RESEARCH ARTICLE



Temporal introduction patterns of invasive alien plant species to Australia

Brad R. Murray¹, Megan L. Phillips¹

School of the Environment, University of Technology Sydney, PO Box 123, Broadway, NSW 2007, Australia

Corresponding author: Brad R. Murray (Brad.Murray@uts.edu.au)

Academic editor: P. Pyšek | Received 23 November 2011 | Accepted 13 March 2012 | Published 16 May 2012

Citation: Murray BR, Phillips ML (2012) Temporal introduction patterns of invasive alien plant species to Australia. NeoBiota 13: 1–14. doi: 10.3897/neobiota.13.2422

Abstract

We examined temporal introduction patterns of 132 invasive alien plant species (IAPS) to Australia since European colonisation in 1770. Introductions of IAPS were high during 1810–1820 (10 species), 1840– 1880 (51 species, 38 of these between 1840 and 1860) and 1930-1940 (9 species). Conspicuously few introductions occurred during 10-year periods directly preceding each introduction peak. Peaks during early European settlement (1810-1820) and human range expansion across the continent (1840-1860) both coincided with considerable growth in Australia's human population. We suggest that population growth during these times increased the likelihood of introduced plant species becoming invasive as a result of increased colonization and propagule pressure. Deliberate introductions of IAPS (104 species) far outnumbered accidental introductions (28 species) and were particularly prominent during early settlement. Cosmopolitan IAPS (25 species) and those native solely to South America (53 species), Africa (27 species) and Asia (19 species) have been introduced deliberately and accidentally to Australia across a broad period of time. A small number of IAPS, native solely to Europe (5 species) and North America (2 species), were all introduced to Australia prior to 1880. These contrasting findings for native range suggest some role for habitat matching, with similar environmental conditions in Australia potentially driving the proliferation of IAPS native to southern-hemisphere regions. Shrub, tree and vine species dominated IAPS introduced prior to 1840, with no grasses or forbs introduced during early colonisation. Since 1840, all five growth forms have been introduced deliberately and accidentally in relatively large numbers across a broad period of time. In particular, a large number of grass and forb IAPS were deliberately introduced between 1840 and 1860, most likely a direct result of the introduction of legislation promoting intensive agriculture across large areas of the continent. Since the 1980s, only three IAPS have been introduced (all deliberately introduced forbs). The decline in IAPS introductions is most likely a reflection of both increased surveillance and biosecurity efforts and the likelihood that many potential IAPS are still within a pre-expansion lag period.

Copyright B. R. Murray, M. L. Phillips. This is an open access article distributed under the terms of the Creative Commons Attribution License 3.0 (CC-BY), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Keywords

Biological invasions, exotic plants, introduction history, invasion ecology, residence time

Introduction

Comparative analyses of alien plant introductions can pinpoint periods in history when different regions of the world have received particularly high numbers of species (e.g. Pyšek et al. 2003; Pyšek and Jarošík 2005). Such knowledge provides an important historic baseline for determining the factors driving the successful spread of introduced plant species (Phillips et al. 2010a). An understanding of temporal introduction patterns can inform management aimed at limiting the ecological, economic and social impacts of invasive alien plant species (IAPS) on native biodiversity.

Detailed knowledge of alien floras in Europe has led to deep insights into temporal patterns of plant introductions across much of the northern hemisphere (Kühn and Klotz 2003; Pyšek et al. 2005; Křivánek and Pyšek 2006; Chytrý et al. 2008, 2009; Lambdon et al. 2008; Hulme et al. 2009; Štajerová et al. 2009; Knapp et al. 2010; Kubešová et al. 2010; Vilà et al. 2010). At present, however, such detailed insights are less developed for some other regions of the world. In particular, despite a small but important body of work, we are only beginning to establish a comprehensive understanding of temporal patterns in the introduction of IAPS to Australia (e.g. Groves and Hosking 1998; Auld et al. 2003; Groves et al. 2005; Cook and Dias 2006; Caley et al. 2008). Given that recent estimates put the management of IAPS in Australia at more than \$4 billion AUD per year (Sinden et al. 2004), developing our understanding of all aspects of invasion success among alien species in Australia is an imperative.

Permanent settlement of Australia by European colonists began in earnest in 1770. Since then, a total of 26,242 alien plant species have been introduced to Australia, with 2,739 of these species having become naturalized and over 130 now considered IAPS (Randall 2007). Plant species native to most major regions of the world including Asia, Africa, South America, North America and Europe have been introduced to Australia (Harris et al. 2007; Phillips et al. 2010b). In addition, alien species spanning a range of plant growth forms have been introduced both accidentally and deliberately (Groves et al. 2005). Answering the question of how traits of IAPS – such as method of introduction, native range and growth form – relate to interspecific variation in introduction times has the potential to yield important historical insight into the likelihood of successful invasion.

In this study, we compared temporal introduction patterns among IAPS introduced to Australia since European settlement. We related among-species variation in introduction times to the method of introduction of species, their native range and plant growth form. Our aim was to provide a detailed picture of when IAPS with different traits were introduced to Australia and to interpret our findings in the context of drivers of biological invasions and historical events during the development of Australia as a nation.

Methods

We constructed a dataset of 132 IAPS in Australia using the latest compendium of the introduced flora of Australia (Randall 2007). This compendium defines invasive species (category '5A') as those that have been introduced to Australia from elsewhere and have spread rapidly, often forming monocultures and generally having serious high environmental and/or agricultural impacts. We excluded 13 of the '5A' species listed in Randall (2007) from our dataset and included three species not listed as '5A' (*Acaciella angustissima, Asparagus aethiopicus, Senecio angulatus*), with our justification for exclusions and inclusions detailed in Appendix 1.

We accessed the 'Australian Census of Cultivated Plants 2009' (Department of Agriculture, Fisheries and Forestry) to obtain the earliest year each of the study species has been recorded in Australia. The Census contains information gathered from public domain sources including over 600 plant nursery catalogues spanning 200 years, botanical and major garden plant species lists, Australian Quarantine and Inspection Service (AQIS) permitted import list, State department vegetation surveys and Commonwealth lists of imported species (R. Ingram, personal communication). We refer to the earliest year on record for each species as year of introduction. Given the high profile of the invasive species in our dataset, the extensive research, information and first-hand knowledge available in the literature for these species, we are confident that earliest records are tightly linked to actual introduction years.

We performed a literature search involving published papers, reports and government websites seeking information on the IAPS covering method of introduction to Australia, native geographic range and plant growth form. Method of introduction was recorded as either deliberate (e.g. ornamentals, forage or grafting plants, lawn species) or accidental (no known purposeful reason for introduction). The native range of each species was categorized as a single geographic region (Africa, Asia, Europe, North America, South America) or as cosmopolitan (i.e. the native range covered more than a single region). Each of the IAPS was placed into one of the following plant growth form categories: (1) Grass - monocotyledonous graminoids with narrow leaves growing from the base including Poaceae (Gramineae), Cyperaceae and Juncaceae; (2) Forb – self-supporting plants that have no woody stem above ground, with leaves and stems that may die down at the end of the growing season to the soil level; (3) Shrub – self-supporting plants that can produce a woody stem aboveground less than 6 m tall; (4) Tree – self-supporting plants with a woody stem more than 6 m tall; and (5) Vine – herbaceous or woody plants that climb or spread vertically or horizontally. Vines are usually considered as plants that must rely on external support to attain height. However, we classified other similar plants such as scramblers and rambling shrubs (which do not always require additional structural support) as one functional group (vines), consistent with Australia's first exotic vine inventory (Harris et al. 2007), as all of these species behave and alter the environments they invade in similar ways, such as climbing over and smothering vegetation.

Results

Numbers of IAPS introduced to Australia have varied substantially over the years since European settlement (Fig. 1). Introduction numbers were comparatively high from 1810 to 1820 (10 species), 1840 to 1880 (51 species) and 1930 to 1940 (9 species). The 1840 to 1880 period was characterised by an especially high number of introductions between 1840 and 1860 (38 species). In contrast, low introduction numbers occurred during 10-year periods directly preceding each of the introduction peaks, with three IAPS introduced between 1800 and 1810, none introduced between 1830 and 1840 and one introduced between 1920 and 1930. Notably, only three IAPS have been introduced to Australia since 1980.

Deliberate introductions of IAPS (104 species) began in 1770 and have continued to the present day (Fig. 1), far outnumbering accidental introductions (28 species). Indeed, deliberate introductions were particularly prominent during early settlement with no IAPS introduced accidentally either prior to 1840, or in fact since 1970. Generally, species native to all geographic regions have been introduced deliberately (Fig. 2a) and accidentally (Fig. 2b) to Australia across a broad period of time, with no particular region dominating any of the introduction peaks. The native ranges of the majority of IAPS extend over a single geographic region, with most species native to South America (53 species), Africa (27 species) and Asia (19 species). Interestingly, only five species were found to be native solely to Europe and only two species native solely to North America, and all seven were introduced prior to 1880. A total of 25 IAPS were found to be cosmopolitan (Table 1), all introduced across a broad period of time (Fig. 2).



Figure 1. Frequency distribution of introduction times of invasive alien plant species in Australia in relation to method of introduction (accidental or deliberate).



Figure 2. Frequency distributions of **a** deliberate and **b** accidental introduction times of invasive alien plant species in Australia in relation to native geographic range. *Rubus fruticosus* is not included in (**a**) as it is an aggregate species consisting of 14 closely-related species (some of which may be hybrids) from a range of different regions.

Shrub, tree and vine species dominated IAPS introduced (all deliberately) prior to 1840, with no grasses or forbs introduced during early colonisation (Fig. 3). Since 1840, all five growth forms have been introduced deliberately (Fig. 3a) and accidentally (Fig. 3b) in relatively large numbers across a broad period of time. One par-

Species	Introduction	Native range
Acaciella angustissima	Deliberate	North America, South America
Andropogon virginicus	Accidental	North America, South America
Annona glabra	Deliberate	Africa, North America, South America
Arundo donax	Deliberate	Asia, Europe
Baccharis halimifolia	Deliberate	North America, South America
Cardiospermum grandiflorum	Deliberate	Africa, North America, South America
Cenchrus ciliaris	Accidental	Africa, Asia
Gloriosa superba	Deliberate	Africa, Asia
Hydrocotyle bonariensis	Accidental	Africa, North America, South America
Ilex aquifolium	Deliberate	Africa, Asia, Europe
Ipomoea cairica	Deliberate	Africa, Asia
Juncus articulatus	Accidental	Africa, Asia, Europe, North America
Macroptilium atropurpureum	Accidental	North America, South America
Neonotonia wightii	Deliberate	Africa, Asia
Olea europaea	Deliberate	Africa, Asia, Europe
Opuntia imbricata	Deliberate	North America, South America

Table 1. Cosmopolitan species, their method of introduction and native geographic ranges.

Parietaria judaica	Accidental	Africa, Asia, Europe
Parkinsonia aculeata	Deliberate	North America, South America
Parthenium hysterophorus	Accidental	North America, South America
Phyla canescens	Deliberate	North America, South America
Prunus cerasus	Accidental	Asia, Europe
Ricinus communis	Deliberate	Africa, Asia, Europe
Salvia coccinea	Deliberate	North America, South America
Tamarix aphylla	Deliberate	Africa, Asia
Verbesina encelioides	Accidental	North America, South America



Figure 3. Frequency distributions of **a** deliberate and **b** accidental introduction times of invasive alien plant species in Australia in relation to plant growth form.

ticular growth-form pattern that emerged showed that a comparatively large number of grasses and forbs were deliberately introduced between 1840 and 1880 and since then (up until 1970) accidental introductions of grasses and forbs have been especially prominent. The small number of IAPS introduced since 1980 have all been forbs.

Discussion

We identified three distinct periods in Australia's recent history when introductions of IAPS were particularly high. We describe a simple null model that relates increased introductions of IAPS to increases in both 'colonization pressure' and 'propagule pressure' (*sensu* Lockwood et al. 2009). For colonization pressure, increased numbers of IAPS introductions under our model are linked to increases in the total number of all alien plant species introduced during peak IAPS periods. Here, the introduction of large numbers of alien plant species raises the likelihood that more invasive species will emerge. Empirical evidence for this idea is scarce, however, recent work in Europe provides some support for our null model. Chytrý et al. (2012) have shown that areas predicted to have an increase in alien species in projected models for future land-use change will also most likely harbour more serious invaders. For propagule pressure, increased IAPS introductions under our model are linked to increases in the number of individuals of each species in each 'release' event and the number of discrete release events. Previous studies provide some support for our null model, with findings in other systems that propagule pressure is a key driver of both establishment success (Lockwood et al. 2005) and invasive spread (Colautti et al. 2006; Johnston et al. 2009) in a range of taxa and across a variety of geographic regions.

For Australia, as is common in many retrospective studies of invasion (Pyšek et al. 2010), data on colonization pressure and propagule pressure for IAPS are hard to come by (but see, for instance, Cassey et al. 2004). To test our null model and in the absence of such data, future studies might explore proxies of these pressures. For example, the total number of recorded alien plant species introduced to Australia from different regions of the world could act as a proxy for colonization pressure (e.g. Phillips et al. 2010a), while occurrence in nursery catalogues could act as a proxy for propagule pressure (e.g. Pemberton and Liu 2009). Previous studies have used measures of human population size as indicators of these pressures and related them to the presence of alien species (Lonsdale 1999; McKinney 2002; Pyšek et al. 2002; Essl et al. 2011). Effectively, increased colonization and propagule pressure of alien plant species is predicted to be a function of human population size. Simply, more people transport more species (colonization pressure) and more individuals of the same species (propagule pressure) by either bringing them into a country or by spreading them around a country.

Support for a link between human population size and the introduction of IAPS in Australia might be obtained if substantial increases in Australia's population coincided with increased introductions of IAPS. We accessed data from the Australian Bureau of Statistics, specifically the database 'Australian Historical Population Statistics 2008' showing changes in Australia's population over time (http://www.abs.gov.au, accessed February 2012). We compared increases in both IAPS and Australia's population during both peaks, and importantly, during periods of low introductions of IAPS directly prior to the peaks. We found that peaks during early European colonization (1810– 1820) and human range expansion across the continent (1840-1860) both coincided with considerable growth in Australia's human population (Fig. 4). The introduction lows in the preceding periods were associated with comparatively smaller increases in Australia's population. These correlated events provide support for the idea that human population increase could be a substantial driver of increased introductions of IAPS via increased colonization and propagule pressure.

Interestingly, unlike the first two peaks in IAPS, the third peak (1930-1940) did not coincide with a marked increase in Australia's population (Fig. 4). During the preceding period (1920-1930), there was a much larger increase in Australia's



Figure 4. Increases in IAPS (numbers of species above arrows) and Australia's human population (number of people below arrows) in relation to time (years shown between arrows) during **a** early European colonization **b** human range expansion and **c** pre-World War II.

population (c. 1 million people) but only one IAPS was introduced. Nevertheless, Australia's population still increased by half-a-million people between 1930 and 1940, which could still explain to some extent the peak in IAPS. The significant low in IAPS introductions between 1920 and 1930 might be a result of economic difficulties during the Great Depression during the 1920s. At such a time, costs associated with importing species might have been avoided. It is also likely that during such financially challenging times that hobbies such as gardening would not have been a high priority, potentially reducing the influx of ornamental plants. Perhaps the subsequent peak in introductions may be accounted for by more targeted introductions of species during pre-World War II immigration to Australia during the 1930s. A goal of future work will be to unravel the pathways of introduction of IAPS, particularly during the 1930-1940

peak, to determine why IAPS introductions peaked during a time when Australia's population showed a comparatively smaller increase.

What explains the predominance of IAPS in Australia that are native to the southern hemisphere? This finding suggests that invaders from other continents might possess a degree of climatic pre-adaptation or habitat matching that facilitates invasiveness (e.g. see Thuiller et al. 2005; Pyšek et al. 2009). Similar biotic and abiotic conditions in southern-hemisphere regions might underpin the proliferation of IAPS from these regions in Australia. Recent work on 26 plant species introduced to Australia has shown that alien species are able to occupy climate niches in their new range that differ substantially from those of their native range (Gallagher et al. 2010). This suggests that climatic pre-adaptation might not be so important for IAPS in Australia, and in fact points to other potential explanations. For instance, introduction-history features (e.g. colonization and propagule pressures) may be stronger in species from these regions because of their geographic proximity (Pyšek et al. 2004). Nevertheless, it is important to note that more than half of the 26 species examined in Gallagher et al. (2010) are not currently considered nationally invasive (category '5A' in Randall 2007); thus, a habitat-matching mechanism is still potentially important for IAPS in Australia.

Our work has shown that most IAPS were deliberately introduced to Australia. This is not surprising, with previous studies documenting the importance of deliberate introductions in the increase in alien plant species in Australia (Kloot 1987; Carr 1993; Groves et al. 2005). We also know that the proportion of deliberate to accidental introductions does not differ significantly between the pool of IAPS and the pool of naturalized non-invasive alien plant species in Australia (Phillips et al. 2010b). Although deliberate introductions dominate the pool of IAPS, this does not mean that alien plant species are any more likely to become invasive via this method of introduction. Interestingly, we found that deliberate introduced accidentally prior to 1840. Species were introduced deliberately by early British colonists most likely as garden ornamentals (*Cytisus scoparius, Ipomoea indica*), food for people and fodder for animals (*Ulex europaeus* and *Opuntia monacantha*, the latter also probably used to make whiskey) and for use in health and medicine (e.g. castor oil from *Ricinus communis*).

