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A risk-based detection survey for the predatory mirid *Macrolophus pygmaeus* in New Zealand

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Research Paper

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Abstract

Macrolophus pygmaeus, a predatory mirid used to manage greenhouse whitefly, was illegally imported into New Zealand, and for a time was reared and sold to commercial tomato growers. We designed and implemented a risk-based detection survey to determine whether *M. pygmaeus* was still present in New Zealand a decade later. The survey was designed to have an 80% chance of detecting a single low density (0.05 per lineal metre of host plants) population within 1 km of known points of introduction. The survey was implemented between 8 and 15 March 2018. Local habitat constraints meant that the planned sampling had to be modified but this was accounted for in the subsequent analysis. No *M. pygmaeus* were found in the samples, but 93 specimens from seven other mirid taxa were detected, validating the sample methods. The survey gives 60% confidence that *M. pygmaeus* was not present at a mean density of 0.05 per lineal metre of habitat. It gives 80% confidence that a population at 0.1 m^{-1} was not present and 90% confidence that no population exists at >0.18 m^{-1}. Though there are no published data on typical field population densities of *M. pygmaeus*, for related species the survey would have had high confidence in detecting any medium to high density population present. Therefore, it is likely that *M. pygmaeus* is no longer present in New Zealand, but if extant within the sampled areas then we have high certainty that it was at low densities compared to other predaceous mirids.

Introduction

Macrolophus pygmaeus (Rambur, 1839) (Hemiptera: Miridae) is a predatory mirid originating from Europe and the Mediterranean (De Backer *et al.*, 2014). It primarily feeds on whiteflies such as greenhouse whitefly (*Trialeurodes vaporariorum* Westwood 1856, Hemiptera: Aleyrodidae), a damaging pest of greenhouse tomato crops. *Macrolophus pygmaeus*, or a close relative *M. melanotoma* (=*M. caliginosus*) with which it has often been confused (Castañé *et al.*, 2013), has been successfully used as a biocontrol agent for whiteflies in European greenhouses (Hart *et al.*, 2002; De Backer *et al.*, 2014) but if prey become rare it feeds on plant tissues instead and may cause damage to some crops (Sanchez *et al.*, 2018). Nevertheless, its high rate of prey consumption – an adult may consume up to 40 whitefly eggs per day and will also consume other pest insects – makes it an attractive candidate biocontrol agent for greenhouse tomato growers in New Zealand (Workman and Davidson, 2007).

Macrolophus pygmaeus was first reported in New Zealand from the Auckland Botanic Gardens in 2007 (Eyles *et al.*, 2008). The incursion was investigated by the then Ministry of Agriculture and Forestry Biosecurity New Zealand, who decided not to attempt eradication. At that time the then Environmental Risk Management Authority granted interim permission for Crown Research Institute to conduct trials using *M. pygmaeus* at their Pukekohe and Mt Albert sites (Workman and Davidson, 2007). The latter was a PC2-level facility so escapes were very unlikely, but there was potential for local establishment at the Pukekohe site. In addition, a commercial biocontrol company in Pukekohe, South Auckland was rearing the predator for sale to tomato growers (Thomas and Bullians, 2009). According to company records it was sold as 'energy bugs' to several commercial greenhouses in Pukekohe, Taupo and Blenheim. At least two small introductions were deliberately made into private gardens in South Auckland, though subsequent observations suggested these almost certainly did not establish (Thomas and Bullians, 2009). In 2009 it was revealed that *M. pygmaeus* had been illegally introduced to New Zealand (Flynn *et al.*, 2010) and searches failed to detect it at the Auckland Botanic Gardens from where it had reportedly been collected (Thomas and Bullians, 2009). The species was no longer authorized for rearing and sale and all known greenhouse and laboratory populations were destroyed. However, *M. pygmaeus* can overwinter outdoors in the United Kingdom (Hatherly *et al.*, 2005) so it was speculated that populations might have escaped from greenhouses and persisted in the New Zealand environment. This

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paper describes a risk-based detection survey carried out to determine whether *M. pygmaeus* is still present in New Zealand. Detecting a relict population of any mirid would be challenging; these are small, fragile, cryptic insects that actively hide from observers (Wheeler, 2001). The survey was based on epidemiological methods developed to prove absence of disease in mammal populations (Martin *et al*., 2007). These methods were adapted to design a risk-based survey with a known confidence in detecting a single small *M. pygmaeus* population. The relative likelihood of presence (risk) across potential sites was estimated as the normalized product of known introduction pressure, climate suitability and habitat suitability (Dupin *et al*., 2011). The efficacies of two sampling methods were then quantified from published studies, and the optimal sampling effort at each site was derived. Unlike random or subjective sampling (e.g. Hedgren and Weslien, 2008) our method allowed for imperfect detection and allowed us to quantify the confidence that a population of a specified size would be detected, and unlike site occupancy approaches (Bailey *et al*., 2014) it did not need to be calibrated from the occupied sites.

