The compatibility of the egg parasitoid guild of *Paropsis charybdis* (Col.: Chrysomelidae) in New Zealand with the predicted phenology of *Eadya daenerys* (Hymenoptera: Braconidae) a proposed new larval biocontrol agent

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Report information sheet

Report title	The compatibility of the egg parasitoid guild of *Paropsis charybdis* (Col.: Chrysomelidae) in New Zealand with the predicted phenology of *Eadya daenerys* (Hymenoptera: Braconidae) a proposed new larval biocontrol agent
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Client	MBIE
SIDNEY output number	61186
Signed off by	Stephen Pawson
Date	15 August 2018
Confidentiality requirement	Publically available
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Published by: Scion, 49 Sala Street, Private Bag 3020, Rotorua 3046, New Zealand. www.scionresearch.com

Executive summary

The problem

Paropsis charybdis (Coleoptera: Chrysomelidae) has been a major pest of Symphyomyrtus *Eucalyptus* species since it arrived in New Zealand from Australia. The study had three objectives: 1) Quantify the present day phenology (seasonal occurrence) of *P. charybdis* presence in the central North Island, 2) Estimate defoliation by *P. charybdis* in *E. nitens* plantations using the crown defoliation index (CDI), and 3) Determine if the phenology of *P. charybdis* and the impact of the current established egg parasitoids attacking *P. charybdis* has changed since it was last studied in detail in the fieldin 2005. Answers to these questions will inform discussion of the potential effectiveness of *Eadya daenerys*, a proposed new larval biological control agent of *P. charybdis* from Tasmania.

This project

P. charybdis phenology, egg parasitism, and defoliation rates were assessed at two *Eucalyptus nitens* plantations in the central North Island during the season of 2016/17. The sites were visited every fortnight for five months. We applied climate modelling (CLIMEX) to examine the compatibility of New Zealand for the establishment of the larval parasitoid *Eadya daenerys*, based on its known geographical origin, in Tasmania, Australia. By comparing the timing when adult *E. daenerys* are present and active in Tasmania, we will be able to estimate what time of year it might attack *P. charybdis* larvae if it is introduced into New Zealand.

Key results

First generation (spring) *P. charybdis* eggs are not controlled by existing egg parasitoids (*Enoggera nassaui* and *Neopolycystus insectifurax)* with 90-100% egg survival observed. Second generation *P. charybdis* egg survival is less than 50% due to parasitism by *E. nassaui* and *N. insectifurax*. *Baeoanusia albifunicle*, a hyperparasitoid of *E. nassaui*, reduces second generation egg parasitism – but its impact has not yet been quantified.

Implications of results for the client

Phenology modelling confirmed that first generation *P. charybdis* larvae are likely to be synchronous with adult *Eadya daenerys* (Hymenoptera: Braconidae). *Eadya daenerys* is day active from mid-November to late December in the field in Tasmania. Climate matching predicts *E. daenerys* could establish in all areas of New Zealand where *P. charybdis* affected eucalypt plantations are grown. The introduction of a larval endoparasitoid into this parasitoid-pest guild would significantly contribute to the suppression of *P. charybdis* by decreasing first generation survival thereby reducing recruitment of the pest into the second generation.

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Introduction

Forestry is a significant contributor to the New Zealand economy, directly contributing $1.9 billion to national GDP (Nixon et al., 2017). *Eucalyptus* spp. form a small but important part of this industry. Eucalypts comprised 1.3% of the total plantation area (FOA, 2017; Yao et al., 2013), and are valued at $671 million (asset value) (Radics et al. unpublished data). Eucalypts are valued for their use in pulp and paper, amenity plantings, shelter, soil erosion control, and as a source of naturally durable wood (Haslett et al., 1984; McKenzie, 1993; Wilcox, 1980).

While a large number of Australian eucalypt feeding insects have arrived in New Zealand (Withers, 2001), the major pest of eucalypt plantations remains *Paropsis charybdis* Stål (Coleoptera: Chrysomelidae), the eucalyptus tortoise beetle. *Paropsis charybdis* established in New Zealand more than 100 years ago (Withers & Peters, 2017) and is a major defoliator of eucalypt trees. *Paropsis charybdis* is known to feed on at least 59 eucalypt species (White, 1973), including *E. nitens* (Deane & Maiden) the most commonly planted species (Withers & Peters, 2017). Adult and larval *P. charybdis* feed on adult foliage, but not on the glaucous waxy juvenile foliage of young heteroblastic (age related change in leaf form) eucalypt species (Edwards, 1982). Repeated defoliation of the tree crown significantly reduces tree growth (and occasionally leads to tree death), which ultimately decreases the economic proposition of growing eucalypt trees (Bain & Kay, 1989; Elek & Baker, 2017).

The high fecundity of female *P. charybdis,* which can lay 1500-2000 eggs in a lifetime, (Styles, 1970) results in rapid population growth. In New Zealand, adults fly from late September to late April, with larvae present from October to April (McGregor, 1989). *Paropsis charybdis* have one spring and one summer generation (Styles, 1970) and then overwinter as adults sheltering beneath bark and in crevices (McGregor, 1989). First instar larvae have a high survival rate in the field, with 77% reportedly reaching the second instar (McGregor, 1989).

Both chemical and biological options to control *P. charybdis* are used in New Zealand and there is an on-going search for insect-resistant germplasm (McGregor, 1989; Wilcox, 1980; Withers & Peters, 2017). Chemical control is currently achieved by aerial application of synthetic pyrethroids (Rolando et al., 2016; Withers et al., 2013) that require a derogation from the Forest Stewardship Council (FSC) for use in FSC certified plantations (Rolando et al., 2016; Withers & Peters, 2017). Chemical control is not considered a sustainable long-term solution (Withers & Peters, 2017).

