Technical Report

Susceptibility of *Eucalyptus bosistoana* families to EVB defoliation across sites and families Feb/March 2019

(Milestone 1.1.11.4)

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EXECUTIVE SUMMARY

In the absence of a PhD student being secured to continue the durable eucalypt health assessment work in time for the 2018-2019 summer season, Ruth McConnochie was contracted to assess paropsine defoliation and EVB (*Paropsisterna varicollis*) incidence on *E. bosistoana* families in February and March 2019. Trees at two sites were assessed using two methods; 1) a visual estimate of % crown defoliation and 2) a quantitative 'occupied leaves per shoot' count of eggs and larvae. Few EVB and only minor paropsine chewing damage was observed at the first selected site, Ngaumu. One hundred and fifty trees were assessed, but the small pest population precluding assessment of family-level susceptibility. At McNeill's, where EVB has been well established for several seasons, 3-17 trees from each of 80 families were able to be assessed, giving a total of 889 trees. Both EVB and *Paropsis charybdis* were present at the site, however the EVB population was significantly larger. Defoliation varied significantly within and between families, ranging from 5% to 90% of the upper third of tree crowns. Average defoliation per family ranged from 27.3% to 63.3%. Defoliation of families from the Won Wron provenance was significantly higher than three of the other provenances. No EVB or *P. charybdis* eggs or larvae were detected on the shoots sampled at either McNeill's of Ngaumu, however small numbers of eggs were collected ad hoc throughout the trials. *Enoggera nassaui* was reared from several EVB egg batches from McNeil's, while *E. nassaui*, *N. insectifurax* and *B. albifunicle* were reared from *P. charybdis* eggs. Large numbers of *Cleobora mellyii* were also observed at the McNeill's site along with some harlequin ladybirds.

An additional ad hoc survey for EVB was conducted at Cravens Rd (Marlborough) while collecting scion material in February 2019. The beetle was detected at the site for the first time, with 2 adult beetles detected on Southern provenance *E. bosistoana* families.

The data presented here provides further evidence that there is sufficient variation in defoliation sustained by different *E. bosistoana* families to warrant continued investigation into potentially heritable differences in susceptibility to paropsine attack. A fully replicated assessment conducted across multiple sites, species and families is still required to definitively inform selection of species and families that are less susceptible, or more tolerant, to EVB attack. This work needs to be conducted to coincide with peak egg laying and larval abundance so that the agent(s) (EVB or *P. charybdis*) causing the damage can be confirmed, and to reduce error in assessing preferences. Such a trial is scheduled to be conducted by a newly appointed PhD student (commencing August 2019) at sites across Marlborough and the lower North Island in the 2019-2020 field season.

INTRODUCTION

The Australian Chrysomelid beetle *Paropsisterna variicollis*, or eucalypt variegated beetle (EVB), was detected in New Zealand for the first time in March 2016 in Te Pohue Forest, Hawkes Bay. A delimitation survey, conducted by SPS Biosecurity, indicated the beetle was already present at sites >50km apart in the Hawke's Bay region (Fig. 1). As a close relative of the well-established pest *Paropsis charybdis*, EVB was immediately identified as a possible threat to eucalypt plantation forestry in New Zealand. Although as yet unquantified, observations to date suggest EVB has the capacity to be more damaging than *P. charybdis* as it appears to be active for a greater proportion of the year, with adults emerging earlier in spring and entering the overwintering state later in autumn. Given this threat, and the initial establishment of the beetle in the dryland east-coast region of the North Island, it is essential to incorporate information on the host-preference of this new pest, and the susceptibility of different eucalypt species, provenances and families, into the future selection of elite eucalypt breeds for establishing a durable hardwood industry.

Figure 1: Locations where EVB was present (red triangles) or absent (yellow circles) during the initial delimitation survey conducted by SPS Biosecurity in April 2016 following detection of the beetle in the Te Pohue Forest (purple circle).

