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Impacts of a biocontrol agent on invasive *Ageratina adenophora* in Southwest China: Friend or foe?

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Abstract

Classical biological control of invasive plants depends on the introduction of host-specific natural enemies. Many natural enemies have provided successful control, but the impacts of herbivorous insects on their hosts are highly variable and context dependent. Under some circumstances, overcompensation can even occur, with plant reproduction temporarily increasing rather than decreasing in response to herbivory. The stem-galling fly *Procecidochares utilis* has been widely introduced to help control *Ageratina adenophora*, a globally significant weed, but its impact has been inconsistent and in places ineffective. Its galling is known to stimulate production of side branches in plants under laboratory conditions. We examined the abundance and impact of *P. utilis* at four *A. adenophora* sites of Southwest China that were invaded at different times and support different densities of the insect. Stems with galls were more likely to be branched and produced more capitula. Furthermore, seed numbers in capitula from galled and un-galled stems were similar, and galling resulted in only slightly reduced seed weights. However, the increase in above-ground vegetative biomass associated with branching resulted in less efficient capitulum production relative to vegetative growth. Clearly *Procecidochares utilis* is not providing effective biocontrol of *Ageratina adenophora* in Southwest China so far. In the long term, galled plants may display reduced competitive ability and lower lifetime reproductive success, but the short term increases in seed production it generates suggest it may be favouring its host's range expansion. The fly has been a successful biocontrol agent in dry area of Hawii, so understanding the factors that determine its effectiveness represent a major challenge for the future.

Keywords: apical dominance; galls; invasive Asteraceae; over-compensation; *Procecidochares utilis*; Tephritidae.

1. Introduction

Invasive species represent an increasing threat to natural and managed ecosystems (Fournier et al., 2019; Pearson et al., 2018; Pyšek et al., 2012; Simberloff et al., 2013). Once they become abundant, transformative non-native plant species can reduce biodiversity (Bellard et al., 2016; Butchart et al., 2010; Rands et al., 2010; Wilcove et al., 1998) and have a serious impact on ecological processes and ecosystem services (Pejchar and Mooney, 2009; Vilà et al., 2011). They can also generate significant socio-economic and management costs (Bradshaw et al., 2016). Classical biological control can produce long term, effective and ecologically safe results (Messing and Wright, 2006; Shea and Possingham, 2000), but the results are less predictable than with physical or chemical methods (Van Wilgen et al., 2013).

The consequences of herbivory for individual plants and for the demographics of plant populations are highly variable and context dependent. They vary, for example, with the extent of competition with other plants, the densities of herbivores and the timing and duration of their feeding (Crawley, 1983; Reese et al., 2016). Plants can often survive even intense herbivory, especially if the duration of the damage is brief (Myers and Sarfraz, 2017). Plant responses to herbivory have been categorized in terms of their capacity for resistance, tolerance or phenological escape (Agrawal, 2000; Gong and Zhang, 2014; Rosenthal and Kotanen, 1994). Their ability for compensatory growth can mean that herbivory has no detectable impact on subsequent reproductive success, at least in the short term (Garcia and Eubanks, 2019; Rosenthal and Kotanen, 1994). One unusual compensatory response is overcompensation, where vegetative growth and/or sexual reproduction increases temporarily in response to herbivory (Agrawal, 2000; McNaughton, 1979). Early evidence for overcompensation was based on plants under controlled conditions (Belsky, 1986), where high resource availability and weak competition made overcompensation easier to detect (Garcia and Eubanks, 2019). However, recent studies have demonstrated that overcompensation can also occur in natural systems (Ramula et al., 2019).

Insect-induced overcompensation has obvious consequences for the chances of success of biocontrol programmes that target invasive plants (Garcia and Eubanks, 2019). For example, two agents (*Neogalerucella calmariensis* (L.) and *N. pusilla* (Duft), Coleoptera) that were released to control invasive *Lythrum salicaria* L. (Lythraceae) in North America generated increased numbers of flowers on the plants they ate (Blossey and Schat, 1997; Russell-Mercier and Sargent, 2015; Thomsen and Sargent, 2017). Overcompensation in terms of flower production among invasive plants is likely to be achieved at the cost of reduced competitive ability and vegetative growth, and have context-dependent consequences (Agrawal, 2000; Garcia and Eubanks, 2019). Any increases in seed production, however temporary, would be especially significant during earlier phases of invasion, when the plants are still extending their ranges and seed production is particularly significant for recruitment (Ramula et al., 2019).