Biological control is a favoured method of control (Withers & Peters, 2017), and multiple introductions of biological control agents have occurred since the 1970s, although some failed (Bain & Kay, 1989). The egg parasitoid *Enoggera nassaui* Girault (Hymenoptera: Braconidae) was first introduced in 1987 from Western Australia (Bain & Kay, 1989), with a subsequent second introduction in 2000 from Tasmania that is more climatically similar to New Zealand (Murphy et al., 2004; Withers et al., 2011). *Enoggera nassaui* controlled the second generation for several decades (Murphy & Kay, 2000), however in 2002 *Baeoanusia albifunicle* Girault (Hymenoptera: Encyrtidae) a hyperparasitoid of *E. nassaui* was found to have self-introduced and established (Murphy, 2002). *Baeoanusia albifunicle* may significantly disrupt the biological control of *P. charybdis* in New Zealand by reducing the effectiveness of *E. nassaui* (Mansfield et al., 2011; Murray et al., 2008). Another *P. charybdis* egg parasitoid, *Neopolycystus insectifurax* Girault (Hymenoptera: Pteromalidae) was found to have self-introduced in 2002 (Berry, 2003). Fortunately *B. albifunicle* does not utilise *N. insectifurax* as a host and Murray and Mansfield (2015) predict that *N. insectifurax* may increase in importance as a control agent with the decline of *E. nassaui*. The generalist southern ladybird *Cleobora mellyi* Mulsant (Coleoptera: Coccinellidae) was introduced in 1977 from Tasmania (Bain & Kay, 1989; Bain et al., 1984) to target eggs and young larvae of *P. charybdis* – it has been ineffectual (Murray et al., 2008). The only predators of *P. charybdis* larvae in New Zealand are generalist pentatomids (Edwards & Suckling, 1980). Currently no natural enemies present in New Zealand provide any effective control of *P. charybdis* larvae such that they reduce *P. charybdis* population growth rates. Recent development of *Eucalyptus* spp. plantations for solid wood (Satchell, 2015) and ground-durable timber (Millen, 2011) has led to the re-examination of larval biological control agents from Tasmania (Withers et al., 2012).

Eadya daenerys Ridenbaugh (Hymenoptera: Braconidae) (Ridenbaugh et al., 2018) is one of several Australian species of *Eadya* that are all larval endoparasitoids of eucalypt feeding chrysomelids (Peixoto et al., 2018). It is currently under consideration as a new biological control agent for release in New Zealand. *Eadya daenerys* is most commonly reared from *Paropsisterna* (referred to as *Pst.*) *agricola* Chapius and *P. charybdis* (Peixoto et al., 2018) in Tasmania, Australian Captial Territory, and New South Wales (Ridenbaugh et al., 2018). Other minor hosts include *Pst. bimaculata* Olivier (de Little, 1982) and *Pst. nobilitata* Erichson (Peixoto et al., 2018). Since no other recorded hosts are present in New Zealand, *E. daenerys* will utilise *P. charybdis* as its primary host. In Tasmania, *E. daenerys* is mostly active from mid-November until mid-December, although Rice (2005) observed some adults in January. *Eadya daenerys* phenology is ideal to target the spring generation of larval *P. charybdis* and has been the subject of intensive non-target host-range testing (e.g., Withers et al., 2015) to establish if it is safe for release into New Zealand (T. Withers et al., unpublished data).

In this paper we examine the phenology of *P. charybdis* to provide further evidence of the pest problem, and its existing egg parasitoid complex in relation to the timing of tree damage. We then apply climate modelling to examine the compatibility of New Zealand for *E. daenerys* establishment and timing of its attack on *P. charybdis* larvae.

Materials and methods

Field Sites

Paropsis charybdis damage, presence of different life stages, and egg parasitism were monitored in two *Eucalyptus nitens* forests in the central North Island of New Zealand. The first site was located in Kaingaroa Forest, owned by Timberlands Limited (GPS NZTM 1883983E 5697119N), and consisted of 576 trees propagated from a sub-sample of the most promising 32 families of the New Zealand eucalyptus breeding co-operative's stock. Established in 2011, the majority originated from Victorian provenances with three of the *E. nitens* families originating from provenances in New South Wales. Trees at Kaingaroa were 5 years old and 3-6 m in height at the time of sampling; never subject to insecticide applications, and most had undergone the transition from juvenile to adult foliage. The second site at Poronui New Zealand, Taupo, owned by Westervelt Company (GPS NZTM 1885166E 5672807N), was established in 1998. The trees are from a small number of seedlots, and have historically suffered from on-going and repeated *P. charybdis* attack (Murphy & Kay, 2000). Approximately 6-12 m in height, all have transitioned to adult foliage; some have coppiced in response to repeated defoliation. In order to maintain tree health and growth within a mixed forest setting, the forest managers are committed to actively manage *P. charybdis* populations with insecticide applications when necessary. Both sites were visited between November 2016 and April 2017, approximately every fortnight (weather dependent). Aerial spraying with an insecticide with the active ingredient alpha-cypermethrin was applied to the Poronui plantations on the 17th and 20th of December 2016, and the 14th of January 2017 at 0.03 kg a.i. in 5 L/ha. No visits to Poronui occurred at these times.

Field Assessments

Paropsis charybdis phenology New Zealand
At each site, ten trees bearing flushing adult foliage were selected ad-hoc. At each tree one branch, up to 3 metres from the base was pulled down gently and visually examined. All *P. charybdis* life stages (eggs, larvae, and adult beetles) observed on the sample branches were recorded.

Paropsis charybdis egg parasitism
All egg batches found on sampled branches were collected and returned to the laboratory where they were placed in Petri dishes and wrapped with Parafilm™. Eggs were kept at 22˚C, with a 14:10 L:D regime and monitored for the emergence of parasitoids. Parasitism was calculated as a percentage of total eggs collected per fortnight for each species.

Eucalyptus appearance and health assessment

The Crown Damage Index (CDI) (Stone et al., 2003) was used to estimate the incidence and severity of foliar feeding by *P. charybdis* (the main insect responsible for insect damage). The damage incidence is the visually estimated proportion of leaves in the crown affected by insects or fungi and the damage severity is the visually estimated proportion of each leaf that is damaged.

On each visit, six different trees were selected ad-hoc to be assessed using the CDI, starting at the edge of the plantation and walking inwards, picking a different starting point at each visit. Each tree was observed from at least 2 angles to obtain a clear assessment of the damage on the tree. Insect and fungal damage were calculated separately for juvenile and adult foliage. The CDI was calculated by multiplying the incidence and severity for each foliage category for both insect and fungal damage. The sum of these incidence/severity products gives the overall tree CDI (Stone et al., 2003).

CLIMEX match climates

Climatic similarities between the Tasmanian locations where *Eadya daenerys* were collected and New Zealand were calculated using an algorithm of Climex version 3.3 called 'match climates regional' (Climex-MCR; Sutherst et al., 2007), following the method of Phillips et al. (2018). Similarities are quantified on a scale of 0 (no match) to 1 (perfect match), and are referred to as 'CMIs' (Composite Match Indices). Most studies to date have adopted a CMI threshold of 0.7 to distinguish between favourable (CMI ≥ 0.7) and unfavourable (CMI < 0.7) climatic conditions for species persistence (Robertson et al., 2008; Senaratne et al., 2006), though there are exceptions (Magarey et al., 2018).