No grass or forb IAPS were introduced during early colonisation but a comparatively large number of these were deliberately introduced between 1840 and 1860. The later introduction of forb and grass IAPS is associated with a period of time in Australia's history when land 'selection' became prominent. Selection allowed settlers to have free choice of government land in some Australian colonies under land legislation acts introduced in the 1860s (Roberts 1924). These acts provided opportunities for intensive agricultural productivity by settlers with limited financial means. As such, much land was opened up to farming and in the process many grasses currently recognized as IAPS were introduced both deliberately and accidentally. In fact, since 1860 and up until 1970, accidental introductions of grasses and forbs have been especially prominent. These growth forms are especially prone to accidental introduction due to their prolific production of many minute seeds, often which have specific adhesive adaptations like awns, hairs and spines

that can stick to animal coats and clothing (Groves et al. 2005). Some species, including *Cenchrus ciliaris* which arrived in Australia in 1875, were initially recorded as accidental introductions to Australia, but were later purposefully spread throughout the country for various reasons such as for use as fodder crops and land stabilisation (Humphreys 1967).

Since the 1980s, only three IAPS have been introduced (all deliberately introduced forbs). The decline in IAPS introductions is most likely due to two factors. First, increased surveillance and biosecurity efforts have been successful in controlling the influx of alien species likely to become IAPS. For example, accidental introductions are much less likely due to seed cleaning techniques and quarantine services ensuring minimal contamination (Mack and Lonsdale 2001). Second, some potential IAPS might still be within a pre-expansion lag period (they are still 'sleeping', Groves 2006). For instance, there may not yet have been enough time to register the spread and impacts of alien species with long juvenile periods as IAPS (e.g. Auld et al. 2003). However, Daehler (2009) has recently reported much shorter lag times for long-lived species than previously estimated from indirect observations. Although the study was based on tropical species, it does provide tantalizing evidence that lag times may not be as long as generally thought, and that current biosecurity efforts halting the introduction of potentially serious invasive plant species are effective.

Acknowledgements

We thank the members of the Biodiversity Research Group at UTS, Carl Arbegast, Petr Pyšek and an anonymous reviewer for providing helpful comments on a draft of the paper. We are grateful to Robert Ingram of the Department of Agriculture, Fisheries and Forestry for kindly providing access to the Census of Cultivated Plants 2009. This paper is dedicated to the memory of Ivy Murray.

References

- Auld B, Morita H, Nishida T, Ito M, Michael P (2003) Shared exotica: Plant invasions of Japan and south eastern Australia. Cunninghamia 8: 147–152.
- Caley P, Groves RH, Barker R (2008) Estimating the invasion success of introduced plants. Diversity and Distributions 14: 196–203. doi: 10.1111/j.1472-4642.2007.00440.x
- Carr GW (1993) Exotic flora of Victoria and its impact on indigenous biota. In: Foreman DB, Walsh NG (Eds) Flora of Victoria, Vol. 1, Introduction, Inkata Press, Melbourne, 256–297.
- Cassey P, Blackburn TM, Sol D, Duncan RP, Lockwood JL (2004) Global patterns of introduction effort and establishment success in birds. Proceedings of the Royal Society of London B 272: S405-S408. doi: 10.1098/rsbl.2004.0199

- Chytrý M, Maskell LC, Pino J, Pyšek P, Vilà M, Font X, Smart SM (2008) Habitat invasions by alien plants: a quantitative comparison among Mediterranean, subcontinental and oceanic regions of Europe. Journal of Applied Ecology 45: 448–458. doi: 10.1111/j.1365-2664.2007.01398.x
- Chytrý M, Pyšek P, Wild J, Maskell LC, Pino J, Vilà M (2009) European map of alien plant invasions, based on the quantitative assessment across habitats. Diversity and Distributions 15: 98–107. doi: 10.1111/j.1472-4642.2008.00515.x
- Chytrý M, Wild J, Pyšek P, Jarošík V, Dendoncker N, Reginster I, Pino J, Maskell LC Vilà M, Pergl J, Kühn I, Spangenberg JH, Settele J (2012) Projecting trends in plant invasions in Europe under different scenarios of future land-use change. Global Ecology and Biogeography 21: 75–87. doi: 10.1111/j.1466-8238.2010.00573.x
- Colautti RI, Grigorovich IA, MacIsaac HJ (2006) Propagule pressure: a null model for biological invasions. Biological Invasions 8: 1023–1037. doi: 10.1007/s10530-005-3735-y
- Cook GD, Dias L (2006) It was no accident: deliberate plant introductions by Australian government agencies during the 20th century. Australian Journal of Botany 54: 601–625. doi: 10.1071/BT05157
- Daehler CC (2009) Short lag times for invasive tropical plants: evidence from experimental plantings in Hawai'i. PLoS ONE 4: e4462. doi: 10.1371/journal.pone.0004462
- Essl F, Dullinger S, Rabitsch W, Hulme PE, Hülber K, Jarošík V, Kleinbauer I, Krausmann F, Kühn I, Nentwig W, Vilà M, Genovesi P, Gherardil F, Desprez-Loustau M-L, Roques A, Pyšek P (2011) Socioeconomic legacy yields an invasion debt. Proceedings of the National Academy of Sciences USA 108: 203–207. doi: 10.1073/pnas.1011728108
- Gallagher RV, Beaumont LJ, Hughes L, Leishman MR (2010) Evidence for climatic niche and biome shifts between native and novel ranges in plant species introduced to Australia. Journal of Ecology 98: 790–799. doi: 10.1111/j.1365-2745.2010.01677.x
- Groves RH (2006) Are some weeds sleeping? Some concepts and reasons. Euphytica 148: 111–120. doi: 10.1007/s10681-006-5945-5
- Groves RH, Hosking JR (1998) Recent Incursions of Weeds to Australia 1971–1995. Cooperative Research Centre for Weed Management Systems, Technical Series No. 3, Adelaide.
- Groves RH, Boden R, Lonsdale M (2005) Jumping the Garden Fence: Invasive Garden Plants in Australia and Their Environmental and Agricultural Impacts. WWF-Australia, Ultimo.
- Harris CJ, Murray BR, Hose GC, Hamilton MA (2007) Introduction history and invasion success in exotic vines introduced to Australia. Diversity and Distributions 13: 467–75. doi: 10.1111/j.1472-4642.2007.00375.x
- Hulme P, Pyšek P, Nentwig W, Vilà M (2009) Will threat of biological invasions unite the European Union? Science 324: 40–41. doi: 10.1126/science.1171111
- Humphreys LR (1967) Buffel Grass (*Cenchrus ciliaris*) in Australia. Tropical Grasslands 1: 123–134.
- Johnston EL, Piola RF, Clark GF (2009) The role of propagule pressure in invasion success. In: Rilov G, Crooks JA (Eds) Biological Invasions in Marine Ecosystems, Springer-Verlag, Berlin, 133–151. doi: 10.1007/978-3-540-79236-9_7

- Kloot PM (1987) The naturalised flora of South Australia. 3. Its origin, introduction, distribution, growth forms and significance. Journal of the Adelaide Botanic Gardens 10: 99–111.
- Knapp S, Kühn I, Stolle J, Klotz S (2010) Changes in the functional composition of a Central European urban flora over three centuries. Perspectives in Plant Ecology, Evolution and Systematics 12: 235–244. doi: 10.1016/j.ppees.2009.11.001
- Křivánek M, Pyšek P (2006) Predicting invasions by woody species in a temperate zone: a test of three risk assessment schemes in the Czech Republic (Central Europe). Diversity and Distributions 12: 319–327. doi: 10.1111/j.1366-9516.2006.00249.x
- Kubešová M, Moravcová L, Suda J, Jarošík V, Pyšek P (2010) Naturalized plants have smaller genomes than their non-invading relatives: a flow cytometric analysis of the Czech alien flora. Preslia 82: 81–96.
- Kühn I, Klotz S (2003) The alien flora of Germany: basics from a new German database. In: Child LE, Brock JH, Brundu G, Prach K, Pyšek P, Wade PM, Williamson M (Eds) Plant Invasions: Ecological Threats and Management Solutions, Backhuys, Leiden, 89–100.
- Lambdon PW, Pyšek P, Basnou C, Hejda M, Arianoutsou M, Essl F, Jarošík V, Pergl J, Winter M, Anastasiu P, Andriopoulos P, Bazos I, Brundu G, Celesti-Grapow L, Chassot P, Delipetrou P, Josefsson M, Kark S, Klotz S, Kokkoris Y, Kühn I, Marchante H, Perglová I, Pino J, Vilà M, Zikos A, Roy DB, Hulme PE (2008) Alien flora of Europe: species diversity, temporal trends, geographical patterns and research needs. Preslia 80: 101–149.
- Lockwood JL, Cassey P, Blackburn T (2005) The role of propagule pressure in explaining species invasions. Trends in Ecology & Evolution 20: 223–228. doi: 10.1016/j.tree.2005.02.004
- Lockwood JL, Cassey P, Blackburn TM (2009) The more you introduce the more you get: the role of colonization pressure and propagule pressure in invasion ecology. Diversity and Distributions 15: 904–910. doi: 10.1111/j.1472-4642.2009.00594.x
- Lonsdale WM (1999) Global patterns of plant invasions and the concept of invisibility. Ecology 80: 1522–1536. doi: 10.1890/0012-9658(1999)080[1522:GPOPIA]2.0.CO;2
- Mack RN, Lonsdale WM (2001) Humans as global plant dispersers: getting more than we bargained for. Bioscience 51: 95–102. doi: 10.1641/0006-3568(2001)051[0095:HAGP DG]2.0.CO;2
- McKinney ML (2002) Influence of settlement time, human population, park shape and age, visitation and roads on the number of alien plant species in protected areas in the USA. Diversity and Distributions 8: 311–318. doi: 10.1046/j.1472-4642.2002.00153.x
- Pemberton RW, Liu H (2009) Marketing time predicts naturalization of horticultural plants. Ecology 90: 69–80. doi: 10.1890/07-1516.1
- Phillips ML, Murray BR, Pyšek P, Pergl J, Jarošík V, Chytrý M, Kühn I (2010a) Plants species of the Central European flora as aliens in Australia. Preslia 82: 465–482.
- Phillips ML, Murray BR, Leishman MR, Ingram R (2010b) The naturalisation to invasion transition: Are there introduction-history correlates of invasiveness in exotic plants of Australia? Austral Ecology 35: 695–703. doi: 10.1111/j.1442-9993.2009.02076.x
- Pyšek P, Jarošík V (2005) Residence time determines the distribution of alien plants. In: Inderjit (Ed) Invasive plants: ecological and agricultural aspects, Birkhäuser Verlag-AG, Basel, 77–96.

- Pyšek P, Jarošík V, Chytrý M, Kropáč Z, Tichý L, Wild J (2005) Alien plants in temperate weed communities: prehistoric and recent invaders occupy different habitats. Ecology 86: 772–785. doi: 10.1890/04-0012
- Pyšek P, Jarošík V, Hulme PE, Kühn I, Wild J, Arianoutsou M, Bacher S, Chiron F, Didžiulis V, Essl F, Genovesi P, Gherardi F, Hejda M, Kark S, Lambdon PW, Desprez-Loustau A-M, Nentwig W, Pergl J, Poboljšaj K, Rabitsch W, Roques A, Roy DB, Solarz W, Vila M, Winter M (2010) Disentangling the role of environmental and human pressures on biological invasions across Europe. Proceedings of the National Academy of Sciences USA 107: 12157–12162. doi: 10.1073/pnas.1002314107
- Pyšek P, Jarošík V, Kučera T (2002) Patterns of invasion in temperate nature reserves. Biological Conservation 104: 13–24. doi: 10.1016/S0006-3207(01)00150-1
- Pyšek P, Jarošík V, Pergl J, Randall R, Chytrý M, Kühn I, Lubomír Tichý, Danihelka J, Chrtek J jun, Sádlo J (2009) The global invasion success of Central European plants is related to distribution characteristics in their native range and species traits. Diversity and Distributions 15: 891–903. doi: 10.1111/j.1472-4642.2009.00602.x
- Pyšek P, Richardson DM, Williamson M (2004) Predicting and explaining plant invasions through analysis of source area floras: some critical considerations. Diversity and Distributions 10: 179–187. doi: 10.1111/j.1366-9516.2004.00079.x
- Pyšek P, Sádlo J, Mandák B, Jarošík V (2003) Czech alien flora and a historical pattern of its formation: what came first to Central Europe? Oecologia 135: 122–130.
- Randall RP (2007) The introduced flora of Australia and its weed status. CRC for Australian Weed Management, Department of Agriculture and Food, Western Australia, University of South Australia, Adelaide.
- Roberts SH (1924) History of Australian Land Settlement, 1788–1920. Melbourne University Press, Melbourne.
- Sinden J, Jones R, Hester S, Odom D, Kalisch C, James R, Cacho O (2004) The economic impact of weeds in Australia, Technical Series no. 8, CRC for Australian Weed Management, Australia.
- Štajerová K, Šmilauerová M, Šmilauer P (2009) Arbuscular mycorrhizal symbiosis of herbaceous invasive neophytes in the Czech Republic. Preslia 81: 341–355.
- Thuiller W, Richardson DM, Pyšek P, Midgley GF, Hughes GO, Rouget M (2005) Nichebased modelling as a tool for predicting the risk of alien plant invasions at a global scale. Global Change Biology 11: 2234–2250. doi: 10.1111/j.1365-2486.2005.001018.x
- Vilà M, Basnou C, Pyšek P, Josefsson M, Genovesi P, Gollasch S, Nentwig W, Olenin S, Roques A, Roy D, Hulme PE, DAISIE partners (2010) How well do we understand the impacts of alien species on ecological services? A pan-European cross-taxa assessment. Frontiers in Ecology and the Environment 8: 135–144. doi: 10.1890/080083

Appendix I

Invasive ('5A') taxa in Randall (2007) that were excluded from our study and the reason for their exclusion.

Taxa	Reason for exclusion
Acacia boliviana	Non-current synonym for <i>Acaciella angustissima</i> which is not listed at all in Randall (2007); <i>Acaciella angustissima</i> is included in our dataset given the 5A status of <i>Acacia boliviana</i>
Crocosmia X crocosmiiflora	An engineered horticultural hybrid, thus quite distinct and not comparable to all other invasive species in our dataset
Myrsiphyllum asparagoides	Non-current synonym for <i>Asparagus asparagoides</i> which is listed as 5A in Randall (2007) and already in our dataset
Panicum maxiumum	Not listed as naturalized (N) in Randall (2007) and no literature evidence for naturalized status; our dataset contains species that are both naturalized and invasive
Pinus elliotii	A gymnosperm, our dataset contains angiosperms only
Pinus radiata	A gymnosperm, our dataset contains angiosperms only
Protasparagus aethiopicus	Non-current synonym for <i>Asparagus aethiopicus</i> which is not listed as 5A in Randall (2007); <i>Asparagus aethiopicus</i> is included in our dataset given the 5A status of <i>Protasparagus aethiopicus</i>
Protasparagus plumosus	Non-current synonym for <i>Asparagus plumosus</i> which is listed as 5A in Randall (2007) and already in our dataset
Salvinia molesta	A fern, our dataset contains angiosperms only
Senecio tamoides	A taxonomic misapplication of <i>Senecio angulatus</i> which is not listed as 5A in Randall (2007); <i>Senecio angulatus</i> is included in our dataset given the 5A status of <i>Senecio tamoides</i>
Sporobolus pyramidalis	Not listed as naturalized (N) in Randall (2007) and no literature evidence for naturalized status; our dataset contains species that are both naturalized and invasive
Tradescantia albiflora	Non-current synonym for <i>Tradescantia fluminensis</i> which is listed as 5A in Randall (2007) and already in our dataset
Turnera subulata	Not listed as naturalized (N) in Randall (2007) and no literature evidence for naturalized status; our dataset contains species that are both naturalized and invasive

RESEARCH ARTICLE



Sources and modes of action of invasive knotweed allelopathy: the effects of leaf litter and trained soil on the germination and growth of native plants

Madalin Parepa¹, Urs Schaffner², Oliver Bossdorf¹

1 University of Bern, Institute of Plant Sciences, Altenbergrain 21, CH-3013 Bern, Switzerland **2** CABI Europe Switzerland, Rue des Grillons 1, CH-2800 Delémont, Switzerland

Corresponding author: Madalin Parepa (parepa@ips.unibe.ch)

Academic editor: I. Kühn | Received 29 February 2012 | Accepted 6 March 2012 | Published 16 May 2012

Citation: Parepa M, Schaffner U, Bossdorf O (2012) Sources and modes of action of invasive knotweed allelopathy: the effects of leaf litter and trained soil on the germination and growth of native plants. NeoBiota 13: 15–30. doi: 10.3897/ neobiota.13.3039

Abstract

Invasive knotweeds, native to Eastern Asia, are among the most dominant plant invaders of European and North American temperate ecosystems. Recent studies indicate that one cause of this dominance might be allelopathy, but the possible sources and modes of action of this allelopathy are insufficiently understood. Here, we asked whether the invasive knotweed *Fallopia* × *bohemica* can exert allelopathic effects on native plants also through its leaf litter, or through persistent soil contaminants, and whether these affect the germination or growth of native plants. In a germination experiment with nine native species neither litter leachate, an aqueous extract of knotweed leaves added to the soil, nor trained soil with a history of Fallopia pre-cultivation suppressed the germination or early growth of natives. A mesocosm study with experimental native communities showed that the presence of *E* × *bohemica*, although not a dominant in these communities, caused significant shifts of life-history strategy in two dominant natives, and that similar effects on the biomass of natives. Our study indicates that knotweed allelopathy acts on the growth rather than germination of natives, and that soil contamination through persistent allelochemicals may not be a significant problem in habitat restoration. It also shows that allelopathic effects can sometimes be subtle changes in life-history and allocation patterns of the affected species.

