Invasional meltdown: pollination of the invasive liana *Passiflora tripartita* var. *mollissima* (Passifloraceae) in New Zealand

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Abstract: Banana passionfruit (*Passiflora tripartita* var. *mollissima*) is an invasive vine in New Zealand where it lacks its natural hummingbird pollinator. We investigated the mating system and reproductive traits that facilitate its spread in the Marlborough Sounds. Flower observations revealed that visitors were almost exclusively introduced honeybees and bumblebees, indicating an invasive mutualism. We investigated the pollination system of banana passionfruit by comparing fruit set, fruit size, seed set, and germination success between hand-selfed, hand-crossed, bagged and open flowers, and inbreeding depression in seedlings grown in competition. Fruit set was reduced by 83% when pollinators were excluded (3.0% fruit set, compared with 18.0% for unmanipulated flowers) indicating reliance on pollinators for reproduction. While banana passionfruit is partially self-compatible, fruit set was significantly reduced in hand-selfed flowers (17.5%) compared with crossed flowers (29.5%), and we found significant pollen limitation (hand-crossed vs unmanipulated, Pollen Limitation Index = 0.39). There was no significant inbreeding depression found in fruit size, seeds per fruit, germination success, seedling growth or seedling survival. Combining these data showed that natural unmanipulated flowers produce more seedlings per flower (1.7) than bagged flowers (0.9), but fewer than hand-selfed (3.0) and hand-crossed (5.3) flowers. Thus, reproduction in *Passiflora tripartita* var. *mollissima* is facilitated by an (imperfect) new association with exotic bees.

Keywords: *Apis*; banana passionfruit; *Bombus*; invasional meltdown; invasive mutualism; *Passiflora mollissima*; pollination; pollen limitation; weedy vine

Introduction

Invasive species are considered to be a serious threat to conservation and cause major modifications to indigenous biodiversity and ecosystem function, especially in isolated areas such as New Zealand that are particularly susceptible because of their high degree of endemism (Williams & West 2000). There are many barriers that must be overcome in order for a non-native species to become invasive (Henderson et al. 2006). Until recently, the emphasis of invasion ecology has been on the negative interactions between invasive species and other resident species (e.g. Parker et al. 2006) rather than the positive or mutualistic interactions that may enable a species to proliferate in an ecosystem. However, the absence of pollinators can limit seed production, which has been shown in some cases to reduce plant density (Parker 1997; Anderson et al. 2011). Consequently, some invasive species are not able to invade and survive in an ecosystem in the absence of mutualists, whether they be native or exotic (Richardson & Pysek 2006). For example, in New Zealand red clover (*Trifolium pratense*) is unable to set seed in the absence of introduced bumblebees (Richardson et al. 2000). Simberloff (Simberloff & Von Holle 1999; Simberloff 2006) called this invasional meltdown, where the spread of one exotic species increases in the presence of another exotic species. To understand what limits the spread of invasive species we must understand factors controlling reproduction, as many exotic plants depend on animal-mediated pollination and seed dispersal (Parker & Haubensak 2002).

Newstrom and Robertson (2005) stressed the need for more research in New Zealand focused on pollination, breeding systems and, specifically, invasive mutualisms. One prime candidate for such research is banana passionfruit, *Passiflora tripartita* var. *mollissima* (Passifloraceae) (Heenan & Sykes 2003), previously known as *P. mollissima* (Webb et al. 1988). The common name banana passionfruit applies to several species in the genus, but in this paper we use it to refer to *Passiflora tripartita* var. *mollissima*. This is one of several introduced vines considered to be serious weeds in New Zealand (Baars et al. 1998). It is a tendril-climbing liana species with large pink flowers with an extended floral tube, and large, yellow, fleshy fruits. Banana passionfruit flowers appear to be very specialised as they have an elongated floral tube (8–9.5 cm long; Webb et al. 1988) leading to a large nectar source. Together with its native pollinator, a hummingbird called the Andean swordbill (*Ensifera ensifera*) whose bill length may reach more than 10 cm, it represents a striking example of pollinator–plant coevolution (Endress 1994).

In its invasive range, including New Zealand, the plants lack their specialised pollinator. The extent to which hummingbirds have been replaced by other pollinators and the ensuing effectiveness of their pollination has been rarely studied. Research in Hawai'i (LaRosa 1992), where banana passionfruit is also invasive, showed that biotic pollinators play a major role in reproduction, and the mating system combines outcrossing with selfing. While flowers are highly self-compatible, natural selfing is infrequent (LaRosa 1992). However, little is known of the mutualistic interactions and reproductive strategies contributing to the proliferation of *Passiflora tripartita* var. *mollissima* in New Zealand. This raises several questions. Given that banana passionfruit has such specialised flowers, how has it become so invasive in New

Zealand without the specific pollinators of its native range? Do banana passionfruit flowers require pollinator visitation in order to reproduce? If so, which pollinator species are visiting these flowers in New Zealand, and are they effectively pollinating flowers?

