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The fickle activity of a fly and a moth: variation in activity of two biocontrol agents of *Chrysanthemoides monilifera*

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The fickle activity of a fly and a moth: variation in activity of two biocontrol agents of *Chrysanthemoides monilifera*

Abstract

Biocontrol agents released to control exotic pests may not have the same spatial distribution as the pest species and may therefore vary in efficacy across the exotic range. These changes in distribution are unlikely to be known until species have had time to fill all preferred niches in the invasive habitat. However, studies of post-release activity of biocontrol agents rarely assess longer-term patterns of establishment in the landscape. *Comostolopsis germana* and *Mesoclanis polana* were released to control *Chrysanthemoides monilifera* spp. *rotundata* (bitou bush) between 29 and 32 years ago. We assessed their activity in foredune and hinddune habitats of coastal beaches across the major distribution of bitou bush and experimentally assessed the effectiveness of *C. germana* at preventing flowering and seed set. Both biocontrol agents were found to be distributed along the 870 km of coastline, representing the core area of infestation. Tip damage by *C. germana* was highly variable but was consistently more effective in the foredune. *Comostolopsis germana* was found to reduce flower production from 15 to 59% with tip damage increasing with latitude. *Mesoclanis polana* did not show differences in activity with latitude and only showed a marginal increase in activity in hinddunes. *Comostolopsis germana* and *M. polana* are reducing the reproductive output of bitou bush but are unlikely to be effective as a sole management strategy particularly in warmer latitudes where more seeds are released.

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3 **The fickle activity of a fly and a moth: Variation in activity of two**4 **biocontrol agents of *Chrysanthemoides monilifera***

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ABSTRACT

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Key words: *Comostolopsis germana*; Bitou Tip Moth; *Mesoclanis polana*; Bitou Seed Fly;

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dunes;

34 **Introduction**

35 Classical biological control uses introduced natural enemies as a means of controlling exotic
 36 species and has had success as a weed control tool in Australia (Briese 2000). Biological
 37 control programs are well established in a number of countries: South Africa has established
 38 75 biocontrol agents for a range of invasive weeds (Moran et al. 2013). By 2000, Australia
 39 had over 60 weeds that had been, or currently were, the targets of a biological control
 40 project (Briese 2004), however, there is uncertainty regarding their long-term efficacy
 41 (Ghosheh 2005). One of the political impediments is that, often, the action of a biological
 42 control agent is slow and may take many years to establish and reach equilibrium within the
 43 host environment which is at odds with the current structure of research funds (Briese
 44 2004; Morin et al. 2009). In 2002, quantitative data on the impact of the biocontrol agent
 45 was available for only 23 weeds at the plant level and 12 weeds at the population level
 46 (Dhileepan 2002), however no study measured the agent's efficacy in slowing weed invasion
 47 or growth nor whether it was equally effective across the whole distribution of the weed.

48 The success of using biological control agents is predicated upon the enemy release
 49 hypothesis and predator-prey theory (Keane and Crawley 2002) where introduced invasive
 50 weed plants experience a release from their enemies, enabling improved growth in their
 51 host habitats. Successful establishment for most biological control agents is investigated for
 52 a few years post-release at release sites (Clewley et al. 2012), however, this may not include
 53 measures of reductions in growth. Mostly, it is a recording of the presence or abundance of
 54 the agent (e.g. Hill et al. 2001, Norambuena et al. 2007, but see Barton et al. 2007). For some

agents which kill plants, evaluation is relatively easy (e.g. salvinia beetle for *Salvinia molesta*, Martin *et al.* 2018; white smut fungus on *Ageratina riparia*, Barton *et al.* 2007), where the reduction in abundance is obvious. However, many biocontrol agents don't kill adults but reduce growth or reproductive output and evaluation is more difficult. Furthermore, as the biocontrol agent establishes and spreads in the landscape, its efficacy may well change. As a result, the distribution, abundance and long-term effectiveness of many biocontrol agents across the distribution of their host weed is unknown. In a meta-analysis only 5 studies assessed effectiveness after 7 years of release (Clewley *et al.* 2012). As these agents usually form part of an integrated approach to weed management, their effectiveness is important to establish across the distribution of the weed, to inform management priorities and actions and how these might need to vary regionally.

