Incursion Management in the Face of Multiple Uncertainties: A Case Study of an Unidentified Nematode Associated with Dying Pines Near Melbourne, Australia

Mike Hodda, David Smith, Ian Smith, Lila Nambiar and Ian Pascoe

Abstract In late November 1999, dying pine trees were observed near the docks in Melbourne. The cause was initially identified as *Bursaphelenchus xylophilus*, the pinewood nematode. However, it was soon discovered that it was another nematode, *Bursaphelenchus hunanensis*, which was associated with the dying pine trees. Very little was known about the biology or pathogenicity of this species, except that it had never before been recorded in Australia. Other dying trees were soon discovered with the nematode, and deciding on an appropriate response became a critical issue. This paper describes the subsequent events in the face of the uncertainty regarding the pathogenicity of the nematode, its origin, its vector, and its biology, particularly dispersal. More general principles can be drawn from this experience regarding the management of incursions of pinewood and other nematodes. There may also be important lessons regarding spread of nematodes associated with wood and insects.

Introduction

This paper is about the response to an apparent incursion of an exotic nematode of uncertain pathological effect. The nematode is from the genus *Bursaphelenchus*, which includes the pinewood nematode (*B. xylophilus*), a major quarantine pest. The nematode was originally thought to be *B. xylophilus*, but was instead the little known species *B. hunanensis*. This paper describes how the situation was handled, particularly in the light of uncertainty about the biological characteristics of the nematode, its pathogenicity, and its relationship to the local fauna. It is important to document such events so that successful and unsuccessful incursions can be compared, the processes of invasion and of becoming a pest can be better understood, and improved responses to future incursions can be planned and implemented. Some

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M. Hodda
CSIRO Entomology, GPO Box 1700, Canberra ACT 2601, Australia
e-mail: mike.hodda@csiro.au

M.M. Mota, P. Vieira (eds.), *Pine Wilt Disease: A Worldwide Threat to Forest Ecosystems*,

general issues in the management of incursions are discussed. The benefit-cost ratio of action is calculated as indicating that erring on the cautious side was justified in this case, so that taking appropriate action to contain and eradicate the potential pest was the correct response. The eradication campaign shows that with an appropriate, adaptive response to incursions in place, eradication of nematodes can be achieved. This, too, is an important result in considering the most appropriate responses to the incursion of exotic nematodes.

The Situation and Initial Response

In January 2000, government officers from a local Council reported the rapid decline of a mature pine tree (*Pinus halepensis*) in the botanic gardens at Williamstown, near the main port of the city of Melbourne, Australia (37°51′S 144°53′E). The tree was reported as having declined rapidly, with the needles turning yellow to brown and the twigs becoming dry and brittle. Symptoms first appeared in early summer and developed over a 4–6 week period. The dead pines retained their needles. These symptoms are very similar to those of Pine Wilt Disease (PWD) (Evans et al., 1996).

In February, wood samples were taken from the tree and submitted to the local diagnostic service. Initially the cause was thought to be a fungal disease, but the only fungi extracted after extensive sampling were *Sphaeropsis* spp. isolated from the branches and trunk. *Sphaeropsis* spp. are known as shoot blight, and were formerly in the genus *Diplodia. Sphaeropsis* spp. are known to cause severe damage only in trees under stress from unfavourable environmental conditions, and often kill only current-season buds, shoots, and 2nd-year cones. Cankers and resin exudations on infected shoots, branches and main stems are also characteristic of *Sphaeropsis* infections. However, there were none of these symptoms, and no other fungal pathogens could be found. Foliage chloride was tested because the tree was near the coast, but this was below the threshold considered harmful for pines.

It was decided that further investigations were warranted for two reasons. First, because the tree was located near a port handling a large volume of international cargo, and there was concern about incursions of exotic pests and pathogens. Second, there are large areas of plantation pine forests located in the state. Pines are the basis of a large plantation softwood industry producing about 10 million cubic metres of timber per year. Furthermore, the only species of the genus *Pinus* in Australia are introduced, and subject to few diseases: this is one of the reasons for the large plantation area. There is thus a strong interest in any disease—local or exotic—which may affect this resource.

Following the failure to find any fungi likely to have killed the tree, the local diagnostic service searched for nematodes. Samples of wood were cut into chips and the nematodes were extracted over several days using Whitehead-Hemming trays (Hooper, 1986). Large numbers of nematodes were found of several different types. One of the types was identified as possibly being *B. xylophilus*, the cause of PWD.

This species had never been found in Australasia before (MacLeod et al., 1994), and is a major quarantine pest (Evans et al., 1996; EPPO, 2003). The tentative diagnosis was forwarded to the federal quarantine authorities, who convened a meeting of the advisory committee on plant health. The committee requested that more nematodes be extracted and forwarded to the main nematode diagnostic laboratory in Australia, at CSIRO Entomology in the city of Canberra.

At the CSIRO laboratory, three types of nematodes were found in the wood samples, but the absence of *B. xylophilus* could be confirmed definitely based on the morphology of the nematodes, within the statistical restrictions of sampling a large volume of material. All of the Aphelenchida present differed from *B. xylophilus* in basic characters, such as stylet length, tail shape and the development of the anus. Two of the nematodes were identified as belonging to the genera *Aphelenchoides* and *Ektaphelenchus*. No species in these genera are pathogenic to trees (Hodda, 2003), so identification to species was not attempted at this stage. The third type of nematode was identified morphologically as probably belonging to the species *B. hunanensis*. There were no adult males present, which made positive identification problematic. Attempts to culture the nematode using the methods devised for *B. xylophilus* (e.g. Bolla and Jordan, 1982; Braasch et al., 1995, 1999a; Hoyer et al., 1998) were unsuccessful, including using both local fungi and fungal cultures used for several *Bursaphelenchus* species, which were obtained from Dr Thomas Schroeder (BBA, Braunschweig, Germany). The diagnosis of *B. hunanensis* raised several issues about the differential diagnosis of the genus *Bursaphelenchus* from *Aphelenchoides* and *Laimaphelenchus*. These issues have also arisen in other studies of aphelenchid nematodes (Braasch, 2004; **?**, **?**). There have been no molecular studies of this species.