Assessments of EVB damage were initiated in January 2017 at durable eucalypt trial sites in close proximity to the initial detection site. A total of 1,591 trees from 11 *Eucalypt* species were assessed across 3 sites; Alexander, McNeill and HBRC / Tutira (Lin et al., 2017). Adult beetles were observed on all species, but defoliation severity varied significantly between and within species, and across the three sites. Defoliation was not observed to exceed 60% of foliage for any species, and some trees of most species sustaining only incidental or light damage. This indicated there may be heritable differences in susceptibility to EVB attack that could be exploited in the current breeding programme, and that they may also be influenced by local site and climate attributes. Defoliation and the incidence of eggs and larvae was greatest on *E. bosistoana*, one of the priority species in the durable eucalypt programme. This species was therefore selected as the focus for further studies into heritable susceptibility traits, although future studies will also assess variation in susceptibility of other species.

A follow-up study was conducted over a three week period in late November / early December 2017, to assess the spread of EVB by surveying 8 durable eucalypt trial sites across the lower North Island (Murray & Kuwabara, unpublished). Ten eucalypt species were assessed and all showed signs of leaf beetle damage. EVB was detected in 4 sites, the three in which it was observed the previous season, as well as the Woodville nursery. *Paropsis charybdis* was present in all sites and more prevalent than EVB on *E. quadrangulata*, but less prevalent than EVB on *E. bosistoana*. EVB larvae were observed on *E. argophloia*, *E. bosistoana, E. camaldulensis, E. longifolia*, *E. quadrangulata,* and *E. tricarpa* while *P. charybdis* were found on these as well as *E. eugenoides*, *E. globoidea* and *E. macrorhyncha.* The predatory ladybird *Cleobora mellyii* was observed feeding on both *P. charybdis* and EVB eggs at the McNeill and Alexander sites. Twenty-six EVB egg batches and 63 *P. charybdis* egg batches were collected for parasitism assessment during the survey. Both the known primary parasitoids (*Enoggera nassaui* and *Neopolycystus insectifurax*) as well as the hyperparasitoid (*Baeoanusia albifunicle*) emerged from *P. charybdis* eggs and parasitism rates were quite high for December. In contrast, only *E. nassaui* was reared from EVB eggs, rates were much lower, and the wasps were observed to be extremely small, reflecting the fact that eggs of EVB are somewhat smaller than those *of P. charybdis.*

Following on from these initial studies, work was planned for the 2018-2019 summer to greatly expand our understanding of the potential heritability of traits influencing leaf beetle damage in general, and EVB damage in particular. The study was intended to extend defoliation assessments across multiple trials, and to incorporate more species and family lines to better understand the interactions between tree genetics and site attributes and their respective influences on pest risk. The trial was also intended to address a major short-fall in the earlier pilot studies by undertaking assessments earlier in spring to obtain data on oviposition preferences. The selection of species and genotypes by female beetles for egg laying is expected to be significantly more informative than observations of adult beetles, as the latter can be highly mobile and may rest or feed on less preferred host plants for short periods of time. Unfortunately, a PhD student could not be secured in time to establish the fully-replicated spring assessments and the study had to be delayed. Here, we report on a reduced assessment of two *E. bosistoana* breeding trials conducted in February and March 2019.

METHODS

Two sites were selected in which to assess paropsine defoliation and the relative incidence of EVB. The first site (see Appendix 1 for site locations), 'Ngaumu', is an *E. bosistoana* breeding trial established in the Wairarapa in September 2012, located at ~240m asl. The trial consists of 140 blocks of 36 trees each. Eighty-two *E. bosistoana* open-pollinated families are represented with 70 replicates per family in an incomplete block design. EVB had not been observed in the trial previously, but the large number of *E. bosistoana* families present made this a priority site for assessing variation in susceptibility to the species as soon as possible.

The second site, 'McNeill's', is an open pollinated progeny trial of 80 *E. bosistoana* families planted in 2012 near Waimarama, Hawkes Bay, with an average annual rainfall of ~1061mm. The trial is laid out in an incomplete block design, as for Ngaumu, with 124 blocks of 36 trees. EVB have been well established in the trial since at least January 2017.

