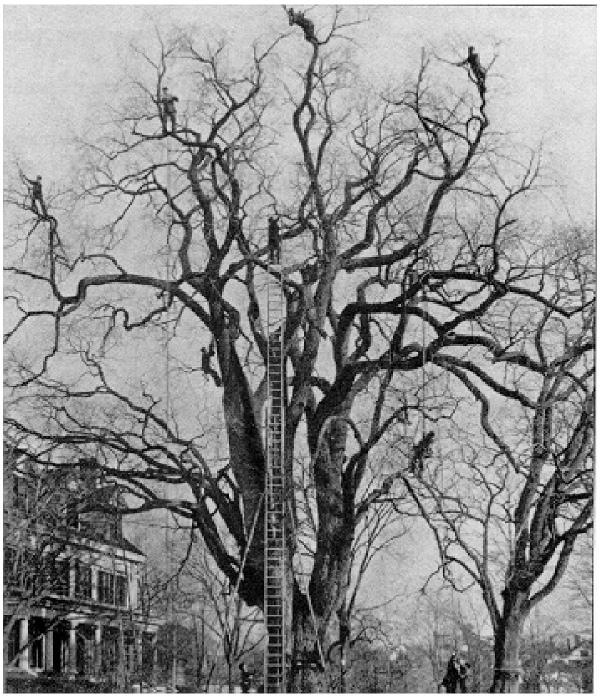
Strategies for the eradication or control of gypsy moth in New Zealand

Travis R. Glare[1], Patrick J. Walsh[2*], Malcolm Kay[3] and
Nigel D. Barlow[1]

[1] AgResearch, PO Box 60, Lincoln, New Zealand
[2] Forest Research Associates, Rotorua ([*]current address Galway-Mayo Institute of Technology, Dublin Road, Galway, Republic of Ireland)
[3]Forest Research, Private Bag 3020, Rotorua



Efforts to remove gypsy moth from an elm, Malden, MA, circa 1891

May 2003

STATEMENT OF PURPOSE

The aim of the report is to provide background information that can contribute to developing strategies for control of gypsy moth. This is not a contingency plan, but a document summarising the data collected over a two year FRST-funded programme on biological control options for gypsy moth relevant to New Zealand, completed in 1998 and subsequent research on palatability of New Zealand flora to gypsy moth. It is mainly aimed at discussing control options. It should assist with rapidly developing a contingency plan for gypsy moth in the case of pest incursion.

Abbreviations

GM	gypsy moth
AGM	Asian gypsy moth
NAGM	North America gypsy moth
EGM	European gypsy moth
Bt	*Bacillus thuringiensis*
Btk	*Bacillus thuringiensis kurstaki*
MAF	New Zealand Ministry of Agriculture and Forestry
MOF	New Zealand Ministry of Forestry (defunct, now part of MAF)
NPV	nucleopolyhedrovirus
LdNPV	*Lymantria dispar* nucleopolyhedrovirus
NZ	New Zealand
PAM	Painted apple moth, *Teia anartoides*
FR	Forest Research
PIB	Polyhedral inclusion bodies

SUMMARY

Gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantriidae), poses a major threat to New Zealand forests. It is known to attack over 500 plant species and has caused massive damage to forests in many countries in the northern hemisphere. The native range of gypsy moth extends across much of Eurasia and North Africa. In the late 1880s it was introduced to the USA and continues to be a major pest in forests in that country.

Recently, an "Asian" strain (AGM) has been recognised which has characteristics that increase the potential for damage. The AGM females can fly (European and North American strains cannot fly), are attracted to light, have a wider host range, are generally larger and have a higher premature eclosion. Eggs of AGM require less exposure to low temperature to hatch and hatch over a longer period. It is currently spreading across much of Asia and Europe, and has been accidentally introduced and possibly eradicated from North America several times.

The finds of viable egg masses of AGM on ships in New Zealand ports alerted this country to the likelihood of an introduction in the near future. In preparation for accidental introduction, we considered environmentally acceptable control and eradication tools for use in New Zealand, conducted research and interacted with experienced overseas experts to determine the most suitable control agents. We also considered methods for monitoring and detection. As larvae and male moths of AGM and the European/North American (EGM/NAGM) strains are very difficult to distinguish morphologically, we investigated molecular methods that could be used for rapid identification. PCR based on ribosomal DNA was used to separate the two strains successfully.

A predictive model of the population behaviour of both European (non-flighted) and Asian (flighted) strains in New Zealand has been produced. The model suggests subtle differences in the long-term behaviour of Asian and European strains of gypsy moth under New Zealand conditions. If predators exist, AGM is predicted to increase more rapidly at first, and then to maintain more widely fluctuating densities and more pronounced outbreaks than the NAGM/EGM. In the absence of predators, then the model predicts that outbreak behaviour will not persist.

Since the first discovery of AGM on ships in New Zealand ports in 1993, a monitoring system based on pheromone trapping has been in place. It is known that gypsy moth males seldom fly more than 800 metres and the minimal trap density of 2 traps/km^2 is based on this knowledge. If an eradication programme is implemented, trapping should take place for two years in the infested zone after eradication has been attempted. High density trapping (up to 16 traps/km^2) should be maintained in the known areas of infestation.

Control and eradication of GM has been attempted in many countries. Historically, common methods of control were destroying egg masses, burning infested bushes, banding around trees to trap caterpillars or spraying with insecticides such as lead arsenate and DDT. Biological control has been extensively investigated. Initially parasites and predators were imported into the USA, but they were largely unsuccessful in reducing the problem. Parasites and predators of GM, although numerous, would be unlikely to be suitable for use in New Zealand as they are generalists and would therefore pose a threat to New Zealand's indigenous fauna.

Several biological or green chemistry control options with lower environmental risks than traditional chemical insecticides have shown success overseas. Diflubenzuron (Dimilin), an insect growth regulator, has been used in the USA and Europe, resulting in population reductions. However, there were some impacts on non-target organisms. The bacterium, *Bacillus thuringiensis kurstaki* (*Btk*), produces a toxin that is specific to Lepidoptera and has been formulated as a pesticide. It has been used extensively against gypsy moth, as well as other Lepidoptera, and is effective and more environmentally benign than most available insecticides. It was recently used in the eradication programme for tussock moth, *Orgyia thyellina*, in Auckland and is currently being used against painted apple moth, *Teia anartoides* (PAM). Non-target effects are restricted to Lepidoptera. *Btk* has been the insecticide of choice in several eradication programmes against GM in North America.

The gypsy moth nucleopolyhedrovirus (LdNPV) is active in most countries where gypsy moth is found and has been produced as a commercial or semi-commercial product in the USA (Gypchek), Canada, (Disparvirus) Czech Republic (Biola) and Russia (Virin-Ensh). It is specific to *Lymantria* spp. and testing in New Zealand and elsewhere has not found an infection in other Lepidoptera. It has been aerially applied in many areas of North America and used throughout Europe. As the virus is produced commercially and applied to large areas of forest, it appears a good candidate for use in New Zealand. The main benefit of the virus over *Btk* and other control measures is increased specificity. However, it is unknown whether sufficient quantities of viral product would be readily available for an emergency response in New Zealand.

Modelling the effect of LdNPV introductions on AGM in NZ suggested that, in the long term and in the presence of predators, a single introduction would result in an average of 20% outbreak density population suppression each year. According to the model, suppression of 80% of peak larval densities in the year following control can be obtained from an application of NPV at the standard rate (2500 GPIB ha^{-1}), if the larval density is extremely high (10^5 ha^{-1}) or extremely low (10 ha^{-1}). This drops dramatically to a 30% reduction at a larval density of 100 ha^{-1}. The effects are qualitatively similar in the absence of predation. Assuming that the default parameters are correct, eradication of a gypsy moth population with NPV is only possible if the population is very low (1 - 5 larvae ha^{-1}) and several sprays are used in rapid succession or the spray concentration is very high (25,000 GPIB ha^{-1}).

The fungus, *Entomophaga maimaiga* (Entomophthorales: Zygomycetes), is a pathogen of gypsy moth, originally described from Japan and introduced to the USA. It has spread through the USA gypsy moth population and has caused a rapid decline in numbers since 1990. The fungus is difficult to cultivate outside the host and no mycoinsecticide formulation is available. It does show potential as a classical biocontrol agent in established GM populations. Although in the field *E. maimaiga* has shown specificity to GM, in the laboratory other Lepidoptera have been infected. Further host range testing is required before safety for

NZ non-target insects is known. The success of *E. maimaiga* in controlling gypsy moth populations in North America indicates it has potential to assist other control agents in combating any gypsy moth outbreak/importation.

A synthetic sex pheromone has been commercially produced for gypsy moth, under the name Disparlure. In addition to its use in monitoring populations, there are two main methods for using the pheromone to reduce GM populations: mass trapping and mating disruption. Mating disruption is of doubtful use as an eradication tool for gypsy moth, but would be useful in population suppression.

Given the complexity of the gypsy moth life system and the need to consider alternative scenarios in the absence of appropriate data (especially on the natural enemies likely to affect the moth in New Zealand), long-term control options need further evaluation. Options include both a greater variety of NPV spraying regimes relative to the timing of outbreak cycles, and the integration of such strategies with the use of other controls such as *Btk* and *E. maimaiga*.

If AGM is found in NZ, eradication will be the first consideration. Eradication has been successful against small, well-defined populations infesting under 100 acres in the USA. After a review of available data we conclude that:

- *Btk* would be the main eradication insecticide, especially for aerial application, with the limitation that it will decimate the lepidopteran population in general. This may temporarily affect some bird life in the spray zone.
- Dimilin (an insect growth regulator) should be considered for ground application to assist in hot spot infested areas.
- Spinosads, new “green chemistry” pesticides are possible agents, but there is relatively little indendepent information on safety available as yet, which would make large-scale urban application difficult.
- LdNPV, such as Gypchek, should be used early in an eradication programme and in environmentally sensitive areas.
- Mass trapping may be a useful addition around the edges of infestations as both a monitoring tool and to trap male GM.

Other agents, such as mating disruption, *E. maimaiga* and parasites are not suitable for eradication alone, but may assist in eradication in conjunction with other agents.

For ongoing control, in the event of the eradication programme failing, we suggest that:

- The nucleopolyhedrovirus LdNPV be introduced as soon as a population is found in NZ.
- The introduction of the fungus *Entomophaga maimaiga* be investigated and if suitable, the fungus be released as soon as practical.
- *Bacillus thuringiensis* could be useful to protect particular areas such as stands of susceptible trees.
- Mass trapping and mating disruption, based on the use of synthetic pheromone, have proven useful in slowing the spread at the front of GM and could form a component of ongoing control and eradication strategies.

TABLE OF CONTENTS

1. GYPSY MOTH

The gypsy moth, *Lymantria dispar* L. (Lepidoptera: Lymantriidae), is a serious pest of forests throughout the northern hemisphere. It attacks over 500 species of flora, including many significant forest trees. In addition to the effects of defoliation on tree health and subsequent loss of wood and indigenous forest areas, gypsy moth has also been an urban pest in many areas. Its urban pest status has not been limited to tree and plant damage; the urticating hairs of the larvae often cause allergic responses in humans. At the height of epidemics, the larvae have even been known to attack grasses. While the native range of gypsy moth extends across much of Eurasia and North Africa, it may have originated in Japan and spread east (Garner and Slavicek, 1996). Although it does not generally kill trees outright, consecutive defoliation or infestations coupled with other diseases may result in the loss of trees. This has been the case in places where pollution or other factors have decreased the ability of trees to withstand repeated defoliation.

Gypsy moth was introduced into the New England region of North America from Europe in 1869 (Forbush and Fernald, 1896). Gypsy moth is thought to have been accidentally released from a colony maintained by Professor L. Trouvelot, who was attempting to breed better silkworm caterpillars by crossing them with GM. The history of gypsy moth as an invasive pest of the USA was reviewed in the book "The Gypsy Moth: Research Towards Integrated Pest Management" (Doane and McManus, 1981). However, much additional research of direct relevance to New Zealand has been completed since this compendium was published. Natural spread of this North American strain has been about 3-5 miles/year, but at times it has been spread much further through human activity. It remains a quarantine pest in parts of the USA and Canada. In many parts of USA it is not considered to be a particularly important forestry or agricultural pest, but it is considered to be an urban pest. In Slovakia, we were told of GM being a problem for oak seedlings, which were completely stripped. This can be very expensive, as it costs between 90-100,000 Sk /ha ($NZ 4500-5000) to plant new forests. Defoliation on trees other than *Quercus* (oaks) may lead to tree death. Presently, GM in Slovakia builds up on oaks then moves readily to vineyards, apples and cherries.

In many countries, the data on outbreaks indicate that for non-flight capable strains, populations reach epidemic proportions about every 6-11 years. Peak populations of gypsy moth occurred Europe in 1993-95. In Slovakia, outbreaks have occurred in 1946-49, 54-56, 63-65, 74-76, 85-87 and 93-95. It has been hypothesized that gypsy moth outbreaks start from "reservoirs" where populations of gypsy moth can always be detected. At present in Slovakia, gypsy moth is restricted to a few focal points, from where the next outbreak will spread. These regions, one in the east at Trebisov and the other in the south-west at Cifare (near Levice), also serve as monitoring sites for the foresters. An increase in the populations here will immediately precede an outbreak.

According to the Slovakian scientists, outbreaks depend on four factors: climate (dry and warm April); host plant quality (*Quercus*) is the preferred host from which GM spreads to other oak and tree species); time since the last outbreak (minimum of four years); and some unknown predisposition of the insects.

Interest in gypsy moth as a pest has recently increased in many countries, due to the recognition of an "Asian" strain with characteristics that increase the potential damage, notably female flight.

The literature on gypsy moth is extensive, and we will not provide an exhaustive treatment of the literature herein. This report is specifically aimed at strategies that would be useful for eradication or control of gypsy moth if it was found in New Zealand. Therefore the emphasis is on methods and agents for control, and on tools and techniques that can assist with predicting and assessing damage potential.

2. ASIAN GYPSY MOTH: BIOLOGY AND DISTRIBUTION

The fear of gypsy moth invading New Zealand, and much of the impetus for overseas research continuing on gypsy moth control, is due to the emergence of an "Asian" strain of GM (AGM). The Asian strain has many characteristics that make it a potentially more devastating pest than the strain defoliating North America and Europe at present. The females can fly (which European and North American strains cannot), are attracted to light, have a wider host range, are generally larger and have a higher premature eclosion. Eggs of AGM require less exposure to low temperature to hatch and hatch over a longer period (Keena, 1996), which increases the chances of introduction to new environments. Some of these characteristics have enabled AGM to survive transport across the equator, increasing the areas at risk from GM invasion including New Zealand. While the biology of the European gypsy moth (EGM) makes it predictable in its dispersal pattern, AGM exhibits traits that will make its distribution much more difficult to predict. Table 1 compares the differences and similarities of the two ‘strains’.

TABLE 1: Comparison of European/North American gypsy moth with the Asian strain

Life Stage	EGM/NAGM	AGM (USSR)
Adult male	Strong flier	Strong flier
	Attracted to pheromone	Attracted to pheromone
Adult female	Flightless	Strong flier (>30km)
		Attracted to light
Larvae	1st instars disperse	1st and 2nd instars disperse
	Uniform colour	Highly variable colour
	Larvae feed in canopy at night and move to resting sites during day	Larvae feed at night and remain on host during day
	Late instars use artificial resting locations when they are provided	Late instars use artificial resting locations when they are provided
Hosts	Oak, birch, poplar, willow and alder	In excess of 600 species identified including conifers and hardwoods[7]
Egg masses	On tree boles, rocks and litter	On foliage, tree boles, rocks and virtually any object associated with lights
Egg phenology	<5% premature eclosion[1]	25% premature eclosion
	Full eclosion usually completed in one season	Full eclosion may take several years if eggs survive
	Vernalisation required	Vernalisation not required
Causes of mortality	NPV[2], *Bt*[3], Fungus[4], 11 parasites (mostly univoltine[5]) and various predators	NPV, *Bt*, Fungus, Microsporidia, numerous parasites and predators

[1] Eclosion = egg hatch.
[2] NPV = Nucleaopolyhedrovirus.
[3] *Bt = Bacillus thuringiensis* is the primary bacterium commercially available for control of insect pests.
[4] Fungus = invasion of insects by fungi results from a spore landing on the cuticle of the insect. The germ tube then penetrates the cuticle directly.
[5] Univoltine = one generation per year.

Until recently, it was believed that all gypsy moths on the North American continent originated from Europe. During 1990 and 1991 a new ‘strain’ of gypsy moth was found in British Columbia and in Oregon and Washington states. This phenotype exhibits differences in reproduction, dispersal and host range.

AGM is found north of 30°N latitude until it reaches the far northern latitudes where taiga or boreal forest begins. In its natural habitat irruptive populations are recorded as occurring every 6-7 years (Schaefer *et al.*, 1984). It is univoltine where it is indigenous. Schaefer *et al.* (1984) record differences in the phenology of AGM with latitude. Although it remained univoltine in the lower latitudes, the time of egg eclosion and adult flight became progressively earlier. This indicates that the AGM has great plasticity in its response to environmental cues. If it entered New Zealand, in the absence of natural enemies and the presence of a mild oceanic climate, it may become multivoltine (several generations per year).

The phenology of the eggs may also be a cause for concern. EGM demonstrate a skewed distribution in egg eclosion (Waggoner, 1984) but all of the eggs are usually hatched or unviable after the first year. They also require vernalisation (a cold shock) before development can be completed. The information on AGM is mainly anecdotal but what we do know is that 25% of the egg mass can hatch during the first year without vernalisation. If the eggs survive to a second year, which is quite possible, then a further 25% of the original egg mass can eclose; after that egg hatch becomes continuous (D. Bridgwater, pers. comm., 10/1992; Humble pers. comm., 4/1992). It is obvious from this information that the AGM eggs can survive for extended periods of time.

Monitoring AGM can be difficult in areas where EGM is also present. The most effective monitoring technique is pheromone trapping. Gypsy moth sex pheromones only attract males of the species. Although a process based on larval head capsules shape has been described (Wallner *et al.*, 1994), no rapid or easy method for morphological distinction has been developed for most life stages, especially males. A method for molecular identification of male GM strains has been developed (see section below) but requires more widespread testing. Fortunately, in New Zealand EGM is not present and has not arrived from Europe or North America. This is mainly due to its mode of dispersal, which is not as effective as AGM for covering long distances. The preference of AGM for lighted areas makes it more likely to ‘hitchhike’ on modern modes of transport such as planes and ships. In the event of AGM entering NZ, either as egg masses or ballooning larvae, pheromone trapping combined with DNA screening should be able to confirm the strain of GM.

It has been observed that indigenous forests in China and eastern Commonwealth of Independent States (CIS) are subject to cyclic irruptive populations of AGM. During 1990 and 1991 the populations of AGM around the ports of Vladivostok, Vostochny and Nakhodkha were very high. Increased trade between the CIS and northwest America presented an opportunity for the pest species to be transported into areas where it could become established. New floodlights were installed in these ports to allow ships to be loaded

---

[6] Microsporidia are protozoan pathogens of gypsy moth, which are ingested and slow-acting.

[7] Of the many *Pinus* spp. AGM is capable of attacking, *Pinus radiata* is a favoured species for oviposition. In addition, eucalypts in Asia have been defoliated by large populations of AGM (R. Wylie, Queensland Forest Service, pers. comm.).

and unloaded at night. Because AGM is positively attracted to light, many of the ships in port were oviposited on by female AGM attracted to the area. It is postulated that this is the reason AGM was transported to the U.S. during this time of high population. One ship entering an American port yielded enough eggs to "fill an oil drum". Merely having egg masses transported into port is not enough to allow the moth to become established. Eggs must also hatch at the appropriate time during transportation to allow the possibility of young larvae ballooning to shore on silk threads. If the eggs had been on board for an extended period of time then continuous hatching may occur.

Forest health staff originally thought that the risk of AGM entering the southern hemisphere by ship was low. The fact that shipping passed through the tropics led MAF to believe that all eggs would hatch before they arrived in New Zealand or die because of high temperatures. Recent information from the U.S. indicates that this assessment was incorrect. A ship examined off the coast of Florida was found to have three egg masses on it. This ship must have spent an extended period of time in tropical waters to get to Florida from an infested area. Max Ollieu (Forest Pest Management, Pacific North West) has said that ships have spent in excess of one year in the tropics and arrived in northern ports with viable egg masses. Extrapolating from this information, any ship that has been in port near an infested area during the previous two years must be regarded as a potential risk.

There was also a problem with port inspectors in the CIS. The level of training was not as high as in many western countries and ship hygiene was not perceived as a priority for vessels leaving port, so the possibility of egg masses being observed before departure was low in these areas. Even ships that had been inspected and passed by the CIS authorities were found by U.S. Inspectors to be infested (Gypsy Moth Exotica 1992). However, recent experience indicates that CIS inspection may have improved. The New Zealand National Institute of Water and Atmospheric Research (NIWA) has leased a CIS research vessel to carry out surveys around the NZ coast. This vessel had been in the port of Vladivostok for a period of eight months and the then Ministry of Forestry (MOF) expressed some concern about it. On the 23 March 1993 this vessel was requested to heave-to 5-8 km off the coast near Tauranga. In the company of MAF staff, a co-author of this report (P.J. Walsh) carried out an inspection of the ship. The ship had been inspected twice before leaving Vladivostok and the crew were aware of the problem and had carried out further inspections en route to New Zealand. No egg masses were found. This increased awareness combined with the fact that the cyclic irruption of AGM around eastern Siberian ports is on the wane reduces our risk rating for these areas. A watching brief is recommended and targeted ships, i.e. ships that have been in port in the infested area up to two years ago, will continue to be inspected before landfall.

The threat of infestation from North America is ambiguous. If AGM is established there then the risk of it entering the southern hemisphere is greatly increased. The information being conveyed after control measures were implemented in North Amercian infested areas is contradictory. Pheromone trapping and molecular analysis (DNA) of the specimens indicate that AGM is no longer present. These data must be tempered by the caveat that only 20% of the individuals captured have been analysed. Unsubstantiated reports from Vancouver have noted AGM flying around the port.

Greater trade links with this part of the world increase the opportunity for accidental AGM introductions. The ability of the AGM egg masses to withstand very low temperatures would allow the moth to be transported to New Zealand by plane. This type of risk did not have to be considered by the Americans when dealing with the CIS, as aerial links are very rare between these areas.

AGM in Japan has been mentioned in several inquiries to Forest Health staff. Defoliating outbreaks of gypsy moth have been recorded for many years in Japan, mainly in Hokkaido. The Japanese gypsy moth is considered to be an indigenous sub-species, *Lymantria dispar praeterea* Karadakoff (Inoue, 1982), and appears to have a more restricted host range (Higashiura, 1989). Gypsy moth in Japan is also subject to destruction of egg masses by a fungus, *Entomophaga maimaiga*. This fungus has been tested for efficacy in the U.S. and during periods of high humidity caused very high mortality of egg masses (News Brief, Journal of Forestry, 1993).

In Canada, AGM egg masses were discovered in the Vancouver port, with one ship having over 3000 egg masses. This prompted a massive eradication campaign in 1992 using the bacterium *Bacillus thuringiensis kurstaki* (*Btk*). However, whether the eradication was successful remains a point of contention. In 1991 AGM (and hybrids of AGM and EGM) were discovered in North Carolina. This also resulted in a massive eradication programme using several biological control agents, mainly *Bacillus thuringiensis kurstaki,* and the infestation declared eradicated in 1997 (USDA, 2003).

Introduction into North America and on ships in New Zealand ports occurred because gravid females deposited eggs on ships and containers. Female AGM may fly up to 100 km (Wallner *et al.*, 1995). It has been shown that in northeastern China females are attracted to lights, where they deposit their eggs. Efforts have been made to reduce the risk of AGM at the port of loading. For example, Wallner *et al.* (1995) showed that modifications to the wavelength of lights can result in lack of attraction of three *Lymantria* pests (nun moth, pink nun moth and AGM). Filters that blocked spectral emissions <480 nm were unattractive to AGM when used at a far east Russian port. Wallner *et al.* (1995) also found differences between the daily flight activity for all three species. Peak activity for AGM was 2300 to 0100 hrs.

Dr Keena (US Forest Service, pers. comm.) is looking at several behavioural and physiological factors that make the AGM more of a potential threat than NAGM. Although results are still being analysed, it appears that F1 hybrids lose flight ability and about 30% of F2 backcross females can fly. Dr Keena thinks that this number may be the average already found in German hybrid populations. F1 hybrids had similar diapause chill requirement, similar size and wing length and developed slightly faster than AGM. Therefore little dilution of AGM population is expected with mating between the races. Dr Keena also found that there is more of a continuum of characteristics amongst GM than just two distinct (NAGM and AGM) races.

In Europe, the spread of AGM is being closely monitored. In discussions with European researchers, notably Dr Julius Novonty (Slovakian Forest Research Institute) and scientists at the BBA, Darmstadt (Germany), said GM capable of flight have been found in Poland, Germany, Northern France and Lithuania. None have been found in Slovakia, Austria, Croatia, Bulgaria, Switzerland, Portugal or Southern France. AGM is still a quarantine pest in some areas of Europe, such as Slovakia.

In comparison with EGM, AGM causes more defoliation, develops faster, grows on marginal hosts, has greater genetic variability, is capable of extended strong flight, prefers to fly before egg laying and has shorter diapause-chill periods. This latter trait may result in multiple generations/year as non-diapausing strains can develop (Hoy 1977).

Given all of the above information, an assessment of the risk to New Zealand from AGM is the same as any of the pestiferous species, e.g. *Hylobius abietis* from Europe, entering our ports. We should remain vigilant but not concentrate all our resources in one area while some nasty little beetle crawls across the dock to infest our plantations.

A summary of the actions initiated to date are:

1. MAF are checking containers, cars and other sources of potential carriers of AGM egg masses.

2. Ships known to have been in infested areas are targeted for inspection.

3. MAF in collaboration with Customs now have the power to order a ship to heave-to 8km from the NZ coastline. This includes offshore islands.

4. If a ship is found to be infested it will not be allowed into New Zealand ports for two years.

5. Before an infested ship is expelled from port, the egg masses should be examined for signs of hatching and live larvae. Records of wind direction/speed during the ship's approach to New Zealand should be taken to ascertain the most likely area of landfall for ballooning larvae. A predictive model using wind speed, direction and landform is available for this purpose.

6. Trapping around New Zealand using pheromone traps has been conducted for several years.

3. HOST PLANT SUSCEPTIBILITY TO GYPSY MOTH

The preferred hosts for GM are oaks, apple, sweetgum, speckled alder, basswood, birch, popular and willow. However, GM larvae are polyphagous and they will feed on almost any plant when the population is sufficiently high (USDA, 1995). A list of over 600 species and their susceptibility to GM is reproduced from the draft environmental impact statement on Gypsy moth management in the United States (USDA, 1995) in Appendix 14.1.1.

Much has been made of the increased risk of AGM over other strains due to a wider host feeding range. Dr Michael Montgomery (US Forest Service, Hamden CT) has compared the plant host range of AGM and NAGM. There are some problems with doing this type of study, such as getting the appropriate stage of plants, but some interesting results have emerged. About 40 plants/trees were tested and the general trend was for the two races to be similar in tastes. However, AGM grew faster and survived better than NAGM on most host plants (Appendix 14.1.2). It is thought that a host species immune to one race is likely to be immune to the other. Feeding trials with New Zealand flora have now been completed (Kay *et al.* 2002 - Appendix 14.1.3). These trials shows a smilar response to those found by Montgomery (Appendix 14.1.2) in terms of relative host acceptance by EGM/AGM, but the field collected AGM was more 'robust', than the 9th generation laboratory cultured Beijing strain.