New Zealand climate data were represented by a 0.05° x 0.05° (about 5 km x 5 km) grid of values (n = 11,471 locations), which are based on measurements made during the period 1960–2004 (Tait et al., 2006). Climate data for Tasmania were obtained from a 0.5° x 0.5° (about 50 x 50 km) grid of values provided with Climex, which are based on measurements made during the period 1961–1990 (Sutherst et al., 2007).

Eadya daenerys phenology

Sampling methods for emergence traps, bucket traps, malaise traps, and field parasitism are detailed in Rice (2005) and Smart (2016). Field parasitism of larvae is recorded as the date of field collection, when those larvae subsequently were shown to be parasitized after lab rearing. Wing catches were made using sweep nets to catch adult *E. daenerys* in eucalypt plantations where they were known to occur, up to 5 metres from the ground.

Results

Paropsis charybdis *phenology New Zealand*

In general the *P. charybdis* season at Kaingaroa was shorter than at Poronui. At Kaingaroa Forest, egg production peaked in mid-November and again in early February (Fig. 1). As expected, peaks in egg production were followed by peaks in the abundance of early instar larvae (first and second), and then by peaks in the abundance of late instar larvae (third and fourth). The number of adults found on sample branches was low throughout the sampling period. At Poronui, egg production first peaked in mid-November. Egg production peaked again after the spraying of the insecticide, at the end of January and into February (Fig. 1). As at Kaingaroa, egg collection peaks at Poronui were followed by peaks in the abundance of early instar larvae, which were followed by peaks in the abundance of late instar larvae. Observations of adults at Poronui were high at the beginning of the season but declined severely after the aerial spraying (Fig. 1). However, the adult population recovered a little after the appearance of adults from the second generation.

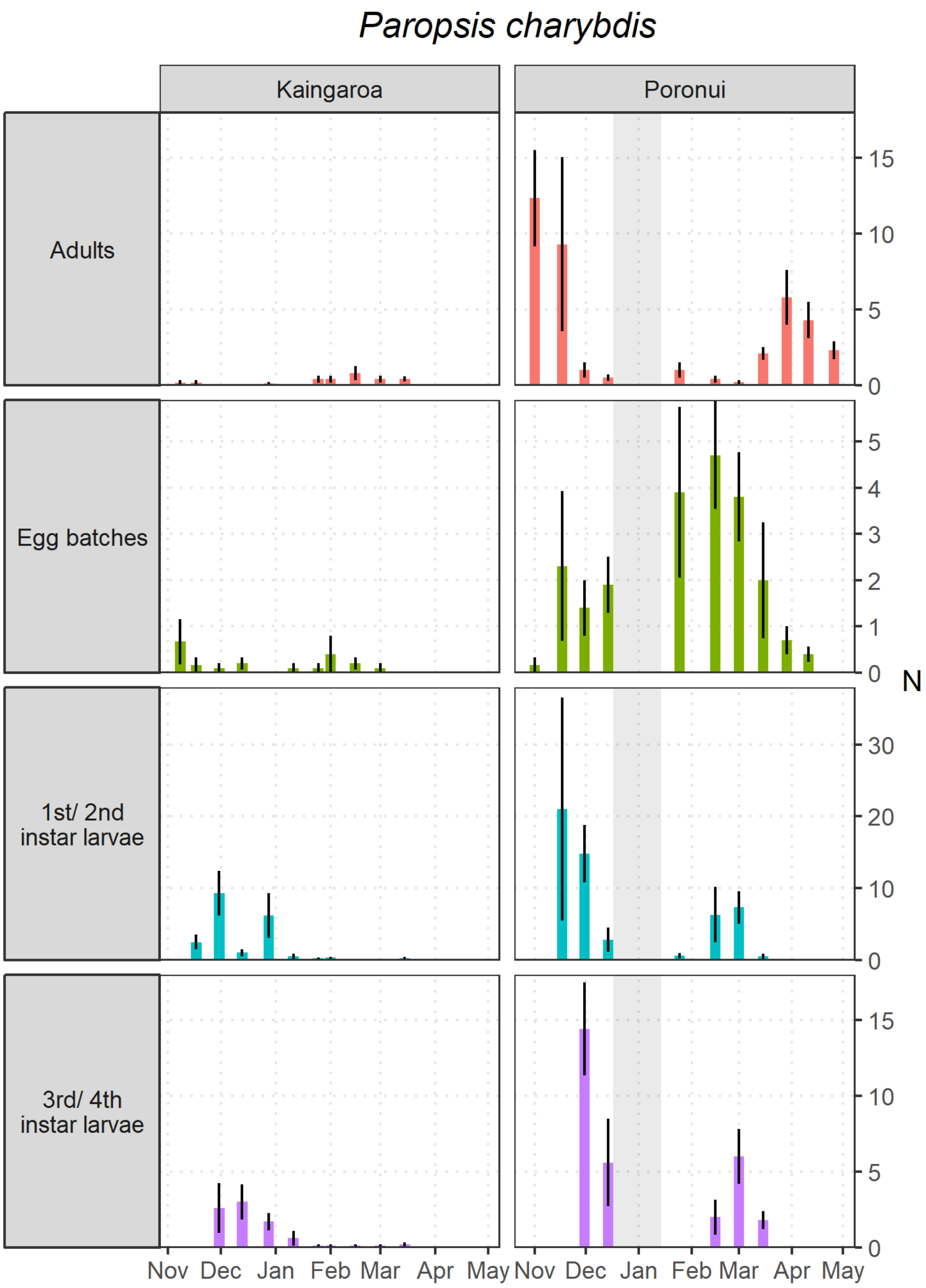

Figure 1. Mean number (± SE) *P. charybdis* life stages present on sample branches at Kaingaroa Forest and Poronui per fortnight 2016-17 season. Greyed out area represents spray period at Poronui Station