B. hunanensis has been recorded in the literature only from Hunan Province, China, where it was associated with dead *Pinus massoniana* Lamb (Yin et al., 1988). The only known records of any species of the genus *Bursaphelenchus* in Australia were as follow.

1. An unidentified species was found on *Hyleops glabratus*, the hoop-pine stitch beetle (Coleoptera, Curculionidae) on 7 July 1972 about a thousand kilometres away in another state (north-eastern NSW). This record was from subtropical wet forest and not associated with dying trees. The record was from the specimen database of the Queensland Museum, Brisbane, but the specimen was no longer in good condition, and the identification was doubtful.
2. A species described as close to *B. sexdentati* was found on *Ips grandicollis* which were attacking *Pinus taeda* in a pine plantation in similar areas to the first report (north-eastern NSW) (Stone, 1990; Stone and Simpson, 1990, 1991).

There were few other records of sampling for nematodes in trees of any sort in Australia (Hodda, 2003), and sampling of other, healthy trees nearby did not find *B. hunanensis*.

The conclusion from the data available at this stage was that *B. hunanensis* was probably exotic, and possibly associated with pathology of the trees in some way. However, neither of these conclusions could be definitive given the data available. This sort of situation may be more common in quarantine situations than is often thought, given the increasing recognition of cryptic species, races, biotypes, pathotypes and other hitherto unknown structure within what were previously considered uniform "species", as well as the vast number of unknown taxa. Nematodes are not the only organisms where this may happen.

Response Stage Two

The advisory committee on plant health decided that further action was justified. Several factors were taken into account in deciding this: that *B. hunanensis* was apparently exotic, the serious quarantine pest status of one species in the genus *Bursaphelenchus*, plus the uncertain pest status of some others, the high monetary value of nearby pine forests, and the value of an immediate response. A detailed evaluation of these factors in the decision is presented in the section "evaluation of response".

The infested tree was isolated, then the above-ground parts were cut down and carefully removed in a sealed truck to the local garbage tip, where it was burnt. The roots were excavated for a radius of about a metre around the stump and to a similar depth, then removed in a sealed truck for deep burial at the same tip.

The next steps were to ascertain if there was any spread of the nematode, and identify any insect vectors involved. Light traps were set up around the area where possible, and checked regularly.

In deciding where to sample for the nematode, data on the flight radius for vectors of *B. hunanensis* were sought. Unfortunately, there is no data available for this species, as is the case for many species of the genus *Bursaphelenchus*, so the data for *B. xylophilus* was used. This raises another issue where the best-known species in a genus is almost invariably the most severe pest, not necessarily the most representative. This issue is discussed further in the section "evaluation of response".

Known and potential insect vectors of *B. xylophilus* include at least 40 species of beetles (Coleoptera): 19 species of the genus *Monochamus* (pine sawyers), 10 species in 9 other genera of the family Cerambycidae, 4 species in genera of Curculionidae, and a species of Buprestidae (Hodda, 2006). The flight range of most of these is, again, unknown, so again the only information available comes from the species that are the most severe economic pests. *Monochamus alternatus* are the main vector in Japan, and generally disperse less than 100 m (Ido and Kobayashi, 1977). In Japan, about 75% of beetles are recaptured within 100 m of release, but dispersal of 2.5 Km has been recorded (Ido and Kobayashi, 1977; Fujioka, 1993; Yoshimura et al., 1999; Takasu et al., 2000). The annual expansion of range for *B. xylophilus* on *M. alternatus* was estimated at 2–15 Km (Togashi et al., 2004). The issue of dispersal is also discussed further in the section "evaluation of response".

Sampling of all trees within a 1 Km radius, with sampling of dead or dying coniferous trees only within a further radius of 4 Km, was decided upon as combining a

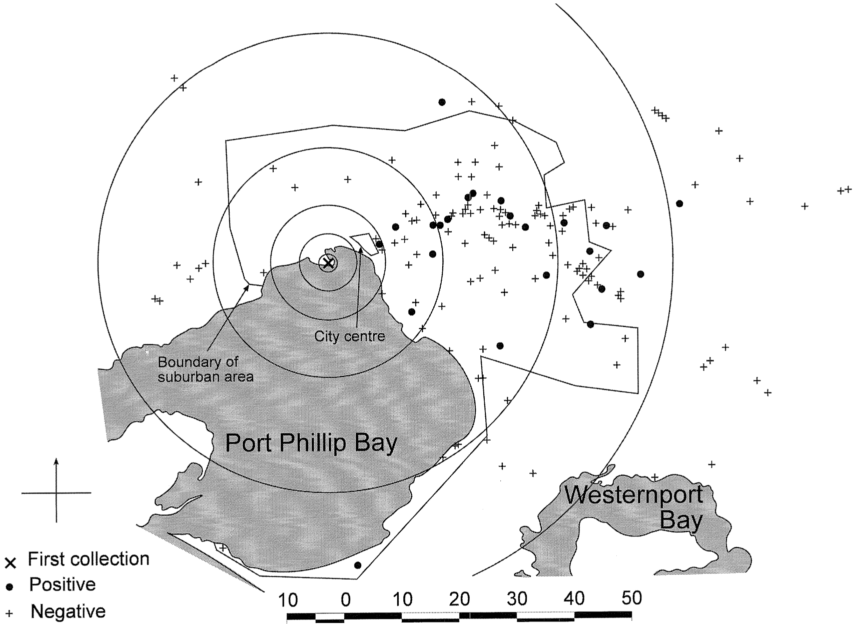

Fig. 1 Trees sampled for nematodes around Melbourne. Concentric circles or arcs correspond to distances of 1, 5, 10, 20, 40 and 60 Km from the first tree

high probability of containing all or most nematodes if vectored by *Monochamus* (Fig. 1). In deciding this, the assumption was that this was a recent introduction. It was assumed that if the introduction caused significant pathology and occurred some time in the past, symptoms would have been noticed before they were. The known pathology of *B. xylophilus* makes this assumption somewhat tenuous. Disease symptoms only occur above a certain temperature, and are more likely if trees are under stress, particularly from drought, high temperatures, chemical compounds or shading (Tanaka, 1975; Rutherford and Webster, 1986; Kaneko, 1989; Rutherford et al., 1990; Evans et al., 1996; Kawaguchi et al., 1999; Mamiya, 1999; Braasch, 2000). Melbourne winters and springs are cool and damp, so few symptoms would be expected until early summer, even if introduction was up to 6 months previously.

