The influence of agent rearing success on weed biocontrol programs in New Zealand

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Rearing problems

Due to recent problems rearing some arthropod weed biocontrol agents in containment we reviewed past rearing to investigate:

1. The extent that rearing failure has influenced the establishment of weed biocontrol agents in NZ
2. Whether factors that influence rearing success can be identified so we can overcome problems
3. Whether we can mitigate problems in other ways (e.g. improving the success rate of small releases of agents that cannot be reared in large numbers)

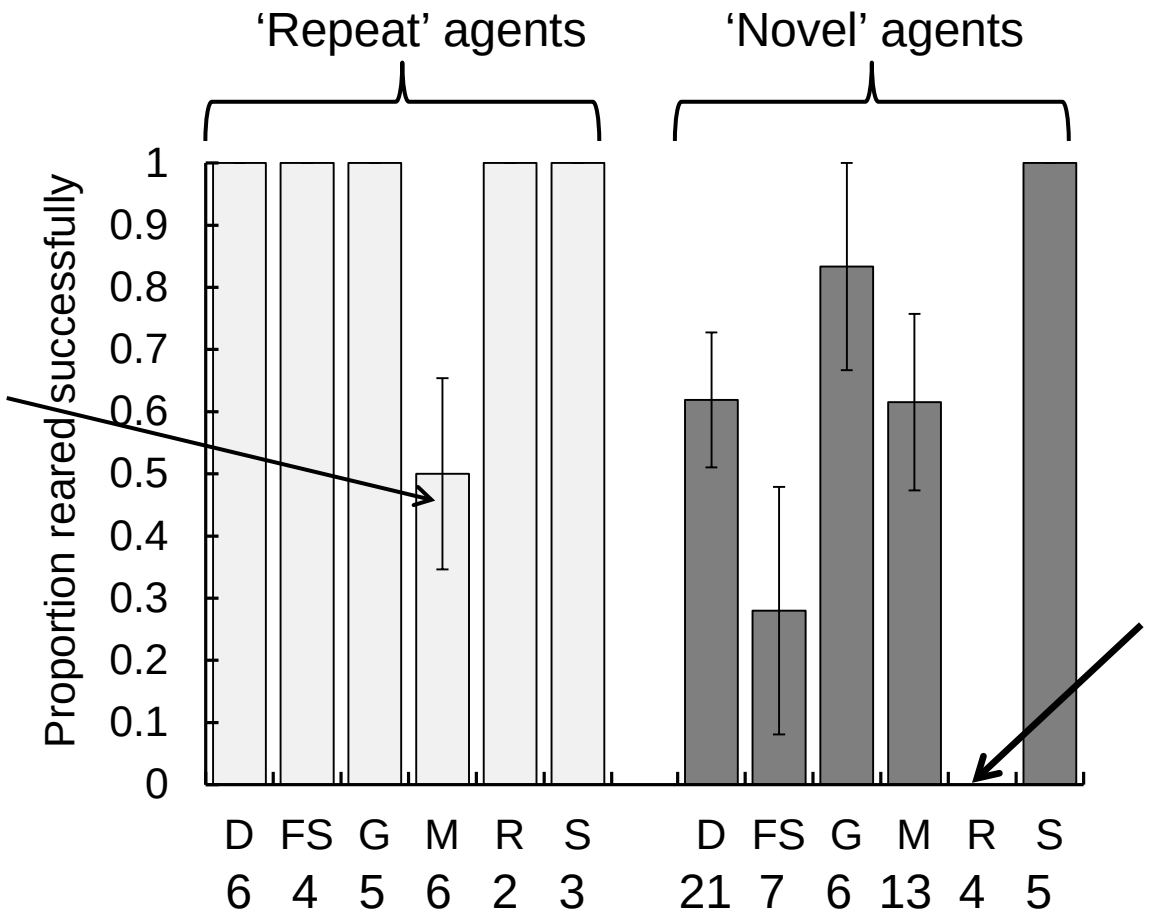
Review of rearing 1925-June 2015

82 arthropod spp. imported into NZ containment, where information on rearing success was available. Of these:

- Failure/low rearing success affected 26/82 (~32%) of candidate agents
- 13 spp. could not be reared at all
- 13 spp. could only be reared in low numbers, limiting No. available for host-range testing or release

Rearing success: larval feeding guild & prior knowledge

Difficulties with some miners, due to plant quality issues



More success with some groups

BUT data potentially misleading: e.g.:

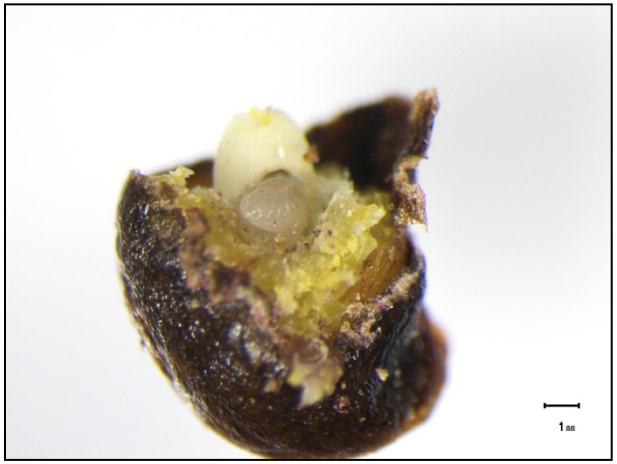
Low ‘ns’ in some guilds

Root/rosette-feeder results biased by 2 *Cheilosia* spp. that did not mate in containment (larval feeding guild irrelevant!)

D = Defoliator; FS = Flower/seed-feeder; G = Gall-former; M = Miner/borer; R = Root/rosette-feeder; S = Sucking/piercing

Causes of total failure

- 6 spp. (4 Lepidoptera; 2 Diptera) would not mate in captivity
- 4 spp. plant quality was a major problem – e.g. barberry plants aborted flowers in containment (disastrous when rearing flower & seed-feeders!)
- 3 spp. had problems with diapause



Causes of low rearing success

Causes often less clear-cut

- Low/sporadic mating success likely:
  - *Allotalanta* spp. (candidate agent for Japanese honeysuckle *Lonicera japonica*)
  - Probably important for OMB sawfly, *Monophadnus spinolae*: haplodiploid sex-determination means unmated ♀ sawflies lay fertile eggs that develop into haploid ♂ but mating essential to produce diploid ♀
    - Mass rearing of *M. spinolae* reportedly difficult due to a 20:1, ♂:♀ sex ratio[1], indicating mating was rare

[1]Gourlay AH et al. 2000. *Proc. X Int. Symp. on Biol. Contr. Weeds*. pp. 709-718.

Causes of low rearing success

- Host plant quality a likely problem for many spp. e.g. *Hadroplontus* (*Ceutorhynchus*) *litura* *"inadequate lighting in quarantine affected plant quality & reduced larval survival*[1]*"*
- Diapause/aestivation a problem with broom shoot moth *Agonopterix assimilella*, which has a period of dormancy over summer, before oviposition begins
- No obvious cause for root-feeders *Colaspis argentinensis* & *Sitona regensteinensis* (hard to see what's going on underground!)

Hadroplontus litura

(Photo: Eric coombs)

Agonopterix assimilella

Colaspis argentinensis

[1]Jessep, CT 1989. A review of biological control of invertebrate pests & weeds in NZ 1874-1987. Technical Communication - CAB IIBC, Wallingford, Oxon, UK, pp. 343-345.

Consequences of low rearing success?

Failure to rear a candidate agent in containment is NOT an insurmountable barrier because:

1. host-range testing can often be done in the native range
   - mated individuals can be collected from the field
   - can culture on plants growing outdoors - easier to maintain plant quality
   - no diapause problems to overcome
2. Can make 'direct' releases – e.g. gorse seed weevil *Exapion ulicis* is hard to rear in large Nos., so ~38,000 imported from England in 1931 & released directly into the field

1984

- Mandatory screening of agents instigated to prevent diseases (e.g. microsporidia) being released into the environment
- Reliably checking 1000s of arthropods difficult:
  - Pre 1984, cumulative totals of ‘direct’ releases averaged *c*. 55,000 individuals per sp.
  - Since 1984, cumulative totals of individuals released in direct releases have not exceeded 377 for any sp., & averaged just ~150