Keywords

Biological invasions, Fallopia × bohemica, litter leachate, plant-plant interactions, soil training

Introduction

Explaining the success of highly invasive plants requires a solid understanding of the mechanisms by which they interfere with native competitors. Oftentimes, invasive plants are superior resource competitors (Grotkopp et al. 2002, Richards et al. 2006, van Kleunen et al. 2010), i.e., they are able to utilize resources quicker (Dawson et al. 2011) or more efficiently than native plants (Funk and Vitousek 2007), or deplete them to a lower level. Still, recent research indicates that interference between invasive and native plants can be more complex than previously thought (Mitchell et al. 2006) and may involve the exudation of allelopathic substances as well as interactions with the soil community (Callaway and Aschehoug 2000, Callaway et al. 2008).

Allelopathy is the chemically mediated interference between co-occurring plants, where secondary compounds exuded by plant roots or leaves affect the germination or growth of neighbouring plants (Inderjit and Nielsen 2003). These effects can be direct, or they can be indirect, mediated by changes in mycorrhiza, bacteria or other soil biota (Mangla et al. 2008, Kaur et al. 2009). If allelochemicals are leaching from plant leaves, the presence of litter alone may exert allelopathic effects on other plants (e.g., Olson and Wallander 2002, Dorning and Cipollini 2006). Likewise, if allelochemicals persist in the soil, the presence of such contaminated soil alone may cause inhibition of other plants (Prati and Bossdorf 2004, Stinson et al. 2006). Although many examples exist of inhibitory effects of plants, plant leachates or extracts on other plants, the rigorous demonstration of allelopathy is not a trivial task and requires careful experimentation (Inderjit and Callaway 2003, Lau et al. 2008).

One of the most aggressive and at the same time least understood group of plant invaders are the clonal knotweeds *Fallopia japonica* and *F. sachalinensis*, and their hybrid *F.* × *bohemica*. Originally introduced from Eastern Asia as ornamentals (Bailey and Connolly 2000), these species have become major environmental threats in the temperate zones of Europe and North America and are now considered among the world's worst invasive species (Lowe et al. 2000). Invasive knotweeds are highly successful competitors that often form dense monocultures in their introduced range. They rapidly regenerate from rhizome fragments and are almost impossible to get rid off once established (Child and Wade 2000). In spite of their spectacular vigour and noxiousness (Aguilera et al. 2010), the reasons for the competitive success and dominance of these species are still not well understood (Weston et al. 2005).

Previous studies have shown that invasive knotweeds contain several potentially allelopathic compounds (Inoue et al. 1992, Vrchotová and Sera 2008, Fan et al. 2009, Moravcová et al. 2011), and it has been demonstrated in field (Siemens and Blossey 2007) or pot experiments (Murrell et al. 2010) that invasive knotweeds can indeed exerts allelopathic effects on the natives. However, all previous studies tested the effects of living knotweed plants, whereas other possible sources of allelopathy, such as leaf litter or trained soil, and their modes of action, were never tested. If knotweed allelopathy can also act from these other sources, this would have important implications for habitat restoration, and it is therefore important to test them in an ecologically meaningful set-up.

Here, we investigated the effects of *Fallopia* × *bohemica* litter leachates and trained soil on a range of native European plant species. We carried out two experiments, one in which we tested for allelopathic effects on the germination and establishment of individual plants, and a second one in which we examined allelopathic effects on experimental communities of several native species. Specifically, we tested the following hypotheses: (1) Litter leachates of *F. × bohemica* reduce the germination and growth of native plants. (2) Native plants germinate and grow less well on soils with a history of *F. × bohemica*.

Materials and methods

Plant materials and soil treatments

Fallopia × bohemica (Bohemian knotweed) is a hybrid between *Fallopia japonica* (Japanese knotweed) and *Fallopia sachalinensis* (Giant knotweed), two tall perennial *Polygonaceae* which were introduced to Europe from Eastern Asia at the beginning of 19th century as ornamentals. Although both parental species are well-known for their vigorous growth and clonal spread, the hybrid appears to even surpass the vigour and rate of spread of its parents (Mandak et al. 2004), and it is expected to eventually become the most abundant and problematic of the invasive knotweeds.

We used plant material from an invasive population of $F. \times$ bohemica (hereafter *Fallopia*) located along the river Birs, close to Delémont, Switzerland (47°22.29'N, 7°21.26'E). This population has already served as a source of plant material for previous studies (e.g. Murrell et al. 2011), and its hybrid identity has been verified through molecular methods (Krebs et al. 2010).

To create a litter leachate of *Fallopia* litter we followed the methods of Dorning and Cipollini (2006), using a tissue-to-volume ratio of 0.1 g/mL, which was shown to be effective in previous studies. We soaked 10 kg of fresh litter in 100 L water for 72h and filtered the liquid through a coarse sieve. The litter was collected and immediately frozen in the fall of 2008, and the leachates were prepared right before the start of the experiments in 2009.

To test for possible allelopathic effects of persistent soil contaminants of *Fallopia*, we used the soil training approach (e.g., Bever 2003, Callaway et al. 2004). In September 2008 we planted one large *Fallopia* rhizome (300-800g fresh weight) into ten 20-L plastic containers filled with a 1:1 mixture of sand and fresh field soil (RICO-TER Erdaufbereitung AG, Aarberg, Switzerland). In the beginning of June 2009, we removed the growing plants, sieved the substrate, and mixed it with an equal amount of fresh sand:soil mixture.

We selected nine native plant species as targets: two grasses (*Lolium perenne*, *Poa trivialis*) and seven forbs (*Filipendula ulmaria*, *Geranium robertianum*, *Geum urbanum*, *Glechoma hederacea*, *Silene dioica*, *Symphytum officinale* and *Urtica dioica*). All of these species commonly occur in habitats invaded by knotweed (Gerber at al. 2008). We used seed material from a regional supplier of wild-collected seeds (Rieger-Hofmann GmbH, Blaufelden-Raboldshausen, Germany).

Germination experiment

To investigate potential allelopathic effects of litter leachates and trained soil on the germination of the target species, we carried out a germination experiment in a greenhouse. In June 2009 we sowed seeds of each of the nine native species in 1-L pots filled with three different substrates: (1) a 1:1 mixture of sand and field soil, (2) the same mixture, but with litter leachate added twice (50 mL/pot each time), before and three days after sowing, and (3) the trained soil described above. To half of the pots in each substrate treatment we added activated carbon (Charcoal Activated, Merck KGA, Darmstadt, Germany) at a concentration of 20 mL/L. Activated carbon (AC) has a high capacity to adsorb organic compounds, and it can therefore be used to test for the presence of allelochemicals in the soil (e.g. Callaway and Aschehoug 2000, Inderjit and Callaway 2003, Prati and Bossdorf 2004).

We sowed 20 seeds into each pot and covered them with a thin layer of identical soil, to avoid increased light absorption of the mixtures that contained AC and were thus slightly darker. There were 10 replicates per species × treatment combination, and thus a total of 540 pots (9 species × 3 substrates × 2 AC treatments × 10 replicates). The pots were arranged in a fully randomized order inside an unheated greenhouse under white shading cloth (60%). During the following eight weeks, we recorded germination every second day. In August 2009, when hardly any further germination could be observed, we thinned down each pot to the largest seedling and allowed this seedling to grow for another six weeks. After that, we harvested the aboveground biomass of the seedlings, dried them at 80°C for 72h, and weighed them.

Community experiment

To examine potential allelopathic effects of litter leachates and trained soil on the growth of established native communities, we carried out an additional mesocosm study in the garden. In June 2009, we planted artificial communities of five native forbs (Geranium robertianum, Geum urbanum, Glechoma hederacea, Silene dioica, Urtica dioica) into 4-L pots filled with the same 1:1 mixture of sand and field soil as above. In each pot, we planted five seedlings (one per species) in a circle, with randomized species order. There were four *Fallopia* treatments: (1) no *Fallopia* (= controls), (2) a piece (6–10 cm, two nodes) of *Fallopia* rhizome planted 5 cm deep in the centre of the pot, (3) 500 mL litter leachate added to each pot right after planting of the natives and the same amount two weeks later, and (4) the regular substrate replaced with trained soil as described above. To half of the pots in each treatment we added AC at a concentration of 20 mL/L. There were 11 replicates for each treatment by AC combination, a total of 88 pots. The pots were placed on root barrier (Plantex® Gold, DuPont, Wilmington, USA) in an experimental garden, with fully randomized order. The experiment lasted from June 2009 to June 2010. To avoid nutrient depletion, we fertilized all pots once in early 2010, using liquid fertilizer (N-P-K ratio: 7-5-6) equivalent to 25 kg N/ha.

During the spring of 2010, we recorded whether and when plants flowered. In June 2010, we cut the aboveground parts of all plants, dried them at 80°C for 72h and weighed them. In addition to flowering time and biomass, we recorded for each species the most feasible measure of reproduction: numbers of flowers for *Geranium*, *Geum* and *Silene*, number of flowering shoots for *Glechoma*, or numbers of inflorescences for *Urtica*. For *Glechoma*, the only species in our experiment that also reproduced vegetatively (stoloniferous spread), we also counted the numbers of runners, and calculated the ratio between runners and flowering shoots as a measure of allocation to vegetative versus sexual reproduction. Finally, we used the biomass data to calculate total community biomass as well as Shannon diversity (using species biomasses as abundances) for each pot.

Statistical analyses

The data from the germination experiment were analysed with linear models that tested the effects of soil treatments (control, litter leachate, trained soil), activated carbon, and their interactions. For germination rates, we used a generalized linear model (GLM) with quasibinomial error distribution, whereas the seedling biomass data were log-transformed and analysed with regular linear models. The two other types of germination data – time to first germination and germination half-time – were extremely ill-distributed and heteroscedastic and we therefore dropped them from the analyses.

The data from the community experiment were analysed with linear models that tested the effects of *Fallopia* treatments (control, rhizome planted, litter leachate, trained soil) activated carbon, and their interactions. First, we analysed total aboveground community biomass and community diversity. Second, we analysed the biomass and reproduction of each species individually. For reproduction, which was always count data, we used GLMs with quasipoisson error distribution, whereas biomass data were log-transformed and analysed with regular linear models. For most species in the community experiment, analyses of flowering time turned out to make little sense, because not enough plants flowered, or all flowered within a short period of time. The only species with a reasonably complete data set for analysis of flowering time was *Silene*, and we therefore restricted analyses of flowering time to this species. The flowering time data were analysed using GLMs with quasipoisson error. Finally, we analysed the clonal:sexual reproduction ratio of *Glechoma* using GLM with quasibinomial error distribution.

Results

Germination experiment

We found significant effects of soil treatments on seed germination in seven of the nine target species (Table 1). However, in all cases these effects were due to *positive* effects

	Germination rate			Seedling biomass		
	Treatment	AC	Treatment × AC	Treatment	AC	Treatment × AC
Species	(d.f. = 2)	(d.f. = 1)	(d.f. = 2)	(d.f. = 2)	(d.f. = 1)	(d.f. = 2)
Filipendula ulmaria	ns	ns	ns	ns	ns	ns
Geranium robertianum	***	ns	ns	ns	ns	ns
Geum urbanum	*	**	ns	ns	ns	ns
Glechoma hederacea	ns	ns	*	***	ns	ns
Lolium perenne	***	ns	ns	ns	ns	ns
Poa trivialis	***	ns	ns	ns	ns	ns
Silene dioica	***	ns	**	**	ns	ns
Symphytum officinale	***	ns	ns	ns	ns	ns
Urtica dioica	***	ns	ns	ns	ns	ns

Table 1. Analyses of variance of the effects of soil treatments (control, *Fallopia* × *bohemica* litter leachates, *Fallopia* × *bohemica* trained soil), activated carbon (AC), and their interaction, on the germination and early growth of nine native European species. *** P<0.001, ** P<0.01, * P<0.05. d.f. = degrees of freedom.

of trained soil on seed germination (Fig. 1), whereas we never observed significant negative effects of litter leachates or trained soil on seed germination (all post-hoc tests non-significant for cases with a negative trend). There was a significant main effect of AC on *Geum*, where the percent of germinating seeds increased from 13 to 36. In two species, we observed a significant treatment by AC interaction. In the control treatment, the addition of activated carbon increased germination of *Silene* from 40% to 60%, but in the other two treatments, it did not have any effects. The interaction was more complex in *Glechoma*, where AC increased germination in the control treatment (5% to 11%) but decreased it in the litter leachate (11% to 5%) and trained soil (14% to 7%) treatments. There were generally much fewer effects of soil treatment effect. In *Glechoma*, litter leachate and trained soil increased seedling biomass by 27% and 100%, respectively, whereas in *Silene* the same treatments decreased seedling biomass by 22% and 27%. There were no effects of AC, or its interaction with the soil treatments, on seedling biomass in any of the species.

Community experiment

In all of our experimental communities, *Silene* became the dominant species (average of 46.2% of the biomass across all treatments), followed by *Glechoma* (32.4%), *Urtica* (8.1%), *Geum* (5.6%) and *Geranium* (2.3%). This ranking was very stable and hardly affected by the treatments. In all of the 22 pots where we had planted *Fallopia* rhizomes, *Fallopia* resprouted, and it eventually constituted an average of 6.76% of the final community biomass. Overwinter survival exceeded 90% for all native species and did not differ across the four treatments. All individuals of *Silene* flowered in 2010, whereas flowering rates were lower in the other species (*Glechoma* 77%, *Geranium*).



Figure 1. The effects of litter leachates and trained soil of *Fallopia* × *bohemica* on the germination rates of nine native European species.

50%, *Urtica* 40%, *Geum* 30%). Out of the 22 planted *Fallopia* plants, none flowered until June 2010.

We did not find a significant main effect of the *Fallopia* treatments or AC on total native biomass (Table 2). However, there was significant interaction between *Fallopia treatments* and AC (Table 2, Fig. 2): while the addition of AC did not affect community biomass in the control or trained soil treatments, it strongly decreased (-45%) community biomass where *Fallopia* rhizomes had been planted, and it tended to increase (+14%) community biomass in the litter leachate treatment. There was a marginally significant (P = 0.065) effect of *Fallopia* treatments on the (biomass-based) Shannon diversity of the native communities, which decreased from 0.8 in the controls to 0.73 and 0.71 in the litter leachate and rhizome treatments, respectively, but increased to 0.92 in the trained soil treatment.

When we analysed the biomass responses of the native species separately, we found that the *Fallopia* treatments significantly affected the biomass of *Glechoma*, which,

Table 2. Native plants community biomass and diversity as well as individual species biomass in response to *Fallopia* × *bohemica* litter leachate and trained soil, with or without activated carbon (AC) added to the soil. Main effect and their interaction tested by factorial ANOVA. The values are *F*-values. ** P<0.01, * P<0.05, (*) P<0.1. d.f. = degrees of freedom.

	Treatment (d.f. = 3)	AC (d.f. = 1)	Treatment × AC (d.f. = 3)
Total native biomass	1.24	0.79	3.29*
Shannon index	2.5(*)	0.01	1.96
Silene dioica	0.19	0.84	0.58
Urtica dioica	2.16	1.13	2.47(*)
Geranium robertianum	1.90	1.64	2.05
Glechoma hederacea	3.03*	10.66**	1.98
Geum urbanum	0.33	0.67	0.27

compared to the controls, had 43% less biomass in the rhizome treatment, but no significant change in the other two treatments. We also found that *Glechoma* biomass was consistently decreased by the addition of activated carbon (average of -33% across treatments). There were no other significant treatment or AC effects on the biomass of any other native species (Table 2).

We also found significant treatment effects on reproductive traits for several of the natives. In all allelopathy treatments, the start of flowering of *Silene* was significantly



Figure 2. The effects of different possible sources of allelopathy of *Fallopia* \times *bohemica* on the total aboveground biomass of a community of five native European species with or without activated carbon (AC) added to the substrate.

(F = 9.92, P < 0.001) delayed, and this effect was ameliorated by AC in the rhizome and trained soil treatments (Fig 3). Moreover, addition of AC significantly increased the numbers of *Silene* flowers across treatments (F = 4.43, P < 0.05). There was also a



Figure 3. The effects of different possible sources of allelopathy of *Fallopia × bohemica* on the flowering phenology of *Silene dioica*, and allocation to vegetative reproduction of *Glechoma hederacea*, with or without activated carbon (AC) added to the soil.

significant (*F*=3.69, *P*<0.05) treatment effect on reproduction in *Glechoma*, where the number of flowering shoots strongly decreased (from 17 to 6) where *Fallopia* rhizomes were planted. The number of runners, however, was not equally affected, which resulted in a significant (*F* = 4.69, *P*<0.05) shift of the ratio *Glechoma* runners: flowering shoots. With *Fallopia* rhizomes and litter leachates, this ratio was greatly increased (Fig. 3). Last, we found a significant (*F* = 4.02, *P*<0.05) treatment by AC interaction for *Urtica* flower biomass: addition of AC increased reproduction in the control and rhizome treatments, but decreased it in the two other treatments.