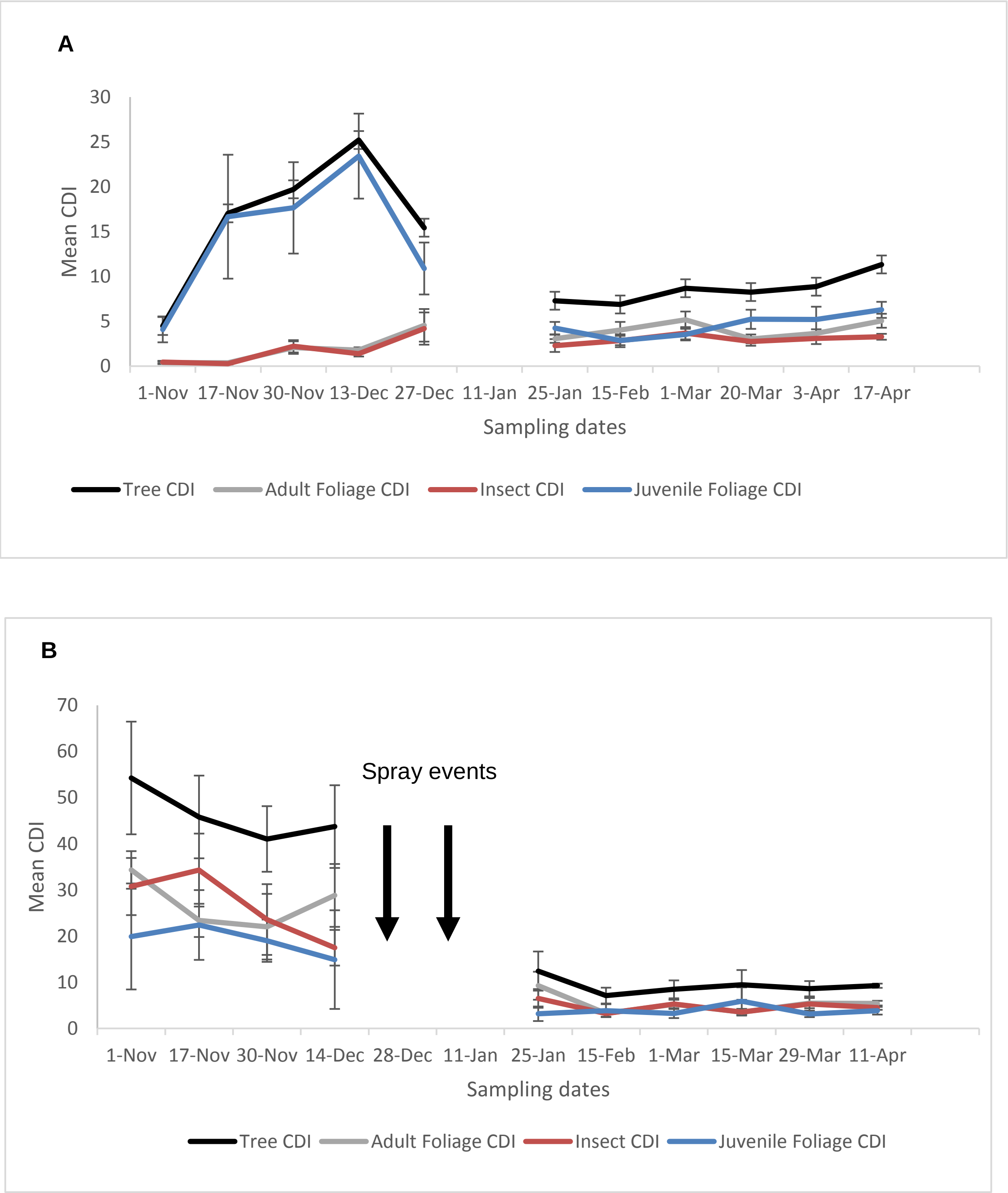

Figure 2. Mean CDI (± SE) scores for the overall tree, insect-specific defoliation, general adult foliage damage, and general juvenile foliage damage during 2016-2017 season at (A) Kaingaroa and (B) Poronui. The difference between Tree CDI and Insect CDI is the mean fungal proportion of damage.

Damage to juvenile foliage was worse than damage to adult foliage at Kaingaroa, peaking during November-December (CDI of 23). By contrast, the CDI score for mature foliage was relatively low and consistent across the whole season (Fig. 2A). At Poronui the scores were reversed with greater damage (and higher CDIs) for mature foliage compared to juvenile foliage. CDI scores for both juvenile and mature foliage were worst during November-December at Poronui, with both scores improving after insecticide spraying in late December-early January (Fig. 2B).

Paropsis charybdis *egg parasitism*

Enoggera nassaui, *Neopolycytus insectifurax* and the hyperparasitoid *Baeoanusia albifunicle* were all observed at Kaingaroa and Poronui (Fig. 3). *Enoggera nassaui* was the first parasitoid to appear at both locations. *Neopolycystus insectifurax* appeared earlier at Kaingaroa (early February) than at Poronui (late February).

There was no parasitism of *P. charybdis* eggs in Kaingaroa at the beginning of the field season (November), and did not increase substantially until mid-January. Parasitism by primary parasitoids from November-December was by *E. nassaui* (Poronui), but was replaced by *N. insectifurax* in late January through to March (Fig. 3) at both sites. *Baeoanusia albifunicle* (hyperparasitoid) appeared at both sites in mid-February.