The other factor in choosing this sampling scheme was feasibility. In a basically urban environment, sampling of all trees within the 1 Km radius was considered likely to be feasible, but sampling every tree within a radius of 5 Km was thought unrealistic. There were no estimates of the number of coniferous trees in the area on which to base this assessment.

To complete the sampling, trees in the 1 Km core area were located visually by driving all local streets. All were then sampled for nematodes by cutting discs from any branches which appeared unhealthy, as well as taking small cores from the trunk at various heights. Whitehead-Hemming trays were used for extraction of nematodes. Using these methods, nematodes were detected in none of the 21 trees located within the 1 Km radius study area, nor in any of the trees sampled in the 1–5 Km radius area. (Cypress trees on the foreshore were dying back due to high chloride in foliage, the result of salt spray.)

Samples from pine forests from other states of Australia were taken by local forestry agencies to confirm that *B. hunanensis* really was exotic. Pines in poor condition were preferentially sampled in Western Australia, South Australia, Tasmania, NSW and Queensland using similar methods to those used in Victoria, and submitted to the CSIRO laboratory in Canberra. None of the 10 samples had any of the species isolated from the original dying tree. This was consistent with the nematodes being exotic, although it was hardly a sufficient number of samples to prove this was so with any degree of certainty. This issue is discussed further in the section "evaluation of response".

At this stage (March 2000), it seemed that there was only a single tree which had been infested. However, in May, a single dying *Pinus* spp. was reported approximately 10 Km from the original tree, and on investigation found to contain *B. hunanensis*. The tree was immediately removed using similar protocols to the tree at Williamstown. No single cause of death other than the nematode could be found, despite extensive testing.

A survey of all dead or dying coniferous trees within a 5 Km radius of the second tree with *B. hunanensis* was commenced immediately, with trees located as before from the roads. In this survey, a further tree containing *B. hunanensis* was located near the edge of the 5 Km radius, and 15 Km from the original tree. It was becoming obvious that the assumptions regarding dispersal were inconsistent with observations, so a new strategy was adopted. Aerial surveys were conducted by helicopter for any coniferous tree with symptoms within a radius of 50 Km of the original tree, and the public of the entire state were invited in print, radio and television to report coniferous trees which had died rapidly. Large areas of largely native (*Eucalyptus* spp.) forest east of Melbourne were also surveyed visually for obviously diseased trees from the main roads. Light and pheromone traps were deployed throughout Melbourne in an attempt to catch any vector, particularly if it too was exotic. The measures continued through June and July.

All 110 trees identified in the aerial surveys and 36 trees reported by the public were investigated for the presence of nematodes. Trees reported by the public were located as far as 500 Km away. A total of 33 trees contained *B. hunanensis*, the furthest 60 Km from the original tree and presumed source at the docks (Fig. 1). All were removed as soon as practicable after large samples of wood from the trunk and branches were removed. These large wooden billets were placed in drums to recover potential vectors.

The exotic cerambycid *Arhopalus rusticus* and the globally widespread *Ips grandicollis*, both potential vectors for PWD (Lieutier and Vallet, 1982; Linit et al., 1983), were found in the light and pheromone traps. A few *A. rusticus* were found in one of the infested trees. No *Monochamus* were found in the light or pheromone traps. No nematodes were found in extensive searches of the exterior and interior of the beetles. *B. hunanensis* was common, but not universal in suspect trees, and no *B. xylophilus* were found.

Response Stage Three

Surveillance was maintained for the following 18 months, particularly for the following two summers (December 2000–March 2001, and December 2001–March 2002). A further 40 trees were tested because of rapid death during the summer of 2000–2001, with only two having *B. hunanensis*. These trees were removed. During the summer of 2001–2002, only 5 trees were tested and none had *B. hunanensis*. *B. hunanensis* was not isolated from any healthy tree, nor was it found in all dying trees.

Pathogenicity of B. hunanensis

Attempts to evaluate the pathogenicity of *B. hunanensis* were conducted in parallel to the eradication campaign. Nematodes extracted from wood were directly inoculated into 3-year-old *Pinus radiata* (the predominant species in local plantations) and *P. halepensis* (the species of the first tree affected). Trees of this age were the only ones available at short notice. Other studies have often found that pathological responses are more likely in young trees (McNamara, 2004). After 3 months, no nematodes could be reisolated from the trees and there were no symptoms.

Direct inoculation of healthy trees with wood plugs from trees known to have the nematode also failed to reproduce the symptoms. No nematodes or fungi could be re-isolated from trees so treated. Attempts to grow the nematode in culture were unsuccessful (see above: stage 1). Other potential causes of pathogenicity were considered but rejected as being the main cause. The state was in the fourth year of drought, but this was unlikely to be a main cause because affected trees were often within groups of otherwise healthy trees. Several of the trees were within parks where the trees received some degree of care and watering. Likewise, although *Diplodia* was isolated from many dying trees, it was not found in all trees. Nor were there symptoms consistent with the usual manifestation of the disease: whole trees were affected rather than single branches, and only one individual in a group of trees was dying rather than all. Other fungi and insects—*Armillaria* spp., *Ophiostoma* spp., *Phytophthora* spp. and *Ips grandicollis*—were rejected as being primary causes of mortality on similar grounds. Salt levels were tested and found outside the generally accepted pathogenic range in most affected trees. Physical wounding, earthworks etc. may have affected some of the trees that died, but were certainly not involved in most, including the first two trees affected.