In this study we aimed to (1) determine the extent to which banana passionfruit relies on out-crossing from biotic pollinators for reproduction, or is self-compatible and capable of autogamy, and the effect this has on fruit production, germination success and seedling growth; (2) establish the primary visitors to banana passionfruit flowers in New Zealand and the extent to which they are enhancing reproductive success through effective pollination; and (3) document the mating system of banana passionfruit and the degree to which reproduction is constrained by pollen limitation and inbreeding depression.

Materials and methods

Two sites in Queen Charlotte Sound, Marlborough, were used for pollination experiments. Each site had 10 replicate patches. Patches were used rather than separate plants due to the inability to distinguish one plant from another, and were separated by at least 15–20 m of clear ground. Site 1 was located on the uphill side of the segment of Port Underwood Road at 41°15.57' S, 174° 3.32' E. Patches 1–7 were located on a dense stand of banana passionfruit on low broadleaved vegetation while Patches 8–10 were located below 'Marina Views' where the vegetation was mostly gorse (*Ulex europaeus*). Site 2 was located along Queen Charlotte Drive in Kaireperepe Bay (41° 16.01' S, 173° 58.97' E) among scrubby mid-successional native tree species regenerating on slips and the edges of broadleaved forest.

Experimental pollinations

Within each patch we selected 40 flowers when the bud began to swell, prior to opening, during a heavy flowering period in December 2005 and January 2006. We marked flowers at the base using a paper tag with cotton tie as well as a colour-coded wire tag to indicate the treatment. We interspersed the 40 flowers among four main treatments. *Selfed* – we bagged ripe buds until open, then hand-pollinated them using anthers of the same flower, before replacing the bag. *Crossed* – we emasculated flowers prior to pollen production and hand-pollinated them using pollen of another flower located at least 100 m away. *Bagged* – we placed a bag (nylon with mesh size of 1 mm) over the bud before it opened to exclude all pollinators from flowers, to determine whether the flower is capable of autonomous selfing. *Natural* – we labelled these flowers and left them unmanipulated to measure natural fruit set rates when accessible to all pollinators. Overall, we selected 200 flowers for each treatment across patches and sites, giving 800 flowers in total. In addition, we selected 40 flowers (20 at each site) to test for asexual (apomictic) fruit production. Selected buds were emasculated prior to pollen production, and placed inside a bag to eliminate pollinators. This study included all of the five treatments specified by Newstrom and Robertson (2005) as being necessary to fully investigate the breeding system of a species.

We revisited the flowers approximately a month later and noted fruit development. The fruit were then left to ripen. We collected fruit when yellow and soft and recorded the length, maximum width, and number of seeds in each fruit. The

sample sizes for fruit ripened in each treatment were: selfed = 23 fruit; crossed = 37 fruit; bagged = 4 fruit; natural = 18 fruit; apomictic = 0 fruit. After the seeds from the first season were counted, we realised that smaller seeds which did not appear fully developed (e.g. with light colouring or no outer casing) were counted in seed counts. However, these were not used for germination experiments, so we conducted a second experiment in summer 2006/07 comparing the mean number of filled (large) seeds per fruit from selfed and out-crossed flowers.

Germination success

We planted the hand-cleaned seeds from the collected fruits to determine whether pollination treatments had any effect on germination success, which may be indicative of inbreeding depression. Each germination experiment was carried out in both the glasshouse and the field as results often differ significantly according to the conditions in which seeds are germinated (Robertson et al. 2006). Where possible, 50 cleaned seeds from each fruit were planted in the glasshouse and 50 in the field. Some fruit that contained fewer than 100 seeds had seeds planted in either the field or the glasshouse. Seeds were planted from as many fruit as possible (glasshouse and field: bagged n = 4 and 3; natural 7 and 10; crossed 17 and 20; selfed 8 and 10 respectively).

