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Asteraceae invaders have limited impacts on the pollination of common native annual species in SW Western Australia's open woodland wildflower communities

Xingwen Loy · Claire E. Wainwright · Margaret M. Mayfield

Abstract The York gum–jam woodlands of southwest Western Australia support diverse annual wildflower communities despite extensive habitat fragmentation, remnant isolation and the invasion of many exotic annual plant species. Few studies have explored the pollinator–plant relationships maintaining these persistently species-rich 'novel' communities. We examine the pollination ecology of five native species common to York gum–jam woodland annual communities to determine whether native pollinators may be mediating impacts of exotic annual plants on native wildflower species. We determined the pollination requirements of native focal species and the diversity and frequency of pollinator visitation to these focal plant species across invasion gradients. We also recorded the pollinator community of a dominant exotic herb in this system: *Arctotheca calendula* (cape

weed). Only two of the five native species examined had significant seed set benefits attributable to insect pollination. One native plant species, *Podotheca gnaphalioides,* had pollinator assemblages that overlapped significantly with exotic *A. calendula*, with some reduction in pollinator visitation evident. One species, *Waitzia acuminata*, was found to benefit from insect pollination only in the larger of two surveyed remnants, which may reflect emerging reproductive polymorphism among geographically isolated populations. We highlight two mechanisms in this system that may buffer pollinator-mediated impacts of exotic species on native species: autonomous seed production, which may be increasingly prevalent in isolated populations, and segregation of pollinator resources among species. Our findings illustrate the ways that pollinator-mediated interactions can affect seed set within plant communities persisting in highly fragmented and invaded agricultural landscapes.

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X. Loy (✉) · C. E. Wainwright · M. M. Mayfield
School of Biological Sciences, The University of Queensland, Brisbane, QLD 4072, Australia
e-mail: xingwen.loy@uq.net.au

C. E. Wainwright
e-mail: claire.wainwright@uq.net.au

M. M. Mayfield
e-mail: m.mayfield@uq.edu.au

Introduction

Biological invasions are considered one of the largest threats to biodiversity worldwide (Gurevitch and Padilla 2004; Wilcove et al. 1998). While negative impacts of exotic plant species on native plant

communities are well-documented, the mechanisms driving these impacts are less clear. Though direct competition with natives is often suggested to be key to mediating impacts of invasion on native communities (Levine et al. 2003), interactions between native and exotic plant species can also be indirect and mediated by third-party mutualists, such as pollinators. Though indirect interactions have been recognised as important to the dynamics among native and exotic species, they have not been as widely studied as direct impacts (White et al. 2006). As seed production in many plant species is pollen-limited (Joar Hegland and Totland 2008; Kelly et al. 2007; Law et al. 2010; Pauw and Bond 2011; Rosenheim et al. 2014), invader-driven disruptions to pollinator–plant interactions that influence seed production may reduce the fitness of seed-limited native species and contribute to changes in local populations and community dynamics.

Exotic plant species can affect local native plant–pollinator relationships in several ways. They may, for example, increase the diversity of available floral resources, leading to increases in local pollinator abundance (Feldman et al. 2004; Ghazoul 2006; Jakobsson et al. 2009). Such pollinator increases may or may not benefit native plants, however, as these new pollinators will not necessarily visit native species and even if they do, may increase heterospecific pollen exchange (transfer of pollen between different plant species) rather than intraspecific pollen transfer (Eaton et al. 2012). In addition, exotic plant species may reduce the pollination success of neighbouring native plant species by outcompeting native plants for available pollination services (Dietzsch et al. 2011; Knight et al. 2005; Waters et al. 2014). In spite of the often shared biogeographic and evolutionary histories of native insect-pollinated plants and their pollinators, pollinator interactions are more often opportunistic than oligolectic in nature and pollinator infidelity is common (Kearns et al. 1998). Even where exotic and native species do not share pollinators, exotic species may indirectly impact native plant pollination by reducing native species densities and thus floral density (through direct competition) (Karron et al. 1995; Kirchner et al. 2005; Steffan-Dewenter et al. 2001) or by driving a reduction in flowering effort per individual (driving a reallocation of resources away from flower production, Suter 2009; Wang et al. 2014). The mechanisms and

impacts of pollinator-mediated interactions between native and exotic species are clearly variable, and the introduction of an exotic species into a native plant community can produce contrasting effects on different native species (Waters et al. 2014). Studying pollinator-mediated interactions between native and exotic plant species across many systems is key to gaining a more complete view of the role pollinating insects play in driving changes in or maintaining native biodiversity.

In this study, we examined insect pollination in five native annual plant species from annual wildflower communities found in semi-arid woodlands of southwest Western Australia. Using natural variation in plant community composition (and invasion extent within communities) across two isolated remnants, we examine whether successful exotic Asteraceae species have pollinator-mediated effects on the reproductive success of locally abundant native annual forbs. Specifically, we asked:

1. Is there evidence that seed production in the native focal species is pollen-limited in this system?
2. Do the native focal species share pollinators with the most common insect-pollinated exotic Asteraceae (cape weed) in this system?
3. How does pollinator visitation to native plant species change between different densities of exotic Asteraceae within remnants?

Methods

Study system

The York gum–jam woodlands of southwest Western Australia experience a Mediterranean-type climate, with cool, wet winters and warm, dry summers. The canopy of these woodlands is characterised by sparse York gum (*Eucalyptus loxophleba* subsp. *loxophleba*) and jam trees (*Acacia acuminata*) with an understorey of sparse shrubs, perennial grasses, and diverse and abundant ephemeral annual flora dominated by Asteraceae species. The annual wildflowers in this system have a growing season that runs from July–November (winter/spring), with flowering and seed production occurring between September and November.

York gum–jam woodlands are found in the Southwest Australian Floristic Region, a hotspot of

biodiversity and endemism, which has suffered severe population declines and extinctions due to widespread land clearing for agricultural production over the last 60 years (Beard 1990). Fertiliser application in farmlands surrounding remaining York gum–jam woodland fragments has increased nutrient availability along woodland borders, which has facilitated the invasion of exotic annual plant species into remnant woodland plant communities (Hester and Hobbs 1992). These invasions have resulted in new plant assemblages that support mixtures of native and exotic plants (Dwyer et al. 2015). These 'novel' communities present ideal systems for studying the small-scale interactions between exotic and native plants in species-rich communities, particularly given their close proximity to native-dominated reference communities.