Visual defoliation assessments were conducted on 17-18 February at Ngaumu and 17-18 March at McNeill's. At each site, 10 trees per family were selected avoiding 'edge' trees where possible. Eucalypt performance across the McNeill trial site is highly variable, therefore only blocks with good survival and growth were eventually selected for the defoliation assessment. (Fig. 2.). For each tree, defoliation (proportion of foliage missing) was estimated for the upper third of each tree crown to the nearest 5%. For at least 3 trees in each family, pole pruners were used to collect a 1 m length of shoot from the mid-section of the crown to count the proportion of leaves bearing 1) eggs and 2) larvae of EVB and *P. charybdis*. During previous assessments similar numbers of larvae have been observed in both the upper and middle sections of tree crowns, but eggs were appear to be more abundant in the mid-section (S. Kuwabara pers. com). Any EVB and *P. charybdis* eggs found were collected into Petri-dishes, stored in a cool box with an ice pack and subsequently sent to Scion, Rotorua, where they were incubated under laboratory conditions to assess parasitism rates.

A third *E. bosistoana* breeding trial in Marlborough (Cravens Rd., planted 2010, 24 *E. bosistona* families, mean annual rainfall 668 mm) was also assessed, ad hoc, during collection of scion material for the breeding programme in late February 2019.

Analysis of Variance (ANOVA) and Tukeys post hoc tests (R version 3.2.2, R Core Development Team).were applied to compare mean defoliation scores between families and provenances (see Appendix 2 for Provenance locations).

Figure 2: Variation in survival and growth at the McNeill's trial site. Blue outlines denote those blocks with sufficiently good survival for inclusion in the defoliation assessment, shaded regions indicate blocks suffering the lowest levels of defoliation (see results)

RESULTS

Ngaumu

Ngaumu trees were in excellent condition with fresh foliage and full crowns. No notable paropsine damage, eggs, larvae or adults were observed on the first 150 trees selected for assessment across the trial. An extensive search of trees across the entire *E. bosistoana* trial revealed only one large group of EVB larvae (Fig. 3) and one single larvae on a second tree. Two adult EVB were observed, and a small number of adult *P. charybdis* were observed, primarily on the adjacent *E. quadrangulata* (Fig. 3). No EVB egg batches were located but a small number of *P. charybdis* egg batches were collected and sent to Scion to determine parasitism levels. Given the lack of insect damage it was decided that further inspection at the site was unnecessary.

Figure 3: Evidence of paropsine beetle damage in the Ngaumu, 2012 *E. bosistoana* breeding trial; (a) EVB larvae collected from *E bosistoana*, (b) typical healthy *E. bosistoana* crown, (c) paropsine chewing damage to adjacent *E. quadrangulata* crown. Photos: Ruth McConnochie.

McNeill's

Overall survival of trees in the 29 blocks selected for assessment was 89%. Visual assessment of defoliation was completed for a total of 889 trees, with the number of trees from each of the 80 families ranging from 3 to 15, as some families were planted with fewer replicates and therefore were not present in all blocks evaluated. Defoliation attributable to paropsine beetles were observed in the upper third of tree crowns across the site. A large population of EVB adults was present, and *P. charybdis* was also present as adults and larvae, but in much lower numbers.

All 80 *E. bosistoana* families assessed sustained highly variable levels of defoliation. Defoliation of individual trees across the 29 blocks assessed ranged from 5-90%. The range of defoliation values between individual trees within families always exceeded 30%, and extended up to 85% (Fig. 5a). Individual trees within 52 of the 80 families suffered >80% defoliation, while at least some trees from each of 39 families (including representatives from all provenances except Won Wron and family 24) sustained <10% defoliation. Despite this variation, mean defoliation differed significantly between families (F=1.578, df = 79, p = 0.0016), ranging from 27.3% to 63.3% (Fig. 4). Significant differences were also detected between provenances (F =3.059, df = 6, p = 0.0057, Fig. 5b), with families from Won Wron provenance sustaining significantly more defoliation, on average, than those from Genoa, Tambo Lower, and Whangarei (original Australian origin unspecified).

