original "benchmark" isolate, NZFS 4178, which is *S. cancrinum*. Again, this result highlights the importance of knowing what species you are working with to ensure the validity of the observations. Until recently, it was thought that only two species were associated with CCD in New Zealand; however, there could be as many as six or even more, which could have implications for management and resistance breeding programmes.

While only three isolates of *Seiridium* have been isolated from *Pinus*, only one (1810035-1) from *Pinus pinaster* was included in the initial ML analysis. There were some difficulties amplifying this region for these specific isolates. The tentative identity of this isolate based on the *BTUB* ML tree is unknown. Recent pathogenicity work was done comparing three *Seiridium* isolates, including the isolate "1810035-1", to *Diplodia pinea* (an important pathogen of pine) and *Diplodia africana* (Dobbie & Carey 2021). The results of that work showed that the *Seiridium* isolates produced small lesions ($\dot{x}$ = 8mm – 12mm) on inoculated *P. radiata* but were much smaller than *D. africana* lesions ($\dot{x}$ = 22mm) or *D. pinea* lesions ($\dot{x}$ = 31mm). These trials will need to be expanded on and a repeat of this work is needed over a longer time frame with other hosts included.

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Subject: Continue to Develop the Bayes Net (BAIPA) Model for predicting Biocontrol Impacts

Authors: Nicolas Meurisse, Bruce Marcot, Owen Woodberry, Barbara Barratt, Jacqui Todd

Introduction

A classical biological control programme can be viewed as the deliberate introduction of an invasive organism (the BCA), where one aims to maximise the invader's ability to suppress a target organism (usually a pest or a weed), while typically ensuring safety to otherwise valued NTS (native or beneficial introduced species). The target organism is often an invasive species itself, hence potential BCAs are commonly searched for in the area of origin of the target (Fig. 1 – selection of BCA). The list of BCA candidates is then refined based on a preliminary evaluation of each candidate's ability to control the target species, balanced with a consideration of the negative effects it could cause in the introduction area.

An assessment of the physiological host range of a candidate BCA is the typical first step in addressing its potential impact on species in the proposed area of introduction. It is usually determined pre-release, based on choice and other response tests evaluating the attacking behaviour and reproductive success of a BCA with selected NTS.

The process of biological control agent species selection for host range testing (Fig. 1 – assessment of BCA in quarantine) is always a critical component in the risk assessment before releases. It has progressively evolved from the traditional "phylogenetic, centrifugal" approach originally developed for weeds, where non-target species (NTS) more closely related to the target species are tested in priority, to a more holistic approach considering both taxonomic and ecological similarities between species (Barratt et al., 1997; van Lenteren et al. 2006; Hajek et al. 2016). Nevertheless, assurance about the specificity of a proposed BCA is typically inferred from the examination of its physiological host range which, of necessity, is undertaken in the laboratory with little evaluation generally possible to determine its ecological host range prior to its release (Barratt et al., 1997; van Lenteren, et al. 2006).

In our accepted paper, we reviewed the main approaches that are currently used for risk assessment of BCAs. Physiological host range testing is often used to discard BCA candidates with a host range wider than just the target. However, the physiological host range of a BCA, as evaluated in the laboratory, may significantly differ from its ecological host range, which requires the consideration of other field constraints, such as habitat or seasonal matching.

We also advocated for the adoption of more comprehensive, ecologically-based, probabilistic risk assessment methods, and provide a new tool based on a Bayesian network model. Bayesian networks (BNs) (Pearl, 1988; Korb & Nicholson, 2011) are an increasingly popular paradigm for reasoning under uncertainty. BNs are directed acyclic graphs, in which nodes represent variables and arcs represent direct probabilistic relations. For a discrete BN, the relationship between variables is quantified by conditional probability tables (CPTs) associated with each node. BNs allow for a wide range of inferences about the modelled system to be made in an efficient way. Users can set the values of any combination of

nodes in the network that they have observed, and this evidence propagates through the network, producing a new posterior probability distribution for each variable in the network. In this context, the reasoning required is a predictive one; given a scenario of a proposed BCA and NTS, a BN model can be used to incorporate evidence about the species, their biological features and environments, to compute quantitative likelihood of impact. Sensitivity analysis (also known as influence runs, see Marcot, 2006) can be performed with a BN to explore the influence of the input variables on the output posterior probability distribution. This is done by sequentially selecting each state of the input variable, updating the BN, and recording the range of the output variable's posterior probabilities in a tornado plot.