In the glasshouse we germinated seeds in trays of potting mix. Each tray was half-filled with fertiliser mix (containing 80% pH-adjusted horticultural bark and 20% Bioblend of Blood and Bone) and segregated into four sections for four different fruit. In each section of the tray, we evenly scattered 50 seeds from one fruit. Consequently, each tray contained 200 seeds from four fruit of randomly selected treatments. We then sprinkled a thin layer of potting mix over the seeds, followed by enough fine shingle to prevent seed desiccation and reduce the growth of mosses and liverworts. We placed the trays in a heated glasshouse from mid-March through winter. Summer temperatures ranged from approximately 15° to 32°C averaging about 22°C, while winter temperatures ranged from 12° to 22°C. The seeds were watered every few days, but received no additional fertiliser. We recorded the number of seeds that germinated each month, removing seedlings to reduce competition effects and to ensure more accurate counts.

In the field, in Kaireperepe Bay near where fruit were collected, we cleared an area of soil of any vegetation and cultivated it so the soil was loose. We put segments of 65-mm-diameter plastic downpipe approximately 7 cm long in the ground to contain the seeds (similar to those used by Robertson et al. 2006). We arranged the tubes of pipe in groups of 20 (5 × 4) covering them with pegged-down wire mesh (6-mm-diameter holes) to stop disturbance and seed predation. Four fruits were randomly selected for each 5 × 4 plot. Each fruit contributed 50 seeds randomly allocated to each of five different tubes. After we had scattered the 10 seeds evenly in each pot, we sprinkled a thin layer of soil and leaf litter over the top to provide cover from desiccation and the surrounding environment. We recorded the number of seeds that germinated each month and removed the seedlings.

Inbreeding effects on seedling growth with competition

We grew selfed and crossed seedlings together in competition to see if there was any evidence for inbreeding depression at the seedling stage. In the glasshouse we planted two trays

of 25 selfed seedlings ($n = 50$) and 25 crossed seedlings ($n = 50$) of equivalent size and maturity from the germination experiment, with a thin covering of grass seed sown on top. We selected seedlings from a range of different fruits. Trays were arranged in rows of 10×5 at 4-cm spacings, alternating between crossed and selfed seedlings. We harvested the above-ground material of seedlings after 118 days by cutting off the stems at the soil level. We recorded the number of leaves on each plant, the length of the stem to the top leaf, and the length of the longest leaflet for each seedling, then each shoot was dried individually and weighed.

Flower visitation observations

We observed insect visitations to flowers in different patches for eight, 5-min periods from 9 January 2006 until 11 January 2006, between 0950 and 1430. We recorded information regarding the visitor species, number of visits to flowers, number of flowers being observed, total time spent by the visitor on each individual flower and overall time on all flowers.

We were also given access to data showing visitations to banana passionfruit flowers at one site (Grampians, Nelson) between 17 November 2005 and 15 December 2005 by the Landcare Research Community Pollination Project. They observed six flowers on four different plants every 2 h starting at 0700 or 0900 (e.g. 0900, 1100, 1300, 1500 hours) for 172 observation periods. Observational data were collected using 'near-instantaneous counts' of the number of individuals from each species present on the six flowers when the observer approached. The methods used are described in more detail on their website (www.landcareresearch.co.nz/research/biocons/pollination/).

Analysis

We used statistical package R (v.2.1.1; R development Core Team 2005) for all statistical analyses. The analyses used match those used by Kelly et al. (2004). We analysed the effect of pollination treatment on fruit set and germination success, using binomial generalised linear models (GLMs). The GLMs examined the effect of pollination treatments on the proportion of fruit set and the proportion of seeds that germinated, including 'patch' and 'site' as block effects where appropriate. For germination, we used a quasi-binomial model with chi-square significance tests to better match the dispersion of the data. A Tukey's Honestly Significant Difference test was then carried out to determine which treatments differed significantly.

The effect of experimental pollination treatments on fruit length, fruit width, and seed count per fruit were all tested separately using ANOVAS with Gaussian error distributions. In the seedling growth experiment, stem length, longest leaflet length and dry weight were also tested with Gaussian

ANOVAS. Stem length and dry weight were log- and square-root-transformed respectively, to improve normality prior to analysis. The response 'number of leaves' produced count data and consequently was analysed using a Poisson GLM using a chi-squared test for significance. The two replicate trays in the seedling growth experiment were started on different dates so 'tray' was added as a main effect to those analyses.

Results

Experimental pollinations

Buds typically ripened 2 days before the flower opened and remained open for approximately a week. Even though *Passiflora tripartita* var. *mollissima* flowers produce a lot of pollen, flowers had to be bagged prior to opening in order to collect enough pollen for cross-pollinations. None of the 40 flowers selected for the apomictic treatment ripened fruit, which shows that banana passionfruit does not use apomixis to produce fruit.