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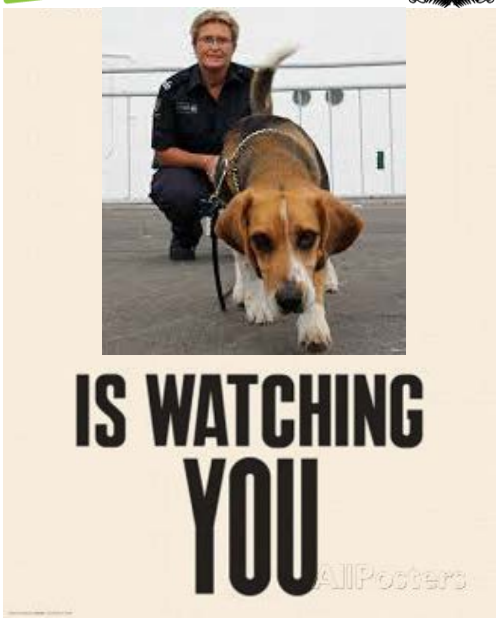
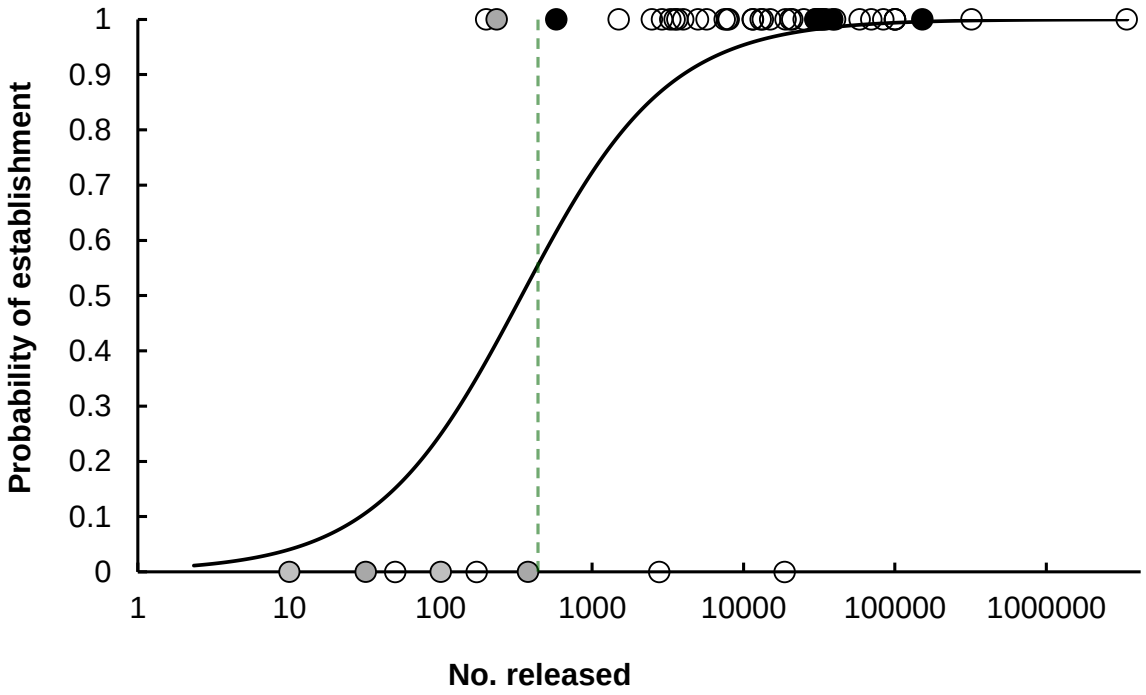


Consequences of small release size: establishment vs cumulative No. released



- Overall 84% success rate
- But only 2/8 spp. established where <500 released nationwide
- 39/41 (95%) established when > 500 released nationwide

○ = agents that were reared in captivity
● = direct releases pre 1984
● = direct releases since 1984
Logistic regression: $\chi^2 = 21.35$, d.f. = 1, $P < 0.001$

Consequences of low rearing success

- Strong correlation between release size & establishment success indicates rearing difficulties contribute to most establishment failures
- Most failures associated with small release size have occurred since mandatory disease-testing in 1984
- Rearing difficulties have likely contributed to the failure to establish 5/34 (15%) agents released 1985–2014
- Estimated cost of developing these ‘fruitless’ agents ~$2.34M
- What can we do to improve things?