Methods

When a detection survey fails to find the target organism this does not necessarily mean it is not there. However, our methodology allows us to quantify the confidence (probability) that apparent absence from the survey reflects true absence. To do so, we must estimate the relative probability of presence ('risk') at each site and quantify the detection efficacy of the sampling methods (Martin *et al*., 2007). Since probability of detection scales with population size we must also specify the target population to approximate a minimum viable unit. This allows survey effort to be allocated optimally across sites to maximize the chance of detection and the confidence in absence if nothing is found (Kean *et al*., 2015).

Survey sites

Table 1 summarizes the sites where *M. pygmaeus* was known or suspected to be present in New Zealand. The close relative *M. melanotoma* moves readily from greenhouses to adjoining crops (Goula *et al*., 1991; Alomar *et al*., 2002; Castañé *et al*., 2004). Greenhouse tomato industry experts with experience of *M. pygmaeus* in Europe believed it unlikely that *M. pygmaeus* might have persisted undetected inside industry greenhouses because of the pest scouting and control carried out there, so the detection survey focused on outdoor environments.

Disposal of spent tomato plants was identified as the main potential pathway for spread from greenhouses. At the rearing facility P2, waste was bagged before disposal, so the local potential for escape was minimal (John Thompson, Bioforce, pers. comm.). Green waste from the Pukekohe and Taupo sites was burned, composted and/or buried on site. Green waste from the Blenheim greenhouse B1 was transported elsewhere for composting.

We could find no published information on *M. pygmaeus* dispersal distances, though other mirids are known to be strong fliers. Nevertheless, industry experts believed that if outdoor populations had established then *M. pygmaeus* would still be present close to the sites from which they escaped, even if their range had spread further. Therefore all sampling was carried out within a 1 km radius of potential introduction points.

Estimating relative risk of sites

The relative risk of each site was estimated from propagule pressure, climate and habitat suitability estimates.

Propagule pressure was estimated as the recorded introduction number plus an additional factor for any green waste disposed of on site. The introduction number was estimated as the number of 'energy bug' jars purchased from the rearing company, based on the records now held by the new owners of that company, or as 1 for the casual release sites P1, P5 and P6. The value for the rearing site P2 was the sum of the units sold multiplied by a risk factor, assumed to be 0.1, that recognizes the additional containment measures that were apparently in place there. The green waste component was the introduction number multiplied by a risk factor, assumed to be 0.5, and was added to the site to which waste was transported.

Previous work projected the potential distribution of *M. pygmaeus* in New Zealand outside greenhouses, based on overseas distributions (Logan, 2012). The model was re-run on fine-scale (4×5 km resolution) interpolated climate data (Tait *et al*., 2006) for 2017 according to the Hadley Centre Coupled Model version 3 under emission scenario A1B (Mullan *et al*., 2008). The resulting map is shown in fig. 1. Climate suitability for each *M. pygmaeus* introduction site was indicated by the ecoclimatic index (EI) of the nearest climate site. Blenheim sites had the highest EI values of 18–20, suggesting that the local climate may be suitable for *M. pygmaeus* to persist outside greenhouses. Pukekohe sites were nearly as suitable, with EI = 14–17. The two Taupo sites were estimated to be much less suitable (table 1).

The third factor assumed to constrain insect establishment was habitat suitability. Although *M. pygmaeus* has a wide prey range (Hatherly *et al*., 2009; Sylla *et al*., 2016), the primary prey of the strain known to have been introduced to New Zealand is greenhouse whitefly (*T. vaporariorum*). Industry experts thought it likely that any extant population of *M. pygmaeus* would still be associated with whiteflies, particularly on tomatoes or other Solanaceae such as potatoes, nightshades and poroporo (*Solanum aviculare* and *S. laciniatum*). When insect prey are rare, *M. pygmaeus* can also feed directly on host plants (Hatherly *et al*., 2009). Overseas, hedgerows and other refuges provide overwintering sites for mirid populations (Alomar *et al*., 2002). Relative habitat suitability was assessed by inspection of high-resolution aerial imagery of each risk site, together with local on-ground observations. Sites were subjectively rated by the presence and abundance of potential host plants and insect prey, especially whiteflies. Most sites were surrounded by grasslands with some crops, hedgerows and roadside strips. Many sites were also close to residential properties which may harbour host plants and poorly controlled whitefly populations. However, three sites had striking local habitats. Sites P3 and P4 were surrounded by very extensive potato fields known to host large seasonal greenhouse whitefly populations (Anthony Stone, Turners and Growers, pers. comm.), though pesticide use has increased in recent years in response to the establishment of the tomato–potato psyllid *Bactericera cockerelli*. Nevertheless, the local habitat was likely to have high relative suitability for *M. pygmaeus*. Similarly, site B1 was surrounded almost exclusively by vineyards, which may harbour whiteflies and other prey, but was also subjected to managed pest control. Table 1 lists the subjective assessments of relative habitat suitability at each risk site. We investigated the implications of alternative values but found their effects on sample design minor, providing the relative rankings were similar to those assumed in table 1.