For pollination treatments, the GLM (Table 1) and Tukey's test revealed a highly significant treatment effect, with bagged flowers producing significantly fewer fruit than the other three treatments (means ± SE: bagged = 3.0% ± 1.1%; crossed = 29.5% ± 5.3%; natural = 18.0% ± 2.9%; selfed = 17.5% ± 4.9%). Therefore, *Passiflora tripartita* var. *mollissima* does require visitation by biotic pollinators to effectively produce fruit. While there was considerable variability across treatments and patches (Fig. 1a), crossed flowers were most likely to be successful, while the lower fruit sets of natural and selfed flowers demonstrated some degree of pollen limitation and inbreeding depression (or self-incompatibility) respectively. The Pollen Limitation Index (PLI) [1 − (natural fruit set / hand-crossed fruit set)] following Larson & Barrett (2000) was 0.39 showing that 39% of fruits that could have been produced were not because of a lack of suitable pollen.

Two ANOVAS showed no significant difference between the length (mean = 6.47 cm; $F_{3,40} = 0.18$, $P = 0.91$) or width (mean = 3.12 cm; $F_{3,40} = 1.26$, $P = 0.30$) of fruit collected from flowers that had received different pollination treatments. Similarly, pollination treatments in the first season failed to significantly affect the number of seeds per fruit (mean = 108 seeds; $F_{3,40} = 0.87$, $P = 0.46$) suggesting that the total number of seeds per fruit is not affected by inbreeding depression or pollen limitation (Fig. 1b). The repeat experiment in the 2006/07 season, which counted only large seeds, also found no significant difference in large seeds per fruit from selfed and cross-pollinated fruits ($F_{1,8} = 1.33$, $P = 0.282$). Therefore, selfed flowers do not contribute fewer seeds per fruit to the seed pool.

Germination success

Overall, the mean germination success across treatments was 39.9% in the glasshouse and 15.3% in the field. The binomial GLM (Table 2) revealed that this difference between glasshouse and field conditions was significant, but that treatment effects were non-significant. This was consistent with the wide variation in germination success (Fig. 1c), particularly for bagged fruits where sample sizes were small due to low numbers of available fruits in this treatment. In our initial model we included interaction effects of site:treatment and location:treatment but both were non-significant and consequently left out of the final model. Overall, there was

Table 1. Effect on fruit set in banana passionfruit (*Passiflora tripartita* var. *mollissima*) of natural, selfed, crossed and bagged pollination treatments across two sites with 10 patches each in the Marlborough Sounds during the 2005/06 season.

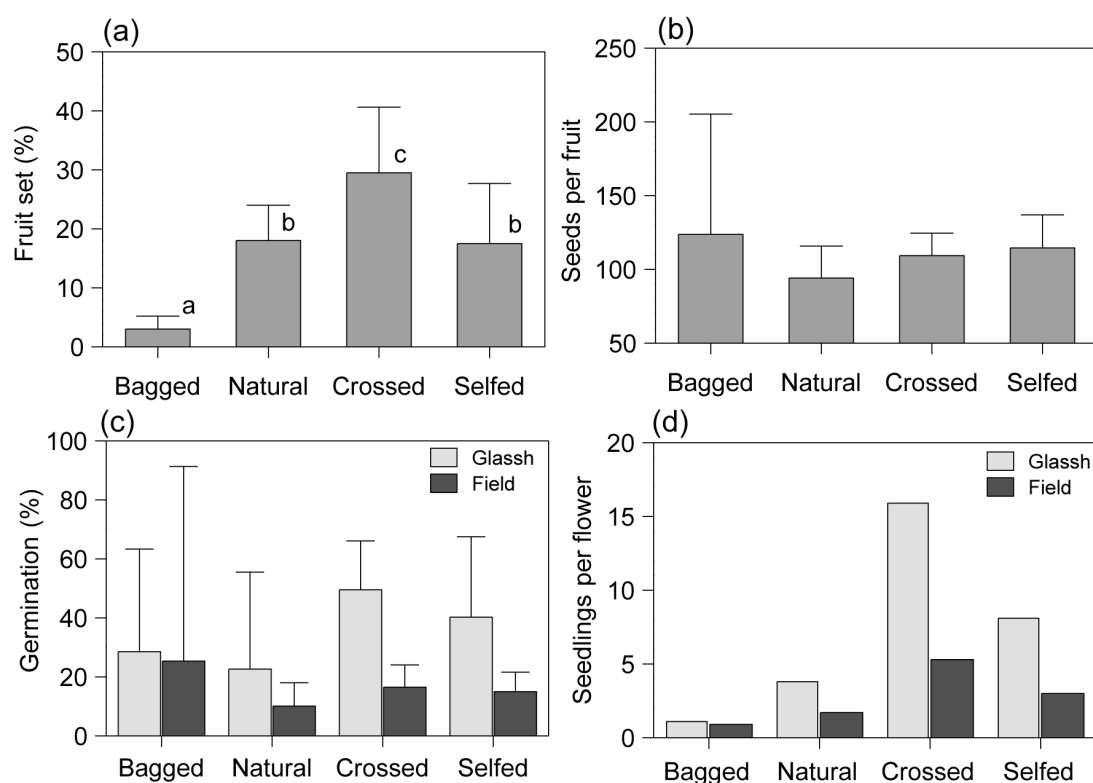| Factor	d.f.	Deviance	F	P
Patch	19	88.61	4.66	< 0.001
Treatment	3	65.78	21.93	< 0.001
Residual	57	64.62		

