Pest management for durable eucalypts: Deliverables Q4 FY16-17

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EXECUTIVE SUMMARY

One of the more unexpected and important results from the phenology study summarised here, was that only one generation of *P. charybdis* was observed in each of the two years monitored. This contrasts with two generations usually observed in the North Island. In the North Island the second generation occurs in late summer (February/March) and can be partially reduced due to the actions of two species of egg parasitoids. Given *P. charybdis* appears to have only one generation in the Marlborough study area, it is possible the phenology of these egg parasitoids is similarly affected, which may reduce their effectiveness and biological control agents and would be worthy of investigation.

Robust developmental data, including developmental thresholds, development duration and stage specific mortality were successfully obtained for all stages of *P. charybdis*. In combination with environmental data from the field sites, these will be used to create population models for *P. charybdis* in the Marlborough region. These data will be combined with results obtained from another part of durable eucalypt pest management programme, on growth impact of defoliation, to help inform models for predicting damaging population numbers as part of IPM monitoring.

Clear differences were seen in the incidence and severity of natural insect pest damage sustained by different *E. boasistoana* families. This indicates there is variation, which if heritable, could provide a basis for selecting for pest tolerance. Interestingly, all except the southern provenance *E. bosistoana* families sustained less or similar levels of overall and chewing damage compared to the single *E. globoidea* family assessed as a representative monocalypt. Of the four feeding guilds assessed, chewers inflicted the greatest damage, as expected, and mining damage was relatively minimal. The least tolerant families were more easily identified than the most tolerant and represented the four families from the southernmost provenance, as well as family 128 from Bungonia. Generally, families 108 followed by 125 and 129 show the greatest tolerance, with high proportions of trees sustaining incidental or no damage from the different feeding guilds. Other families were more difficult to rank due to greater variability between the individual trees assessed, but all showed some apparently tolerant individuals which will be investigated further.

INTRODUCTION

This document reports on work completed as required for the June 30 2017 milestones associated with project 1.11.2 Pest management of durable eucalypts. The two deliverables are:

1: Pest phenology (insect life-cycle) study complete
2: Family susceptibility/tolerance identified or ranked for E. bosistoana

The report contains a summary of data from two draft thesis chapters in preparation by University of Canterbury PhD candidate Huimin Lin and submitted to supervisor Dr Tara Murray. All field work, data collection and preliminary analysis pertaining to the deliverables has been completed. Data analysis and the draft thesis chapter will continue to be revised and refined for several more months until final thesis submission. As data presented here form part of the PhD thesis and associated publications, the contents should not be distributed outside the SWP TST unless permission is first gained from the author.

DELIVERABLE 1: PEST PHENOLOGY

Background

Understanding the biology and phenology of insect pests is key to developing an effective, and both economically and environmentally sustainable, pest management strategy. Outbreaks of insect pests leading to devastating production loss can be reduced or avoided if the event can be reliably predicted. The eucalypt defoliators found in New Zealand have not been extensively studied in the South Island but are expected to show different life-cycle dynamics compared to populations in the North Island due to differences in local environmental conditions, especially temperature. Modelling is a good way to study outbreak risk potential for insect pests in different regions. Once developed, these models can be populated with local seasonal climate data and used as part of integrated pest management (IPM) plans to monitor and manage pests on an annual basis. This may include, for example, determining if and when to apply chemical control or whether to rely on biological control based on the expected population growth of an insect pest and the impacts a population of that size is predicted to have in a particular region.

Methods

Field phenology

Pest phenology was evaluated in a stand of *Eucalyptus bosistoana* at a site near Lake Grassmere, Marlborough (Fig. 1). Trees were planted in 2010 on a north facing slope approximately 64m asl. The site receives < 600 mm rainfall per annum. Approximately 237 trees were assessed for the presence and abundance of each life stage of the four most common insect defoliators observed in the study site; *Paropsis charybdis* (eucalyptus tortoise beetle), *Opodiphthera eucalypti* (gum emperor moth), *Strepsicrates macropetana* (eucalyptus leafroller) and *Phylacteophaga froggatti* (eucalyptus leaf-blister sawfly). The species intentionally represented different insect orders (coleoptera, lepidoptera, hymenoptera) and feeding guilds (chewers, minders, leaf rollers). Observations were repeated approximately monthly for two growing seasons (2015-2017) to determine, in particular, the peak abundance and duration of each life-stage and number of generations completed per year for each pest species.