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Table 1. Known sites of introduction of *Macrolophus pygmaeus* into New Zealand

Site ID	Risk details	Number of introductions or jars bought	Relative climate suitability	Relative habitat suitability	Relative risk of presence
Pukekohe					
P1	Auckland Botanic Gardens. The alleged site of initial collection. Searches there found none.	1	17.3	1	0.000
P2	Commercial rearing facility and primary source.	1331	17.0	5	0.011
P3	Large commercial greenhouse. Green waste is burned on site.	9712	14.7	10	0.559
P4	Large commercial greenhouse. Green waste is burned at site P3.	3400	14.7	10	0.117
P5	Private garden. Likely site of a casual release.	1	17.0	2	<0.001
P6	Private garden. Likely site of a casual release.	1	12.3	2	<0.001
P7	Research facility with minimal containment.	200	15.8	3	0.004
P8	Research facility with a high level of containment.	0	15.2	1	0.000
Taupo					
T1	Commercial greenhouse. Green waste is burned or buried on site.	57,250	2.4	3	0.098
T2	Commercial greenhouse. Green waste is composted on site.	11,900	0.1	1	<0.001
Blenheim					
B1	Commercial greenhouse. There is some doubt about whether this received any *M. pygmaeus*.	13,100	20.1	3	0.184
B2	Green waste from site B1 is composted here.	0	18.0	1	0.027

Introductions are based on the records of the commercial distributor, climate suitability is from a CLIMEX model (Logan, 2012) and habitat suitability is a subjective visual assessment of each site. The relative risk for each site was obtained as the normalized product of propagule pressure (introductions plus a green waste factor), climate and habitat.

The products of the estimates of propagule pressure, climate suitability and habitat suitability for each site were normalized (scaled to sum to one) to give the relative risk of each site. More than half the estimated relative risk of presence was accounted for by site P3, with sites P4, T1 and B1 comprising most of the remainder (table 1).

Sampling methods

We evaluated many methods used to sample mirids, including bagging (Wilson and Room, 1982; Zalom *et al.*, 1993; Deutscher *et al.*, 2003), visual inspection (Wilson and Room, 1982; Adams *et al.*, 1984; Deutscher *et al.*, 2003), suction sampling (Zalom *et al.*, 1993; Kharboutli and Allen, 2000; Alomar *et al.*, 2002; Wade *et al.*, 2006) and passive samplers such as malaise traps, sticky traps and suction traps (Wheeler, 2001). Shake cloth sampling and sweep netting were determined to be the most effective and efficient methods for our survey.

Shake cloth sampling involves bending 1 m of host plant material across a bucket, tray or groundsheet and shaking it vigorously to dislodge insects for collection or counting. This method has been widely used for monitoring mirid populations in cotton, for example, and has been well tested and quantified (Boivin and Stewart, 1983; Deighan *et al.*, 1985; Zalom *et al.*, 1993; Kharboutli and Allen, 2000; Deutscher *et al.*, 2003). However it is more suitable for counting than collecting mirids because the adults may fly away before they can be captured. Shake cloths may also be difficult to use under wet conditions (Threlfall *et al.*, 2006). Trials in Australia showed that scout experience made little difference to the numbers of predators detected in this way (Deutscher *et al.*, 2003). The average time to perform a sample in cotton was 3–5 min, regardless of the size of the crop (Deutscher *et al.*, 2003; Wade *et al.*, 2006). Mirid density estimates using shake sheets did not vary significantly with time of day (Wade *et al.*, 2006).

Experimental calibration of a similar method in Canadian pipfruit orchards detected between 20 and 80% of mirids present, depending on their lifestage and species (Boivin and Stewart, 1983). These values are similar to those reported for detecting all predatory species in Australian cotton by shaking: 58, 30 and 78% depending on the time of year (Deutscher *et al.*, 2003). In soybeans, Deighan *et al.* (1985) found shake cloths detected 86–93% of *Geocoris* sp. present (predatory bugs similar to mirids), but in strawberries this method detected only 52% of *Lygus* sp. mirids present (Zalom *et al.*, 1993). Considering these and other measurements for shake cloth efficacy, we assumed that shake cloths would detect a mean of 50% of *M. pygmaeus* present on sampled plants, but we investigated the range 20–90% in an uncertainty analysis (see below).

Sweep netting is also widely used for collecting and monitoring mirids in Australia and North America (Wilson and Room, 1982; Adams *et al.*, 1984; Snodgrass *et al.*, 1984; Deighan *et al.*, 1985; Wipfli *et al.*, 1992; Kharboutli and Allen, 2000; Threlfall *et al.*, 2006). Standard practice is to use a 40 cm diameter net, sweeping back and forth through the outer vegetation of host plants in a continuous movement to prevent flying insects from escaping