Crofton weed, *Ageratina adenophora* R. King & H. Robinson (Asteraceae) (previously known as *Eupatorium adenophorum* Sprengel) is among the world's most serious weeds (Heystek et al., 2011; Wan et al., 2010). Its native distribution covers Mexico and Costa Rica in Central America, from where it has been spread to over 30 tropical and subtropical countries. In non-native areas it is an ecological hazard and can also cause serious economic losses to forestry and agriculture (Heystek et al., 2011; Wan et al., 2010). The plant has been introduced to Asia, where in the 1940s it expanded into Yunnan Province, China from Burma (Myanmar). It is now distributed throughout Southwest China, including Yunnan, Sichuan, Guizhou, Guangxi and Xizang Provinces and the Chongqing region (Sun et al., 2004; Wan et al., 2010).

Ageratina adenophora is a perennial herb or subshrub with a life span of 12 to15 years (Sun et al., 2004; Sun et al., 2005). The plant can reproduce through seeds and also spreads vegetatively via rhizomes. In Southwest China, numerous terminal capitula with white flowers are produced from the end of February, followed by seed set in April and May. One individual can produce over 10,000 small seeds (Heystek et al., 2011; Sang et al., 2010). They are mainly dispersed by the wind but can also be transported by water (Heystek et al., 2011; Wan et al., 2010). Most seeds germinate in the rainy season of the same year in which they are produced (Dang et al., 2008). The ant *Dorylus orientalis* Westwood (Hymenoptera: Formicidae) has been recorded to damage *A. adenophora* (along with a variety of crop species) in Southwest China, but the plant does not routinely support any native phytophagous insects (Niu et al., 2010). A fungal pathogen, *Alternaria alternata* (Fr.) Keissler was isolated from *A. adenophora* in Southwest China which induced damage to the plant (Chen et al., 2005; Qiang et al., 2006; Wan et al., 2001), however no effective control was generated at the time (Wan et al., 2001). The leaf-spot fungal pathogen, *Passalora* *ageratinae* Crous and A.R. Wood, an agent used for the control of the weed in South Africa (Buccellato et al., 2012, 2019), has not been recorded from China.

Procecidochares utilis Stone (Diptera, Tephritidae) is a host-specific fly that induces galls on both main stems and side branches of *A. adenophora.* Its galls act as resource sinks that can reduce the vegetation growth and reproductive vigour of galled plants (Bess and Haramoto, 1972; Erasmus et al., 1992; Van Staden and Bennett, 1991a, 1991b; Wang et al., 2006). It was introduced from its native Mexico to Hawaii as a biocontrol agent and has subsequently been released in at least seven further countries, where it has become widely established (Bess and Haramoto, 1972; Heystek et al., 2011). Within this large area, the effectiveness of control by *P. utilis* has been highly variable (Heystek et al., 2011; Sharma Poudel et al., 2020). This has been attributed to the fly's inability to reach high densities in some areas, in part because it can suffer from high rates of attack from parasitoids (Heystek et al., 2011; Wan et al., 2010; Zhang et al., 2008). In Hawaii, *A. adenophora* populations in drier locations were reduced or even eliminated by the fly, but it had little effect in areas where precipitation was high (Bess and Haramoto, 1972). These differences in impact reflect the plant's vegetative growth being inhibited in some places (Buccellato et al., 2012; Erasmus et al., 1992) but not affected elsewhere (Wan et al., 2010). Similarly, galling by the fly can reduce seed numbers per capitulum (Erasmus et al., 1992) or have no effect (Wan et al., 2010). Seed vigour and germination rates may or may not be depressed (Erasmus et al., 1992; Wan et al., 2010).