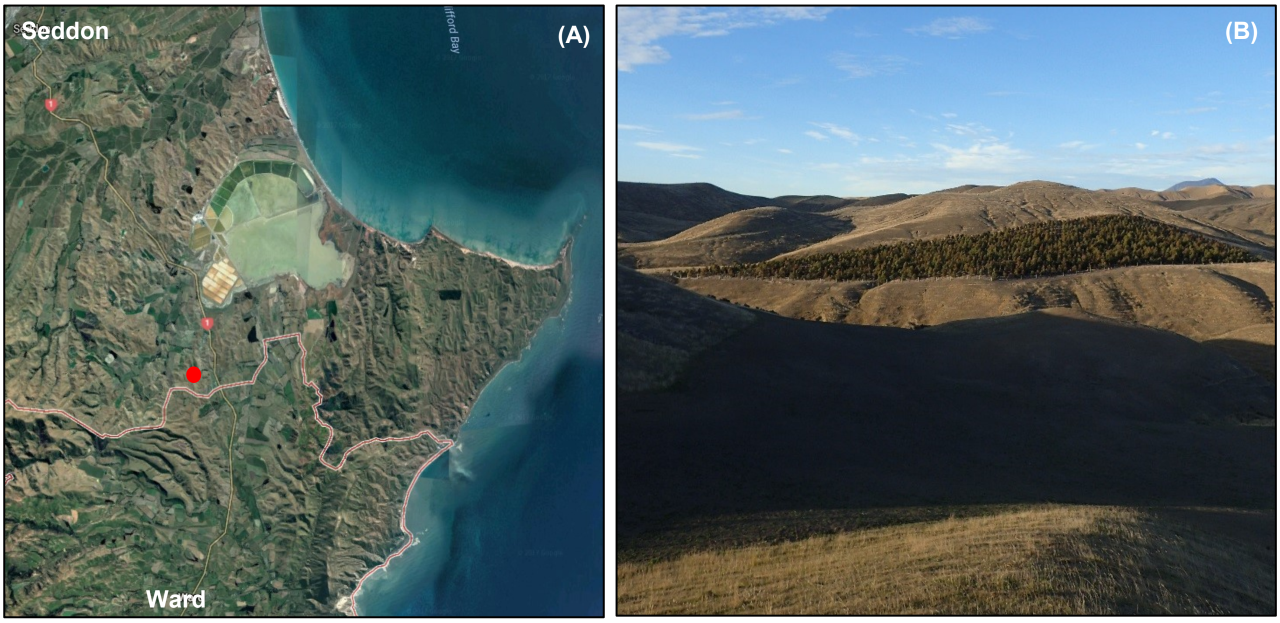


Figure 1: A) Location (red circle) of *E. bosistoana* study site between Seddon and Ward in Marlborough. B) Close up of stand planted October 2010 on North facing slope.

On each sampling occasion, 3-5 shoots were inspected per tree and for each of the four insect species the number of eggs and numbers of each larval stage were recorded. This is a variation on the Occupied Leaves per Shoot (OLS) method (Nahrung et al 2008).The number of *P. charybdis* adults were also recorded as they feed actively on *Eucalyptus* foliage, while the adults of the other three species do not. For *S. macropetana*, number of leaf rolls per shoot were counted and larvae classified as early, mid and late instar based on body size flowing Mauchline et al. (1999). For *Ph. froggatti,* the number of mines per shoot was counted and the larval stage (based on head capsule width) or presence of a pupa within each mine was determined by shining a light through the leaf.

Tree height, DBH, tree form, number of expanded vs. young leaves (on the assessed shoots) and a damage assessment score (for the first season, see deliverable 2 for method) was also recorded for each tree. Air temperature and humidity were recorded hourly using loggers installed at the centre of the site and all other climate data (e.g. rainfall) were obtained from the nearby weather station installed in a second *E. bosistoana* trial approximately 3.3km to the NW.

Development rates

Rate of development was assessed in detail for *P. charybdis* in a controlled laboratory study. Eight hundred eggs were split into 40 groups of 20 and divided between four growth cabinets (10 reps each) at 8°C, 15°C, 20°C and 28°C. Each feeding group was supplied fresh *E. bosistoana* foliage *ad libitum* and monitored daily until pupation. Upon moulting to 3rd instar, feeding groups were reduced to 3-5 larvae per cup to ensure foliage did not run out before it could be replenished. Survival and development duration for eggs, each larval instar, pre-pupae and pupae were recorded.