Study species

Five native focal species and one common exotic species were selected for this study (Fig. 1): *Goodenia berardiana* (Gaudich.) Carolin (Goodeniaceae), *Podolepis lessonii* (Cass.) Benth. (Asteraceae), *Podotheca gnaphalioides* Graham (Asteraceae), *Rhodanthe manglesii* Lindl. (Asteraceae), *Waitzia acuminata* Steetz (Asteraceae) and one exotic Asteraceae, *Arctotheca calendula* (L.) Levyns. The native focal species are common and regularly co-occur with each other and various exotic annual species in York gum–jam woodland annual communities. Exotic plants in York gum–jam woodlands are predominantly annual Asteraceae (potentially insect-pollinated) and Poaceae (wind-pollinated). *A. calendula* (cape weed) is a major agricultural weed in the region and can form near monocultures in and around remnant native vegetation including York gum–jam woodland remnants. While we did not assess pollinator dependency in this species, we conducted pollinator observations to determine if this species and the native focal species share insect visitors.

The focal species were studied in the growing season of 2013 in communities from two reserves located 55 km apart and more than 4 km from any other natural remnant: Bendering (32°23′7.88″S, 118°23′5.66″E) and Kunjin reserves (32°21′19.31″S, 117°45′42.32″E). Bendering reserve covers approximately 16 km^2, with York gum–jam woodlands covering less than 3 km^2. Kunjin reserve covers

2.4 km^2 of which only 0.2 km^2 is York gum–jam woodland. Due to variability in the composition of the annual assemblages in these two reserves, data were only collected in both reserves for *W. acuminata* and *R. manglesii*. *P. lessonii* was surveyed only in Kunjin, while *G. berardiana*, *P. gnaphalioides* and the exotic *A. calendula* were surveyed only in Bendering.

Measuring invasion: landscape invasion levels and invasion parameters

The York gum–jam woodlands found in Bendering and Kunjin reserves span a gradient of mild to severe biotic invasion. To survey pollinator communities of native focal species across invasion gradients, we marked out four to eight 5 m × 5 m study plots for each focal species spanning the natural variation in exotic cover in each reserve. Each focal species was surveyed from at least four plots in each reserve where it was studied (Online Resource Table 1). Where possible, we surveyed multiple focal species within the same plots for efficiency and located plots so as to include large populations of each species.

We used estimates of available floral resources and/or plant community composition as explanatory variables in analyses exploring the differences in pollinator visitation rates. Within each plot, we estimated the extent of exotic invasion by counting species abundances in five arbitrarily located 30 cm × 30 cm quadrats and determining the relative abundance of exotic species (includes annual grasses and forbs—mostly Asteraceae). In addition, within each plot, we recorded fluctuations in the proportion cover of open flowers belonging to various non-grass invasive and native species for each day that we conducted pollinator observations in a given plot (detailed below).

Assessing pollinator dependency

To determine if native focal species benefited from insect pollination, we set up simple pollen limitation experiments. For all focal species except *P. gnaphalioides,* we arbitrarily selected 12 plants in each plot and assigned them one of four treatments representing all combinations of pollinator exclusion and manual pollen addition (Online Resource Table 1), resulting in three replicates of each 'treatment' per plot per focal
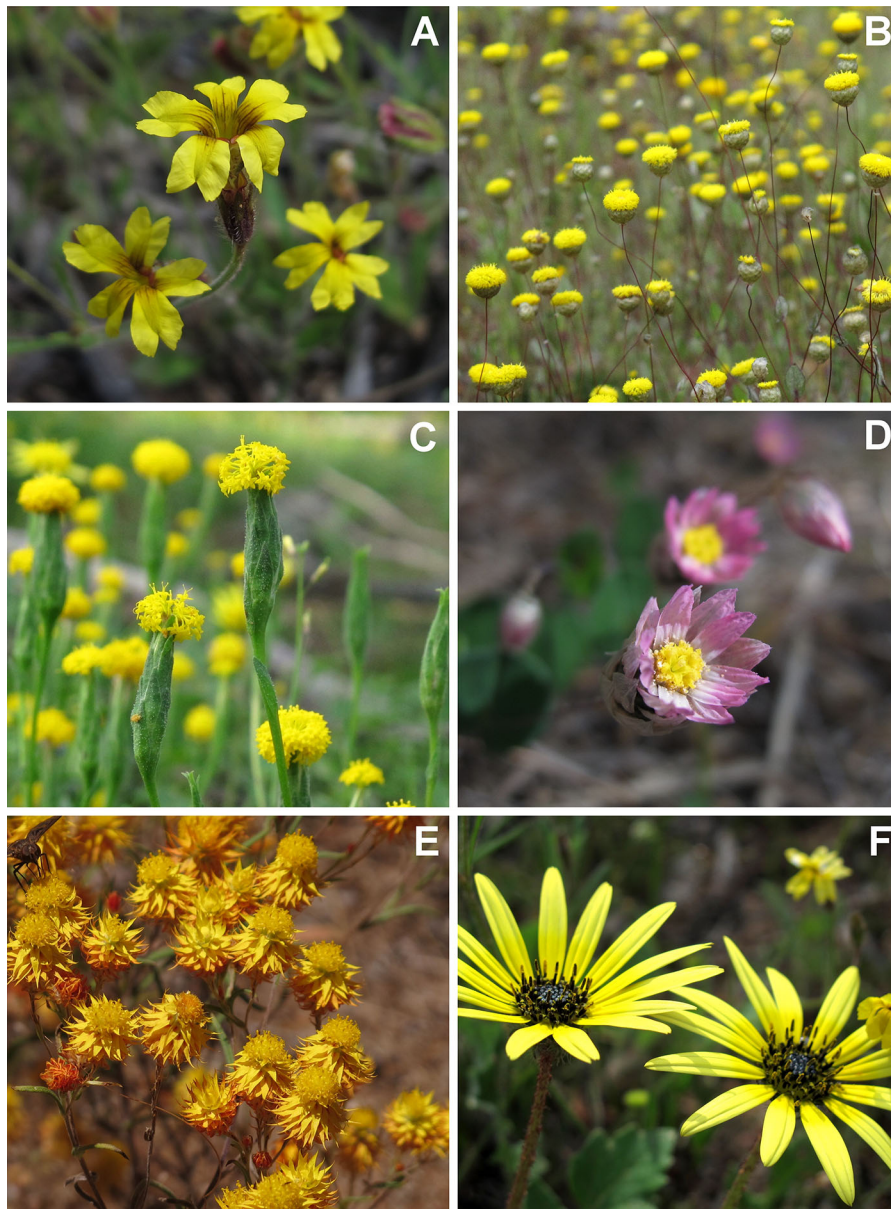
Fig. 1 Five native focal species and one common exotic species selected for this study: **A** *Goodenia berardiana*, **B** *Podolepis lessonii*, **C** *Podotheca gnaphalioides*, **D** *Rhodanthe manglesii*, **E** *Waitzia acuminata* and **F** an exotic Asteraceae, *Arctotheca calendula* (cape weed). Photos by Xingwen Loy