A retrospective case study demonstrates the use of the model for predictions of the behaviour of a BCA in an actual ecological setting, and to predict its potential impact on a NTS. The tool enables identification of the key driving influences on the overall impact of the BCA on the NTS, and in this case shows how the outcome of interactions predicted for the natural environment can differ from laboratory-based predictions.

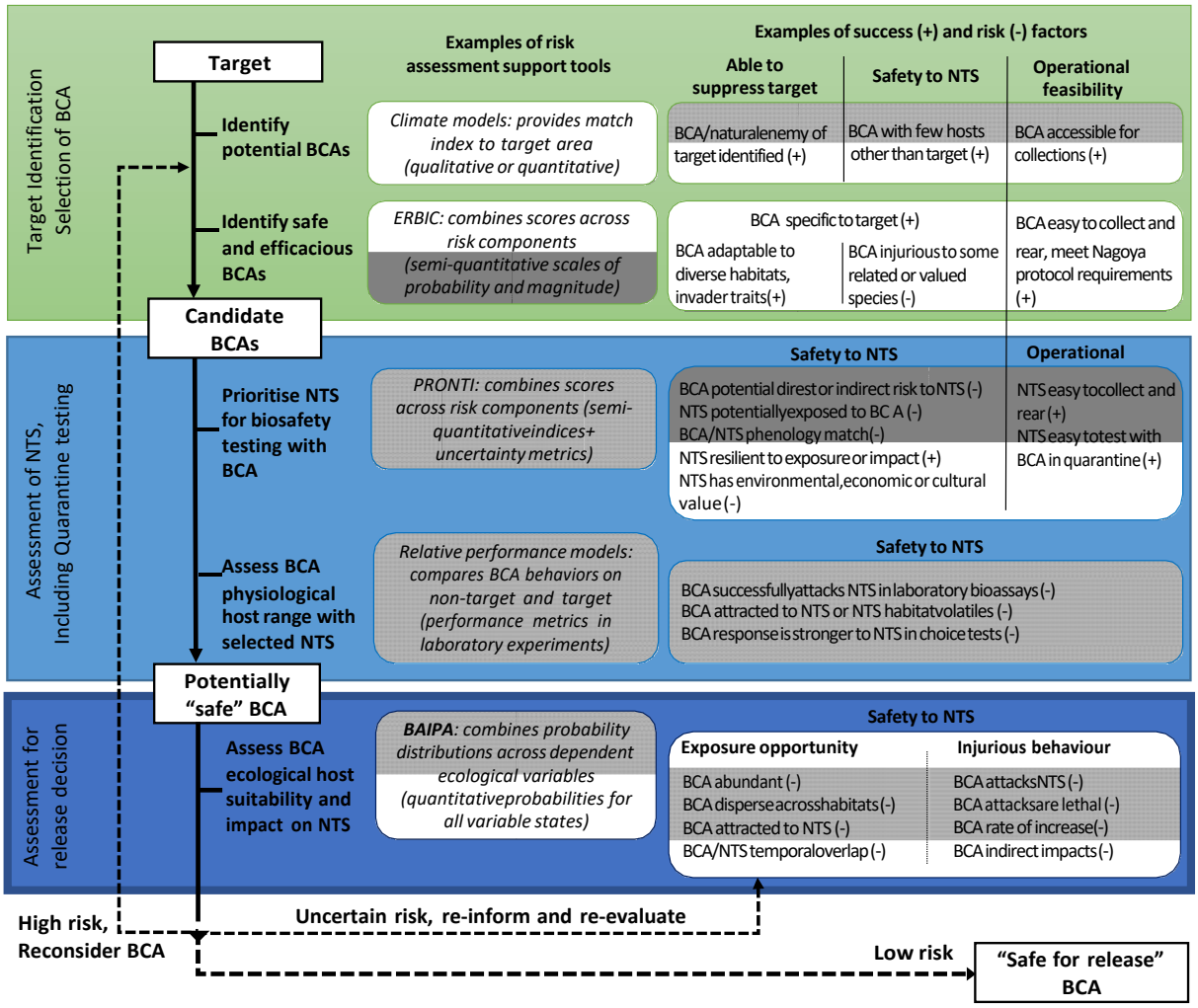

Fig. 1. General evaluation components in the risk assessment of a biological control agent (BCA). Components related to the initial selection of candidate BCAs are shown in the top part of the diagram (green-coloured box). This evaluation usually consists of a pre-import analysis of the biology and behaviour of the agents in the area of origin of the pest or weed. Components related to the assessment of candidate BCAs for the area of introduction are shown in the bottom part of the diagram (blue-coloured boxes). This part of the evaluation usually consists of the selection of non-target species (NTS) and testing in quarantine conditions (assessment of the physiological host range of the candidate BCA), and a general evaluation for release (assessment of the ecological host range and potential impact of the candidate BCA should it be released in the natural environment). The Biocontrol Adverse Impact Probability Assessment (BAIPA) aims to assess whether a BCA is low risk (safe for release), high risk

(unsafe for release), or the risk level is too uncertain (more information is required) for each NTS identified to be at risk. ERBIC = Evaluating Environmental Risks of Biological Control Introductions into Europe; PRONTI = Priority Ranking of Non-Target Invertebrates (Todd et al., 2015).

The "Biocontrol Adverse Impact Probability Assessment" (BAIPA) Model

We propose here a new tool to assess the potential negative ecological impacts of candidate biological control agents (BCAs) on individual, at-risk non-target species (NTS). The "Biocontrol Adverse Impact Probability Assessment" (BAIPA), uses a probabilistic, Bayes Net-based, model to combine nine evaluation components to assess the probability that an introduced BCA will reduce the population of a specified NTS in a specified habitat (Fig. 2). The model evaluates key species interactions, such as the frequency of encounters between the BCA and the NTS (based on local species abundances and the possibility of spatial and temporal overlap), and the local frequency of successful attacks (based on likely interactions between the BCA and the NTS), and considers potential indirect effects to estimate the overall probability for population impact (Table 1 in Appendix). All evaluations are based on an extrapolation from the situation where the BCA successfully establishes and controls the original target species in all habitats where the target occurs.

