Research reports

Scenario tree risk analysis of zero detections and the eradication of yellow crazy ant (*Anoplolepis gracilipes* (Smith)), in New South Wales, Australia

B.C. Dominiak[A,B], **K. Gott**[A], **D. McIver**[C], **T. Grant**[D], **P.S. Gillespie**[E], **P. Worsley**[A], **A. Clift**[F] and **E.S.G. Sergeant**[G]

[A] New South Wales Department of Primary Industries, PMB 21, Orange, New South Wales 2800, Australia.

[B] The Department of Biological Sciences, Macquarie University, NSW 2109, Australia.

[C] New South Wales Department of Primary Industries, PMB 2, Grafton, New South Wales 2460, Australia.

[D] New South Wales Department of Primary Industries, PO Box 823, Murwillumbah, New South Wales 2484, Australia.

[E] New South Wales Department of Primary Industries, Orange Agricultural Institute, Forest Road, Orange, New South Wales 2800, Australia.

[F] Faculty of Agriculture, University of Sydney, New South Wales 2006, Australia.

[G] AusVet Animal Health Services Pty Ltd, PO Box 3180, South Brisbane, Queensland 4101, Australia.

Abstract

Yellow crazy ant (YCA) *Anoplolepis gracilipes* (Smith) is ranked among the world's worst invasive species. Following the detection of this ant on Goodwood Island in northern New South Wales, Australia in 2004, an eradication program was initiated. The last detection was made in January 2006 and the declaration of freedom from the pest was made in January 2008, based on the traditional two-year period without a detection. However, although this eradication criteria is widely used, the two year timeframe is an arbitrary period with little or no scientific basis. Here, in addition to describing the eradication, we present a scenario tree analysis of zero detections to predict the level of confidence that the pest would have been detected if it was still present. Following a two year period with no detections, the scenario tree analysis indicated that there was a probability of absence of 0.999 998 under an assumed incursion pressure of one incursion every ten years. After eradication, the scenario tree analysis also indicated that as few as 20 randomly located visual inspections in the high risk area every three months was sufficient to maintain >0.95% probability of freedom. The analysis was also used to assess the merits of different surveillance techniques.

Introduction

Tramp ants are common stowaways on international freight, and are frequently found near airports, sea ports and facilities receiving imported commodities (Harris and Baker 2007). They create an insidious problem worldwide that threatens biodiversity especially in the Pacific Islands (Cranston 2010). Yellow crazy ant (YCA), *Anoplolepis gracilipes* (Smith), is a tramp ant with a world wide tropical and subtropical distribution. YCA is a pest of agriculture, domestic and natural environments (Young *et al.* 2001). YCA is one of the world's 100 most invasive species (Anon. 2008) and one of the world's six worst ant invaders (Holway *et al.* 2002). Polygyny and the lack of intra-specific aggression among workers mean that the ants can form super-colonies or networks of closely connected colonies that, once established, monopolize the entire site to the exclusion of other ant species (Vanderwoude and Abbott 2006). A small proportion of females in a colony become sexually reproductive queens however colony dispersal is primarily by budding rather than nuptial flights. Dispersal over distances greater than a few metres is largely due to human assisted relocation of part of a colony containing at least one queen (Vanderwoude and Abbott 2006). The life cycle has been estimated to take 76–84 days (Rao and Veresh 1990).

YCA is notable for its frantic activity when disturbed, and characteristic erratic walking style on long legs and waving of long antennae (Anon. 2008). YCA has broad and agricultural impacts usually by displacing a broad range of creatures (Lewis *et al.* 1976, Haines *et al.* 1994, Holway *et al.* 2002, Abbott 2005). YCA is a general scavenger and predator spraying formic acid on their prey. These victims include small isopods, myriapods, earthworms, molluscs, arachnids and insects in the litter and canopy of trees. YCA will also attack large land crabs, birds, mammals and reptiles (Lewis *et al.* 1976, O'Dowd *et al.* 1999).