Three factors are important in understanding the long-term ability of biocontrol agents to control weed populations. First, there is a need to determine if the abundance of the biocontrol agent is continuous and equally common across the distribution of the weed. Biocontrol agents are released at a number of sites, with the hope that they will spread through the landscape and establish across the distribution of the weed. The success of this is rarely measured. Given a change in the climatic envelope of many weeds in their invasive range relative to their native habitat (Broennimann *et al.* 2007; Gallagher *et al.* 2010), biocontrol agents sourced from native habitats may not establish across the distribution of the weed in the invaded range, particularly if areas differ in climate from the native range. A mismatch in the distribution of invasive species and their biocontrol agents may lead to

poorer control using biocontrol agents, particularly at range edges and within new climatic regions of occupancy.

Secondly, biocontrol agents may show microclimatic preferences even within the distribution of the weed such that the activity of the biocontrol agent is variable across an environmental gradient (e.g. Ireson et al. 2003; Norambuena et al. 2007). And finally, in evaluating the efficacy of any biocontrol agent we need to understand the magnitude of impact in reducing growth or reproduction and how this varies across the distribution of the weed. This enables clear measures of effectiveness and guides future management

Our study investigated the distribution of two biocontrol agents which were released over 20 years ago to control *Chrysanthemoides monilifera* spp. *rotundata* (bitou bush): *Comostolopsis germana* and *Mesoclanis polana*. Bitou bush is a perennial woody shrub from South Africa. In 2002, bitou bush was estimated to occupy over 80% of the New South Wales (NSW) coastline and was the dominant plant over 400 km, particularly in the northern coastal regions (Thomas and Leys 2002). Its impact on invaded range ecosystems and species richness has been well established (Lindsay and French 2004; Ens et al. 2009; French et al. 2010; Mason et al. 2012).

To reduce the abundance of seeds being stored in the soil, six biocontrol agents have been released (Downey et al. 2007). Only two appear to be widely established. *Comostolopsis germana*, a tip feeding moth (Geometridae), was released from 1989 and *Mesoclanis polana*, a seed eating fly (Tephritidae), was released from 1996 (Edwards et al. 1999). *C. germana* feed in the apices of bitou bush and several generations can develop each season (Julien et al. 2012). Between 1990 and 1997 *C. germana* was released in 72

locations, however the exact extent of the distribution of *C. germana* has not been established (Downey et al. 2007). Holtkamp (2002) undertook a small scale study at Botany Bay using a chemical exclusion technique to assess the efficacy of *C. germana* and showed that seed production was reduced by more than 50% and sometimes by more than 80%.

Mesoclanis polana, the seed fly, feed in the capitula of bitou bush flowers and deposit eggs into flowers where they enter the ovary (Julien et al. 2012). *M. polana* was released in 1996 and over two years spread rapidly over 1200 km along the eastern coastline (Edwards et al. 1999). Between May 2001 and April 2002, the average attack rate on seeds was 23% from surveys at 5 sites (Stuart et al. 2002). Edwards et al. (2009) provided evidence that seed destruction by *M. polana* had steadily increased up to 2004 since its release in northern NSW to an average of 58% of seeds attacked.

Edwards et al. (2009) surveyed eight sites from 1996 to 2004 and found that the levels of seed damage increased over the eight-year study period where they detected lower attack rates at more southern sites possibly due to the cooler climate not providing optimal conditions for *M. polana*. In its native country, South Africa, *M. polana* is found north of 31°S and was considered well suited for northern New South Wales which shares the same latitude. However, in Australia, *M. polana* has extended further south to approximately 37°S and seed destruction rates ranged from 86% to 2% along the east coast (Edwards et al. 2009). In the early years of establishment of this agent, there was likely to be variation in the effectiveness of this biocontrol agent over the distribution of the weed. As biocontrol agents establish, such early preferences may be overcome through local adaptation, and agents may become effective over wider climatic areas.

We used surveys across the main distribution of bitou bush as well as an experimental manipulation at one site to investigate the efficacy of these two agents in acting on bitou bush. We use this information to develop a model of the differential impacts of the biocontrol agents across the main distribution of bitou bush.

Methods

Distribution of *C. germana* and *M. polana*.

Measurements of the presence of *C. germana* in bitou bush growing tips were undertaken at 14 dune sites at the back of beaches along a 900km stretch of the NSW coast within the existing Bitou Bush containment line; north of Sussex Inlet (35.1500° S, 150.5667 ° E) up to the Byron Local Government Area (28.6431°S, 153.6150°E) from 2013-2015 (Table S1). To the north and the south of the containment line, eradication of populations is sought through a range of mechanical and chemical control programs (Winkler et al. 2008)).