BAIPA aims to support management decisions in biological control programmes. The outcome from BAIPA (component 9 in Fig. 2) indicates that the probability of a reduction in the population of the selected NTS following release of the BCA is either minimal (supporting a decision to release), too great (supporting a decision not to release), or too uncertain (supporting a requirement for more information on the BCA and/or NTS to enable a technically justified decision to be made). BAIPA is built on a discrete BN comprising the nine evaluation components described in Table 1 (Appendix). A case study is presented below to assess the potential negative impact on a native weevil species associated with the introduction of a BCA targeting a pest weevil in New Zealand.

Table 1. *Model components in BAIPA.* The high-level structure of the Bayesian network model is shown in Fig. 2. A full data dictionary, including detailed nodes and states definitions, is provided in Table S1. BCA = biological control agent; TS = target species; NTS = non-target species. The model is run separately for each combination of BCA, TS, NTS and habitat.

Model component	**Description**	**Input required from assessor**
1. TS/NTS Habitat & Abundance	States the abundance of the TS and NTS populations within the considered NTS habitat.	Size and stability of the TS population in habitat (1) Size and stability of the NTS population in habitat (1) Spatial proximity to nearest TS habitat (if TS absent) (1)
2. BCA Long-distance Dispersal	Evaluates the frequency at which BCA individuals disperse outside their habitat of introduction.	Long-distance passive dispersal ability of the BCA (2) Long-distance active dispersal ability of the BCA (2)
3. Short- & Medium-range Attraction	Evaluates whether BCA individuals are attracted to the NTS within the considered NTS habitat.	Direct attraction of BCA to NTS (medium-distance) (2) Indirect attraction of BCA to NTS (medium-distance) (2) Direct attraction of BCA to NTS (short-distance) (2) Indirect attraction of BCA to NTS (short-range) (2)
4. BCA Habitat & Abundance	Evaluates the abundance of the BCA population within the considered NTS habitat.	No input required, determined by other factors in the model