The tests for pathogenicity were therefore inconclusive. *B. hunanensis* could not be confirmed as associated with symptoms, except statistically, and Koch's Postulates were not satisfied. However, no other single potential cause, biological, chemical or physical could be unambiguously identified either. Because of the nature of the disease, doubts have been expressed as to whether Koch's Postulates can ever be used to demonstrate the pathogenicity of *B. xylophilus* (McNamara, 2004).

In the present case, the most likely explanation may be that a complex of factors was involved. This is discussed further below.

Distribution of Trees Containing **B. hunanensis**

A summary of the trees from which *B. hunanensis* was isolated is presented in Fig. 1. The pattern resembles a plume from the first tree and presumed origin at Williamstown. Most trees containing the nematode were in an arc between bearings of 60° and 120° from the first tree, with the mean direction about 70–90° (East-Nor-East to East). This is the general direction of prevailing winds.

Another feature of the figure is that there are few infested trees within 10–15 Km of the presumed origin. In the direction of the plume, shorter distances correspond with the city centre (where there are few trees) and the open waters of the bay. The furthest tree was about 62 Km away, but most were within 20–50 Km. None of the trees outside the immediate environs of Melbourne contained *B. hunanensis*.

The main exception to the general pattern was a tree located about 52 Km away at bearing 170°. In this general direction this is the closest landfall.

The pattern is generally consistent with a single dispersal flight by wind-borne or wind-aided vectors. However, the distances involved are considerably greater than are normally associated with vectors of *B. xylophilus*. Within the context of preparedness for, and control measures following, possible incursions by nematodes from the genus *Bursaphelenchus*, the fact that at least some species of the genus can be transported these distances is of particular note. The identity of the vector would be most interesting, but as *B. hunanensis* now appears to have been eradicated, the vector will probably never be known. The rapid decline in trees containing the nematode over the years subsequent to its first isolation, and the inability to find any insects with nematodes, indicated that whatever the vector was, it was inefficient in terms of the percentage of vectors carrying nematodes and possibly also the number of nematodes carried on the vector.

However inefficient in terms of numbers, the distances an organism can be carried is more significant in terms of quarantine. This is especially so if there are other vectors which may be more efficient, but only travel short distances. The short-distance efficient vector could then transmit nematodes from foci created by the inefficient, but long-distance vector some distance away from initial infestations.

Most trees had relatively few *B. hunanensis* (less than 10 individuals per 10 g of wood), but some had high numbers of nematodes within them (up to 7000 individuals per 10 g of wood). The vast majority of nematodes were juveniles. These are population characteristics consistent with non-breeding, inviable populations. The nematodes may have found few appropriate hosts, habitats, food sources, or appropriate vectors beyond their initial ones.

Discussion

Initial Analysis of Risk

The likely impact of an exotic organism relative to the cost of the options of eradication, containment, control or doing nothing is important information in considering the best response to an incursion. The likely impact can be estimated by considering each part of the invasion process separately: the likelihood of success in invasion, the consequences of a successful invasion, and the success and cost of mitigation strategies if the organism becomes established. The likelihood of success in invasion can be further divided into processes of arrival, establishment, spread and persistence. Consequences can be divided into direct effects on humans, crops or beneficial organisms, indirect effects such as loss of ecosystem services or trade restrictions, and the cost of control or management strategies, including the costs of what is precluded by the pest or management strategy.

The problem with estimating the risk this way is that a lot of data is required: not all may be available, and quality of different parts may vary widely (Stohlgren and Schnase, 2006). This situation is common with nematodes (Hockland et al., 2006). Thus there is an estimation process with some degree of uncertainty. The role of uncertainty is discussed in the following section.

In the initial consideration of the risk posed by *B. hunanensis*, the basic information was evaluated as follows. This was the situation after *B. hunanensis* had been found in a single dying tree, with no other information except the report of *B. hunanensis* from the type locality.

- ***Cost of successful establishment of B. hunanensis***. The worst case scenario is similar to that for *B. xylophilus*. In Japan, mortality (without mitigation measures) of *P. radiata* (the most common species in Victoria) was about 80% (Mamiya, 2004). Spread has been about 2–15 Km yr^{-1} (Togashi et al., 2004), and the value of *P. radiata* plantations is about AUD15 000 to AUD 30 000 Ha^{-1} (Southern Tablelands Farm Forestry Network, pers comm.). With these figures, the total value of potential losses ranges from about AUD 0.2–12 million in the first year, and 0.35–19 billion over 20 years.

  Another way of estimating is using the national turnover of forest products (AUD 18 billion: Parsons et al., 2006) or returns for exports (AUD 2 billion, Australian Bureau of Statistics, 2005), and the proportion of forests potentially affected (30%: Mamiya, 2004). Using these figures, the estimate of potential effects are AUD 6 billion in annual turnover, and 0.7 billion annually in exports. At the initial stage, the only record of *B. hunanensis* on *P. halepensis* was the one we had, so pathogenicity in our situation was uncertain, discussed further below in section using other species data. The costs of trade restrictions, amenity value etc. would have to be added to these figures. Damage to ecosystems may also occur, though quantification is lacking (Batabyal and Beladi, 2006; Perrings et al., 2000).

- ***Containment***. This was not considered a feasible option, given the urban setting with large, unregulated movement of plant and other material. It was also considered an unlikely because of the spread in most countries in which it had been introduced (Webster, 1999, 2004; Yoshimura et al., 1999).
- ***Eradication***. Cost unknown but thought relatively small because of the sparse distribution of potential hosts in the urban area. Note that relative costs and feasibility of containment and eradication may invert in a less-inhabited, heavily-forested environment, or one where access was restricted.
- ***Control costs if established***. Based on the experiences of China and Japan, complete control appeared unlikely, and mitigation the best likely to be achieved (Evans et al., 1996; Mamiya, 1988; Yang, 2004). Furthermore, in the local context, mitigation measures were likely to be difficult because of large areas and low workforce numbers. The costs of this option were thought likely to be high.