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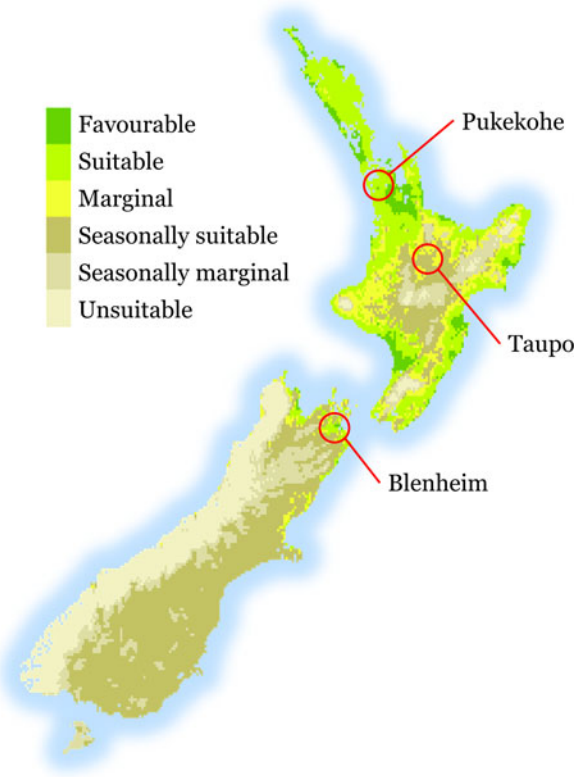

Figure 1. Projected climate suitability for *Macrolophus pygmaeus* in New Zealand, outside of greenhouses, based on a CLIMEX model (Logan, 2012). The three survey areas are indicated.

the net (Threlfall *et al.*, 2006). Mirid surveys have used between 10 and 30 sweeps per sample, with 20 being the most common standard. After sweeping, the handle is rotated to close the mouth of the net; mirids can then be carefully counted or collected directly from the walls of the net. Sweeping is significantly faster than shake cloths and may collect more adults per unit of sample time (Adams *et al.*, 1984). Twenty sweeps, covering approximately 20 lineal metres, can be performed and processed in 3–6 min (Threlfall *et al.*, 2006). It also has the advantage of capturing specimens for verification and identification. There appears to be little difference in the efficacy of sweep netting between experienced collectors (Threlfall *et al.*, 2006).

One study showed that a damaging mirid infestation in cotton would appear as two mirids caught in 20 m of sweeping, or three observed in 1 m of shaking (Threlfall *et al.*, 2006). This suggests that on a per metre basis, sweeping is only 3.3% as effective as shaking for detecting mirids. Similarly, data reported by Wilson and Room (1982) suggest that sweeping detects only around 3.5% of the Hemipteran individuals present in Australian cotton fields. In American cotton fields sweeping detected 0.8–1.4% of the *Lygus* sp. mirids that would be found by shaking the same area (Kharboutli and Allen, 2000). In soybean, sweep netting was around 10% as effective as shaking at collecting *Geocoris* spp. (Deighan *et al.*, 1985). For the design of the survey, it was assumed that sweeping would collect 3% of the *M. pygmaeus* that would be found by shaking the same area, and the range 0.8–10% was investigated. Note that the low efficacy of sweeping compared to shaking is compensated for by the ability to cover a much larger area in the same time.

Table 2. Characteristics of the population of *M. pygmaeus* that the survey aimed to detect

Level	Unit	Potential number per level	Target infestation
1	Site (1 km radius)	12	1
2	20 m lineal transects per site	varies	5%
3	1 m transect sections	20	50% (=10)
4	*M. pygmaeus* adults or nymphs per 1 m section	many	2

Optimizing the survey design

To quantify the probability of detecting relict *M. pygmaeus* we first had to specify the target population on a hierarchy of spatial scales (Kean *et al.*, 2015). To be conservative, the survey targeted a low density population existing at only one of the 12 potential introduction sites. Each site consisted largely of lineal features (crop rows, hedgerows, roadside strips etc.) and the two proposed sampling methods are well adapted to such features. Therefore, we classified each site first as a set of potential 20 m potential sweep net transects, with the target population occupying 5% of such transects at the single hypothesized infestation site. Further, each sweep net transect was divided into twenty 1 m shake cloth samples, and the target population assumed to occupy 50% of these within infested sweep transects. Finally, each infested shake cloth sample was defined as containing two *M. pygmaeus* individuals (table 2). This was intended to approximate a minimum viable population, and was well below the density at which the impacts of mirids are visible (e.g. Threlfall *et al.*, 2006).

Given a number of random sweep net and shake cloth samples taken at each site, standard formulae (e.g. Kean *et al.*, 2015) could be used to estimate the probability of detecting the target population. Starting at the smallest spatial scale, the probability of detection in an infested shake cloth sample was $1-(1-d)^n$, where d is the probability of detecting a particular *M. pygmaeus* present (=50%, see above) and n is the number present (=2, as specified by the target population, table 2). From this, there was a 75% chance of detecting at least one *M. pygmaeus* in a shake cloth sample infested by the target population. However, the target population occupied only half of the potential 1 m samples within an infested 20 m transect, so the probability of detecting at least one *M. pygmaeus* by shake cloth sampling an infested transect was $p_{\text{shake}} = 75\% \times 50\% = 37.5\%$. Now assuming that the number of potential transects was large compared to the number actually sampled (as it was for the optimal sampling plan) the probability of detecting the target infestation in an infested site was $P_{\text{shake}} = 1-(1-p_{\text{shake}} \times t)^{N_{\text{shake}}}$ where t is the proportion of transects infested (5%, from the target population specification) and N_{shake} is the number of transects sampled as 1 shake cloth per transect. Sweep nets are only around 3% as effective as shake cloths for detecting a particular mirid (see above) but the target population specified 20 mirids per infested sweep transect. With $d = 3\% \times 50\%$ and $n = 20$, the probability of detecting at least one *M. pygmaeus* by sweeping an infested transect was $p_{\text{sweep}} = 26.1\%$. Now $P_{\text{sweep}} = 1-(1-p_{\text{sweep}} \times t)^{N_{\text{sweep}}}$. Using both sampling methods, the probability of detecting the target population in an infested site was $P_{\text{site}} = 1-(1-P_{\text{shake}}) \times (1-P_{\text{sweep}})$. The chance of that site being infested was determined by its relative