The ability of *P. utilis* to generate changes in its host plant's architecture may contribute to the variability of its impact. Galling by the fly induces the production of side branches, which can support additional capitula. This means that even if galling initially has a negative impact on reproduction, compensatory growth of side branches can result in increases in capitula production, though their size and quality can be lower than that on gall-free plants (Buccellato et al., 2012; Erasmus et al., 1992). The relative significance of vegetative growth and reproduction by seed varies between sites. The impact of *P. utilis* on *A. adenophora* individuals can therefore potentially range from negative, through neutral to even positive, depending on the densities of the insect, the ecological context in which the plant and insect are interacting and the timing of assessment of reproductive performance relative to when the insects gall the plants.

Here, we examine the relationship between *A. adenophora* and *P. utilis* across an invasion front in Southwest China to ascertain the insect's likely contribution towards slowing the expansion of the plant. The specific questions we address are: (1) How variable are *P. utilis* gall densities at four sites across its host's invasion front? And (2) is the plant's reproductive output influenced by the insect?

2. Materials and Methods:

2.1 Locations and sampling

Ageratina adenophora is spreading northwards and eastwards by about 20 km per year in China (Wan et al. 2010), with roads acting as important conduits for the spread of the plant (Lu and Ma, 2006). Chinese National Road 108 runs south to north across Sichuan Province, and the range of *Ageratina adenophora* is expanding from the south along it. Four roadside sites were sampled, from Pingdi (PD) in the South to Hangyuan (HY) about 500 km further north (Table 1). *A. adenophora* populations are not continuous along the roadside, but the plant can reach high densities in areas where they are present. The southern site at PD has had the plant established since at least the 1980s (Yang et al., 1996) whereas the plant was first recorded at HY in 2002 (Luo et al., 2006. Our 2018 visual surveys from vehicles suggest that HY lies at the current northern range extent of *A. adenophora* in Sichuan Province.

Individual plants of *A. adenophora* were hard to distinguish within dense populations, because the rhizomatous spread of the plant allows individuals to occupy areas up to more than one metre across, with several flowering stems. An area-based sampling method was therefore employed. 50 plots of area 0.0625 m^2 (25cm x 25cm) were sampled in a linear fashion within each of the four sites in April 2018. The plots were spread along the roadside within each site, with the plots separated by at least 50 metres from each other. All *A. adenophora* plants in each plot were uprooted using a garden fork and taken back to the laboratory for later examination. Main stems were recognized on the basis of ascending from the ground, from each of which there were often side branches.

The capitula within each plot were removed quickly to prevent the seeds from escaping. The length of main stems and their associated side branches were measured to the nearest 0.1cm with a ruler. Side branch lengths were summed for each main stem. The numbers of *Procecidochares utilis* galls were recorded for each main stem. Measurements of gall size were not made, but there was no apparent geographical variation in this character. The branches (without capitula) with their leaves and galls were dried for 48h at 65°C to constant weight. Above-ground dry biomass (i.e. excluding roots) was then weighed using an electronic balance (Deching Baijie Electric Co., Ltd., China).

Comparisons of seed numbers per capitulum were made using seeds from capitula located on branches with or without galls, sampled from fourteen plots (1m x 1m) at sites HY and PD. These sites represented the most recent and longest-established populations respectively. To avoid any plant architecture effects, only capitula on larger side branches arising from the lower half of the main stems were sampled. The collections were made in April 2017, when the seeds were just reaching maturity, to prevent any chance of seed shedding. The presence of galls on each branch was recorded, and two entire capitula were selected at random from each galled and un-galled branch to count the seeds they contained.

Seed quality comparisons were based on samples taken in May 2019, by which time the capitula contained mature seeds. Twelve to fifteen 1m^2 plots were sampled at each of the four sites. Two branches, one with galls and another without, were sampled for each plot. The seeds remaining in the capitula were removed from the sampled branches and stored in silica gel to remove moisture before being weighed in groups with an electronic balance (SHIMADZU, with measurements to 0.1mg).