4. MODELLING GYPSY MOTH IN NEW ZEALAND

Barlow *et al.* (2000) (Appendix 14.5) have examined population density data and produced a model which predicts the spread of both European (nonflighted) and Asian (flighted) strains in New Zealand. The model suggests subtle differences in the long-term behaviour of Asian and European strains of gypsy moth under New Zealand temperatures, in which the outbreak cycles become asynchronous. The Asian strain is predicted to increase more rapidly at first, and then to maintain more widely fluctuating densities and more pronounced outbreaks than the European strain, given the presence of predators functionally equivalent to those elsewhere in the moth's range.

If gypsy moth establishes in New Zealand in the absence of the predators that characterise it elsewhere, then the model predicts that outbreak behaviour will not occur. There may be initial outbreaks over the first few years but these will rapidly damp to a more stable population, whose mean density may be similar to that in the presence of predators but in which damaging outbreak densities will no longer be realised. Theoretically, permanent outbreak cycles cannot be generated through an interaction involving only a herbivore and its resource; a third agent is required, such as predators, parasitoids or disease. In practice, should fluctuations due to weather disturb the predicted equilibrium in the absence of predators to the extent that densities reach damaging levels, then the model predicts that the immediate effect of a spray is similar to the behaviour in the presence of predation (as described above).

5. RISK ASSESSMENT FOR NEW ZEALAND

In New Zealand, there are major concerns about the introduction of GM. Several ships in New Zealand ports have been found to have viable egg masses and a live male in a Hamilton-based trap. These concerns are heightened as the eggs were of the more virulent Asian strain (AGM) not the North American strain (NAGM).

The finding of viable AGM egg masses on ships in New Zealand ports has raised awareness of the danger of this pest reaching our shores. Risk assessments by the then Ministries of Forestry (MOF) and Agriculture (MAF) and the Forest Research (FR) concluded that it is almost inevitable that gypsy moth will reach New Zealand and that it would be a devastating pest (Cowley *et al.* 1993; Horgan, 1994). A more recent assessment has been made by Kay *et al.* (2002) based on climatic and host preference. They found it was difficult to use climate matching with the programme CLIMEX to fully model gypsy moth population spread in the Northern Hemisphere and occurrence of oak, the preferred host plant was an important factor in colonisation. Gypsy moth found many New Zealand native trees unpalatable, even though related Northern hemisphere species would support gypsy moth survival and growth (Kay *et al.* 2002). Despite the reduced number of species which would be affected by gypsy moth, it is still considered a threat to New Zealand, especially to naturalised Northern Hemisphere species.

The risk assessments carried out for New Zealand have examined only the pathways by which GM could be introduced into this country. The requirements for establishment of GM in this country are not fully understood. Although a suitable climate and the availability of known host trees indicate that the prerequisites for establishment are present in this country, the threat to New Zealand is not clear. No host range testing has been performed for this insect using New Zealand grown trees. Feeding trials in the US indicate that GM can complete its life cycle on eucalypts and some pines. This is not a measure of threat, merely of risk of establishment or biological survival probabilities.

A measure of relative performance on different species of tree will allow the population model (see next section) to become more robust. Even if a particular tree grown in New Zealand is found to be a good host, i.e. it produces adults with high fecundity, it is still necessary to place this knowledge within the framework of management regimes and the climatic zones within which the particular host tree is grown. It has already been mentioned that in Slovakia outbreaks are centred on woodlots containing *Quercus*. This does not mean that *Quercus* is the only good host for GM, merely that it is grown in sites where a combination of factors contribute to the build up of a population over time. These factors include host quality, time, local climate and possibly changes in dominant genotypes in local populations. It would be necessary to place the results of any gypsy moth feeding and performance trials in a similar context for New Zealand.

In the case of gypsy moth introduction into New Zealand, an environmentally sound method of eradication and/or control will be required. As the pest has been in the USA and Canada for over 100 years, there is a lot of research and practical control experience available.

Risk assessment of control agents is a complicated issue. It not only involves the direct threat to non-target organisms, but also possible food chain effects from loss of an insect species which may be a food source for another organism. In addition, when considering the risk of pathogens, the effect of defoliation by GM on non-target insects needs to be weighed up against the effects of pathogens on non-target insects.

6. INTERCEPTIONS, DETECTION AND DELIMITATION OF POPULATIONS: MONITORING GM POPULATIONS IN NEW ZEALAND

The first egg masses of AGM on a ship entering a New Zealand port were found in 1993. Due to increased awareness and surveillance, during the years since the first interception, there have been multiple discoveries of egg masses of GM on ships and containers. In one case a dead larva was found on an imported car. A gypsy moth detection programme utilising pheromone traps has been initiated in New Zealand at 11 ports. The density of traps at each port is 2/km^2 in a 7 km radius around the ports. Figure 1 shows interceptions of viable egg masses of GM in New Zealand. The trapping programme started in 1993, and traps are inspected from 1 November to 30 April each season, once every ~10 working days. For the 2002/03 season, 1066 traps were placed around ports and high risk sites throughout New Zealand, including 17 traps placed around Hamilton City.

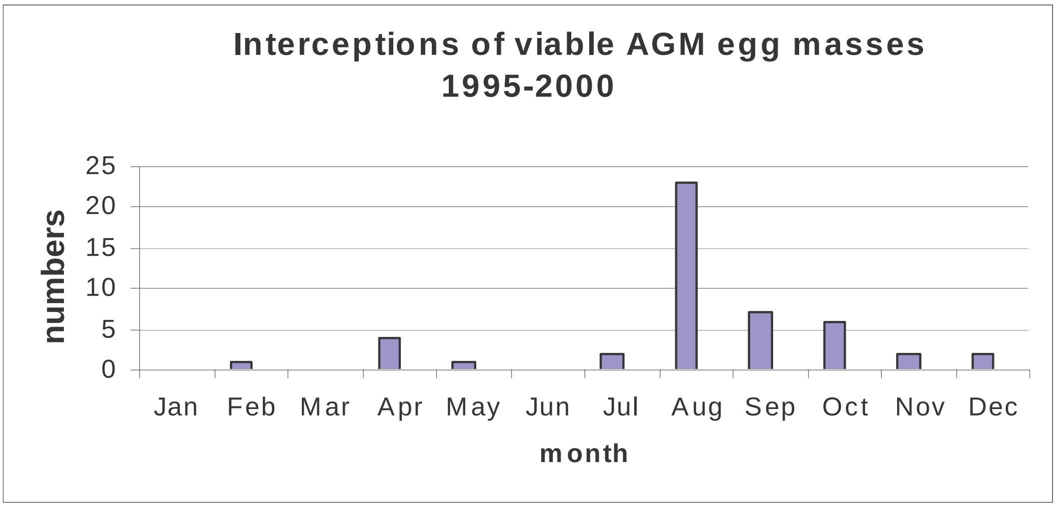


Figure 1: Interceptions of vaiable egg masses in New Zealand (from Kay *et al.* 2002-Appendix 14.1.3).

The decision to trap within a 7 km radius of the ports is based on a recommendation made by the United States Asian Gypsy Moth Science Advisory Panel in 1993. A trap density of 2/km^2 is the absolute minimum that may be used for detecting an onshore population of gypsy moth. Estimated probabilities for **NOT** detecting a population of 10, 100 and 1000 gypsy moth males are given in Table 2. This is based on work done in the United States (Elkinton and Carde, 1980).

TABLE 2: Probabilities of NOT detecting a gypsy moth population at different trap densities.

	Gypsy moth numbers (males)/km^2		
Traps/km^2	10	100	1000
2	91%	41%	0.01%
10	51%	0.12%	6.48×10^{-28}%

As can be seen from Table 2, detection trapping gives a minimal probability of detecting a population unless the insect is there in good numbers. This emphasises the need for good offshore quarantine protocols to prevent the insect reaching New Zealand shores.

To this end the Ministry of Forestry (now MAF) sent quarantine staff to the areas of perceived highest risk, the Russian Far East ports of Vladivostok, Vostochny and Nadhodka. MOF staff studied the measures taken by Russian authorities to ensure the ships were clear of egg masses. These measures were approved and ships are certified to be clear of infestation at their port of departure. If a ship enters a New Zealand port from a high risk area, a certificate of cleanliness is required before it is allowed to dock. Even then it is inspected for egg masses. If the ship does not have a certificate of cleanliness it is required to heave to 8 km offshore where it is inspected by a team of MOF, MAF and FRI staff. This distance offshore is based on knowledge collected for the Asian gypsy moth Pest Risk Assessment (Cowley *et al.*, 1993).

If a moth is trapped onshore then it is imperative that the population is delimited. Delimitation has already been mentioned in this report in terms of the barrier trapping utilised by Slovakian scientists to determine where their main populations of gypsy moth are. Delimitation for a pest incursion is somewhat different as it is important to clearly define where the population is and get an estimate of local moth numbers.

It is known that gypsy moth males seldom fly more than 800 metres and the minimal trap density of 2 traps/km^2 is based on this knowledge. Combined with the fact that the best pheromone dispensers have an attractiveness of 1200 metres, intertrap distance is important in finding and delimiting populations.

In the event of a male moth being trapped onshore an immediate deployment of traps for delimitation should be undertaken. The following figures are based on the moth capture being the centre of the delimitation.

- 16 traps/km^2 should be deployed in a 2 km radius
- 8 traps/km^2 should be deployed in the 2-4 km radius
- 2 traps/km^2 should be deployed in the 4-7 km radius

If an eradication programme is implemented, trapping should take place for two years in the infested zone after eradication has been attempted. High density trapping should be maintained in the known areas of infestation. After two years with no moths being caught it may be safe to announce successful eradication.

In 2003, a single male GM moth was caught in a surveillance trap in Hamilton. A delimitation and search survey was initiated immediately by MAF and a technical advisory group established. The trapping programme deployed pheromone baited traps at the following densities within three specified zones:

Zone 1: 0 to 2 km radius: 16 traps per square km
Zone 2: 2 to 4 km radius: 8 traps per square km
Zone 3: 4 to 7 km radius: 2 traps per square km

As a precaution additional traps have been placed alongside main arterial routes in and out of Hamilton to a 30 km radius (Appendix 14.9). Ground surveys have also been conducted. At the time of writing, three reports at weekly intervals on the search for any GM had been issued, covering the response to date (Appendix 14.9).

7. MOLECULAR IDENTIFICATION OF STRAINS

Much of the risk assessment and subsequent planning in New Zealand for gypsy moth invasion has been based on the Asian strain, which has been characterised overseas as having a number of potentially more damaging traits than the European or North American strains (see section 2). However, rapid identification of the Asian strain from European or North American flightless strains can be difficult at the morphological level. Larvae posses inconsistent differences in body coloration (Keena, 1994) and can be identified on the basis of head capsule coloration only by experts (Wallner *et al.*, 1995). However, pheromone-trapped male moths may pose problems (Garner and Slavicek, 1996). Obviously, rapid response will depend on rapid strain identification. It may also be useful to determine the country of origin of any GM insects found in New Zealand.

Molecular biology has a range of techniques which have been used to show species and subspecies variation in many different insects. As these techniques examine DNA directly, larvae and adult stages can be treated the same way, which also allows larval identification, not possible using morphological techniques. In New Zealand, molecular techniques have been developed by Karen Armstrong and colleagues (Armstrong, 2000; Armstrong and Cameron, 2000; Armstrong *et al.,* 2003) which allow rapid identification of gypsy moth and other lymantriids. These will be invaluable in rapidly determining the species of larval and even adult interceptions.

Several studies have attempted to use molecular techniques to identify strains of gypsy moth. Early attempts to apply molecular identification to gypsy moth focussed on mitochondrial (mtDNA) markers (e.g. Harrison and Odell, 1989; Davis *et al.*, 1994; Bogdanowicz *et al.*, 1993). Mitochondrial markers were thought to be excellent markers for determining the history of colonisation and introduction (Harrison and ODell, 1989). The evidence of mtDNA suggests ancient divergence between Asian populations of gypsy moth. Although sequencing of mitochondrial regions allowed separation of the strains, the difficulty in isolating mtDNA and the maternal inheritance of mtDNA, which results in lack of detection of hybrids (Pfeifer *et al.*, 1995; Bogdanowicz *et al*., 1993), reduced the value of this approach. It is possible hybrids could be introduced into New Zealand rather than a pure lineage.

Pfeiffer *et al.* (1995) used amplification of ribosomal DNA (rDNA), specifically the ITS2 region to investigate differences between strains. They found that by using the specific polymerase chain reaction (PCR) primers HITS1 and LITS2 to amplify ITS2 along with subsequent restriction enzyme digestion, North American, Asian and hybrid strains could be detected in blind tests. Furthermore, other *Lymantria* species could also be distinguished.

Garner and Slavicek (1996) used RAPD-PCR to identify polymorphic DNA between the two strains and used locus-specific primers to amplify a marker, designated FS-1 which could be used to distinguish between the two strains. This technique is sometimes referred to as sequence-characterised amplified regions or SCARs. The specific primers used to amplify FS-1 resulted in detection of marker fragments for Asian, hybrid and North American populations. However, small percentages of hybrid alleles were found in North American populations (Garner and Slavicek, 1996), where there is no evidence Asian strain has ever established (Melody Keena, pers. comm.). Also, in some east Europe populations, FS-1 linked to the flighted Asian strain has been detected in populations where flight has never been observed (Julius Novonty, pers. comm.). The estimate by Garner and Slavicek (1996) was 96% accuracy for FS-1. Overall, Garner and Slavicek (1996) conclude that the two strains are genetically very similar, contributing to the paucity of observed useful genetic markers. Schreiber *et al.* (1997) identified a further three RAPD markers specific for either Asian or North American L. dispar. The FS-2 and FS-3 markers are specific for the North American strain, and the FS-4 marker is specific for *L. dispar* from far east Asia. The 3 RAPD-amplified fragments were cloned and sequenced, and locus-specific primers were developed for the FS-3 marker. These markers, in combination with other markers already available, will be useful for increasing the accuracy of identification of suspect moth specimens. While molecular methods are constantly under development, no method is yet thought to be 100% accurate in detecting Asian, hybrid and other strains, possibly because the characteristics of each strain are not related directly with phylogeny. The methods of Bogdanowicz *et al.* (1993) and Schreiber *et al.* (1997) are currently used for quarantine analyses by the USDA to determine strain of GM.

In New Zealand, the nuclear rDNA and RAPD markers, as used by Pfeffier *et al.* (1995) and Garner and Slavicek (1996), were tested with samples of gypsy moth from Slovakia, the far east and USA (Appendix 14.6). Glare *et al.* (unpubl. data) targeted the ribosomal DNA (rDNA) region as it is a repeating sequence, and therefore has a high copy number. This makes it easier to amplify this region using PCR. In addition, rDNA is of fundamental importance for making proteins and so is highly conserved. Amplification of rDNA of the ITS1 region and subsequent digestion by the enzymes *Hae*III and *Tru*91 allowed distinction between gypsy moth strains (T.R. Glare, N.K. Richards and D.G. Hall, unpubl. data). This was similar to the results of Pfieffer *et al.* (1995) after amplification of ITS2. ITS1 was amplified using a reverse HITS1 primer of Pfieffer *et al.* (1995) and TW81 of Curran *et al.* (1994). Molecular techniques currently exist that have a high probability of distinguishing Asian and non-flight strains, although unambiguous markers have yet to be found. Analysis of ITS1, as in Appendix 14.6, needs to be performed on a much larger sample of gypsy moth before its usefulness can be judged. This work is being extended to include sequencing both ITS1 and the CO1 gene of mtDNA.

8. CONTROL AND ERADICATION: OVERSEAS EXPERIENCE

The history of control attempts in the USA date from about 1890, when the gypsy moth population exploded in Medford, Massachusetts. Common methods of control were destroying egg masses, burning infested bushes, banding around trees to trap caterpillars or spraying with insecticides. Lead arsenate was a common chemical control after 1893 (McManus and McIntyre, 1981). Biological control was initiated early in the century with the importation of natural enemies, primarily parasites. These failed to stop the spread of gypsy moth. Importing control agents was neither simple nor inexpensive. Some agents established but did little, some refused to breed and others vanished without a trace after release (USDA, 1995). Chemical methods were used extensively in 1920s and 1930s due to the need to reduce highly visible populations. Forest spraying however proved ineffective. In 1923, the USDA established a barrier zone along the Hudson River, where infestations to the east were to be controlled and those to the west were to be eradicated. This general philosophy has been maintained, however the barrier zone has slowly shifted west with the moving gypsy moth front. Spot populations in the western USA are still routinely eradicated.

In 1940-1950 DDT was the chemical of choice for GM control. Eventually around 1960, environmental concerns and pressure from the public made the use of DDT impossible. Subsequently, chemicals with less widespread action were routinely used and replaced by the next generation insecticide until the latest generation of specific insecticides, such as insect growth regulators (e.g. Dimilin).

The move from chemicals back to biological control has been steady since the 1960s. A variety of natural agents are known to kill gypsy moths. These agents include over 50 insect parasitoids and predators in Asia and Europe. Small mammals are often important gypsy moth predators, especially at low population densities. Birds are also known to prey on gypsy moths but at least in North America this does not significantly affect populations. A nucleopolyhedrovirus (NPV) often causes the collapse of outbreak populations and recently an entomopathogenic fungus species has caused considerable mortality of populations in North America.

In addition, the bacterium *Bacillus thuringiensis* produces toxins which include strains effective against gypsy moth. *Bacillus thuringiensis* (*Bt*) is the most widely used biological control in the world for insect pests. Research on the use of *Bt* and the NPV of GM began in the USA in the 1970s and has continued since. This has been supported by the move to control methods with less effect on non-target organisms. In the USA, three approaches are taken to GM, depending on the area of infestation: eradication, suppression and slow-the-spread. From 1980 to 1993, eradication was attempted at over 380 sites and over 550 individual eradication projects were carried out. In addition, eradication has been attempted with AGM introductions in both the eastern USA and Vancouver (see section 2).

A cautionary note regarding control attempts is sounded by the fact that around 1900, control efforts in Massachusetts were considered so successful, the programme was abandoned. By 1906, gypsy moth had spread so far that eradication was not possible (USDA, 1995).

9. CONTROL AGENTS

Broad spectrum chemicals, widely used for GM control before 1970, have largely fallen out of favour. This has been caused by many factors, most notably the unacceptable environmental damage caused by non-specific chemicals and the recognition of many parasites, predators and pathogens capable of naturally reducing GM populations. Presently the most widely used "chemical" control appears to be diflubenzuron

9.1 Diflubenzuron

Diflubenzuron (Trade name: Dimilin) is an insect growth regulator that interferes with the synthesis of chitin. The treated larvae cannot complete moulting, its body wall ruptures from internal pressure, and eventually it dies. In the USA and parts of Europe, Dimilin has been used in suppression projects by means of ground or aerial application. Two formulations are available, Dimilin 25W, a wettable powder containing 25% diflubenzuron, and Dimilin 4L, a liquid containing 480 g/l diflubenzuron. In the USA, forestry use was removed from the 25W product label, leaving only 4L for use against GM (USDA, 1995). Recommended dose rates are 7-28 g diflubenzuron /4.67-23.34 l/ha . In the USA, no more than two applications are allowed per year and there is also a restriction on total active ingredient applied in any year (USDA, 1995). It is usually applied twice by air for eradication projects. Reduced dose rates have been used successfully in suppression projects (USDA, 1995 and references within).

According to some studies, diflubenzuron can give effective control of GM. It had a 98% success rate in six states in the USA during 1989-1990 for foliage protection and 80% reduction in populations below 500 eggs mass per acre (Twardus and Machesky, 1992; USDA, 1995) Between 1990 and1994, 40% of GM treated area was with Dimilin.

There are, however, negative aspects of diflubenzuron use. It can have an adverse effect on non-target organisms, such as arthropods and aquatic crustaceans. For example, Schaaf and Vogt (1995) investigated the side effects of Dimilin on non-target organisms in spray trials against GM. Larvae of *Chrysoperla carnea* (Neuroptera, Chrysopidae) died after exposure to leaves sprayed 4 months earlier and the number of species of Collembola were reduced in the soil from treated areas. However, the population density of mites (Prostigmata, Oribatida, Mesostigmata) was higher in the treated soil due to lower interspecific competition. The USDA review of ecological risks of control options stated that diflubenzuron affects far more non-target species than other control methods, and for a longer time after spraying. The effects can also be downstream, affecting small mammals and birds dependent upon a range of invertebrates for food. Frequent applications, not recommended in the USA, would increase the residue risk.

9.2 Spinosad

Spinosad is one the so-called “green chemistry” pesticides recently developed. It is based on the metabolites metabolites (spinosyns), produced during fermentation by the actinomycete *Saccaropolyspora spinosa*. This family of unique metabolites was identified and developed by Dow AgroSciences as a selective, environmentally-friendly insecticide known as spinosad or Naturalyte. Products are composed of a mixture of spinosyn A and spinosyn D. Spinosad works by both direct contact and ingestion by insects and kills rapidly. There are a number of products based on spinosyns available from Dow Agrosciences. Tracer-Naturalyte™, Success-Naturalyte™, SpinTor-Naturalyte™ and Conserve™ SC are the four main products currently available commercially; these are formulated for different agricultural, ornamental and forestry applications against various pest insects. Gypsy moth is one of the label listed targets of Success™ and toxicity for gypsy moth has been demonstrated in the field by Wanner *et al.* (2000), showing similar activity and persistence to the insecticide permethrin. Success™ is registered as an insecticide in New Zealand. However, in considering Spinosad as pesticide for use against painted apple moth, O'Callaghan and Glare (2001) found only limited independent literature supporting the environmental safety, making it difficult to suggest these products for use in eradication. In general, spinosad products have few impacts on beneficials, for example, predator species, but non-target safety has, perhaps, been overstated in some reports and product information. Direct toxicity has been found to bees and certain parasites, but there is little data on many non-target organisms and few ecosystem level studies. The USA EPA allocated spinosad very low mammalian toxicity ratings. It is possible that spinosads will prove to be important environmentally friendly pesticides in the future, but further independent data on safety will be required for any highly public eradication campaign.

9.3 Bacillus thuringiensis kurstaki

Bacillus are spore-forming bacteria, typically rod-shaped. *Bacillus thuringiensis* (*Bt*) form a protein crystal next to the spore during sporulation, which is a toxin to many insect species. The first attempts to use *Bt* for insect control were, coincidentally, against GM, in the late 1920s in the USA. It is produced by liquid fermentation. Strains of *Bt* are now used in several biological insecticides and probably account for 95% of all products based on microbes sold for pest control.

9.3.1 Strains

Many different strains of *Bt* have been isolated throughout the world which are toxic to a variety of insect and other species. A lepidopteran-active strain, *Bacillus thuringiensis kurstaki* (*Btk),* was isolated in 1962 and is lethal to the GM larval stage (Reardon *et al.*, 1994). It is the most widely used control agent for GM in the world and has been used in the eradication campaigns in the USA and Canada. It is also registered as a control agent in New Zealand, mainly for use against leafrollers, and has been used in the eradication programme for tussock moth in Auckland.

The most common isolate is HD-1, originally from the pink bollworm (Reardon *et al.*, 1994). This strain is used in Dipel and Foray, *Btk* based insecticides often used against GM. There appears to be no difference between the susceptibility of AGM and other GM to *Btk* (Norman DuBois, pers. comm.).

9.3.2 Disease cycle

The crystal of *Btk* is a bipyramidal protein matrix of large molecules of inactive proteins, which are not toxic to the insect until solulised in the gut of the insect. Therefore, *Btk* must be eaten to be effective and the protein solulised by the gut to act. Unlike most insect pathogens, *Bt* does not recycle in a population very effectively and is more commonly used as a single treatment "chemical" for knockdown. Although the cells can multiply in the insect haemolymph, it does not usually reach high numbers and after death does not rupture the insect integument. Infected insects usually fall to the ground, and the toxins are quickly inactivated by normal environmental soil factors (Reardon *et al.*, 1994). Although spores can survive in soil for extended periods of time, natural epizootics caused by *Bt* have never been observed in GM populations. Because it does not recycle effectively in insect populations it must be applied each year.

9.3.3 Standardisation

A difficulty with *Bt* has been standardising the method used to express efficacy. Initially a spore count was used, but since the spores are not directly related to toxins, there was often little relationship to mortality. An international unit devised by the Pasteur Institute was accepted by most in 1966, based on *Bt. thuringiensis*. Depletion of the material used as a standard led to this being altered. A second standard, based on *Btk* HD-1 was accepted in 1972 (Reardon *et al.*, 1994). Potency of *Bt* is determined by parallel bioassays of a product and standard on artificial diet with 4 day old cabbage looper, *Trichoplusia ni* larvae (Beegle *et al.*, 1986). The formula is

$$\frac{LC_{50}\text{ (HD-1-S-1980)}}{LC_{50}\text{ (product)}} \quad \text{X } 16{,}000\text{ IU/mg} = \text{potency of the product in IU/mg}$$

where IU = International Units.

9.3.4 Host range

There is extensive literature on the specificity of *Btk* showing that it does not pose a danger to non-Lepidopteran insects (see review in Glare and O'Callaghan, 2000). It has also been extensively safety tested for mammalian toxicity, with no effects noted (e.g. O'Callaghan and Glare, 2002 and references therein). This was confirmed in New Zealand during the Tussock moth eradication programme, where a suspension of *Btk*, Foray 48B, was used (Hosking *et al.*, 2003).

Btk is relatively non-specific among Lepidoptera. A study by Peacock *et al.* (1998) using laboratory bioassays found significant mortality in 27 of 42 species tested using Foray 48B. Interestingly, it was difficult to predict which species would succumb, as species within a single genus showed both susceptibility and resistance. However Johnson *et al.* (1995) examined non-target effects of *Btk* and thought that, although the bacterial strain would kill many lepidopteran species in the laboratory, the short half-life in the environment would limit dangers to non-target Lepidoptera.

Some field studies have shown that species diversity, as expected, reduces after spraying of *Btk* (Miller, 1990; Sample *et al.*, 1993). The flow-on effect of such reductions in general Lepidoptera on birds must also be considered (Peacock *et al.*, 1998). Although its specificity to Lepidoptera is preferable to a broad spectrum insecticide, the death of non-target larvae, especially rare species, makes its use in ecologically-sensitive areas unacceptable. Resistance to *B. thuringiensis* has also been reported for some insects (e.g. McGaughey, 1985). In Forestry Canada, a group is looking at the environmental impact of biological control agents, especially *Bt* against non-target Lepidoptera. One of the issues the group has been assessing is how altering the availability of Lepidoptera affects the food chain, such as the effects on birds.

An example given was the Spruce budworm, which makes up over 90% of food for certain birds; therefore reducing budworm numbers may affect bird numbers.

Butler *et al.* (1995) investigated the effect of application of *Btk* against GM in a forest area of West Virginia on macrolepidoptera and other arthropods in 1990-92. Treatment caused a significant decline in macrolepidopterous larvae in the year of treatment, especially among those species that were in the oak canopy at the time of application. There appeared to be no significant difference between abundance in treatment and control plots for those species occurring in mid- and late-season, some weeks or months after treatment. No difference between treated and control plots was shown in abundance for most species in 1992, the post-treatment year. Non-Lepidoptera appeared to suffer no negative effect from treatment.