Beaches were sampled if they had bitou bush present in foredunes and hinddunes and only had *C. germana* in growing tips. Beaches found to also have *Tortrix* sp. (Leaf Rolling Moth), a recently established agent, were not sampled. Foredune samples, were taken from primary vegetation zones that were colonised by shrubs and ground plants. Hinddune samples were taken from the tertiary vegetation zone, which were colonised by shrubs, trees and ground plants.

At each beach, four branches with a stem diameter of 10-15 mm, were taken from each of four randomly-selected bushes from both the fore-dune and the hind-dune. The

bushes were a minimum of 5 m apart. All tips from the four branches were counted and classified according to their level of damage. We used three subjective categories: None where there was no damage to the tip (which was considered viable), Light where the tip showed low level of damage but was considered to be still viable and growing, Severe where damage was more obvious and the likelihood of continued growth was low (considered inviable). The proportion of damaged tips in each category was calculated for each replicate plant. Only one author (KB) classified tips to ensure comparisons amongst these subjective classifications were consistent.

To sample activity of *M. polana*, samples of the soil seed bank were collected from similar sites during 2017-2018. Following a pilot study to determine appropriate sampling intensity, six soil samples were taken from both the fore-dune and hind-dune at all 14 beaches. A quadrat of 250 mm x 250 mm x 60 mm deep was placed into the sand so that the quadrat top was level with the sand. The sand within the quadrat was collected and later sieved to retrieve the seeds. The seeds were counted and examined to locate any exit holes of the larvae of the tip fly.

Efficacy of *C. germana* in reducing flowering and growing

An exclusion experiment was conducted at Windang Beach (34.51°S, 150.86°E) in both the foredune and hinddune habitats. Thirty-two sample points were identified, 16 in the hinddune and 16 in the foredune. Half of the samples at each habitat were sprayed with Yates Success Ultra pesticide at concentrations of 5 ml per 100 mls (active ingredient; spinetoram) at approximately 2 weekly intervals over a period of 3 months prior to

flowering. Spraying commenced in December 2014, and plants were sampled in March 2015 during the main flowering period. A 1m² quadrat was placed over the sample area and a photograph was taken. The flowers within the quadrat were counted using this photograph allowing a more accurate count as flowers could be marked off as they were counted avoiding duplications.

Analysis

Differences in probability of tips having damage (light and severe categories) and severe tip damage by *C. germana* between habitats (foredune and hinddune) and latitude were compared using a binomial logistic model (JMP Pro 11). We calculated the average proportion of tips damaged per plant for each site and used an ANCOVA to determine the effect of habitat and latitude. One point was an outlier (91% damage in a mid-latitude site) and caused the data to be non-normal. We excluded this from the analysis. Variation in seed abundance and in the proportion of seeds damaged by *M. polana* with latitude and habitat were compared using ANCOVA. Where necessary, Tukeys HSD tests were undertaken to determine where significant differences lay. For the exclusion experiment, differences in floral output between treatments and habitats were analysed with 2 factor ANOVA (JMP Pro 11). Tukeys HSD tests were used to identify where differences lay.

Results

Tip Damage

The effect of latitude on the probability of a plant having a tip damaged by *C. germana* varied with habitat (interaction term: $\chi^2_1 = 6.287$, $p = 0.012$). We undertook single logistic regressions for each habitat and found that the probability of a plant having a tip damaged by *C. germana* did not vary with latitude in the hinddune ($\chi^2_1 = 0.827$, $p = 0.363$), but increased with increasing latitude in the foredune ($\chi^2_1 = 6.183$, $p = 0.013$). The probability of a plant having a tip that is severely damaged only varied with habitat ($\chi^2_1 = 11.189$, $p < 0.001$) with foredunes having a greater probability than hinddunes.

The effect of latitude on the average % of tips damaged per quadrat at sites differed between foredunes and hinddunes (interaction term: $F_{1,23} = 7.34$, $p = 0.013$; Fig. 1). In foredunes the average % of damage increased with increasing latitude ($F_{1,11} = 18.17$, $p = 0.001$) but did not change in the hinddune ($F_{1,12} = 0.82$, $p = 0.38$). For every degree of latitude south, the average % of damage increased by 5%. The average % of tips that were severely damaged per quadrat at sites was higher in foredunes than hinddunes ($F_{1,23} = 14.03$, $p = 0.001$, Fig. 2) but did not vary with latitude ($F_{1,23} = 4.03$, $p = 0.057$) although there was a tendency to increase with latitude.