Block location may also have influenced defoliation levels. It was observed that those blocks with the lowest mean defoliation (>45%) all occurred on the western edge of the site, and slightly lower down the hill slope (shaded regions Fig. 2).

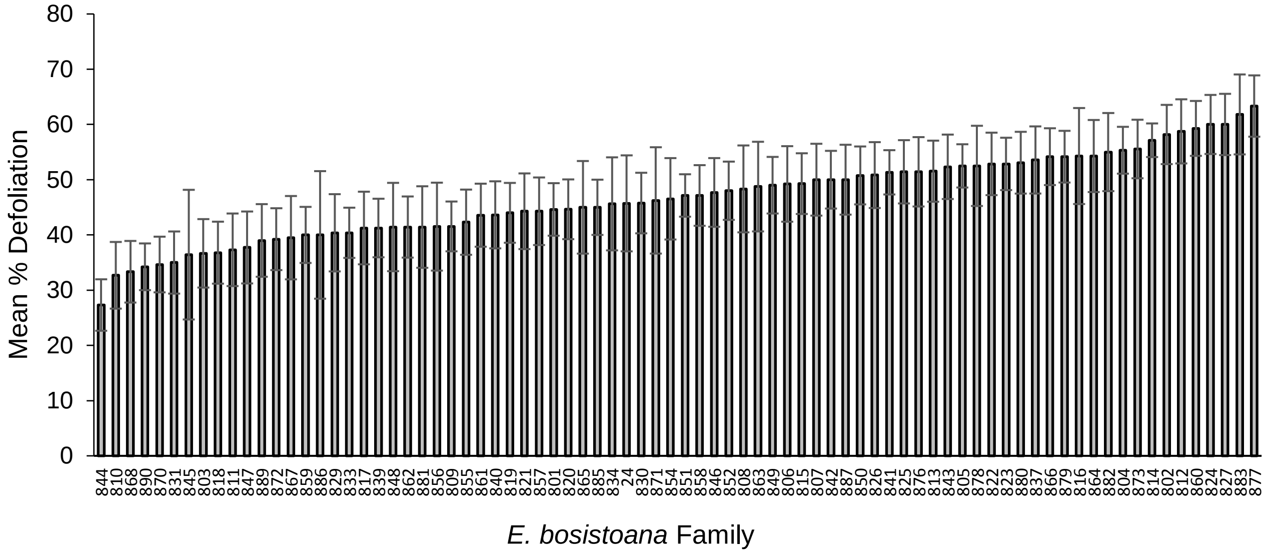


Figure 4: Mean (± SE) leaf beetle defoliation assessed for 80 *E. bosistoana* families at the McNeill's 2012 progeny trial in Hawkes Bay, March 2019.