The native range is suspected to be sub-Saharan Africa or Asia (Holway *et al.* 2002). Although the distribution of YCA remained fairly static for much of the twentieth century, the number of incursions at new locations has increased in recent decades (Vanderwoude and Abbott 2006). YCA has been found in Papua New Guinea (Baker 1976, Young 1996), the Seychelles (Haines and Haines 1978), India (Rao *et al.* 1991), New Zealand (Gunawardana and Sarty 2007), China (Zheng *et al.* 2008), Christmas Island (O'Dowd *et al.* 1999, Slip 2002) and is widespread throughout the tropics (Way and Khoo 1992).

YCA has been intercepted in Australian ports at least 161 times since 1988. Approximately 40% of interceptions have been in New South Wales (NSW) ports. Nationally, the number of interceptions has increased and the last five years accounted for 93% of all interceptions recorded from 1988 to 2002 (Hughes 2008). Multiple breaches and incursions have occurred on the Australian mainland (Department of Environment and Heritage 2006). YCA was first recorded in East Arnhem Land in the early 1980s (Majer 1984) and currently has spread across an area of approximately 2500 km^2 (Young *et al.* 2001, Hoffman and Saul 2010). It has been suggested that the original incursion occurred in the 1950s (Hoffman and Saul 2010). There have been many small establishments of YCA in Queensland including suburbs of Cairns, Brisbane, Caboolture, Hervey Bay and Townsville (Anon. 2008, Brown 2008a,b). In NSW, YCA was reported as being detected at Mullumbimby and Port Botany (Sydney), however despite extensive surveys, no further YCA were detected. These reports are regarded as incursions but non-establishments (Dominiak *et al.* 2010). YCA has not been reported from any of the other Australian states (Shattuck and Barnet 2001). In Queensland, small incursions might be eradicated and the two year period without a positive YCA detection has been used as the basis of eradication for three sites in Cairns (Brown 2008c). This two year period has also been applied to other exotic pests and diseases (Jones 1991, Froud 2003, Pascoe 2003, Vanderwoude *et al.* 2010). In NSW during July 2004, YCA were found near an Australian Quarantine Inspection Service (AQIS) mosquito monitoring trap located at Goodwood Island wharf (Dominiak *et al.* 2010). Local residents claimed the pest had been present for several years. In 2005, invasion of YCA into NSW was listed as a Key Threatening Process under the *Threatened Species Conservation Act 1995*. YCA is listed as a notifiable pest under Proclamation P172 of the *Plant Diseases Act 1924*. Hughes (2008) reported that climatic modelling indicated that YCA was capable of inhabiting most of northern and north-eastern Australia, and down the east coast of Queensland into coastal and inland parts of northern NSW. Goodwood Island is in the lower reaches of the desirable climatic zone for YCA establishment. Further south and in New Zealand, YCA was detected at the Port of Auckland in 2003 (Froud 2003) and twice in 2005 (O'Connor 2005a).

This paper describes the NSW response to YCA detected at Goodwood Island, the eradication program undertaken to eliminate the pest, and the use of scenario tree analysis of zero detections to provide confidence that the pest was actually absent.

Materials and methods

Location – Goodwood Island

Goodwood Island is a river delta island located approximately 5 km from the mouth of the Clarence River, on the far north coast of NSW, Australia. The island is very flat and generally no more than two metres above sea level. It is approximately 7 km long and varies in width from 0.3 to 2 km. Sugarcane production is the dominant land use and covers two-thirds of the western (upstream) end of the island. Remnant native vegetation occurs on the eastern one third. Road access to the island is from the northern side. There are approximately ten private residences and a caravan park on the island. Recreational fishermen launch boats from any accessible areas along the southern shoreline. The towns of Iluka and Yamba are five kilometres downstream on opposite banks at the mouth of the river.

The Port of Yamba is a commercial wharf located midway along the island's southern shore and handles import and export trade to Norfolk and Lord Howe Islands, and other Pacific destinations. Treated hardwood poles are shipped from Goodwood Island wharf to various Pacific nations including New Zealand and the Philippines.