9.3.5 Products

There are many products based on *Btk* that are suitable for use against gypsy moth. Reardon *et al.* (1994) list four companies which produce formulations of the HD-1 strain for use against GM (see Table 3).

TABLE 3: Some *Btk* formulations used for control of gypsy moth.

Valent BioSciences	Aqueous flowable suspensions: Dipel 6AF and 8AF Non-aqueous emulsifiable suspensions: Dipel 4L, 6L, 8L and 12L
	Aqueous flowable suspensions: Foray 48B and Foray 76B
Ecogen inc.	Oil flowable: Condor OF
Sandoz Crop protection	Aqueous flowable suspensions: Thuricide 32LV, 48LV and 64 LV

There are possibly other products, especially in Eastern Europe. Over 100 *Bt*-based biopesticides are listed in Glare and O'Callaghan (2000). However, Dipel and Foray are the most commonly used products in Western Europe and North America.

9.3.6 Use

Most of the use of *Bt* has been aimed at population suppression rather than eradication. Between 1980 and 1994, approximately 1.7 million ha were treated with *Bt* in the eastern USA for insect control. Over the period, use against gypsy moth ranged from 6.4% to 79.5% of the total area (Reardon *et al.*, 1994). Compared to other Lepidoptera, Lymantriidae were not very susceptible to *Bt*. The efficacy of *Bt* needs to be 90-95% for effective control.

When spraying NAGM and AGM with *Btk*, the US Forest Service uses approximately 90 billion IUs/acre, then re-treat 5-6 days later. This dose is around the LD_{75} for NAGM (Dr Norman DuBois, pers. comm.). In the view of the Forest Service, one application at a high dose would be easier and more cost-efficient than two applications, but emergence of AGM occurs over several weeks and would therefore require more than one application to cover the hatchlings. For *Bt* a droplet size of 75-125 mm VMD (volume mean diameter) gives fairly good distribution and is large enough to contain a lethal dose (DuBois *et al.*, 1994). Falchieri *et al.* (1995) showed that droplets from 9-149 droplets/cm^3 containing *Btk* decreased the LD_{50} and the length of time after spray increased. LD_{50} decreased from 14.1 BIU/l to 3.1 BIU/l between 48 and 144 hours after spraying. Reardon *et al.* (1994) recommended doses of 40-90 billion IU/ha applied undiluted in volumes of 1.8 to 4.7 l/ha. They saw the benefit of no dilution as increasing the pay load for aircraft performing the spray application.

In Germany, Dipel and Foray 48B are registered for use. Both have been successfully applied against GM in Germany. They are also used against the other major *Lymantria* pest species, the nun moth, *L. monacha.* The recommended rate of application of Dipel is 900 g/ha, although this is considered to be too low to reduce defoliation in many cases (Lagenbusch and and Horst, BBA, Germany pers. comm.). For ground spray the normal rate is 4 l/ha. Currently, Dipel is used at 3 1/ha for the nun moth and 2 l/ha for GM.

Total defoliation of trees in Germany can occur when the population is around 600 GM larvae/tree. As ground water levels are decreasing in Europe, trees cannot take the stress of defoliation as well as they previously could. In German forests where control has been attempted in the past few years, approximately 50% of attacked areas in the Hessen, Western Germany and Baden-Wurtenburg were treated with *Bt*, while the remainder were treated with Dimilin. In Bavaria, 90% of the controlled areas were treated with Dimilin.

Schaaf and Vogt (1995) reported on a comparison of using the insecticide Dimilin 25 WP and the *Btk* Dipel ES to control a second year outbreak of GM in oak and beech forest in Baden-Wurttemberg, Germany. *Btk* stopped feeding of larvae more quickly than Dimilin, and no damage at all was found in the *Btk*-treated plot, whereas some damage was found in the Dimilin treated area. Naturally-occurring NPV caused high moralities in the untreated areas.

In Slovakia *Btk* is used at a rate of 2 l/ha to control GM, which is sufficient to keep populations below outbreak levels. *Btk* (Foray) was used to treat 100 ha where an outbreak was predicted, and no outbreak occurred. *Btk* is used as prophylactic treatments against 2nd instar GM.

There have been many studies on the effects of droplet size, density and larval instar. *Btk* has been shown to be more effective against first and second instars than later instars (Reardon *et al.*, 1994). Droplets of known size (100, 200 and 300 µm) and density (1, 5 and 10 drops/cm^2) of *Btk* were sprayed onto leaf disks of oak foliage and fed to larvae. LT_{50} increased as droplet density and size decreased and as larval size increased (Maczuga & Mierzejewski 1995). Droplet densities of 5 and 10 drops/cm^2 were effective (>90% mortality) against 2nd and 3rd instars. Fourth instars had high mortality rates at 5 and 10 drops/ cm^2 and at the 200 and 300 µm droplet sizes.

The relationship between dose and death is complicated by the fact that high concentrations can be a deterrent to the feeding of GM larvae. Therefore, choice of dose is not simply a function of increasing *Btk* density per drop, but a balancing act between drop and dose density. Higher densities of small (50-150 µm) drops were more effective than a lower density of bigger drops for the same amount of *Btk* applied (Bryant and Yendol, 1988).

9.3.7 Environmental factors

The effectiveness of *Btk* is dependent on environmental conditions. To reduce failure due to environmental constraints, two applications are used. Temperatures of less than 80^{o}F (26.7^{o}C), humidity greater than 50% and wind speed of 2-10 mph (3-16 kph) are recommended for applications of *Btk* (Reardon *et al.*, 1994). Anderson *et al.* (1992) compared aerial-applied spray deposits in an oak forest with predicted deposits using a canopy and penetration model of the US Forest Service and found extreme variation.

Persistence of *Bt* is generally considered low. Reardon *et al.* (1994) reviewed some of the literature detailing the effects of sunlight, leaf temperatures, drying, rain effects, microbial degradation and leaf chemistry on *Bt* survival, noting that solar radiation appears to be the most important. In uninterrupted sunlight, the half-life appears to be around 3.7 hours, while field estimates range from 12-32 hours. Although the half-life of *Bt* on foliage is usually considered to be about 30 hours, Johnson *et al.* (1995) showed that at times, *Btk* was toxic on foliage for up to 30 days after spraying. However, this was in protected environments. A deposit of 75 IU/cm^2 from an application of 90 BIU/ha can give 4-6 days activity at LD$_{50}$ level (Reardon *et al.*, 1994).

Environment will also affect the behaviour of the target pest; for example cold temperatures keep GM larvae to the lower branches of trees, away from the area reached by helicopter applications.

Diet of gypsy moth can influence the effectiveness of *Btk.* Hwang *et al.* (1995) found a 100 fold variation in *Btk* toxicity (Foray 48B) in gypsy moth reared on 5 different aspens (*Populus tremuloides*). This variation is possibly related to condensed tannins (Appel and Schultz, 1994) and phenolic glycosides, with the latter strongly enhancing *Btk* in artificial diet trials. Previous studies have shown that dietary constituents, such as tannins, nicotine and simple phenolics can influence *Bt* toxicity.

Feeding inhibition can occur at high doses of *Bt,* but is not related to droplet size or dose per unit area (Falchieri *et al.*, 1995).

9.3.8 Mammalian safety

Products containing *Bt* have been used in pest control efforts for decades with few associated mammalian health issues. Numerous laboratory studies testing the infectivity and toxicity of *Bt* have shown few adverse effects on small mammals (summarised in e.g. McClintock *et al.*, 1995; Siegel, 2001; O'Callaghan and Glare, 2002). Proven cases of *Bt* causing clinical infections in humans remain extremely rare and are all associated with non-industrial strains. The production of *B. cereus*-type-diarrhoeal toxins by *Bt* isolates has raised concerns that *Bt* may cause gastroenteritis in humans but no link has been found to date between use of *Bt* and episodes of diarrhoea. Epidemiological studies conducted in association with large scale aerial *Bt kurstaki* applications in the US, Canada and New Zealand found no increased incidence of illness. A recent study (Bernstein *et al.* 1999) on the immune responses of farm workers found increases in human antibody levels following exposure to *Bt* products. Tests with *Bt* toxin-expressing transgenic plants have shown no adverse effects on mammals.

In September 1996, a study by Auckland Healthcare Services (A+) into the health risks of Operation Ever Green found that generally no special precautions need to be taken because of aerial application. "However, people with severe or unstable asthma, or other respiratory conditions, immune conditions, allergies, or others who have concerns, such as pregnant women, can reduce exposure to the spray by staying inside with windows and doors shut during the spray operation". A health risk monitoring of the aerial spray programme to eradicate the white-spotted tussock moth throughout the eastern suburbs of Auckland found no increase in ill health resulting from the spray. (Aer'aqua Medcine 2001). Similarly, a 2002 Health Assessment for the painted apple moth eradication found that *Btk* had not been implicated in infection nor does it posed a health risk to people (Auckland Health Board, 2002).

9.3.9 New Zealand perspective

In New Zealand *Btk* (Foray 48B) has already been used successfully in the eradication programme against the Lymantriidae, *Orgyia thyellina* (tussock moth) (Hosking *et al.*, 2003; section 10). Interestingly, Reardon *et al.* (1994) concluded that aerial application of *Btk* provided good foliage protection, but population reduction was highly variable. However, *Btk* has been the insecticide of choice in several eradication programmes against GM in North America. *Btk* (Foray 48B) is also currently being used as the main agent for eradication of the painted apple moth in Auckland during 2000-2003 (see section 10).

The Auckland Health Board's Public Health Protection service undertook a health risk assessment of the 2002 aerial spray programme for eradicating the Painted Apple Moth from West Auckland.

The main findings of the report (Auckland District Health Board, 2002) are:

- The spray can cause temporary irritation to the eyes, nose, throat and skin
- The spray has a strong, unpleasant odour which comes from the fermentation process involved in the spray's manufacture. Some people respond to strong odours, but the odour does not indicate any toxicity
- People with pre-existing severe allergies to specific components in the spray (mainly to such things as soy, corn or starch which could be used in the fermentation broth) should avoid the spray. Residents are highly unlikely to develop new allergies even if exposed directly to the spray. Advice on allergies can be obtained through the MAF health service.
- People with asthma and chronic skin conditions may find their symptoms worsen, and should ensure that their medication and management plans are up to date. This should be sufficient for most people with these conditions
- The inactive ingredients include remnants of the culture broth, and 7 additives which stabilise the product and assist with spraying. They are all approved for use in foods, cosmetics and/or medicines and are of low toxicity.
- There is no indication that Btk and Foray 48B causes infections, diarrhoea, birth defects, thyroid conditions, miscarriages, premature labour, or auto-immune disorders.

9.4 Virus (Nucleopolyhedrovirus)

A pathogen which has been successful in controlling pest outbreaks in northern hemisphere forests is the gypsy moth nucleopolyhedrovirus (LdNPV). This virus is active in most countries where gypsy moth is found and has been produced as a commercial or semi-commercial product in the USA, Canada, Czech Republic and Russia (see section 9.4.6). The virus often contributes to population decline. For example, in China, a NPV routinely causes 50% natural mortality among GM populations (Yan *et al.*, 1994). It was not found in the USA until 1907, after an accidental introduction by unknown means (Hajek *et al.*, 1995c).

9.4.1 Biology

LdNPV is a multi-encapsulated NPV. Viral rods are contained in a large (1-10 µm) polyhedral inclusion body (PIB). Polyhedra are produced in the nucleus of the host cells, hence the name.

There are many "strains" of the virus, some of which are more virulent than others. Molecular comparison of a NPV from gypsy moth with other NPVs using the *pol* gene sequence has shown that LdNPV is genetically distinct from the two major groups of NPVs, and is presently the only published NPV which does not fall into the two groups (Zanetto *et al.*, 1993). One strain which is especially virulent to gypsy moth, the "Abby" strain, has been patented by USDA scientists.

9.4.2 Disease cycle

The virus typically attacks 4th and later instars in the field, but can infect younger instars. Virus-diseased caterpillars tend to climb, so are often found high in trees attached by the central legs forming an inverted V shape, when dead. The cuticle after death is extremely fragile and can easily be ruptured to rain virus particles down. Within are the PIBs, which contain the infective viral rods.

The LdNPV is not a very virulent NPV. Shapiro *et al.* (1994) found the Connecticut strain had an LC_{50} of 18,115 PIB/ml against second instar GM. Some NPVs have LC_{50} of below 100 PIB/ml. Differences have been seen in susceptibility of GM populations in North America to LdNPV. For example, a Pennsylvania population, used as standard for many years, appears to be the most susceptible to LdNPV. Differences of up to 9x have been detected in susceptibility, both within and between wild type and laboratory cultures, which is similar to the differences in susceptibility between NAGM and AGM found by Dr Podgwaite (US Forest Service) in Hamden. The Asian gypsy moth is 10x more susceptible to LdNPV than a standard New Jersey NAGM strain.

9.4.3 Survival and spread

An important aspect of a viral pathogen and pesticides based on virus is the environmental survival. LdNPV will recycle in the GM population and following application of the virus, there can be 5-6 years population suppression, depending on climatic conditions.

German scientists found that peak virus mortality was at time of pupation, which resulted in within-season defoliation but population reduction. NPV mortality can be over 70%, enough to reduce defoliation, but little virus survives in the population at this level for next year.

Virus-only treatment can result in defoliation, but with a larger proportion of the population virus infected in the next year (greater residual virus). A discrepancy between North American GM researchers (T.R. Glare, 1992 travel report) is whether the gypsy moth virus recycles after application. Clearly, virus will recycle if there is a population in which to recycle.

At present, biological limitations to the spread of the virus are expected to be the presence of susceptible hosts. There is no evidence that the virus can infect hosts other than *Lymantria* (see section 9.3.4). In the field, in the absence of natural hosts, UV radiation inactivates the formulated virus over time (Doane and McManus, 1981). The virus can persist in soil for years under ideal conditions. To the authors' knowledge there have been no studies on the persistence of *Ld*NPV in water, although it has been shown that *Ld*NPV does not persist in or infect aquatic vertebrates or fish (Doane and McManus, 1981).

Several recent studies have shown that virus transmission is not a simple proportional rate of horizontal transmission, but declines as both pathogen and host increase in numbers (D'Amico *et al.*, 1996; Dwyer and Elkington, 1995). D'Amico *et al.* (1996) found higher transmission rates at lower densities. While NPV can be spread by ballooning first instars, Dwyer and Elkington (1995) found that ballooning accounted for the rate and direction of spread only in the first weeks of an artificial GM outbreak. After that, spread was more rapid than ballooning and less directional. Rainfall also plays a role in spreading the virus within a tree (D'Amico and Elkington, 1995). Cook *et al.* (1995) studied virus in both the upper and lower canopies, and found that measurement of the lower canopy gave a good estimate of the whole infected population.

9.4.4 Host range and safety

Overseas, extensive research has shown that the virus attacks only *L. dispar* and possibly another Lymantriidae species (Barber *et al.* 1993), indicating that any danger of use in New Zealand is likely to be restricted to Lepidoptera. New Zealand has no Lymantriidae species (Dugdale 1988), the closest families taxonomically are Noctuidae or Hepialidae. Host range studies in New Zealand have not found any Lepidoptera susceptible to LdNPV (Glare *et al.*, 1995; Appendix 14.7).

The virus has been used in areas with sensitive environments, such as botanical gardens and rare butterfly habitats (e.g. eradication programme in North Carolina). The Gypchek product (see section 9.3.6) has been extensively tested for environmental and mammalian safety and was passed by the EPA in the 1970s. There appears to be no danger to humans from the virus itself, although there is some problem of skin irritants from the hairs of caterpillars which are included in the product. The product consists of viral infected and some uninfected caterpillar ground up and formulated. People who work with the virus and products are more likely to be affected than members of the public (USDA, 1995). There is also the occasional contaminant in the product. The presence of unknown bacteria would require careful consideration before spray application over urban areas.

9.4.5 Culturing

The virus does not grow outside of host cells and can only be cultured in insect cells in the laboratory with a great deal of effort (e.g. Lynn, 1994).

9.4.6 Commercialisation

There are several "products" based on LdNPV strains. All are only semi-commercial ventures, produced by government associated organisations. Most are looking for commercial partners. Isolates of this virus have been produced for gypsy moth control in the USA, Canada, Czech and Slovak Republics and the former USSR, as described below.

- **Gypchek (USA)**. The USDA began development of LdNPV in the 1950s as an alternative to chemical controls. In 1978 the virus product Gypchek was first registered as a biological pesticide (Reardon and Podgwaite, 1992, 1994). Gypchek is labelled for use under supervision of the US Forest Service. Gypchek is aerially applied on around 20,000 acres of forest annually. Early development of the virus involved several institutes and small companies contracting to produce the virus, but all these ventures failed. Although efforts are continuing to develop Gypchek into a fully commercial venture, we are unaware of any company involvement currently. The preparation is made from *in vivo* infected larvae in a facility near the Hamden US Forest Service laboratories.

- **Disparvirus (Canada)**. The virus is also under development in Canada by Forestry Canada, under the name "Disparvirus". The production system appears similar to Gypchek. Forestry Canada are seeking commercial partners to further develop the viral product. The Canadians had expected to have Disparvirus registered in Canada by 1995, but to the authors’ knowledge no such registration has been announced. Approximately 1300 ha were sprayed with the virus in Canada between 1982-1992.

- **Biola (Czech Republic).** Production of LdNPV in the Czech Republic is undertaken on a cooperative farm, ZD Chelcice Agricultural cooperative, at Chelcice, a small town in the southern Czech Republic. The factory of the Agricultural cooperative have been producing biological control agents since about 1984, but have only begun the production of insect pathogens in around 1996. Of insect pathogens, the laboratory is concentrating on viruses, and were working on production of codling moth GV, *Lymantria dispar*, *L. monacha* and *Neodiprion* NPVs.

- **Virin-Ensh (Russia).** Information on the Russian product is fragmentary and no sample of the virus has been examined under this programme. A USA/USSR joint report (Ignoffo *et al.*, 1983) compared Virin-Ensh with Gypchek. Liquid Virin-Ensh was reported in that document to have a concentration of no less than 10^9 PIBs/ml and had a guaranteed storage period of over 1 year at less than 15°C. Anecdotal information from Europe suggested the product was prepared from field collected larvae and could contain an unacceptable level of contaminants for use in Germany. Surprisingly, Virin-Ensh was pathogenic to other Lepidoptera, whereas Gypchek was not in the same tests (Martignoni, 1983), suggesting either a contaminant in the product and/or a very different strain of LdNPV. Virin-Ensh had an LC_{50} of 210.3 ± 82.02 compared to $5.5\text{-}47.4 \pm 2.28\text{-}16.59$ PIB x 10^3 when directly compared in laboratory bioassays against *L. dispar* (Shapiro, 1983).

9.4.7 Production and application

(i) Gypchek

The Gypchek production facilities near the US Forest Service laboratory in Hamden, CT, started producing LdNPV in 1977 and 50,000 acres were treated in 1979. Presently, about 15-20,000 acres/year are treated with virus in the USA. If production could be increased, there would be sufficient market for 30 000-50 000 acres. However, under the present system of the US Forest Service producing Gypchek, supplies are limited. That quantities are limited is recognised by end-users and demand is probably limited by this knowledge. Therefore the market for Gypchek in USA could potentially be larger.

The preparation is produced from infected larvae, grown and infected at Cape Cod in an APHIS facility, which has an automated production line for GM. Purified virus is sent from Hamden and inoculated with around 10^6 PIBs into 6 ml diet cups containing 15 larvae of NAGM. Approximately 14 days later, larvae are assumed infected and frozen for transport to Hamden. Approximately 70-80% of larvae in the product are virus killed. Gypchek is based on a mixture of virus genotypes.

In the production facility near Hamden, frozen larvae are thawed, blended in 3:1 water:larvae, filtered through a 100 mesh and cheesecloth, then centrifuged in a specialised centrifuge to remove much of the water. The rest of the water is removed over 3 days by lyophilizing. The dried product is then ground in coffee grinders. The powder is packaged and stored at 4°C, with an extended shelf life of over 1 year. The whole process is very technology-oriented and it would be expensive to set up the "factory".

The approximate yields are around 2-4.5 x 10^9 PIBs/larva from large larvae. However, the Cape Cod colony is getting smaller and the females have no 6th instar, so yields are down. The preparation is currently around 2 x 10^9 PIBs/larva. One hundred and sixty kg of cadavers gives between 5-5.5 kg of virus powder and it takes about 10 g of final virus powder for 1 acre. The final dry preparation consists of 14-19% virus PIBs, with the remainder insect bits and bacteria.

(ii) Biola

For Biola production, *L. dispar* eggs are in storage and can be used to produce virus in small quantities at one month’s notice. Virus is produced in insects, which are ground to form the basis of the product. The strain of gypsy moth used is the Otis strain from the USA. Quality counts were by light microscope. The virus is not produced on a commercial scale at present, but the estimated cost of a product would be between 600-1000 Ks/ha (approximately $NZ 30-50). Problems are being experienced in getting a concentrated and clean virus preparations (as we found on comparing Gypchek at 10^{10} PIBs/GM to Biola at a label concentration of 10^9, which was actually 10^8 PIBs/GM, see Appendix 14.8). Some fresh material was sent to Banska Staivnica at the time of our visit. Although the label stated that Biola contained 4.82 x 10^9 PIBs/g, this material was below specifications for the level of PIBs. Counts indicated the material contains about 10x less PIBs/g than stated on the label. Also, in comparison to the USDA product, Gypchek, the Biola product contained a lot more insect material.

One infected larvae produces about 10^9 PIBs, so 7-10 larvae are used per gram of product. Larvae are harvested about 10-14 days after inoculation with the virus, mixed with Siloxid, blended, then airdried at 20-25°C. The product is then stored at -20°C. Lack of industrial centrifugation above 10-15K rpm means that a concentrated product cannot be produced. Quantities of Biola recommended for spraying are 2 applications of 5 x 10^{11} PIBs/ha. According to Dr Pultar, preparation presently is between 5 x 10^9- 1 x 10^{10} PIBs/g..

Production of virus in the Czech Republic is thought to be good of quality by German scientists. A Russian product is thought to be suspect, as the production method uses field-infected larvae and therefore can contain many other organisms in addition to the GM virus, LdNPV. The Biola material has been used extensively by Dr Novonty and also by German researchers.

9.4.8 Quality control

(i) Gypchek

Quality control on each batch includes virulence checks, using bioassays against NAGM larvae and toxicity tests. Generally for incorporation of virus into diet experiments, 5×10^5 PIBs is a high dose, and should give 100% kill. For foliage treatment a higher dose is needed, around 10^8 PIBs for NAGM. Occasionally, the preparation has been contaminated with bacteria such as *Bacillus* spp., which can be harmful in large doses. Consequently, mice toxicity studies are routinely done on the final product.

(ii) Biola

In our tests, the Biola product was not up to label specifications, therefore the dose used was about 10x more dilute than that of the Gypchek treatments. Allowing for this, the new Gypchek and Biola products could be about the same potency (Appendix 14.8). The old Gypchek, which was collected from the USA in 1992, travelled for several weeks before refrigeration and kept at 4^oC for 4 years, retained virulence, and was equivalent to the Biola product (about 10 fold decrease on the new Gypchek).

9.4.9 Present use of LdNPV in the USA

In the USA, larvae are present May-June. Application is by spray, either from planes or ground applicators. Many carriers and stickers have been developed. A molasses-lignosulfonate mixture has been used extensively and several new products, such as carrier "244" from Novo Nordisk have been trialed. Industry estimates are that 4×10^{11} PIBs/acre are needed for control.

In addition to ongoing control in forests, there are one-off requirements for virus. An example is the attempted eradication of an AGM introduction into North Carolina in 1993. The AGM (actually hybrid AGM and European non-flying strain) were introduced to North Carolina by defense force movements between Germany and the USA. Once discovered, both *Btk* and NPV were used to eradicate the pest. Approximately 6000 acres were sprayed with Gypchek. For the virus, two applications of 2×10^{11} PIBs/acre were used, 3 days apart. Because the virus is more specific than the bacteria, it was used in areas thought to have beneficial or endangered insects. The eradication in North Carolina was declared successful in 1997.

Recently, aerial spray applications were carried out in southern Ontario to evaluate NPV (Payne *et al.* 1996). Mature oak (*Quercus rubra* and *Q. alba*) forest plots were sprayed with a repeated application of 5×10^{10} PIBs/ha per application, and applications at $5 \text{ X } 10^{10}$ and 10^{11} PIB/ha, all with volume application rates of 5 litres/ha per application.

9.4.10 Enhancement

Shapiro and others (Shapiro and Dougherty 1994; Shapiro *et al.* 1994; Shapiro and Robertson 1992; Shapiro 1992) have found that some optical brighteners enhance LdNPV efficacy up to 2000x. The USDA holds a patent for optical brighteners that act as viral enhancers. These optical brighteners work with other viruses and host systems to improve virus virulence. Trials have demonstrated at least 10x increase in efficacy in the field in some cases. However, the enhancement works for both inoculated and latent viruses and may increase the host range of virus. Glare, Jackson and Giddens (unpubl. data) conducted a small study on whether addition of Tinopal, an optical brightener, would allow infection of 4 leafroller species with LdNPV. Although no infection was found, this was a very inadequate test to examine the effects of optical brighteners on host range, and caution is still required with the widespread use of viral enhancers.

Other viral enhancing agents have been discovered recently and it may be possible in the future to improve viral efficacy, especially of "weak" viruses such as LdNPV.

9.4.11 New Zealand perspective

As the virus is produced commercially and can be applied to large areas of forest, it appears a good candidate for use in New Zealand. The main benefit of the virus over *Btk* and other control measures is its specificity. *Btk* is already registered in New Zealand, mainly for use against horticultural pests. Although the virus has not been registered for use, under our FRST programme sufficient information has been obtained to apply for registration, if required.

For experimental purposes, the virus has been obtained from the semi-commercial production in the USA (USDA, Hamden, CT) under the trade name “Gypchek”. The virus has been imported under permit no. R94/BIO/187 and is now stored in a contained manner at AgResearch, Lincoln. It may also be obtained from Forest Canada, Sault Ste. Marie, Ontario, who are developing a commercial product from the virus or from Dr Pultar, Czech Republic. Purified forms may be obtained from USDA, Beltsville laboratories, where researchers are looking at production of the virus in insect tissue culture (e.g. Lynn, 1994). The purified form of the virus will not survive in the field and is only useful for laboratory studies, such as DNA isolations.

If required for use in eradication of a gypsy moth infestation, sourcing sufficient virus from overseas will be dependent on occurrence of GM outbreak in the northern hemisphere. We had intended sourcing the virus from Hamden, USA or Czech/Slovakia. The Russian product would be unsuitable for use in New Zealand due to quality control concerns. However, the USDA production is presently limited and sufficient quantities may not be available at the time required by New Zealand. Biola would not be available in sufficient quantities for eradication purposes. Large-scale production would be difficult without several months advanced notice. The Biola product has some problems in standardised quantity, but virulence was good (Appendix 14.8).

Although the virus appears to be as effective as *Btk,* virus is more expensive to produce than *Btk.* Since worldwide production of the virus is presently limited, it would be difficult to find large amounts for use in New Zealand. It is expected that the increase in production in Canada and the development of virus production technology in Czech Republic and Russia will alleviate this problem.

