



Pathogen genomics allows us to infiltrate the enemy camp and gather vital intel

Gathering intelligence or 'intel' on an enemy is vital if you want to win your battles. For pathogens, gathering intel about the genes present in an organism's genome through genomic analysis can be valuable for beating a disease. Genomic analysis of pathogens across research areas is changing pathology research; the COVID-19 pandemic is a current example of how we have rapidly become reliant on this technology and the information that it can provide to identify and characterise different variants. It is a similar situation for plant pathogen genomics.

A recent study by researchers at Massey University and Scion has demonstrated the utility of pathogen genomics for identifying key proteins involved in *Dothistroma septosporum* infection of *Pinus radiata*¹. Using a novel bioinformatic workflow, these proteins (effectors) can be used to test the defences or screen plants of interest, such as *P. radiata*. Included in this computer-based workflow are methods that identify key features in the gene sequences based on what is known from characterised infection-related genes in other fungal plant pathogens. It also identifies at what stage these genes are involved or expressed in the infection process and if they are significant. Together, these methods identified a short list of 30 candidate effectors.

To test this intel, the model species, *Nicotiana benthamiana* and *Nicotiana tabacum* (tobacco) were used to understand the function of these effectors. These plants are fast growing and easy to manipulate in the laboratory. Infiltrating purified proteins into the large, flat leaves is very easy and allows testing for a disease response. In this study, both plant species were used to screen the 30 candidate effectors from *D. septosporum*.

Of these, five produced a necrotic lesion, indicative of cell death and a hypersensitive response, in *N. tabacum*. A hypersensitive response indicates that a strong immune response was triggered, which leads to localised cell death. In *N. benthamiana*, one effector was able to produce a consistent, strong response. This effector did not produce a response in *N. tabacum*.

While using model plants is useful for indicating which effector proteins cause defence responses, using the true pathogen host, *P. radiata*, is essential for understanding the actual role of the effector proteins in host defence and disease. As a first step to achieving this, a reliable, straight-forward methodology was developed to deliver effector proteins into pine needles. This method involves production of large amounts of *Dothistroma* protein in a non-standard host (*Pichia pastoris*; a yeast), for



Dothistroma septosporum on *Pinus radiata* needles.

purification and then vacuum infiltration of the purified protein into *P. radiata* tissue culture shoots. The pine shoots are then returned to their growth medium and monitored for symptom development, a response to the protein.

Results from screening a single *Dothistroma* effector protein, Ds70057, showed consistent symptom development (cell death in this case) in all three *P. radiata* genotypes tested. These results were identical to those observed with the *Botrytis cinerea* effector protein (positive control). This effector protein was also effective at inducing cell death in *N. tabacum*. The ability of the *Dothistroma* effector proteins to induce cell death in both angiosperm and gymnosperm hosts in this study, where this is likely the first method developed for the screening of pines or other gymnosperms, demonstrates that screening using effectoromics (studying effector biology using both genomics and plant-based analyses) to identify host resistance or susceptibility genes may be possible even in gymnosperm trees.

The use of effectoromics to gather intel and screen for disease resistance or susceptibility and accordingly select or deselect lines for breeding has been applied in crops such as wheat, to eliminate diseases such as Septoria blotch of wheat. From this study, we found that it is critical to fully understand the lifecycle of these pathogens to successfully understand the role of effector proteins in the disease cycle and win the war against diseases.

This work was conducted by Lukas Hunziker and Mariana Tarallo, PhD students at Massey University and was funded by Scion SSIF and FGR funding through the Resilient Forests programme. Lukas is now working at Curtin University as a Research Associate and Mariana is currently in the third year of her PhD studies.

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¹ Hunziker, L., Tarallo, M., Gough, K., Guo, M., Hargreaves, C., Loo, T. S., McDougal, R. L., Mesarich, C. H., and Bradshaw, R. E. (2021). Apoplastic effector candidates of a foliar forest pathogen trigger cell death in host and non-host plants. *Sci Rep*, 11(1), 19958. doi:10.1038/s41598-021-99415-5

Insects travelling the world

Thousands of insect species are moving globally through trade or travel. Some of these species have already or will eventually establish in New Zealand. While many of them will have no noticeable impact, others such as the pine bark beetle, *Ips grandicollis*, introduced to Australia in the 1940s where it attacks *Pinus radiata* and vectors blue stain fungi, may have a large economic, environmental and social impact. Although New Zealand's biosecurity is highly respected internationally, we are a small country, which means that our resources are limited when it comes to monitoring all insects moving globally. Noticeably, our system is not impermeable as evidenced by an average of seven new records of introduced insect species establishing within New Zealand each year (since the 1980s, based on Edney-Browne et al. 2018² and updated records).