5. Temporal Window	Evaluates the level of activity of the BCA during the period when susceptible life stages of the NTS are present, within the considered NTS habitat.	Seasonal match between the NTS and the BCA [2,3] Reproductive phenology of the BCA [2]
6. NTS-BCA Encounters	Evaluates the frequency of encounters between the BCA and the NTS within the considered NTS habitat.	No input required, determined by other factors in the model
7. Direct Impacts	Evaluates whether the introduction of the BCA has a direct negative impact on the NTS population within the considered habitat.	Frequency of attacks when BCA encounters NTS [2] Mortality frequency of NTS after attack [2] Frequency of non-lethal attacks that affect fitness of NTS [2]
8. Indirect Impacts	Evaluates whether the introduction of the BCA has an indirect negative impact on the NTS population within the considered habitat.	Indirect impact potential of BCA on NTS [2,4]
9. Impacts	Evaluates whether the introduction of the BCA has an overall negative impact on the NTS population within the considered habitat.	No input required, determined by other factors in the model

[1] Species abundance inputs will be directly informed by the "fact sheets" summarising the identity and local abundance of the organisms considered in the assessment. It may take the form of a probability distribution to consider uncertainty. The outcome of the assessment will be more informative to the assessor if assumptions are made on these inputs under the form of "worst cases" or "what if" scenarios.
[2] Species ecological and biological inputs will be entered by the assessor under the form of a probability distribution based on the information provided by the applicant, eventually completed by additional information or knowledge gathered by the assessor. A "default" probability distribution can be used in case no information is available at all. This "default" distribution can be uniform or may depend on the type of organism investigated.
[3] The temporal match between the NTS and the BCA can be directly informed by the user or estimated from the known seasonal activity patterns of both the NTS and BCA.
[4] Evaluating the outcome of indirect interactions between a BCA and a NTS can be complex. It is therefore recommended that, first, assumptions of no possible indirect impacts, and, second, assumptions of realisation of the most severe impact from all possible outcomes, are tested. This is equivalent to testing best and worst case scenarios for indirect interactions.