Figure 1. Effect (means and 95% CIs) in banana passionfruit (*Passiflora tripartita* var. *mollissima*) of pollination treatments at two sites in the Marlborough Sounds. (a) Fruit set (% of flowers), different letters indicate significantly different means. (b) Seeds per fruit; there was no significant treatment effect. (c) Germination (%) in glasshouse (light bars) and field (dark bars); again treatment effects were not significant. (d) Overall germinated seedlings per flower (proportion fruit set x average seeds per fruit x proportion of seeds that germinated) in glasshouse (light bars) and field (dark bars).

no evidence for consistent effects of pollination treatments on germination. Because we were particularly interested in inbreeding depression, we ran a separate test including only selfed vs crossed treatments, but again the treatment effect was non-significant (quasibinomial GLM, $F_{1,151} = 0.656$, $P = 0.42$).

Armbruster and Reed (2005) stressed the importance of combining the components of life stage into an index that measures overall fitness in order to determine accurately the effect of inbreeding depression on a species. The number of germinated seedlings produced per flower (Fig. 1d), determined by combining fruit set, seed set and germination success, indicated that crossed flowers gave rise to nearly twice as

many seedlings as selfed flowers. The trends were the same in the glasshouse and field with natural flowers producing more seedlings per flower (1.71 in field) than bagged flowers (0.93), but fewer than hand-selfed (3.02) and crossed (5.31) flowers.

Inbreeding effects on seedling growth with competition

All selfed and crossed seedlings survived when grown in close proximity to one another and in competition with grass. There was no significant treatment effect after 118 days in any of the four growth characteristics measured (Fig. 2): leaves per seedling ($F_{1,96} = 3.47$, $P = 0.062$), main-stem length ($F_{1,96} = 1.25$, $P = 0.267$), leaflet length ($F_{1,96} = 0.75$, $P = 0.389$), or shoot dry weight ($F_{1,96} = 0.02$, $P = 0.884$). This experiment showed no evidence of inbreeding depression affecting the survival or growth of seedlings, even in the presence of environmental stress as a result of competition. The largest seedling (a selfed seedling) grew to 95.5 cm tall with 21 leaves and a dry weight of 3.6 g, despite the crowded conditions.

Pollinator visitation observations

At their Nelson site, Landcare Research recorded that introduced honeybees (*Apis mellifera*) and bumblebees (*Bombus* spp.) were the predominant visitors to banana passionfruit flowers, with bumblebees more common (82 *Apis* and 124 *Bombus*). Native bees were only observed visiting flowers on 2 of the 172 observation periods, and no bird visits were recorded. In

Table 2. Effect on germination success of banana passionfruit (*Passiflora tripartita* var. *mollissima*) seeds of testing location (glasshouse vs field), site (Waikawa vs Kaireperepe) and treatment (selfed, crossed, bagged and natural fruit). Chi-squared test for significance used on quasi-binomial GLM.

Factor	d.f.	Deviance	P (X^2)
Location	1	309.865	< 0.001
Site	1	0.462	0.859
Treatment	3	96.629	0.085
Residuals	73	1121.4	

our observations, honeybees and bumblebees were the only flower visitors. Birds never visited during our observation periods. A few bellbirds were seen robbing flowers at other times, but physical damage to flowers consistent with robbing was rare, and some of that was bite marks consistent with mammals (perhaps possums) rather than birds. We made no night observations so visits by nocturnal species such as moths would not have been recorded. However, the flowers do not show any of the features typical of moth-pollinated flowers (dark colour, scented, small) so we think moths are unlikely to have been important.