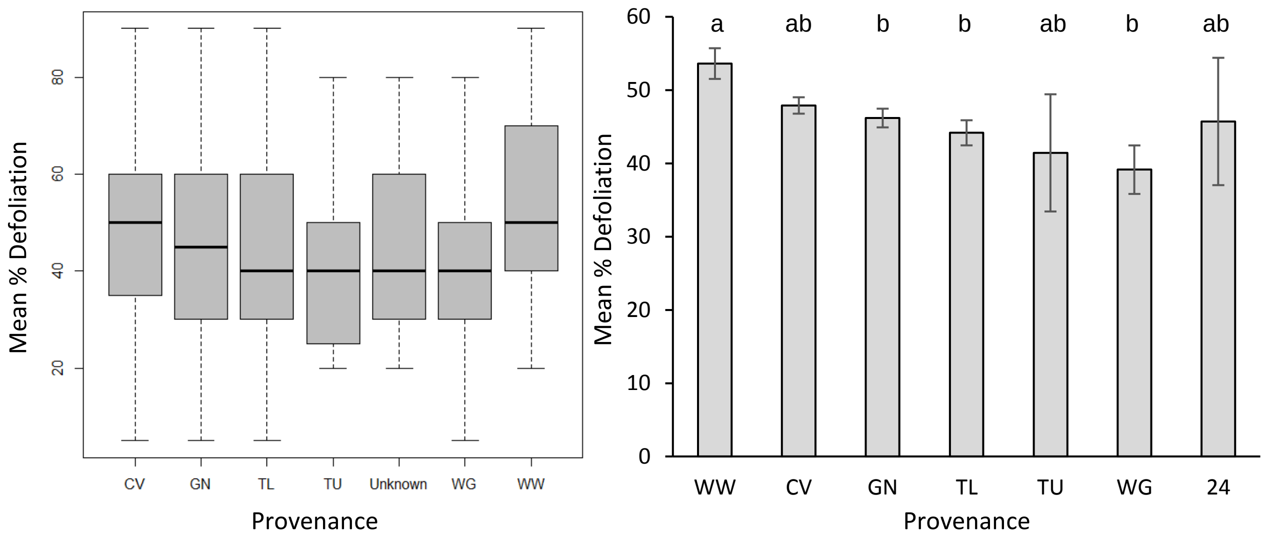


Figure 5: Variation (a) and Mean (± SE) (b) defoliation of *E. bosistoana* families by provenance (WW = Won Wron, *n* = 94; CV = Cann Valley, *n* = 340; GN = Genoa, *n* = 262; TL = Tambo Lower, *n* = 150; TU = Tambo Upper, *n* = 7; WG = Whangarei (original origin unknown), *n* = 29, '24' and 'unknown' = family 24 near Genoa, *n* = 7). Letters above bars indicate significant differences between means (p<0.05).

Shoot samples were collected from 6 blocks (216 trees) to assess the number of EVB and *P. charybdis* eggs and larvae, however none were detected. Collection of eggs were subsequently made ad hoc throughout the trial, wherever they could be found. Nine EVB and 9 *P. charybdis* batches were located. *Neopolycystus insectifurax* emerged from three of the *P. charybdis* batches, and *Enoggera nassaui* and its hyperparasitoid *Baeoanusia albifunicle* emerged from five. Five of the EVB batches were unparasitised and 4 contained *E. nassaui* (Withers & Pugh, 2019).

The presence of many predatory *Cleobora mellyi* adults, and small numbers of Harlequin ladybirds (*Halmus chalybeus*) were also noted during tree assessment both in January (A. Pugh pers. com.) and March (R. McConnochie pers. com.).

Cravens Road

Inspection of *E. bosistoana* branches felled for collection of scion material at Cravens Rd., Marlborough, revealed two EVB adults. Both beetles were found in plots along the road edge of the trial, and found on trees from ‘southern’ provenance families (Family 138 in plot 22, and family 133 in plot 5). Neither of the trees from which the beetles were collected were heavily defoliated and crown health was generally very good across the whole trial. This represents the first observation of EVB in Marlborough and the southern-most detection to date (Fig. 6).