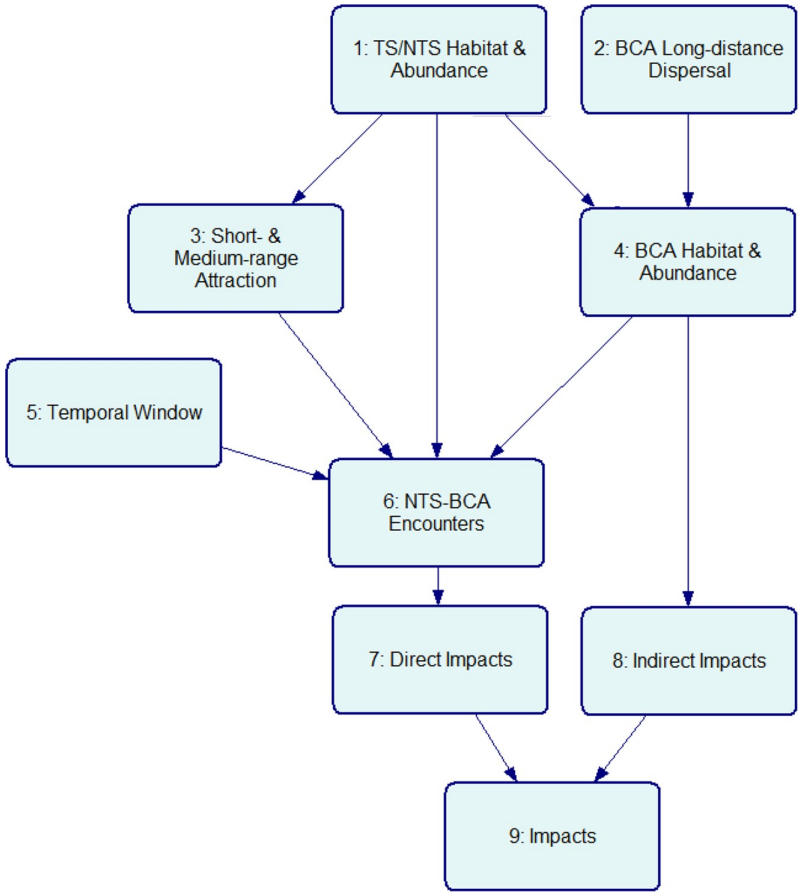

Fig. 2. High-level structure of the Bayesian network model used to assess the non-target impacts of biological control agents (BCAs). The "Biocontrol Adverse Impact Probability Assessment" (BAIPA) tool comprises nine interconnected Bayesian Network model components to assess the ecological overlap between a BCA and a non-target species (NTS) (components 1 to 6), and their physiological matching and potential for direct and indirect impacts (components 7 to 9). Data on the target species (TS) is also used.

Case study and application

The lucerne pest, *Sitona discoideus* (Coleoptera: Curculionidae), a weevil first recorded in New Zealand in 1974, rapidly became recognised as a serious pest affecting lucerne (alfalfa, *Medicago sativa*), a perennial legume (Goldson et al., 1984). The introduction in 1982 of a hymenopteran endoparasitoid, *Microctonus aethiopoides* (Braconidae: Euphorinae), successfully reduced *S. discoideus* populations providing benefits to farmers (Goldson et al., 1993). Concerns arose when it was discovered that 19 species of non-target weevils were attacked in the field, 14 of which are native species (Barratt & Johnstone, 2001; Barratt et al., 2007, 2010). The non-target parasitism associated with the introduction of *M. aethiopoides* in New Zealand provides an interesting case of a BCA initially expected to establish only in the receiving environment (lucerne crops and pastures), but which has now established populations in natural ecosystems (mainly native tussock grasslands that are habitats for native weevil species).

Here we retrospectively assessed the impact of *M. aethiopoides* on the native weevils in the genus *Nicaeana* (Curculionidae: Entiminae), in low grazing intensity pastures (the habitat of BCA introduction) and mid-altitude native grassland environments (a "refuge" habitat for the NTS). Permanent populations of *M. aethiopoides* are generally established in pasture habitats, where they successfully control the target *S. discoideus*. In low grazing intensity pastures, *S. discoideus* (and *M. aethiopoides*) coexist with resident populations of native weevils, including species in the genus *Nicaeana,* such as *N. cinerea* and *N. cervina*. *Sitona discoideus,* and these endemic *Nicaeana* weevils are also established in native tussock grassland, distant from pasture environments. Additional knowledge required as user input in BAIPA is provided in Table S2.

Results of running BAIPA for the case study

Fig. 3 shows the BAIPA BN for the described case study, simplified to show only the key variable components.[1] The BN calculates the probability that the parasitic wasp *M. aethiopoides* (the BCA), will have an impact on populations of native weevils in the genus *Nicaeana* (the NTS), given the set of inputs. For this case study example, the BAIPA BN predicts there will be a 10% probability of *BCA impact on NTS population* (i.e., an arguably substantial population reduction of *Nicaeana* weevils) in native grassland, which is divided between a 6% probability of *BCA direct Impact on NTS population* and a 5% probability of *BCA indirect Impact on NTS population*.