Latitude influenced seed densities in the soil, with decreasing numbers of seeds with increasing latitude ($F_{1,164} = 9.94$, $p = 0.0019$) but there was high variation ($R^2 = 0.11$, Fig. 3). Equal densities of seeds were recorded in hinddunes and foredunes (av. = 37.85 ± 36.15 seeds per sample, $F_{1,164} = 0.01$, $p = 0.911$). Seed densities per m^2 averaged $605 \text{ seeds} \pm 651(\text{sd}) m^{-2}$; the high standard deviation indicating high patchiness in seed numbers in the soil amongst quadrats at sites. Latitude did not influence seed predation by *M. polana* ($F_{1,164}$

= 0.26, $p = 0.611$), however, hinddunes had a greater proportion ($22.0 \pm 22.4\%$) of seeds with exit holes than foredunes ($14.01 \pm 17.7\%$) ($F_{1,164} = 5.25$, $p = 0.023$, Fig. 4).

C. germana reduced flower production by 30%. The effect of spraying on flower production varied with dune position ($F_1, 28 = 10.59$, $p = 0.003$). In the foredune, the treated plants had more flowers (average = 49.13 ± 35.32) than the control plants (average = 19.63 ± 14.41) while in the hinddune there was no consistent pattern in flowering (overall average = 30.81 ± 14.78 ; Fig. 5).

Discussion

Both *C. germana* and *M. polana* were distributed along the entire sampled coastline, representing the core area of bitou infestation, however their activity and effectiveness was not evenly distributed across this range. For the tip moth, *C. germana*, activity increased with latitude and the moth was most active and effective along the foredune. The seed fly, *M. polana*, on the other hand, was slightly more effective in the hinddune but had attacked a similar proportion of seeds along the coast. The result of the activity of these two biocontrol agents is a reduction in seed added to the soil each year but there are differences in the quantity of seeds likely to be viable in the soil in different habitats (Fig. 6). The greatest reduction in seeds arriving into the soil was in the southern areas of the distribution within foredune habitats where the activity of the *C. germana* was greatest, reducing flowering and resulting in a significant decline in flowers available to the *M. polana*. In this habitat the released biocontrol agents halve the potential available seeds

that would arrive into the soil. This model includes all activity levels of *C. germana* from light to severe even though we considered that some of the lightly attacked tips may still have the capacity to grow. We included all because we assumed that the presence of *C. germana* would eventually result in the tips experiencing severe damage, although at the time of sampling the percentage of tips with severe damage was less than 0.2%. Should tips survive the presence of *C. germana*, then the number of potential flowers, and therefore seeds, will be higher.

The high activity of *C. germana* on the foredune was unexpected as this habitat is much more exposed and drier. We consider three possible explanations for this pattern. There may have been high growth in hinddune plants associated with shadier, moister habitat which may have compensated for moth activity resulting in a decline in percentage tip damage, however, without growth data, we cannot evaluate this possibility. The second possibility is that the hinddunes house a greater abundance of insectivorous species, such as ants or birds and *C. germana* is subjected to higher predation levels. The third, and perhaps least likely is that the highly exposed habitats of the foredune are preferred habitat for this moth. This is unlikely given the less exposed habitats from which the moth was collected in their native range (Adair and Scott 1989).

Similar mechanisms may well explain the reduced effectiveness of *C. germana* in the northern populations where warmer, subtropical conditions may have stimulated high growth of bitou bush. For every decrease in 1 degree of latitude there was a 5% reduction in the proportion of tips colonised. While this may be because the climatic envelope for this

biocontrol agent is for cooler climates, this does not equate to what is known of the native range and was not evident in the early stages of release.

While *M. polana* is distributed along the coast, overall it is less effective at reducing the potential of the weed. It was only present in about 20% of seeds in the soil. Like *C. germana*, its activity was highly variable but did not show the same latitudinal increase. In fact in the early years following release of this agent, Edwards et al. (2009) identified a decline in activity with increasing latitude. This suggested that the southern areas were well outside the natural climatic envelope of this species in South Africa. After 20 years of presence in Australia, such latitudinal patterns are no longer evident, suggesting this species has acclimated and extended its climatic envelope and its introduced range.