2.2 Data analysis

Descriptive statistics included the proportion of plots with galls of *P. utilis* and the densities of galls per stem, the numbers of stems, side branches and capitula present, the numbers of seed per capitulum, the dry weights of the seeds and the branches.

Between-site differences of gall densities, branch numbers, capitulum numbers, gall numbers and vegetative growth, flower production among sampled sites were tested using Generalized Linear Models (GLMs) with a quasi-Poisson distribution in R 3.4.4 (R Development Core Team, 2013). Spearman correlations were also carried out in R. The differences in the likelihood of stem branching between sampled sites were assessed by Chi square tests of the frequencies of branched main stems in R.

To take into account our split-plot design, the relationship between type (galled vs non-galled side branches) and seed mass was initially tested in R using a linear mixed model, with gall presence and site as fixed effects and plot as a random effect.

However, as there was no effect of plot (p=1), this variable was removed, and the effects of type and site tested in a linear model. Normality and homoscedasticity of residuals were checked using Shapiro-Wilks test and Levene's test.

MetaWin 2.1 software (Rosenberg et al., 2000) was used to generate Hedge's d statistics that assessed the extent of compensatory responses of *A. adenophora* to the galling of *P. utilis* (Garcia and Eubanks, 2019). 95% confidence intervals for Hedge's d were bootstrap-generated. The responses of individual stems were recorded in terms of branch numbers and the branch lengths.

3. Results

3.1 Ageratina adenophora vegetative growth and reproductive investment

Ageratina adenophora was present at high densities, with an overall average of more than 10 main stems per plot (equivalent to 160 stems m^{-2}) with above-ground dry vegetative biomass of almost 200 g per plot (3.2 kg m^{-2}) (Table 2).

Plots that had a higher above-ground vegetative biomass had more main stems and more side branches (Spearman correlations, plots from all sites combined, rho = 0.393, $p < 0.001$ and rho = 0.651, $p < 0.001$, respectively). The highest vegetative biomass and density of plant stems were recorded at site PD, where the plant population has been established longest (Table 2). However, stem densities and vegetative biomass at site HY, where the plant has most recently become established, were similar to those of the two intermediate sites (Table 2). Consequently, there was no clear relationship between vegetative features and how recently the plant populations had become established.

Capitulum densities were extremely high, especially at site PD, where they reached over 2000 per plot (equivalent to 32000 capitula m^{-2} and over 200 capitula per main stem) and around 12 capitula per gram of vegetative biomass. Capitulum numbers in relation to vegetative biomass did not vary significantly between sites (Table 2). Plots containing a higher vegetative biomass and more stems generally contained more capitula, but there was some variation between sites (Supplementary Table 1).

3.2 Densities of Procecidochares utilis galls

Procecidochares utilis galls were recorded in a majority of the *Ageratina adenophora* plots at all four sites and were especially widespread at site PD, where 88% of the plots had galls present (Fig. 1). Within plots, the proportion of stems supporting gall development ranged from 12-15% at three of the sites, but galling rates were twice this at site PD (Fig. 1). The number of galls present on the main stems and their associated branches ranged from zero to ten, but most galled stems supported a single gall (312 out of 507 galled stems). The highest gall densities per stem were at site PD, where there were significantly higher numbers of galls per plot, per unit vegetative biomass, per main stem and per stems plus associated branches than elsewhere (Supplementary Table 2). Other sites did not vary significantly in any measures of gall abundance.

The relationships between the numbers of galls present in the plots and features of their host plants are summarized in Table 3. Plots that contained a higher vegetative biomass, more stems, and more stems plus their side branches, supported more galls. The number of galls on individual stems was independent of the number of stems in the plots at most of the sites (Spearman Correlations, $p>0.05$), although a significant negative relationship was present at site HY (rho=-0.300, $p=0.035$).

3.3 The extent of branching

The numbers of side branches present on individual main stems ranged from 0 to 29.

Overall, 41.5% of the main stems had side branches (N stems = 2369), but the proportion of branched stems increased progressively from north to south, with about 30% of the stems branched at northern site HY and 60% branched at the southern site PD (Fig. 2).