Preliminary Results

Pictorial graphs describing the phenology of each pest species are being drawn. The field abundance data for each life stage observed (Fig. 2) will be used to determine how many generations occurred per season for each pest species. Year one (2015/2016) was very dry relative to year two (2016/2017) which was in contrast extraordinarily wet. This may account for the some of the differences observed in insect populations between years. Peak egg laying by *P. charybdis* was missed in both years, despite monitoring starting a month early the second year. The first generation of young larvae peaked in October-November both years but few late instar larvae were observed and no second generation was detected (Fig. 2a). Eggs of *O. eucalypti* were observed in November 2015 and November to January in 2016/2017(Fig. 2b) but numbers were low and distribution among trees was patchy. The presence of mid-instar larvae in December and February suggests two small generations may occur annually but the population does not appear to be strongly synchronised, with eggs and young larvae appearing for 3 months of the year. *Phylacteophaga froggatti* (Fig 2c) was detected at much lower abundance than the other three pest species. In the first season in particular, *Ph. froggatti* did not show strong phonological synchrony, with both early and late instars observed at low levels in December and January. *Strepsicrates macropetana* was present in greater abundance than *Ph. froggatti* and *O. eucalypti*. A single generation was observed with a clear cohort progression visible in the second season; with early instars appearing in November, mid-instars peaking in January, and late instars following in February (Fig 2d).

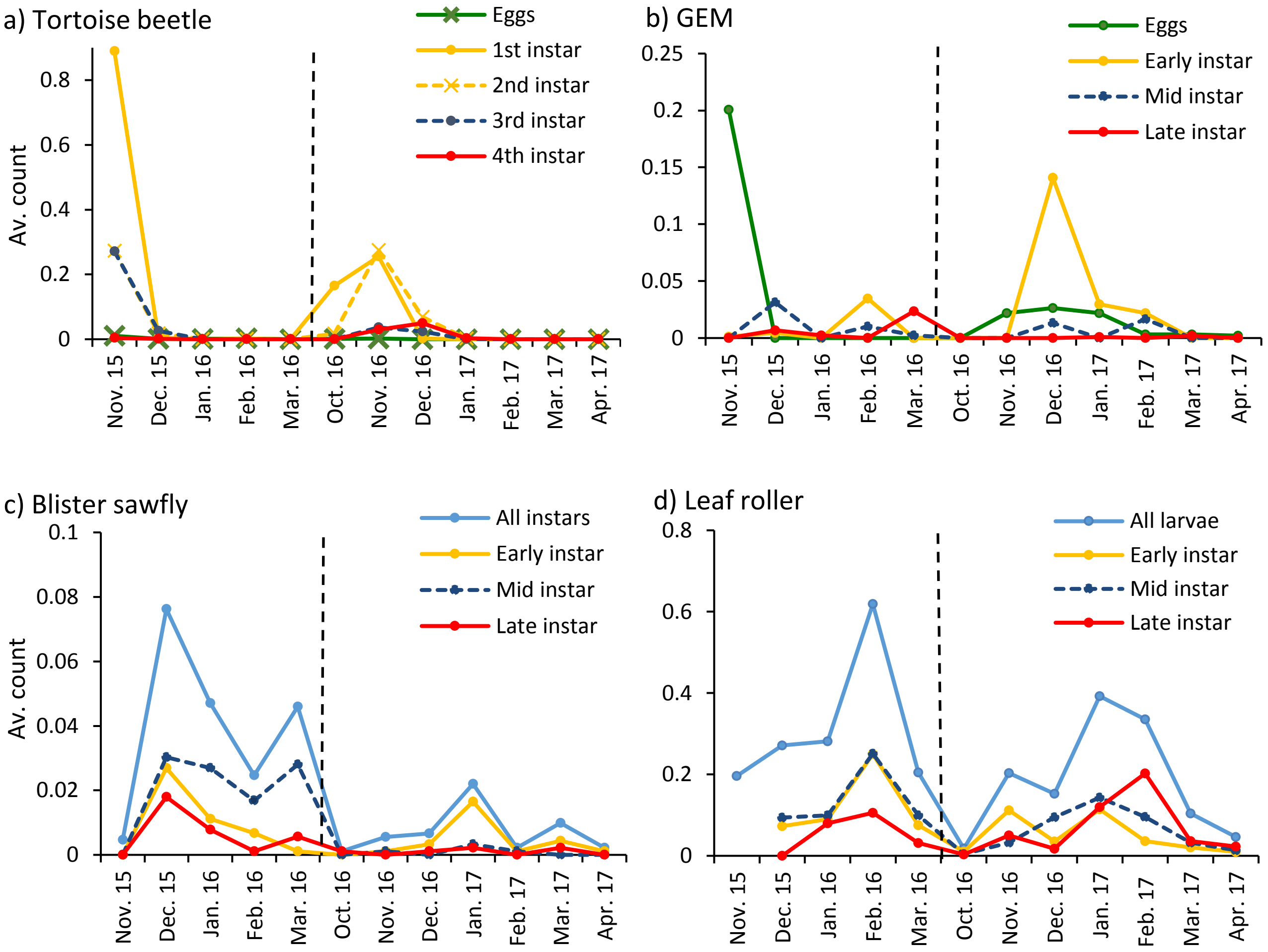


Figure 2: Average abundance per shoot for eggs and/or larvae of four key pests, assessed monthly for two growing season on *E. bosistoana*. Dashed line denotes the break between seasons. a) *P. charybdis* (eucalyptus tortoise beetle), b) *O. eucalypti* (Gum emperor moth), c) *Ph. froggatti* (leaf blister sawfly), d) *S. macropetana* (eucalypt leaf roller).