We observed 29 honeybees and bumblebees visiting banana passionfruit flowers. At times there were too many simultaneous visitors to record the two taxa separately, but among confirmed identifications honeybees were more common (7 of 9 visitors). The visitation rate for both bees combined averaged 3.7 ± 0.9 visits per flower per hour (mean $\pm$ SE), and 64.1 ± 20.6 seconds of visit per flower per hour (s $fl^{-1} h^{-1}$). Banana passionfruit flowers are open for 5–7 days. If we assume bee visitation for 8 h a day (about half of the

summer daylength of 15 h) and that stigmas stay receptive as long as flowers are open, each flower may receive 180 bee visits totalling over 50 min over its lifetime. However, these bees may not always be effective pollinators: on some occasions we observed bumblebees entering the floral tube of the flower, to access nectar, without touching the stigma.

Discussion

Our main aim was to determine the extent to which pollinators are necessary for successful reproduction of banana passionfruit in New Zealand. Many lianas are able to propagate effectively using vegetative reproduction, which enhances their ability to exploit favourable conditions (Laurance et al. 2001). *Passiflora tripartita* var. *mollissima* failed to produce any fruit from the 40 flowers selected to test for apomictic reproduction, suggesting it is unable to produce seed asexually. However, stems that are broken off are able to reroot and grow (Williams & Buxton 1995). This provides protection against damage during tree-fall and may reduce the effectiveness of purely mechanical control efforts (Warshauer et al. 1983). For example, the density of banana passionfruit is high along roads, such as Queen Charlotte Drive, that are trimmed periodically by mechanical flails. Such mechanical treatment does not kill banana passionfruit and may even enhance its growth by disadvantaging the native woody vegetation, as *Passiflora tripartita* var. *mollissima* is a successful coloniser after disturbance (LaRosa 1992).

The overall fruit production was low with 18%, 29.5%, and 17.5% of flowers producing fruit in unmanipulated, hand-crossed and hand-selfed treatments respectively. However, this was compensated for by the fact that each fruit had on average 108 seeds. Removing pollinator visitations to flowers significantly reduced fruit set to 3%. This implies that the species relies strongly on pollinators for successful flower fertilisation and fruit production, consistent with research from Hawai'i by LaRosa (1992), which also shows that pollinators are important. Flower morphology is the likely barrier to autogamy, as inflorescences are large with high herkogamy (anther–stigma separation). The low fruit-set in autogamous flowers is mainly due to pollen not reaching the stigma, as hand-selfed flowers had nearly six-fold higher fruit set than bagged flowers. However, there is some degree of self-incompatibility as selfed flowers were shown to have fruit set that was 40% lower than for crossed flowers. This may be due to partial self-incompatibility in *Passiflora tripartita* var. *mollissima*, which has been found in several other *Passiflora* species (Varassin et al. 2001). Alternatively, it may be the result of early-acting inbreeding depression leading to embryo abortion (Keller & Waller 2002; Parker & Haubensak 2002). Given that banana passionfruit seems to rely so heavily on pollinators, it is of particular interest that visitations to flowers were almost always from introduced honeybees and bumblebees, indicating an invasive mutualism.

We have established that insect visitors play an important role in fruit production, which raises the question of how effectively bees are pollinating these large ornithophilous flowers (Endress 1994). Banana passionfruit is currently thriving in many areas around New Zealand, despite control efforts in many cases, which suggests fairly good pollinator performance. However, significantly fewer fruit were produced by unmanipulated flowers than those that were cross-pollinated. The Pollen Limitation Index of 0.39 indicates that 39% of fruits that could have been produced failed due to a lack of
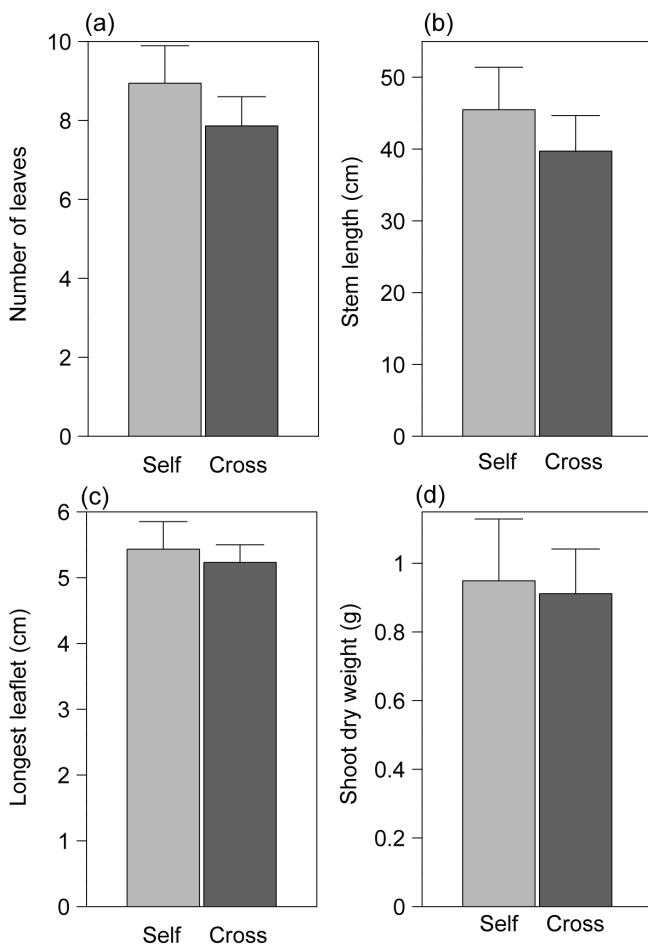
Figure 2. Four indicators of fitness in selfed and crossed seedlings of banana passionfruit (*Passiflora tripartita* var. *mollissima*) (means with 95% CIs, *n* = 50 of each) grown for 118 days in competition with each other and with grass. All results were non-significant showing no effect of inbreeding depression on offspring vigour. (a) Leaves per seedling, (b) length of main stem, (c) length of longest leaflet, and (d) above-ground dry weight. All 100 seedlings survived till harvest.