3.4 The relationship between branching and capitulum numbers

Overall, plots with more side branches supported the development of larger numbers of capitula (with significant correlations also present at three of the four individual sites) and plots where there were more side branches per main stem also produced more capitula (with significant correlations at two of the four individual sites) (Supplementary Table 1).

3.5 The relationship between galls, branching and capitulum numbers

Stems with galls averaged about twice as many side branches as stems that had not been galled (Table 4). Stems with greater numbers of galls also had increasing numbers of side branches (Supplementary Table 3). Site PD had a far higher proportion of galled stems than the other sites (Fig. 1) and also had a higher proportion of main stems that were branched (Fig. 2). At all four sites there was a consistent positive relationship between the number of galls present on a stem and the number of side branches (Table 5). The effects of *P. utilis* galling for each main stem were assessed in the plot where galls were present. The galled stems had more side branches (Supplementary Table 4). The plant responses to galling, as measured by Hedge's d, are presented in Fig. 3. The values for Hedge's d are within the range recorded for reproductive (0.8676 to 1.3294) and vegetative (0.9334 to 1.2359) overcompensation by Garcia and Eubanks (2019).

Plots that included larger numbers of galls also contained more capitula, but when capitulum numbers are compared relative to the amount of vegetative biomass present, there was a negative relationship with gall abundance (Table 3). An explanation for this is that as the numbers of galls increased, so did the extent of branching. This led to the production of more capitula, but because of the extra vegetative growth required to form the branches, the ratio of vegetative to reproductive investment declined.

3.6 Seed numbers and seed weights

The numbers of seeds inside capitula collected from the large side branches were recorded at sites HY and PD. In total, 3695 branches were sampled (111.97 ± 61.36 branches per plot, mean ± SD). A total of 3050 side branches had no galls and 645 branches had *P. utilis* galls present. At both sites about 50 seeds were present in each capitulum, with no differences in the numbers of seeds in capitula collected from galled and un-galled branches (Table 6).

Seed weight was slightly higher overall in capitula obtained from stems that had not been galled, but no difference was detected at three of the four individual sites. The weights of the seeds differed much more strongly between sites ($F_{[3,109]} = 6.71$, $P = 0.0003$). However, when the differences between each pair of populations were tested, only site PD had lower weight (Table 6).

4. Discussion

We investigated the continuing expansion of *A. adenophora* populations northwards along Chinese National Road 108 in Sichuan Province and the impact that the gall-forming fly *P. utilis* may be having on this expansion. Seed production is likely to be essential for the establishment of new populations of the plant, but this is supplemented by vegetative spread leading to the establishment of the dense stands that make the plant a significant economic and ecological problem. This densification of existing stands was reflected in the higher stem densities and increased within-plot vegetative biomass present in the southernmost population PD, where the plants have been established longest. There were also higher densities of the gall-forming fly *P. utilis* at this site and more branching of the stems than elsewhere.

Compensatory growth of *A. adenophora* in response to simulated herbivory (Zhu and Sang, 2008) and insect galling (Buccellato et al., 2012; Erasmus et al., 1992) has been explored previously under controlled conditions. Under our field conditions the galled stems were more likely to branch than undamaged stems. *Procecidochares utilis* lays eggs on the branch near the terminal vegetative bud, and groups of their larvae burrow into the young stems where they induce galls (Bennett and Vanstaden, 1986). Increased branching is likely to reflect a relaxation of apical dominance (Agrawal, 2000; Buccellato et al., 2019; Rosenthal and Kotanen, 1994) resulting from high levels of cytokinins generated within the galls (Van Staden and Bennett, 1991b).