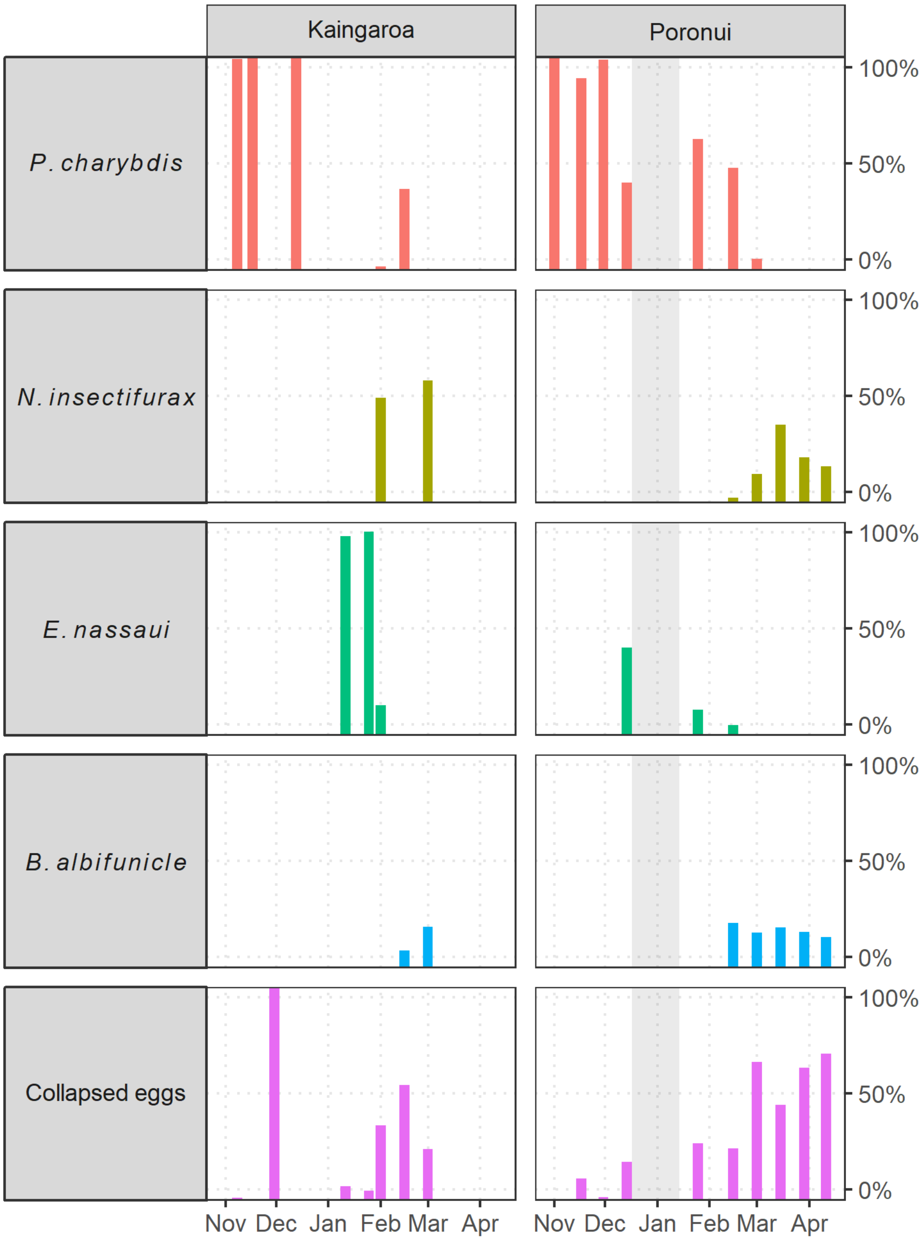

Figure 3. Percent hatch of *P. charybdis* eggs per fortnight, and egg parasitism by *Enoggera nassaui, Baeoanusia albifunicle or Neopolycytus insectifurax* from eggs collected during 2016-2017 season at (A) Kaingaroa and (B) Poronui. Greyed out area represents spray period at Poronui Station

Egg parasitism at Kaingaroa was highest in the month of January (95%). The percentage of collapsed eggs (undetermined cause of death) increased over the summer, peaking in February (54%). *Enoggera nassaui* was dominated by its hyperparasitoid, *B. albifunicle*, in early February (Fig. 3). Parasitism of *P. charybdis* at Poronui was low during the early part of the breeding season (November). As expected *Enoggera nassaui* was the first parasitoid to appear, and remained at low densities throughout the monitoring period. From late January to early April, *N. insectifurax* become the dominant parasitoid, reaching up to 36% parasitism (Fig. 3). Both the hyper-parasitoid *Baeoanusia albifunicle* and the primary egg parasitoid *N. insectifurax* appeared at Poronui at the same time in late January-early February. Parasitism at Poronui was highest in the month of March. The percentage of collapsed eggs increased as the season progressed, peaking in April (68%). Abundances of *Enoggera nassaui* were surpassed by the hyperparasitoid *B. albifunicle* in early February (Fig. 3).

Phenology and suitability of *Eadya daenerys* in New Zealand

The modelled potential distribution of *E. daenerys* in New Zealand shows that much of the country is climatically suitable (CMI >0.7) for its establishment (Fig. 4). The climatically suitable areas include all the major eucalypt growing regions of New Zealand. Only minor potential *Eucalyptus* growing areas in Northland and the East Coast of the North Island drop below this 0.7 threshold.