9.5 Entomophaga maimaiga

Relatively few fungal pathogens are known to attack gypsy moth (Majchrowicz and Yendol, 1973; Soper *et al.*, 1988). The fungus, *Entomophaga maimaiga* (Entomophthorales: Zygomycetes), is a pathogen of gypsy moth originally described from Japan (Soper *et al.*, 1988). It is morphologically indistinguishable from *Entomophaga aulicae*, a common Lepidoptera pathogen in North America and elsewhere, which does not attack gypsy moth. *Entomophaga maimaiga* has recently become a very effective classical control agent of gypsy moth in the USA, although the origins of the present outbreak are somewhat mysterious (Hajek *et al.*, 1995c). A fungus, probably *E. maimaiga*, was imported to the USA by Speare and Colley in 1910-11 and released but apparently failed to establish. In 1984-1986, Soper and colleagues also imported and released *E. maimaiga*, again apparently without establishment (Hajek *et al.* 1995c). It was eventually recovered in the USA in 1989 (Andreadis and Weseloh, 1990; Hajek *et al.*, 1990), although this was around Boston and not at the release sites. Subsequent surveys established the fungus in 10 northeastern states and Ontario by 1990. The distribution of the fungus has continued to expand. Whatever the origins of the USA outbreak of *E. maimaiga*, it has contributed significantly to suppression of populations since 1990. There have been many "releases" of the fungus throughout the gypsy moth range in the USA. It is apparently the same species which contributes to natural control of gypsy moth in Japan (Soper *et al.*, 1988) and China (Yan Jingjun, pers. comm.). It continues to cause natural epizootics in gypsy moth populations throughout northeastern USA and Canada.

9.5.1 Characteristics and disease cycle

The fungus is a member of the *E. aulicae* complex of Entomophthorale, a group of morphologically indistinguishable species and strains that are virulent against other caterpillars. Isozyme and more recently DNA comparisons have demonstrated that the gypsy moth pathogenic strains are a distinct species, with little intraspecific variation. The fungus is difficult to culture and can be regarded as an obligate pathogen outside the laboratory.

Entomophaga maimaiga is known to attack only larvae. The fungus overwinters by the production of resting spores (azygospores), which have an obligate dormant period (Reardon and Hajek, 1993). The resting spores are thick walled and capable of surviving in soil for periods of years. In spring, when conditions become favourable, the resting spores produce the infective, pear-shaped conidia. The conidia are actively discharged and once in contact with a susceptible larva will produce a germ tube, which penetrates the host cuticle with enzymatic assistance. Within the host, the fungus reproduces in the heamolymph in a wall-less protoplast stage, using the nutrients in the insect blood. Eventually the fungus takes out vital organs and the host dies, basically containing only fungus within the cadaver. It takes less than 1 week to kill larvae at 20-25°C (Shimazu and Soper, 1986). Around the time of larval death, the protoplasts develop into walled hyphal bodies, which in turn develop into conidiophores after death, which grow out through the cuticle to produce and discharge conidia. Alternatively, resting spores are produced internally. Sometimes, both spore types are produced.

Once established in a gypsy moth population, the fungus produces both spore types, conidia and resting spores, depending on conditions. The conidia are fragile and produced in quantities to initiate rapid epizootics if susceptible hosts are present. Presence of susceptible hosts and environmental conditions are limiting factors on infection (e.g. Hajek *et al.*, 1990).

Moisture is important to the sporulation and germination of conidia. Conidial production is usually greatest on days of rainfall (Weseloh and Andreadis, 1992), while resting spores germinate in the days after significant rainfall (Reardon and Hajek, 1993). Larvae killed by the fungus often remain attached to trees, increasing the distribution of discharged conidia or resting spores.

Reardon and Hajek (1993) estimated that under optimal moisture conditions, *E. maimaiga* in North America can undergo 4-9 multiplicative cycles within one generation of the gypsy moth.

9.5.2 Culturing and production

In the laboratory, the fungus is cultured in liquid culture (Grace's tissue culture medium + 5% Fetal Bovine serum) as protoplasts. The fungus grows relatively poorly in culture. Therefore *in vitro* work is difficult and most experimental research is done with material produced *in vivo*. It can be induced to sporulate on egg yolk medium (Hajek *et al.*, 1995b), but doesn't sporulate well or quickly. It can take all night to collect spores in water and the spores germinate quickly, so they need to be refrigerated every 2-3 hours after collection for use in bioassays. Conidia can be produced more easily in a host, by injecting larvae with 23 gauge needle and leaving 5-6 days. Using larger larvae gives better results. High conidial inoculation levels cause septicemia in GM, rather than sporulation.

Resting spores from the field germinate readily (Hajek *et al.* 2001). In the field about 60% of resting spores germinate each year, which leaves some residual for the next season even if no more are produced. First instar GM are in the soil, 2-3rd instars are in trees, while 4-6th instars are back in soil and lower branches. It is in the soil that larvae contact inoculum from germinating resting spores. If soil containing resting spores has been moved to initiate an epizootic, it is best to wet the soil each week.

Long term culturing or storage of the fungus can lead to loss or instability of virulence (Hajek *et al.*, 1995b) so the fungus needs routine passage through the host to maintain condition.

9.5.3 Host range

Interest in the host range of *E. maimaiga* has been high in the USA due to the number of regional groups trying to release strains. Evidence is accumulating that the fungus is restricted to Lepidoptera (Reardon and Hajek, 1993; Vandenburg, 1990) and may attack only Lymantriidae in the field. Hajek *et al.* (1996) sampled 1511 larvae of 52 species of Lepidoptera (7 families) and found only 2 individuals (1 of 318 forest tent caterpillars (*Malacosoma disstria*) and 1 of 96 *Catocoala ilia*) became infected with the fungus. These collections were from areas where 40.8-97.5 % of the gypsy moth populations were infected with *E. maimaiga*.

Hajek *et al.* (1995a) also examined susceptibility in the laboratory, using exposure to conidia and injection of protoplasts directly into larvae. A total of 78 species of Lepidoptera were examined and 23.6% of species had deaths significantly greater than controls after treatment with *E. maimaiga* conidia. A total of 33.3% of tested larvae supported fungal sporulation. Lymantriidae were the most susceptible. The susceptibility of larvae was related to the surface setae. Infection was rare for surfaces with few setae while larvae with fine hairs were more likely to be infected. Many more insects were susceptible when protoplasts were injected directly into the haemocoel than when exposed to spores.

The laboratory results contradict those from field exposure, leading Hajek *et al.* (1995a) to conclude that laboratory bioassays were not a good predictor of field susceptibility. The risk of non-target infections among lepidopterans appears higher for the fungus than for the virus. However, when the limited period of exposure (about 10 weeks in spring-summer) and dose required to cause mortality in non-hosts is taken into account, few Lepidoptera would be expected to be at risk. In New Zealand, with no Lymantriidae, there would be only limited risk, so host range studies would need to be conducted as with the NPV (Glare *et al.,* 1995). Mammalian safety testing has not been conducted in the USA, as the strain(s) was accidentally released, but should be a priority in New Zealand.

9.5.4 Use as a mycoinsecticide

The fungus has not been commercialised, but has been introduced into areas in the USA by moving the resting spore stage in soil (Reardon and Hajek, 1993). The infective conidia are fragile and unsuited to production and storage for mass inoculation. At present, a system for using dried and powdered hyphae has not been successful with *E. maimaiga*, although such a process has been developed for other *E. aulicae* strains (Nolan, 1993). Therefore introduction of the fungus into new areas as only been attempted using *in vivo*-produced resting spores or infected cadavers. Generally this has involved relocation of soil containing resting spores from one habitat to another (Reardon and Hajek, 1993). Such a process is operationally restricted and expensive for any form of mass inoculation. Hajek and Roberts (1991) demonstrated that field-collecting soil containing resting spores and keeping the soil moist was the most effective method to initiate disease in gypsy moth populations.

9.5.5 New Zealand perspective

The fungus has been imported to quarantine and is presently held as protoplasts in glycerol in the AgResearch Insect Pathogens Culture Collection at -80°C. All information gathered indicate the fungus would not be suitable for use as a pesticide in eradication procedures, but would have potential for release as a control agent should gypsy moth establish here. The fungus is difficult to maintain in the laboratory and cannot be mass produced, but has maintained itself in gypsy moth populations once introduced.

Prior to release, it must be established that New Zealand insects are not endangered, so host range experiments against indigenous Lepidoptera which have a larval stage during spring and summer need to be carried out. It would be wise to examine effect against mammals, as this has not been tested previously. Very few entomophthoralean fungi have been found to pose any danger to mammals and none in the *E. aulicae* group have been implicated in mammalian health issues.

The success of *E. maimaiga* in controlling gypsy moth populations naturally in North America (e.g. Hajek *et al.*, 1993) indicates it has potential to assist other control agents in combating any gypsy moth outbreak/importation.

9.6 Protozoa

Several protozoan pathogens of gypsy moth have recently been described and may have potential in Europe and the USA. Dr Joe Maddox (USDA, Illinois) reported on over 10 different microsporidans from gypsy moth in Europe. Gypsy moth populations in Western and Central Europe naturally harbor several microsporidian species that have been placed in what are now considered to be three genera, *Nosema*, *Vairimorpha* and *Endoreticulatus* (Leellen Solter *et al.* the impact of mixed infection of three species of microsporidia isolated fr om the gypsy moth, *Lymantria dispar* L. (Lepidoptera: Lymantriidae, http://www.nal.usda.gov/ttic/ tektran/data/000013/83/0000138352.html). These microsporidia are largely specific to Lymantriidae, although they can also attack other Lepidoptera feeding on the same leaves. *Nosema lymantriae* can be transferred transovarially, and will remain in a population for 2-3 years. *Nosema* needs to be used two years in advance of an outbreak to establish in the population.

One of the few documented attempts to use a microsporidian against field GM populations was reported by Novotný (1988). He introduced 1 x 10^{11-12} spores/ha and obtained mortality of up to 63% in larvae and 79% at adult emergence. It was difficult to separate out the effects of different pathogens in action and control mortality was around 50-60%. Novotný (1988) concluded that the microsporidia have prospects as biocontrol agents for GM.

Microsporidia cannot be produced outside living cells and any production would require insect hosts, so would therefore be relatively expensive. The microsporidians would be unsuited to use in New Zealand during eradication and, given the lack of complete specificity, would require host range testing before recommendation for release in established GM populations in New Zealand.

9.7 Predators and parasites

There are many parasites and predators which attack gypsy moth in the northern hemisphere. In some places, natural population regulation is thought to be due to the action of a group of parasites and/or predators. For example, in Austria parasites are effective agents, where a complex of 5 important and 10 useful parasites and predators control gypsy moth, presently without intervention. Outbreaks are rare and Austrian scientists have had to resort to importing gypsy moth to infest trees in their study area. The parasites generally attack the larval stage and there are few egg parasites recorded. Eggs are the overwintering stage of GM, so without egg parasitism, the parasites overwinter on alternative hosts. In China, over 40 parasite and predators are recorded (Yan *et al.*, 1994, and Yan Jingjun, pers. comm.). In the USA, interest in parasite control of gypsy moth has continued for many years and at least 10 have been introduced and successfully established.

GM is only in the larvae stage for 6-8 weeks and parasites and predators require other hosts to maintain them throughout the year. Often, alternate hosts are also pest species. Parasites would be unsuitable for use in New Zealand due to the requirement for alternate hosts and lack of specificity.

9.8 Pheromone use

A synthetic sex pheromone has been commercially produced for gypsy moth, under the name disparlure. In addition to its obvious use in monitoring populations, there are two main methods of use of the pheromone in population reduction: mass trapping and mating disruption. This has been used against gypsy moth populations in the USA as part of the slow-the-spread programme (Suckling and Gibb, 2002 and reference therein).

In mass trapping, the disparlure is used to attract male moths to traps before they have the chance to mate with females. Different trap designs are used for different expected population densities. In "delta" traps, a sticky substances coats the insides only, while in others a sticky strip is used in conjunction with an insecticide dichlovos (2,2 dichcloroethenyl dimethyl ester phosphoric acid). With these latter traps, "milk carton" types are marketed as Vaportape II (Hercon Environmental Company, Emiigsville, PA). Delta traps have a capacity of about 15 moths, while the milk carton type vaportape II is used for larger populations.

These traps are the same as those used for monitoring populations. For mass trapping, these traps are used in an intensive grid pattern at a rate of at least 23 traps/ha (USDA, 1995). Mass trapping is usually used in conjunction with other methods of control. In the USA between 1967 to 1993, mass trapping was used as the primary treatment in 20% of eradication efforts (USDA, 1995), but it is usually only used on small areas. It is best used where the egg mass density is below 25 egg masses/ha. The method is labour-intensive, but relatively harmless to non-target organisms.

Mating disruption relies on saturating the area with pheromone so females cannot find a mate. It has been accomplished by ground application using a slow release tape impregnated with pheromone, which is manually attached to trees. The use of pheromones in mating disruption for European gypsy moth was reviewed by Reardon *et al.* (1998). They concluded that mating disruption should only be used to manage isolated or area-wide low density populations of EGM. Kolodny-Hirsch *et al.* (1990) evaluated the tape and found it requires further research before use, but the practise has become more common since (Suckling and Gibb, 2002). Aerial application is still under development. Out of all the GM eradication attempts in the USA up to 1993, only six used mating disruption as the primary treatment for eradication (USDA, 1995). Suckling and Gibb (2002) suggest pheromone use during eradication would be a useful technique to use in combination with other techniques, such as sterile male release or biopesticide applications, as pheromones target the adult males while biopesticides target larval stages and sterile male release targets adult females. In trials to test its efficacy, innudative releases of pheromone resulted in higher mating success for gypsy moth in areas of low population (Schwalbe, 1980).

9.9 Agent combinations

There have been few experimental comparisons of the use of more than one microbial agent against GM populations. In one trial in Germany, *Btk* alone gave 90% control (resulting in 25-30% damage), while the virus reduced feeding by an estimated 40%. Combinations of 0.1x virus and 0.1x *Btk* worked as well as *Btk* treatment alone. However, the forest area was denser and more diverse and GM populations were less in the areas treated with combinations of pathogens, so there is some doubt about the validity of the comparisons (Jurg Huber, BBA, Germany, pers. comm.). In the eradication of AGM in North Carolina, both *Btk* and Gypchek (LdNPV) were used. The virus was used in a few, environmentally sensitive areas and *Btk* in the majority. However, both were not reported to be sprayed against the same population.

Suckling and Gibb (2002), among others, advocate the use of mating disruption with another treatment, such as *Btk.*

The use of two or more agents against a target population would have some obvious benefits:

- Reduce the risk of agent failure by using agents with different basic infection methods.
- Possible additive or even synergistic effect.
- Introduce recycling agents, such as virus or fungus, with non-recycling agents, such as Dimilin or *Btk.*

10. STRATEGIES FOR ERADICATION IN NEW ZEALAND

About 10 years after Trouvelot accidentally introduced GM, the first outbreaks began in his neighbourhood and in 1890 the State and Federal Government began their attempts to eradicate the gypsy moth. These attempts ultimately failed and since that time, the range of gypsy moth has continued to spread. Every year, isolated populations are discovered beyond the contiguous range of the gypsy moth but these populations are eradicated or they disappear without intervention. It is inevitable that gypsy moth will continue to expand its range in the future, including reaching New Zealand. There needs to be developed a robust strategy for eradication of gypsy moth if/when it reaches New Zealand. There is evidence that the country is becoming well-prepared for eradication, with the experience of Operation Ever Green and the painted apple moth eradication programmes.

10.1 Summary of Operation Ever Green

The finding of the white spotted tussock moth, *Orgyia thyellina* (Lepidoptera: Lymantriidae), in Auckland in April 1996 was unexpected since the anticipated threat was from gypsy moth. After a necessarily rapid assessment of pest potential, it was decided to attempt eradication. As the pest was found in the major city of Auckland, control options appeared limited. Eventually *Btk* was selected as the only appropriate agent for aerial and ground sprays, because of environmental and human safety.

The programme was named Operation Ever Green and its success story has been documented by Hosking *et al.* (2003), which gives an excellent, concise summary of the programme. Some of the key factors contributing to the success of Operation Ever Green and the lessons learnt from this programme (mainly from Hosking *et al.* 2003) are summarised below.

10.1.1 Key factors for success

- The timing of detection was helpful as the third generation diapausing eggs found in April were not expected to hatch until spring, allowing time during winter to make decisions and put together an operations team.
- *Btk* has been widely studied and data were available on its persistence and efficacy. Throughout the programme research continued to evaluate and develop strategies to minimise the number of sprays and apply them at times of maximum effectiveness.
- It was essential to have good research and modelling skills available to develop the technical side of the spraying programme. Again, research throughout the programme, such as bioassays on plants exposed during spraying operations, was important to give feedback and adjust spraying protocols.
- The insect rearing facilities to provide live female moths for monitoring and the subsequent development of a synthetic pheromone were critical to the success of Operation Ever Green. The insect rearing programme also enabled efficacy trials and host risk assessments, both of which gave valuable feedback.
- Without a doubt, the communications strategy was a key element in the success of Operation Ever Green. Founded on the principle of openness, the strategy was designed to ensure that all affected parties had full access to all available information, whether good or bad, at no personal cost. This was particularly important when providing health

information that could be interpreted by the public. The co-operation of health professionals, including medical practitioners within the spray area, contributed to an effective communications strategy.
- Operation Ever Green developed an integrated team approach.

10.1.2 Lessons from Operation Ever Green

- Using GPS technology to map important sites within the spray zone would have been useful to track progress and assist with decision making in the programme.
- Better knowledge of the biology of the insect may have meant that predicting the timing of egg hatch and hence the number of spray applications required could have been more accurate.
- While the Science Advisory Group that was an integral part of the operations team made a major contribution to Operation Ever Green's success, the Science Panel established to evaluate and monitor the science input was only partially successful. Major difficulties were the distance of the Science Panel from the operation and the interdepartmental politics involved.
- Although the communications strategy was largely successful, towards the end of the programme when the number of spray applications were considerably extended, opposition groups emerged, such as S.T.O.P. (Society Targeting Overuse of Pesticides) and G.A.S.P. Effectively communicating the adverse effects of lymantriid moths, such as allergic reactions from urticating hairs on the moth and the economic and environmental damage, may help to quell opposition to future eradication campaigns.
- The costly importation of a DC6 for spraying operations was necessitated by the reluctance of the CAA to allow low level flights (<500 m) above cities. Eventually the multi-engine DC6 was allowed to fly at a height that enabled sufficient coverage with *Btk*.

10.1.2 Painted apple moth eradication programme

In mid-1999, another lymantriid moth, *Teia anartoides*, was found established in Glendene and neighbouring suburbs in west Auckland. The painted apple moth (PAM) is an occasional pest in its native range in Australia, but potentially a threat to New Zealand forestry and agriculture. Since then, a programme has been underway, supervised by MAF, to eradicate the insect. MAF has taken a less aggressive approach to the painted apple moth than the white spotted tussock moth, because it spreads more slowly and it was decided to try alternatives to aerial spraying first. Initially ground spraying with several insecticides (Lorsban, Chlorpyrifos and Decis have all been used) was attempted, but the moth persisted. Spraying of Foray 48B (*Btk*) from the air using both helicopter and fixed wing aircraft is now underway (as of May 2003).

Due to more organised public opposition to aerial spraying since operation Ever Green, a targeted and limited spray programme of fewer than 600 ha was initially attempted, but the moth has subsequently been found outside the spray zone and the zone has been extended. Little can be summaried about the spray programme at present, as it is ongoing, except that it appears to be significantly reducing the population with no live moths caught in the month of March 2003, for the first time since trapping started. Climatic variables will have contributed to this reduction, but it is possible that eradication can be achieved by aerial application of Btk and ground spraying. Host (vegetation) removal is also underway in some areas.

No pheromone is currently available for PAM, but colonies are maintained by HortResearch, Auckland, and Forest Research, Rotorua, which provide flightless females to use in traps for monitoring purposes. Originally a Technical Advisory group (TAG) and a Community Advisory group (CAG) were convened, the former at times numbering over 30 researchers, government offical and other local and national representatives. The TAG group has since been cut down to a few specialists, while the CAG is no longer directly involved.

10.2 Modelling the use of NPV for the biological control of Asian gypsy moth invading New Zealand

A full report on modelling the effect of NPV used under different strategies is given in Appendix 14.5 (Barlow *et al.*, 1997). In summary:

- In the long term and in the presence of predators, introducing NPV to a disease-free population as a single application (2500 GPIB ha^{-1}) at the optimum time of year (mid-October), with no further augmentation, gives an average reduction of 20% in outbreak densities over a 50 year period. In the absence of predators, NPV reaches equilibrium with a 40 - 50% reduction in gypsy moth density.

- In the medium term, the addition of NPV gives more substantial suppression but the extent depends on the timing of the application relative to the outbreak cycle. The best time is during the increase phase but before the peak is reached, giving a 70% reduction in the following two outbreaks over a 15 year period. However, the result is sensitive to the timing and if NPV is applied too late, at the peak of the outbreak (which may be only one year later), the results can be very much poorer.

- In the short term (the year following control), suppression depends on the application rate of the spray and, because of the recycling of infection within the population, on the larval density at the time of application. The latter effect distinguishes NPV from *Btk* or other pesticides, whose proportional effect is usually density-independent. Thus, 80% suppression of peak larval densities in the year following control can be obtained from an application of NPV at the standard rate (2500 GPIB ha^{-1}), if the larval density is extremely high (10^5 ha^{-1}) or extremely low (10 ha^{-1}). This drops dramatically to a 30% reduction at a density of 100 ha^{-1}. The effects are qualitatively similar in the absence of predation.

- In the short term, timing of application within a year is also important. A spray at the time of predicted maximum larval abundance, gives twice the percentage suppression in peak larvae the following year as a spray when densities are lowest.

- Applying NPV repeatedly when threshold egg mass densities are reached results in spraying, on average, every 7 years, with a reduction in peak outbreak densities of 40 - 70% (variable from outbreak to outbreak because of the differing amounts of disease already present). The result is insensitive to a wide range of thresholds, from half to double the Ontario Ministry for Natural Resources recommendation of 1250 egg masses ha^{-1}.

- Assuming that the default parameters are correct, eradication of a gypsy moth population with NPV is only possible if the population is very low (1 - 5 larvae ha^{-1}) and several sprays are used in rapid succession or the spray concentration is very high (25,000 GPIB ha^{-1}).

- The model's behaviour, in terms of whether or not it generates sustained outbreak cycles, is sensitive to several of the parameter values and to the model's age-class structure. This suggests that simple models, which ignore the effects of age-classes, time lags, egg diapause and temperature, may give quite misleading results. On the other hand, it is impossible to consider all components of the highly complex life system in detail because the data do not exist to parameterise them (in spite of attempts to do so in several published models). The present model seeks a middle course.

- Given the complexity of the gypsy moth life system, and the need to consider alternative scenarios in the absence of appropriate data (especially on the natural enemies likely to affect the moth in New Zealand), there is a need for further evaluation of long-term control options which include both a greater variety of NPV spraying regimes relative to the timing of outbreak cycles, and the integration of such strategies with the use of other controls such as *Entomophaga maimaiga*.

10.3 Strategies for eradication

If GM is found in New Zealand, the first response should be an eradication programme. In the USA and Canada, "eradication" has been performed over 550 times and is thought successful against small, well defined populations in areas less than 100 acres in size (USDA, 1995). As with the white spotted tussock moth, decisions will need to be made between available control options. While these decisions have a large political and public response component, sufficient knowledge exists such as is contained in this document to determine possible successful strategies, which can then be modified to suit the public/political climate. A summary of the relative risk of the various strategies is given in Table 4.

- *Btk* would be the main eradication insecticide, especially for aerial application. *Btk* has the benefits of being widely available, used previously in many eradication procedures and kills gypsy moth effectively. The tussock moth programme has already established the safety of use over urban areas in New Zealand. Drawbacks are that it will decimate the lepidopteran population in general which may affect some bird life temporarily in the spray zone.
- Dimilin should be considered for ground application to assist in hot spot infested areas. Lack of an effective knockdown chemical severely limited ground-based control efforts in the tussock moth programme. This chemical has potential non-target effects, so its use should be limited to specific ground applications.
- LdNPV, such as Gypchek, should be used early in an eradication programme. If the infested area is an important lepidopteran conservation area, it may need to be the primary method for eradication. There may be benefits from using more than one agent in aerial and ground applications (see section 9.8). In any case, the virus needs to be introduced to the population early to establish and recycle, in case eradication is unsuccessful. Except for possible

contaminants in the formulation of virus, there appear to be no non-target problems associated with use.

- Mass trapping may be a useful addition around the edges of infestations as both a monitoring tool and to trap males.
- Mating disruption may be a useful addition to other strategies in some situations.
- Other agents, such as *E. maimaiga* and parasites, are not suitable for eradication.

Data is available on *Btk* and viral spray densities and timing of sprays required for eradication. In general, in the USA, two to three applications 2-7 days apart are used in eradication procedures. This contrasts with the 48+ sprays performed for tussock moth eradication, which was largely caused by lack of knowledge of egg hatch and life cycle in Auckland. The model (Appendix 14.5) should predict GM egg hatch duration under different climatic conditions in New Zealand and so reduce the chances of failure. A more predictable eradication programme will also increase public confidence in biosecurity science.

However, control of AGM will be more difficult than NAGM or EGM. Egg hatch of AGM will be over a longer period than non-flight GM, making the targeting of sprays against selected instars more difficult. Both virus and *Btk* are more effective against younger instars which makes spray timing important. The view of some scientists at Darmstadt is that the virus will not be a successful eradication agent for GM, but is very good for ongoing suppression. High doses of *Btk* will be more successful for eradication.

Research indicates that the virus is specific to *Lymantria* spp. (Lepidoptera: Lymantriidae) while the bacterium, *B. thuringiensis*, is not specific and would be expected to kill some native New Zealand Lepidoptera. For this reason and to have more than a single potential control agent, the virus is being considered for use in New Zealand.

10.4 Strategies for control

If GM establishes in New Zealand and eradication is unsuccessful, then ongoing population suppression will be required. If GM arrives in NZ without its natural control agents, as is most likely, then the population will go through substantial outbreaks. Early introduction of a range of agents, suitable for use in NZ, will be required. We suggest that:

- The nucleopolyhedrovirus LdNPV be introduced as soon as a population is found in NZ. This virus has been shown to have no obvious non-target risks and should maintain itself in populations in NZ, eventually leading to suppression at low levels, as shown by the model in Appendix 14.5. While obtaining enough virus for eradication in NZ may be problematic, sufficient quantities to introduce into a population should not be difficult to obtain.

- The introduction of the fungus *Entomophaga maimaiga* should be investigated and if suitable, released as soon as is practical. This fungus has become a major suppression factor in much of the North American population after its first epizootic in 1989 near Boston. While the fungus has not been tested for non-target safety in NZ, there is no indication from overseas studies that this will be a problem.

- *Bacillus thuringiensis* is a single action insecticide in most cases and would not be useful as an ongoing control agent unless applied by spray against each target population. It could be useful to protect particular areas such as stands of susceptible trees.

- Mass trapping, and mating disruption, based on the use of synthetic pheromone, may be useful in slowing the spread of GM.

- Parasites and predators from other countries are unlikely to be of use, due to their broad host range and requirement for hosts often not present in NZ.