species. *P. gnaphalioides* did not occur at high enough densities to assign three individual plants to each treatment in each plot (there were generally 10 or fewer *P. gnaphalioides* individuals in each plot), so we arbitrarily selected four large individuals and assigned the four treatments to four inflorescences on each of these plants.

We selected and assigned a single inflorescence one of four treatments: unmanipulated natural pollination ("open"), natural and hand cross-pollination ("open + hand-pollination"), exclusion of all insect pollinators ("exclusion"), or pollinator exclusion with hand cross-pollination controls ("control"). We marked "open" inflorescences with a piece of

coloured yarn but they were otherwise unmanipulated, allowing pollination to occur naturally. We also marked "open + hand-pollination" flowers with a piece of yarn tied to the base of the inflorescence but added pollen from another individual from the same site to supplement the amount of pollination received naturally. We enclosed inflorescences in the pollinator "exclusion" treatment in mesh bags constructed of white organza fabric, with holes no wider than 1 mm, which allowed airborne pollen to pass over the flowers but excluded insect pollinators. We attached these bags while flowers were still in the early budding phase to ensure no visitation had occurred pre-treatment. We enclosed "control" treatment inflorescences in mesh bags but also hand-pollinated them with pollen from other individual inflorescences in the study plot once flowers bloomed. We performed hand-pollination by rubbing an inflorescence from a different conspecific plant on the appropriate test flower ("open + hand-pollination" and "control"). Our experimental design follows the standard approach for assessing plant species reliance on insect pollinators and pollen limitation in the field (Kearns and Inouye 1993; Steenhuisen and Johnson 2012; Abdala-Roberts et al. 2014).

Once seeds of all experimental flowers reached maturity, we collected and counted fully developed seeds and undeveloped ovules in each inflorescence in the four focal Asteraceae (*P. lessonii, P. gnaphalioides, R. manglesii and W. acuminata*). We used these values to calculate the proportion of developed seeds (developed seeds/total ovules) and used this proportion as a proxy for the rate of successful pollination. For *G. berardiana* (Goodeniaceae), it was not possible to determine the number of undeveloped seeds, and hence for this species, we used total seed counts for analysis. "Open-hand" treatments that received all potential pollinating agents (insects, wind and hand-pollination) were expected to have higher proportions of developed seeds than other treatments. If wind alone is inadequate for maximal pollination and insect pollination improves seed production in a focal species, the proportion of developed seeds was expected to be higher in the "open" treatments (wind and insects) than the pollinator "exclusion" treatments (wind only). If pollen was still limiting seed set despite pollination by locally available insect pollinators, the proportion of developed seeds in the "open" treatments (wind and insects) was expected to be

lower than in the "open-hand" treatments (wind, insects and hand-pollination) (Kearns and Inouye 1993; Knight et al. 2005). Control treatments in this study helped account for unexpected effects of exclusion treatments and to test the efficacy of our hand-pollination technique (Kearns and Inouye 1993).

Pollinator observations

We conducted pollinator observations to determine which, if any, insects were common flowers visitors of each native focal species and exotic *A. calendula*. We also determined if the relative abundance of exotic plant species and native focal conspecifics in communities, as well as changes in the proportion cover of open flowers of native or exotic flowers through time, was correlated with potential pollinator diversity and visitation rates. We conducted seven to ten 10-min observations on each focal species in the 5 m × 5 m study plots for 7–9 days (detailed in Online Resource Table 1). We distributed the observations for each species roughly over the 2-month-long flowering season and across three times of day: morning (from 0900 to before 1100 h), midday (from 1100 to before 1400 h) and afternoon (from 1400 to 1700 h). For each native focal species in a given study plot, we conducted only one observation in a single day and made at least three observations in each time slot per species. Pollinator observations of *A. calendula* were conducted in five of the 5 m × 5 m study plots at Bendering reserve in a fashion consistent with observations conducted on native focal species.

At the start of each 10-min pollinator observation period, we estimated the proportion cover of open flowers from all annual species, native and exotic, within the study plot. Within the plot, we then randomly chose an observation area of 20 cm × 50 cm and recorded the number of blooming flowers of the relevant focal species in that area. Where possible, multiple focal species were observed at the same time for efficiency. Only insects that behaved in a manner consistent to gathering nectar or pollen from flowers were considered as potential pollinators and their visits counted. Potential pollinators (henceforth referred to as "pollinators") were categorised into recognisable taxonomic units (morphospecies). We recorded the total number of visits by each pollinator morphospecies during each observation. It was not possible to keep track of individual

pollinators once they left the observational area. Following observations, we collected voucher specimens of observed pollinators for identification. As some insect species observed in the field were impossible to distinguish on the wing (Halictid bees and Syrphid flies), morphospecies in this study provide a conservative estimate of pollinator species richness in this system.

Data analysis

Pollen limitation experiments

We analysed the seed production among pollination treatments using generalised linear mixed-effects models (GLMMs), using the Gaussian function for seed count data in *G. berardiana* and specifying binomial errors and a logit link function for proportion data in the other focal species. The GLMMs consisted of a fixed factor of experimental treatment (four levels: "open", "open-hand", "exclusion" and "control") and two variables included as random effects: study plot and reserve (for species with experimental plots in both Kunjin and Bendering reserves). For *P. gnaphalioides*, we also included 'plant' as a random effect, as the four experimental treatments for this focal species were nested within individual plants. We performed post hoc multiple pairwise comparisons between treatments with generalised linear hypothesis testing assessed using Wald tests.