The conclusion had to be that a response was probably warranted because of the economic importance of pine forests, and further, that to attempt eradication was probably the best response. This was what was implemented.

The main factor in the decision was the large potential cost of a nematode with effects similar to *B. xylophilus*. Even though the probability that *B. hunanensis* would have such effects was very low, the high cost meant that action was warranted. This is a general feature of risk management: if the unmitigated negative effect is very large, then action is required even if the likelihood is very low (Gigerenzer, 2002).

Uncertainty

Uncertainty is a common feature of many quarantine situations: an incursion by definition involves an organism in a place that it does not normally occur. Where the organism is a relatively well-known exotic, the outcome can be predicted with reasonable certainty in some situations.

- There may have been previous incursions of the organism.
- The new landscape is highly modified so that it is very similar in many ways to the landscape which is the origin of the exotic. The commonest examples are many agricultural landscapes, which have been deliberately modified to at least partially mimic the origin of a particular crop (and many of its pests).
- The organism has been involved in numerous documented incursions into other new places, and so there are numerous precedents.

In many other situations, like that described for *B. hunanensis* in Australia, the organism and its response in a new environment are inadequately known, so the outcome of an incursion cannot be predicted with certainty. Whether it is exotic or not may even be uncertain. In these cases there needs to be a prediction as to whether the organism will become a pest, with a measure of the uncertainty of the prediction.

There are several ways to estimate the likelihood that an exotic nematode will become a pest. The simplest calculates the percentage of known species that are pests. For animals in general this is about 1% (Williamson, 1996; Williamson and Fitter, 1996). For nematodes, the figure may be about 2% (about 200 species are recognized as pests (Nickle, 1992), out of 11 050 species described at a similar time (Andrassy, 1992)). More sophisticated estimates might involve the percentage of exotics that are pests (Dark, 2004; Rejmanek and Randall, 2004), but this is not known for nematodes.

Of most relevance is whether uncertainty should increase or decrease the reason to respond. There are reasons to do both.

Reasons that a high degree of uncertainty should increase the risk—and consequently the likelihood of a response—include the simple statistical observation that a high degree of uncertainty generally means that there will be a wider range of possibilities. This means that there is a greater chance of severe negative effects. The possibility of severe negative effects (disasters) generally means a high level of concern, almost irrespective of the probability (Vitousek et al., 1996; Mack et al., 2000; Gigerenzer, 2002).

There is also the possibility that there will be strong selective pressures for the evolution of pathotypes, races or biotypes in pests, and that these will be unknown. Recent evidence suggests that evolution may occur more rapidly than previously thought (Rodriguez-Trelles and Rodriguez, 1998; Bradshaw and Holzapfel, 2006; Umina et al., 2005; Franks et al., 2007). The genus *Bursaphelenchus* has long been considered likely to evolve rapidly (Giblin-Davis, 1993; Evans et al., 1996). The importance for quarantine of such characteristics of many pest species is becoming increasingly recognised (Brasier, 2001; Kohn, 2004).

Reasons that a high degree of uncertainty should lessen the risk include the observation that, in nematodes at least, knowledge about a particular species may be related to pest status. Pest species are studied more than those that are benign precisely because of their economic effects, so a higher proportion of pest species may be known than benign. Conversely, pests may be a smaller proportion of the species about which we know little—and are consequently highly uncertain—than they are among the well-known species. Hence, there may be a lower probability that a poorly known or unknown nematode will be a pest than there is for known species. If this is the case, there should be less justification for action for poorly-known or unknown species.

There are also reasons for the observation of pests frequently being better-studied than benign nematodes meaning that action is justified. This is because many quarantine pests are not major pathogens in their native range. The best examples of this in nematodes are some of the biggest quarantine threats: *B. xylophilus* and the potato cyst nematodes (PCN) *Globodera rostochiensis* and *G. pallida* (Mamiya, 1984; Wingfield, 1987; Lehman, 2004; Hockland et al., 2006). *B. xylophilus* was not studied in its native range until after it was recognized as the cause of PWD elsewhere (Mamiya, 1984). Likewise the PCN (Franco et al., 1998). Thus species which become pests out of their native range may actually be less likely to be well-studied because they are unlikely to be major problems in their native range.

It is also possible that a species being poorly known may have no effect on the probability that it will become a pest in a new environment. This is because characteristics in the native range are often poor at predicting whether an animal will become an invasive pest in another place (Williamson, 1996; Kolar and Lodge, 2001; Marchetti et al., 2004; Arim et al., 2006; Inderjit and Drake, 2006). Whether this applies to nematodes requires research: many of the worst nematode pests are apparently the same everywhere (e.g. Mota et al., 2006).

The fact that the host is also out of its native range may also increase the probability of pathological interactions. It is has been frequently observed that *B. xylophilus* only affects many species out of their native range (Furuno et al., 1993; Mamiya, 2004). All species of *Pinus* are exotic to Australia.

Estimation of Impact Using Known Relatives

One way to predict the possible effects of an organism about which little is known is to use the nearest relative about which the information exists. This may lead to an over estimate of the adverse effects of the poorly-known species because that species is likely to be a pest, because, as suggested above, pests are more likely to be studied than their benign relatives due to their economic impact. Unless there are more studies of free-living nematodes, this will remain a factor in decision making, but it should be acknowledged more than currently.

Whatever the bias, estimation of impact using known relatives may give an excellent estimate of the "worst-case scenario". For many risk analysis methodologies, the worst case is the most significant (Gigerenzer, 2002).

Eradication

There have been few examinations of the options available for nematode quarantine, and few documented cases of eradication. Emphasis in nematode quarantine is mostly on prevention of incursions through hygiene and disinfestation (Hockland et al., 2006). When incursion management is discussed, it is generally with regard to containment (e.g. McNamara and Smith, 1998; Whitehead and Turner, 1998; Watson, 2004; Hockland et al., 2006), only very occasionally with regard to eradication (e.g. Marshall, 1998).