Goodwood Island wharf is categorized as a secure site by NSW Maritime Authority. Access is restricted and gates are locked at all times. The surveillance and eradication program was undertaken with the full cooperation of the Port of Yamba authorities and stevedoring operations. This cooperation ensured site access, clean up of the site, awareness and monitoring against further spread and on-going passive surveillance for YCA.

Survey methods and schedule

Surveys were of two types (Table 1). A visual inspection involved an inspection of an area, sampling any suspect ants. No food lures were used and no GPS coordinates were taken. The other method was the use of attractive baited monitoring traps, using tuna cat food, jam or peanut butter as food lures; fish meal has been observed to be the most effective YCA attractant. Sarty et al. (2007) reported a preference for tuna cat food between October to March, and a carbohydrate preference for the cooler months. Baited sites were reinspected within four hours of food lure deployment and GPS coordinates taken. Intense surveys (on a 5 m grid) were conducted near the infested area to assess the populations in the known infested area. Delimiting surveys using visual inspections to define the perimeter of the infestation were conducted on a broader grid, from 100–300 m, depending on site accessibility through cane fields.

Foraging activity of YCA declines in temperatures below 25°C (O'Dowd *et al.* 1999). On most occasions, YCA surveys were conducted when the maximum daily temperature was above this level. In November 2004, ant activity was high but declined markedly after fipronil baiting. The wharf area was inspected intensely and regularly. The entire Goodwood Island was surveyed in March 2005. The neighbouring Gourd Island was inspected in October 2005. Goodwood Island and surrounds were intensely surveyed in February 2006 and again in subsequent years (Table 1).

Ant identification and tracing possibly infested consignments

Suspect samples were sent to the Agricultural Scientific Collections Unit (ASCU) of Orange Agricultural Institute, Orange, NSW, for identification. ASCU identification staff have been trained to international standards to identify YCA and had considerable experience following the red imported fire ant surveillance program (Dominiak *et al.* 2007, 2010). Deliveries from Goodwood Island were traced to timber and transport businesses, and waste transfer stations in Grafton and Maclean, the two largest Clarence River towns upstream of Goodwood Island. Surveys at these locations did not detect the pest.

Eradication procedures

Surveys of Goodwood Island, the surrounding islands and mainland, revealed that YCA was restricted to a riparian corridor about 150 m along and 100 m behind the wharf area. Subsequently a public awareness campaign was conducted in the district and remained active for the duration of the program. Official declaration of YCA as a notifiable pest under the *NSW Plant Diseases Act 1924* required land-owners and occupiers to report any detections (New South Wales Gazette 2004).

Permits for Presto® (active ingredients 0.01% fipronil) and IGR Grain Protectant® (5 g kg^{-1} s-methoprene) were obtained from the Australian Pesticides and Veterinary Medicines Authority. In September 2004, chemical treatments of the infested area at Goodwood Island were commenced. Mowing road verges and removing waste improved general access for bait applicators. Bait stations, each containing approximately 100 g of fipronil ant bait (Presto), were placed within the wharf perimeter fence. Fipronil bait was broadcast on land adjoining the wharf but no fipronil bait was placed within five metres of the river. There were subsequent broadcast applications with fipronil bait on the land

Table 1. Summary of numbers of sites baited and visually inspected in the three risk areas from September 2005 to March 2010.

Month	Visual inspection			Bait			Number of positive detections
	High risk	Medium risk	Low risk	High risk	Medium risk	Low risk	
Sept 2005				187			1
Nov 2005				40			0
Jan 2006				80			1
Feb 2006		201	98	265			0
Mar 2006				58			0
July 2006	20						0
Nov 2006	115			0			0
Apr 2007	98			0			0
Mar 2008	296			0			0
Mar 2010				200			0

adjoining the wharf at weekly intervals between November 2004 and May 2005. S-methoprene bait was broadcast monthly in summer and autumn and three monthly in winter to treat areas within 5 m of the river.