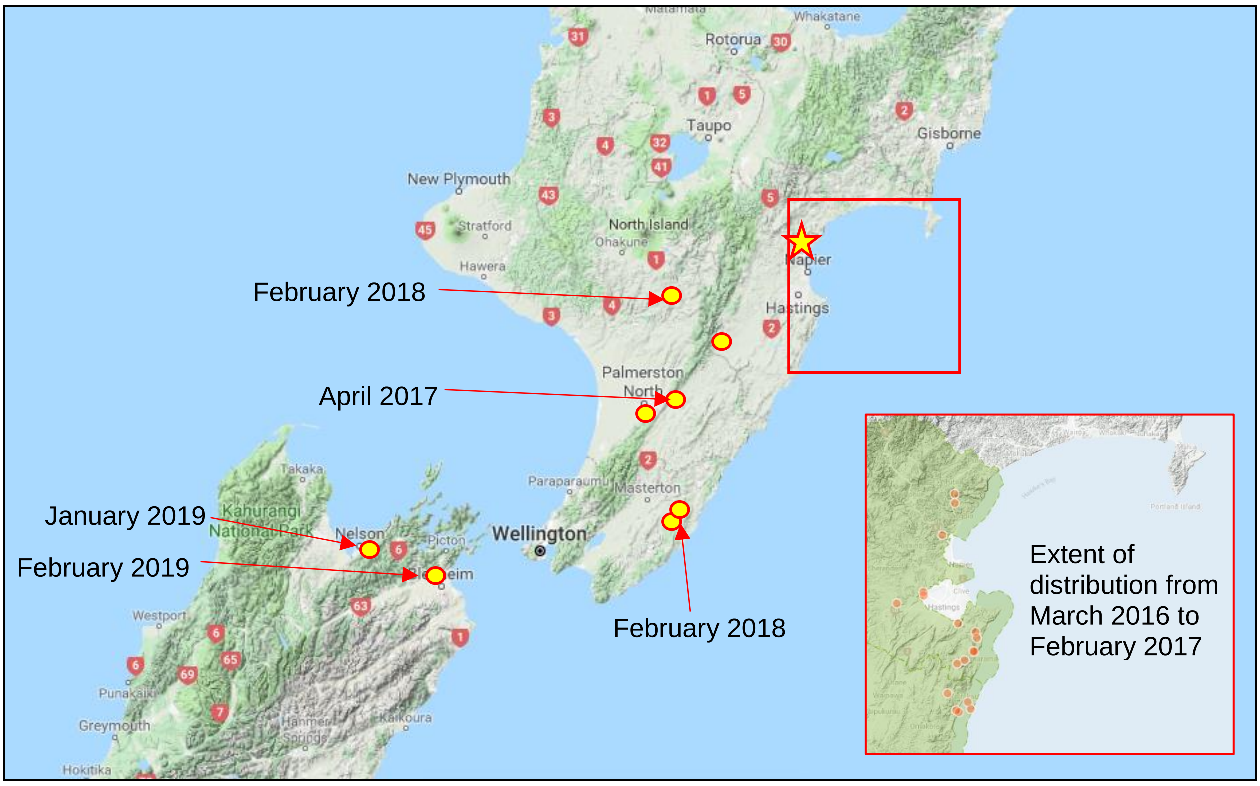


Figure 6: Current known distribution of *Paropsisterna variicollis* (EVB) in New Zealand as of May 2019. Inset shows iNaturalist observations from the Hawke’s Bay region where the beetle was initially detected in March 2016 (yellow star) in March/April 2016. The February 2019 record represents the most recent detection at the Cravens Rd. *E. bosistoana* field trial in Marlborough.

DISCUSSION

Although EVB were detected at Ngaumu, the population is still small and as a result has had little impact to date. Given the on-going population growth in the Hawkes's Bay and continued expansion into new regions, the Ngaumu population is expected to increase over the next 2-3 growing seasons. The limited damage at the site in February 2019 precluded any assessment of EVB preferences for, or susceptibility of, different *E. bosistoana* families to defoliation. As this trial includes the largest number of *E. bosistoana* families (82) it will remain a focus for future assessment and comparison to the performance of the same families across other sites in the Wairarapa, as well as Marlborough and Hawke's Bay.

EVB was much more prevalent at the McNeill's site, where it appears to have become well established shortly after its initial detection in March 2016. Significant differences in mean defoliation were observed between families, and all families included some individual trees that sustained very low levels of chewing. This supports earlier work in Marlborough that indicated there may be heritable differences in susceptibility to leaf beetle attack, which could be incorporated into selection of future breeding stock (Lin, 2018). Further assessment of the substantial variation within and between families is required and must include quantitative assessment of egg (during peak spring oviposition) and larval abundance across multiple sites. Findings from the current study suggest tree position within trials may also influence defoliation, and this needs to be incorporated in to future assessment.