It is very noteworthy that eradication of nematodes can work, as reported here. It should be considered as an option in at least some nematode incursions. The circumstances under which eradication will be the best option require further investigation.

Cost of Delay

There is a cost in delaying action in the face of uncertainty. In either of the options of eradication or containment, delay inevitably increases the radius of the area which needs to be treated. Cost is, of course, generally related to area, and area is related to the square of the radius, so cost increases rapidly. The chance that a suitable habitat may be located for establishment increases in a similar way.

In the context of the incursion of *B. hunanensis*, a second flight of a vector similar to the presumed original flight was estimated to cost AUD 1 million. This is a substantial cost of delay, and justified at least commencing responses before all data were available.

The correct identification of the nematodes to at least genus by the local identification service also facilitated a quick response. Maintaining a degree of awareness of nematodes and some expertise in nematode identification at a local level proved valuable in the present case.

Value of Systematics

Having expertise or diagnostic tools for both local and exotic species is an important part of being able to respond rapidly (discussed above). There are two issues: one is the general difficulty of all but experts in differentiating nematode species (Eyualem and Blaxter, 2003; Powers, 2004; Sturhan, 1996). The other is that only a small percentage of all nematode species have been described, and an even smaller percentage of species have had intra-specific variability recorded. This means that it is often difficult to diagnose species, either on the basis of evidence or by judgement. This difficulty is relevant in quarantine situations when the organism found is nearly, but not exactly, like a known species, either local or exotic. Is it then the same or different? This is a frequent occurrence in parts of the world like Australia where knowledge of the fauna remains poor, and undescribed species are common. This is a reason to maintain general taxonomists with general knowledge of important groups of organisms.

Apart from being able to provide the most rapid response, having expertise within the country avoids release of partial information: the first report of this incursion was in fact a report from NZ, based on material sent for identification of potential beetle vectors (Ridley et al., 2001; Smith et al., 2007). However, in the absence of tools or expertise for exotic species, a personal network with overseas experts can be a surrogate, so that an unfamiliar species can be referred to someone familiar with it.

Whatever the expertise available, a robust phylogenetic systematic framework in which to place both known and unknown species, is of considerable value in reducing uncertainty and improving predictions in quarantine situations such as that described here. An unknown species needs to be placed into an existing taxonomic framework to determine the closest relatives, where they are found, and how distant is the relationship. In the case of all of the three species found initially at Williamstown, there was uncertainty caused by the desperate need for revision of the entire phylum Aphelenchida (Hunt, 1993).

Having a robust phylogenetic classification is particularly important for unknown or poorly-known species. It allows the nearest relative to be identified, and used to predict the otherwise unknown risks associated with the species (as discussed above). If material is insufficient to allow identification to species, then a phylogenetic classification enables the maximum information to be gleaned from identification to species group, genus, sub-family, family or superfamily.

In the events described herein, this issue arose in the identification of *B. hunanensis* and the genera *Aphelenchoides* and *Bursaphelenchus*. There are considerable morphological overlaps between the females of these genera, and nematodes with a long stylet, long dorsally overlapping oesophageal glands, a short post-uterine sac (PUS), and without a vulval flap could be in either genus (Baujard, 1980; Hunt, 1993). There are other characters to separate males, but males are not known in all species of *Aphelenchoides*, nor are they present at all times in *Bursaphelenchus* spp. (Hunt, 1993; Ryss et al., 2005).

It is better still if there have been attempts to map traits—such as parasitism, hosts, or vectors—onto phylogenies. This allows estimation of the likelihood that a new species will have a greater or lesser potential for pathogenicity. For example, pathogenicity may have evolved several times within a genus, so that a new species in the genus has a heightened chance of being pathogenic. Similarly, a large number of species within a genus may be pathogenic, but from only one evolutionary event. If pathogenicity is absent within the larger Family or Ordinal grouping, then the chances that a new species of uncertain placement within the genus, Family or Order may be less likely to be pathogenic.

There is some debate about the origins of pathogenicity within the genus *Bursaphelenchus*. Some hypothesise that pathogenicity has evolved in an all-or-nothing fashion at most twice in the genus of about 75 species (including *B. cocophilus*: Giblin-Davis et al., 2003; McNamara, 2004; Ryss et al., 2005). Others hypothesise that there is a gradualistic transition between non-pathogenic and pathogenic species, with some species partly pathogenic or only pathogenic under certain circumstances (Braasch et al., 1999b; Kanzaki and Futai, 2006; Michalopoulos-Skarmoutsos et al., 2004). The evidence is currently equivocal, but may become clearer if there is a bacterial complex involved in causing disease (Han et al., 2003, 2006; Zhao et al., 2003).

The genome of *B. xylophilus* is highly plastic, with consequent potential for changes in pathogenicity (Evans et al., 1996; Jones et al., 2005; Kikuchi et al. 2004, 2005, 2006, 2007). Two forms of *B. xylophilus* within North America, are recognised by some: one has a round tail, usually occurs in *Pinus* spp. and is mostly associated with disease; the other has a more pointed or mucronate tail, occurs mostly on fir or spruce (occasionally on pine and other conifers) and is usually benign (Bolla et al., 1986, 1987). Populations maintained as laboratory cultures can change in pathogenicity and biochemical composition over time (Bolla et al., 1986, Kiyohara and Bolla, 1990).

How many other species in the genus *Bursaphelenchus* share this plasticity is unknown. However, it is possible that plasticity is a characteristic of the genus. *Bursaphelenchus* has had a spectacular evolutionary radiation and is the second-largest genus within the Order Aphelenchida: only *Aphelenchoides* with over 200 species is larger (Hodda, 2003).

It is important to note that systematic resources as discussed in this section cannot be generated instantly in the case of an urgent need when there is an incursion.