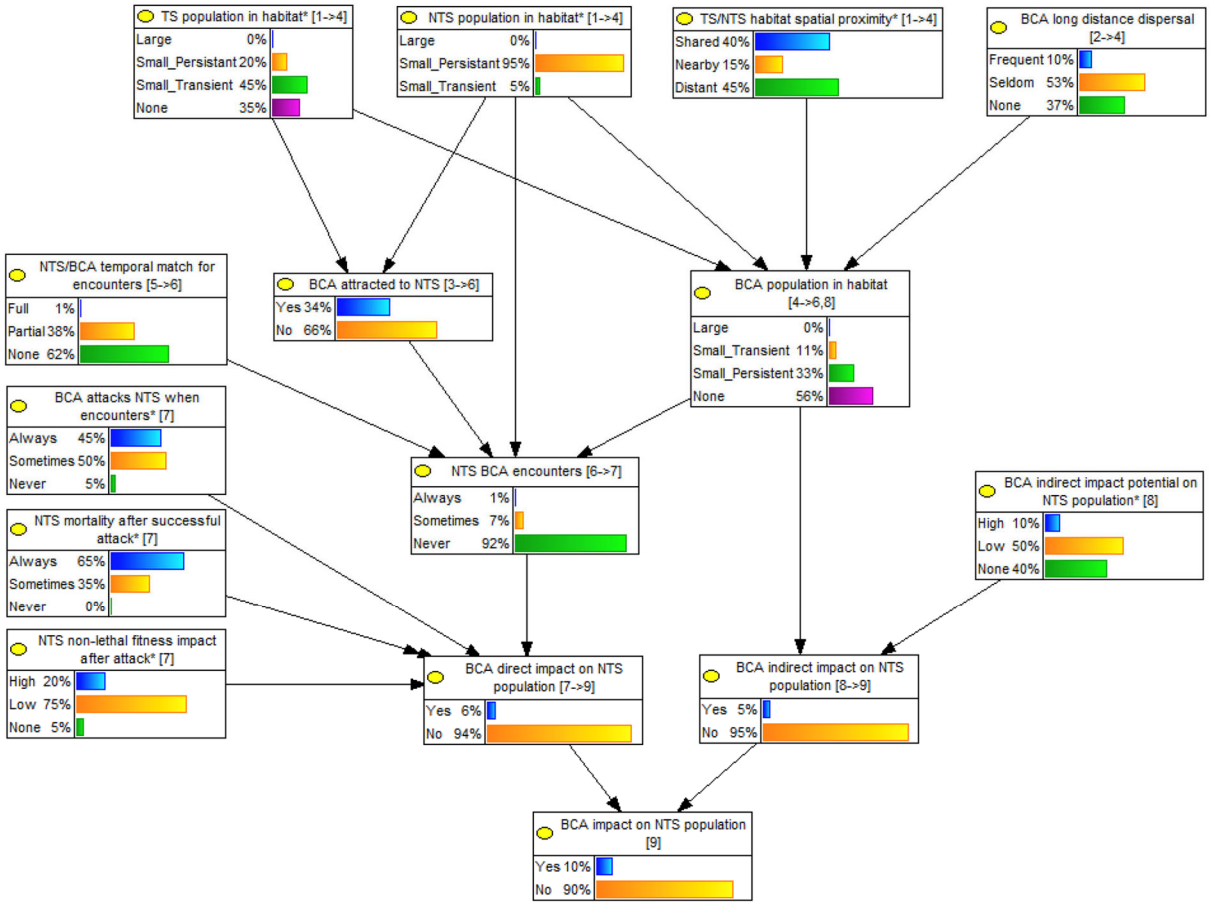

Fig. 3. "Biocontrol Adverse Impact Probability Assessment" (BAIPA) Bayesian network model: Case study of the biological control agent (BCA) *M. aethiopoides* impact on non-target species (NTS), native weevils in the genus *Nicaeana* (Curculionidae: Entiminae), in mid-altitude grassland environments. The BN predicts there will be a 10% probability of *BCA impact on NTS population*, divided between a 6 % probability of *direct impact on NTS population* and a 5% probability of *indirect impact on NTS population*. Data on the target species (TS), *Sitona discoideus*, is also used. The numbers in square brackets associated to the model nodes (each individual box) refer to the model components that use them (see Fig. 2 and Table 1). Key input variables shown in the diagramme are indicated by an asterisk. A complete description of the model structure and definitions of all variable and states are provided in Table S1.

BAIPA, a new tool to support release decisions of biological control agents

Current models and tools support the selection of appropriate BCAs based on their potential to suppress the target and predicted safety to NTS based on quarantine screening. They help to prioritise at-risk NTS for further assessment, often in quarantine laboratory trials to evaluate the BCA host attraction and

[1] For the detailed model components, see Table S1.

physiological host range. BAIPA complements these existing tools by incorporating all gathered knowledge on the BCA and NTS interactions and enabling for comprehensive assessment of the adverse impact of a BCA on populations of at-risk NTS. We aim for BAIPA to assist decision making for release of BCAs by providing:

- reproducible evaluations of the ecological host range of a BCA, including the probability of *in situ* encounters with NTS in different habitats, and an evaluation of the BCAs impact at the population level;
- a method to incorporate information from various sources, including quantitative, qualitative, and expert knowledge;
- a display of the order of events that result in potential impacts on the NTS, including their quantification (conditional probabilities) and the propagation of uncertainties (variability, errors, etc.) associated with the model structure and parameter estimates; and
- a transparent and consistent decision-support tool, to help visualisation of all input factors, intermediate calculations, and outcome probabilities in the model, with capacity for sensitivity analysis and scenario testing.