TABLE 4: Qualitative relative risk estimate by tactic (from Ecological Risk Assessment, Gypsy moth management in the United States: a cooperative approach. Draft environmental impact statement. USDA, 1995).

Endpoint	Ecological Indicator	Diflubenzuron	*Btk*	*NPV*	Disparlure
Change in forest health	Forest productivity, tree growth rates, mass production	Minimal	Minimal	Minimal	Minimal
	Successional state, stand age, species composition of tree and understory species	Minimal	Minimal	Minimal	Minimal
	Susceptibility to fire	Minimal	Minimal	Minimal	Minimal
	Incidence of disease, tree mortality rates, degree of insect damage	Minimal	Minimal	Minimal	Minimal
Change in non-target species	Species richness of mammals, birds, reptiles, amphibians, fish, invertebrates	High (Decrease in numbers of invertebrate species)	High (Decrease in numbers of invertebrate species)	Minimal	Minimal
	Population densities of groups of special concern (spring-emerging lepidopterans, summer-emerging native lepidopterans, insect predators and parasites, pollinators, amphibians, mollusks	High (Decrease in lepidopteran populations throughout growing season, possible reduction in parasitoid species)	High (Decrease in lepidopteran populations feeding on leaves during spring)	Minimal	Minimal
	Population densities of game species: wild turkey, deer, black bears, trout, salmon	Low (Decrease in fish population, especially in the forested residential ecosystem)	Minimal	Minimal	Minimal
	Population densities of organisms eaten by game fish: crustaceans, aquatic insects, small fish	High (Large decreases in populations of aquatic invertebrates; small population decrease in fish)	Minimal	Minimal	Minimal
Change in water quality	Water temperature, dissolved oxygen concentration	Minimal	Minimal	Minimal	Minimal

11. FUTURE RESEARCH REQUIREMENTS

- Further modelling of the interaction of all major control agents, for both eradication and control. The present model has used virus as the agent for population suppression, but the use of virus only is not recommended.
- A source of virus and other agents will be needed. The possibility of producing virus in New Zealand needs to be considered if other products cannot be produced in time.
- New Zealand registration of gypsy moth virus.
- *Entomophaga maimaiga:* Research is required to examine the specificity of the fungus in laboratory bioassay against New Zealand Lepidoptera, to ensure environmental safety, and to incorporate overseas and New Zealand data to develop strategies for use in both eradication and control situations against *L. dispar*. In addition, safety tests against mice to establish tier 1 mammalian toxicity may be required, as such tests have not been completed in the USA (where it was accidentally introduced). As it is difficult to grow in culture, it appears better suited to classical control introductions than an inoculative, mycoinsecticide approach. As such, it would not be considered for eradication programmes in New Zealand but if gypsy moth establishes, it could be a useful introduction to lessen gypsy moth impact.
- Investigation of pheromones and other pathogens and parasites, in the event GM establishes in New Zealand.

12. INTERACTIONS AND ACKNOWLEDGEMENTS

This programme is the result of collaboration between Dr Patrick Walsh and Malcolm Kay, FRASS and Forest Research Institute, Drs Travis Glare and Nigel Barlow of AgResearch. Funding was provided by the Foundation for Research, Science and Technology under programmes C10 434 and C10 540 and by the Forest Health Research Collaborative. Much of the report is based on discussions and interactions with key GM researchers around the world. In particular we would like to thank Drs John Podgwaite, Jane Carter, Bill Wallner, Melody Keena, Norman DuBois and Mike Montomergy (US Forest Service, Hamden CT); Dr Ann Hajek (Cornell University, Ithaca, NY, USA); Drs Gisbert Zimmermann, Jurg Huber (director), Horst Baton and Gustav Langenbruch (BBA, Darmstadt, Germany); Dr Julius Novotny (Forest Research Institute, Banska Staivnica, Slovakia); Dr Furher, Dr Axel Schof and Gernot Hoch (Institut fur Forstentomologie, Forstpathologie und Forstschultz, Universtat fur Bodenkultur, Wien, Austria); Drs Martin Shapiro, Ed Doughety, Jean Adams and Dwight Lynn (Insect Pathology Laboratories, USDA/ARS, BARC-West,Beltsville MD, USA); and Dr John Cunningham, Dr William Kaupp, Peter Ebling, Stephen Holmes, Dr Dave Tyrrell and Dr G.M. Howses (Forest Pest Management Institute, Forestry Canada, Queen Street, Sault Ste. Marie, Ontario, Canada). Some of the information in this report was supplied from unpublished research and meetings which these people made available to us.

We especially thank Dr Sue Zydenbos for extensive editorial work on the report, Dr Maureen O'Callaghan for comments on a draft and Mandy Barron for editorial assistance on an earlier version.

13. REFERENCES

Aer'aqua Medicine (2001). Health Surveillance following Operation Ever Green: A programme to eradicate the white-spotted tussock moth from the eastern suburbs of Auckland. Report to the Ministry of Agriculture and Forestry, May 2001. Prepared by Aer'aqua Medicine Ltd.

Appel, H.M. and Schultz, J.C. (1994). Oak tannins reduce effectiveness of thuricide (*Bacillus thuringiensis*) in the gypsy moth (Lepidoptera: Lymantriidae). *J. Econ. Entomol.*. 87: 1736-1742; 36

Anderson, D.E., Miller, D.R., Wang, Y., Yendol, W.G., Mierzejewski, K. and McManus, M.L. (1992). Deposition of aerially applied *Bt* in an oak forest and its prediction with FSCBG model. *J. Appl. Meteor.* 31: 1457-1466.

Andreadis, T.G. and Weseloh, R.M. (1990) Discovery of *Entomophaga maimaiga* in North American gypsy moth, *Lymantria dispar*. *Proc. Nat. Acad. Sci. USA 87:* 2461-2465.

Armstrong, K.F. (2000). DNA diagnostic procedures for the identification of selected species of *Lymantria* and *Orgyia* moths from intercepted egg masses. MAF Operational Research Final Report MBS301. 23 p.

Armstrong, K.F.; Cameron, C.M. (2000). Molecular kit for species identification: Lymantriidae. MAF Operational Research MBS301. 32 p.

Armstrong K. F., Mchugh P., Chinn W., Frampton E. R. and Walsh P. (2003) Tussock moth species arriving via imported used vehicles: confirmation by DNA analysis. New Zealand Plant Protection Journal 56 (submitted).

Auckland Health Board, (2002). Health risk assessment of the 2002 aerial spray eradication programme for the painted apple moth in some western suburbs of Auckland. Copy available at http://www.adhb.govt.nz/akphp/Services/AppleMoth/Overview.htm.

Barber, K.N., Kaupp, W.J. and Holmes, S.B. (1993). Specificity testing of the nuclear polyhedrosis virus of the gypsy moth, *Lymantria dispar* (L.)(Lepidoptera: Lymantriidae). *Can. Entomol. 125*: 1055-1066.

Barlow, N.D., Caldwell, N.P., Kean, J.M. and Barron, M.C. (2000). Modelling the use of NPV for the biological control of Asian gypsy moth *Lymantria dispar* invading New Zealand. *Agr. For. Entomol.* 2: 173-184.

Beegle, C.C., Couch, T.L., Alls, R.T., Versoi, P.L. & Lee, B.L. (1986). Standardization of HD-1-S-1980: U.S. standard for assay of lepidopterous-active *Bacillus thuringiensis*. *Bull. Entomol. So. Amer.* 32, 44-45.

Bernstein, I.L., Bernstein, J.A., Miller, M., Tierzieva, S., Bernstein, D.I., Lummus, Z., Selgrade, M.J.K., Doerfer, D.L., and Seligy, V.L. (1999). Immune responses in farm workers after exposure to *Bacillus thuringiensis* pesticides. *Environ. Health Perspect.* 107: 575-582.

Bogdanowicz, S.M., Wallner, W.E., Bell, J., Odell, T.M., Harrison, R.G. (1993) Asian Gypsy Moths (Lepidoptera: Lymantriidae) in North America: evidence from Molecular Data. *Annal. Entomol. Soc. America*, 86(6), 710-715.

Bryant, J.E. and Yendol, W.G. (1988). Evaluation of the influence of droplet size and density of *Bacillus thuringiensis* against gypsy moth larvae (Lepidoptera: Lymantriidae). *J. Econ. Entomol.* 81: 130-134.

Butler, L., Zivkovich, C. and Sample, B.E. (1995). Richness and abundance of arthropods in the oak canopy of West Virginia's Eastern Ridge and Valley Section during a study of impact of *Bacillus thuringiensis* with emphasis on macrolepidoptera larvae. *Bulletin Agricultural and Forestry Experiment Station, West Virginia University.* No. 711, 19 pp..

Cook, S.P., Webb, R.E. and White, G.B. (1995). Estimates of gypsy moth (Lepidoptera: Lymantriidae) mortality at two sample heights following application of gypsy moth nuclear polyhedrosis virus. *J.Entomol. Sci. Vol.* 30(4): 507-510.

Cowley, J.M., Bain, J., Walsh, P.J., Harte, R.T., Baker, R.T., Hill, C.F., Whyte, C.F. and Barber, C.J. (1993). Pest Risk Assessment for Asian Gypsy Moth, *Lymantria dispar* L. (Lepidoptera: Lymantriidae). New Zealand Forest Research Insititute. Report prepared for MAF, NZ. 39pp

Curran, J., Driver, F., Ballard, J.W.O. and Milner, R.J. (1994). Phylogeny of *Metarhizium*: analysis of ribosomal DNA sequence data. *Mycol.l Res.* 98: 547-552.

D'Amico, V. and Elkinton, J.S. (1995). Rainfall effects on transmission of gypsy moth (Lepidoptera: Lymantriidae) nuclear polyhedrosis virus. *Environ. Entomol.* 24(5): 1144-1149.

D'Amico, V., Elkinton, J.S., Dwyer, G., Burand, J.P. and Buonaccorsi, J.P. (1996). Virus transmission in gypsy moths is not a simple mass action process. *Ecology* 77(1): 201-206.

Davis, R.E., Kelly, T.J., Masler, E.P., Thyagaraja, B.S., Rote, C.A. and Imberski, R.B. (1994). Complete Base Sequence for the Mitochondrial Large Subunit Ribosomal RNA of the Gypsy Moth *Lymantria dispar*. *Insect Molecular Biology*, 3(4): 219 - 228.

Doane, C.C. and McManus, M.L. (1981). *The gypsy moth: research towards integrated pest management*. Forest Service Technical Bulletin 1584, USDA Washington D.C. 757 pp.

Dubois, N.R., Mierzejewski, K., Reardon, R.C., McLane, W. and Witcosky, J.J. (1994). *Bacillus thuringiensis* field applications: effect of nozzle type, drop size and application timing on efficacy against gypsy moth. *J. Environ. Sci. Health* B29: 679-695.

Dugdale, J., (1988). Lepidoptera- annotated catalogue, and keys to family-group taxa. *Fauna of New Zealand 14*: 264 pp.

Dwyer, G. and Elkinton, J.S. (1995) Host dispersal and the spatial spread of insect pathogens. *Ecology* 76(4): 1262-1275.

Elkinton, J.S. and Carde, R.T. (1980). Distribution, dispersal, and apparent survival of male gypsy moths as determined by capture in pheromone-baited traps. *Environ. Entomol.* 9: 729-737.

Falchieri, D., Mierzejewski, K. and Maczuga, S. (1995) Effects of droplet density and concentration on the efficacy of *Bacillus thuringiensis* and carbaryl against gypsy moth larvae (*Lymantria dispar* L.). *J. Environ. Sci. Health* B30(4): 535-548.

Forbush, E.H. and Fernald, C.H. (1896). The gypsy moth. *Porthetria Dispar* (Linn.). Boston: Wright & Potter. 495 p.

Garner, K.J., Slavicek, J.M. (1996). Identification and Characterisation of a RAPD-PCR Marker for Distinguishing Asian and North American Gypsy Moths, *Insect Molecular Biology*, 5(2) 81 - 91.

Glare, T.R. (1994). Use of pathogens to control Gypsy Moth, *Lymantria dispar*, in northeastern USA and Ontario, Canada, Travel Report, 32 pp.

Glare, T.R., Barlow, N.D. and Walsh, P.J. (1998). Possible agents for use in New Zealand for the eradication or control of gypsy moth, *Lymantria dispar*. *Proceedings of the 51th New Zealand Plant Protection Conference*, 224-229.

Glare, T.R., Newby, E.M. and Nelson, T.L. (1995). Safety testing of a nuclear polyhedrosis virus for use against gypsy moth, *Lymantria dispar*, in New Zealand. *Proceedings of the 48th New Zealand Plant Protection Conference* 264-269.

Glare, T.R. and O'Callaghan M. (2000). Bacillus thuringiensis: Biology, Ecology and Safety. John Wiley and Sons, Chichester. 350 pp.

Gypsy Moth Exotica. (1992). An irregularly issued US/Canada informational report on the Asian gypsy moth. July.

Hajek, A.E. (1994). Natural enemies: tools for integrated pest management. Field identification of the gypsy moth nuclear polyhedrosis virus (NPV). USDA Forest Service, Northeastern Area NA-PR-01-94. 2 pp.

Hajek, A.E., Bulter, L. and Wheeler, M.M. (1995a). Laboratory bioassays testing the host range of the gypsy moth fungal pathogen *Entomophaga maimaiga*. *Biological Control*. 5: 530-544.

Hajek, A.E., Butler, L., Walsh, S.R.A., Silver, J.C., Hain, F.P., Hastings, F.L., Odell, T.M. and Smitly, D.R. (1996) Host range of the gypsy moth (Lepidoptera: Lymantriidae) pathogen *Entomophaga maimaiga* (Zygomycetes: Entomophthorales) in the field versus laboratory. *Environ. Entomol.* 25(4): 709-720.

Hajek, A.E., Carruthers, R.I. and Soper, R.S. (1990). Temperature and moisture relations of sporulation and germination by *Entomophaga maimaiga* (Zygomycetes: Entomophthoraceae), a fungal pathogen of *Lymantria dispar* (Lepidoptera: Lymantriidae). *Environ. Entomol.* 19: 85-90.

Hajek, A.E., Humber, R.A. and Elkinton, J.S. (1995c). Mysterious origin of *Entomophaga maimaiga* in North America. *Amer. Entomol.* 41: 31-42.

Hajek, A.E., Shimazu, M. and Humber, R.A. (1995b). Instability in pathogenicity of *Entomophaga maimaiga* after long-term cryopreservation. *Mycologia*. 87: 483-489.

Hajek, A.E., Larkin, T.S., Carruthers, R.I. and Soper, R.S. (1993). Modeling the dynamics of *Entomophaga maimaiga* (Zygomycetes: Entomophthoraceae), epizootics in gypsy moth (Lepidoptera: Lymantriidae) populations. *Environ. Entomol.* 22: 1172-1187.

Hajek, A.E. and Roberts, D.W. (1991). Pathogen reservoirs as a biological control resource: introduction of *Entomophaga maimaiga* to North American gypsy moth, *Lymantria dispar*, populations. *Biological Control* 1: 29-34.

Hajek, A.E. and St. Leger, R.J. (1994). Interactions between fungal pathogens and insect hosts. *Ann. Rev. Entomol.* 39: 293-322.

Hajek, A.E., Wheeler, M.M., Eastburn, C.C. and Bauer, L.S. (2001). Storage of resting spores of the gypsy moth fungal pathogen, *Entomophaga maimaiga*. *Biocon. Sci. Technol*. 11: 637-647

Harrison, R.G. and Odell, T.M. (1989). *Mitochondrial DNA as a Tracer of Gypsy Moth Origins,* General Technical Report.

Henry, J.M., Raina, A.K. and Ridgway, R.L. (1990). *Isolation of High-Molecular-Weight DNA from Insects. Analytical Biochemistry*, 185, 147 - 147-150.

Higashiura, Y. (1989). Survival of eggs in the gypsy moth *Lymantria dispar*. 1. Predation by birds. *J. Animal Ecol.*, 58: 403-412.

Horgan, G., 1994. Economic impact of Asian gypsy moth. Report prepared for the N.Z. Ministry of Forestry, August 1994, 20 pp.

Hosking, G, Clearwater, J., Handiside, J., Kay, M., Ray, J. and Simmons, N. (2003). Tussock moth eradication- a success story from New Zealand. *International Journal of Pest Management* 49: 17-24.

Howse, G.M. and Applejohn, M.J. (1993). Survey Bulletin, Forest Insect and Disease Conditions in Ontario Summer 1993. Second of three bulletins issued each year by Forest Insect and Disease Survey Unit of Forestry Canada. 14 pp.

Hoy, M.A. (1977). Rapid response to selection for a nondiapausing gypsy moth. *Science USA* 196: 4297, 1462-1463.

Hudson, G.V., (1928). *The butterflies and moths of New Zealand*. Ferguson and Osborne Limited, Printers and Publishers.

Hwang, S.Y., Lindroth, R.L., Montgomery, M.E. and Shields, K.S. (1995). Aspen leaf quality affects gypsy moth (Lepidoptera: Lymantriidae) susceptibility to *Bacillus thuringiensis*. *J. Econ. Entomol.* 88(2): 278-282.

Ignoffo, C.M., Martignoni, M.E. and Vaughn, J.L. (eds.)(1983). A comparison of the US (Gypchek) and the USSR (Virin-Ensh) preparations of the nuclear polyhedrosis virus of the gypsy moth, *Lymantria dispar*. Results of research conducted under project V-01.0705, Microbiological control of insect pests, of the US/USSR joint working group on the production of substances by microbiological means.

Inoue, H. (1982). Lymantriidae. Moths of Japan. Vol. 1. Text (ed. By H. Inoue, S. Sugi, H. Kuroko, S. Moriuti and A. Kawabe), pp. 628-638. Kodansha, Tokyo.

Johnson, K., Scriber, J.M., Nitao, J.K. and Smitley, D.R. (1995). Toxicity of *Bacillus thuringiensis* var. *kurstaki* to three nontarget Lepidoptera in field studies. *Environ. Entomol.*24(2): 288-297.

Kay, M. Matsuki, M. Serin, J. and Scott, J.K. (2002). A risk assessment of the Asian Gypsy moth to key elements of the New Zealand flora. Forest Health Collaborative Report, New Zealand, 32 pages.

Keena, M.A. (1994). Summary of Research Findings for European Populations. May 20, 1994 and "Information on DNA analysis of samples sent from the quarantine". - results reported on analysing egg masses from Switzerland using FS1 marker, studying the origin of German material.

Keena, M.A. (1996). Comparison of the hatch of *Lymantria dispar* (Lepidoptera: Lymantriidae) eggs from Russia and the United States after exposure to different temperatures and durations of low temperature. *Annals of the Entomological Society of America* 89 (4): 564-572.

Keena, M.A.; Wallner, W.E.; Grinsberg, P.S. and Cardé, R.T. (2001). Female flight propensity and capability in *Lymantria dispar* (Lepidoptera: Lymantriidae) from Russia, North America and their reciprocal F_1 hybrids. *Environ. Entomol.* 30: 380-387.

Kolodny-Hirsch, D.M., Webb, R.E., Olsen, R. and Venables, L. (1990). Mating disruption of gypsy moth (Lepidoptera; Lymantriidae) follwoing repeated ground application of racemic disparlure. *J. Econ. Entomol.* 83: 972-1976.

Liebhold, A.M., Gottschalk, K.G., Muzika, R., Montgomery, M.E., Young, R., O'Day, K., and Kelly, B. (1995). Suitability of North American tree species to the gypsy moth: a summary of field and laboratory tests. USDA General Technical Report NE-211, USDA Forest Service, Delaware, Ohio, USA.

Lynn, D.E. (1994). Enhanced infectivity of occluded virions of the gypsy moth nuclear polyhedrosis virus for cell cultures. *J. Invertebr. Pathol.* 63: 268-274.

Maczuga, S. and Mierzejewski, K.J. (1995) Droplet size and density effects of *Bacillus thuringiensis kurstaki* on gypsy moth (Lepidoptera: Lymantriidae) larvae. *J. Econ. Entomol.* 88(5): 1376-1379.

Majchrowicz, I. and Yendol, W.G. (1973). Fungi isolated from gypsy moth. *J. Econ. Entomol.* 66: 823-824.

Martignoni, M.E. (1983). *In vivo* host specificity of VIRIN-ENsh . In: A comparison of the US (Gypchek) and the USSR (Virin-Ensh) preparations of the nuclear polyhedrosis virus of the gypsy moth, *Lymantria dispar*. (Ignoffo, C.M., Martignoni, M.E. and Vaughn, J.L., eds.). Results of research conducted under project V-01.0705, Microbiological control of insect pests, of the US/USSR joint working group on the production of substances by microbiological means. pp 43-49.

McClintock, J.T., Scahffer, C.R., and Sjoblad, R.D. (1995). A comparative review of the mammalian toxicity of *Bacillus thuringiensis*-based pesticides. *Pestic. Sci.* 45: 95-105.

McGaughey, W.H., (1985). Insect resistance to the biological insecticide *Bacillus thuringiensis*. *Science* 229: 193-194.

McManus, M.L. and McIntyre, T. (1981). Introduction. In *The gypsy moth: research towards integrated pest management* (Doane, C.C. and McManus, M.L., eds.). Forest Service Technical Bulletin 1584, USDA Washington D.C. 1-7.

Miller, J.C. (1990). Field assessment of the effects of a microbial pest control agent on non-target Lepidoptera. *Amer. Entomol.* 36: 135-139.

Morse, J.G. and Simmons, G.A. (1979). Simulation model of gypsy moth introduced into Michigan forests. *Environ. Entomol.* 8(2): 293-299.

NEWS Briefs (1993). *Journal of Forestry* (U.S.). January.

Nolan, R.A. (1993). An inexpensive medium for mass fermentation production of *Entomophaga aulicae* hyphal bodies competent to form conidia. *Can. J. Microbiol*. 39: 588-593.

Novotny, J., (1988). The use of nucleopolyhedrosis virus (NPV) and microsporidia in the control of the gypsy moth (*Lymantria dispar*, Linne). *Folia Parasitol.* 35: 199-208.

O'Callaghan, M. and Glare, T.R. (2001). Environmental and health impacts of Spinosad. Report for the Ministry of Agriculture and Forestry. 61 pp.

O'Callaghan, M and Glare, T.R. (2002). The mammalian safety of *Bacillus thuringiensis.* Biotechnology of *Bacillus thuringiensis* and its environmental impact. (R.J. Akhurst, C.E. Beard and P.A. Hughes, eds.). *Proceedings of the 4th Pacific Rim Conference*, CSIRO, Canberra, 254-261.

Payne, N.J.; Cunningham, J.C.; Curry, R.D.; Brown, K.W. and Mickle, R.E. (1996). Spray deposits in a mature oak canopy from aerial applications of nuclear polyhedrosis virus and *Bacillus thuringiensis* to control gypsy moth, *Lymantria dispar* (L.). *Crop Protection*, 15: 425-431.

Peacock, J.W., Schweitzer, D.F., Carter, J.L. & Dubois, N.R. (1998). Laboratory assessment of the effects of *Bacillus thuringiensis* on native Lepidoptera. *Environ. Entomol.*, 27, 450-457.

Pfeifer, T.A., Humble, L.M., Ring, M., Grigliatti, T.A. (1995). *Characterisation of Gypsy Moth Populations and Related Species Using a Nuclear DNA Marker*. *Can. Entomol.*, 127: 49 - 58

Reardon R.C., Dubois, N. and McLane, W., (1994). *Bacillus thuringiensis* for managing gypsy moth: a review. USDA, Tech. Trans. FHM-NC-01-94, 32 pp.

Reardon, R. and Hajek, A. (1993). *Entomophaga maimaiga* in North America: a review. AIPM, Technology transfer. USDA Forest Service, Northeastern Area, NA-TP-15-93. 22 pp.

Reardon, R.C., Leonard, D.S., Mastro, V.C., Leonhardt, B.A., McLane, Talley, S., Thorpe, K.W. and Webb, R.E. (1998). Using mating disruption to manage gypsy moth: a review. USDA Forest Service FHTET-98-01, 85 pp.

Reardon R.C. and Podgwaite, J.D., (1994). Summary of efficacy evaluations using aerially applied Gypchek against gypsy moth in the USA. *J. Environ. Sci. Health*, B29(4): 739-756.

Reardon, R. and Podgwaite, J. (1992). The gypsy moth nuclear polyhedrosis virus product, Gypchek. AIPM technology transfer. USDA Forest Service, North east area. 9 pp.

Sambrook, J., Fritsch, E.F. and Maniatis, T., (1989). Molecular Cloning. 2nd edition. Cold Springs Harbour Laboratory Press, Cold Spring Harbour.

Sample, B.E., Bulter, L., Zivkovich, C. and Whitmore, R.C. (1993). Evaluation of *Bacillus thuringiensis* and defoliation effects of native Leidoptera. USDA Forest Service, Northeastern Area, Forest Health Protection, Appalachian Integrated Pest Management, AIPM Tech. Transfer: *Bacillus thuringiensis.* 12 pp.

Schaaf, C. and Vogt, H. (1995). Investigations in gypsy moth, *Lymantria dispar* (Lepidoptera, Lymantriidae), outbreak regions under different control strategies. [Untersuchungen in Gradationsgebieten des Schwammspinners *Lymantria dispar* L. unter Berucksichtigung verschiedener Bekampfungsmassnahmen.] *Mitteilungen der Deutschen Gesellschaft fur Allgemeine und Angewandte Entomologie* 10: 1-6, 123-128.

Schaefer, P.W., R.M. Weseloh, X. Sun, W.E. Wallner, and J. Yan. (1984). Gypsy moth, *Lymantria (=Ocneria) dispar* (L.) (Lepidoptera: Lymantriidae), in the People's Republic of China. *Environ.l Entomol.* 13(6): 1535-1541.

Schreiber, D.E.; Garner, K.J.; Slavicek, J.M. (1997): Identification of three randomly amplified polymorphic DNA polymerase chain reaction markers for distinguishing Asian and North American gypsy moths. *Annals of the Entomological Society of America* 90: 667-674.

Scott, R.R., (1984). New Zealand Pest and Beneficial Insects. Lincoln University, College of Agriculture.

Siegel, J.P. (2001). The mammalian safety of *Bacillus thuringiensis*-based insecticides. *J. Invert. Pathol.* 77: 13-21.

Shapiro, M. (1983). Comparative infectivity of Gypchek L-79 and VIRIN-ENsh to *Lymantria dispar*. In: A comparison of the US (Gypchek) and the USSR (Virin-Ensh) preparations of the nuclear polyhedrosis virus of the gypsy moth, *Lymantria dispar*. (Ignoffo, C.M., Martignoni, M.E. and Vaughn, J.L., eds.). Results of research conducted under project V-01.0705, Microbiological control of insect pests, of the US/USSR joint working group on the production of substances by microbiological means. pp 38-42.

Shapiro, M. and Dougherty, E.M. (1994). Enhancement in activity of homologous and heterologous viruses against the gypsy moth (Lepidoptera: Lymantriidae) by an optical brightener. *J. Econ. Entomol*. 87: 361-365.

Shapiro, M., Im, D.J., Adams, J.R. and Vaughn, J.L. (1994). Comparative effectiveness of *Lymantria* nuclear polyhedrosis and cytoplasmic polyhedrosis viruses against the gypsy moth (Lepidoptera: Lymantriidae). *J. Econ. Entomol*. 87: 72-75.