The increase in branching in response to galling by *P. utilis* resulted in the production of more capitula, but capitula on galled stems were more costly for the plants to produce because of the extra investment in vegetative biomass that was required to produce the side branches. In terms of the energy budgets of the plants, this suggests that galling resulted in a greater, but less efficient, commitment of resources to reproduction and above-ground vegetative growth. The extent to which this was reflected in changes in below-ground growth and energy stores is unknown. The increase in reproductive investment generated by the galls, as well as the direct energetic costs of supporting gall development (Van Staden and Bennett, 1991a), suggests that the competitive abilities of the plants may be reduced by the flies. However, this impact may be insignificant for inter-specific competition because of the physiological characteristics of the invasive plants that make them particularly vigorous. Photosynthetic rates and nutrient use-efficiencies of *A. adenophora* in Chinese sites were higher than those of sympatric native species in China (Feng, 2008a, b; Lei et al., 2011; Zheng et al., 2012) and also higher than those recorded for the plant in its native range (Feng et al., 2011; Lei et al., 2011; Wang et al., 2013). The plant's prolonged growing season also allows a greater accumulation of photosynthates than sympatric native species in China (Gao et al., 2013; Wang et al., 2013) and in addition, a self-reinforcing mechanism has been found that favours invasion by *A. adenophora*, because high densities of *A. adenophora* increase the levels of available N, P and K in the soil (Wan et al., 2010).

Rates of seed production are particularly important for the dynamics of range expansion by invading species (Tabassum and Leishman, 2018). In situations such as those in Sichuan, where the distribution of the plant is spreading northwards, this suggests that galling by the fly has the potential to speed the expansion of its host. Any consequences of increased seed production are not likely to have been reduced by negative effects of galling on the numbers of seeds in the capitula or their quality because the former was unaffected by galling and there was only a minor reduction in seed size. Additionally, germination rates in this species have been found to be independent of seed weight (Datta et al., 2017) and the between-site variation in seed weights we recorded was much greater than the impact of galling. Under controlled conditions, lower rates of germination of seeds from heavily galled *A. adenophora* have nonetheless been reported (Wan et al., 2010; Wang et al., 2006).

Procecidochares utilis has been introduced widely to control *A. adenophora*, but its impact on the plant has been inconsistent (Heystek et al., 2011, Sharma Poudel et al., 2020). In Hawaii, successful control was only achieved at sites where 50% or more of the stems were galled and densities of galls on each stem were high (Bess and Haramoto, 1972). Galling rates at our study populations were lower than this, only reaching even 40% at the longest-established site. The lack of any negative impact of *P. utilis* galling on the plant in Southwest China is therefore consistent with results from elsewhere, but the likely benefits for the plants from the activity of the fly may be exceptional. Transnational migration of biocontrol agents into areas where they have not been deliberately introduced is often beneficial (Langa et al., 2020) but this has not been the case with the arrival of *P. utilis* in China.

5. The wider story – beyond our study species

Over-compensation by plants in response to herbivory may be more frequent than usually realized (Garcia and Eubanks, 2019). The responses of plants to herbivory are dependent on ecological context (Wise and Abrahamson, 2005), and models suggest that responses to herbivory are highly dependant on available resources (Wise and Abrahamson, 2005). Increased photosynthetic rates, resource uptake rates and nutrient transport rates, with more nutrients allocated into meristems are features of regrowth after damage (Agrawal, 2000; Rosenthal and Kotanen, 1994; Wise and Abrahamson, 2005). Compensatory responses to herbivory have been recorded in many terrestrial plants (Agrawal, 2000; McNaughton, 1979), including some invasive species (Liao et al., 2016), and may be almost ubiquitous. Our results suggest that particular care is needed during assessment of the impact of candidate biocontrol agents that alter the architecture of their hosts.

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FIGURE LEGENDS

Fig. 1 The abundance of *Procecidochares utilis* galls in four populations of *Ageratina adenophora*. Open bars indicate the proportion of plots with galls and solid bars indicate the proportion of stems with galls. The sample sizes are provided above the bars. HY, MN, XC and PD represent the sampled populations, i.e Hanyuan County, Mianning County, Xichang City and Pindi Town.

Fig. 2 The percentage of branched *Ageratina adenophora* stems. Significant differences are indicated by different letters on the bars (based on Chi square tests of frequencies). The stem sample sizes are the same as in Fig. 1.

Fig. 3 The extent of overcompensation in response to galling in terms of summed side branch lengths and side branch numbers. Sample sizes for the two measures were the same and are in parentheses (numbers of ungalled stems, numbers of galled stems).