Discussion

Understanding the mechanisms of interference between successful invasive plants and their native competitors is key to explaining and ultimately managing plant invaders. Here, we experimentally examined whether one of the world's worst plant invaders, the invasive knotweed $F \times bohemica$, can exert allelopathic effects on natives also through its leaf litter or trained soil. We found little effects on the germination or biomass of natives, but both *Fallopia* litter extracts and trained soil caused significant life-history shifts in the dominant native species.

Germination experiment

Exposing native species seeds to *Fallopia* litter leachate and trained soil did not have any negative impact on their germination or early growth. In fact, trained soil even significantly increased germination rates of most native species. It is possible that the pre-cultivation of soil with *Fallopia* generally stimulated the soil microbial community, with positive consequences for seed germination, either because seeds have a greater chance of encountering the mutualists required for germination, or because more abundant soil microbes improve the water conditions in upper soil layers (Franzluebbers 2002). In any case, our results indicate that neither litter leachates nor trained soil of *Fallopia* × *bohemica* inhibit the germination of native competitors, as has been shown for other plant invaders (Dorning and Cipollini 2006, Prati and Bossdorf 2004, Yang et al. 2007), and they corroborate results of previous experiments investigating *Fallopia* trained soil effects on native plant germination (Gerber et al. 2005).

Community experiment – community level

By setting up artificial native communities, we were able to evaluate the allelopathic potential of *Fallopia* in an ecologically meaningful set-up. In the community experiment, we found that neither planted *Fallopia* rhizomes, nor litter leachates or trained soil had a negative effect on the total biomass or diversity of the native community.

Moreover, in none of the *Fallopia* treatments did addition of AC lead to increased native plant biomass or diversity, which would have indicated allelopathic effects. On the contrary, in the presence of *Fallopia* rhizomes, addition of AC even significantly *de*creased native plant biomass, which indicates beneficial chemical interactions between soil organisms and the native community, or between different native plant species, which were disrupted by AC. Taken together, our study provides no evidence for allelopathic effects of *Fallopia* at the level of the whole native community.

In our study, even the planted Fallopia rhizomes did not have allelopathic effects on native plants. This result is inconsistent with a previous study in which we found strong allelopathic effects of planted Fallopia (using the same knotweed genotype) on native community biomass (Murrell et al. 2010). We can think of two main explanations for this inconsistency: First, there were great differences in Fallopia dominance among the two experiments. In the previous study, even though it was much shorter than the present one, Fallopia not only grew larger in absolute terms, but it was the dominant species and constituted more than half of the community biomass. In the present study it constituted only some 10% of the total biomass, and one of the main reasons for this difference could be that different substrates were used in the two experiments. In the previous study we used a commercial potting substrate, which likely provided very favourable conditions for rhizome growth, whereas the present study used a natural, loamy field soil, which was much heavier and denser, and therefore probably less penetrable to *Fallopia* rhizomes, than the potting substrate. It is possible that in less favourable substrate, Fallopia is a weaker competitor and simply cannot afford to produce allelopathic compounds (Herms and Mattson 1992). The second reason for the lack of allelopathic effects of Fallopia rhizomes could be that the field soil used in the this study sustains a richer or functionally different soil microbial community which has a greater ability to uptake and detoxify allelopathic compounds (Inderjit 2005, Lankau 2010, Kaur et al. 2009) and therefore prevented allelopathic effects in our study.

We should stress that the first argument, lack of impact because of small size, applies only to the rhizome treatment, but not to the litter leachate and soil training. For the litter leachates, we followed the successful methods of previous studies (Dorning and Cipollini 2006), and the resulting leachate was clearly highly concentrated. For the soil training, we used fairly large rhizomes, and there was a dense network of *Fallopia* roots in the pots after the training period. Thus, both treatments appeared to be rather strong and we have no reason to suspect they may have been too weak to elicit allelopathic effects.

Community experiment - species level

Even though the total biomass of the native community was unaffected by the allelopathy treatments, such stability at the community level could mask underlying responses at the level of individual species. When we analysed the biomass responses of each species separately, the only species that showed a significant response to the experimental treatments was *Glechoma*, which had reduced biomass in the presence of *Fallopia* rhizomes or litter leachate. The reduction of biomass was stronger in the presence of AC, which suggests that AC may in fact have neutralized allelopathic compounds of *Glechoma* (rather than *Fallopia*) and thus reduced its competitive ability. Since *Glechoma* is one of the dominant species in the community, and the patterns of biomass change of *Glechoma* were similar to those of the whole community, it appears that biomass responses at community-level were largely driven by the responses of *Glechoma*.

In two of the natives, the dominant species Silene dioica and Glechoma hedera*cea*, we looked also beyond biomass and reproduction and investigated allelopathic effects on key life-history traits, and we found that these were indeed strongly affected by the Fallopia treatments. In Glechoma, the only stoloniferous species in our experiment, with a clear dimorphism between shoots that become (vertical) flowering shoots and such that become (horizontal) runners, shoot allocation to runners was strongly increased both in the presence of Fallopia rhizomes and litter leachate. Such increased investment into runners with fast lateral growth can be interpreted as a switch towards a guerrilla strategy of growth (De Kroon and Hutchings 1995, Cheplick 1997), a behaviour frequently reported in clonal plants, including Glechoma (Price and Hutchings 1996), as response to competition or stress (Koivunen et al. 2004). Such changes in growth strategy can strongly influence plant population structure and dynamics (Doust 1981). In *Silene*, all three *Fallopia* treatments significantly delayed the time of flowering. Potentially, such induced changes in flowering phenology could cause temporal mismatches between native plants and their pollinators (Hegland et al. 2009).

In both cases where we found these shifts in life-history strategy, addition of AC tended to counteract these effects, which suggests that in both cases treatment effects must indeed be chemically mediated. Since the treatments generally did not affect plant biomass, the observed changes in allocation or phenology are not just allometric consequences of changes in plant size. For the chemical mechanisms behind these effects, there are several potential candidate classes of substances, including stilbenes, resveratrolosides and proanthocyanidins, which have been found in *Fallopia* and which were previously shown to be allelopathic in bioassays (Fan et al. 2010). As many of these compounds have antimicrobial and antifungal properties (Daayf et al. 1995, Kumagai at al. 2005) it is possible that at least part of the observed effects act indirectly, through changes in soil biota.

Conclusion

Our experiments show that *Fallopia* allelopathy acts on the growth of natives rather than their germination. Persistent soil contaminants appear to have rather limited effects on later life-history stages and this should not increase the efforts of restoring

habitats after removing the invader. We also demonstrated that allelopathic effects can sometimes be subtle changes in life-history traits, which would be overlooked by a simple focus on plant biomass.

Acknowledgements

This research was supported by Swiss National Science Foundation grant 31003A_122408. We would like to thank one anonymous reviewer for the helpful comments.

References

- Aguilera AG, Alpert P, Dukes JS, Harrington R (2010) Impacts of the invasive plant *Fallopia japonica* (Houtt.) on plant communities and ecosystem processes. Biological Invasions 12: 1243–1252. doi: 10.1007/s10530-009-9543-z
- Bailey JP, Connolly AP (2000) Prize winners to pariahs A history of Japanese knotweed s.l. (Polygonaceae) in the British Isles. Watsonia 23: 93–110.
- Bever JD (2003) Soil community feedback and the coexistence of competitors: conceptual frameworks and empirical tests. New Phytologist 157:465–473. doi: 10.1046/j.1469-8137.2003.00714.x
- Callaway RM, Aschehoug ET (2000) Invasive plants versus their new and old neighbors: A mechanism for exotic invasion. Science 290:521–523. doi: 10.1126/science.290.5491.521
- Callaway RM, Thelen GC, Rodriguez A, Holben WE (2004) Soil biota and exotic plant invasion. Nature 427: 731–733. doi: 10.1038/nature02322
- Callaway RM, Cipollini D, Barto K, Thelen G, Hallett SG, Prati D, Stinson K, Klironomos J (2008) Novel weapons: Invasive plant suppresses fungal mutualists in America but not in its native Europe. Ecology 89: 1043–1055. doi: 10.1890/07-0370.1
- Cheplick GP (1997) Responses to severe competitive stress in a clonal plant: differences between genotypes. Oikos 79: 581–591. doi: 10.2307/3546902
- Child LE, Wade PM (2000) The Japanese knotweed manual. Packard, Chichester.
- Dawson W, Fischer M, van Kleunen M (2011) The maximum relative growth rate of common UK plant species is positively associated with their global invasiveness. Global Ecology and Biogeography 20: 299–306. doi: 10.1111/j.1466-8238.2010.00599.x
- Daayf F, Schmitt A, Belanger RR (1995) The effects of plant extracts of *Reynoutria sachalinensis* on powdery mildew development and leaf physiology of long English cucumber. Plant Disease 79: 577–580. doi: 10.1094/PD-79-0577
- De Kroon H, Hutchings MJ (1995) Morphological plasticity in clonal plants the foraging concept reconsidered. Journal of Ecology 83: 143–152. doi: 10.2307/2261158
- Dorning M, Cipollini D (2006) Leaf and root extracts of the invasive shrub Lonicera maackii inhibit seed germination of three herbs with no autotoxic effects. Plant Ecology 184: 287– 296. doi: 10.1007/s11258-005-9073-4

- Doust LL (1981) Population-dynamics and local specialization in a clonal perennial (*Ranunculus repens*). I. The dynamics of ramets in contrasting habitats. Journal of Ecology 69: 743–755. doi: 10.2307/2259633
- Fan PH, Hay AE, Marston A, Hostettmann K (2009) Allelopathic potential of phenolic constituents from *Polygonum cuspidatum* Sieb. & Zucc. (Polygonaceae). Planta Medica 75: 928.
- Franzluebbers AJ (2002) Water infiltration and soil structure related to organic matter and its stratification with depth. Soil & Tillage Research 66: 197–205. doi: 10.1016/S0167-1987(02)00027-2
- Funk JL, Vitousek PM (2007) Resource-use efficiency and plant invasion in low-resource systems. Nature 446: 1079–1081. doi: 10.1038/nature05719
- Gerber E, Krebs C, Murrell C, Moretti M, Rocklin R, Schaffner U (2008) Exotic invasive knotweeds (*Fallopia* spp.) negatively affect native plant and invertebrate assemblages in European riparian habitats. Biological Conservation 141: 646–654. doi: 10.1016/j.biocon.2007.12.009
- Gerber E, Krebs C, Murrell C, Schaffner U (2005) Assessing the ecological and economic impact of the invasive plant species Japanese knotweed, *Fallopia japonica*. Annual Report 2004, CABI Bioscience Centre, Delémont.
- Grotkopp E, Rejmanek M, Rost TL (2002) Toward a causal explanation of plant invasiveness: Seedling growth and life-history strategies of 29 pine (*Pinus*) species. American Naturalist 159: 396–419. doi: 10.1086/338995
- Hegland SJ, Nielsen A, Lazaro A, Bjerknes AL, Totland O (2009) How does climate warming affect plant-pollinator interactions? Ecology Letters 12: 184–195. doi: 10.1111/j.1461-0248.2008.01269.x
- Herms DA, Mattson WJ (1992) The dilemma of plants to grow or defend. Quarterly Review of Biology 67: 283–335. doi: 10.1086/417659
- Inderjit, Callaway RM (2003) Experimental designs for the study of allelopathy. Plant Soil 256: 1–11.
- Inderjit, Nilsen ET (2003) Bioassays and field studies for allelopathy in terrestrial plants: Progress and problems. Critical Reviews in Plant Sciences 22: 221–238.
- Inderjit (2005) Soil microorganisms: An important determinant of allelopathic activity. Plant Soil 274: 227–236.
- Inoue M, Nishimura H, Li HH, Mizutani J (1992) Allelochemicals from *Polygonum sachalin*ense Fr. Schm. (Polygonaceae). Journal of Chemical Ecology 18: 1833–1840. doi: 10.1007/ BF02751107
- Kaur H, Kaur R, Kaur S, Baldwin IT, Inderjit (2009) Taking ecological function seriously: soil microbial communities can obviate allelopathic effects of released metabolites. PLoS ONE 4:e4700. doi: 10.1371/journal.pone.0004700
- Koivunen S, Saikkonen K, Vuorisalo T, and Mutikainen P (2004) Heavy metals modify costs of reproduction and clonal growth in the stoloniferous herb *Potentilla anserine*. Evolutionary Ecology 18: 541–561. doi: 10.1007/s10682-004-5143-7
- Krebs C, Mahy G, Matthied D, Schaffner U, Tiebre MS, Bizoux JP (2010) Taxa distribution and RAPD markers indicate different origin and regional differentiation of hybrids in

29

the invasive *Fallopia* complex in central-western Europe. Plant Biology 12: 215–223. doi: 10.1111/j.1438-8677.2009.00219.x

- Kumagai H, Kawai Y, Sawano R, Kurihara H, Yamazaki K, Inoue N (2005) Antimicrobial substances from rhizomes of the giant knotweed *Polygonum sachalinense* against the fish pathogen *Photobacterium damselae* subsp *piscicida*. Zeitschrift für Naturforschung C-A Journal Of Biosciences 60: 39–44.
- Lankau R (2010) Soil microbial communities alter allelopathic competition between *Alliaria petiolata* and a native species. Biological Invasions, 12: 2059–2068. doi: 10.1007/s10530-009-9608-z
- Lau JA, Puliafico KP, Kopshever JA, Steltzer H, Jarvis EP, Schwarzländer M, Strauss SY, Hufbauer RA (2008) Inference of allelopathy is complicated by effects of activated carbon on plant growth. New Phytologist 178 (2): 412-423. doi: 10.1111/j.1469-8137.2007.02360.x
- Lowe S, Browne M, Boudjelas S, De Poorter M (2000) 100 of the World's Worst Invasive Alien Species. Invasive Species Specialist Group. www.issg.org/booklet.pdf
- Mangla S, Inderjit, Callaway RM (2008) Exotic invasive plant accumulates native soil pathogens which inhibit native plants. Journal of Ecology 96: 58–67.
- Moravcová L, Pyšek P, Jarošík V, Zákravský P (2011) Potential phytotoxic and shading effects of invasive *Fallopia* Polygonaceae) taxa on the germination of native dominant species. NeoBiota 9: 31–47. doi: 10.3897/neobiota.9.1266
- Mitchell CE, Agrawal AA, Bever JD, Gilbert GS, Hufbauer RA, Klironomos JN, Maron JL, Morris WF, Parker IM, Power AG, Seabloom EW, Torchin ME, Vazquez DP (2006) Biotic interactions and plant invasions, Ecology Letters 9: 726–740. doi: 10.1111/j.1461-0248.2006.00908.x
- Murrell C, Gerber E, Krebs C, Parepa M, Schaffner U and Bossdorf O (2010) Invasive knotweed affects native plants through allelopathy. American Journal of Botany 98: 38–43. doi: 10.3732/ajb.1000135
- Olson BE, Wallander RT (2002) Effects of invasive forb litter on seed germination, seedling growth and survival. Basic and Applied Ecology 3: 309–317. doi: 10.1078/1439-1791-00127
- Prati D, Bossdorf O (2004) Allelopathic inhibition of germination by *Alliaria petiolata* (Brassicaceae). American Journal of Botany 91: 285–288. doi: 10.3732/ajb.91.2.285
- Price EAC, Hutchings MJ (1996) The effects of competition on growth and form in Glechoma hederacea. Oikos 75: 279–290. doi: 10.2307/3546251
- Richards CL, Bossdorf O, Muth NZ, Gurevitch J, Pigliucci M (2006) Jack of all trades, master of some? On the role of phenotypic plasticity in plant invasions. Ecology Letters 9: 981– 993. doi: 10.1111/j.1461-0248.2006.00950.x
- Siemens T J and Blossey B (2007) An evaluation of mechanisms preventing growth and survival of two native species in invasive Bohemian knotweed (*Fallopia × bohemica*, Polygonaceae). American Journal of Botany 94: 776–783. doi: 10.3732/ajb.94.5.776
- Stinson KA, Campbell SA, Powell JR, Wolfe BE, Callaway RM, Thelen GC, Hallett SG, Prati D, Klironomos JN (2006) Invasive plant suppresses the growth of native tree seedlings by disrupting belowground mutualisms. PLoS Biology 4: 727–731. doi: 10.1371/journal. pbio.0040140

- van Kleunen M, Weber E, Fischer M (2010) A meta-analysis of trait differences between invasive and non-invasive plant species. Ecology Letters 13: 235–245. doi: 10.1111/j.1461-0248.2009.01418.x
- Vrchotová N, Sera B (2008) Allelopathic properties of knotweed rhizome extracts. Plant, Soil and Environment 54: 301–303.
- Weston LA, Barney JN, DiTommaso A (2005) A review of the biology and ecology of three invasive perennials in New York State: Japanese knotweed (*Polygonum cuspidatum*), mugwort (*Artemisia vulgaris*) and pale swallow-wort (*Vincetoxicum rossicum*). Plant & Soil 277: 53–69. doi: 10.1007/s11104-005-3102-x
- Yang RY, Mei LX, Mei LX, Tang JJ, Chen X (2007) Allelopathic effects of invasive *Solidago canadensis* L. on germination and growth of native Chinese plant species. Allelopathy Journal 19: 241–247.