Shapiro, M. and Robertson, J.L. (1992). Enhancement of gypsy moth (Lepidoptera: Lymantriidae) baculovirus activity by optical brighteners*.* *J. Econ. Entomol*. 85: 1120-1124.

Shapiro, M. (1992). Use of optical brighteners as radiation protectants for gypsy moth (Lepidoptera: Lymantriidae) nuclear polyhedrosis virus. *J. Econ. Entomol*. 85: 1682-1686.

Shimazu, M. and Soper, R.S. (1986). Pathogenicity and sporulation of *Entomophaga maimaiga* Humber, Shimazu, Soper and Hajek (Entomophthorales: Entomophthoraceae) on larvae of the gypsy moth, *Lymantria dispar* L. (Lepidoptera: Lymantriidae). *Appl. Entomol. Zool.* 21: 589-596

Soper, R.S., Shimazu, M., Humber, R.A., Ramos, M.E. and Hajek, A.E. (1988). Isolation and characterization of *Entomophaga maimaiga* sp. nov., a fungal pathogen of gypsy moth, *Lymantria dispar*, from Japan. *J. Invertebr. Pathol.* 51: 229-241.

Suckling, D.M. and Gibb, A.R. (2002). Use of pheromones and other attractants to combat insect incursions. *In* Defending the Green Oasis: New Zealand Biosecurity and Science (S.L. Goldson and D.M. Suckling, eds.). NZ Plant Protection Society, 31-44.

Twardus, D.. and Machesky, H.A. (1992). Gypsy moth suppression in the Northeast: summary of the treatment monitoring database, 1989-1990 NA-TP-08-92. Morgantown, WV. USDA Forest Service Northeastern Area State and private Forestry, 11 pp.

USDA (1995) Gypsy moth mangement in the United States: a cooperaive approach. (Draft environmental impact statement). USDA Forest Service, Washington DC.

USDA (2003). Asian Gypsy Moth. www.ceris.purdue.edu/napis/a-facts/fsgma.html (accessed 7/04/2003).

Vandenberg, J.D. (1990). Safety of four entomopathogens for caged adult honey bees (Hymenoptera: Apidae). *J. Econ. Entomol*. 83: 755-759.

Waggoner, P.E., (1984). The hatching of Gypsy Moth eggs, a phenological model. *Agricultural and Forest Metereology*, 33, 53-65.

Wallner, W.E., Grinberg, P.S. and Walton, G.S. (1994). Differentiation between gypsy moth (Lepidoptera: Lymantriidae) populations by spectral color discrimination of head capsules. *Environ. Entomol*. 23: 659-664.

Wallner, W.E., Humble, L.M., Levin, R.E., Baranchikov, Y.N. and Carde, R.T. (1995) Response of adult Lymantriid moths to illumination devices in the Russian Far East. *J. Econ. Entomol.* 88(2): 337-342.

Walsh, P.J., (1993). *Asian Gypsy Moth: The Risk To New Zealand*. New Zealand Forestry, 38(2): 41 - 43.

Walsh, S. P., Metzger, D. A and Higuchi., R. (1991). *Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material*, *Biotechniques* 10(4): 506-513.

Wanner, K.W., Helson, B.V. and B.J. Harris (2000). Laboratory and field evaluation of spinosad against the gypsy moth, *Lymantria dispar. Pest Management Science.* 56: 855-860.

Weseloh, R.M. and Andreadis, T.G. (1992). Epizootiology of the fungus *Entomophaga maimaiga*, and its impact on gypsy moth populations. *J. Invertebr. Pathol.* 59: 133-141.

Yan J., Pemberton, R.W., Yao D., Liu H. and Li G. (1994). Studies on the natural enemies of gypsy moth, *Lymantria dispar* (Lep: Lymantriidae) in Dayi, Sichuan Province. *For. Res.*, 7: 269-276.

Zanotto, P.de A.; Kessing, B.D.; Maruniak, J.E., (1993). Phylogentic relationships among Baculoviruses: evolutionary rates and host associations. *J. Invertebr. Pathol*. 62: 147-164.

14. APPENDICES

Appendix 14.1 Gypsy moth host plants (USA)

14.1.1 Plant list of susceptibility to gypsy moth

From: USDA (1995) Gypsy Moth Management in the United States: a cooperative approach. Draft environmental impact statement. USDA Forest Service and Animal and Plant Health Inspection Service.

This appendix shows the susceptibility of plant species to feeding by gypsy moth caterpillars, considering preference and weight gain of both European and Asian strains. The index number provides a general ranking, recognizing that preference varies between the strains (Liebhold *et al.*, 1995):

1—susceptible
2—resistant
3—immune

Genus and species	Common name	Susceptibility index
Abelia grandiflora	glossy abelia	3
Abies amabilis	Pacific silver fir; silver fir; lovely fir; amabilis fir	2
Abies balsamea	balsam fir; Canada balsam; eastern fir	3
Abies balsamea var. *phanerolepis*	balsam fir; bracted balsam fir	3
Abies bifolia	Rocky Mountain subalpine fir	3
Abies bracteata	bristlecone fir; Santa Lucia fir; silver fir	2
Abies concolor	white fir; concolor fir; silver fir	2
Abies fraseri	Fraser fir; southern balsam fir; southern fir	3
Abies grandis	grand fir; lowland white fir; lowland fir; balsam fir	2
Abies holophylla	needle fir; Manchurian fir	2
Abies lasiocarpa	subalpine fir; alpine fir; balsam fir; white balsam fir; Rocky Mountain fir	2
Abies lasiocarpa var. *arizonica*	corkbark fir	2
Abies lowiana	California white fir, white fir; Sierra white fir;	2
Abies magnifica	California red fir; red fir; silvertip; golden fir	2
Abies procera	noble fir; red fir; white fir	2
Acacia baileyana	Bailey acacia, cootamundra wattel	2
Acacia farnesiana	huisache; sweet acacia; Texas huisache; cassie	2
Acacia greggii	Gregg catclaw; catclaw acacia; Texas catclaw; devilsclaw; long-flowered catclaw	2
Acacia koa	koa acacia	2
Acacia longifolia	golden wattle; Sydney golden wattle	2
Acacia spp.	acacia	2
Acacia tortuosa	huisachillo; catclaw; twisted acacia; Rio Grande acacia	2
Acacia wrightii	Wright catclaw; Texas catclaw; Wright acacia	2
Acer barbatum	Florida maple; sugar maple; hammock maple	2
Acer campestre	hedge maple; English field maple	2
Acer circinatum	vine maple	2
Acer dasycarpum	silver maple; cut-leaf maple	2
Acer ginnala	amur maple	3
Acer glabrum	Rocky Mountain maple; dwarf maple; mountain maple; Sierra maple	2
Acer grandidentatum	canyon maple; bigtooth maple; sugar maple; Uvalde bigtooth maple	2
Acer japonicum	fullmoon maple	2
Acer leucoderme	chalk maple; white-bark maple	2

Acer macrophyllum	bigleaf maple; Oregon maple; broadleaf maple	2
Acer negundo	boxelder; ash-leaved maple; boxelder maple; Manitoba maple	2
Acer nigrum	black maple; black sugar maple; hard maple; rock maple	2
Acer palmatum	Japanese maple	2
Acer pensylvanicum	striped maple; moosewood	3
Acer platanoides	Norway maple	2
Acer pseudoplatanus	planetree maple; sycamore maple	2
Acer rubrum	red maple; scarlet maple; swamp maple; soft maple	2
Acer saccharinum	silver maple; soft maple; river maple; silverleaf maple	3
Acer saccharum	sugar maple; hard maple; rock maple	2
Acer spicatum	mountain maple; moose maple	3
Acer tartaricum	tartarian maple; Tartar maple	2
Achras emarginata	wild-dilly	2
Aesculus californica	California buckeye	3
Aesculus glabra	Ohio buckeye; fetid buckeye; stinking buckeye; American horsechestnut	2
Aesculus hippocastanum	horsechestnut; common horsechestnut	3
Aesculus octandra	yellow buckeye; sweet buckeye; big buckeye	3
Aesculus pavia	red buckeye; scarlet buckeye; woolly buckeye; fire-cracker-plant	2
Aesculus sylvatica	painted buckeye; dwarf buckeye; Georgia buckeye	2
Ailanthus altissima	ailanthus; tree of heaven; Chinese tree-of-heaven; copaltree	2
Albizia julibrissin	silktree; mimosa; mimosa-tree; powderpuff-tree	3
Aleurites fordii	tung-oil-tree; tungtree	2
Alnus maritima	seaside alder	1
Alnus oblongifolia	Arizona alder; Mexican alder; New Mexican alder	1
Alnus rhombifolia	white alder; Sierra alder	2
Alnus rugosa	speckled alder; smooth alder; tag alder; gray alder; hoary alder; hazel alder	1
Alnus serrulata	hazel alder; smooth alder; common alder; tag alder; black alder	2
Alnus sinuata	Sitka alder; mountain alder; wavyleaf alder	2
Alnus tenuifolia	mountain alder; thinleaf alder; river alder	1
Alvaradoa amorphoides	Mexican alvaradoa	2
Amelanchier alnifolia	western serviceberry; saskatoon serviceberry; serviceberry; juneberry; western shadbush	2
Amelanchier arborea	downy serviceberry; Allegheny serviceberry; shadblow; apple shadbush	2
Amelanchier canadensis	thicket serviceberry; oblongleaf juneberry	2
Amelanchier laevis	Allegheny serviceberry; downy serviceberry; smooth serviceberry	2
Amelanchier spp.	serviceberry	2
Amyris elemifera	torchwood; candlewood; sea amyris	2
Annona glabra	pond-apple; alligator-apple	2
Aralia spinosa	devils-walkingstick; Hercules-club; prickly-ash; angelica-tree	3
Arbutus arizonica	Arizona madrone; madrona; Arizona madrono	2
Arbutus menziesii	Pacific madrone; madrone; madrona	1
Arbutus texana	Texas madrone; madrona	2
Arbutus unedo	strawberry madrone; strawberrytree	2
Ardisia escallonioides	marlberry; marbleberry	2
Ardisia japonica	Japanese ardisia; marlberry	3
Arecastrum romanzoffianum	queen palm; plumy coconut	3
Asimina triloba	pawpaw; common pawpaw; pawpaw apple; false-banana	2
Avicennia nitida	black-mangrove; blackwood	2
Betula alba:	European white birch; white-barked canoe birch; cut-leaved birch	2
Betula alleghaniensis	yellow birch; gray birch; silver birch; swamp birch	3
Betula caerulea	blueleaf birch	1
Betula eastwoodiae	Yukon birch	1
Betula lenta	sweet birch; black birch; cherry birch	2
Betula nigra	river birch; red birch; black birch; water birch	1
Betula occidentalis	water birch; red birch; black birch; spring birch; paper birch	2
Betula papyrifera	paper birch; canoe birch; white birch; silver birch	1
Betula pendula	European birch; European white birch; cut-leaf weeping birch; blueleaf birch	1
Betula populifolia	gray birch; grey birch; white birch; wire birch; fire birch; oldfield birch	1
Betula pumila	swamp birch; bog birch	1

Betula verrucosa	European white birch	1
Bourreria ovata	Bahama strongback; Bahama strongbark; strongback	3
Broussonetia papyrifera	paper mulberry; common paper mulberry	3
Bumelia lanuginosa	gum bumelia; woolly buckthorn; chittamwood; swiftwig-gum; gum elastic; buckthorn	2
Bursera simaruba	gumbo-limbo; West-Indian-birch; gum-elemi	2
Callitris glaucophylla	white cypress-pine	3
Calocedrus decurrens	incense-cedar	3
Calycanthus floridus	common sweetshrub; Carolina allspice; hairy (Caroline) allspice	3
Calyptranthes pallens	pale lidflower; spicewood; white spicewood	2
Calyptranthes zuzygium	myrtle-of-the-river; spicewood	2
Canella winterana	canella; cinnamonbark; wild-cinnamon	2
Canotia holacantha	canotia; Mohave thorn; crucifixion-thorn	2
Capparis cynophallophora	Jamaica caper; capertree; Jamaica capertree	2
Caragana arborescens	peatree; peashrub; Siberian peashrub; Siberian pea tree	2
Carica papaya	papaya; pawpaw	2
Carpinus caroliniana	American hornbeam; blue-beech; water-beech	2
Carya aquatica	water hickory; bitter pecan; swamp hickory; bitter water hickory	2
Carya cordiformis	bitternut hickory; bitternut; swamp hickory; pignut; pignut hickory	2
Carya floridana	scrub hickory; Florida hickory	2
Carya glabra	pignut hickory; pignut	2
Carya illinoensis	pecan; sweet pecan	2
Carya laciniosa	shellbark hickory; big shellbark hickory; king nut hickory; big shagbark hickory	2
Carya leiodermis	pignut hickory; swamp hickory	2
Carya myristiciformis	nutmeg hickory; swamp hickory; bitter water hickory	2
Carya ovalis	red hickory; small pignut; sweet pignut	2
Carya ovata	shagbark hickory; shellbark hickory; upland hickory; scalybark hickory	3
Carya pallida	sand hickory; pignut hickory; pale hickory; pallid hickory	2
Carya spp.	hickory	2
Carya texana	black hickory; bitter pecan; Buckley hickory; pignut hickory	2
Carya tomentosa	mockernut hickory; mockernut; white hickory; whiteheart hickory	2
Caryota urens	toddy palm; white palm; fishtail palm; wine palm	3
Castanea dentata	American chestnut; chestnut	2
Castanea ozarkensis	Ozark chinkapin; Ozark chestnut	2
Castanea pumila	Allegheny chinkapin	2
Castanopsis chrysophylla	giant chinkapin; golden chinkapin; giant evergreen chinkapin	1
Casuarina equisetifolia	horsetail casuarina; beefwood; Australian pine; horsetail-tree	2
Casuarina stricta	coastbeefwood	2
Catalpa bignonioides	southern catalpa; common catalpa; catawba; Indian-bean; cigartree	3
Catalpa speciosa	northern catalpa; hardy catalpa; western catalpa; catawba	3
Catalpa spp.	catalpa; hardy catalpa	3
Ceanothus arboreus	feltleaf ceanothus; island myrtle; Catalina ceanothus	3
Ceanothus integerrimus	deer brush	3
Ceanothus maritimus		2
Ceanothus spinosus	greenbark ceanothus; spiny ceanothus; redheart ceanothus; California-lilac	3
Ceanothus spp.	ceanothus	3
Ceanothus thysiflorus	blueblossom; blue-myrtle; blue-brush; blueblossom ceanothus	3
Cedrus atlantica	atlas cedar	2
Cedrus deodara	deodar cedar	2
Cedrus libani	Cedar of Lebanon	2
Celtis laevigata	sugarberry; southern hackberry; Mississippi hackberry; Texas sugarberry	3
Celtis occidentalis	hackberry; northern hackberry; sugarberry; nettletree	3
Celtis tenuifolia	Georgia hackberry; dwarf hackberry; upland hackberry	3
Cephalanthus occidentalis	buttonbush; buttonball bush; honey-balls; globeflowers	2
Cercidium floridum	blue paloverde; Texas paloverde; paloverde	2
Cercidium microphyllum	yellow paloverde; littleleaf hornbean; foothill paloverde; littleleaf paloverde	2
Cercis canadensis	eastern redbud; redbud; Judas tree	3
Cercis occidentalis	California redbud; western redbud; Arizona redbud	3
Cercocarpus betuloides	birchleaf cercocarpus; birchleaf mountain-mahogany; alderleaf cercocarpus	2

Cercocarpus breviflorus	hairy cercocarpus; Wright mountain-mahogany; hairy mountain-mahogany	2
Cercocarpus intricatus	little leaf mountain-mahogany	2
Cercocarpus ledifolius	curlleaf cercocarpus; mountain-mahogany; curlleaf mountain-mahogany	2
Cercocarpus montanus	alderleaf cercocarpus; alderleaf mountain-mahogany; mountain-mahogany; true mountain-mahogany	2
Cereus giganteus	saguaro; giant cactus; pitahaya	2
Chamaecyparis lawsoniana	Port-Orford-cedar; Port-Orford white-cedar; Oregon-cedar; Lawson cypress	3
Chamaecyparis nootkatensis	Alaska-cedar; Nootka cypress; Alaska yellow-cedar; Sitka cypress	3
Chamaecyparis thyoides	Altantic white-cedar; Atlantic cedar; white-cedar; southern white-cedar	3
Chilopsis linearis	desert-willow; desert-catalpa	3
Chionanthus virginicus	fringetree; fringe tree; old-mans-beard	2
Chrysobalanus icaco	cocoplum; icaco coco-plum; smallfruit cocoplum; Everglades cocoplum	2
Chrysophyllum oliviforme	satinleaf	2
Cinnamonum camphora	camphor-tree	1
Citharexylum fruticosum	fiddlewood; Florida fiddlewood	2
Citrus aurantifolia	limes key lime	2
Citrus limon	lemon	3
Citrus sinensis	orange; navel orange; sweet orange	2
Cladrastis lutea	yellow-wood	2
Clethra alnifolia	sweet pepperbrush; summersweet clethra	3
Clethra spp.	clethra; pepperbush	3
Cliftonia monophylla	buckwheat-tree; titi; black titi	2
Coccoloba diversifolia	pigeon-plum; doveplum; tie-tongue	2
Coccoloba uvifera	seagrape; grape-tree	2
Coccothrinax argentata	Florida silverpalm; Biscayne-palm; brittle thatch; thatchpalm	3
Cocos nucifera f	coconut; coconut palm	3
Colubrina reclinata	soldierwood	2
Conocarpus erectus	button-mangrove; buttonwood; silver buttonwood	2
Cordia sebestena	geiger-tree	3
Cornus alternifolia	alternate-leaf dogwood; blue cornel	3
Cornus drummondii	roughleaf dogwood	3
Cornus florida	flowering dogwood; dogwood; cornel; boxwood;	2
Cornus nuttallii	Pacific dogwood; flowering dogwood; mountain dogwood;	3
Cornus racemosa	gray dogwood	3
Cornus rugosa	roundleaf dogwood; roundleafed cornel;	3
Cornus spp.	dogwood; cornel ;	3
Cornus stolonifera	red-osier dogwood; American dogwood; redstem dogwood; kinnikinnik	3
Corylus americana	American hazelnut; American filbert; wild hazelnut	1
Corylus avellana	European hazelnut; European filbert	1
Corylus avena		1
Corylus cornuta	beaked hazelnut; beaked filbert; western hazelnut	2
Corylus rostrata	beaked hazelnut	1
Cotinus coggygria	smoketree; common smoketree	1
Cotinus obovatus	American smoketree; smoketree; chittamwood; yellowwood	1
Cotoneaster pyracantha	firethom; everlasting thorn	1
Cowania mexicana	cliffrose; Stansbury cliffrose; quininebush	2
Crataegus berberifolia	barberry hawthorn; bigtree hawthorn; barberryleaf hawthorn	1
Crataegus boyntonii	Biltmore hawthorn; Boynton hawthorn	1
Crataegus brachyeantha	blueberry hawthorn; blue haw; pommette blue	1
Crataegus coccinea	scarlet hawthorn; scarlet haw	1
Crataegus crus-galli	cockspur hawthorn; hog-apple; cockspur-thorn; Newcastle thorn	1
Crataegus douglasii	black hawthorn; Douglas hawthorn; river hawthorn	1
Crataegus induta	downy hawthorn; turkey hawthorn	1
Crataegus marshallii	parsley hawthorn; parsley-leaf hawthorn	1
Crataegus mollis	downy hawthorn	1
Crataegus monogyna	oneseed hawthorn; singleseed hawthorn; English hawthorn; European hawthorn	2
Crataegus opaca	riverflat hawthorn; English hawthorn; May hawthorn; May haw; apple haw	1
Crataegus oxyacantha	English hawthorn	1
Crataegus pedicellata	scarlet hawthorn	1

Crataegus pruinosa	frosted hawthorn; waxy-fruit thorn	1
Crataegus pyracantha	firethorn; white thorn	1
Crataegus saligna	willow hawthorn	1
Crataegus spathulata	littlehip hawthorn; small-fruit hawthorn; pasture hawthorn	1
Crataegus spp.	hawthorn	1
Cunninghamia lanceolata	China fir; blue Chinese fir	3
Cupressocyparis leylandii	Leyland cypress	3
Cupressus arizonica	Arizona cypress	3
Cupressus bakeri	Baker cypress; Siskiyou cypress; Modoc or MacNab cypress	3
Cupressus goveniana	Gowen cypress	3
Cupressus guadalupensis	Guadalupe cypress; Forbes' cypress; Tecate cyress:	3
Cupressus macnabiana	MacNab cypress	3
Cupressus macrocarpa	Monterey cypress	3
Cupressus sargentii	Sargent cypress	3
Cydonia japonica	common flowering quince; dwarf Japanese quince; Japan quince	2
Cydonia vulgaris	quince	2
Cyrilla racemiflora	swamp cyrilla; swamp ironwood; leatherwood:	2
Dalea spinosa	smokethorn; smoketree; indigobush	2
Diospyros texana	Texas persimmon; black persimmon; Mexican persimmon	3
Diospyros virginiana	persimmon; common persimmon; eastern persimmon; possumwood	3
Dipholis salicifolia	willow bustic; bustic; willow-leaf bustic; cassada	2
Drypetes lateriflora	Guiana-plum	3
Elaeagnus angustifolia	Russia-olive; oleaster	3
Elaeagnus hortensis	oleaster	2
Elliottia racemosa	elliottia; southern plume	2
Enallagma latifolia	black-calabash	3
Eriobotrya japonica	loquat; loquat tree	2
Erythrina herbacea	southeastern coralbean; eastern coralbean; Cherokee-bean	2
Ethretia anacua	anaqua	3
Eucalyptus botryiodes	bastard mahogany; bangalay	2
Eucalyptus camaldulensis	longbeak eucalyptus; camal eucalyptus; redgum	2
Eucalyptus camphora	eucalyptus	3
Eucalyptus cinerea	silver dollar eucalyptus	1
Eucalyptus diversifolia	eucalyptus	3
Eucalyptus globulus	bluegum eucalyptus; Tasmanian bluegum; bluegum	2
Eucalyptus gunnii	cider gumtree	1
Eucalyptus leucoxylon	white ironbark	2
Eucalyptus polyanthemos	redbox eucalyptus; redbox-gum; Australian beech; silver dollar gum	2
Eucalyptus pulchella	white peppermint	2
Eucalyptus rudis	desert gum	2
Eucalyptus sideroxylon	red ironbark	2
Eucalyptus spp.	eucalyptus; gum-tree	2
Eucalyptus tereticornis	horncap eucalyptus; gray or slaty gum	2
Euonymus atropurpureus	eastern burningbush; burningbush; eastern wahoo; strawberry-bush	2
Euonymus europaeus	European spindletree; European euonymus	2
Euonymus japonicus	Japanese euonymus; evergreen euonymus	2
Euonymus occidentalis	western burningbush; wahoo; western wahoo;	2
Euonymus verrucosa	spindle tree	2
Exostema caribaeum	princewood; Caribbean princewood	2
Exothea paniculata	inkwood; butterbough	2
Fagus grandifolia	American beech; beech	2
Fagus sylvatica	European beech	2
Fatsia japonica	Japanese fatsia; Japanese aralia	3
Ficus aurea	Florida strangler fig; golden fig; strangler fig; wild fig	2
Ficus benjamina	Java fig; Java willow; Benjamin fig	3
Ficus carica:	fig; common fig	2
Ficus elastica	India-rubber fig; rubber plant; India rubber tree;	2
Firmiana platanifolia	Chinese parasoltree	2

Forestiera acuminata	swamp-privet; forestiera; common adelia; whitewood	3
Fraxinus americana	white ash; Biltmore ash; Biltmore white ash	3
Fraxinus anomala	singleleaf ash; dwarf ash	3
Fraxinus caroliniana	Carolina ash; water ash; Florida ash; pop ash; swamp ash	3
Fraxinus cuspidata	fragrant ash; flowering ash	3
Fraxinus excelsior	European ash	2
Fraxinus greggii	Gregg ash; littleleaf ash; dogleg ash	3
Fraxinus latifolia	Oregon ash	3
Fraxinus nigra	black ash, swamp ash; basket ash; brown ash; hoop ash; water ash	3
Fraxinus pennsylvanica	green ash; red ash; Darlington ash; white ash; swamp ash; water ash	3
Fraxinus profunda	pumpkin ash; red ash	3
Fraxinus quadrangulata	blue ash	3
Fraxinus spp.	ash	3
Fraxinus texensis	Texas ash	3
Fraxinus uhdei	Hawaiian ash	3
Fraxinus velutina	velvet ash; Arizona ash; desert ash; Modesto ash; leatherleaf ash; smooth ash; Toumey ash	3
Garrya fremontii	Fremont silktassel; silk-tassel	3
Gaultheria shallon	salal, shallon	2
Ginkgo biloba	ginkgo; maidenhair tree:	3
Gleditsia aquatica	waterlocust	3
Gleditsia texana	honeylocust; Texas honeylocust	3
Gleditsia triacanthos	honeylocust; sweet-locust; thorny-locust	3
Gordonia lasianthus	loblolly-bay; tan bay; gordonia; bay; holly-bay	2
Grevillea 'noellii'	grevillea	3
Grevillea robusta	silk-oak; silky oak	3
Guaiacum sanctum	roughbark lignumvitae; holywood lignumvitae; lignumvitae	2
Guettarda elliptica	elliptic-leaf velvetseed; Everglades velvetseed; velvetseed	2
Guettarda scabra	roughleaf velvetseed	2
Gyminda latifolia	falsebox; false boxwood; West Indies falsebox	2
Gymnanthes lucida	oysterwood; crabwood	3
Gymnocladus dioicus	Kentucky coffeetree; coffeetree	3
Hakae spp.		2
Halesia carolina	Carolina silverbell; silver bell; snowdrop-tree; opossum-wood	3
Hamamelis virginiana	witch-hazel; common witch-hazel; southern witch-hazel	1
Heteromeles arbutifolia	toyon; Christmas berry; Califomia-holly; hollyberry	2
Hibiscus rosa-sinensis	Chinese hibiscus	2
Hibiscus tiliaceus	sea hibiscus; mahoe; tree hibiscus	2
Hippomane mancinella	manchineel	3
Ilex aquifolium	English holly	3
Ilex cassine	dahoon; dahoon holly; Alabama dahoon; Christmas-berry	3
Ilex coriacea	large gallberry; tall inkberry; gallberry; bay-gallbush	3
Ilex decidua	possurnhaw; deciduous holly; winterberry	3
Ilex glabra	inkberry; gallberry	3
Ilex krugiana	tawnyberry holly; Krug holly; southern holly	3
Ilex montana	mountain winterberry; mountain holly	3
Ilex opaca	American holly; holly; white holly	3
Ilex verticillata	common winterberry; black-alder; winterberry	3
Ilex vomitoria	yaupon; cassena; Christmas-berry; evergreen holly	3
Jasminum nudiflorum	winter jasmine	3
Juglans californica	southern California walnut; California walnut; California black walnut	2
Juglans cinerea	buttemut; white walnut; oilnut	2
Juglans hindsii	northern California walnut; Hinds walnut; California black walnut	2
Juglans major	Arizona walnut; Arizona black walnut	2
Juglans microcarpa	little walnut; Texas walnut; Texas black walnut; river walnut	2
Juglans nigra	black walnut; eastern black walnut; American walnut	2
Juniperus ashei	Ashe juniper; mountain-cedar; rock-cedar; post-cedar; Mexican juniper	3
Juniperus californica	California juniper	3
Juniperus coahuilensis	redberry juniper; roseberry	3