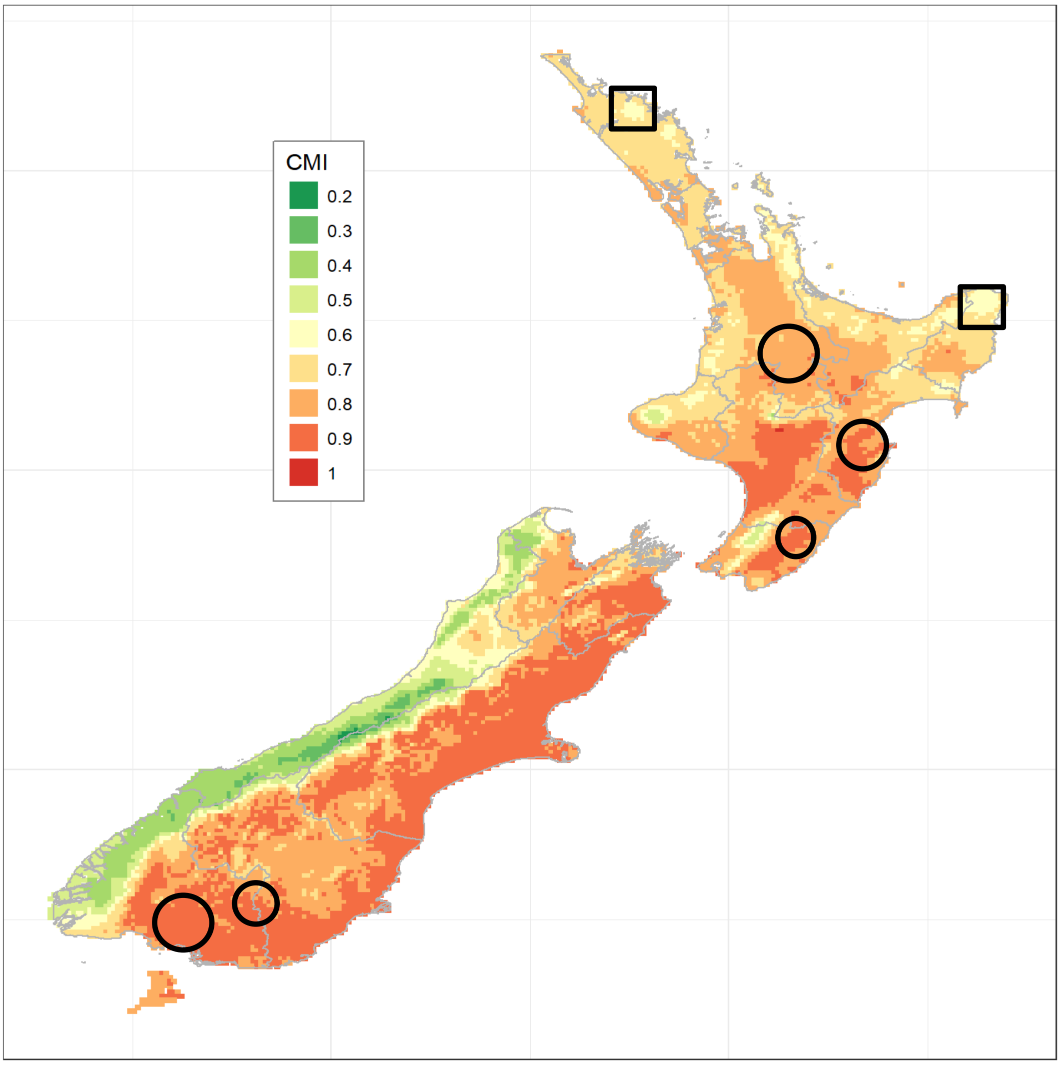

Figure 4. Climate match between *Eadya daenerys* distribution in Tasmania compared to all of New Zealand. The algorithm calculates a 'composite match index' (CMI) for each New Zealand location, which can range from zero (no match) to one (perfect match). The colour gradient indicates CMI values. CMIs >0.7 are generally interpreted as an indication that the two climates are sufficiently similar for a species to persist at that location. Black circles represent the main *Eucalyptus* growing areas. Square boxes highlight minor growing areas that do not meet the 0.7 threshold.

Eadya daenerys phenology

The phenology of *E. daenerys* has been monitored for several years in Australia, predominantly in Tasmania. The phenology pattern is stable across years, with a clear peak in November and December, with shoulder windows of appearance in October and January. In addition, some historical sampling noted in Ridenbaugh et al. (2018) found specimens from November only on the Australian mainland from Australian Capital Territory and New South Wales from 1930, 1958, 1959, 1960, 1961.

Table 1. The phenology of *Eadya daenerys* from nine seasons using multiple sampling methods from Tasmania. Emergence tent (E), Parasitism (P), Bucket trap (B), Wing catch (W), Malaise trap (M). Red text indicates sampling method was used but did not find any *E. daenerys* in that time period.

Year	Sept	Oct	November (early)	November (late)	December (early)	December (late)	Jan	Feb	Mar
2000/01	E	E	E P	E P	E P	E P	E P	E P	_
2001/02	_	E P M	E P B M	E P B M	E P B M	E P B M	E P M	E P[1]	P
2011/12	_	_	P W	P W	P W	P W	_	_	_
2012/13	_	_	_	_	P W	P W	_	_	_
2013/14	_	_	W	W	W	W	_	_	_
2014/15	E	E	E	E	E P W	E	_	_	_
2015/16	_	_	M	W M	W M	W M	M	_	_
2016/17	W	W	M	W M	W	_	_	_	_
2017/18	_	_	_	_	W	_	_	_	_

[1] It is not clear if this was the sampling date like other recorded field parasitism, or date of emergence in the lab.

Discussion

Paropsis charybdis phenology in New Zealand 2016/17

Eucalyptus is grown throughout New Zealand, with the two most significant regions being the central North Island and Southland (see MPI 2017). Susceptible species in all areas are affected by *P. charybdis*. This pest's population varies annually (e.g. McGregor, 1989), however a consistent pattern remains, where the first generation peaks in November-December and the second generation peaks in February-March. This pattern was observed at both Kaingaroa and Poronui for the 2016-17 season in the central North Island. This same trend has previously been seen at other central North Island sites. For instance at Kahu Farms, near Whakamaru, sampled by McGregor (1989) who monitored marked shoots (see Appendix 1) and trapped prepupae as they fell from the trees. Frass trapping by Murphy & Kay (2000), as a proxy for *P. charybdis* population densities at Poronui, was not an accurate method to detect first generation of *P. charybdis* but showed a slightly improved ability to detect the onset of the second generation, probably due to differences in sampling protocol.

Tree health

The population density of *P. charybdis* at Kaingaroa was much lower than at Poronui, perhaps due to the former being a younger plantation that has not yet been extensively invaded by beetles. Damage to juvenile leaves was driven by fungal damage at Kaingaroa, while at Poronui about a third of juvenile leaf damage was attributable to minor insect pests such as *Eriococcus coriaceus* and *Strepsicrates* spp. *Eucalyptus nitens* has previously been shown to be particularly susceptible to leaf spots such as those caused by *Kirramyces eucalypti* (Cooke & Massee), *Mycosphaerella cryptica* (Cooke), and *M. nubilosa* (Cooke) (Hood et al., 2002). After infecting individual leaves, sometimes affecting over 70% of leaf area, the leaves are shed. The adult foliage CDI score remained relatively constant across the season at Kaingaroa with seldom more than 20% of leaf area eaten by *P. charybdis*.

Overall tree CDI scores were much higher (worse) at Poronui. From November through December, there was significant defoliation of adult foliage, and this resulted in the forest manager's decision to spray insecticide as an emergency pest management measure (Rolando et al., 2016). The spraying of the aerial insecticide between mid-December and early January 2017 rapidly reduced the *P. charybdis* population, and was accompanied by an improvement in overall tree CDI score. An improvement in leaf retention was observed, as well as the return of red-coloured new growth to the upper crown of the trees following spraying (E. Peters, pers. observations).

After the spray applications at Poronui, adult and larval populations of *P. charybdis* remained low for the rest of the growing season. Adult *P. charybdis* were reduced by at least 35%, and early and late instar larvae by at least 50%; however egg numbers continued to increase (Fig. 1). This indicates that the spray was effective against the active life stages, but not eggs. Withers et al. (2013) found that the same active ingredient (alpha-cypermethrin) is highly efficacious, causing 100% mortality of *P. charybdis* adults and larvae at the same active ingredient concentration as was sprayed at Poronui. Further, unparasitised eggs of *P. charybdis* are also susceptible to alpha-cypermethrin, while egg batches containing developing *E. nassaui* appear to protect the egg parasitoid to some degree and permit successful emergence (Withers, unpublished data).

The continued appearance of both egg parasitoid species after spray application indicates that the insecticide is either not lethal to the egg parasitoids, or more likely, the *P. charybdis* eggs confer protection to the developing egg parasitoid inside. Loch (2005) found similarly that the egg parasitoid *Anaphes nitens* (Girault) was protected inside the eggs of *Gonipterus scutellatus* Gyllenhal (Col.: Curculionidae) in the field when sprayed with alpha-cypermethrin at the slightly lower concentration of 24 g a.i./ha in southwestern Australia. Tree architecture, which can create spray refugia, and the timing of the spray applications will also influence the outcomes on pest and beneficial insects.