Knowing the Local Fauna

It is important in quarantine situations such as with *B. hunanensis* that the local fauna be reasonably well described and sampled. Without descriptions of many local species at all, it may be uncertain whether an interception is a species occurring locally or not. In the absence of formal descriptions, voucher material and samples which can be checked rapidly are useful. It may be impossible to obtain material to tell whether an organism occurs locally or not within the time frame needed for quick decisions on action (as discussed below: sampling problems). It is worth knowing the local fauna. This is a worldwide problem for a wide range of nematodes (e.g. Bello et al., 2005 in Spain; Bongers et al., 2003 in Costa Rica; Braasch and Enzian, 2004 in Europe; Queneherve and van den Berg, 2005 in French West Indies).

Before the incursion of *B. hunanensis*, the Australian fauna of Aphelenchida associated with pines and insects was almost totally unknown, with two records only (Queensland Museum, Stone 1990; Stone and Simpson, 1990, 1991). Considerable efforts have now gone into rectifying this situation (e.g. **? ?**, Zhao et al. 2006a, b, 2007). Decisions as to the best action after *B. hunanensis* was first found would have been much easier if the information and collections available now had been available then.

Sampling

Three sampling issues arose in responding to the incursion of *B. hunanensis*: the area necessary to search, the way to search, and how to tell when *B. hunanensis* is absent. It was necessary to sample for the absence of *B. hunanensis* in two circumstances: to verify that it really was absent from other areas and states of Australia, and to verify eradication.

The area to search for *B. hunanensis* was partly dependent on estimates of the dispersal of possible vectors (discussed in the next section), and partly on practical considerations (what resources were available and the ways that the nematode could be found).

As the initial search area was fairly small (1 Km radius), and potential hosts for the nematode (coniferous trees) were sparse, comprehensive manual searching was feasible, both in terms of logistics (one team in a vehicle was easy to obtain), and in terms of manually sampling every tree. In the outer (5 Km radius) area, there were no estimates of the number of trees, unlike in a forest, but locating and sampling every potential host could have been a very large task. There is no estimate of the percentage of trees sampled, but the targeting of diseased trees only made the task feasible. When the area was expanded enormously after *B. hunanensis* was found outside the initial search area, targeting of diseased trees only made what could have been an enormous task feasible. Targeted diseased trees were also easy to identify from the air, and so made the use of a helicopter possible. Aerial searching is often overlooked as a possibility for sampling large areas because of difficulties in identification from the air (Mullerova et al., 2005).

Throughout the searches for *B. hunanensis*, completely covering the area of the incursion was more important than improving the detection rate within the area. This was because of the high level of uncertainty surrounding the vector(s), the importance of restricting spread outside the urban area, and the need to minimise the area from which eradication would be required. Missing nematodes that subsequently dispersed further increases the cost of eradication enormously (as discussed above). The fact that initial estimates of the radius of dispersal were very much too low is discussed further below.

In considering the lessons from the experience with *B. hunanensis*, one of the best lessons was that relying on reports from the public was relatively effective and efficient. Within the suburban environment, a high proportion of the dying trees were reported, and even in rural areas, trees were reported from hundreds of kilometres away.

The other major issue in searching for *B. hunanensis* was sampling to verify that it was in fact exotic, and that it had been eradicated. Sampling for the absence of an organism is often very costly because sampling effort for a given reliability increases inversely with abundance. One lesson here was that ensuring total absence is not necessary if a minimum viable population density can be estimated (Anderson, 2005). The sampling effort to ensure that abundance is below that which is viable may be considerably less than that required to ensure that the population is essentially zero (with the same degree of reliability). Estimates of minimum viable populations are, of course, subject to considerable uncertainty, as discussed above. Minimum viable populations for *B. xylophilus* have been estimated (Togashi, 1985; Togashi and Shigesada, 2006).

Dispersal

The dispersal distance is key information for many parts of dealing with an incursion such as that of *B. hunanensis*. From the feasibility of eradication or the area that needed searching to estimating the minimum viable population, dispersal distance was among the most important pieces of information.

Hence considerable effort was justified in trying to locate the vector. The fact that a vector was not found increased the uncertainty involved in all actions, but perhaps not by much. Potential vectors may behave very differently in different environments. For example, a species of *Dendroctonus* spp. disperses approximately 16 Km in North America (Smith, 1971), but up to 35 Km in China (Zhang et al., 2002), and attacks trees in a different way (Yan et al., 2005). Vector behaviour may also be affected by the phoretic nematodes (Aikawa et al., 2003).

Whatever the vector, a main conclusion from the results reported here is that the nematode dispersed considerably farther than originally estimated. Part of this may have been due to assistance of the vector by wind: the distribution of trees with the nematode is a classic plume in the direction of prevailing winds. Part of the reason for the great distance of dispersal may have been due to the plume being over water or urban areas for shorter distances from the presumed origin (Fig. 1). Part of the reason for the greater distances may be simply that some vectors can travel these distances. Most studies on this topic have concentrated on dispersal by vectors of *B. xylophilus* which are efficient in causing PWD (e.g. Linit et al., 1983; Wingfield and Blanchette, 1983; Kobayashi et al., 1984; Sato et al., 1987). However, *B. xylophilus* also has a non-disease cycle (Wingfield, 1987), and may also have other vectors. These vectors may feed in different ways or be attracted to dead trees only, so that they are not efficient in transmitting PWD, but can nevertheless disperse the nematode (e.g. Arakawa and Togashi, 2002). There may also be vectors which carry few nematodes—and are therefore unlikely to be detected—but which can travel considerable distances. The rapid spread of *B. xylophilus* after the incursion in Portugal in 1999, and in other countries also suggests that dispersal distances may be greater than current estimates. Likewise, studies from Australia on other aphelenchids associated with dead and dying trees (*Aphelenchoides, Ptychaphelenchus* and *Laimaphelenchus*), have shown that these nematodes may disperse over large areas (Hodda and Falez, 2008). These studies were made subsequent to the incursion of *B. hunanensis*.