Pollinator assemblages of native focal species and *A. calendula*

To determine how pollinator assemblages varied among the five focal species and if they shared pollinators with exotic *A. calendula*, we used multivariate permutational analysis of variance (PERMANOVA). This was based on a resemblance matrix constructed using Bray–Curtis similarity values (Anderson et al. 2008). This analysis used Type III sums of squares with 9999 permutations, with plant species identity as a single fixed factor. Pollinator assemblages of each plant species were characterised at the plot-level using the total visitation rates of each visiting pollinator morphospecies as a proxy for abundance, which we square root transformed prior to analysis to meet the assumptions of multivariate homogeneity of variance. We then used principle coordinates ordination (PCO) to plot dissimilarities in pollinator assemblages among focal species in two-dimensional space (Anderson et al. 2008).

Pollinator visitation and pollinator species richness

We used a two-step modelling process to assess if the composition of plant communities and/or available floral resources within plots affects plant–pollinator interactions in each of the focal species. First, we modelled the probability of observing at least one insect visit (binary response) using GLMMs with binomial errors and a logit link function. Second, when insect visitation occurred, we modelled the number of visits (excluding all 'absence' data) using GLMMs with Poisson errors and a log link function. This two-step approach was required due to the large number of observations with no visitors. Over-dispersion was evident in the Poisson GLMMs so in addition to 'plot', 'observation' was also included as a random effect to capture variation in visitation that was not adequately described by the Poisson distribution (Bolker et al. 2009).

For each response variable, we constructed 12 biologically feasible candidate models of varying complexity (Online Resource Table 2) and selected the most parsimonious model explaining the largest amount of data using weights based on corrected Akaike's information criteria (AICc) (Anderson et al. 2000; Hurvich and Tsai 1989; Wagenmakers and Farrell 2004). We considered the simplest model with the number of flowers observed during an observation period (model 2) as the null model for each species. We determined that a model was superior to all others when it had an Akaike weight of at least 0.25, and this value had to be at least twice as large as the second best model. Where no single model was clearly superior to all others or no models were superior to the null (model 2), we concluded that none of the constructed models adequately described the observed pollinator–plant interactions in the focal species. Where a model emerged superior, we then investigated which fixed-effects were statistically significant based on Wald tests (Bolker et al. 2009). For analyses with visitation data from *W. acuminata,* we did not include model 9 (interaction between proportion cover of native Asteraceae flowers and focal conspecific flowers) as peak flowering for this species occurred after that of most other native Asteraceae species.

Using the visitation data (excluding absences), we also modelled pollinator species richness per observation (number of morphospecies) using the same approach described above for numbers of visits. In addition, we modelled total species richness of pollinators (observed over the entire study period) as a function of the estimated relative abundance of exotic plants in each plot using linear regression.

Unless stated otherwise, we conducted all statistical analyses in R (R Development Core Team 2012) and constructed mixed-effects models using the *glmer* function from the package 'lme4' (Bates and Maechler 2009). We performed multiple comparisons between pollen limitation experiment treatments using the 'multcomp' package (Hothorn et al. 2008). We conducted model selection with the *model.sel* function in the R package 'MuMIn' (Burnham and Anderson 2002). We analysed variations in pollinator assemblage among focal species using the statistical package Primer v6 + PERMANOVA (Primer-E Ltd 2008).

Results

Pollen limitation

We found significant differences in seed production among experimental treatments for *P. gnaphalioides* and *W. acuminata* but not the other species (Fig. 2; Online Resource Table 3). For *P. gnaphalioides*, the proportion of developed seeds from flower-heads in 'exclusion' treatments was significantly lower than those in the 'open' treatments but seed production in the 'control' treatments ("exclusion + hand-pollination") and "exclusion" treatments was not significantly different. The proportion of developed seeds in flower-heads from "open" and "open + hand-pollination" treatments was also not significantly different for this species.

For *W. acuminata*, treatments had different impacts on seed production in Kunjin and Bendering reserves. In Kunjin reserve, seed set was significantly higher in the "open + hand-pollination" treatment than in the "exclusion" treatment but the seed set in the "exclusion" and "control" treatments was not significantly different (Fig. 2; Online Resource Table 3). In contrast, seed set in Bendering reserve in the "open", "open + hand-pollination" and "control" treatments was all significantly higher than in the "exclusion" treatment.

Pollinator assemblages

Pollinator communities were largely distinct among native focal plant species (Fig. 3; p(perm) < 0.01; Online Resource Table 4). The potential pollinators visiting exotic *A. calendula* overlapped significantly with those of *P. gnaphalioides* but no other focal
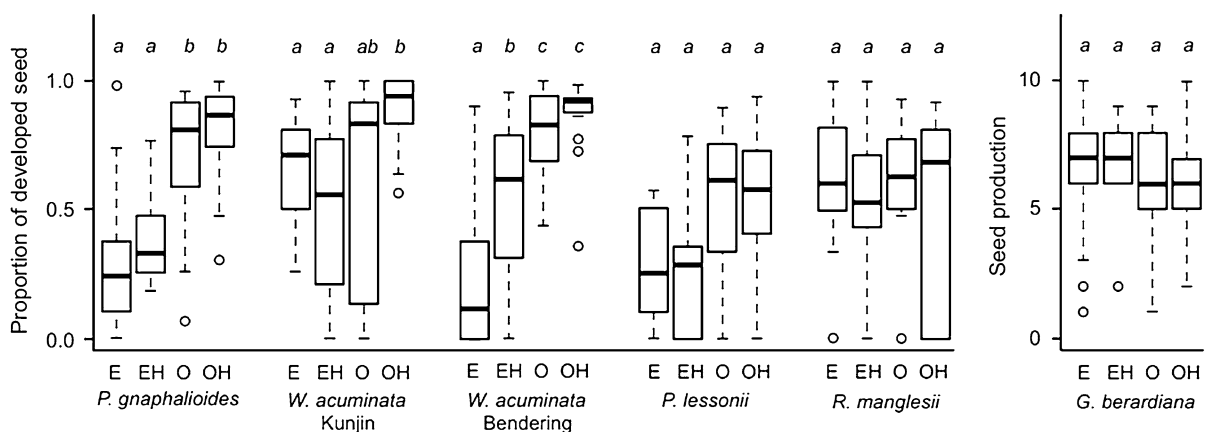

Fig. 2 Seed production from treatments in the pollen limitation experiments conducted on each focal species (*E* exclusion, *EH* control, *O* open, *OH* open + hand-pollination). The *y* axis shows the proportion of developed seed (the number of developed seed/total ovule count) for the four focal Asteraceae species, and the total seed count (number of developed seed) for *G. berardiana*. *Box and whisker plots* show median values (*solid horizontal line*), 50th percentile values (*box upper and lower limits*), 90th percentile values (*whiskers*), and outliers (*circles*)

species. Very few insects were observed to visit *G. berardiana* and *R. manglesii*. Insect morphospecies observed visiting each of the other native focal species and *A. calendula* are listed in Online Resource Table 5.