There was a degree of variation in the average abundance of *P. charybdis* larvae observed feeding on the different *E. bosistoana* families in the study. The four southern provenance families (133, 134, 135, 138) were preferred with some consistency (Fig. 3), while 108 and 116 (Marulan provenance) and 999 (*E. globoidea*) were least preferred in both years.

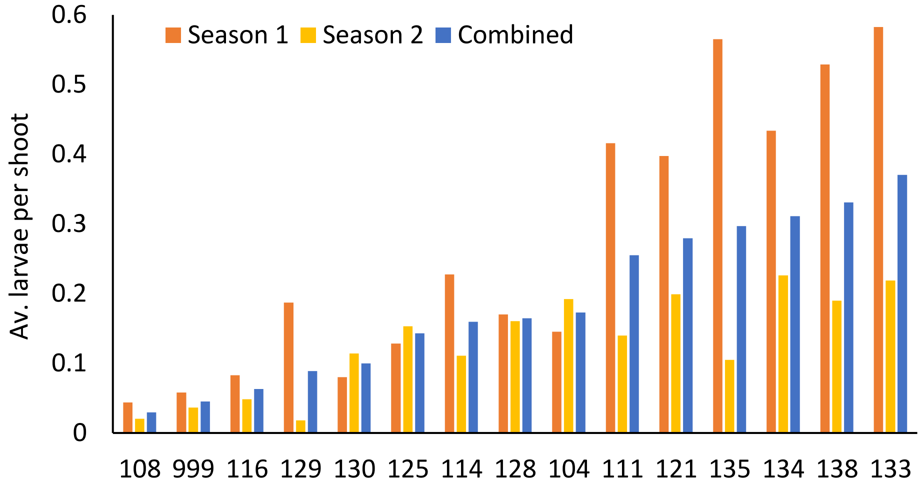


Figure 3: Ranking of *E. bosistoana* families by average abundance of *P. charybdis* larvae counted per shoot over two growing seasons. Season 1 = November 2015-March 2016, Season 2 = October 2016-April 2017. See Figure 5 for family provenance. 999 represents a single family of *E. globoidea*.

Developmental data (Fig. 4) has been used to calculate a developmental threshold temperature (y intercept) and degree days required for eggs, larval instars 1-4, pre-pupae and pupae. Development rate showed a strong linear relationship with temperature for all stages (Fig. 4b).