LETTER TO THE EDITOR



Plant pathogens as biocontrol agents for Cirsium arvense – an answer to Müller and Nentwig

Michael G. Cripps¹, Graeme W. Bourdôt², Karen L. Bailey³

Lincoln University, John Burton Building, Christchurch 7647, New Zealand **2** AgResearch Limited, Private Bag 4749, Christchurch 8140, New Zealand **3** Agriculture & Agri-Food Canada, 107 Science Place, Saskatoon, Saskatchewan, S7N 0X2, Canada

Corresponding author: Michael G. Cripps (michael.cripps@lincoln.ac.nz)

Received 29 March 2012 | Accepted 3 April 2012 | Published 16 May 2012

Citation: Cripps MG, Bourdôt GW, Bailey KL (2012) Plant pathogens as biocontrol agents for *Cirsium arvense* – an answer to Müller and Nentwig. NeoBiota 13: 31–39. doi: 10.3897/neobiota.13.3137

Recently, Müller and Nentwig (2011) reviewed the plant pathogens that have been considered for biological control of the weed *Cirsium arvense* (L.) Scop. (Canada thistle, Californian thistle, creeping thistle), and concluded that the prospects have been largely overestimated. The premise of their conclusion is that no bioherbicide products have achieved marketability, which they surmise is due to lack of host specificity, effectiveness, and issues with application. While it is true that no microbial products have achieved marketability for this weed, we believe their reasoning for this is erroneous, and likely due to lack of distinction between two biocontrol approaches, specifically classical biocontrol, and innundative biocontrol (often referred to as the biopesticide approach). These two different types of biocontrol have different goals, and are applied in different ways.

Generally, in classical biocontrol, coevolved insects or pathogens from the native range of a weed are imported and released in regions where the weed has been introduced, and has become invasive (McFadyen 1998; Watson 1991). Classical biocontrol is permanent, and when successful, requires little or no continued management input. The goal is not eradication, but rather to suppress weed populations to a level where they are no longer problematic. When importing natural enemies (insects or microbes), assurance of safety to non-target plants is paramount, and often requires a high degree of host specificity (Barton 2004; Berner and Bruckart 2005).

Copyright Michael G. Cripps et al. This is an open access article distributed under the terms of the Creative Commons Attribution License 3.0 (CC-BY), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

In the introduction to their paper, Müller and Nentwig (2011) summarise the insect biocontrol agents that have been released (classical biocontrol agents) (Cripps et al. 2011), and noted a gap in the review literature, the potential of pathogens as biocontrol agents. However, since they failed to explain the two biocontrol approaches it is worth noting that to date no microbial pathogens have been intentionally released as classical biocontrol agents for *C. arvense*, although there is a current active program searching for potential pathogens suitable for release as classical agents against this weed. Recent foreign exploration has been focused in north-western China, a little explored region of the native range of *C. arvense*. Potential classical biocontrol pathogens include the white blister rust, *Pustula* cf. *spinulosa* and more aggressive strains of the rust, *Puccinia punctiformis* (Li et al. 2011). Ongoing tests with these, and other pathogens, will determine if they are suitable for release as classical biocontrol agents (H. Hinz, personal communication).

Other than the recent initiative to find classical biocontrol pathogens, all of the microbes researched to date, including all those reviewed by Müller and Nentwig, have been considered for innundative control, or microbial agents that could be utilised as bioherbicides. This is a different type of biocontrol compared to the importation and release of natural enemies from the native range. In contrast, innundative biocontrol typically does not involve importing new species, but rather utilises species already present, often cosmopolitan generalist microbial pathogens. Innundative biocontrol involves the mass release or application of a pathogen, typically in the same manner as conventional pesticides. The goal is short term (e.g. growing season), but substantial control, that will often require repeated applications.

Specificity of bioherbicides

Müller and Nentwig insistently raised the issue of specificity and claimed that: "*The primary reason for the failure of most of the tested plant pathogens against C. arvense is the missing host specificity*". There are many constraints in the development and registration of bioherbicies, but least among these is specificity (Auld and Morin 1995). The most successful bioherbicides of the past 30 years (e.g. DeVine, COLLEGO, BioMal, Chontrol) are not host-specific, but capable of infecting a relatively broad range of plant species (Bailey 2010; Charudattan 1990). Despite their broad host range, a thorough understanding of the biology and epidemiology of the pathogens has allowed their use without any adverse effects to non-target plants, or environmental hazards. Bioherbicides are intended to be used in selected sites, with care taken to avoid areas with potentially susceptible non-target plants, as indicated on the product label. The pathogens selected for development into bioherbicide products do not naturally spread far from their site of application, which is why they need to be spread, typically by using conventional pesticide application equipment, in order to be effective.

Sclerotinia sclerotiorum is an example of a fungal pathogen with a broad host range, but suitable for use as a bioherbicide to control *C. arvense* in permanent pastures. Un-

fortunately, Müller and Nentwig (2011) misconstrued some of the studies with this pathogen in their statement: "...*as its spores spread easily, its use on pastures may cause hazards after changes of land use and for adjacent areas, even if a safety zone is allowed.*" Contrary to their interpretation, the risk analysis revealed that the use of this pathogen in pastures in New Zealand presents little or no risk to adjacent downwind crops due to the spore-trapping ability of the pastures combined with the ubiquitous nature of this pathogen and its relatively high ambient atmospheric spore density (Bourdôt et al. 2011).

Another example of a broad spectrum fungal pathogen capable of controlling *C. arvense* in certain situations such as cereal crop production is *Phoma macrostoma* (Zhou et al. 2004). This pathogen is applied to the soil as a pre-emergent bioherbicide capable of controlling many herbaceous weeds, but does not harm most Poaceae species, making it an attractive alternative for broadleaf weed control where common phenoxy herbicides are no longer desirable or permitted for use (Bailey et al. 2011a). Safety to non-target plants is assured when used in selected situations since the infective propagule applied is mycelium that has limited mobility and weak persistence (Bailey et al. 2011b).

In their conclusion, Müller and Nentwig mentioned the possibility of unintended impacts on native, non-target thistle species. This indeed was a problem with the classical biocontrol insect, *Rhinocyllus conicus* (Gassmann and Louda 2001); however, there is no reason to believe that the prudent use of microbial pathogens as bioherbicides would have similar non-target impacts. This further emphasises the need to clearly distinguish the type of biological control intended when discussing host specificity requirements, and potential non-target effects.

Pathogens with broad spectrum activity are currently preferred from a commercial developmental perspective, since host-specific pathogens have limited market potential. For instance, the host-specific rust pathogen, *Puccinia thlaspeos*, was registered as a bioherbicide for control of *Isatis tinctoria* (dyer's woad) (US-EPA 2002), but never became a commercial product due to lack of investor interest in such a specialised product (Bellgard 2008). Nevertheless, if demand was great enough, and the product produced cheaply enough, a bioherbicide based on a host-specific pathogen could be a viable product. Given that *C. arvense* is a particularly troublesome weed of global significance (Tiley 2010), a bioherbicide based on a host-specific pathogen could be a viable product, particularly if alternatives to synthetic herbicides are desired. The host-specific rust pathogen, *Puccinia punctiformis*, has long been considered a potential biocontrol agent of *C. arvense* (Frantzen 1994), and conceivably could be formulated into a product in much the same way as *P. thlaspeos*, using dried, mulched, rust-infected plants (Thomson and Kropp 2004).

Müller and Nentwig (2011) also mentioned problems with "application possibilities", although they did not elaborate on what they meant by this. We imagine they might be referring to the inability to culture inoculum of the fungus, *P. punctiformis*. Whilst the strict host specificity and obligate biotrophic nature of *P. punctiformis* are constraints on its development as a bioherbicide, it does not rule out the possibility of augmenting the effect of natural infestations of this fungus on *C. arvense*. Augmentation of the effect of this pathogen has been hampered by an incomplete understanding of how systemic infection is initiated (Cripps et al. 2009; Frantzen 1994). Nevertheless, enhancement of systemic rust disease has been achieved by spreading its spores during strategic mowing operations (Demers et al. 2006), and it is plausible that this effect could be enhanced further by mowing during rainfall (Bourdôt et al. 2011a).

Effectiveness of bioherbicides

Müller and Nentwig claimed that "the varying and low virulence of the pathogens pose a problem (e.g., Alternaria cirsinoxia, Sclerotinia sclerotiorum, Phomopsis cirsii) as constant levels of virulence must be ensured for a successful inhibition of the growth of the target weed." In the case of *A. cirsinoxia*, lack of efficacy, due to the inability of the pathogen to infect young leaves, and defensive mechanisms of the plant, were reasons this pathogen was considered unsuitable for bioherbicide development (Bailey 2004). However, the efficacies of *S. sclerotiorum*, *P. cirsii*, and many other pathogens, are not a limiting factor for their development into bioherbicides. Müller and Nentwig's assumption that low and varying efficacy has prevented bioherbicides from commercialisation lacks a standard for comparison. The efficacy of synthetic herbicides might be considered a benchmark for measuring the effectiveness of bioherbicides could replace or compete with.

Considerable variation among synthetic herbicides for controlling *C. arvense* has been well documented (Donald 1990). As a recent example, Enloe et al. (2007) compared several standard commercial herbicides for control of *C. arvense* and reported that control ranged from 34 to 97 percent. Variation in the efficacy of particular herbicides for the control of *C. arvense* also varies with many factors including phenology of the weed (Miller and Lym 1998), season of application (Wilson et al. 2006), environmental factors (Hunter and Smith 1972), and resistant ecotypes (Donald 1990). Thus, although reliability in the efficacy of a weed control product is highly desirable, it is often not realised in practice, even with currently marketed synthetic herbicides.

In particular, the variability in efficacy of a potential bioherbicide product based on *Sclerotinia sclerotiorum* was compared with two phenoxy herbicides, MCPA and MCPB. It was shown that the bioherbicide was no more variable in its efficacy under field conditions than the two synthetic herbicides and on average just as effective as MCPA (Bourdôt et al. 2007). Similar responses were observed in field trials using *P. macrostoma* and the standard herbicide mixture of 2,4-D, mecoprop and dicamba such that both products provided greater than 80% control of dandelion in 75% of the trials (unpublished data, K. Bailey).

While considerable variation is often reported in the discovery and proof-of-concept stages for a potential bioherbicide, much of the variation can be reduced by selecting particular aggressive strains, and by amending the final formulation with adjuvants that enhance and protect the pathogen (Ash 2010; Weaver et al. 2007). This was demonstrated for *P. cirsii* as selected fungal strains were shown to be highly pathogenic (Leth et al. 2008), and adjuvants reduced the long necessary dew period that limited its potential (Leth and Andreasen 2000).

Another important point that Müller and Nentwig (2011) failed to emphasise is that *C. arvense* is difficult to control by any means, including biological and synthetic herbicides (Donald 1990; Tiley 2010). Control of *C. arvense* often requires multiple herbicide applications over several years (Donald 1990; Donald 1992), and can be improved by integrating herbicides with other techniques such as cultural (e.g. tillage and mowing), (Beck and Sebastian 2000) and biological control (Sciegienka et al. 2011). Thus, while bioherbicides on their own may not be the solution to control of *C. arvense*, their efficacy is not worse than other current control measures, and certainly not the reason for their absence.

Why are there no bioherbicides for Cirsium arvense?

With so much potential, why are there no bioherbicides for control of this important weed? The general dearth of bioherbicides has been addressed by other authors (Hallett 2005), and in essence the reasons are bureaucratic and economic issues. The bureaucratic issues lie primarily with the onerous and costly government registration procedures that require bioherbicides to provide the same information necessary for synthetic herbicides such as human and environmental toxicology reports (Bailey 2010). However, more recently, some government agencies have relaxed their registration procedures for biologically-based and other reduced-risk pesticides relative to the procedures required for new conventional pesticides in order to promote the development of lower-risk products (Bailey et al. 2010). The major impediment to the development of bioherbicides is the assurance of profitability to commercial investors. And the main limitation to profitability is the cost of production, which for fungi typically involves solid-state or liquid fermentation on some sort of nutrient medium (Weaver et al. 2007). Similarly, the most common reason for discontinuation of previously marketed bioherbicides is that the cost of production is too high, and the market too small to justify production costs.

It is important to remember that contrary to classical biocontrol where agents (insects or pathogens) are released for common good without proprietary right, the intention of innundative biocontrol is the development of a bioherbicide product that can be sold for profit in a competitive market (Auld 1991). This competitive herbicide market is currently dominated by cheaply-available glyphosate and phenoxy herbicides, which has been suggested as a primary reason for the absence of new synthetic herbicide modes of action over the past 20 years (Duke 2012). Bioherbicides offer increased safety and sustainability, however, these values are more difficult to quantify. In order for bioherbicides registered for use against *C. arvense* to reach the market place there will likely need to be increased demand for alternative products through legislation restricting synthetic herbicides, and by placing greater value on the safety and sustainability aspects of bioherbicides (Boyetchko and Rosskopf 2006; Charudattan 2001).

Conclusions

Müller and Nentwig (2011) can be commended for undertaking a review of an important subject not thoroughly dealt with in other recent reviews, and for covering most microbial pathogens that have been considered for biocontrol of *C. arvense*. However, we disagree with their conclusion that microbial control of this weed has been largely overestimated. We believe their conclusion is based on erroneous reasoning and a disconnection with contemporary perspectives on bioherbicides. The unfortunate consequence of such an ill-informed review is that readers are left with the false impression that bioherbicides are not a viable option for *C. arvense* control, and possibly other weeds as well. Contrary to Müller and Nentwig's reasoning, we have argued that specificity and efficacy of microbial biocontrol agents are not major limitations to their development as bioherbicides for *C. arvense*. Our perspective is that bioherbicide development is an underdeveloped approach with great potential, rather than overestimated.

References

- Ash GJ (2010) The science, art and business of successful bioherbicides. Biological Control 52: 230–240. doi: 10.1016/j.biocontrol.2009.08.007
- Auld BA (1991) Economic aspects of biological weed control with plant pathogens. In: TeBeest DO (Ed) Microbial control of weeds. Chapman and Hall, London, 262–273. doi: 10.1007/978-1-4615-9680-6_14
- Auld BA, Morin L (1995) Constraints in the development of bioherbicides. Weed Technology 9: 638–652.
- Bailey KL (2004) Microbial weed control: an off-beat application of plant pathology. Canadian Journal of Plant Pathology 26: 239–244. doi: 10.1080/07060660409507140
- Bailey KL (2010) Canadian innovations in microbial biopesticides. Canadian Journal of Plant Pathology 32: 113–121. doi: 10.1080/07060661.2010.484195
- Bailey KL, Boyetchko SM, Langle T (2010) Social and economic drivers shaping the future of biological control: A Canadian perspective on the factors affecting the development and use of microbial biopesticides. Biological Control 52: 221–229. doi: 10.1016/j.biocontrol.2009.05.003
- Bailey KL, Pitt WM, Falk S, Derby J (2011a) The effects of *Phoma macrostoma* on nontarget plant and target weed species. Biological Control 58: 379–386. doi: 10.1016/j.biocontrol.2011.06.001
- Bailey KL, Pitt WM, Leggett F, Sheedy C, Derby J (2011b) Determining the infection process of *Phoma macrostoma* that leads to bioherbicidal activity on broadleaved weeds. Biological Control 59: 268–276. doi: 10.1016/j.biocontrol.2011.06.019
- Barton J (2004) How good are we at predicting the field host-range of fungal pathogens used for classical biological control of weeds? Biological Control 31: 99–122. doi: 10.1016/j. biocontrol.2004.04.008