In December 2004, a population was found in a rock wall, but due its proximity to the water, could only be treated with S-methoprene bait and fipronil bait stations. This rock wall population, and another at the far eastern end of the wharf area, persisted for several months. In January 2005, the uptake of bait was estimated to be 95% in the wharf area and 80% in the rock and waterfront area.

Targeted treatment was required on 30 January 2006 when NSW Maritime Port Services Officer discovered a large YCA nest under a pile of discarded telegraph poles near the eastern end of the wharf. A licensed pest exterminator was contracted to work with the port authority to treat each pole and the ground beneath it as each pole was moved so that an immediate kill of ants in the exposed nests could be achieved. The control treatment occurred on 31 January 2006 and the poles were piled on site and burnt on 8 February 2006. This was the last detection of YCA on Goodwood Island and the last pesticide treatment. The summary of surveillance using visual inspections and baited monitoring stations is provided in Table 1.

Statistical analyses

Two methods of assessing eradication are presented below.

Method 1: Eradication – International Standards for Phytosanitary Measures (ISPM) The International Plant Protection Convention has established International Plant Protection Convention No. 9, the guidelines for pest eradication programs (International Plant Protection Convention 1998). This describes four main components of a pest eradication program which may be accepted in claiming that a pest is not longer present in an area. Surveillance, containment and treatment are the three main activities entailed in the eradication process, followed by verification of absence.

The Guidelines recommend that the verification procedure should use criteria established at the beginning of the programme and be supported by adequate documentation of program activities and results. Section 3.3 states that:

'the minimum period of time of pest freedom to verify eradication will vary according to the biology of the pest, but should take into consideration factors such as sensitivity of detection technology, ease of detection, life cycle of the pest, climatic effects and efficacy of treatment'.

For the Goodwood Island YCA infestation, a period of two years of nil detections through active surveillance was adopted as the minimum requirement. The last detection of YCA occurred on 31 January 2006 and hence the two year period elapsed on 31 January 2008.

Method 2: Scenario-tree analysis to demonstrate freedom Surveillance data for the period September 2005 to March 2010 was analysed using scenario-tree analysis (Martin *et al*. 2007). Scenario tree analysis provides a logical and transparent framework for quantifying the probability of detection (and consequently probability that the population/area is free) of a pest or disease if it was present at a specified level (design prevalence) in a defined population or geographic area. The method allows for adjustment of infestation and detection probabilities based on variations in risk of pest occurrence and for targeting of surveillance towards higher risk locations. It also allows for incorporation of historical data, discounted for the probability of new incursions occurring over time and the combination of data from multiple surveillance activities (components).

Based on figures provided by Goodwood Island authorities of produce imported annually, we estimated that the expected incursion rate for YCA in 2004 was one incursion per year. Therefore, for the first scenario, the monthly probability of introduction was assumed to be 1/12 = 0.083 (i.e. one incursion per year) while the second scenario assumed one incursion every 10 years (1/120 = 0.0083 per month).

The first scenario simulated the high-risk situation that occurred prior to the incursion in 2004, whereas the second scenario simulated the more realistic situation for the period considered, with measures in place to reduce the likelihood of further incursions, following the initial detection. A 'site' size of 25 m^2 was used for both baiting and visual detection components. The prior probability of freedom (or pest absence) at the start of the surveillance period was set at 0.5.

For this analysis, the surveillance area was divided into high risk (the immediate port area), medium risk (the rest of Goodwood Is.) and low risk (the adjoining islands and mainland coast). Separate scenario trees were developed for both baiting and visual inspections and data were analysed for monthly time periods. Model outputs included component sensitivities (probability of detection if infestation was present at the design prevalence or above) for each detection component (baiting and visual inspection), as well as an overall system sensitivity (probability that YCA would be detected if present at the design prevalence) and probability of freedom (probability that YCA was not present at the design prevalence) for each month during the surveillance period. When calculating the probability of freedom for each month, the prior probability of freedom was the posterior probability of freedom for the previous month, discounted by the probability of introduction during the current month. Model inputs were provided as probability distributions, to represent uncertainty about their true values, resulting in corresponding distributions of values for model outputs. The scenario tree model was developed using Excel (Microsoft Corporation) and PopTools (Hood 2006) and was run for 1000 iterations.