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Table 3. Default values and realistic ranges for model parameters

Uncertain parameter	Best guess	Realistic range	Notes
Relative risk factor associated with rearing facility P2	0.1	0.0 to 0.2	An industry expert believed the chance of escape was very low
Relative risk factor associated with green waste disposal	0.5	0.0 to 1.0	For most sites the waste is disposed of on site
Time required per sweep net sample (min)	5	3 to 10	Based on Australian literature
Time required per shake cloth sample (min)	5	3 to 10	Based on Australian literature
Time required between samples at a site (min)	5	3 to 10	Estimate
Probability of detecting each mirid present in a shake sample	50%	20–90%	Based on published estimates from Australia and North America
Probability of detection by sweep net relative to shake cloth	3%	0.8–10%	Based on published estimates from Australia and North America

risk value R_{site} (table 1), so the overall probability of detecting the target population across all sites was $s = \sum(R_{site} \times P_{site})$. This is the sensitivity of the survey, or the confidence that apparent absence indicates true absence.

The time required per sweep or shake sample was estimated as 5 min (see above), and we allowed a further 5 min between samples. Therefore, the time required for sampling a site, not including preparation, breaks, moving between sites and follow-up diagnostics, was estimated as $\sum(N_{shake} + N_{sweep}) \times 10$ min.

The sampling model was programmed in a Microsoft Excel spreadsheet. The Solver add-in was then used to determine the number of sweep net and shake samples to take at each site to achieve a target survey sensitivity (by default $s = 80\%$) while minimizing the sampling time required. Field experience by the authors suggested that around four shake samples should be taken per sweep sample to balance their different efficacies for adults and nymphs, so we also required that $N_{shake} \approx 4 \times N_{sweep}$ for each site.

We investigated the effects of uncertainty by assuming a triangle distribution for each parameter, specified by the minimum, best guess and maximum values (table 3). Random deviates were sampled from these distributions and the survey sensitivity and time required were examined across 10,000 Monte Carlo random simulations using the PopTools add-in for Excel.

In practice, local habitat constraints meant that the prepared sampling design could not be followed exactly, so the sensitivity of the survey was re-estimated using the actual samples collected.

Sample collection, processing and diagnostics

All sites were sampled in fine weather in late summer, when mirid populations are expected to be at their seasonal peak. The adjoining sites P3 and P4 were sampled on 8–9 March 2018. Our estimated relative risks suggested that if *M. pygmaeus* were present in New Zealand there was around a 65% probability that it would be in one of these two sites (table 1). A scientist with experience in sampling predatory mirids in Australian cotton crops (S.M.) trained and led a team of six local tomato industry crop scouts. The team sampled an area of prime habitat with many volunteer tomato plants and abundant whiteflies at the green waste disposal site at P3. The remaining samples were taken in likely habitat for *M. pygmaeus* consisting largely of roadside hedges and the adjacent potato fields. The P3 and P4 roadsides were mostly mown grass but sweep samples were taken from roadside hedges and weedy edges of the potato fields. The potato crops were found to be unsuitable for sampling with shake cloths because of their close planting and dense unbroken canopy, so extra sweep samples were taken instead. Low numbers of whitefly were present in the potato fields. Other insects were present but no other abundant prey for *M. pygmaeus* were seen. Approximately 50 person hours were spent preparing the sampling equipment and taking a total of 87 sweep net samples from these sites.

Table 4. Summary of planned and actual samples taken

Site	Date sampled	Number of sweep net samples		Number of shake cloth samples	
		Design	Actual	Design	Actual
P3	8–9 March	31	65	124	0
P4	8–9 March	15	32	60	0
T1	15 March	5	37	20	0
B1	15 March	20	23	80	80
B2	15 March	1	1	4	4
B3	15 March	0	5[a]	0	0
Total		72	163	288	84

[a]Approximated from 20 sweep net samples of isolated trees and shrubs and three 20 m sweep samples of nearby hedges.

Site T1 was sampled on 15 March 2018. Here the habitat consisted of weedy ungrazed pasture adjacent to the glasshouses, a home garden on site, and weedy pasture on reserve land across the road. The low stature of the vegetation meant shake samples were not appropriate, so additional sweep samples were taken.