Paropsis charybdis parasitism

Murphy & Kay (2000) monitored *P. charybdis* phenology and egg parasitism in a number of sites in the central North Island, prior to the arrival of *N. insectifurax* and *B. albifunicle*. The phenology still showed the same general trend as presented here of low parasitism by *E. nassaui* in the first generation (grouped as October-December) and high parasitism in the second generation (January-March). The lack of first generation egg parasitism was also found a later by Jones & Withers (2003), after *N. insectifurax* and *B. albifunicle* had established. Mansfield et al. (2011) conducted the most detailed study of *P. charybdis* egg parasitism over three consecutive summers in the central North Island. Observations by Mansfield et al. (2011) were consistent our results showing nil parasitism during the first generation October and November 2016 period (Fig. 2). This stable phenology over multiple studies in the last two decades shows consistently inadequate biological control of the spring generation larvae of *P. charybdis* in New Zealand. It is this gap that we expect *Eadya daenerys* to target.

Our results also support the conclusion of Murray & Mansfield (2015) that the arrival of the egg hyperparasitoid, *B. albifunicle* has to a certain degree limited the effectiveness of *E. nassaui*. However, the other egg parasitoid, *N. insectifurax*, now provides a very useful level of control over the second generation of *P. charybdis* between February and April. *Neopolycystus insectifurax*, which is not susceptible to *B. albifunicle,* provides no control for the first generation of *P. charybdis*, except for the role it plays in reducing the population size of overwintering adults.

Mansfield et al. (2011) and Murray & Mansfield (2015) concluded that there was no strong evidence that the accidental introduction of *N. insectifurax* had improved the biological control of *P. charybdis* beyond the control achieved by *E. nassaui*, as parasitism levels during the first generation did not increase. This situation was also seen at the field sites monitored in the 2016/17 season in this study. While *N. insectifurax* is a less effective biocontrol agent than *E. nassaui* in New Zealand, the fact that it is unable to host the hyperparasitoid supports the possibility that it will compensate for the biocontrol suppression of *E. nassaui* from hyperparasitism (Mansfield et al., 2011; Murray & Mansfield, 2015). It was observed at both Kaingaroa and Poronui that combined parasitism of *N. insectifurax* and *B. albifunicle* in *E. nassaui* -attacked eggs overall resulted in total parasitism of 51% of *P. charybdis* eggs in the second generation. Furthermore the high propensity for eggs to collapse in the second generation is likely to have been as a result of over stinging (super-parasitism) or failure of the egg parasitoids to develop. Duffy (2008) found parasitized egg batches of *Paropsis atomaria* (Olivier) had twice the failure rate of unparasitised egg batches. It appears that *N. insectifurax* is indeed still usefully compensating for the reduced parasitism by *E. nassaui* at both sites later in the season.

The arrival of *B. albifunicle* in New Zealand was predicted to negatively impact the biological control of *P. charybdis* by *E. nassaui*. Murray & Mansfield (2015) projected that *E. nassaui* could become even more scarce in the spring due to high winter mortality and repression by *B. albifunicle*. These factors would then further increase the time needed for *E. nassaui* to reach sufficient levels to control *P. charybdis* populations. Murray & Mansfield (2015) determined that *B. albifunicle* had the potential to reduce the effective parasitism of *E. nassaui* in the field to between 5 and 16%. However this does not seem to have occurred in the central North Island, with *E. nassaui* parasitism first appearing in late November, which is consistent with results of prior studies (Murphy & Kay, 2000). By December, 41% of *P. charybdis* eggs at Poronui were parasitized by *E. nassaui* (Fig. 3). This implies that while parasitism by *E. nassaui* may have been reduced in the spring, population levels have not been as severely suppressed by *B. albifunicle* attack later in the season as Murray & Mansfield (2015) predicted.

Our methods to monitor *P. charybdis* and their biocontrol agents in the field had one potential limitation. *P. charybdis* mainly feed in the top of the crowns of tall trees, as they prefer newly expanding adult leaves, but due to practical reasons we were only able to monitor branches that we could access from the ground with a hooked pole, thereby limiting us to approximately 2.5-3 m from the ground.

Climate matching

One method of assessing whether an organism will establish in a new area is through climate matching. It is generally accepted that a match of >0.7 between the home and new location is a good indicator that the organism is likely to establish (Sutherst et al., 2007). This assumes that other biotic factors are also favourable, e.g., suitable host is present. Climate matching between *E. daenerys* native Tasmanian range (Peixoto et al., 2018) and New Zealand, indicates the majority of New Zealand is a suitable climatic match for its establishment apart from the Southern Alps (Fig. 4). Importantly, this includes the major eucalyptus growing regions in the North and South Islands.

Phillips et al. (2018) tested the utility of the CLIMEX-MCR algorithm for assessing whether New Zealand's climates could be suitable for the establishment of various non-native ants, and found that the algorithm was a useful tool to predict establishment. Magarey et al. (2018) made a preliminary evaluation of its usefulness for predicting weed distributions in the USA and also concluded the algorithm was probably useful in making such predictions. To date, no studies have comprehensively tested the validity of Climex-MCR or any other method for predicting species' potential distributions in New Zealand. Thus our results should be treated cautiously despite previous successful establishment of other biocontrol agents from Tasmania in New Zealand.

Likely phenology match for *Eadya daenerys* to spring larval generation

A successful relationship between a host and its specialist parasite requires a parasitoid to be synchronised with its host, especially early in the season when host population growth is initiated. This starting time sets the progression of life stages throughout the rest of the season. A parasitoid that emerges too early will be unable to find hosts in the right life stage to exploit (Rowbottom et al., 2013). While it is impossible at this time to predict exactly how a newly introduced biocontrol agent will adapt its phenology in a new geographic range, natural enemies that come from areas with similar climates are more likely to succeed in the place to which they are introduced (Hoelmer & Kirk, 2005). Mismatching phenology in weed biological control agents has resulted in some issues of unexpected non-target host use (Harman, 1999; Paynter et al., 2008). Therefore examining expected phenology of a new biological control agent in its proposed new range is an important aspect of choosing the right agent, and ensuring the conditions are optimal for their establishment. Previous successful introductions to New Zealand of biological control agents from Tasmania, *Cleobora mellyi, Cotesia urabae* and a Tasmanian strain of *E. nassaui* (Withers et al., 2011) already suggest that climate will not be a limiting factor in successful establishment of *E. daenerys*. In addition, the longevity of field-caught *E. daenerys*, up to 44 days (mean 16.3 days for females, 12.6 for males) (Smart, 2016), also shows they will be active as adults for the duration of the first *P. charybdis* generational peak (Fig. 1, Table 1). While we must be extremely careful in interpreting these data as a prediction for what will be realised in New Zealand, they provide promising insights into how *E. daenerys* could target the currently-uncontrolled first generation larvae of *P. charybdis*.