1. Avoid: Prioritize candidate agents which are likely to be easiest to rear

Our analysis indicates some guilds are easier to rear BUT:

- Ability to predict rearing success limited
- Inability to rear an agent has not prevented host-range testing from being done & agents can be established by direct-releases
- Some highly successful agents in NZ belong to feeding guilds that have proven difficult to rear (e.g. root/rosette-feeders)
- Prioritizing candidate agents that are easiest to rear could be counter-productive &, we believe, should continue to be a relatively minor factor when selecting candidate agents

2. Fix: Develop improved rearing techniques

Potential improvements identified to tackle main causes of failure:

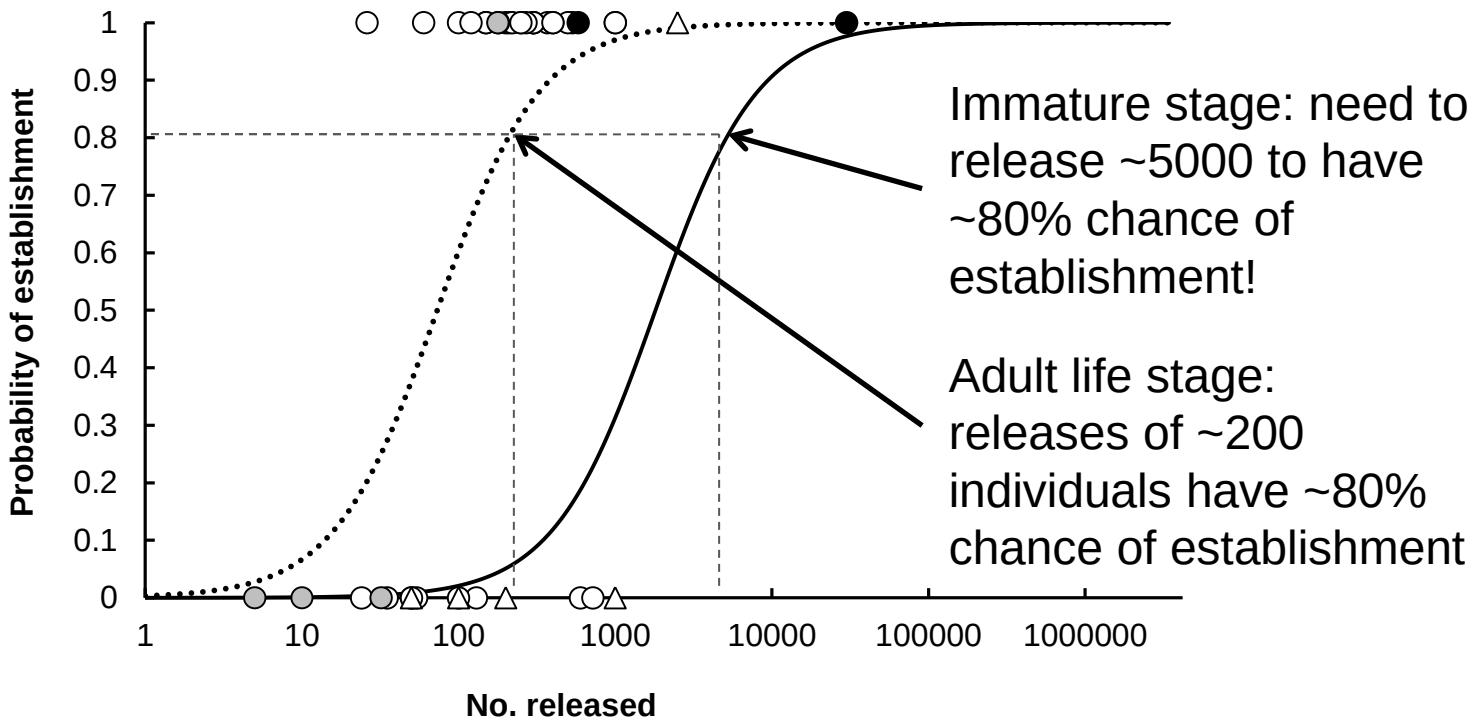
- Enhance probability of mating in containment, e.g.:
  - air movement may help ♂ moths to follow a pheromone plume to a ♀;
  - natural light/dusk may be needed (*c.f.* abrupt lights on/off)
  - hand pairing techniques could be tried
- Improve food/plant quality e.g.:
  - improve internal quarantine procedures to prevent pest outbreaks
  - grow plants in natural light
  - Rear on living plants vs cut stems
  - cross-pollinate plants by hand to avoid abscission of unfertilized flowers
- Resolve diapause problems e.g.:
  - If critical photoperiod unknown, keep in total darkness & cold (~4°C) for ≥ 3 months before exposing them to temperatures & day length that they would naturally be exposed to at the end of dormancy in the native range

3. *Circumvent*: Improve techniques to increase establishment success of agents released in low numbers

There will always be some agents that prove difficult, if not impossible, to rear - so can we improve the success rate of small releases?