Results

The surveillance undertaken during the period of interest in the three risk areas is summarized in Table 1. Stylized diagrams of the scenario trees for baiting and visual detection components are shown in Figure 1 and inputs for the model are summarized in Table 2.

Monthly values for component sensitivity for baiting (undertaken exclusively in the high-risk area) ranged from 0.89 to 1.0, depending on the number of baited sites, whereas component sensitivity for visual inspection ranged from 0.18 to 1.0, depending on the number and location of inspections. Overall probability of area freedom was virtually 1 in any month when baiting was undertaken, but declined in months with no baiting activity, because of the probability of a new incursion occurring (see Figure 2). The decline in probability of freedom was rapid for the higher-risk first scenario, but more gradual for the second scenario, where the probability of an incursion was much lower (Figure 2).

The second last YCA was detected in September 2005 and the mean system sensitivity (probability of detection) at this time was >0.999 for both scenarios. Following continued surveillance, the probability of absence was 0.978 in December 2005. In the following month January 2006, the last YCA was detected. After surveillance for 14 months, the probability of absence in April 2007 was 0.9996 for scenario 2. By the two year period in March 2008, the probability of absence was 0.999 998 and following additional inspections in March 2010, the probability of absence was 0.999 942, for scenario 2.

Discussion

In terms of eradication, the two-year period with zero detections at Goodwood Island through both baited and visual surveys fulfils standard eradication requirements (Jones 1991, Froud 2003, Pascoe 2003). The small size of the infestation may have minimized the chance of colony survival when subjected to the stress of chemical treatments. The likelihood of eradication was enhanced because the locality appears to be in the lower range of climatic suitability for establishment of

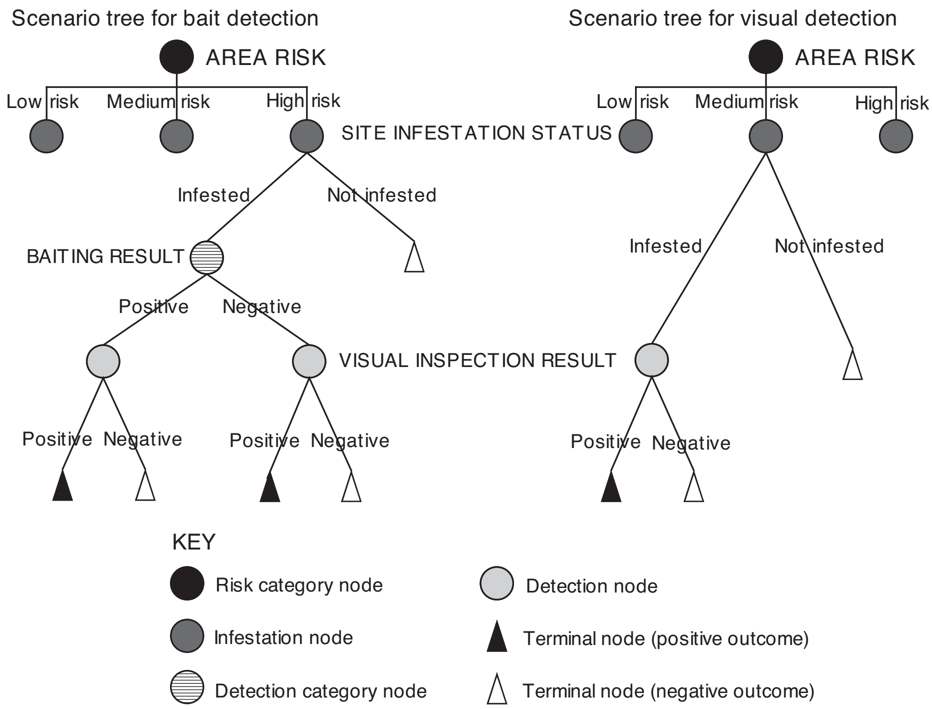

Figure 1. Diagrammatic representation of scenario trees for bait detection (left) and visual inspection (right) for yellow crazy ants (tree structure is replicated below each of the Site Infestation Status nodes but not shown to simplify the diagrams).