Sites B1 and B2 were sampled on 15 March 2018 by another applied scientist with research experience of mirids in Australian cotton (S.H.). Site B1 consisted of a commercial glasshouse complex which was surrounded by a vineyard. Within the vineyard were shelter belts, plantings of exotic and native shrubs and trees which were associated with residential dwellings, and boundaries and roadsides as well as weedy areas that contained solanaceous weeds. While no tomato plants were located at site B2 the weedy areas on the boundary of the site contained solanaceous weeds. Sweep and shake samples were taken as planned at both sites. There was some suggestion that the *M. pygmaeus* recorded to have been sold to a Blenheim

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Table 5. Optimal sampling effort required to achieve different levels of detection survey sensitivity

Confidence in detection	70%	75%	80%	85%	90%	95%
Number of sites to sample	3	4	5	5	7	8
Total sweep net samples	48	58	72	95	163	117
Total number of shake cloth samples	192	232	288	380	652	1170
Estimated total sampling time (h)	40	49	60	80	136	215

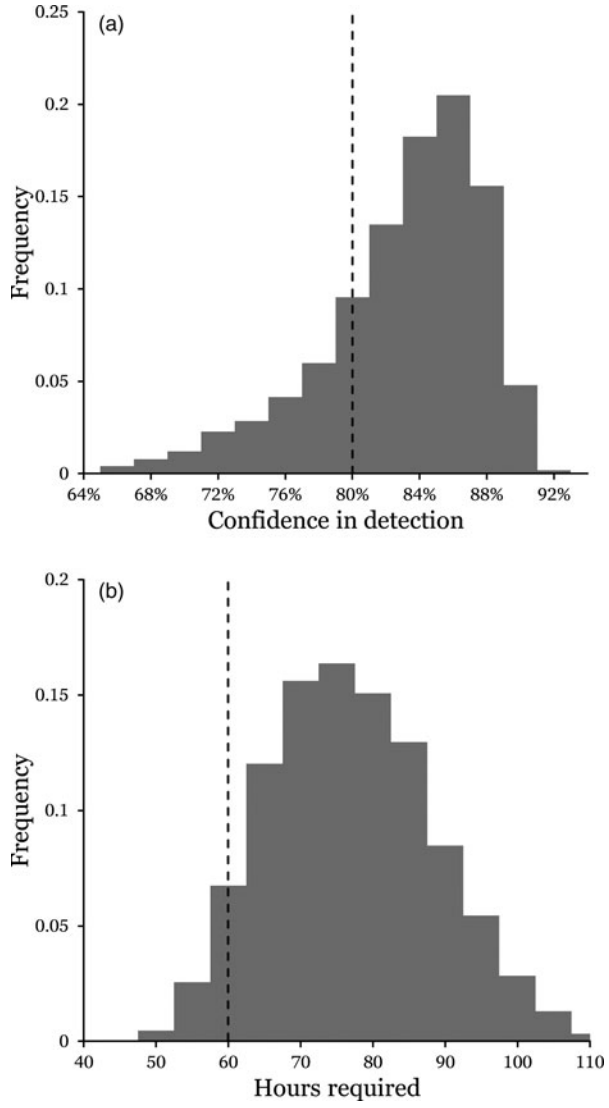

Figure 2. Frequency histograms of survey sensitivities (left) and sample times (right) arising from 10,000 Monte Carlo simulations of the uncertainties in the sampling design (table 3). Dashed lines show the results expected from the best guess parameters.

grower may have been ordered by a nearby operator whose property has since been converted to a commercial retail centre. This site was denoted B3 and additional sampling was done there in carpark landscaping and a large boundary hedge containing solanaceous weeds. Potential mirid prey, such as whitefly, aphids, leaf miners and small lepidopteran larvae were observed at all three sites B1-3.

All insects collected in samples were bulked by sample into sealed plastic pottles containing 30–50 ml 70% ethanol. Pottles were labelled and placed in a chilly bin. Once back at the laboratory they were refrigerated at 5°C until sorting under a binocular microscope. All mirids were removed, separated into labelled vials containing 5 ml 70% ethanol and stored at room temperature.

Mirids in the samples were then identified morphologically using descriptions by Eyles and Schuh (2003) and Eyles *et al.* (2008). Where there was any doubt, we were prepared to extract DNA from 2–3 legs per specimen and sequence the CO1 region for comparison with previously published sequences. In practice, DNA identification was not required.

Results

Optimal survey design

The optimal sampling design for achieving 80% sensitivity is shown in table 4. Greater sensitivity might be achieved with greater effort, but achieving ≥90% survey sensitivity would require exponentially increasing effort (table 5). Monte Carlo simulations suggested the sampling design was robust to uncertainties in the parameters, with most potential cases exceeding the target 80% confidence in detection (mean = 81%, fig. 2). However, the time required was likely to be longer than the 60 h indicated by the best guess parameters (mean = 72 h).

Detections in samples

No *M. pygmaeus* were found in any of the samples. However, 93 specimens of at least seven other mirid species were detected, mostly from site T1. The potato mirid *Calocoris norvegicus* (Gmelin) was found only in samples taken from potato fields at sites P3 (ten specimens) and P4 (11 specimens). Site T1 yielded three specimens of the tomato mirid *Engytatus nicotianae* (Koningsberger) which is morphologically very similar to the target *M. pygmaeus*. The other mirids collected were the fern mirid *Felisacus elgantulus* (one specimen at site P3), *Halormus velifer* (ten at T1), *Mecenopa albiapex* (21 at T1) and *Polyozus* sp. (two at each of P3 and P4). *Xiphoides* spp. mirids were collected at all sampled sites (total of 33 specimens) and were the only mirids present in the Blenheim samples B1 and B2.