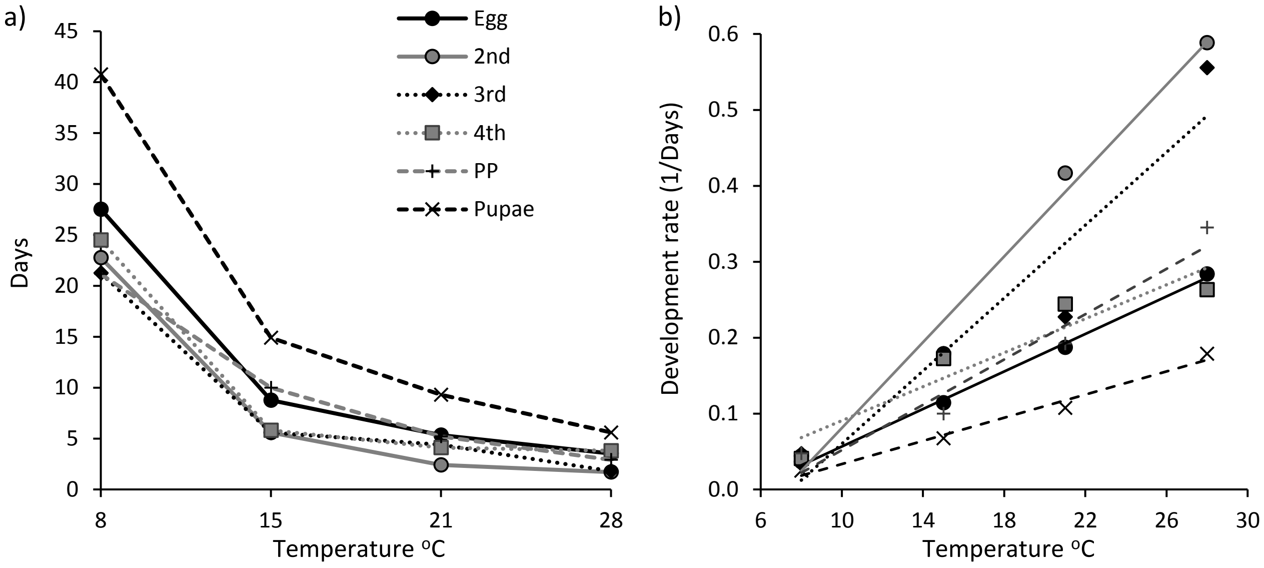


Figure 4: Average development duration (days) and development rate (reciprocal of duration) for egg, 2nd, 3rd and 4th larval instars, pre-pupal (PP) and pupal stages of *P. charybdis* at 8°C, 15°C, 20°C and 28°C. R^2 = 0.99, 0.98, 0.90, 0.90, 0.95, 0.98 for egg, 2nd, 3rd, 4th, PP and P respectively.

In the final thesis chapter, phenology data will be combined with defoliation data (collected for the study detailed under *Deliverable 2*) to determine the most damaging insect species and stages and their seasonal occurrence at this Marlborough dryland site. A population growth model will be attempted for *P. charybdis* based on the temperature dependant development rates determined from the lab study and stage specific mortality data derived from the lab and the field abundance assessment. Egg and larval abundance will be used to determine a final ranking of feeding preference by tree family for each pest species.

DELIVERABLE 2: FAMILY TOLERANCE RANKED FOR *E. BOSISTOANA*

Background

In their natural environment, many eucalypts are known to sustain substantial levels of insect herbivory with apparently minimal mortality or reduction in growth rate, while others are severely affected. Insects have also been shown to have significant differences in their preferences for feeding on different eucalypt species, or even genetically distinct families. This is often thought to be related to concentrations of plant secondary metabolites, which are important in constitutive defence and vary between & within *Eucalyptus* species (Eyles et al. 2013). Considering this natural variation, the need to manage insect pests in New Zealand eucalypt plantations may be much reduced if species or families can be identified that are unpalatable to the relatively limited suite of established eucalypt pests. Given there is also ongoing potential for new pest incursions (Withers 2001) it is essential that the most insect tolerant genotypes available are selected as the basis for developing an industry that will remain sustainable in the future. Recent studies have demonstrated that screening existing breeding trials to eliminate the most insect susceptible material can be a feasible and more cost effective strategy than adding a new trait into the breeding process *per se* (Henery 2011, Elek and Wardlaw 2013, Boshier and Buggs 2015).

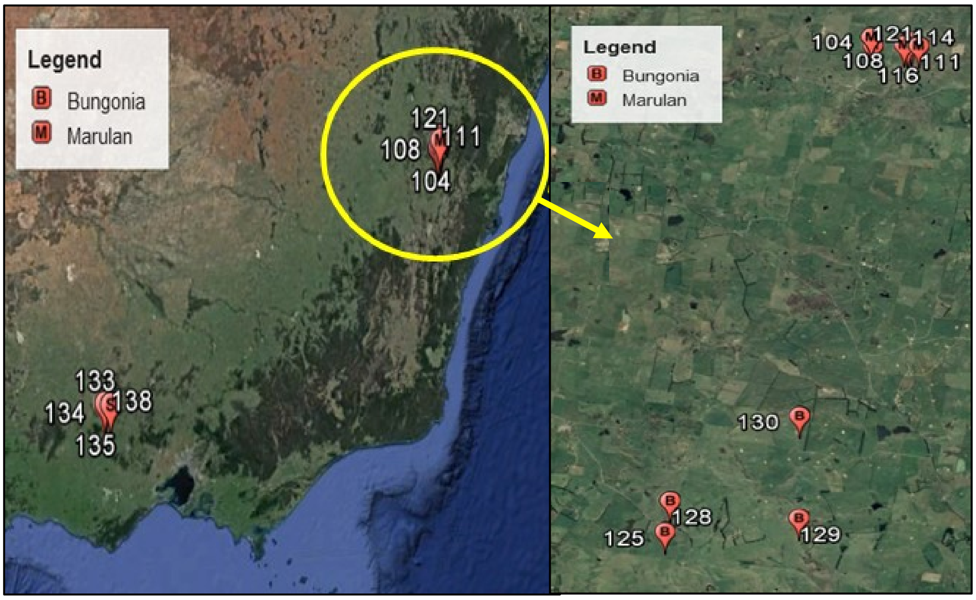
Methods

Assessment of natural defoliation

To assess variation in insect resistance and tolerance within *E. bosistoana* families, an extensive health assessment was conducted at a site near Lake Grassmere, Marlborough (Fig. 1). Approximately 237 trees were assessed representing 14 *E. bosistoana* families (seeds collected from known mother trees, Fig. 5) and one family of *E. globoidea* which was included for comparison as it is the only monoclaypt represented in the durable eucalypt trials. Monocalypts are often considered to be less susceptible to insect pests (Li 1994, Stone et al. 1998).