Table 2. Input parameters for a scenario-tree model for estimating system sensitivity and probability of freedom for visual inspection and baiting for yellow crazy ants at Goodwood Island. Parameters were input either as a fixed (most likely) value or as a Pert probability distribution (a smoothed triangular distribution defined by specified minimum, most likely and maximum values and commonly used to express uncertainty about expert opinion).

		Distribution parameters		
Parameter description	Distribution type	Minimum	Most likely	Maximum
Design prevalence (proportion of sites infested)	Fixed value		0.0001	
Bait detection sensitivity (probability of detection by baiting given that the baited site is infested)	Pert	0.7	0.85	0.95
Visual Inspection sensitivity at bait negative sites	Pert	0.05	0.1	0.2
Visual inspection sensitivity (without baiting)	Pert	0.6	0.8	0.9
Proportion of sites high risk	Fixed value		0.0004	
Proportion of sites medium risk	Fixed value		0.064	
Proportion of sites low risk	Fixed value		0.936	
Relative risk for high risk area	Pert	200 000	500 000	1 000 000
Relative risk for medium risk area	Pert	5 000	10 000	15 000
Relative risk for low risk area	Fixed value		1	

YCA (Harris and Baker 2007). Holway *et al.* (2002) reported colony expansion by budding alone ranged from 37–402 m y^{-1} in the Seychelles and this method of colonization alone greatly limits colony expansion. We infer that the combination of the two chemicals were successful in eradicating this YCA colony in this particular environment.

Eradication of a non endemic species is often declared either on an *ad hoc* basis or on setting arbitrary thresholds that the species is not present (Regan *et al.* 2006). Traditional methods of statistical analysis do not have the ability to assess zero counts, so simulation techniques have been developed to interpret nil trap catches in fruit fly eradication programs (Clift and Meats 2004, Barclay *et al.* 2005). The scenario tree analysis provides virtual certainty that YCA would be detected if present and consequently that the area is 'free' of YCA, at a level of one infested site for every 10 000 sites (each 25 m^2) in the surveillance area at the time of most inspections. This is mainly because of the large amount of baiting undertaken, primarily in the high-risk area. However, ongoing surveillance, particularly in the high-risk area, is essential to maintain this level of assurance in the absence of measures to minimize the risk of re-introduction. Alternatively, if measures are in place to minimize the likelihood of additional incursions, lower levels of surveillance on a periodic basis would be sufficient to maintain a high probability of continuing freedom. For example, as few as 20 randomly located visual inspections in the high risk area every three months (assuming no detections) is sufficient to rapidly achieve and maintain >0.95% probability of freedom (results not shown).

Another important outcome of this approach was the confirmation of the much greater value of baiting or inspections in the high risk area compared to the low and medium risk areas. For example, 299 visual inspections in the medium (201) and low (98) risk areas resulted in a component sensitivity of 0.177, compared to only 20 inspections in the high-risk area with a component sensitivity of 0.675. This difference reflects the much higher level of risk (mean 500 000 fold) associated with the high risk port area, relative to the low risk area of adjoining islands and mainland. The model was sensitive to estimated relative risk among areas, with mean system sensitivity reduced from >0.999 to about 0.73 for reductions in relative risk for medium and high risk areas by 10-fold and 100-fold respectively.