Visitation probability, rates and diversity

Local plant community and floral resource composition appeared to influence visitation probability and rate in only one focal species: *P. gnaphalioides*. Although the proportion cover of open *W. acuminata* flowers and exotic Asteraceae (model 7, Online Resource Table 2) best described the probability of visitation to *W. acuminata*, there was no statistically significant relationship between any fixed factors and visitation probability (Online Resource Table 6).

For *P. gnaphalioides*, the relative abundance of exotic Asteraceae plants (model 12, Online Resource Table 2) best explained the probability of a single visit occurring, with visit incidences becoming rarer as exotic Asteraceae abundances increased (Online Resource Table 6). Where insect visitation did occur, visitation rate was best explained (significantly) by the interaction between the proportion cover of exotic

Asteraceae flowers and focal conspecific flowers (Fig. 4; model 7, Online Resource Tables 2 and 6).

Only the pollinator assemblages visiting *P. gnaphalioides* and *W. acuminata* were sufficiently diverse to statistically explore pollinator diversity. However, none of the tested models were clearly superior in explaining the number of pollinator morphospecies detected visiting either *P. gnaphalioides* or *W. acuminata* (Online Resource Table 7). Across all study plots, we found no significant relationship between total pollinator richness for either species and the relative abundance of exotic Asteraceae plants or exotic grasses in communities.

Discussion

In this study, we found extensive variation in the impact exotic annual plants have on the pollination of five common native annual plant species in the heavily invaded and fragmented York gum–jam woodlands of SW Western Australia. These communities seem dominated by self-pollinating native species, with
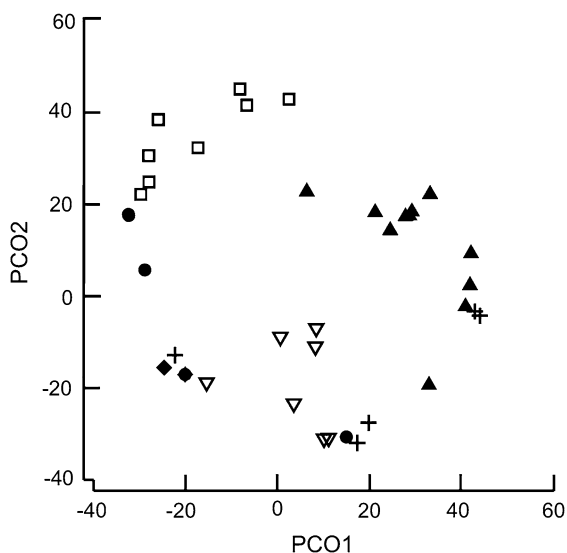
Fig. 3 Principle coordinates ordination (PCO) based on the visit rates of pollinator morphospecies to each native focal species and *A. calendula*. (*plus symbol*) *A. calendula*, (*filled diamond*) *G. berardiana*, (*inverted triangle*) *P. gnaphalioides*, (*square*) *P. lessonii*, (*filled circle*) *R. manglesii*, (*filled triangle*) *W. acuminata*
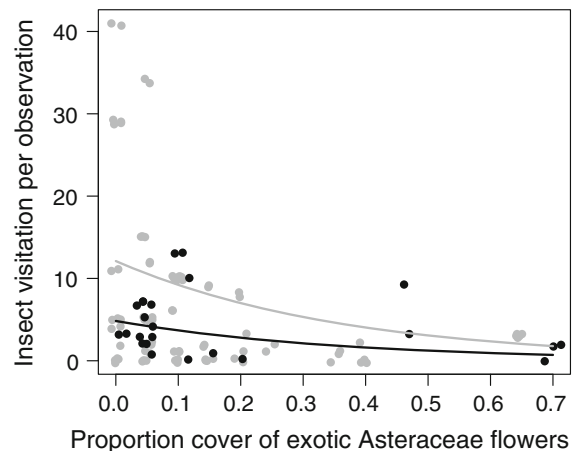
Fig. 4 Relationship between the number of insect visits per day to *P. gnaphalioides* and the proportion cover of exotic Asteraceae flowers in study plots. *Data points* represent the number of insect visits to *P. gnaphalioides* flowers within the 20 cm × 50 cm observation areas during each 10-min pollinator observation per day, when the proportion cover of *P. gnaphalioides* conspecific flowers was above (*black*) and below (*grey*) the mean of 0.19. *Lines* show the relationship between number of visits and exotic flower cover, at the mean of *P. gnaphalioides* conspecific proportion cover values above (0.45, *black*) and below (0.11, *grey*) the overall mean proportion of *P. gnaphalioides* conspecific flower cover of 0.19

the pollinator assemblages of only one of five native focal species, *P. gnaphalioides*, overlapping significantly with that of a common co-occurring exotic species (*A. calendula*), possibly reducing pollinator visitation to the native. For one focal species, habitat fragmentation may have more substantial impacts on pollination than invasion extent within reserves, a finding that highlights the complex ways that land use changes can impact native plant-pollinator dynamics of species persisting in habitat remnants. Though exotic plants do have direct negative impacts on the native wild flower species in this system (Lai et al. 2015), they do not seem to be driving consistent pollinator-mediated reductions of seed set in common native species.

Is there evidence that seed production in native focal species is pollen-limited?