Figure 5: Provenance locations within Australia for the 14 *E. bosistoana* families assessed for insect tolerance. Bungonia is approximately 10km south of Marulan and 800km North of the 'southern' provenance.

Family	Provenance
104	Marulan
108	Marulan
111	Marulan
114	Marulan
116	Marulan
121	Marulan
125	Bungonia
128	Bungonia
129	Bungonia
130	Bungonia
133	Southern
134	Southern
135	Southern
38	Southern



Each tree was assessed on four occasions from November 2015 – March 2016 and given four health scores representing natural damage caused by the different guilds of insect herbivores as follows: 1) overall insect damage (all insects), 2) chewing damage (eucalyptus tortoise beetle and gum emperor moth), 3) mining damage (leaf blister sawfly), 4) leaf roll damage (eucalyptus leaf roller). Health scores were based on a modified version of the Crown Damage Index (CDI) (Stone et al. 2003). The CDI score is determined as Incidence x Severity / 100; where *incidence* is the estimated proportion of damaged leaves in the tree crown, and *severity* (Fig. 6) is the average proportion of damage to each leaf. Incidence was determined by visually assessing the whole tree crown for damage types 1, 2 and 4, but only the lower 1/3rd of the crown for type 3 as mining damage usually occurs only in this part of the crown. Severity was based on inspection of individual leaves from 3-5 shoots per tree from the upper, middle and lower portions of the crown (lower and middle only for mining damage).

In the second season methods were refined such that a more rapid assessment could be made. Trees were assessed only twice (January and April 2017) and given a single defoliation score for each of the damage types 1 – 4 as above. Defoliation was recorded as a) little or no damage, b) light damage, c) moderate damage, d) severe damage. Tree height and DBH were measured on each sampling occasion in both seasons to compare relative growth rates of trees sustaining different levels of damage.

Data Analysis

For each of the eucalypt families assessed, an average score was calculated for each damage type (1-4) and for growth increment over the two seasons. Families were ranked from lowest to highest susceptibility (level of damage observed) and tolerance (growth increment) to insect herbivores. Post hoc Cumulative Link Mixed Models (CLMM) were fitted to investigate the effects of each damage type separately on tree growth over the two seasons and determine tolerance to insect damage by family, provenance and location within provenance.