The recent detections of YCA in Australia and New Zealand has prompted many Pacific Rim countries to improve awareness and surveillance methods of BioSecurity agencies to minimize the chance of other Pacific countries becoming

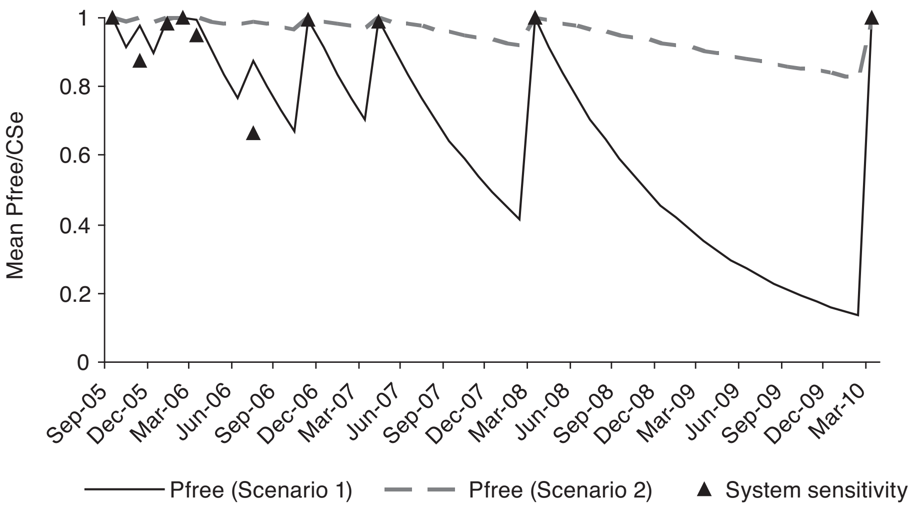

Figure 2. System sensitivity and probability of area freedom from yellow crazy ants based on analysis of combined baiting and visual inspection data for high (1 per year) and low (1 per 10 years) probability of introduction for the period September 2005 to March 2008.

infested by tramp ants. Ant identification training and enhanced surveillance techniques have been provided for many Pacific nations to improve the chances of early detection (O'Connor 2005b, Gunawardana and Sarty 2007). The International Plant Protection Convention revised phytosanitary measures, scientific needs of organizations and their capacity to minimize the introduction of invasive ant species (Newman 2004). Subsequently the Pacific Invasive Ant Surveillance program was developed, focusing on high-risk sea and airport surveillance in Pacific island countries (Sarty 2007).

For the immediate future, the two year period of zero detections is likely to remain the international standard for declaring area freedom. However, the scenario tree method of analysing zeros may become a future standard for providing statistical confidence of pest absence. What remains unclear is the appropriate level of confidence for surveillance. The standard insect disinfestation probability is Probit 9 or 0.999 97. Bliss (1934) originally described the Probit method of transforming the percentage of pests killed by a pesticide. This method is a demonstrated acceptable level for some pests such as fruit fly (Meats and Clift 2005). However, is the Probit standard an appropriate standard for evaluating surveillance of zero population levels. At Goodwood Island, the probability of absence immediately before the second last and the last detections was 0.999 and 0.978 respectively. These are clearly inadequate as YCA was subsequently detected. After 14 and 26 months of inspections the probability of absence (0.999 6 and 0.999 998 respectively) compared well with the Probit 9 standard (0.999 97). While Probit 9 is a standard for fruit fly disinfestation, is Probit 9 an appropriate standard for surveillance?

For any pest or surveillance situation, scenario tree analysis may help identify the more effective surveillance methods, and identify what method and frequency of surveillance would provide a higher level of confidence for absence. The scenario tree analysis could be used to predict the number of inspections required to maintain a specific level of confidence for an assumed incursion pressure. Any method to shorten the period of trade restriction and provide confidence that a pest is absent will be a positive contribution to global trade. There have been advances in statistical methods of analysing zero detections in animal industries (Martin *et al.* 2007, Sutherst and Bourne 2009). Ramsey *et al.* (2009) used the Bayesian approach to estimate the degree of confidence in the success of the feral pig eradication program when no more pigs were detected. Solow *et al.* (2008) used a Bayesian model to predict when to terminate an eradication program based on the probability that all individuals had been removed. Regan *et al.* (2006) used an economic model to predict when the expected costs of surveillance outweighed the expected benefits. In relation to plant pests, modelling has started on the invasion and eradication of invasive weeds (Cacho *et al.* 2006) and some insect pests such as fruit fly (Clift and Meats 2004). However more plant and insect based pest models need to be developed to provide confidence in the analysis techniques.

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