We found that restricting insect access to flowers had little to no impact on seed set in all but two native species, *P. gnaphalioides* and *W. acuminata*, suggesting that autonomous self-pollination may be a common pollination strategy in this flora. This is somewhat surprising given the showy floral morphology of the species examined in this study (Fig. 1). The main role of insect pollinators in this system seems likely to be in mediating gene flow (though direct tests are needed to confirm this) rather than seed production. Alternatively, it is possible that exclusion treatments failed to keep out extremely small insects (e.g. thrips) that ameliorated pollen limitation in the absence of larger pollinators. Furthermore, hand-pollination treatments for some species appeared to be ineffective. While pollinators clearly did not affect seed production in *G. berardiana* and *R. manglesii* (Fig. 2), our hand-pollination treatments may have had a negative effect on seed production in *R. manglesii*, though this did not have significant impacts and appears to have been inconsistent. There was no evidence that *P. gnaphalioides* suffers from pollen limitation in Bendering based on comparisons of "open + hand-pollination" and "open" insect-visited flowers. We did, however, observe much lower seed production in flowers from which insects were excluded (both bagged treatments). This suggests that insect pollinators do provide seed production benefits in this species but our hand-pollination technique appears to have been ineffective, limiting our ability

to confirm pollinator dependency. Though not significant, seed production patterns in open and bagged treatments for *P. lessonii* were similar to those for *P. gnaphalioides*. A lack of significant differences between experimental treatments in *P. lessonii* may reflect high variance among plants rather than a complete lack of dependence on insects, and a larger sample size may be needed to elucidate possible pollen limitation in this species (Fig. 2).

For *W. acuminata*, proportional seed set from "control" and "open + hand-pollination" experimental treatments was fairly consistent between the reserves but seed set from "exclusion" and "open" treatments differed substantially. Excluding pollinators to *W. acuminata* resulted in significantly reduced seed set in Bendering but not Kunjin, and this species may be more reliant on insect pollinators for seed set in Bendering. Population-level differences in pollinator reliance have been noted for other species elsewhere, and have been interpreted as indication of local adaptations of breeding systems (Kalisz et al. 2004). This likely occurs when pollinator services are deficient over numerous generations, driving selection for increased selfing rates, thereby producing spatial variation in pollinator dependency within a species (Kalisz et al. 2004; Moeller and Geber 2005). Naturally available pollinator services may have been very inconsistent in Kunjin reserve, as evidenced by the large variance in seed set from the "open" treatments. When natural levels of pollen deposition were supplemented by hand-pollination, the variance in seed production was reduced substantially (compare 'open' and "open + hand-pollination" treatment for Kunjin; Fig. 2) indicating that our hand-pollination treatment did have an effect for this species. Though more *W. acuminata* individuals were included in the pollen limitation experiment in Bendering (7 study plots) than Kunjin (4 study plots), at Bendering, variation in seed set between individuals receiving 'open' treatments within study plots was consistently lower than in Kunjin (not shown), and thus, this difference was not due to differences in sample size but reflects real differences in seed production between these two sites.

Bendering and Kunjin reserves are highly isolated woodland fragments (55 km apart and 4 km to any other natural remnant) in a matrix of agriculture, it is perhaps not surprising to find evidence of some differentiation in selfing rates within species across this landscape, especially for species that are reliant on

insect pollinators for maximised seed set. Admittedly, determining whether *W. acuminata* populations truly exhibit population-level differences in breeding systems will require tests on genetic differentiation that extend beyond the scope of this study.

Do the native focal species share pollinators with the most common insect-pollinated exotic Asteraceae in this system? How does pollinator visitation to native plant species change along gradients of invasion within remnants?

Exotic Asteraceae appeared to affect pollinator interactions in only one of our focal species, in line with several recent studies showing that pollinator–plant relationships can be resilient to extensive exotic plant invasion (Bartomeus et al. 2008a; Kaiser-Bunbury et al. 2011; Carvalheiro et al. 2014).

We found that insect visitation to *P. gnaphalioides* was less likely in communities with higher relative abundances of exotic Asteraceae plants. This may reflect competition for pollinators between exotic Asteraceae, including *A. calendula*, and *P. gnaphalioides*. Certainly, our results show extensive overlap in the potential pollinators visiting *P. gnaphalioides* and *A. calendula*, and reductions in daily insect visitation rates to *P. gnaphalioides* with increasing proportion cover of exotic Asteraceae flowers (Fig. 4). Although the significant negative relationship between increasing densities of *P. gnaphalioides* flowers and visitation rates also suggests strong intraspecific competition for pollinators among *P. gnaphalioides* individuals, at very low conspecific densities, the benefits of reduced intraspecific competition may be overwhelmed by stronger interspecific competition with *A. calendula*. Thus, individuals of *P. gnaphalioides* will likely have the highest pollination success at low conspecific densities, provided that exotic competitors for pollinators are also few. Nevertheless, changes in pollinator visitation rates do not always affect realised changes in seed production, and competition for pollinators between species may sometimes significantly impact plant fecundity only when available pollinator services are already limiting (Lázaro et al. 2014).

Among the remaining native focal species, neither the relative abundance of exotic Asteraceae plants nor proportion cover of blooming exotic Asteraceae flowers appeared to affect pollinator relationships.

This could be because most native focal species in this study, *G. berardiana*, *P. lessonii* and *R. manglesii*, have flowers or florets that are much smaller or are morphologically distinct from *A. calendula* and most other exotic Asteraceae species in this system; differences that may, in combination with differences in floral rewards, promote visitation by distinct insect taxa. Similarity in floral traits between species has been shown to be more important in eliciting pollinator-mediated interactions than geographic origin (Carvalheiro et al. 2014). Thus, while some studies show that exotic species can significantly impact pollination of co-flowering natives (Dietzsch et al. 2011; Knight et al. 2005; Waters et al. 2014), here we find limited evidence for strong impacts. Furthermore, the observed effects of exotic Asteraceae on visitation in *P. gnaphalioides* may be attributed to superficial similarities in inflorescence colour, floret size, plant size and flowering times (pers. obs), rather than plant species origin per se.

Although analyses reveal that potential pollinators visiting *W. acuminata* were largely distinct from those seen visiting other native species and exotic *A. calendula*, the common visitors to *W. acuminata* appeared to be generalist foragers. The segregation of pollinator assemblages between *W. acuminata* and other focal species may be due in part to differences in flowering phenology, as *W. acuminata* is reliably one of the latest blooming annual species in these woodlands (pers. obs). Common insect visitors of *W. acuminata* were also observed visiting the few individuals of earlier-flowering focal species that persisted late into the season. This may indicate that the delayed flowering of *W. acuminata* provides at least some release from competition for pollinators by more attractive and/or rewarding species that complete flowering earlier in the season. Temporal segregation of flowering to avoid competition for pollinators has been found in other systems as well (Pleasants 1980; Raine et al. 2007). However, the greater variety and distinct assemblage of visitors to *W. acuminata* may also be attributed to temporal differences the reproductive biology of pollinators, which may or may not play a role in pollinator-mediated plant interactions (Olito and Fox 2015).