- Analysis performed using data from the 1st release of each agent to investigate the probability of establishment of a single release of an agent vs release size (first releases tend to be the best documented & done at the "safest sites")
- This analysis was also able to investigate whether there was any difference between releasing immature stages versus adult stages

Improving success of direct releases



○ = agents that were reared in captivity; △ = releases of immature stages

● = direct releases pre 1984

● = direct releases since 1984

Release size: $\chi^2 = 11.69$, d.f. = 1, $P < 0.001$

Interaction life stage: release size: $\chi^2 = 8.96$, d.f. = 1, $P < 0.01$

Threshold release size?

- 27/43 agents (*c*. 63%) established at the 1st release site
- Release size ranged from 5 – 30,000
- Significant correlation between establishment success & release size:
  - Only 1/9 (11%) releases of ≤ 50 adults established
  - Most (86%) releases of >200 adults established

Just 5 releases of immature stages but limited data indicates MUCH higher release sizes needed for immature stages to establish, so best ro release adults, if possible

Improving success of direct releases: non-destructive disease testing

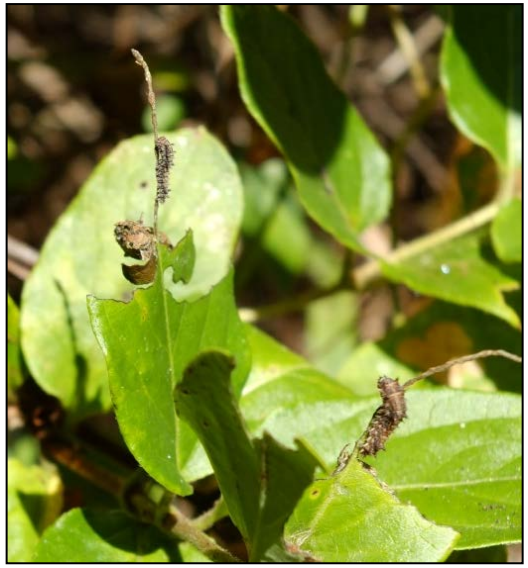
- A subsample of ~10% of an agent population is normally destructively appraised for the presence of pathogenic organisms BUT for small shipments of agents destined for direct release the proportion sacrificed for pathogen testing >> 10%, to improve replication
- For small releases, a minor increase in release size should have a tangible impact on chance of establishment
- If reliable molecular techniques could be developed to test rapidly & non-destructively for key pathogens (e.g. in agent frass) then the size of direct agent releases could be increased by a potentially significant amount

Improving success of direct releases: picking the best release site

- Selecting climatically favorable release sites may improve establishment success e.g. heather *Calluna vulgaris* is most invasive in subalpine scrub in Tongariro NP (challenging climatic conditions)
- Heather beetle *Lochmaea suturalis* establishment rates were low in subalpine parts of TNP, but it established readily at lowland sites, probably due to higher overwintering survival
- Prudent to release agents that cannot be mass-reared at most favorable sites before trying to establish them in localities with harsher climates (even if that is where control is most needed!)

Conclusions

- Inability to rear has become a bigger issue since disease testing became mandatory, constraining size of direct field releases
- Some potentially simple solutions to rearing issues identified, but likely that there will always be some agents that prove hard/impossible to rear (particularly those that refuse to mate in captivity)
- Small releases can succeed! Innovative direct field release methods may improve success rate



Establishing the Honshu white admiral *Limenitis glorifica* in NZ

Wouldn't mate in captivity: collected mated ♀♀ in Japan (Sep 2014) & caged them to obtain eggs (initially in Japan, then in NZ containment)

Offspring from each ♀ reared separately (disease testing) at long day length (to prevent diapause), but low temperature to slow development so most butterflies emerged in November (≈May in Japan, when spring generation emerges there)

Released 178 adults at a "dream site" in Waikato 31 Oct - 6 Dec 2014: established & estimated to have spread over 7km^2 after just 15 months

1st ever successful establishment of an agent that could not be reared in captivity in NZ !

Acknowledgements

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Happy & successful rearing!