- Beck KG, Sebastian JR (2000) Combining mowing and fall-applied herbicides to control Canada thistle (*Cirsium arvense*). Weed Technology 14: 351–356. doi: 10.1614/0890-037X(2 000)014[0351:CMAFAH]2.0.CO;2
- Bellgard S (2008) Innundative control using bioherbicides. In: The biological control of weeds book. Landcare Research, NZ.
- Berner DK, Bruckart WL (2005) A decision tree for evaluation of exotic plant pathogens for classical biological control of introduced invasive weeds. Biological Control 34: 222–232. doi: 10.1016/j.biocontrol.2005.04.012
- Bourdôt GW, Baird D, Hurrell GA, De Jong MD (2006) Safety zones for a Sclerotinia sclerotiorum-based mycoherbicide: Accounting for regional and yearly variation in climate. Biocontrol Science and Technology 16: 345–358. doi: 10.1080/09583150500531966
- Bourdôt GW, Hurrell GA, Saville DJ (2007) Variation in the efficacy of a mycoherbicide and two synthetic herbicide alternatives. In: Julien HH et al. (Eds) XII th International Symposium on Biological Control of Weeds. CAB InternationalWallingford, UK, La Grande Motte, Montpellier, France, 507–511.
- Bourdôt GW, Hurrell GA, Skipp RA, Monk J, Saville DJ (2011a) Mowing during rainfall enhances the control of *Cirsium arvense*. Biocontrol Science and Technology 21: 1213–1223. doi: 10.1080/09583157.2011.608119
- Bourdôt GW, Saville DJ, De Jong MD (2011b) Evaluating the environmental safety of broad host range bioherbicides (invited paper). Pest Technology 5 (Special issue 1): 34–40.
- Boyetchko SM, Rosskopf EN (2006) Strategies for developing bioherbicides for sustainable weed management. In: Singh HP, Batish DR, Kohli RK (Eds) Handbook of sustainable weed management, 393–430.
- Charudattan R (1990) Pathogens with potential for weed control. In: Hoagland RE (Ed) ACS Symposium Series 439. American Chemical Society, Washington, DC, 132–154. doi: 10.1023/A:1011477531101
- Charudattan R (2001) Biological control of weeds by means of plant pathogens: Significance for integrated weed management in modern agro-ecology. Biocontrol 46: 229–260.
- Cripps MG, Edwards GR, Waipara NW, Bourdôt GW, Saville DJ, Fowler SV (2009) Does transmission of the rust pathogen, *Puccinia punctiformis*, require stem mining vectors? Biocontrol Science and Technology 19: 447–454. doi: 10.1080/09583150802699420
- Cripps MG, Gassmann A, Fowler SV, Bourdôt GW, McClay AS, Edwards GR (2011) Classical biological control of *Cirsium arvense*: Lessons from the past. Biological Control 57: 165–174. doi: 10.1016/j.biocontrol.2011.03.011
- Demers AM, Berner DK, Backman PA (2006) Enhancing incidence of *Puccinia punctiformis*, through mowing, to improve management of Canada thistle (*Cirsium arvense*). Biological Control 39: 481–488. doi: 10.1016/j.biocontrol.2006.06.014
- Donald WW (1990) Management and control of Canada thistle (*Cirsium arvense*). Reviews of Weed Science 5: 193–250.
- Donald WW (1992) Herbicidal control of *Cirsium arvense* (L.) Scop. roots and shoots in no-till spring wheat (*Triticum aestivum* L.). Weed Research 32: 259–266. doi: 10.1111/j.1365-3180.1992.tb01885.x

- Duke SO (2012) Why have no new herbicide modes of action appeared in recent years? Pest Management Science 68: 505–512. doi: 10.1002/ps.2333
- Enloe SF, Lym RG, Wilson R, Westra P, Nissen S, Beck G, Moechnig M, Peterson V, Masters RA, Halstvedt M (2007) Canada thistle (*Cirsium arvense*) control with aminopyralid in range, pasture, and noncrop areas. Weed Technology 21: 890–894. doi: 10.1614/WT-07-004.1
- Frantzen J (1994) An epidemiological study of *Puccinia punctiformis* (Str.) Röhl as a steppingstone to the biological control of *Cirsium arvense* (L.) Scop. New Phytologist 127: 147–154. doi: 10.1111/j.1469-8137.1994.tb04269.x
- Gassmann A, Louda SM (2001) *Rhinocyllus conicus*: initial evaluation and subsequent ecological impacts in North America. In: Wajnberg E, Scott JK, Quimby PC (Eds) Evaluating indirect ecological effects of biological control. CABI Publishing, Wallingford, Oxon, 147–183.
- Hallett SG (2005) Where are the bioherbicides? Weed Science 53: 404–415. doi: 10.1614/ WS-04-157R2
- Hunter JH, Smith LW (1972) Environment and herbicide effects on Canada thistle ecotypes. Weed Science 20: 163–167.
- Leth V, Andreasen C (2000) Alginate helps *Phomopsis cirsii* Grove to overcome short dew periods. In: BIO-Y2K Combined Millenium Meeting, Grahamstown, South Africa, 563–564. doi: 10.1111/j.1365-3180.2008.00666.x
- Leth V, Netland J, Andreasen C (2008) *Phomopsis cirsii*: a potential biocontrol agent of *Cirsium arvense*. Weed Research 48: 533–541.
- Li H, Wan H, Evans HC, Ellison C, Hinz HL, Zhang Y, Zhang F (2011) Biological control of Canada thistle, *Cirsium arvense*. CABI Annual Report 2010.
- McFadyen REC (1998) Biological Control of Weeds. Annual Review of Entomology 43: 369–393. doi: 10.1146/annurev.ento.43.1.369
- Miller BR, Lym RG (1998) Using the rosette technique for Canada thistle (*Cirsium arvense*) control in row crops. Weed Technology 12: 699–706.
- Muller E, Nentwig W (2011) Plant pathogens as biocontrol agents of *Cirsium arvense -* an overestimated approach? NeoBiota 11: 1–24. doi: 10.3897/neobiota.11.1803
- Sciegienka JK, Keren EN, Menalled FD (2011) Interactions between two biological control agents and an herbicide for Canada thistle (*Cirsium arvense*) suppression. Invasive Plant Science and Management 4: 151–158. doi: 10.1614/IPSM-D-10-00061.1
- Thomson SV, Kropp BR (2004) Production of *Puccinia thlaspeos* "woad" strain inoculum using traditional farming equipment. Phytopathology 94: S155–S155.
- Tiley GED (2010) Biological Flora of the British Isles: *Cirsium arvense* (L.) Scop. Journal of Ecology 98: 938–983. doi: 10.1111/j.1365-2745.2010.01678.x
- US-EPA (2002) *Puccinia thlaspeos* 'strain woad'. US Environmental Protection Agency, Office of Pesticide Programs.
- Watson AK (1991) The classical approach with plant pathogens. In: TeBeest DO (Ed) Microbial control of weeds. Chapman and Hall, London, 3–23. doi: 10.1007/978-1-4615-9680-6_1
- Weaver MA, Lyn ME, Boyette CD, Hoagland RE (2007) Bioherbicides for weed control. In: Upadhyaya MK, Blackshaw RE (Eds) Non-chemical weed management. CAB International, 93–110.

- Wilson RG, Martin AR, Kachman SD (2006) Seasonal changes in carbohydrates in the root of Canada thistle (*Cirsium arvense*) and the disruption of these changes by herbicides. Weed Technology 20: 242–248. doi: 10.1614/WT-05-052R1.1
- Zhou LC, Bailey KL, Derby J (2004) Plant colonization and environmental fate of the biocontrol fungus *Phoma macrostoma*. Biological Control 30: 634–644. doi: 10.1016/j.biocontrol.2004.03.002

LETTER TO THE EDITOR



Highest degrees of host specificity even in the inundative biocontrol approach. Response to Cripps et al.

Wolfgang Nentwig¹

I Institute of Ecology and Evolution, University of Bern, Switzerland

Corresponding author: Wolfgang Nentwig (wolfgang.nentwig@iee.unibe.ch)

Received 14 May 2012 | Accepted 14 May 2012 | Published 16 May 2012

Citation: Wolfgang Nentwig (2012) Highest degrees of host specificity even in the inundative biocontrol approach. Response to Cripps et al. NeoBiota 13: 41–42. doi: 10.3897/neobiota.13.3381

The response to the long letter, Cripps et al. (2012) wrote against Müller and Nentwig (Plant pathogens as biological agents of *Cirsium arvense* – an overestimated approach? NeoBiota 11:1–24, 2011) can be summarised in three points:

- (1) There are two approaches for biocontrol, classical biocontrol and inundative biocontrol. It is usually accepted that both differ in goals and requirements. In our literature review on the efficiency of various pathogens to control *Cirsium arvense*, we did not consequently mention for which approach which pathogen has been proposed since many articles we reviewed did not discuss this aspect. Moreover, there is a tendency to propose agents with insufficient target selectivity for the inundative method. In fact, both techniques do not represent discrete categories, but rather a continuum.
- (2) Nevertheless, Cripps et al. state that inundative biocontrol agents do not need to be specific. Instead of importing "new species" (they probably mean "alien species" and want to refer to classical biocontrol), they define inundative biocontrol as usage of "already present, often cosmopolitan generalist microbial pathogens". They recommend this application, call it safe and sustainable, but do not discuss why already present pathogen species shall be applied or why they are not effective. Moreover, this statement shows an astonishing lack of sensitivity to spreading species which are considered to be cosmopolitan, thus ignoring potential safety problems with non-targets.

(3) The most interesting point in this letter, however, concerns the well-known fact that plant pathogens face a lot of problems in reaching the biocontrol market. Cripps et al. list some of them (lack of investor interests, "bureaucratic issues", "onerous and costly government registration procedures", cost of production, small size of the market, competition by herbicides) which all may contain a grain of truth. In our review we only briefly touched this topic but mentioned another crucial point which refers, in the case of *Cirsium arvense*, to an obvious lack of host specificity or loss of virulence. We are still convinced on the importance of this conclusion and do not agree with Cripps et al. that host-specificity is not necessary.

Our review is restricted to *Cirsium arvense* and we did not draw any conclusion to other weeds or to biocontrol in general. Biocontrol may have a great future and may indeed be underresearched, but scientists have to be more than careful to avoid unsuitable species introductions. The spread of *Harmonia axyridis* in Europe, even after years of negative experience in the US still a declared inundative biocontrol agent, was a fall from grace which may not happen again. Therefore, it is highly recommendable to demand highest degrees of host specificity even in the inundative approach.

RESEARCH ARTICLE



Increase of an introduced bird competitor in old-growth forest associated with restoration

Leonard A. Freed¹, Rebecca L. Cann²

Department of Biology, University of Hawaii at Manoa, 2450 Campus Road, Dean Hall 2, Honolulu, Hawaii 96822
Department of Cell and Molecular Biology, University of Hawaii at Manoa, 1960 East-West Road, Honolulu, Hawaii 96822

Corresponding author: Leonard A. Freed (lfreed@hawaii.edu)

Academic editor: Daniel Sol | Received 15 February 2011 | Accepted 17 May 2012 | Published 23 May 2012

Citation: Freed LA, Cann RL (2012) Increase of an introduced bird competitor in old-growth forest associated with restoration. NeoBiota 13: 43–60. doi: 10.3897/neobiota.13.2946

Abstract

Many successful invasions involve long initial periods in which the invader exists at low densities followed by sudden population increases. The reasons for such time-lags remain poorly understood. Here we document a sudden increase in density of the introduced Japanese white-eye (*Zosterops japonicus*) in a restoration area contiguous with old-growth forest at Hakalau Forest National Wildlife Refuge on the Island of Hawaii. The refuge, with very high density of native birds, existed in a pocket of low whiteeye density that persisted for at least 20 years since the late 1970s. The refuge began an extensive native trees restoration project in 1989 within a 1314 ha abandoned pasture above old-growth forest. This area was soon colonized by white-eyes and their population grew exponentially once the trees had grown tall enough to develop a canopy. This increase was in turn followed by significantly more white-eyes in the open and closed forests adjacent to the restoration area. Competition between white-eyes and native species was documented on study sites within these forests. Density data indicate that competition was more widespread, with loss of tens of thousands of native birds in the 5371 ha area surveyed. Our results are consistent with the view that ecological barriers may delay the population increase of invaders and that human-derived activities may help invaders cross these barriers by creating new ecological opportunities. Control of white-eye numbers may be essential for recovery of native species.

Keywords

Introduced bird, restoration, exponential population growth, propagule pressure, biotic resistance, competition, time-lag

Introduction

When introduced into novel environments, some invaders increase rapidly in the new location, while others experience time-lags of various lengths (Roughgarden 1986, Williamson 1996, Blackburn et al. 2009). The causes of the time-lags are not well understood (Crooks 2005), but some explanations have been advanced (summarized by Crooks and Soule 1999). Most explanations focus on demographic factors (e.g. Allee effects; Taylor and Hastings 2005, Tobin et al. 2007), and evolutionary factors (genetic changes for adapting to the novel environment or increased competitive ability; Sakai et al. 2001, Kolbe et al. 2004, Hufbauer and Torchin 2007). Nevertheless, it is also possible that some invaders remain at low numbers due to biotic or environmental factors, like diffuse competition or unfavorable climatic conditions. These cases can be detected when the factors limiting population growth no longer exist. The goal of this study is to document an avian invasion where the population exhibited a time-lag that lasted over 20 years and then suddenly increased in numbers when environmental conditions changed due to a native trees restoration project. That invasion eventually led to increased density off-site with strong negative impacts to native bird species.

Restoration is a human-derived environmental change that is known to provide ecological opportunities in habitat for plants and animals (D'Antonio and Meyerson 2002), similar to what happens during succession (Connell and Slayter 1977). Consequently, restoration can also attract invasive animals. While the existence of ecological opportunities should trigger the increase of the invader population, in restoration areas time-lags may arise associated with growth of plants, analogous to animals that become more abundant in later seral stages of succession. In addition, nearby mature habitat can be affected if the propagule pressure from the restoration area is strong enough to overwhelm biotic resistance (Hollebone and Hay 2005, Lockwood et al. 2005, von Holle and Simberloff 2005).

This paper focuses on the invasion of Hakalau Forest National Wildlife Refuge (Mauna Kea, Island of Hawaii) by the Japanese white-eye (*Zosterops japonicus*), a passerine bird native to Asia. The white-eye was intentionally introduced to the Island of Oahu in 1929 to control insects (Caum 1933). It was later introduced to the Island of Hawaii in 1937 (Foster 2009), where Baldwin (1953), Dunmire (1962), and Banko and Banko (1980) documented an explosive increase in numbers and range. During the Hawaii Forest Bird Survey conducted in the late 1970s, the white-eye was the most common bird in the state and was found in every forest (Scott et al. 1986). There was no documented lag time for its increase after the introduction.

Nevertheless, on Mauna Kea, in the land that eventually became Hakalau Forest National Wildlife Refuge in 1985, the white-eye remained at low densities documented in the late 1970s. The refuge has the highest density of native birds on the island (Scott et al. 1986), so it is possible that the low density was maintained by biotic resistance in the form of diffuse competition from the community of native birds (MacArthur 1972, Pianka 1974, Case 1990). The white-eye overlaps multiple foraging substrates with each native species (Freed et al. 2008a), as assumed by the theory of diffuse competition (MacArthur 1972). In 1989 and following years, over 400,000 *Acacia koa* seedlings were planted in abandoned pasture above the old-growth forest. Seedlings were about 4 months old and 20 cm high, but by 5 years some trees had achieved heights of 8 m (Scowcroft and Jeffrey 1999), with a canopy that sustained arthropods and provided sufficient cover for thermal protection during cold nights at upper elevation.

Our goal is to describe how the refuge restoration area brought a sudden increase in white-eye density, and to ask whether this sudden increase may explain the recent increase of the invader in adjacent open and closed forests with native birds. Documenting the increased density of white-eyes throughout the refuge is particularly relevant given the diverse evidence that the species is negatively affecting native birds (Freed et al. 2008a, 2009; Freed and Cann 2009, 2012), and the possibility that the problems extend well beyond our study areas. We deal with this by also documenting changes in density of native species throughout the refuge.

Methods

Hakalau Forest National Wildlife Refuge (Fig. 1) consists mainly of old-growth ohialehua/koa (*Metrosideros polymorphal Acacia koa*) forest (Freed 2001). Bird surveys were conducted in the three main areas with transects indicated in Fig 2. The 3373 ha open forest area was formerly a cattle ranch so the understory is a matrix of patches of ferns and woody plants surrounded by expansive swatches of introduced pasture grasses. However, the old-growth forest remains in the form of many ohia-lehua trees, including large specimens, and large koa trees that provide nesting sites and foraging substrates for all species of native and introduced birds (Freed 2001, Freed et al. 2008a). Ohia-lehua comprise over 90% of the forest canopy (Freed et al. 2008a), and arthropods have been collected and analyzed from twigs and foliage (Freed et al. 2007). Below the open forest is the 1998 ha closed forest area with similar canopy trees and bird species, but with more native understory plants. Above part of the open forest area is the 1314 ha restoration area that had been extensively cleared for pasture more than 100 years ago (Tomonari-Tuggle 1996).

Estimates of white-eye density and variance from survey data were obtained from Camp et al. (2009) for years 1987–2007 in the restoration and open forest areas, and from 1999–2007 in the closed forest area, the initial time that area was included in surveys. We analyzed the data as follows. First, for all three areas, we tested for temporal autocorrelation using the "acf" function in S-Plus. This estimates the autocovariance function by summing the lagged products and dividing by the length of the series. For autocorrelation, all covariances are further divided by the geometric mean of the variances. The function produces 95% confidence limits for the different lags. No lags excluded 0 in any area. Then, for all areas, we compared exponential and linear trend models, because range expansion models frequently begin with exponential growth (Crooks and Soule 1999). We examined the distribution of residuals to determine the



Figure 1. Maps of distribution and abundance of the endangered Hawaii akepa (Loxops coccineus coccineus) (left) and introduced Japanese white-eye (right) in the windward Hawaii study area from the Hawaii Forest Bird Survey conducted in the late 1970s (Scott et al. 1986). Darker colors indicate higher density. The pocket of low white-eye density contains the southern portion of Hakalau Forest National Wildlife Refuge. That pocket contains most of the Mauna Kea population of the akepa and other native birds. The small high density area of akepa \mathbf{A} (401–800 birds/km²) almost perfectly matches a very low density of white-eyes 11-50 and 51-100 birds/km²). The habitat around the area is identical old-growth forest, so habitat selection is not involved. The area to the north where the akepa is vanishingly rare **B** (1–10 birds/km²) corresponds to an area with very high white-eye density (400–800, 801–1600 birds/ km²). The isolated akepa populations to the south **C** on Mauna Loa (101–200 birds/km²), are in areas with low density of white-eyes (11-50, 51-100 birds/km²). These data contrast with Mountainspring and Scott (1985), who documented a positive relationship between white-eye and akepa densities. The dark rectangular section of high white-eye density in the lower right contains Hawaii Volcanoes National Park, from which the akepa and many other native birds disappeared during the years 1940-1970 when the white-eye increased explosively (Dunmire 1962, Baldwin 1953, Banko and Banko 1980). Avian malaria may also have played a role (van Riper et al 1986). Modified from Scott et al. (1986), with permission from the Cooper Ornithological Society.

fit of the model to the data. See Freed and Cann (2010) for an example where this procedure was mistakenly not followed for trend analysis of native Hawaiian birds. For the closed forest area we used non-linear regression, without transforming the dependent variable, so that Akaike information criterion (AIC) could be used to distinguish



Figure 2. Portion of Hakalau Forest National Wildlife Refuge that was surveyed, relative to aerial photograph **A** and configuration of restoration, open forest, and closed forest areas **B** based on Camp et al. (2010). Black circles on (B) indicate study sites where birds were captured in mist-nets. Open forest sites are on Transect 1 which are numbered sequentially to the north. N and S indicate northern and southern sections of open forest area. The lower N is state-owned mixed native and introduced forest area has large trees (Freed 2001) with mainly introduced grass understory. Modified from Camp et al. (2010), with permission from the University of California Press.

the models (Burnham and Anderson 2002). S-Plus 8.2 (TIBCO Software) was used for all statistical analyses.