Juniperus communis	common juniper; dwarf juniper; prostrate juniper	3
Juniperus deppeana	alligator juniper; checker-bark juniper; western juniper	3
Juniperus erythrocarpa	redberry juniper; red-fruited juniper	3
Juniperus flaccida	drooping juniper; weeping juniper; Mexican drooping juniper	3
Juniperus monosperma	oneseed juniper; cherrystone juniper; West Texas juniper	3
Juniperus occidentalis	western juniper; Sierra juniper	3
Juniperus osteosperma	Utah juniper; bigberry juniper	3
Juniperus pinchotii	Pinchot juniper; redberry juniper	3
Juniperus scopulorum	Rocky Mountain juniper; Rocky Mountain cedar; redcedar; Colorado redcedar	3
Juniperus silicicola	southern redcedar; redcedar; sand-cedar; coast juniper	3
Juniperus virginiana	eastern redcedar; redcedar; red juniper; savin	3
Krugiodendron ferreum	leadwood; black-ironwood	2
Laguncularia racemosa	white-mangrove; white buttonwood; buttonwood	2
Larix decidua	European larch	1
Larix laricina	tamarack; eastern larch; American larch; Alaska larch; hackmatack	1
Larix Iyallii	subalpine larch; alpine larch; timberline larch; tamarack	1
Larix occidentalis	western larch; hackmatack; Montana larch; mountain larch	1
Leitneria floridana	corkwood	2
Lindera benzoin	spicebush	3
Liquidambar styraciflua	sweetgum; redgum; sapgun; starleaf-gum; bilsted	1
Liriodendron tulipifera	yellow-poplar; tuliptree; tulip-poplar; white-poplar	3
Lithocarpus densiflorus	tanoak; tan oak; tanbark oak	1
Lyonia ferruginea	tree lyonia; staggerbush; titi; rusty lyonia	2
Lyonothamnus floribundus	Lyontree; Catalina-ironwood; Iyonothamnus; Santa-Cruz-ironwood	2
Lysiloma bahamensis	Bahama lysiloma	2
Maclura pomifera	Osage-orange; bodark; bodock; bowwood; hedge-apple; horse-apple	3
Magnolia acuminata	cucumbertree, cucumber magnolia; mountain magnolia	3
Magnolia ashei	Ashe magnolia; sandhill magnolia	3
Magnolia fraseri	Fraser magnolia; mountain magnolia; earleaf cucumbertree	3
Magnolia grandiflora	southern magnolia; evergreen magnolia; bull-bay; big-laurel	3
Magnolia macrophylla	bigleaf magnolia; umbrella-tree; large-leaf cucumbertree	3
Magnolia pyramidata	pyramid magnolia; southern cucumbertree; mountain magnolia	3
Magnolia soulangeana	saucer magnolia; rustica rubra	3
Magnolia tripetala	umbrella magnolia; umbrella-tree; elkwood	3
Magnolia virginiana	sweetbay; swampbay; southern sweetbay; laurel magnolia	3
Malus angustifolia	southern crab apple; narrowleaf crab apple; wild crab apple	1
Malus coronaria	sweet crab apple; American crab apple; wild crab	1
Malus diversifolia	Oregon crab apple; Pacific crab apple; western crab apple; wild crab apple	1
Malus glabrata	sweet crab apple; Biltmore crab apple; wild crab	1
Malus ioensis	prairie crab apple; wild crab apple; Iowa crab	1
Malus spp	apple	1
Melaleuca decussata	lilac melaleuca	1
Melaleuca quinquenervia	cajeput-tree; punktree; bottlebrush	2
Melia azedarach	chinaberry; umbrella chinaberry; chinatree; pride-of-India	2
Mespilus germanica	medlar; showy mespilus; European medlar	2
Metasequoia glyptostroboides	dawn redwood	2
Metopium toxiferum	Florida poisontree; poisonwood; West Indies poisontree	1
Morus alba	white mulberry; silkworm mulberry; Russian mulberry; weeping mulberry	3
Morus microphylla	Texan mulberry; Texas mulberry; Mexican mulberry; mountain mulberry	3
Morus nigra	black mulberry	3
Morus rubra	red mulberry; moral	3
Morus tartarica	Tartarian mulberry	2
Myrica californica	Pacific bayberry; California bayberry; Pacific waxmyrtle; western waxmyrtle; California waxmyrtle	2
Myrica cerifera	southern bayberry; southern waxmyrtle; bayberry; candleberry	2
Nyssa aquatica	water tupelo; tupelo-gum; cotton-gum; sourgum	3
Nyssa ogeche	Ogeechee tupelo; sour tupelo-gum; Ogeechee-lime; sour tupelo	3
Nyssa sylvatica	black tupelo; blackgum; sourgum; pepperidge; tupelo	3
Nyssa sylvatica var. *biflora*	swamp tupelo, blackgum; swamp blackgum	3

Olea europaea	olive; common olive	3
Olneya tesota	tesota; desert ironwood; Arizona-ironwood	2
Osmanthus americana	devilwood; wild-olive	3
Ostrya knowltonii	Knowlton hophornbeam; western hophornbeam; wolf hophornbeam	2
Ostrya virginiana	eastern hophornbeam; hophornbeam; American hophornbeam; hornbeam; leverwood	1
Oxydendrum arboreum	sourwood; sorrel-tree; lily-of-the-valley-tree	2
Parkinsonia aculeata	Jerusalem-thorn; horsebean; Mexican paloverde	2
Paulownia tomentosa	royal paulownia; empress-tree; princess-tree; paulownia	3
Paurotis wrightii	paurotis-palm; paurotis	3
Persea americana	avocado; zutano avocado; alligator-pear	2
Persea borbonia	redbay; shorebay	2
Photinia arbutifolia	toyon; Christmas berry	2
Photinia glabra	Japanese photinia	2
Photinia serrulata	Chinese photinia; Chinese medlar	2
Photinia spp.	toyon; photinia	3
Picea abies	Norway spruce	2
Picea breweriana	Brewer spruce; weeping spruce	2
Picea englemannii	Engelmann spruce; Columbian spruce; mountain spruce; silver spruce; white spruce	2
Picea glauca	white spruce; skunk spruce; Canadian spruce; cat spruce	2
Picea glauca var. *conica*	dwarf Alberta spruce; dwarf white spruce	2
Picea mariana	black spruce; bog spruce, swamp spruce; shortleaf black spruce	2
Picea polita	tigertail spruce	2
Picea pungens	blue spruce; Colorado blue spruce; Colorado spruce; silver spruce	2
Picea rubens	red spruce; yellow spruce; West Virginia spruce; eastern spruce	2
Picea sitchensis	Sitka spruce; coast spruce; tideland spruce; yellow spruce	2
Picea spp.	spruce	2
Picramnia pentandra	bitterbush; Florida bitterbush	2
Pinckneya pubens	pinckneya; fevertree; Georgia-bark; fever-bark	2
Pinus albicaulis	whitebark pine; scrub pine; white pine	2
Pinus aristata	bristlecone pine; hickory pine; foxtail pine	2
Pinus attenuata	knobcone pine	2
Pinus balfouriana	foxtail pine	2
Pinus banksiana	jack pine; scrub pine; gray pine; black pine; Banksian pine	2
Pinus cembroides	Mexican pinyon; nut pine; Mexican stone pine	2
Pinus clausa	sand pine; scrub pine; spruce pine	2
Pinus contorta	lodgepole pine; shore pine; beach pine	2
Pinus coulteri	Coulter pine; bigcone pine; pitch pine	2
Pinus discolor	border pinyon	2
Pinus echinata	shortleaf pine; shortleaf yellow pine; yellow pine	2
Pinus edulis	pinyon; two-leaf pinyon; two-needle pinyon	2
Pinus elliottii	slash pine; yellow slash pine; swamp pine; pitch pine	2
Pinus engelmannii	Apache pine; Arizona longleaf pine	2
Pinus flexlis	limber pine; white pine, Rocky Mountain whitepine	2
Pinus glabra	spruce pine; cedar pine; Walter pine; bottom white pine	2
Pinus halepensis	Aleppo pine	2
Pinus jeffreyi	Jeffrey pine; western yellow pine; bull pine; black pine; ponderosa pine	2
Pinus lambertiana	sugar pine; California sugar pine	2
Pinus leiophylla var. chihuahuana	Chihuahua pine; yellow pine	2
Pinus longaeva	intermountain bristlecone pine	2
Pinus monophylla	singleleaf pinyon; pinyon; nut pine	2
Pinus monticola	western white pine; mountain white pine; Idaho white pine; silver pine	2
Pinus mugo	mugo pine; mountain pine; Swiss mountain pine	2
Pinus muricata	bishop pine; prickle-cone pine; Santa Cruz Island pine	2
Pinus nigra	Austrian pine; European black pine	2
Pinus palustris	longleaf pine; swamp pine; longleaf yellow pine; southern yellow pine	3
Pinus pinea	Italian stone pine	3
Pinus ponderosa	ponderosa pine; western yellow pine; yellow pine	2

Pinus ponderosa var. *arizonica*	Arizona pine; Arizona ponderosa pine; yellow pine	2
Pinus pungens	Table Mountain pine; mountain pine; hickory pine	2
Pinus quadrifolia	Parry pinyon; four-needle pinyon; nut pine	2
Pinus radiata	Monterey pine; insignis pine	2
Pinus resinosa	red pine; Norway pine	2
Pinus rigida	pitch pine	3
Pinus sabiniana	Digger pine; bull pine; gray pine	2
Pinus serotina	pond pine; marsh pine; pocosin pine	2
Pinus spp.	pine	2
Pinus strobiformis	southwestern white pine; Mexican white pine; border white pine	2
Pinus strobus	eastern white pine; northern white pine; white pine	2
Pinus sylvestris	Scotch pine; Scots pine	2
Pinus taeda	loblolly pine; oldfield pine; shortleaf pine	2
Pinus thunbergiana	Japanese black pine	3
Pinus torreyana	Torrey pine; Del Mar pine; Soledad pine	2
Pinus virginiana	Virginia pine; Virginia scrub pine; spruce pine; Jersey pine; scrub pine; poverty pine	2
Pinus washoensis	Washoe pine	2
Piscidia piscipula	Florida fishpoison-tree; Jamaica-dogwood; Florida fishfuddletree	2
Pistacia texana	Texas pistache; American pistachio; wild pistachio	1
Pistacia vera	pistachio	1
Planera aquatica	water-elm; planertree	2
Platanus occidentalis	sycamore; American sycamore; American planetree; buttonwood	3
Platanus orientalis	Oriental planetree	2
Platanus racemosa	California sycamore; western sycamore; California planetree	3
Platanus wrightii	Arizona sycamore; Arizona planetree	3
Populus alba	white poplar; silver poplar	2
Populus angustifolia	narrowleaf cottonwood; black cottonwood; mountain cottonwood; narrowleaf poplar	1
Populus balsamifera	balsam poplar; balm; balm-of-Gilead; bam; tacamahac	1
Populus deltoides	eastern cottonwood; eastern poplar; southern cottonwood	2
Populus fremontii	Fremont cottonwood; cottonwood	2
Populus grandidentata	bigtooth aspen; largetoothed aspen; aspen; poplar; popple	1
Populus heterophylla	swamp cottonwood; black cottonwood; river cottonwood	1
Populus nigra var. *italica*	Lombardy poplar	1
Populus palmeri	eastern cottonwood; eastern poplar; Palmer cottonwood	1
Populus sargentii	plains cottonwood; great plains cottonwood; sargent cottonwood	1
Populus spp.	cottonwood; poplar	1
Populus tremuloides	quaking aspen; trembling aspen; golden aspen	1
Populus trichocarpa	black cottonwood; western balsam poplar; cottonwood; balsam cottonwood	1
Populus wislizenii	Rio Grande cottonwood; valley cottonwood	1
Prosopis juliflora	honeylocust; mesquite; algaroba	2
Prosopis pubescens	screwbean mesquite; screwbean	2
Prunus alleghaniensis	Allegheny plum; sloe plum; sloe; Allegheny sloe; northern sloe	2
Prunus americana	American plum; wild plum; red plum; river plum; yellow plum	2
Prunus angustifolia	Chickasaw plum; sand plum	2
Prunus avium	mazzard; common sweet cherry; English cherry	2
Prunus caroliniana	Carolina laurelcherry; laurel cherry; cherry-laurel	2
Prunus domestica	garden plum; plum; Damson plum	2
Prunus emarginata	bitter cherry; quinine cherry; wild cherry	2
Prunusfremontii	desert apricot	2
Prunus glandulosa	flowering almond; dwarf flowing almond; almond cherry; wild peach	2
Prunus hortulana	hortulan plum; wild goose plum; Miner plum; wild plum	2
Prunus japonica	Japanese plum	2
Prunus laurocerasus	cherry laurel; English laurel	2
Prunus Iyonii	Catalina cherry	2
Prunus maritima	beach plum	2
Prunus mexicana	Mexican plum; bigtree plum; inch plum	2
Prunus munsoniana	wildgoose plum; Munson plum	2

Prunus myrtifolia	West Indies cherry; myrtle laurel cherry; laurelcherry	2
Prunus nigra	Canada plum; red plum; horse plum; wild plum	2
Prunus padus	European bird-cherry; black serviceberry	2
Prunus pensylvanica	pin cherry; wild red cherry; fire cherry; northern pin cherry; pigeon cherry; bird cherry	3
Prunus persica	peach; nectarine; heavenly white nectarine; Tilton apricot	2
Prunus pissardi	purple-leaved prune	2
Prunus pumila	sand cherry	2
Prunus serotina	black cherry; wild black cherry; rum cherry; mountain black cherry	2
Prunus spinosa	sloe; blackthorn	2
Prunus spp.	cherry; plum	2
Prunus subcordata	Klamath plum; Sierra plum; Pacific plum; western plum; wild plum	2
Prunus umbellata	flatwoods plum; black sloe; hog plum; sloe	2
Prunus virginiana	chokecherry; common chokecherry; black chokecherry; California chokecherry	2
Pseudophoenix sargentii	buccaneer-palm; Florida cherrypalm; Sargent cherrypalm	3
Pseudotsuga macrocarpa	bigcone Douglas-fir; bigcone-spruce; hemlock	2
Pseudotsuga menziesii	Douglas-fir; red-fir; Oregon-pine; Douglas-spruce	2
Psidium guajava	guava; common guava; guayaba	2
Ptelea trifoliata	hoptree; common hoptree; wafer-ash	2
Punica granatum	pomegranate	2
Pyracantha coccinea	scarlet firethorn; everlasting thorn; fire thorn	2
Pyrus angustifolia	narrowleaf crab apple	1
Pyrus arbutifolia	red chokecherry; red chokeberry; chokeberry	2
Pyrus communis	pear; common pear	2
Pyrus malus	wild apple; common apple	1
Quercus agrifolia	coast live oak; California live oak	1
Quercus alba	white oak; stave oak	1
Quercus arizonica	Arizona white oak; Arizona oak	1
Quercus austrina	Durand oak; Durand white oak; bluff oak	1
Quercus bicolor	swamp white oak	1
Quercus chapmanii	Chapman oak; Chapman white oak; scrub oak	1
Quercus chrysolepis	canyon live oak; California live oak: canyon oak; goldcup oak; live oak; maul oak	1
Quercus coccinea	scarlet oak; black oak; Spanish oak	1
Quercus douglasii	blue oak; California blue oak; iron oak; mountain white oak; mountain oak	1
Quercus durandii	Durand oak; Durand white oak; bluff oak; white oak	1
Quercus ellipsoidalis	northern pin oak; jack oak; black oak; Hill oak	1
Quercus emoryi	Emory oak; black oak; blackjack oak	1
Quercus engelmannii	Engelmann oak; evergreen white oak; mesa oak; Engelmann spruce	1
Quercus falcata	southern red oak; Spanish oak; water oak; red oak	1
Quercus gambelii	Gambel oak; Rocky Mountain white oak; Utah white oak; white oak	1
Quercus garryana	Oregon white Oak; Oregon oak; Garry oak; post oak; Brewer oak; shin oak	1
Quercus hemisphaerica	laurel oak; Darlington oak	1
Quercus hypoleucoides	silverleaf oak; white-leaf oak	1
Quercus ilicifolia	bear oak; scrub oak	1
Quercus imbricaria	shingle oak; laurel oak	1
Quercus incana	bluejack oak; cinnamon oak; sandjack; bluejack; shin oak; turkey oak	1
Quercus kelloggii	California black oak; black oak; Kellogg oak	1
Quercus laevis	turkey oak; Catesby oak; scrub oak	1
Quercus laurifolia	laurel oak; Darlington oak; diamond-leaf oak; swamp laurel oak	1
Quercus lobata	valley oak; California white oak; valley white oak; water oak	1
Quercus Iyrata	overcup oak; swamp post oak; swamp white oak; water white oak	1
Quercus macrocarpa	bur oak; mossy cup oak; blue oak; mossy-overcup oak; scrub oak	1
Quercus margaretta	sand post oak; small post oak; dwarf post oak; post oak	1
Quercus marilandica	blackjack oak; blackjack; barren oak; black oak; jack oak	1
Quercus michauxii	swamp chestnut oak; basket oak; cow oak	1
Quercus muehlenbergii	chinkapin oak; yellow chestnut oak; chestnut oak; frock chestnut oak	1
Quercus myrtifolia	myrtle oak; scrub oak	1
Quercus nigra	water oak; possum oak; spotted oak	1
Quercus nuttallii	Nuttall oak; red oak; Red River oak; pin oak	1

Quercus oblongifolia	Mexican blue oak	1
Quercus oglethorpensis	Oglethorpe oak	1
Quercus pagoda	cherrybark oak; swamp red oak; bottomland red oak	1
Quercus palustris	pin oak; swamp oak; water oak; swamp Spanish oak; Spanish oak	1
Quercus phellos	willow oak; pin oak; peach oak; swamp willow oak	1
Quercus prinus	chestnut oak; basket oak; rock chestnut oak; rock oak; tanbark oak	1
Quercus rubra	northern red oak; red oak; common red oak; gray oak; eastern red oak; rnountain red oak	1
Quercus shumardii	Shumard oak; Shumard red oak; spotted oak; Schneck oak; Schneck red oak; southern red oak	1
Quercus stellata	post oak; iron oak	1
Quercus suber	cork oak	1
Quercus undulata	Rocky Mountain shin oak; wavyleaf oak	1
Quercus velutina	black oak; yellow oak; quercitron oak; yellow-bark oak; smooth-bark oak	1
Quercus virginiana	live oak; Virginia live oak	1
Quercus wislizenii	interior live oak; highland live oak; Sierra live oak	1
Rapanea guianensis	Guiana rapanea	2
Reynosia septentrionalis	darling-plum; red-ironwood	2
Rhamnus californica	California buckthorn; coffeeberry; California coffeeberry; pigeonberry	3
Rhamnus caroliniana	Carolina buckthorn; Indian-cherry; yellow buckthorn; tree buckthorn; yellowwood	3
Rhamnus cathartica	European buckthorn; common buckthorn; European waythorn	3
Rhamnus frangula	glossy buckthorn; alder buckthorn	3
Rhamnus purshiana	cascara buckthorn; cascara; cascara sagrada; bearberry; chittam; coffeetree	2
Rhizophora mangle	mangrove; red mangrove	2
Rhus copallina	shining sumac; dwarf sumac; winged sumac; wing-rib sumac; flameleaf sumac	2
Rhus corallina	mountain sumac	1
Rhus cotinus	smoketree; common smoketree	2
Rhus glabra	smooth sumac; scarlet sumac; common sumac; Rocky Mountain sumac; red sumac	1
Rhus integrifolia	lemonade sumac; sourberry; lemonade-berry; mahogany sumac	2
Rhus typhina	staghorn sumac; velvet sumac	l
Ribes uva-crispa	English gooseberry	2
Robinia neomexicana	New Mexico locust; New Mexican locust; southwestern locust	3
Robinia pseudoacacia	black locust; common locust; yellow locust; white locust	3
Robinia spp.	locust	2
Robinia viscosa	clammy locust	3
Rosa bracteata	Macartney rose	2
Rosa eglanteria	sweetbriar; sweetbriar rose	2
Rosa setigera	prairie rose; climbing prairie rose	2
Rosa spp.	rose	1
Roystonea elata	Florida royalpalm; Cuban royalpalm; royalpalm	3
Sabal palmetto	cabbage palmetto; common palmetto; Carolina palmetto; palmetto; cabbage-palm	3
Salix alaxensis	feltleaf willow	1
Salix alba	white willow; European white willow	1
Salix alba var. *tristis*	golden weeping willow	1
Salix amygdaloides	peachleaf willow; peachleaved willow; almond willow; peach willow; southwestern peach willow	1
Salix babylonica	weeping willow; Babylon weeping willow; Napolean willow	2
Salix bebbiana	Bebb willow; beak willow; long-beak willow; diamond willow	1
Salix bonplandiana	Bonpland willow; Toumey willow; red willow; polished willow	1
Salix caroliniana	Coastal Plain willow; Ward willow; southern willow; Harbison willow	1
Salix cordata	heartleaf willow; heart-leaved willow	1
Salix discolor	pussy willow; glaucous willow; silvery pussy willow	1
Salix eriocephala	pussy willow	1
Salix fragilis	crack willow; brittle willow; snap willow	1
Salix hookerana	Hooker willow; coast willow; Yakutat willow; bigleaf willow	1
Salix interior	sandbar willow; coyote willow; acequia willow; basket willow; gray willow; sandbar willow	1
Salix laevigata	Bondpland willow; red willow; Toumey willow; polished willow	1
Salix lasiandra	Pacific willow; whiplash willow; black willow; red willow; western black willow; yellow willow	1
Salix lasiolepis	arroyo willow; white willow	1
Salix lucida	shining willow; shiny willow	1
Salix mackenzieana	Mackenzie willow	1

Salix nigra	black willow; swamp willow; Goodding willow; western black willow; Dudley willow	1
Salix pentandra	laurel willow; bay willow; bayleaf willow	2
Salix scouleriana	Scouler willow; fire willow; black willow; mountain willow; Nuttall willow	1
Salix spp.	willow	1
Salix taxifolia	yewleaf willow; yew willow	1
Salix viminalis	basket willow; osier; common osier; silky osier	1
Sambucus callicarpa	Pacific red elder; Pacific elder; coast red elder; redberry elder; red elderberry	2
Sambucus canadensis	American elder; common elderberry; common elder; blackberry elder	3
Sapindus drummondii	western soapberry; wild chinatree; cherioni	2
Sapindus marginatus	wingleaf soapberry; Florida soapberry	2
Sapindus saponaria	wingleaf soapberry; Florida soapberry; southern soapberry; Mexican soapberry; wild chinatree	2
Sapium sebiferum	tallowtree; Chinese tallowtree	3
Sassafras albidum	sassafras; white sassafras	2
Schinus molle	peppertree; California peppertree; Peru peppertree	1
Sequoia sempervirens	redwood; coast redwood; California redwood	2
Sequoiadendron giganteum	giant sequoia; sequoia; bigtree; Sierra redwood	2
Sideroxylon foetidissimum	false-mastic; mastic; wild-mastic; wild-olive	2
Simarouba glauca	paradise-tree; bitterwood	2
Sophora affinis	Texas sophora; coralbean; pink-sophora; Eves-necklace	3
Sophora japonica	Japanese pagoda-tree	3
Sophora secundiflora	mescalbean; frigolito; coralbean; Texas-mountain-laurel	2
Sorbus americana	American mountain-ash; mountain-ash; roundwood	1
Sorbus aucuparia	European mountain-ash; Rowan-tree	1
Spiraea bumalda	Bumalda spirea; spirea	3
Stewartia koreana	Korean stewartia; stewartia	3
Stewartia ovata	mountain stewartia; mountain-camellia; angel-fruit stewartia	2
Swietenia mahagoni	West Indies mahogany; mahogany	2
Symphoricarpos albus	snowberry; waxberry; common snowberry	3
Symplocos tinctoria	sweetleaf; horse-sugar; common sweetleaf; yellowwood	2
Tamarix parviflora	small-flower tamarisk	2
Taxodium distichum	baldcypress	3
Taxodium mucronatum	Montezuma baldcypress; Mexican cyress	3
Taxus brevifolia	Pacific yew; western yew	3
Taxus floridana	Florida yew	3
Thrinax microcarpa	key thatchpalm; silvertop palmetto; prickly thatch; brittle thatch; brittle thatch palm	3
Thrinax parviflora	Jamaica thatchpalm	3
Thuja occidentalis	northern white-cedar; white-cedar; eastern arborvitae; American arborvitae; eastern white-cedar	3
Thuja orientalis	oriental arborvitae; Chinese arborvitae	3
Thuja plicata	western redcedar; giant western arborvitae; Pacific redcedar; giant-cedar; arborvitae; canoe-cedar	3
Tilia americana	American basswood; American linden; basswood	2
Tilia caroliniana	Carolina basswood; Florida basswood; basswood; Carolina linden; Florida linden	2
Tilia cordata	littleleaf linden; small-leaved linden; small-leaved European linden	2
Tilia europaea	European linden; common linden	2
Tilia floridana	Florida basswood; Carolina basswood	2
Tilia heterophylla	white basswood; beetree; linden; beetree linden	2
Torreya californica	California torreya; California-nutmeg	3
Torreya taxifolia	Florida torreya; stinking-cedar	3
Torrubia longifolia	Iongleaf blolly; Brace blolly; roundleaf blolly; beeftree; beefwood	2
Toxicodendron vernix	poison-sumac; poison-dogwood; poison-elder; thunderwood	1
Trema micrantha	FIorida trema	2
Tsuga canadensis	eastern hemlock; Canadian hemlock; Canada hemlock; hemlock spruce; common hemlock	2
Tsuga caroliniana	Carolina hemlock	2
Tsuga heterophylla	western hemlock; Pacific hemlock; west coast hemlock	2
Tsuga mertensiana	mountain hemlock; black hemlock; alpine hemlock; hemlock spruce	2
Ulmus alata	winged elm; wahoo elm; cork elm; wahoo	2
Ulmus americana	American elm; white elm; water elm; soft elm; Florida elm	2
Ulmus campestris	English elm, European elm	2
Ulmus crassifolia	cedar elm; basket elm; red elm; southern rock elm	2

Ulmus glabra	Scotch elm; wych elm	2
Ulmus montana	Scotch elm	2
Ulmus parvifolia	Chinese elm; lacebark	2
Ulmus pumila	Siberian elm; Asiatic elm; dwarf Asiatic elm, Pekin elm	2
Ulmus racemosa	rock elm; cork elm	2
Ulmus rubra	slippery elm; red elm; gray elm; soft elm	3
Ulmus serotina	September elm; red elm	2
Ulmus spp.	elm	2
Ulmus thomasii	rock elm; cork elm	2
Umbellularia californica	California-laurel; California-bay; Oregon-myrtle; Pacific-myrtle; pepperwood; spice-tree	3
Vauquelinia californica	Torrey vauquelinia; Arizona-rosewood	2
Veitchia merrillii	Manila palm	3
Viburnum acerifolium	mapleleaf viburnum; dockmackie; maple-leaved arrowwood	3
Viburnum ellipticum	western blackhaw; oval-leafed virburnum	2
Viburnum lantana	wayfaringtree	2
Viburnum lentago	nannyberry; sheepberry; sweet viburnum; blackhaw; sheepberry	3
Viburnum opulus	European cranberrybush; highbush cranberry; cranberry tree	3
Viburnum prunifolium	blackhaw; stagbush; sweethaw	2
Viburnum pubescens	downy viburnum; hairy nannyberry; downy arrowwood	2
Viburnum rhytidophyllum	leatherleaf viburnum	3
Viburnum spp.	viburnum; wayfaringtree	3
Viburnum tomentosum	doublefile viburnum	3
Washingtonia filifera	California washingtonia; California-palm; fanpalm; California fanpalm; desert-palm	3
Ximenia americana	tallowwood; hogplum	3
Zanthoxylum americanum	common prickly-ash; toothache-tree; northern prickly-ash; prickly-ash	2
Zanthoxylum clava-herculis	Hercules-club; pepperbark; southern prickly-ash; toothache-tree; tingle-tongue	2
Zanthoxylum fagara	lime prickly-ash; wild-lime-tree; wild-lime	2
Zanthoxylum flavum	West Indies satinwood; yellowheart; satinwood; yellowwood	2

14.1.2 Suitability of hosts for Asian and North American populations of the gypsy moth

Michael E. Montgomery
US Forest Service, NEFES, 51 Mill Pond Road, Hamden CT 06514
(unpublished report)

Introduction: The suitability of several host plants was compared for gypsy moth from Asia (Siberia, Russia), and North America (Massachusetts). The hosts tested included species native to Asia, Europe and eastern and western North America. First instar gypsy moth larvae were used for the bioassay because this stage is most sensitive to host plant quality. Larvae were reared from hatch for 10 days. Data recorded were the initial and final weight of the larvae, survival, and percentage molted to the second instar. The tests were conducted in the USDA Forest Service Quarantine Laboratory at Ansonia. Connecticut

Results: The Russian larvae were larger at the end of the 10-day rearing period for 39 of the 40 species where both biotypes survived. The Russian larvae also developed faster with more larvae in the second instar at the end of the 10 days. The hatch weight of the Russian larvae averaged 0.70 mg compared to 0.63 mg for the North American larvae. To account for the larger initial size and the fact that growth is exponential, relative growth rates (RGR = ($\mathit{In}W_i/\mathit{ln}W_f$)/time)) were calculated to obtain mg gain in weight/mean body weight/day. These values paralleled the absolute body weights; RGR was also greater for the Asian population for 39 of 40 species. The RGR of the two populations were well correlated (r=0.90) with the RGR of the Asian being twice as great on average. The greatest differences in RGR occurred on *Pseudotsugae menziesii*, *Robinia pseudoacacia, Acer rubrum,* and *Prunus serotina*, hosts of intermediate suitability, where RGR of the Asian larvae was from 4.5 to 5.8 times greater.