suitable pollen. This is similar to PLI's for native New Zealand ornithophilous-flowered species (mean 0.38, range 0.15 – 0.90) and slightly below the average (0.49, ranging between 0.00 and 0.94) for 21 New Zealand species that are insect-pollinated (Robertson et al. 2008). Compared with a global compilation of 363 pollen limitation cases (Knight et al. 2005), the ln(hand cross/natural) odds ratio of 0.494 in this study ranks at about the 68th percentile, showing a slightly above average degree of pollen limitation. Given that *Passiflora tripartita* var. *mollissima* has specialised ornithophilous flowers but is pollinated by insects in New Zealand, it is interesting that pollen limitation is not stronger; several studies have shown fruit set and/or seed set to be significantly lower when inflorescences of large-flowered species were visited by insects rather than birds (Anderson 2003; Robertson et al. 2005).

There are several characteristics, outlined in Robertson et al. (2005), that determine whether a flower will be effectively pollinated by visiting species, one of which is the frequency of visitation (Anderson 2003). Our observations showed relatively frequent flower visitations, using similar methods to Robertson et al. (2005) who observed visitors to native mistletoe flowers (*Peraxilla colensoi* and *P. tetrapetala*). They observed much lower visitation rates by native bees, the predominant visitors to flowers. In one year (l996/97) their visitation rates were $1.25 – 9.40$ s fl^{-1} h^{-1} compared with the 64.1 s fl^{-1} h^{-1} that we observed. Thus, the fact that banana passionfruit flowers are still pollen-limited presumably reflects poor pollinator effectiveness (Kelly et al. 1996; Robertson et al. 2005). Honeybees are effective pollinators in many species of plant (Kearns & Inouye 1997) and carry large pollen loads (Buchmann & Nabhan 1996). However, honeybees are only about 12 mm long and bees that are too small for a flower will transfer few, if any, pollen grains (Kearns & Inouye 1997). Janzen (1968) observed that queen bumblebees (*Bombus medius*) visiting banana passionfruit flowers often failed to touch even the anthers en route to the nectar source. We also observed this, which suggests that the primary reason banana passionfruit is pollen limited is because of a size mismatch between the flowers and visiting bees. While both honeybees and bumblebees frequently visited flowers in this study, we did not compare pollinator effectiveness between those taxa, so cannot predict how pollination would be affected by a decline in the density of one bee (e.g. following a parasite outbreak, such as varroa bee mite *Varroa destructor*).

Across plant species generally, birds visit flowers less often than insects, but process flowers faster and may be more effective at pollen transfer. For example, Robertson et al. (2005) found insects outnumbered birds by 2–3 orders of magnitude as flower visitors to New Zealand mistletoes (*Peraxilla* spp.) but birds were still effective pollinators. Lange and Scott (1999) observed hummingbird visitation rates to *Penstemon pseudospectabilis* to be only 0.30 ± 0.23 SD visits fl^{-1} h^{-1} and about 3.6 visits per day. However, for *Passiflora tripartita* var. *mollissima* there were no observations of birds pollinating flowers, consistent with the absence of birds in New Zealand with long enough tongues to access the nectar legitimately. We also saw little evidence of nectar robbing (slit corollas), probably because the pendent flowers are difficult for birds to perch near, and the stiff base of the corolla tube would be difficult to pierce.