Thus, BAIPA can be seen as an additional tool to help decision-makers assess the risk of releasing BCAs into new environments. As with other risk assessment tools, BAIPA can be used as a component of more complete biocontrol risk assessment frameworks (e.g., Fig. 1; Paula et al., 2021). Further discussion of the important attributes offered by BAIPA are given below.

Management and implications of uncertainties

BAIPA provides a value-neutral risk analysis framework that decision makers can use in risk management, such as by helping articulate decision criteria expressed as probabilities of negative impact outcomes that would be acceptable or unacceptable in comparison to benefits (Heimpel & Cock, 2018). Outcomes from the BAIPA BN are not intended to dictate decisions on BCA introductions, but instead help inform a science-based decision. It is a matter of policy, management and communication to define acceptable probability levels of BCA impacts on NTS. Instead, the BN model serves to document current knowledge, to illustrate the key causal factors leading to the outcomes, and to depict the implications of how variations or uncertainties in the inputs propagate throughout the causal web of conditions and relations. BAIPA considers uncertainties associated with quantitative predictions, addressing general guidelines for biosecurity risk assessment (e.g. International Standard for Phytosanitary Measures (ISPMs) 2, 3, 11), and other expert recommendations (e.g. Kaufman & Wright, 2017, for biological control). In this respect, BAIPA complements existing tools developed under international guidelines and codes of conduct relevant to the introduction of BCAs. Other examples of international and national organizations that regulate or advise on the release of BCAs, are documented in Lockwood, Howart, and Purcell (2001), van Lenteren et al. (2006) and Barratt and Ehlers (2017).

BAIPA coupled with sensitivity and scenario testing provides the user with the capability to determine which factors are the most influential, that is, could most affect outcomes of BCA impacts on NTS populations and that are least understood. Such information can be invaluable for prioritizing future field monitoring and targeted research effort.

Conclusion

For the purpose of risk assessment of potential impacts of biological control agents (BCAs) on non-target species (NTS), we found that the Bayesian network (BN) modelling approach provides the following significant advantages: (1) it displays outcomes as probabilities which work well in a risk management framework, (2) it explicitly shows the propagation of uncertainties and their implications for predictions; (3) through sensitivity and influence analysis, it provides an easy way to determine the relative influence of potential alternative management actions and prior conditions on outcomes; and (4) models can be run efficiently for sets of scenarios of alternative environmental and management conditions. The new Biocontrol Adverse Impact Probability Assessment tool presented here, BAIPA, currently incorporates a BN model that assesses the risk for NTS to be negatively affected by the release of a BCA.

BAIPA has been developed with the potential to be turned into a designed-for-purpose web tool available for risk assessors and may support national regulatory authorities, such as the Environmental Protection Authority in New Zealand or the Animal and Plant Health Inspection Service in the USA, in their decision-

making to release BCAs. Eventually, model outcomes from BAIPA can be incorporated into a structured decision-making framework which may include a formal comparison between multiple risks and benefits associated with the potential or planned release of the BCA. It is important that such decisions are based on risk assessments that incorporate the most relevant ecological information within a logical, coherent, and transparent ecological framework. Hence, the accuracy of predictions will primarily depend on the veracity of assumptions of how BCAs respond to their new environments.

When the publication (Meurisse et al. in press) is in the public domain next year, the complete scientific paper will be distributed to SWP.

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APPENDICES

Appendix 1

“Risk analysis frameworks used in biological control and introduction of a novel Bayesian network tool.”

A manuscript accepted into special edition of “Risk Analysis” published by the Society for Risk Analysis.