Significant differences in defoliation were also observed between provenances. In particular, the Won Wron provenance sustained higher mean defoliation than the Tambo and Genoa provenances further North. In a previous comparison of *P. charybdis* defoliation on 17 *E. bosistoana* families from three provenances not included in the current study, Lin (2018) also found evidence for a provenance effect. Both visual estimates of crown defoliation and variations on the Crown Damaged Index (CDI) method indicated higher levels of defoliation on trees from the 'Sothern' provenance (west of Melbourne), relative to the Bungonia and Marulan provenances (located between Canberra and Wollongong). A similar trend was found for larvae abundance over the season. Although these studies suggest a latitudinal trend for susceptibility to paropsine defoliation its significance is not yet clear. The Southern provenance is located ~300km NW of Won Wron at a similar latitude to the Cann Valley provenance. Despite being located part way between Tambo and Genoa, the Cann Valley provenance did not exhibit significantly lower defoliation relative to Won Wron. Further investigation into climatic and altitudinal differences between these source locations may help to explain drivers behind the differences observed to date.

The proportion of *E. bosistoana* exhibiting > 50% defoliation was much lower (52.8%) than that predicted by Lin et al (2107) (80%) based on defoliation data collected in January 2017 from three Hawke's Bay sites, including McNeill's. This may be partially attributed to differences in the timing of the assessment and tree height. Lin et al., (2017) found a significant effect of tree height on defoliation, and the predictive model was therefore based on the average tree height in the trial (5m). Tree height was not assessed in the 2019 season. An increase in natural enemies at the site could also be reducing damage, although this has not been quantified. Scion staff reported many EVB eggs and adults and extensive chewing on *E. bosistoana* at the site in early January, but trees were still in good condition, experiencing better flush-growth than has been seen in some years (Ben McNeill, pers. com). Approximately 30% of EVB eggs collected by Scion at that time were parasitised by *E. nassaui* (Withers & Pugh, 2019) and high numbers of the predatory beetle, *C. mellyii*, were observed in both January and March. Another predatory ladybird, *Harmonia axyridis,* first detected in New Zealand in 2016, has also become established at the site and is present in low numbers. Neither *N. insectifurax* nor the hyperparasitoid *B. albifunicle* have been found parasitising EVB, but both continue to be detected in *P. charybdis* eggs.

In January 2019 EVB was detected in the South Island (Nelson) for the first time (Toni Withers pers. com.). The subsequent detection, reported here, at the Craven's Rd. durable eucalypt trial in February 2019, represents the southern-most extent of the beetle's range expansion to date. As such, almost all of the durable eucalypt trials currently established in dryland sites (orange and yellow areas in Appendix 1) are now within the range of EVB. There is no reason to expect the beetle will not continue to extend into the remaining trials in mid Canterbury, especially given it has been observed to be more active than *P charybdis* in cooler temperatures. It is interesting to note the two beetles detected in Marlborough were both on trees from ‘Southern’ provenance families that were previously found to be more susceptible to *P. charybdis* attack as noted above (Lin, 2018).