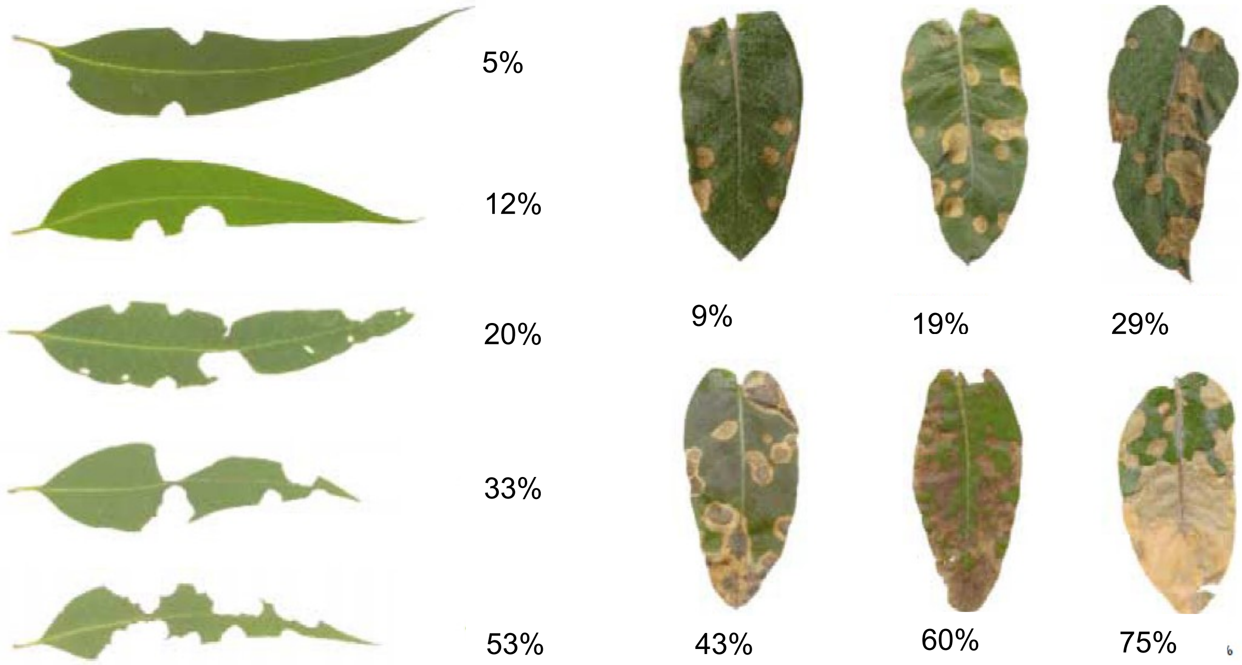


Figure 6. Examples of variation in the estimated severity of damage for chewing damage (left) and mining damage (right) (figures from Stone *et al*., 2013).

Preliminary Results

Only preliminary results are presented here as the results of the CLMM analysis are yet to be fully interpreted. Ranking families from lowest to highest damage based on severity scores in season one showed clear differences in the level of susceptibility of *E. bosistoana* families to insect herbivores. Damage was variable both within and between families, but five families (Fig. 7) sustained substantially higher levels of damaged than all others. Four of these five families (133, 134, 135, 138) were from the Southern-most provenance. The most tolerant families were 108, 130 and 104.

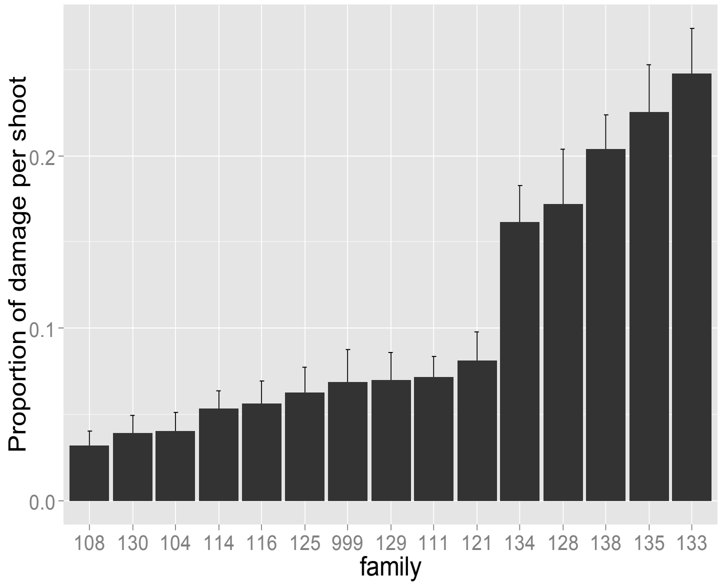


Figure 7: Ranking of *E. bosistoana* families from lowest to highest damage incurred based on average levels of insect herbivore damage sustained per shoot. Note family 999 represents *E. globoidea* as a comparison to a monocalypt species.

Rapid assessment using the percentage defoliation score in season two also showed variability in over-all and guild specific defoliation across the families assessed (Fig. 8). The highest levels of damage for all insects combined and the chewing guild were again seen in the four families from the southern-most provenance. Lower levels of damage were inflicted by mining and leaf rolling insects and family rankings were similar, but weak, compared to the chewing guild. The exception was in the rankings for mining damage, with the *E. globoidea* family conspicuously free of this type of damage. Overall, 10 *E. bosistoana* families included a proportion of trees (3-32%) that suffered no or very incidental damage from insect herbivores, and all but two of these families included more of these 'tolerant' trees than the *E. globoidea* family. All 10 families included a proportion of trees (24-66%) that sustained no defoliation from the chewing insects (*P. charybdis* and GEM). Although leaf rolling damage was generally less severe than chewing, with few trees represented in the severe category, only 3 families had > 30% of trees with no damage. Mining damage was absent from 33-80% of trees from all families.

Post hoc pairwise comparisons (with Tukeys adjustment for multiple comparisons) based on results of CLMM, indicate family, provenance, collection location within provenance, and tree height all have some effect on damage level. In all cases chewing damage appears to be significantly higher on the four Southern provenance families. Further analysis and interpretation of these relationships is underway.