For biosecurity, knowledge is power. Internationally, there is a great deal of data we can learn from. We analysed 1,899,573 interception events from border inspections (e.g. passenger baggage, cargo, containers and vessels) in nine regions or countries³. The countries and regions analysed included New Zealand, Australia, South Korea, Japan, Canada, mainland USA, Hawaii, the UK and a region comprising 52 countries as part of the European and Mediterranean Plant Protection Organization (EPPO). The NZ section of the data, provided by Biosecurity New Zealand (MPI), covered border interceptions from 2000-2017. There were 71,588 interception events, for 1477 insect species. Of the regions analysed, NZ had the highest number of interception events relative to import values, emphasising the importance placed on biosecurity in NZ.

However, the combined international dataset had 8,716 species - about six times more species than those detected by NZ alone. This doesn't necessarily mean that those species intercepted elsewhere never travel to NZ, but alternatively that we failed to record interceptions of them. This could be because travelling individuals of these species are in relatively low numbers or,

as a non-mutually exclusive explanation, because we have a reduced capacity to intercept or identify these species. Indeed, even in the combined international dataset, 44% of the species recorded had only one interception event, informing that there are many more species moving globally for which we have no records. Many species are difficult to detect, often concealed inside objects or using pathways not frequently inspected. Also, similar to other countries, 53% of interceptions in NZ were not identified to species level, so it's possible that some of the partially identified specimens were different species.

Each country has its own set of priorities and constraints and these are reflected in the over or underrepresentation of taxonomic groups intercepted. For example, both NZ and Australia showed a clear focus on ants, and, not surprisingly given the importance to us, NZ intercepted a large range of fruit flies. The NZ data set also revealed proportionally high interception rates of the high-profile brown marmorated stink bug relative to other countries. On the other hand, NZ intercepted a relatively low diversity of Coleoptera (beetle) species relative to other countries. For example, populations of the granulate ambrosia beetle were detected in Auckland in 2019. This species had only been recorded once in the NZ 2000-2017 border dataset; however,



Image: Roanne Sutherland (Scion)

Recent arrival in New Zealand.
Xylosandrus crassiusculus (granulate ambrosia beetle).

nearly 100 border interceptions of this species had been made by other countries over a similar timeframe. While it is possible that this number has been inflated by local contamination at some international ports of entry, this relatively high number demonstrates the potential this species has to be associated with international pathways.

Setting aside the differences in border inspection and associated practices by each country, there are still strong positive correlations between countries in the species interception frequencies *within* taxonomic groups. Highly intercepted species were generally highly intercepted by most countries, which affirms the trends seen in our own data while providing additional information for risk assessment on pathways or groups of species. Several of the most frequently intercepted species were thrips, such as the western flower thrips, already established in NZ, or tropical mealybugs not established in NZ. Thrips were commonly associated with cut flowers and other plant parts and mealybugs with fruit. The most frequently intercepted moths and beetles were stored food pests.

This research was only possible with the collaboration of multiple international researchers and government agencies. The research collaboration was supported by the National Socio-Environmental Synthesis Center (SESYNC) in the USA, with local funding from the Biological Heritage National Science Challenge and Te Pūnaha Matatini. Together with other countries we can improve our knowledge of the global movement of insects and identify opportunities for improved data sharing in the future. The data analysed can be used in biosecurity risk assessment, both in terms of identifying species with the potential to arrive via trade and travel, and also in terms of understanding the reliability of the data - for some insect groups the data will provide more coverage than for others. This is important when refining phytosanitary measures, surveillance, and developing readiness plans for different insect species to ensure fewer insects make it into New Zealand, and if they do, we are ready with a response.

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² Edney-Browne, E., Brouckhoff, E. G., and Ward, D. (2018). Establishment patterns of non-native insects in New Zealand. *Biological Invasions*, 20(7), 1657-1669.

³ Turner, R.M., Brouckhoff, E.G., Bertelsmeier, C., Blake, R.E., Caton, B., James, A., MacLeod, A., Nahrung, H.F., Pawson, S.M., Plank, M.J., Pureswaran, D.S., Seebens, H., Yamanaka, T., and Liebhold, A.M. 2021. Worldwide border interceptions provide a window into human-mediated global insect movement. *Ecological Applications*, p.e02412.