For the open forest area, we also used a randomization test for a stepwise increase during 1988–1999 and 2000–2007. The time periods were based on mist-netting that indicated a stepwise increase beginning in 2000 (Freed et al. 2008a). For this test we eliminated the extreme outlier in 1987 (Fig. 3), justified because this was the first year that surveys were conducted on the refuge, and the outlier value was not corroborated by mist-netting in the open forest site (Freed et al. 2008a). We ran 10,000 permutations and used a two-sided test.

We determined the order of increase between areas in two ways. For the restoration area and the open forest area, we compared slopes of regressions of density on year for 1988–2000. This time period was selected because it occurred before the stepwise increase from mist-netting. A significant slope in the restoration area but not in the open forest area would establish order. For the open forest area and closed forest area, we compared sequential differences in density from 1999 to 2000 and to 2001 in relation to the standard errors of the estimates. We determined if the differences between years were greater than two standard errors of those differences, indicating growth, or no growth if the differences were less than two standard errors.

We use functional data analysis (Ramsey and Silverman 1997, Murtaugh 2007) to document change in numbers of native birds between 2000 and 2007, using survey data from Camp et al. (2009). This type of analysis uses a function of the data, rather than all of the raw data, for statistical analysis that is readily interpretable. For example, growth of individuals can be analyzed through repeated measures analysis of variance, regression of size over time, or estimation of parameters of growth models like the von Bertalanffy. An even simpler function of growth data is the change between an early point and a later point in time. The change can be evaluated by the difference in values and variation associated with each value. We apply this simple function to survey data, using values from the beginning and end of a time interval, to determine if a significant change in numbers had occurred during that interval.

This function tests the same hypothesis as regression, change over time, and it is particularly relevant for documenting the consequences of an environmental change. Unlike regression, the functional data analysis is not constrained by constant change per unit time, or by the location of the intercept relative to the data. There is no theory in population dynamics that predicts constant rate of change. The analysis can also estimate the magnitude of change using basic mathematical properties of mean and variance of sums and differences of random variables (DeGroot and Schervish 2002), and can calculate 95% confidence intervals of the differences in values at each end of the time interval. We use this approach to compare the number of species with significant changes compared with the two from piecewise regression (Freed and Cann 2010).

The two endpoints we compare are densities in 2000 and 2007. The year 2000 is the first year of an environmental change (Freed et al. 2008a; Freed and Cann 2009, 2012). The year 2007 is the last year of available data. To guard against anomalously large or small values at endpoints, we average the endpoints with values from surrounding years, using years 1999, 2000, and 2001 to calculate the mean density of the initial endpoint, years 2006 and 2007 for the mean density at the end of the series. Each density value has a variance, which integrates spatial variability, uncertainty of detection, detections at station center, and model fit from Program Distance (Buckland et al. 1993). Camp et al. (2009) reported the square root of that variance as standard error, which is really the standard deviation, so we square that value to calculate the variance of each point and then use properties of variance of sum of random variables to calculate the variance at each end of the time series. Then the difference in mean density was calculated along with variance of the difference. For each species, we calculated 95% confidence intervals from variance of the difference in density to assess significance of change in density.

We illustrate with this method the decline in the open forest area of the iiwi (*Vestiaria coccinea*), currently a species of concern in the process of being listed as endangered. The density in 2000 was 23.80 birds/ha, bracketed by 19.59 in 1999 and by 17.02 in 2001, for a mean density of 20.14. The density in 2007 was 7.77 birds/ha bracketed by 15.92 in 2006, for a mean density of 11.85. The standard deviations

were 0.0641, 0.890, and 0.722 for years 1999-2001 and 0.0766 and 0.0634 for years 2006–2007. Squaring these gives the variances. Then, the sum of variances for each set of years is calculated using the following property of variance of the sum of independent random variables: $Var(a_1X_1 + ... + a_nX_n) = a_1^2Var(X_1) + ... + a_n^2Var(X_n)$, where the a are coefficients. The mean is the sum of random variables with the a equal to 1/n, where n is the sample size. For the iiwi, the means are the sum of independent random variables $X_1/3 + X_2/3 + X_2/3$ for 1999–2001 and $X_1/2 + X_2/2$ for 2006–2007. Therefore the variance of the mean random variables are (0.0041 + 0.7921 + 0.5213)/9 = 0.1464for 2000 and (0.0059 + 0.0040)/4 = 0.0002 for 2007. Then, the difference between the means, 20.14 - 11.85 = 8.29 becomes a new random variable representing the decline. The variance of the difference between independent random variables is the same as the variance of the sum. Thus the variance of the difference is 0.1464 + 0.0002= 0.1466. Taking the square root gives the standard error of the difference as 0.3829. Twice the standard error on either side of the 8.29 birds/ha difference between 2000 and 2007 gives the 95% confidence interval of (7.52, 9.06). Because the confidence interval does not include 0, we can conclude that a significant decline has occurred in the open forest area. The extent of the decline can be estimated by multiplying the 8.29 birds/ha change by the 3373 ha area.

We use this same methodology to document the white-eye increase in the closed forest area. For the open forest area, we use the difference in means established by the randomization test, and then calculate the variances associated with the standard deviations from all years during 1988–1999 and 2000–2007. Then the 95% confidence interval was calculated as specified for 2000 and 2007.

For community-wide changes in each survey area, we took covariance among species into account. We used the variance of densities of all 8 native species during 2000–2007 plus the covariance of densities from all pairs of native species those same years, according to the standard statistical formula Var(X+Y) = Var(X) + Var(Y) + 2Cov(X,Y), extended to 8 species. From this total, the 95% confidence interval of variances of the difference in density in the community was calculated.

Results

Restoration area. White-eyes increased in the restoration area, especially from 1999 to 2007 (Fig. 3a). They had low or 0 density when seedlings were first planted in 1989, and were mainly present in the few remnant trees along predominantly dry streams following the mountain slope in the area. The linear growth model for the entire series was significant (slope = 0.27 ± 0.06 , R² = 0.57, p = 0.0007), but was dismissed because it had significant lack of fit. This was indicated by a run of 7 negative residuals (sign test, p = 0.01). The exponential growth model for the entire series was also significant (parameter = 0.14 ± 0.03 , R² = 0.66, p = 0.0001), with sufficient fit indicated by runs of no more than 4 positive or negative residuals. The increase did not really start until 1998 (Fig. 3a).



Figure 3. Long-term patterns of Japanese white-eye density with standard error in: **a** restoration area **b** open forest area, and **c** closed forest area. Fitted values from the best model (deviation) are shown in a) and c). Median of the entire series is shown in a) and b). For b), medians for 1987–1999 and for 2000–2007 are shown in dotted lines.

Open forest area. White-eyes also increased in the open forest area where they previously existed at lower density (Fig. 3b). Linear regression did not reveal the increase (slope = 0.01 ± 0.05 , R² = 0.004, p = 0.78). However, residuals had significant lack of fit, indicated by 2/13 positive residuals during 1987–1999 and 6/8 positive residuals during 2000–2007 (test of proportions, p = 0.023). The exponential regression model had adequate fit (runs of maximum length 3) but was not significant (parameter = 0.01 ± 0.02 , R² = 0.035, p = 0.49). Nevertheless, the randomization test clearly indicated a higher mean density during years 2000–2007 (critical value for 0.975 quantile = 0.97, observed difference = 1.05), consistent with a stepwise increase that could not be modeled with linear or exponential regression.

Closed forest area. White-eye density also increased in the closed forest area, where the birds had previously existed at low density (Fig. 3c). Both linear and exponential models were supported (linear: slope = 0.13 ± 0.03 , R² = 0.74, p = 0.003; exponential: parameter = 0.05 ± 0.01 , R² = 0.77, p = 0.002), had adequate fit by runs of signed residuals, and were indistinguishable (Δ AICc exponential = 0, linear = 0.16).

Order of increase among areas. During years 1988–2000, density increased in the restoration area (slope = 0.6, se = 0.06, p = 0.04), but not the open forest area (slope = 0.08, se = 0.08, p = 0.33). Thus the increase began in the restoration area before the open forest area. Changes in density during the years 1999–2001 were significant in the open forest area (1.90 \pm 0.79, 2.82 \pm 0.85), but not in the closed forest area (0.01 \pm 0.42, 0.03 \pm 0.85). The overall order of increase was from the restoration to the open forest to the closed forest area (Fig. 3).

Changes in native birds in the forested areas. Figure 4 shows significant declines in native birds the same years that the white-eye was increasing in both the open and closed forest areas. The declines in the closed forest area were smaller than those estimated in the open forest area (Fig. 4, paired t = 2.15, df =7, p = 0.034). However, the high correlation between changes in density in the two forest areas (cor = 0.93, p = 0.0003), including white-eyes, indicates that the pattern of changes among native species and white-eyes was similar in the open and closed forest areas. Table 1 shows that tens of thousands of native birds perished even under the most optimistic scenario. Based on mean 2000 densities times 3373 ha, there were approximately 166,927 native birds in the open forest area. The loss of 61,289 birds represents a 37% decline, ranging from 51 to 22%. The closed forest area had 86,653 birds with a loss of 9091, representing a 10% decline. The white-eye was the only bird that significantly increased in each area (Fig. 4, Table 1).



Figure 4. Change in densities with standard error of native birds and Japanese white-eyes in the open forest area (black bars) and closed forest area (white bars), between 2000 and 2007. Native passerine species present throughout the closed forest area and the southern section of the open forest area were Hawaiian honeycreepers: Hawaii akepa (*Loxops coccineus coccineus*), Hawaii creeper (*Oreomystis mana*), akiapolaau (*Hemignathus munroi*), Hawaii amakihi (*Hemignathus virens virens*), iiwi (*Vestiaria coccinea*), and apapane (*Himatione sanguinea*); Monarchine flycatcher: Hawaii elepaio (*Chasiempis sandwichensis ridgwayi*); and Thrush: omao (*Myadestes obscurus*).

Table 1. Changes in numbers between 1999–2001 and 2006–2007 in open and closed forest (Fig. 1), based on changes in density multiplied by area (ha). Endangered species (E), species of concern (SOC). Confidence intervals for species are based on two standard errors of changes in density. Confidence intervals for community change are based on two standard errors of the sum of variances and twice the sum of covariances of all native species densities from 2000 through 2007.

Species	Change in Numbers	Lower 95% CI	Upper 95% CI
Open Forest			
Hawaii akepa (E)	-2,081	-3,080	-1,083
Hawaii creeper (E)	-968	-2,506	570
Akiapolaau (E)	-169	-405	69
Hawaii amakihi	-14,329	-17,587	-11,070
Iiwi (SOC)	-27,969	-32,442	-23,496
Apapane	-13,823	-16,602	-11,043
Hawaii elepaio	-1,400	-3,329	530
Omao	-550	-1,191	91
Community change:	-61,289	-85,892	-36,686
Japanese white-eye	3,542	5,083	2,001
Closed Forest	·		
Hawaii. akepa (E)	440	-515	1,395
Hawaii creeper (E)	-320	-1,990	1,351
Akiapolaau (E)	-180	-432	72
Hawaii amakihi	-3,177	-10,557	-7,305
Iiwi (SOC)	-3,576	-6,833	-320
Apapane	-2,737	-4,707	-767
Hawaii elepaio	539	-1,083	2,162
Omao	-80	-615	456
Community change:	-9,091	-12,778	-5,404
Japanese white-eye	1,738	2,829	647

Discussion

Land that became Hakalau Forest National Wildlife Refuge existed for years in a pocket of low white-eye density, especially in the southern end with the intact bird community (Scott et al. 1986). There are several reasons why the invader remained at such low densities. Because white-eyes exhibit substantial overlap in the foraging niche with all native species, invader numbers may have remained low due to biotic resistance. Alternatively, conditions in the forested areas may have been sub-optimal for the invader. Calculation of the deterministic rate of population growth was 0.97 with an assumption of breeding success of 0.6 (Freed et al. 2008a), indicating that limited immigration might have been necessary to maintain numbers. Nevertheless, when the pasture land above the forest became a restoration area, the white-eye colonized that area and eventually grew exponentially in it. The bird then increased in density in the contiguous open forest area followed by the more distant closed forest area. Substantial

decline in native birds in the open forest area and lesser declines in the closed forest area followed. There are many issues concerning numbers and impacts.

Increase of white-eye numbers. The restoration is a clear example of a human activity that increases ecological opportunities for an introduced species that otherwise should have remained at low numbers. The restoration also offered opportunities for three native species (Camp et al. 2010). The Hawaii amakihi (*Hemignathus virens virens*), the most generalized native bird, was growing exponentially at comparable densities with the white-eye. The iiwi and apapane (*Himatione sanguinea*) populations were growing linearly and had much lower densities than the white-eye and amakihi during 2004–2007 (Camp et al. 2010). But unlike the white-eye, these three native species were significantly declining throughout the old-growth forest after 2000 (Fig. 4).

The increase of white-eyes in the open forest area seems to be due more to immigration from the restoration area than to *in situ* reproduction. This is because juvenile survival dropped from 0.27 to 0 for all years during the increase (Freed et al. 2008a). The order of increase excludes the closed forest area as the source of necessary propagules. Given no other land use change near the refuge, it is most likely that whiteeyes came from the restoration area. The exponentially growing population may have exceeded carrying capacity each year after 1999, set in part by native species whose populations were also increasing in the restoration area. The excess white-eyes over carrying capacity may have immigrated into the old-growth forest below.

However, the 0 juvenile survival of white-eyes was not associated with lower mass (Freed et al. 2008a), and is thus contrary to the general pattern in birds of lower mass resulting in lower juvenile survival (Medeiros and Freed 2009). Juveniles produced in the open forest area may have dispersed along a path of least resistance toward the lower density closed forest area. The increase in that area otherwise contradicts the assumption that the white-eye prefers open habitats in Hawaii (Scott et al. 1986) and in the Bonin Islands (Kawagami and Higuchi 2003), although preference for one habitat does not preclude preference for other habitats. The exponential increase in the closed forest at Hakalau either confirms the renowned ability of the entire family Zosteropidae for range and niche expansion (Scott et al 2003, Moyle et al. 2009), or simply resulted from dispersal of juveniles from the open forest or restoration areas.

The relatively small magnitude of the increase in white-eye density in the open forest area may have been severely underestimated. The area is very large (3373 ha) and includes introduced forest and forest with more open structure in the northern sections (Fretz 2002) (Fig. 2). Endangered species have their highest density in the southern section (Scott et al. 1986, Hart 2001). The closed forest area, where endangered species also have high density, is contiguous only with the southern section. There was a discrepancy between the white-eye densities in the two areas during 2004–2005 estimated from survey and mist-netting data. The survey data from the two areas indicated a density in the open forest area 1.4 times that in the closed forest area. However, capture rates in mist-nets were five-fold greater in the open forest study sites than in the closed forest site (Freed and Cann 2009), all in the southern portion of the refuge (Fig. 2). In addition, preliminary trend analysis indicated that the white-eye was increasing while the endangered Hawaii akepa was declining in the southern-most transects 1–4 (Fig. 2), where akepa density was highest (Fig. 1), but this pattern disappeared when densities of the two species from the entire open forest area were analyzed (Freed et al. 2008a). These diverse data imply that most of the white-eye increase was in the southern portion of the open forest area, with an extent of increase much higher than the overall 1.05 bird/ha. It is critically important that future analysis of survey data treat the southern portion of the open forest area separately.

Impacts of white-eyes on native species. Competition between species is indicated by change in condition, survival, and/or numbers of one species as the other increases or decreases in numbers (Keddy 2001). There is some evidence that changes in native species were caused by competition with white-eyes. Mountainspring and Scott (1985) documented competition between white-eyes and several species of native birds from survey data collected during the late 1970s from the Hawaiian Forest Bird Survey (Scott et al. 1986). They detected negative correlation in density between white-eyes and native species, with adjustment for differences in habitat between study areas. Freed et al. (2008a) showed that the white-eye was replacing the akepa in the open forest study sites, but not in the closed forest site with lower white-eye captures in mist-nets (Freed and Cann 2009). Figure 1 shows more spatial evidence of negative correlation between akepa and white-eye densities.