Realized survey sensitivity

The sampling design was derived to give 80% confidence of detecting a single small population with an equivalent of 0.05 target mirids per lineal m of habitat (table 2). When the closed canopy of the potato crops at sites P3 and P4 prevented the use of shake cloths, additional sweep net samples were taken instead. Nevertheless, the lower sensitivity of this method meant the targeted probability of detection at these sites could not be achieved

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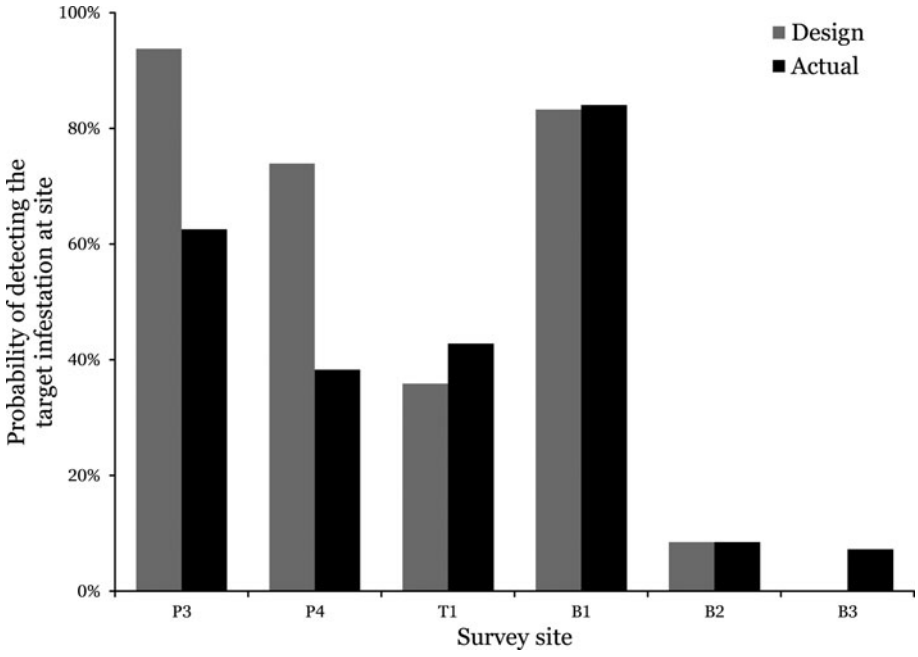

Figure 3. Comparison of the probabilities of detecting the target population at each site, for the designed and actual surveys.

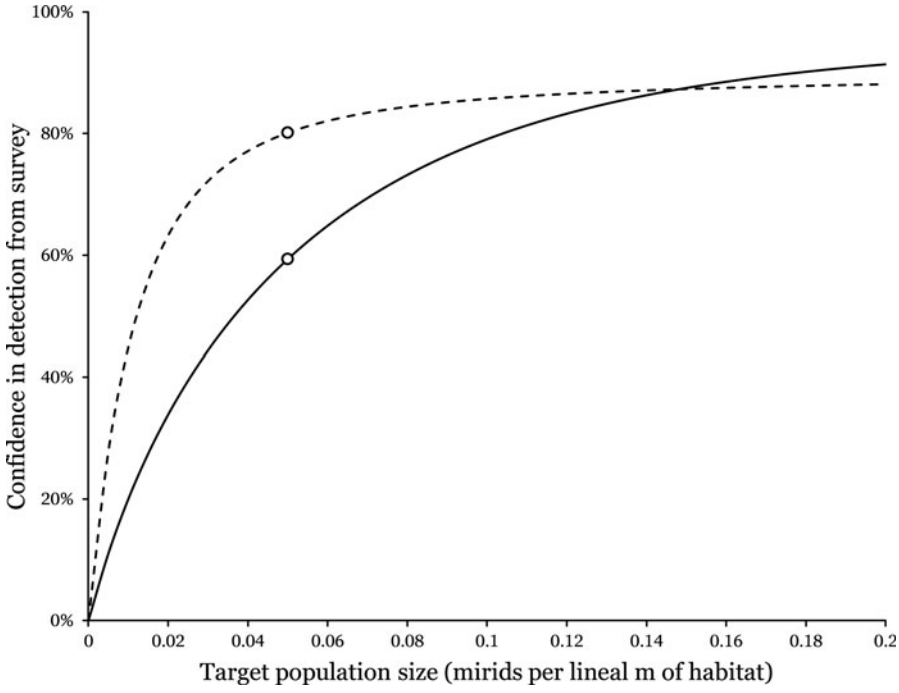

Figure 4. Sensitivity of the designed (dashed line) and performed (solid line) surveys for detection of *M. pygmaeus* populations of different sizes. The dots show the population size which the survey targeted.

(fig. 3). Given the high relative risk associated with these sites, this meant that 80% confidence of detection for the target infestation could not be achieved. From the actual samples taken, the realized probability of detecting the target infestation was close to 60%.