For *P. gnaphalioides* and *W. acuminata*, we did not find a significant relationship between the total richness of potential pollinators and the extent of local exotic invasion. Other studies have found that

pollinator species richness can remain unchanged even when invasive plant species interfere strongly with pollinator visitation rates (Bartomeus et al. 2008b). Additionally, even where pollinator communities suffer due to an invasive plant species dramatically modifying vegetation structure and local abiotic environments, pollinator richness can rapidly recover once invasion is controlled, while recovery of pollinator abundances can be more gradual (Fiedler et al. 2012). This suggests that although pollinator abundance and visitation rates could be lower in heavily invaded study plots, pollinator community richness may be more resilient to exotic plant invasion than expected. Due to the difficulty of making species-level identifications for even common pollinators (for example, all syrphid flies were regarded as one morphospecies in the field), it was not possible to statistically assess how invasion impacted species-level visitation rates in this study.

Conclusion

This study highlights two mechanisms that appear to contribute to the resilience of pollinator relationships in native plant populations and communities in this extensively fragmented and invaded agricultural landscape. Firstly, our study shows that many common native species in this system utilise distinct pollinator groups, which may allow them to avoid competition for pollinators with introduced exotic species exploiting generalist pollinators. Nevertheless, pollinator-mediated interactions with exotic co-flowering Asteraceae may negatively impact at least one of the native species, *P. gnaphalioides*. By interfering with the reproduction of this common species, pollinator-mediated effects of exotic Asteraceae may exacerbate population declines in heavily invaded communities thus influencing community dynamics. Secondly, alternative mechanisms for seed production such as autonomous self-pollination may ameliorate short-term or localised negative impacts of pollinator limitation caused by competition with exotic species or severe habitat fragmentation. With regard to the latter, it may be that annual native populations in very small and isolated habitat remnants, such as *W. acuminata* in Kunjin reserve, may have, over time, developed an increased capacity for autonomous seed production, resulting in population-level differences

in pollinator dependency between remnants. This study illustrates variability in reproductive strategies within and among annual native insect-pollinated plant species. In spite of apparent resilience in many native plant-pollinator relationships in this system, we found that the influence of exotic plants can extend beyond direct interactions with some native species. Though exotic plants seem to rarely lead to direct declines in seed production in the native species extant in this flora, many other indirect interactions may influence the dynamics of native and exotic plants in this system. The lack of apparent impacts of exotics on the pollination dynamics in this system may also contribute to the explanation as to why so many native species persist in these communities, despite extensive invasion, fragmentation and isolation.

References

Abdala-Roberts L, Marrufo-Zapata D, Arceo-Gómez G, Parra-Tabla V (2014) Pollen limitation, fruit abortion, and autonomous selfing in three populations of the perennial herb *Ruellia nudiflora*. Plant Species Biol 29:25–33. doi:10.1111/j.1442-1984.2012.00392.x

Anderson DR, Burnham KP, Thompson WL (2000) Null hypothesis testing: problems, prevalence, and an alternative. J Wildl Manag 64:912–923. doi:10.2307/3803199

Anderson MJ, Gorley RN, Clarke KR (2008) PERMANOVA + for PRIMER: Guide to software and statistical methods. PRIMER-E, Plymouth

Bartomeus I, Bosch J, Vilà M (2008a) High invasive pollen transfer, yet low deposition on native stigmas in a *Carpobrotus*-invaded community. Ann Bot 102:417–424

Bartomeus I, Vilà M, Santamaría L (2008b) Contrasting effects of invasive plants in plant–pollinator networks. Oecologia 155:761–770. doi:10.1007/s00442-007-0946-1

Bates D, Maechler M (2009) R package: lme4, linear mixed-effects models using S4 classes

Beard JS (1990) Plant life in Western Australia. Kangaroo Press, Kenthurst

Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MHH, White J-SS (2009) Generalized linear mixed models: a practical guide for ecology and evolution. Trends Ecol Evol 24:127–135. doi:10.1016/j.tree.2008.10.008

Burnham KP, Anderson DR (2002) Model selection and multimodel inference: a practical information-theoretic approach. Springer, New York

Carvalheiro LG, Biesmeijer JC, Benadi G et al (2014) The potential for indirect effects between co-flowering plants via shared pollinators depends on resource abundance, accessibility and relatedness. Ecol Lett 17:1389–1399. doi:10.1111/ele.12342

Dietzsch A, Stanley D, Stout J (2011) Relative abundance of an invasive alien plant affects native pollination processes. Oecologia 167:469–479. doi:10.1007/s00442-011-1987-z

Dwyer JM, Hobbs RJ, Wainwright CE, Mayfield MM (2015) Climate moderates release from nutrient limitation in natural annual plant communities. Global Ecolog Biogeogr 24(5):549–561. doi:10.1111/geb.12277

Eaton DAR, Fenster CB, Hereford J, Huang S-Q, Ree RH (2012) Floral diversity and community structure in *Pedicularis* (Orobanchaceae). Ecology 93:S182–S194. doi:10.1890/11-0501.1

Feldman TS, Morris WF, Wilson WG (2004) When can two plant species facilitate each other's pollination? Oikos 105:197–207. doi:10.2307/3547899

Fiedler AK, Landis DA, Arduser M (2012) Rapid shift in pollinator communities following invasive species removal. Restor Ecol 20:593–602. doi:10.1111/j.1526-100X.2011.00820.x

Ghazoul J (2006) Floral diversity and the facilitation of pollination. J Ecol 94:295–304. doi:10.2307/3599633

Gurevitch J, Padilla DK (2004) Are invasive species a major cause of extinctions? Trends Ecol Evol 19:470–474. doi:10.1016/j.tree.2004.07.005

Hester AJ, Hobbs RJ (1992) Influence of fire and soil nutrients on native and non-native annuals at remnant vegetation edges in the Western Australian wheatbelt. J Veg Sci 3:101–108. doi:10.2307/3236003

Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in general parametric models. Biom J 50:346–363. doi:10.1002/bimj.200810425

Hurvich CM, Tsai C-L (1989) Regression and time series model selection in small samples. Biometrika 76:297–307. doi:10.1093/biomet/76.2.297

Jakobsson A, Lázaro A, Totland Ø (2009) Relationships between the floral neighborhood and individual pollen limitation in two self-incompatible herbs. Oecologia 160:707–719. doi:10.2307/40310073

Joar Hegland S, Totland Ø (2008) Is the magnitude of pollen limitation in a plant community affected by pollinator visitation and plant species specialisation levels? Oikos 117:883–891. doi:10.1111/j.0030-1299.2008.16561.x

Kaiser-Bunbury CN, Valentin T, Mougal J, Matatiken D, Ghazoul J (2011) The tolerance of island plant–pollinator networks to alien plants. J Ecol 99:202–213. doi:10.1111/j.1365-2745.2010.01732.x

Kalisz S, Vogler DW, Hanley KM (2004) Context-dependent autonomous self-fertilization yields reproductive assurance and mixed mating. Nature 430:884–887

Karron JD, Thumser NN, Tucker R, Hessenauer AJ (1995) The influence of population density on outcrossing rates in *Mimulus ringens*. Heredity 75:175–180

Kearns CA, Inouye DW (1993) Techniques for pollination biologists. University Press of Colorado, Niwot

Kearns CA, Inouye DW, Waser NM (1998) Endangered mutualisms: the conservation of plant-pollinator interactions. Annu Rev Ecol Syst 29:83–112. doi:10.2307/221703

Kelly D, Ladley JJ, Robertson AW (2007) Is the pollen-limited mistletoe *Peraxilla tetrapetala* (Loranthaceae) also seed limited? Aust Ecol 32:850–857

Kirchner F et al (2005) Effects of local density on insect visitation and fertilization success in the narrow-endemic *Centaurea corymbosa* (Asteraceae). Oikos 111:130–142. doi:10.1111/j.0030-1299.2005.14022.x

Knight TM et al (2005) Pollen Limitation of plant reproduction: pattern and process. Annu Rev Ecol Evol Syst 36:467–497. doi:10.2307/30033813

Lai HR, Mayfield MM, Gay-des-Combes J, Spiegelberger T, Dwyer JM (2015) Distinct invasion strategies operating within a natural annual plant system. Ecol Lett 18:336–346

Law W, Salick J, Knight T (2010) The effects of pollen limitation on population dynamics of snow lotus (*Saussurea medusa and S. laniceps*, Asteraceae): threatened Tibetan medicinal plants of the eastern Himalayas. Plant Ecol 210:343–357. doi:10.1007/s11258-010-9761-6

Lázaro A, Lundgren R, Totland Ø (2014) Experimental reduction of pollinator visitation modifies plant–plant interactions for pollination. Oikos 123:1037–1048. doi:10.1111/oik.01268

Levine JM, Vilà M, D'Antonio CM, Dukes JS, Grigulis K, Lavorel S (2003) Review paper. Mechanisms underlying the impacts of exotic plant invasions. Proc Biol Sci 270:775–781. doi:10.2307/3558605

Moeller DA, Geber MA (2005) Ecological context of the evolution of self-pollination in *Clarkia xantiana*: population size, plant communities, and reproductive assurance. Evolution 59:786–799. doi:10.2307/3449026

Olito C, Fox JW (2015) Species traits and abundances predict metrics of plant–pollinator network structure, but not pairwise interactions. Oikos 124:428–436. doi:10.1111/oik.01439

Pauw A, Bond WJ (2011) Mutualisms matter: pollination rate limits the distribution of oil-secreting orchids. Oikos 120:1531–1538. doi:10.1111/j.1600-0706.2011.19417.x

Pleasants JM (1980) Competition for bumblebee pollinators in Rocky Mountain plant communities. Ecology 61:1446–1459. doi:10.2307/1939053

Primer-E Ltd (2008) Primer 6, version 6.1.11 + PERMANOVA version 1.0.1. Primer-E Ltd., Plymouth

R Development Core Team (2012) R: A language and environment for statistical computing. R foundation for Statistical Computing, Vienna

Raine N, Pierson A, Stone G (2007) Plant–pollinator interactions in a Mexican *Acacia* community. Arthropod-Plant Interactions 1:101–117. doi:10.1007/s11829-007-9010-7

Rosenheim JA, Williams NM, Schreiber SJ (2014) Parental optimism versus parental pessimism in plants: how common should we expect pollen limitation to be? Am Nat 184:75–90. doi:10.1086/676503

Steenhuisen S-L, Johnson SD (2012) Evidence for autonomous selfing in grassland *Protea* species (Proteaceae). Bot J Linn Soc 169:433–446. doi:10.1111/j.1095-8339.2012.01243.x

Steffan-Dewenter I, Munzenberg U, Tscharntke T (2001) Pollination, seed set and seed predation on a landscape scale. Proc R Soc Lond 268:1685–1690. doi:10.1098/rspb.2001.1737

Suter M (2009) Reproductive allocation of *Carex flava* reacts differently to competition and resources in a designed plant

mixture of five species. Plant Ecol 201:481–489. doi:10.1007/s11258-008-9499-6

Wagenmakers E-J, Farrell S (2004) AIC model selection using Akaike weights. Psychon Bull Rev 11:192–196. doi:10.3758/BF03206482

Wang P, Weiner J, Cahill JF, Zhou DW, Bian HF, Song YT, Sheng LX (2014) Shoot competition, root competition and reproductive allocation in *Chenopodium acuminatum*. J Ecol 102:1688–1696. doi:10.1111/1365-2745.12313

Waters SM, Fisher SE, Hille Ris Lambers J (2014) Neighborhood-contingent indirect interactions between native and exotic plants: multiple shared pollinators mediate reproductive success during invasions. Oikos 123:433–440. doi:10.1111/j.1600-0706.2013.00643.x

White EM, Wilson JC, Clarke AR (2006) Biotic indirect effects: a neglected concept in invasion biology. Divers Distrib 12:443–455. doi:10.1111/j.1366-9516.2006.00265.x

Wilcove DS, Rothstein D, Jason D, Phillips A, Losos E (1998) Quantifying threats to imperiled species in the United States. Bioscience 48:607–615. doi:10.2307/1313420