REFERENCES

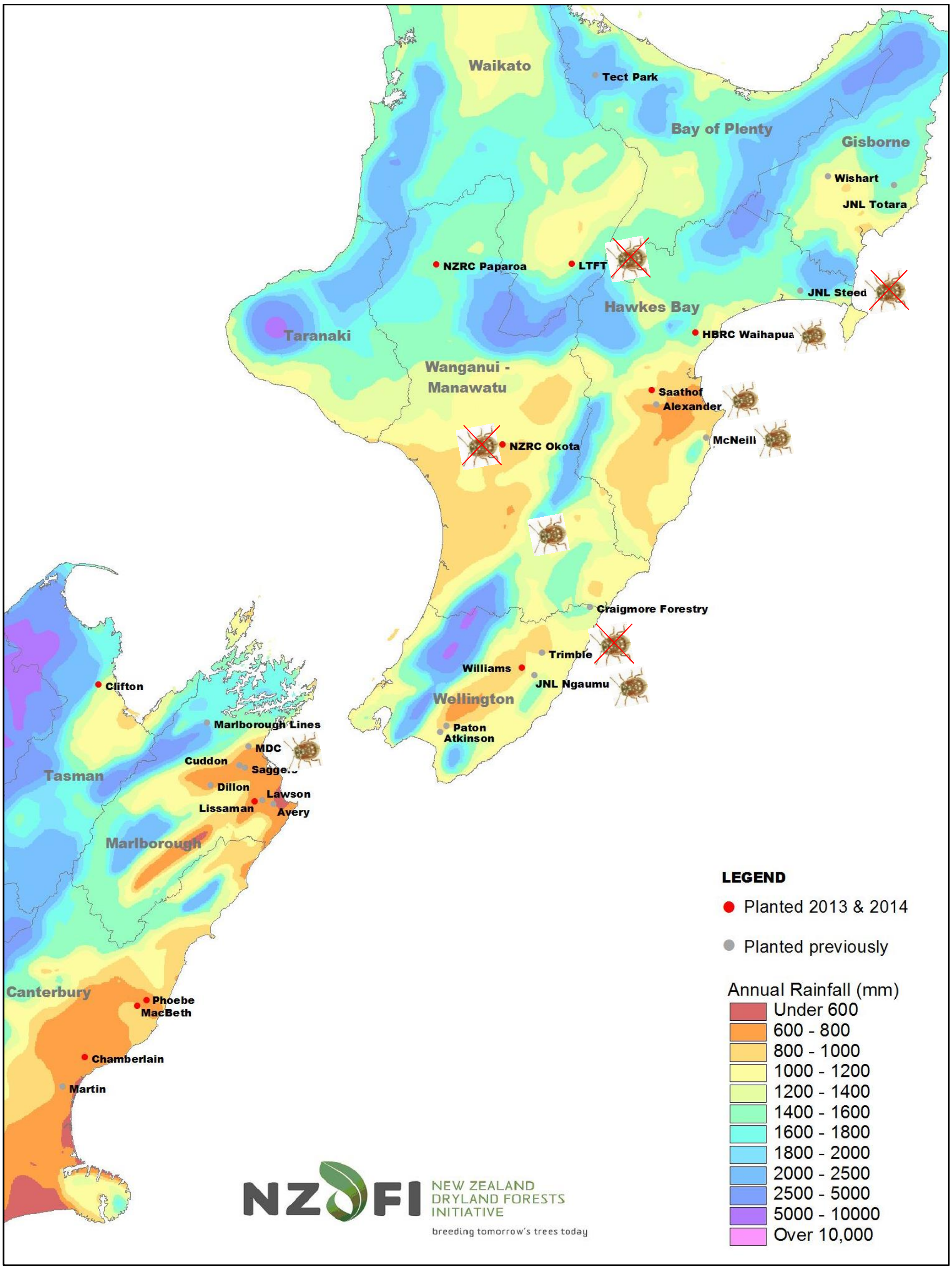
Lin, H. (2018) Risk and impact of insect herbivores on the development of dryland *Eucalyptus* forestry in New Zealand. Unpublished PhD Thesis, University of Canterbury.

Lin, H., Murray, T.J., & Mason, E.G**.** (2017) Incidence of and defoliation by a newly introduced pest, *Paropsisterna variicollis* (Coleoptera: Chrysomelidae), on eleven durable *Eucalyptus* species in Hawke’s Bay, New Zealand. *New Zealand Plant Protection*, *70*, 45-51.

Withers, T.M. & Pugh, A. (2019) SWP File Note. EVB egg parasitism 2018/19.

APPENDICES

Appendix 1: Locations of NZDFI durable eucalypt trials where EVB has been detected (beetle symbol) or has been searched for and not detected (crossed symbol). MRC = Craven's Rd site. Extensive searches have not been conducted for EVB in other Marlborough or Canterbury sties but none have been observed during frequent visits for other activities date.



Appendix 2: Provenance source locations (Victoria, Australia) for *E. bosistoana* families assessed at McNeill's, 2018. WW = Won Wron, TL = Tambo Lower, TU = Tambo Upper, CV = Cann Valley, GN = Genoa, 24 = Family 24. Approximate locations of the 'Southern' (red star) and 'Marulan' and 'Bungonia' (yellow star) provenances discussed in Lin (2018).