However, the survey sensitivity depends on the hypothesized target population. A more abundant *M. pygmaeus* population would have a higher chance of detection. Figure 4 shows the probability of detecting the designed (dashed line) and performed (solid line) surveys in relation to the target population size. While the performed survey only had a 60% chance of detecting the target population of 0.05 m^{-1} over all, it had an 80% chance of detecting a population of twice the size. Above about 0.15 mirids m^{-1}, the actual survey marginally outperforms the designed survey because it used more sweep samples which cover more ground, despite their lower sensitivity compared to shake samples.

Discussion

We used epidemiological methods to design a survey giving reasonable confidence that a single small extant *M. pygmaeus* population within a 1 km radius of previously occupied greenhouses would be detected. The target population size was somewhat arbitrary since we were unable to find any published data on typical densities of *M. pygmaeus* outside of greenhouses. Within

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European greenhouses where they are managed as biocontrol agents *M. pygmaeus* abundance may be as high as one per two tomato leaflets (Castañé *et al.*, 2004) but field populations outside greenhouses are likely to be much lower than this. For comparison, other predatory mirids reach a seasonal peak of 0.5–2 per cotton plant in China (Lu *et al.*, 2008), or 1.5 per strawberry plant in California (Zalom *et al.*, 1993). The potato mirid *Closterotomus* (formerly *Calocorus*) *norvegicus* peaks at around 12 m^{-2} in white clover seed crops in Canterbury, NZ, but may occur at up to 40 m^{-2} in field edges (Schroeder and Clifford, 1996). The target density of the *M. pygmaeus* survey was 0.05 per lineal m of crop, or around 0.1 m^{-2} depending on crop spacing. Compared to the published densities for other mirids, this represents a very low density population since we intended it to approximate a minimum viable population size.

The physical constraints of local environments meant the survey could not be carried out exactly as planned at all locations, resulting in only 60% confidence that the absence of *M. pygmaeus* in the samples indicated the target population was truly absent. Nevertheless, the performed survey gave greater confidence that a more abundant population was not present. We can have 80% confidence that a population of twice the target abundance would have been detected. Similarly, we can be 90% certain that a population of four times the target abundance would have been found. Compared to published densities of other mirids these still represent low densities, so we have high certainty that no medium to high density population was present. In addition, several other mirids were found in the samples, validating the sample methods and suggesting that if *M. pygmaeus* was present then it must have been considerably rarer than these taxa.

These conclusions are dependent on some key assumptions. Most importantly, we assumed that any wild population of *M. pygmaeus* would still be evident within 1 km of a documented release site. This assumption was based on advice from industry experts with experience managing *M. pygmaeus* populations in European greenhouses. A hypothesis of low dispersal distance is supported by the observation that densities of the closely related *M. melanotoma* are highest in the outer rows of seasonal field crops in northern Spain (Alomar *et al.*, 2002), though this might simply reflect the distribution of their whitefly prey. Genetic markers suggest that there has been limited mixing of *M. pygmaeus* populations across Europe since the last glaciation (>10,000 years ago), with notable differences in genetic structure persisting even between some local populations in close physical proximity (Sanchez *et al.*, 2012). Nevertheless, *M. pygmaeus* regularly self-colonizes open greenhouses in Europe (Castañé *et al.*, 2004), suggesting that it is mobile in the search for prey. The study results would only be invalidated if the species had spread widely and then become locally extinct within 1 km of greenhouse sources. Under this scenario, regarded as unlikely by the experts we consulted, it would be infeasible to prove *M. pygmaeus* absent at a national scale.

It was assumed that we know all the locations where *M. pygmaeus* might have been introduced in New Zealand. Most of this knowledge is based on the sales records of the commercial biocontrol distributor, but since the organism had been illegally imported and deliberately misrepresented to authorities (Flynn *et al.*, 2010) we cannot be certain how accurate or complete these records are. Ultimately we cannot be certain that *M. pygmaeus* was not introduced at additional locations, but if the number of propagules, climate or habitat suitability (i.e. relative risk) of these sites is low then the impact on the confidence of absence would be minimal.

The risk-based survey proved a useful, and we believe novel, approach to determining the status of a biocontrol agent. Aside from the results, the survey had considerable value in helping to structure our knowledge of the biology and local history of the target organism, helping to identify areas where data are poor or lacking. By quantifying different sampling techniques we were able to partially compensate for unforeseen local conditions that constrained the use of one such technique. Monte Carlo analysis ensured the survey was robust to uncertainties and identified that the time required may have been underestimated. And the sampling design allowed us to interpret the absence of detections from the sampling and quantify the confidence that this reflects true absence of the population. Therefore we recommend this approach for detection surveys.

In conclusion, the survey gave high confidence that *M. pygmaeus* was not present in one of the sampled areas at a medium to high density relative to other mirids. We can be less certain that it was not present at low density, though a lack of published information makes it difficult to estimate the minimum viable population for this organism. The survey suggests that *M. pygmaeus* was unlikely to have persisted in the environment outside the greenhouses where it was formerly present, and we have moderate confidence that it is no longer present in New Zealand.

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