Conclusion

The first appearance of early instar larvae of *P. charybdis* in New Zealand closely overlaps that of the appearance of adult *E. daenerys* in climatically similar Tasmania. Damage to eucalypts caused by *P. charybdis* can be serious, and the first generation of larvae currently have no major natural enemies in New Zealand. Climatic matching indicates that most of New Zealand, and importantly the major eucalypt growing areas, is a good match with Tasmania, meaning the proposed biocontrol agent *E. daenerys* is likely to establish. This suggests this proposed new parasitoid will both successfully establish in the major eucalypt growing regions, and be well-timed to make a significant impact upon the larvae of the first *P. charybdis* generation, which currently avoid substantial egg parasitism by *E. nassaui* or *N. insecitfurax*.

Acknowledgements

Elise Peters was supervised by Mari Suontama and Toni Withers (Scion), and funded by the Specialty Wood Products Partnership while undertaking a work placement from Waikato University which formed a portion of her Bachelor in Applied Science. We thank Rebekah Smart for field and lab assistance. We gratefully acknowledge Marika Fritzche (Timberlands Ltd) for arranging access to Kaingaroa forest, and Steve Smith, Westervelt Co, for access to Poronui Station. We are grateful to Andrew Dunningham for advice with CLIMEX, and Craig Phillips for completing the modelling. The CLIMEX modelling portion of this research was funded by Scion and AgResearch as part of the Better Border Biosecurity (B3) research collaboration (www.b3nz.org). We thank Carl Wardhaugh and Stephen Pawson (Scion) for their assistance and constructive feedback. We also thanks Nicolas Meurisse (Scion) for assistance with graph creation. Scion received funding from the NZ MPI Sustainable Farming Fund contracts 12-039 and 407964, and NZ Ministry of Business Innovation and Employment Strategic Science Investment Funding. Industry funding co-partners Southwood Exports Limited and Oji Fibre Solutions NZ Ltd (previously Carter Holt Harvey fibre solutions) are gratefully acknowledged, as are the FOA, and the NZ Farm Forestry Association who have also contributed cash and in-kind assistance to the project. Funding providers had no influence over experimental design or decision to publish results.

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Appendix 1.

From McGregor PG (1989) page 48

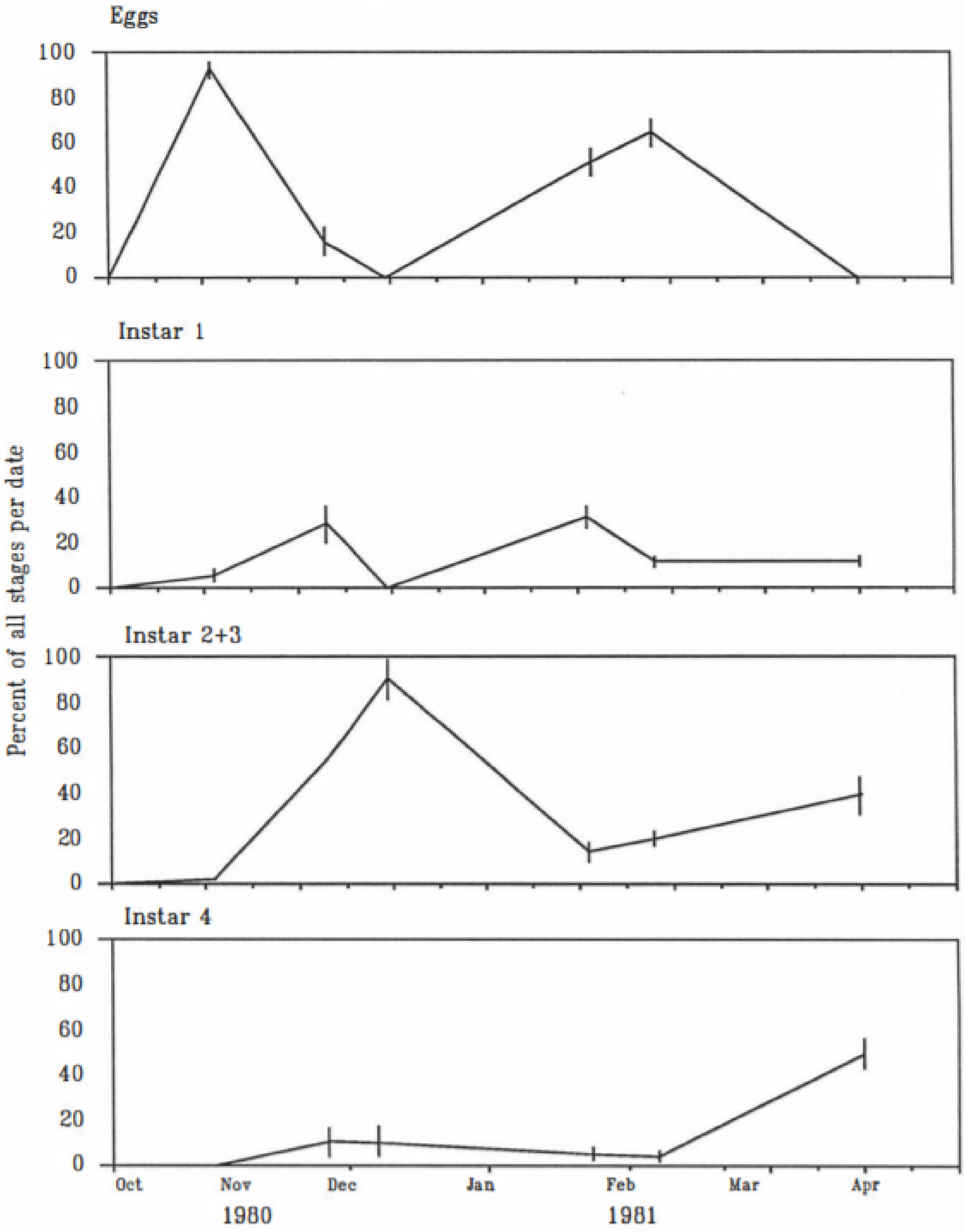

Fig. 4.10 Abundance of *P.charybdis* stages at Kāhu Farms 1980-81, shoot samples. Mean number per shoot expressed as % of all stages, for each date. Vertical bars = 95% binomial confidence limits.