To gain a fuller perspective on the effects of pollen limitation on the establishment of progeny, multiple fitness traits from a range of life history stages must be examined (Brennan et al. 2005). If selfed seeds show inbreeding depression then the actual impact of pollen limitation may be higher than data on pollen limitation or fruit set would suggest (Robertson et al. 2008). In longer lived plant species, inbreeding depression may be so strong that selfed offspring are essentially doomed, as shown for two New Zealand trees (Robertson et al. 2011). However, we found little to indicate that banana passionfruit suffers much inbreeding depression. While self-pollination did decrease fruit set by 40%, there was no significant treatment effect on fruit length, fruit width, seed count per fruit, germination, or seedling growth rates. It seems that once the fruit had begun developing it made little difference whether the pollen originated from the same, or a different, plant in terms of subsequent fitness of the seeds. This has been observed in many plant species suggesting that plants do not produce a fruit unless it has a minimum number of seeds (Burd 1994).

Byers and Waller (1999) stated that measurements of inbreeding depression may differ in different environmental conditions. Consequently, the germination experiment was conducted in both the field and glasshouse following Armbruster & Reed (2005). However, there was no significant treatment effect on total percentage germination success regardless of environmental conditions.

The final test for inbreeding depression looked at the growth of crossed and selfed seedlings when put under competition with grass, as stress often reveals the effects of inbreeding depression (Byers & Waller 1999; Armbruster & Reed 2005). The environment can affect the expression of inbreeding depression through intrinsic factors, such as the density of inbred individuals, or through extrinsic factors such as lack of nutrients or water (Cheptou 2006). Consequently, we grew seedlings close together in the trays with limited resources. However, the selfed and crossed seedlings all survived, and there was no significant reduction in fitness in selfed seedlings in any of the traits measured, at least within the first four months. Thus, inbreeding depression poses no known hindrance to the growth and spread of *Passiflora tripartita* var. *mollissima*. This is probably why it is invasive, as during its colonisation in New Zealand the population would have undergone a significant bottleneck (Nielsen et al. 2007).

By combining results from the different reproductive stages we can gain a fuller understanding of factors constraining the reproduction of banana passionfruit in New Zealand. The combined effects of pollen limitation and inbreeding (Fig. 1d) showed that in optimal conditions, i.e. when the stigma is saturated with crossed pollen and seeds germinated in a glasshouse, *Passiflora tripartita* var. *mollissima* produced over 15 seedlings from one flower. Out-crossed flowers whose seeds were germinated in the field on average produced over five seedlings per flower, and unmanipulated flowers produced 1.7 seedlings per flower in the field so successful flowers can make a lot of seed. Bagged flowers produced only 0.9 seedlings per flower, which suggests that in the absence of exotic pollinators banana passionfruit reproduction to the germinated seedling stage would fall by half.

In indigenous populations of banana passionfruit, flowers reproduce predominantly by outcrossing and show a high degree of self-incompatibility (LaRosa 1992). This may be in order to reduce pollen carryover from flowers on the same plant, or it may be in response to inbreeding depression. However, in New Zealand and Hawai'i – where *Passiflora tripartita* var. *mollissima* is also invasive – the species has a greater ability to self-pollinate, as is common for island colonists (Baker 1955, 1967). Nielson et al. (2007) stated that new colonisers

(particularly on islands) with a self-incompatibility system to prevent selfing may experience selection for a partially leaky system to enhance reproductive assurance. This is probably the case for banana passionfruit in New Zealand. A partial breakdown in the self-incompatibility complex would allow *Passiflora tripartita* var. *mollissima* to endure the small population size and loss of native pollinators it experienced during the process of introduction, through establishment and naturalisation, to invasiveness. While banana passionfruit relies mostly on outcrossing in its native habitat (LaRosa 1992), it seems in New Zealand it has developed a mixed mating system that combines out-crossing with selfing.

Conclusion

Banana passionfruit is invasive in New Zealand at least partly because mutualisms with exotic generalist pollinators allow the production of large quantities of viable seeds. Few fruits are produced autogamously, and our results show that in the absence of pollinators each flower would only produce 0.9 seedlings per flower. Therefore, without the help of exotic bees to facilitate its reproduction the invasive capacity of banana passionfruit would be reduced by half. However, banana passionfruit does suffer pollen limitation and reduced germinability, so bees are probably not as effective as the hummingbird pollinators in its native range. This is demonstrated by the three-fold higher number of seedlings produced per flower in the field by hand-crossed than natural flowers. Nevertheless, banana passionfruit produces abundant flowers and scores of viable seeds per fruit, assisted by a newly established mutualism with exotic bees in the New Zealand environment.

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