The early high levels of activity recorded for *M. polana* were not evident in our study, although there is still a high variation from site to site. Our study showed reduced viable seed abundances of between 14-22% with slightly improved reductions in the hinddune. Higher values were recorded by Edwards et al. (2009) who found seed damage ranged from 2% to 58%. The lowering of activity may well be, at least partially, due to the method of estimating fly activity. While Edwards et al. (2009) assessed seeds attacked while attached to the plant (predispersal), our estimates are post-dispersal and are therefore an accumulation of all years, assessing the whole seed bank. Empty seed husks may disintegrate faster than whole seeds and may not be included, suggesting that we may have under-estimated seed fly activity. However the alternative, is that activity has declined in response to a reduction in the availability of flowers, associated with the activity of the tip moth. There may well be a negative interaction between the effectiveness of the tip moth and the seed fly.

While our model predicts a difference in seed abundances in the soil affected by biocontrol agent activity, our study found no difference between soil seed bank numbers in fore and hinddunes. A number of explanations may account for these differences. Firstly, there may be an increase in the numbers of seeds produced in hinddunes associated with the milder and more protected conditions in the hind resulting in an increase in flowers, improved pollination or more successful seed set, irrespective of the biocontrol agents' activity. There is very little information to evaluate this point. Our manipulation experiment, however, did not suggest this as sprayed plants (plants without agent activity) did not flower more in hinddunes. Secondly, post dispersal seed losses may be higher in hinddunes than foredunes resulting in losses that eliminate the differences in biocontrol activity. Seed viability of seeds in the soil declines to a low level after about 5 years (K. French unpublished data) perhaps associated with decomposition processes. These protected areas are likely to have higher soil water and lower temperatures at soil level (Lindsay and French 2004) which are likely to be important in promoting fungal activity that attacks seeds. Further work is needed to elucidate seedbank longevity and survival.

There has been a clear reduction in the soil seed bank of bitou bush over the past 30 years. We recorded on average 600 seeds m^{-2} in the soil seed bank which is an order of magnitude lower than 2-3000 seeds m^{-2} recorded on the far south coast of NSW (Weiss 1984), suggesting that the activities of the biocontrol agents has caused an accumulated seedbank to be around 20-30% of original pre-biocontrol release numbers. Mason et al. (2007) recorded very low densities at 110 seeds m^{-2} suggesting declines to 5-10% of original abundances. Such reductions in propagule pressure in invaded habitats may not be enough

for long-term control; Stuart et al. (2002) suggested that the 20-30% declines in seed production for *M. polana* were not enough to reduce spread of bitou bush.

This study has identified that for good control of bitou bush in coastal dune communities, integrated management is essential. Both biocontrol agents add a level of control, despite the potential for the moth to negatively influence the fly. In those locations where *C. germana* is less effective it is essential that other control methods such as aerial spraying and hand weeding are routinely adopted. Additionally, these results suggest that both agents may be an effective way of reducing the impact of bitou bush in small localised populations such as steep coastal cliffs that are very exposed and difficult to access.

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Fig. 1. The relationship between latitude and the percentage of tips damaged by *Comostolopsis germana* for bitou bush plants on foredunes (squares) and hinddunes (triangles). One site in the foredune has been removed as it had exceptionally high % tip damage (91%) while occurring at mid latitude.

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399 Fig. 2. Average ($\pm$ sd) percentage of tips of bitou bush plants severely damaged by
400 *Comostolopsis germana*.

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Fig. 3. The relationship between the number of seeds of bitou bush per soil sampled from beaches and latitude.

Fig. 4. The average ($\pm$ sd) proportion of bitou bush seeds attacked by *M. polana* in foredune and hinddunes habitats along the east coast of Australia.

Fig. 5. Average number of flowers ($\pm$ sd) in 1m² quadrats in March in control (unsprayed) and sprayed areas treated for 3 months treated with the insecticide, spinetoram.

Fig. 6. A model of the effect of two biocontrol agents on bitou bush seed availability in foredune and hinddune habitats along the coast of eastern Australia.

453 **Supplementary Information**

454 Table S1. Locations of sampling for biocontrol agents for bitou bush.

Location	Latitude (S)	Longitude (E)
South Ballina	28.876	153.585
Broken Head	28.695	153.613
Evans Head	29.103	153.432
South West Rocks North	30.876	153.030
Hat Head	30.927	153.080
Killick Beach	31.080	153.046
Fingal Beach (Nelson Bay)	32.746	152.170
Anna Bay (Nelson Bay)	32.780	152.115
Red Head Beach	33.014	151.720
Tuggerah Beach	33.321	151.517
Port Phillips	33.977	151.225
Cronulla	34.032	151.177
Puckeys Estate	34.399	150.904
Windang Beach	34.517	150.880

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