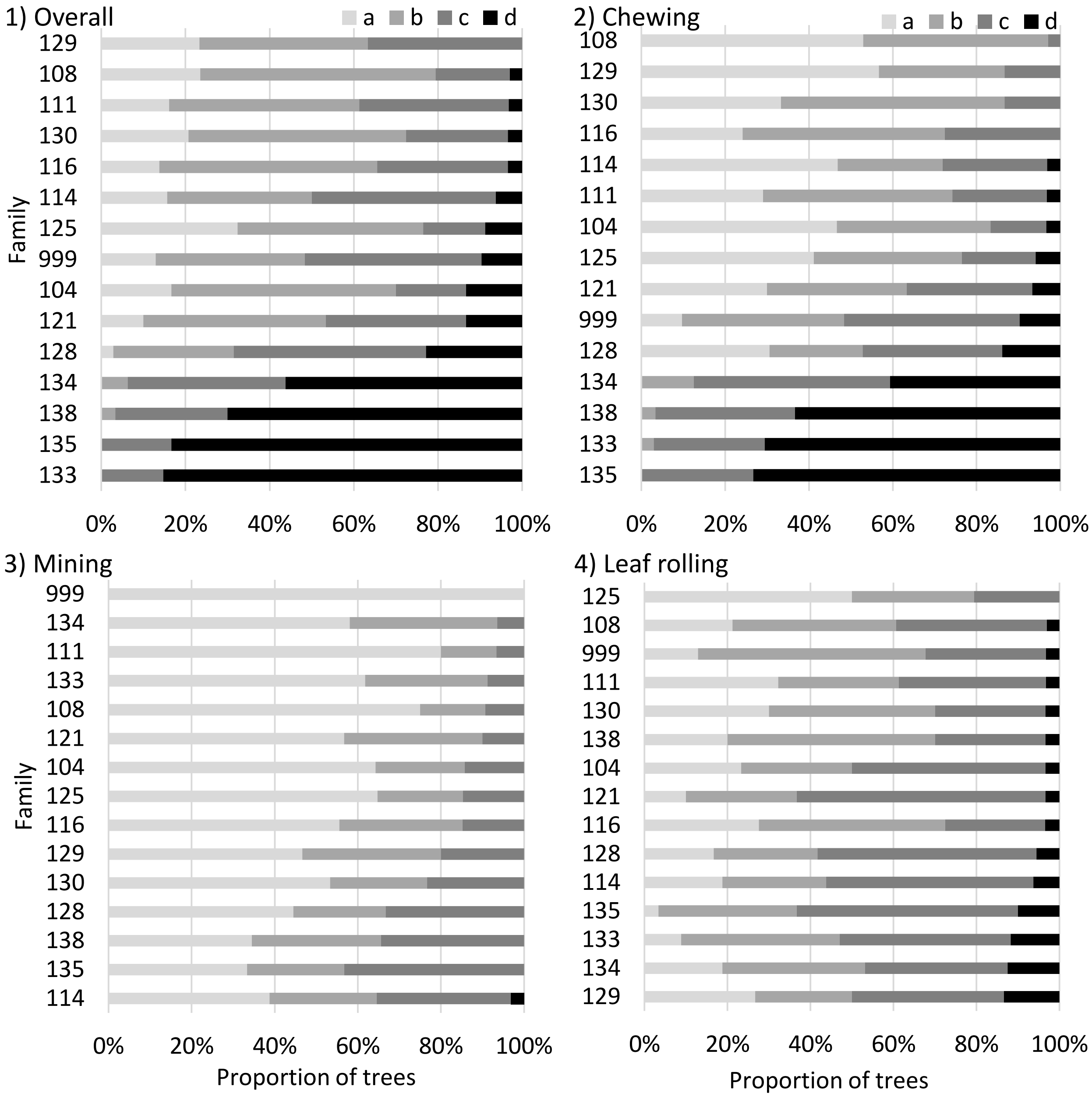


Figure 8: Average proportion of trees sustaining damage of different levels of severity where a = little or no damage, b = light damage, c = moderate damage, d = severe damage. Data are for January and April assessments combined. 1) Overall (all insects), 2) Chewing (eucalyptus tortoise beetle, gum emperor moth), 3) Mining (leaf blister sawfly), 4) Rolling (eucalyptus leaf roller).

CONCLUSIONS

One of the more unexpected and important results from the phenology study summarised here, was that only one generation of *P. charybdis* was observed in both summers. This contrasts with two generations usually observed in the North Island. In the North Island the second generation occurs in late summer (February/March) and can be partially reduced due to the actions of two species of egg parasitoids. Given *P. charybdis* appears to have only one generation in the Marlborough study area, it is possible the phenology of these egg parasitoids is similarly affected, which may reduce their effectiveness and biological control agents and would be worthy of investigation.

Robust developmental data, including developmental thresholds, development duration and stage specific mortality were successfully obtained for all stages of *P. charybdis*. In combination with environmental data from the field sites, these will be used to create population models for *P. charybdis* in the Marlborough region. These data will be combined with results obtained from another part of durable eucalypt pest management programme, on growth impact of defoliation, to help inform models for predicting damaging population numbers as part of IPM monitoring.

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