The piecewise regression used by Freed and Cann (2010) showed declines in all native species in the open forest area during 2000–2007, two of which were significant (akepa and iiwi). Here we add two more species (amakihi, apapane) with significant declines during the white-eye increase (Fig. 4). White-eyes spend as much time in ohia-lehua foliage as the amakihi and apapane (Freed et al. 2008a).

In previous studies, we identified changes in condition of native birds in our open forest sites that could be produced only by a factor that influenced foraging substrates used by each native species. Changes included lower mass, lower fat, shorter legs, and shorter bills (Freed et al. 2008a,b; Freed and Cann 2009), which generated lower juvenile and adult survival. In addition, we identified changes in timing, duration, and symmetry of molt of all native species, with increasingly lower survival during the winter months of 2000–2004 (Freed and Cann 2012). These changes in molt have been experimentally induced by severe food limitation in other birds (Murphy et al. 1988, Swaddle and Witter 1994). It is difficult to imagine an alternative factor that reduced arthropods from the foliage and branches of ohia-lehua trees, and hidden in lichen-covered branches of koa trees, all foraging substrates used by white-eyes (Freed et al. 2008a).

None of the skeletal changes in native birds in the open forest sites were observed in the closed forest site during 2004–2005, where white-eye capture rates were only 20% of open forest rates (Freed and Cann 2009). During 2005, prevalence of non-normal molt was lowest in the closed forest site, but became highest in 2006 (Freed and Cann 2012). This reversal was associated with increased density of white-eyes in the closed forest area and decreased density in the open forest area between 2005 and 2006 (Fig. 3).

The impact of direct competition in the decline of native species cannot be ascertained without experimental evidence, but alternative explanations are unconvincing. Avian malaria kills native birds, but it is too cool at upper elevations for avian malaria to be endemic (Freed et al. 2005). Introduced yellow-jacket wasps (Paravespula pensylvanica) compete for food with native birds (Gambino et al. 1987), but these were controlled after 2000 (Freed and Cann 2009). Parasitoid wasps, escaped from biological control, could reduce native arthropods, but these were more prevalent at lower than at upper elevations (Peck et al. 2008). We documented an explosive increase in chewing lice (from a different introduced bird), which can increase food requirements to replace heat lost from degraded plumage (Freed et al. 2008b). However, signs of food limitation began in 2000 and worsened in 2002 (Freed et al. 2008a, Freed and Cann 2012), before lice became widespread in the community in 2003 (Freed et al. 2008b). Mean monthly air temperatures did not change between 1993-1999 and 2000-2006, indicating that climate change was not responsible for reduced productivity of arthropods (Freed and Cann 2012). If any of these alternatives had caused food limitation and lower survival in all native species, then they should have caused comparable food limitation and declines in white-eyes given the similarity in foraging substrates. In particular, the dynamic of molt and white-eyes between study sites and areas during 2005 and 2006 suggests that a third factor was not involved. Such factors, that favor one species over another to resemble competition, are frequent alternatives to competition (Keddy 2001).

Our data also presage connections between biotic resistance, diffuse competition, and a reversal of diffuse competition through propagule pressure. Biotic resistance relevant to this system is diffuse competition, which arises when multiple native species overlap different parts of the niche of a potential invader (MacArthur 1972). For an invader at low density, such competition in a tightly interacting native community can prevent increases in density (Case 1990). The overlap of multiple foraging substrates with each native species likely provided the diffuse competition that kept the white-eye at low density in the old-growth forest. However, when the population increased, based on the opportunities offered in the restoration area, this competition was overwhelmed by propagule pressure, as documented for other systems (von Holle and Simberloff 2005, Hollebone and Hay 2005, Colautti et al. 2006, Lockwood et al. 2005, Simberloff 2009). A removal experiment that reduces the density of white-eyes may help determine if limiting propagule pressure enables diffuse competition to be restored.

Conclusion

The pocket of low white-eye density that persisted for decades is now filling up with white-eyes from the restoration area. That area is a complete counterexample to restoration areas constraining invasion (Bakker and Wilson 2004). The entire bird community was at the threshold of food-limitation before the increase (Hart 2001). A moderate but sustained increase in white-eyes could then create major food limitation, although that increase may have been much higher in the southern portion of the refuge. Competitive superiority combined with propagule pressure was involved in

the increase in old-growth forest, while ecological opportunism was the basis for the increase in the restoration area. These are usually considered as alternatives for invasion success (Sax et al. 2007, Sol et al. 2012).

The U.S. Fish and Wildlife Service, in formulating its final comprehensive conservation plan in late 2010 (http://www.fws.gov/hakalauforest/planning.html), emphasized habitat restoration efforts on the refuge. Evidence presented here indicates that this plan will provide more ecological opportunities for white-eyes, and this will likely further increase the negative impacts on native birds in the forest below. The plan assumes that avian malaria is the greatest long-term problem for the birds (Benning et al. 2002, Atkinson and LaPointe 2009), and we have shown that epizootics occur in the open forest area (Freed et al. 2005). However, white-eye competition could be a more serious and immediate problem for Hawaiian forest birds, especially if the impacts are in the most intact native communities, as found by Herbold and Moyle (1986) for introduced fish.

A white-eye removal experiment must be performed to determine conclusively if competition is the cause of current food limitation in native birds and extensive declines. An experiment in New Zealand identified positive responses from multiple native species when an introduced bird was controlled (Tindall et al. 2007). Native Hawaiian birds have become so food-limited that they can neither breed successfully nor molt efficiently, and will certainly not be able to mount the expected energetically expensive immune response to malaria (Freed et al. 2005). The outcome of the experiment should serve to inform the future of management of Hawaiian forest birds at Hakalau and perhaps at other forested locations in Hawaii with native birds.

Acknowledgements

We gratefully respect the dedication of many students, volunteers, and interns who assisted with the field research. T. Smith, D. Sol, and anonymous reviewers gave useful comments on the manuscript. We were supported over the years by grants from the John D. and Catherine T. MacArthur Foundation (World Environment and Resources Program), National Science Foundation, Environmental Protection Agency (Science to Achieve Results, R82-9093), and Australian Research Council Discovery-Project Grant DP451402 to LAF (Partner Investigator; H. McCallum, Chief Investigator).

References

- Atkinson CT, LaPointe DA (2009) Introduced avian diseases, climate change, and the future of Hawaiian honeycreepers. Journal of Avian Medicine and Surgery 23: 53–63.
- Bakker JD, Wilson SD (2004) Using ecological restoration to constrain biological invasion. Journal of Applied Ecology 41: 1058–1064.
- Baldwin PH (1953) Annual cycle, environment and evolution in the Hawaiian honeycreepers (Aves: Drepaniidae). University of California Publications in Zoology 52: 285–398.

- Banko PC, Banko WE (1980) Historical trends of passerine populations in Hawaii Volcanoes National Park and vicinity. Second conference on scientific research in national parks 8: 108–125.
- Benning TL, LaPointe D, Atkinson CT, Vitousek PM (2002) Interactions of climate change with biological invasions and land use in the Hawaiian Islands: Modeling the fate of endemic birds using a geographic information system. Proceedings of the National Academy of Science USA 99: 14246–14249.
- Blackburn TM, Lockwood JL, Cassey P (2009) Avian invasions: the ecology and evolution of exotic birds. Oxford University Press, Oxford, 305 pp.
- Buckland ST, Anderson DR, Burnham KP, Laake JL (1993) Distance sampling: estimating abundance of biological populations. Chapman and Hall, London, 446 pp.
- Burnham KP, Anderson DR (2002) Model selection and multimodal inference, 2nd ed. Springer-Verlag, New York, 488 pp.
- Camp RJ, Pratt TK, Gorresen PM, Jeffrey JJ, Woodworth BL (2009) Passerine bird trends at Hakalau Forest National Wildlife Refuge, Hawai'i. Hawaii Cooperative Studies Unit Technical Report HCSU-011, University of Hawaii at Hilo.
- Camp RJ, Pratt TK, Gorresen PM, Jeffrey JJ, Woodworth BL (2010) Population trends of forest birds at Hakalau Forest National Wildlife Refuge, Hawaii. Condor 112: 196–212.
- Case TJ (1990) Invasion resistance arises in strongly interacting species-rich model competition communities. Proceedings of the National Academy of Science USA 87: 9610–9614.
- Caum EL (1933) The exotic birds of Hawaii. Occasional Papers of the BP Bishop Museum 10: 1–55.
- Colautti RI, Grigorovich IA, MacIsaac HJ (2006) Propagule pressure: a null model for biological invasions. Biological Invasions 8: 1023–1037.
- Connell JH, Slatyer RO (1977) Mechanisms of succession in natural communities and their role in community stability and organization. American Naturalist 111: 1119–1144.
- Crooks JA (2005) Lag times and exotic species: the ecology and management of biological invasions in slow-motion. Ecoscience 12: 316–329.
- Crooks JA, Soule ME (1999) Lag times in population explosions of invasive species: causes and implications. In: Sandlund OT, Schei PJ, Viken AV (Eds) Invasive Species and Biodiversity Management. Klywer Academic Publishers, Dordrecht, 103–125.
- D'Antonio C, Meyerson LA (2002) Exotic plant species as problems and solutions in ecological restoration: a synthesis. Restoration Ecology 10: 703–713.
- DeGroot MH, Schervish MJ (2002) Probability and statistics, 3rd ed. Addison-Wesley, Boston, 816 pp.
- Dunmire WW (1962) Bird populations in Hawaii Volcanoes National Park. Elepaio 22: 65–70.
- Foster JT (2009) The history and impact of introduced birds. In: Pratt TK, Atkinson CT, Banko PC, Jacobi JD, Woodworth BL (Eds) Conservation Biology of Hawaiian Forest Birds. Yale University Press, New Haven, 312–330.
- Freed LA (2001) Significance of old-growth forest to the Hawaii akepa. Studies in Avian Biology 22: 173–184.
- Freed LA, Cann RL (2009) Negative effects of an introduced bird species on growth and survival in a native bird community. Current Biology 19: 1736–1740.

- Freed LA, Cann RL (2010) Misleading trend analysis and decline of Hawaiian forest birds. Condor 112: 213–221.
- Freed LA, Cann RL (2012) Changes in timing, duration and symmetry of molt are associated with extensive decline of Hawaiian forest birds. PLOS One 7: e29834.
- Freed LA, Cann RL, Bodner GR (2008a) Incipient extinction of a major population of the Hawaii akepa owing to introduced species. Evolutionary Ecology Research 10: 931–965.
- Freed LA, Cann RL, Diller KL (2009) Sexual dimorphism and the evolution of seasonal varation in sex allocation in the Hawaii akepa. Evolutionary Ecology Research 11: 731–757.
- Freed LA, Cann RL, Goff ML, Kuntz WA, Bodner GR (2005) Increase in avian malaria at upper elevation in Hawaii. Condor 107: 753–764.
- Freed LA, Fretz JS, Medeiros MC (2007) Adaptation in the Hawaii akepa to breed and moult during a seasonal food decline. Evolutionary Ecology Research 9: 157–167.
- Freed LA, Medeiros MC, Bodner GR (2008b) Explosive increase in ectoparasites in Hawaiian forest birds. Journal of Parasitology 94: 1009–1021.
- Fretz JS (2002) Scales of food availability for an endangered insectivore, the Hawaii Akepa. Auk 119: 166–174.
- Gambino P, Medeiros AC, Loope LL (1987) Introduced vespids *Paravespula pensylvanica* prey on Maui's endemic arthropod aauna. Journal of Tropical Biology 3: 169–170.
- Hart PJ (2001) Demographic comparisons between high and low density populations of Hawaii Akepa. Studies in Avian Biology 22: 185–193.
- Herbold B, Moyle PB (1986) Introduced species and vacant niches. American Naturalist 128: 751–760.
- Hollebone AL, Hay ME (2007) Propagule pressure of an invasive crab overwhelms native biotic resistance. Marine Ecology Progress Series 342: 191–196.
- Hufbauer RA, Torchin ME (2007) Integrating ecological and evolutionary theory of biological invasions. In: Nentwig W (Ed) Biological Invasions. Springer, Berlin, 79–96.
- Kawagami K, Higuchi H (2003) Interspecific interactions between the native and introduced white-eyes in the Bonin Islands. Ibis 145: 583–592.
- Keddy PA (2001) Competition (2nd Edition). Kluwer Academic Publishers, Dordrecht, 552 pp.
- Kolbe JJ, Glor RE, Schettino LR, Lara AC, Larson A, Losos JB (2004) Genetic variation increases during biological invasion by a Cuban lizard. Nature 431: 177–181.
- Lockwood JL, Cassey P, Blackburn TM (2005) The role of propagule pressure in explaining species invasions. Trends in Ecology and Evolution 20: 223–228.
- MacArthur RH (1972) Geographical Ecology: Patterns in the Distribution of Species. Harper & Row, Publishers, New York, 269 pp.
- Medeiros MC, Freed LA (2009) A fledgling-mass threshold greatly affects juvenile survival in the Hawaii akepa. Auk 126: 319–325.
- Mountainspring S, Scott JM (1985) Interspecific competition among Hawaiian forest birds. Ecological Monographs 55: 219–239.
- Moyle RG, Filardi CE, Smith CE, Diamond J (2009) Explosive Pleistocene diversification and hemispheric expansion of a "great speciator". Proceedings of the National Academy of Science USA 106: 1863–1868.

Murphy ME, King JR, Lu J (1988) Malnutrition during the postuptial molt of white-crowned sparrows: feather growth and quality. Canadian Journal of Zoology 66: 1403–1413.

Murtaugh PA (2007) Simplicity and complexity in ecological data analysis. Ecology 88: 56-62.

- Peck RW, Banko PC, Schwarzfeld M, Euaparadorn M, Brinck KW (2008) Alien dominance of the parasitoid wasp community along an elevational gradient on Hawai'i Island. Biological Invasions 10: 1441–1455.
- Pianka ER (1974) Niche overlap and diffuse competition. Proceedings of the National Academy of Science USA 71: 2141–2145.
- Ramsey JO, Silverman BW (1997) Functional data analysis. Springer-Verlag, New York, 310 pp.
- Roughgarden J (1986) Predicting invasions and rates of spread. In: Mooney HA, Drake JA (Eds) Ecology of Biological Invasions of North America and Hawaii. Springer-Verlag, New York, 179–188.
- Sakai AK, Allendorf FW, Holt JS, Lodge DM, Molofsky J, With KA, Baughman S, Cabin RJ, Cohen JE, Ellstrand NC, MaCauley DE, O'Neil P, Parker IM, Thompson JN, Weller SG (2001) The population biology of invasive species. Annual Review of Ecology and Systematics 32: 305–335.
- Sax DF, Stachowicz JJ, Brown JH, Bruno JF, Dawson MN, Gaines SD, Grosberg RK, Hastings A, Holt RD, Mayfield MM, O'Connor MI, Rice WR (2007) Ecological and evolutionary insights from species invasions. Trends in Ecology & Evolution 22: 465–471.
- Scott JM, Mountainspring S, Ramsey FL, Kepler CB (1986) Forest bird communities of the Hawaiian Islands: their dynamics, ecology, and conservation. Studies in Avian Biology 9: 1–431.
- Scott SN, Clegg SMB, S. P., Kikkawa J, Owens IPF (2003) Morphological shifts in island-dwelling birds: the roles of generalist foraging and niche expansion. Evolution 57: 2147–2156.
- Scowcroft PG, Jeffrey J (1999) Potential significance of frost, topographic relief, and *Acacia koa* stands to restoration of mesic Hawaiian forests on abandoned rangeland. Forest Ecology and Management 114: 447–458.
- Simberloff D (2009) The role of propagule pressure in biological invasions. Annual Review of Ecology and Systematics 40: 81–102.
- Sol D, Bartomeus I, Griffin AS (2012) The paradox of invasion in birds: competitive superiority or ecological opportunism? Oecologia 169: 553-564.
- Swaddle JP, Witter MS (1994) Food, feathers and fluctuating asymmetries. Proceedings of the Royal Society B: 255: 147–152.
- Taylor CM, Hastings A (2005) Allee effects in biological invasions. Ecology Letters 8: 895–908.
- Tindall SD, Ralph CJ, Clout MN (2007) Changes in bird abundance following common myna control on a New Zealand island. Pacific Conservation Biology 13: 202–212.
- Tobin PC, Whitmire SL, Johnson DM, Bjornstad ON, Liebhold AM (2007) Invasion speed is affected by geographical variation in the strength of allee effects. Ecology Letters 10: 36–43.
- Tomonari-Toggle MJF (1996) Bird catchers and bullock hunters in the upland Mauna Kea forest: a cultural resource overview of the Hakalau Forest National Willdlife Refuge, Island of Hawaii. Prepared for the US Fish and Wildlife Service. International Archeological Institute, Inc., Honolulu.

- van Riper III C, van Riper SG, Goff ML, Laird M (1986) The epizootiology and ecological significance of malaria in Hawaiian land birds. Ecological Monographs 56: 327–344.
- von Holle B, Siimberloff D (2005) Ecological resistance to biological invasion overwhelmed by propagule pressure. Ecology 86: 3212–3218.

Williamson M (1996) Biological Invasions. Chapman & Hall, London, 256 pp.