Interactions and Disease Complexes

The pathology of the tree deaths observed is worthy of discussion. As outlined above, the studies on pathology of *B. hunanensis* were inconclusive, and a disease complex was suggested as a possible explanation.

It has been suggested recently that a bacterium (*Pseudomonas fluorescens*) is associated with pathology by nematodes in pines (Han et al., 2003; Zhao et al., 2003). This bacterium may cause symptoms whether associated with *B. xylophilus* or *B. mucronatus* (Han et al., 2006). The ability of other species of *Bursaphelenchus* to carry the bacterium requires further testing, but may be possible. A toxin may also be associated with some strains of the bacterium (Oku, 1988, 1990).

There is also the possibility that *B. hunanensis* is only pathogenic under some circumstances. This may be the case with several species of the genus *Bursaphelenchus* other than *B. xylophilus*. These may include *B. leoni, B. sexdentati* and *B. hellenicus* (Braasch, 2000; Caroppo et al., 2000; Skarmoutsos and Michalopoulos-Skarmoutsos, 2000; Michalopoulos-Skarmoutsos et al., 2004; Kanzaki and Futai, 2006). The pathogenicity of *B. mucronatus* in some circumstances is disputed (Kruglik, 2001; Giblin-Davis et al., 2003; Kulinich, 2004; McNamara, 2004).

It is also possible that the pathogenicity only occurred in trees where several factors weakened their resistance to disease. Such factors may have included genetic susceptibility and environmental stress (drought, high temperatures, chemicals or shading), which can increase susceptibility to PWD (Tanaka, 1975; Rutherford and Webster, 1986; Kaneko 1989; Rutherford et al., 1990; Evans et al., 1996; Kawaguchi et al., 1999; Mamiya, 1999; Braasch, 2000), as well as a range of diseases (Manion, 1981; Nilsen and Orcutt, 1996; Orcutt and Nilsen, 2000). Trees carrying *B. xylophilus* can be asymptomatic for long periods before the disease manifestation is triggered (Bergdahl and Halik, 1999).

How the risk and consequences of a disease complex are evaluated in quarantine situations requires further investigations.

Cost-Benefit Analysis

In the worst case scenario, the ultimate cost of an incursion of a nematode with a similar effect on local pine plantations as *B. xylophilus* in Japan can be estimated at about \$3 billion per year. Softwood forestry is worth about AUD18 billion yr^{-1} (Parsons et al., 2006), and losses average 30% yr^{-1} in Japan (Mamiya, 2004). The total cost of the eradication campaign was approximately AUD 0.2 million. If *B. hunanensis* was associated with timber losses of the same magnitude as *B. xylophilus*, then the benefit to cost ratio of the eradication campaign over 30 years (the rotation cycle of the trees planted at the time of the incursion), would be about 1 million to 1, a very good ratio. Put another way, it is worth conducting 1 million eradication campaigns where the benefits are uncertain if this results in stopping one pest which will cause damage of the magnitude of that estimated for *B. xylophilus*. The figure of 1 million is a worst-case scenario, but even if this is over-estimated by several orders of magnitude, many eradications where there is uncertainty over the level of threat are justified. The conclusion that action was justified is robust to considerable uncertainty if there is the possibility of a major effect, however remote. We believe that there was the possibility of a major effect in this case, as discussed above.

Many estimates of the probability that an exotic animal will become a pest are about 1% (Williamson, 1996). Much research is directed at being able to predict the phylogenetic, ecological or molecular characteristics of the 1% of species that will become pests, but at present predictions are still unreliable (Williamson, 1996; Kolar and Lodge, 2001; Marchetti et al., 2004; Arim et al., 2006; Inderjit and Drake, 2006). If the chance of an exotic organism such as *B. hunanensis* becoming a pest are 1 in 100, then the eradication campaign was even more justified.

Conclusions

There are many questions about this incursion that will never be answered because of the successful eradication campaign. However, the experiences and suggestions that follow from the events surrounding the incursion of *B. hunanensis* may prove useful in other quarantine incursions, particularly regarding nematodes, and most particularly involving nematodes associated with trees and insects. The main summary points are as follow.

1. More research is needed on which nematodes and other organisms become pests, the conditions under which it is most likely to occur, and the ecosystems most subject to invasion.
2. There are many research issues around predicting the characteristics of an unknown or poorly-known species, and how representative are the species about which most is known.
3. A robust phylogenetic systematic framework within which to place nematode species has considerable value in terms of telling new species from variants of existing species. It is also useful in allowing inadequate material to be identified to the best taxonomic level possible. This sort of information cannot be generated instantly, but relies on existing expertise.
4. Studies mapping the origins of particular traits, particularly the origins of parasitism, may be very useful.
5. A good knowledge of the local fauna, and access to knowledge about the faunas of other areas are important in deciding whether a species is exotic or not. This information is lacking for many groups of nematodes for many parts of the world.
6. The means, speed and distance of dispersal are critical things to know for this group of nematodes.
7. Dispersal of Aphelenchida generally, and *Bursaphelenchus* in particular, may be further than currently assumed on basis of current studies of efficient vectors in pine wilt disease.
8. There is considerable value in an immediate response to incursions by nematodes with the ability to disperse considerable distances. The value is likely to be greater than the extra costs associated with uncertainty about the organism's identity or characteristics. A low cost of eradication because of a rapid response and sparse potential new habitat can have major effect on the economics of eradication (see point 14).
9. Training of staff likely to be involved in detection of exotic organisms in the early stages of incursions proved very useful in this case.
10. The lowest viable populations of exotic invaders is very useful information, if available.
11. Just because an organism is not a major pest in its native environment, doesn't mean that it will not be a pest in a new environment.
12. The presence of exotics that may be part of disease complexes complicates the options and management of incursions.
13. Eradication of exotic nematodes can be successful, and a valid response to incursions.
14. Cost-benefit ratios of eradication programmes for nematodes can be very favourable under almost any assumptions of their cost or the probability of the exotic nematode becoming a pest.

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