These tests were designed to discern differences in host suitability between geographic populations of gypsy moth. Caution must be used in drawing conclusions from the data about differences in the relative suitability between the host species tested for the gypsy moth. The tests were done so that the phenology and condition of the host plant would be ideal for growth and survival of the gypsy moth. Growth on some species such as *Carya tomentosa* and *Fraximus pennsylvanica* was better than expected, perhaps because the foliage was more mature than the larvae would encounter in nature. Growth on *Larix occidentalis* was poorer than expected, likely because the larvae severed the tender shoots and thus were without fresh food for part of the feeding period.

Summation: The data indicate that Asian populations of gypsy moth can be expected to grow faster and survive better than gypsy moth currently established in North America. Greatest differences will likely occur on host tree species such a Douglas fir that are of intermediate suitablility; however, host species immune to one population likely will be immune to the other.

WEIGHTS (MG) OF NEWLY HATCHED LARVAE AFTER 10 DAYS ON HOST

Host species	Geographic Population	
	North American	Asian
Carya tomentosa	21.5	32.6
Betula populifolia	9.6	28.9
Quercus velutina	10.6	27.7
Fagus grandifloia	10.5	26.5
Rosa rugosa	10.3	19.6
Hamamelis virginiana	4.81	8.5
Prunus serotina	3.8	17.6
Sassafras albidum	5.1	15.8
Eucalyptus cinera	5.0	11.2
Populus grandidentata	6.0	12.0
Cotoneaster apiculata	4.7	13.2
Quercus rubra	5.9	11.2
Rhus glabra	6.6	10.0
Myrica pensylvanica	8.0	7.1
Salix x *blanda*	3.0	11.6
Corylus avellana	5.7	8.8
Acer pegunda	3.9	8.3
Pseudotsuga menziesii	1.6	9.2
Betula nigra	4.6	5.4
Carpinus caroliniana	3.4	6.0
Arctostaphylos densiflora	3.0	6.4
Vaccinum corymbosum	2.4	5.7
Larix occidentalis	3.0	4.8
Pinus monticola	2.5	4.7
Photina glabra	1.8	5.0
Ulmus parvifolia	1.9	4.4
Populus fremontii	1.5	4.7
Acer rubrum	1.1	5.0
Robinia pseudoacacia	0.8	4.0
Betula lenta	1.4	3.7
Fraxinus pennsylvanica	1.3	2.9
Acer ginnaia	1.3	2.6
Aesculus glabra	1.1	2.9
Stewartia koreana	dead	1.9
Taxodium distichum	1.5	2.3
Spirea bumalda	1.0	2.1
llex verticillata	0.7	1.2
Elaeagnus augustifolia	0.9	1.2
Oxydendrum arboreum	0.9	1.1
Phellondendron amurense	dead	0.9
Amelanchier canadensis	0.8	1.0
Celtis occicentalis	0.8	dead
Viburnum tomentosum	0.4	1.0
Morus alba	0.1	dead
Halesia carolina	dead	dead
Sophora japonica	dead	dead

Appendix 14.2 New Zealand risk assessments of gypsy moth

14.2.1. A Risk Assessment of the Asian Gypsy Moth to Key Elements of the New Zealand Flora

M. Kay[1, 2], M. Matsuki[1, 3], J. Serin[1] and John K. Scott[1]
[1]CSIRO European Laboratory, Campus International de Baillarguet, Montferrier-sur-Lez, 34980 France, [2]Forest Research, Private Bag 3020, Rotorua, New Zealand, [3] CSIRO Entomology, GPO Box 1700, Canberra, ACT 2601, Australia.

Forest Research report, 2002.

Summary

1. Viable egg masses of Asian gypsy moth (AGM) (*Lymantria dispar*), are continuously intercepted on cargo from Asia, so there is a possibility of its establishment in Australia and New Zealand. The potential risk of establishment of this extremely polyphagous moth was assessed from a study of the insect's host range and potential geographic distribution.
2. We predicted potential geographic range of AGM based on climatic conditions and found AGM should be able to persist in SE and SW Australia and New Zealand.
3. Using three populations of gypsy moth and 75 plant species, we also conducted laboratory trials to examine the ability of AGM larvae to complete development on native plants from Australia and New Zealand.
4. Larval performance on at least five species of Australian native plants was as good as that on AGM’s preferred host species (*Q. humilis* and *Q. robur*). However, larval performance on Australasian *Nothofagus* species was poor.
5. Given the acceptibility of some Australian plants and a climate suitable for the establishment of AGM, this insect should be treated as a quarantine threat, but more particularly to naturalised Northern Hemisphere host plants.

Key words Lymantria dispar, *Asian gypsy moth*, *host range*, *biosecurity*, Eucalyptus, Nothofagus, Callistemon, Acacia, *predicted distribution, CLIMEX, Australia, New Zealand*

Introduction

As an island, New Zealand is seen to be ecologically predisposed to invasion by organisms from surrounding landmasses. As a country reliant on trade, New Zealand is particularly exposed to a wide variety of organisms which normally would be unlikely to survive long distance dispersal. With its diverse naturalised exotic flora and a mild climate, the potential for invertebrate herbivores to establish in New Zealand is considered to be high (Penman 1998). Endemism in the indigenous flora is high but the habitat is greatly disturbed from its original forested state. The depauperate tritrophic fauna suggests that the establishment of potentially invasive species in New Zealand would largely be dependent upon the availability of suitable food.

The prominent plant families of New Zealand's protection forest (the Nothofagaceae and Podocarpaceae) are confined, biogeographically, almost exclusively to the Southern Hemisphere, where they dominate the cool-temperate forests of the circum-Pacific southern landmasses. The five taxa of *Nothofagus* in New Zealand represent a major component of the more than 6 million hectares of remaining indigenous forest. As pure or mixed forests they account for about 84% of the total indigenous forest of the South Island and 40% of the North Island (Wardle 1984). *Nothofagus* forests are distinct from their surrounding vegetation in almost every possible way (McQuillan 1993). Typically they are relatively simple floristically and often form a mono-specific canopy with little understory diversity. All New Zealand species can be found in mono-specific stands, which, with the exception of *N. truncata*, may be extensive. However, species ranges do overlap and mixtures of two or three species are common (Ogden *et al.*1996).

It is well known that plants are not passive targets for herbivores and that phytophagous insects are presumed to have co-evolved with their host plants so that over time and geographic association, plants accumulate a distinctive community of phytophages. Insect diets tend, therefore, to be conservative, with related insects tending to feed on related plants, even though it can be shown that they can thrive on other 'non-host' plants. It follows that insects from the Northern Hemisphere – unless they are very broadly polyphagous – would find the New Zealand endemic flora an unsuitable food resource, but would easily establish a bridgehead on the naturalised Northern Hemisphere plant species, many of which form the basis of New Zealand's primary-product-based economy.

Lymantria dispar (L.) (Lepidoptera: Lymantriidae), the Asian gypsy moth (AGM), represents just such a polyphagous forest defoliator, with a proven invasive ability in comparatively complex habitats (Campbell 1979). This insect is continually intercepted at New Zealand ports on trade goods entering the country (MAF 2000, NZFRI Records) and it is the subject of a large, continual, research effort for a number of nations (Walsh 1993, Lipa and Kolk 1995, Roy *et al.*1995, Savotikov *et al.*1995). Gypsy moth is capable of completing its development on over 600 species of woody plant (Leibhold *et al.*1995). Host selection by female gypsy moth, however, is constrained by the limited dispersal of females of the European strain (EGM) and the cold-adapted behaviour, which favours oviposition on structures which may eventually be covered by a thermal blanket of snow. Host selection therefore is primarily a larval role, usually of early instar larvae 'ballooning' on silken treads.

A number of methods have been used to evaluate the risk of gypsy moth to forests. The best, but obviously limited, of these are probably the evaluations of defoliation to tree species within infested forests (*eg* Campbell & Sloan 1977, Mauffette & Lechowicz 1984). Other assessments have relied on laboratory based larval trials of defoliation (*eg* Wallner & Walton 1979), host-preference (*eg* Barbosa *et al.*1979), survival and/or growth (*eg* Miller & Hanson 1989, Stoyenoff *et al.*1994) in an effort to predict defoliation scenarios for tree species outside infested areas. Understandably in all previous plant host trials, gypsy moth has never been exposed to *Nothofagus*, although this genus is taxonomically closely related to its primary host, *Quercus*.

Although the concept of co-evolution has its detractors, it forms the basis of the tried and proven '*phylocentric*' testing sanctioned by practioners of weed biocontrol to determine host-specificity, usually of monophagus agents (Wapshere 1974). This report records the methods and a risk assessment, based on phylocentric larval feeding trials, of the genus *Nothofagus* (and two examples of the genera *Pinus* and *Podocarpus)* as potential hosts of AGM. As Australia is our biggest and nearest trading partner, an establishment in New Zealand would probably be the most likely stepping-stone for entry into Australia, or *vice versa*. All of the *Nothofagus* trials were therefore run in conjunction with Dr. Matsuki's trials on *Eucalyptus spp.* and other Australian endemics and the overall assessment is complemented with a *Climex* model of habitat suitability for AGM in both countries.

Methods

The trials reported here consisted of 'no-choice' tests, where newly eclosed first instar larvae were caged with cut foliage of one plant species in a two-chamber system. (This kept the stem of the plant specimen in a lower (595 ml) water-filled container, while allowing a dry enclosed upper (395 ml) arena around the foliage for larvae).
Ten larvae were released into the upper arena and when the majority were in the fourth instar they were equally divided into two identical arenas to avoid overcrowding. Five replicates were run for each plant used. Oaks (seedling *Quercus robur* and mature tree *Q. humilis*) were used as a controls. Foliage was replaced as required.

Because of the uncertainty as to the integrity of the species (Roy *et al.*1995), gypsy moth is considered a biosecurity risk throughout the world and therefore, despite its occurrence in France, the trials reported here were run in a quarantine glasshouse at the CSIRO Entomology European Laboratory at Montpellier, France, utilising natural lighting, daylength, and minimum temperatures, but with a controlled maximum temperature of 22°C.

The Gypsy Moth

The AGM larvae used in the trials were reared from eggs sourced from;

a Bavarian State Forest Research colony (a non-diapausing strain of AGM originating from the Beijing area, China),
a wild population at Khabarovsk, supplied by the Russian Far East Forest Research Institute (FEFRI) and
a USDA colony at Montpellier (AGM originating from Alsace, NE France).

The Plants

The foliage of 15 species and two hybrids of *Nothofagus* was sourced from;

Scotland - The Royal Botanic Garden Edinburgh,
England - Wakehurst Place –Kew Gardens Arboretum,
Hilliers Arboretum,
Nymans Garden,

Wales - Tintern, UK Forestry Commission,
Ireland- Mount Congreve Estate
Mount Usher Garden
Fota Estate
France - Arboretum National de Barres
La Roche Fauconniere

The foliage of two *Pinus* species and two *Podocarpus* species was sourced from the Bedgebury Pinetum, UK Forestry Commission.

The assays

Four trials were established;
24th April - Beijing AGM with 7 *Nothofagus spp.* (3 NZ & 4 S.American)

11 May - FEFRI AGM with 14 *Nothofagus spp.* (4 NZ & 10 S.American)

26th May - Beijing AGM with, 12 *Nothofagus spp.* (5 NZ, 5 S.American & 2 Australian), 2 *Pinus spp* and 2 *Podocarpus spp.*

31st May – NE France AGM with 5 *Nothofagus spp.* (4 NZ & 1 S.American),

Foliage was couriered to the laboratory *almost* as required (at approximately 10 day intervals) and stored at 5° until used.
Herbarium specimens of the plants used were taken back to ***Forest Research*** to verify identities.

The Experiments

As many site:species combinations of foliage as possible were trialed, with a maximum of five site-specific replicates used for some species. Where host species were replicated in a number of trials, the foliage utilised came from the same specimen trees and all trees (except *N. truncata*) were mature specimens. The two specimens of *N. truncata* used were small establishing trees, about 10 years old. Where the seed origin of the host trees was unknown the assumption is made that botanical collectors for arboreta chose the 'best' available specimens – probably from the 'easiest'(lower altitude) sites. This appears to be the case for those of known origin.
Because of logistical problems with foliage supplies, many site:species combinations were degraded to species trials, in which the affected larvae were fed only one species but the foliage came from a number of sites. The majority of foliage fed to these larvae however, came from the named site. Occasionally the supply of foliage of a species from any site ran out. Larvae in this situation were transferred to 12°C in a controlled climate cabinet with 14hr photoperiod in an effort to delay development until foliage became available. The time spent at 12°C was recorded and deducted from the larval developmental time.In some instances consecutive foliage deliveries from some sites were irretrievably lost and those treatments were abandoned. For all trials larval mortality, development time, pupal weight (5 days after pupation) and sex were recorded for each treatment.

The trials were laid out using a randomised block design with five blocks. Because we had unbalanced data sets, we used restricted maximum likelihood analysis (REML: Corbeil and Searle 1976) to estimate means. All statistical analyses were conducted using GENSTAT (Genstat 5 Committee 1993).

Miscellaneous Observations

Other observations made during the course of the trials included the flush times of host plants and the determination of threshold temperatures for larval feeding and pupal development, using two climate control cabinets (10° and 20°C, 14 hr photoperiod) within the quarantine area. The larval feeding rate was determined by recording frass production from matched pairs of larvae (*i.e* larvae having the same frass production at ambient temperatures prior to entry into the controlled climate). Pupal development was recorded as time till adult emergence at the given temperature. Pupal weight loss was recorded for one early cohort to determine the best time to record this parameter and a threshold for male adult emergence, with respect to pupal weight, was determined. Potential fecundity was recorded as egg counts from the dissection of a number of emerging females restrained in tubes.

Range Prediction

The methods utilised in the production of the *Climex* model for the prediction of a potential geographic range within Australia and New Zealand are described with the model in Appendix 1 and paraphrased here.

Firstly, we developed a model that predicts the distribution of AGM within the known distribution in Asia. The overall known distribution is reproduced in Figure 1(App. 1) based on Giese and Schneider (1979), who reconstructed to a common scale and projection, historical maps to show a distribution of *Lymantria dispar* across Eurasia. Within CLIMEX, various growth and stress factors were adjusted, iteratively, to maximize the match to the known distribution and to the known phenology of the insect (both AGM and EGM depending on the region, Asia and Europe respectively). The parameter values and how they were derived are shown in Appendix 1.

Secondly, the model was tested against the known distribution of EGM in North America because AGM is not yet well established outside its native range. Further adjustment was to be considered if the model did not include all areas occupied by EGM. Thirdly, the model was used to predict the distribution of AGM in Australia and New Zealand.

Some AGM individuals develop without diapause and this has been used to develop continuous laboratory cultures (Hoy 1977). Thus, we also predicted distributions and growth index in New Zealand and Australia with and without diapause.

The full details of Dr. Matsuki’s trials on *Eucalyptus spp.* and other Australian endemics are included as his report to AFFA, in Appendix 2.

Results

The assays

The three populations of gypsy moth used in the trials showed similar feeding responses to different hosts across the trials, with few minor differences (Table 1). The larvae of the Siberian population were able to complete development on *E. melliodora* and *E. grandis*,

Table 1. A summary of four trials showing average survivorship, pupal weights and development times for female gypsy moth on species of *Nothofagus*, *Pinus* and *Quercus*.
Rankings 1, 2, 3 are equivallent to Liebhold *et al.*(1995) 'susceptible', 'resistant' and 'immune' respectively.

	trial 1					trial 2					trial3					trial 4					
	% survival	**F pup wt**	**Devl. Time**	**GR**	**Rank**	**% survival**	**F pup wt**	**Devl. Time**	**GR**	**Rank**	**% survival**	**F pup wt**	**Devl. Time**	**GR**	**Rank**	**% survival**	**F pup wt**	**Devl. Time**	**GR**	**Rank**	**Ave. Rank**
Nothofagus. alessandri						0.00				**3.00**											**3.00**
N. alpina	74.00	719.90	47.70	15.09	**2.00**	81.00	930.53	36.99	25.30	**1.67**											**1.70**
N. antarctica						93.00	907.40	37.01	24.52	**1.67**											**1.70**
N. betuloides	84.00	802.10	43.09	18.61	**2.00**	80.00	958.83	43.13	22.23	**1.67**											**1.80**
N. cunninghamii											68.00	470.15	51.66	9.10	**2.70**						**2.70**
N. dombeyi	32.00	1224.70	53.13	24.36	**1.67**	71.00	991.47	43.69	22.69	**1.67**	88.00	925.40	46.62	19.85	**1.30**						**1.50**
N. fusca	22.00	467.20	63.71	7.33	**3.00**	24.00	525.37	60.09	8.74	**3.00**	24.40	520.90	65.72	7.93	**3.00**	34.00	485.10	63.96	7.58	**2.70**	**2.90**
N. glauca											70.00	603.40	50.73	11.89	**2.00**						**2.00**
N. leonii						28.00	906.20	54.48	16.63	**2.33**											**2.30**
N. menziesii	0.00				**3.00**	0.00				**3.00**	<4				**3.00**	0.00				**3.00**	**3.00**
N. moorei						0.00				**3.00**											**3.00**
N. nitidia											26.00	598.90	53.49	11.20	**3.00**						**3.00**
N. obliqua	90.00	1147.20	40.55	28.29	**1.00**	91.00	1107.67	37.35	29.66	**1.30**	34.00	827.65	48.51	17.06	**2.30**	70.00	938.30	44.31	21.18	**1.70**	**1.60**
N. obliqua macro						90.00	971.40	46.57	20.86	**1.67**											**1.70**
N. pumilio						94.00	949.10	33.64	28.21	**1.67**											**1.70**
N. solandri	64.00	525.50	52.78	9.96	**2.30**	30.00	670.40	44.74	14.98	**2.70**	48.00	534.73	56.22	9.51	**2.70**	33.00	633.80	55.56	11.41	**2.30**	**2.50**
N. solandri cliffortioides											52.00	593.05	55.69	10.65	**2.70**	19.00	700.30	59.55	11.76	**2.70**	**2.70**
N. truncata						0.00				**3.00**											
Pinus radiata											74.00	904.60	52.31	17.29	**1.67**						**1.70**
P. contorta											22.00	1002.50	64.57	15.53	**2.00**						**2.00**
seedling oak (*Q. robur*)	100.00	1012.70	46.44	21.80	**1.00**	56.00	1453.90	42.16	34.49	**1.00**	100*	1012.7*	46.44*	21.8*	**1.00**	60.00	1181.10	44.67	26.44	**1.00**	**1.00**
mature oak (*Q. humilis*)	90.00	1194.50	43.89	27.23	**1.00**	4.00	1820.40	50.88	35.78	**2.30**	0.00										**2.30**
cut mat. Oak(*Q. humilis*)	83.00	1281.60	43.26	29.63	**1.00**																
1.00	>90	>911.43		>19.62		>50.4	>1308.51		>31.04		>90	>911.43		>19.62		>54	>1062.99		>23.8		
2.00																					
3.00	<50	<506.35		<10.9		<28	<726.95		<17.24		<50	<506.35		<10.9		<30	<590.55		13.22		

while those of the two other populations could not complete development on these species. Larvae of the Siberian population developed slightly faster, and mean pupal weights were greater than those of the two other populations across a number of different host plant species (Table 2).

Table 2. Effect of AGM population origin on pupal weight and larval duration. SEs are for comparing all means in each sex and variable combination. The non-diapausing strain from Bavarian State Forest Research Institute was originally collected from China.

	Pupal mass (mg)			**Larval duration (days)**		
	China	**Siberia**	**France**	**China**	**Siberia**	**France**
Male						
E. melliodora	-	-	-	-	-	-
E. grandis	-	240.5	-	-	46.0	-
E. nitens	251.3	224.6	216.9	70.0	75.6	55.0
E. sideroxylon	294.9	309.1	-	50.8	44.3	-
C. maculata	458.0	486.9	347.3	37.2	31.5	37.6
SE	58.1			3.3		
Population effect	$P = 0.006$			$P = 0.01$		
Population × species effe	$P = 0.59$			$P < 0.0001$		
Female						
E. melliodora	-	529.4	-	-	61.0	-
E. grandis	-	530.5	-	-	50.0	-
E. nitens	-	441.0	-	-	71.9	-
E. sideroxylon	463.4	531.0	366.9	74.0	49.1	47.0
C. maculata	831.0	905.6	734.4	44.4	36.2	48.9
SE	148.2			6.4		
Population effect	$P = 0.03$			$P < 0.0001$		
Population × species effe	$P = 0.99$			$P = 0.01$		

For the evergreen plant species the trials occurred over the period of transition from old foliage to new. There was however no marked differences in growth rates of larvae feeding on *Nothofagus* foliage of different ages. Observations made during the feeding trials showed that later instars had mixed preferences for old and new foliage within the evergreen species. Larvae on the less suitable *N. cunninghamii*, *N. nitidia* and *N. menziesii*, preferred newly flushed foliage. The abscision of old foliage at bud burst in *N. fusca* and *N. solandri* varieties did not allow a choice.

We found no difference in larval performance of AGM between freshly cut and stored foliage of *Q. humilis* (REML testing for the foliage type: $P = 0.2$ for pupal weight and $P = 0.3$ for larval duration. REML testing for the foliage type × sex interaction: $P = 0.2$ for pupal weight and $P = 0.1$ for larval duration).

Survivorship and pupation

Asian gypsy moth showed a wide range of responses to the native plant species from Australia and New Zealand (Table 3).

Table 3. Plant species unsuitable for development of AGM larvae.

No feeding	Feeding but no molting	Molting but no pupation
E. kartzoffinana	*E. acaciafolia*	*E. neglecta*
E. cinerea	*E. glaucascens*	*E. nicholii*
E. cordata	*E. globulus globulus*	*E. obliqua*
E. globulus pseudoglobulus	*E. globulus maidenii*	*E. ovata*
E. polyanthemos	*E. laevopinea*	*E. archeri*
E. rubida	*E. regnans*	*E. parvifolia*
E. viminalis	*E. robertosonii*	*Eucalyptus* sp1
Callistemon viminalis	*E. siebrii*	*Eucalyptus* sp2
Melaleuca squamea	*Callistemon citrinus*	*Acacia dealbata*
Grevillea juniperina	*Callistemon macropunctatu*	
Hakea laurina	*Acacia melanoxylon*	
Nothofagus alessandri		
Nothofagus moorei		
Podocarpus totara		
Podocarpus halli		

Neonate larvae failed to initiate feeding and subsequently died on *N. alessandri, N. moorei, P. halli, P. totara* and 13 Australian endemic species including the important commercial timber *E. globulus pseudoglobulus*. Larvae grew but failed to molt on 10 other species including another timber species, *E. regnans*. On nine further species (including *E. obliqua*) larvae developed to between the 3rd to the 6th instar, but failed to pupate. Larvae failed to progress past the 3 rd instar on *N. menziesii* from five different sites but on foliage from a sixth site produced two small male pupae. The limited trial with *N. truncata* also produced only small male pupae.

On six *Nothofagus* taxa from Australasia and 16 *Eucalyptus* spp., pupae were smaller than those on the control hosts of, *Q. humilis* and *Q. robur*. However, in some trials pupae reared on South American *Nothofagus spp.* and *E. gunnii*, *E. risdonii, E. urnigera*, *Corymbia maculata*, and *Callistemon brachyandrus* were of similar size, or larger, than those reared on *Q. humilis* and *Q. robur* (Tables 1 & 4).

The 'ranking' and consistency of the growth rates and pupal weights for individual *Nothofagus* species from various sites over all trials is shown in Figures 1 & 2 . There was little difference between *N. solandri* and *N. solandri cliffortioides* – the most palatable New Zealand species - but all of the New Zealand beech species would fall within the 'resistant' or 'immune' categories of Liebhold *et al.* (1995). For this study a comparison with the oak controls, used in the manner of Montgomery (1991) arrived at comparative host rankings, derived from;

Susceptible (1)– parameters of pupal weight, survival and growth rate >90% of control,
Resistant (2)- parameters of pupal weight, survival and growth rate 50 – 90% of control and
Immune (3)- parameters of pupal weight, survival and growth rate < 50% of control,
as shown in Table 1 .

Table 4. Test plants suitable for development of AGM. The overall rank is based on pupal weight and larval duration of both male and female and survivorship. SEs for comparing pupal weight are 50 (male) and 155 (female) and for comparing larval duration are 5 (male) and 4 (female).

	pupal mass (mg)		**larval duration (days)**		**survivorship**	**overall rank**
species	**male**	**female**	**male**	**female**		
Q. robur	**512**	**1229**	**25**	**35**	**66**	**1**
C. brachyandrus	480	1054	42	40	100	2
C. maculata	460	891	20	32	68	3
E. risdonii	507	1162	41	43	82	4
*N. procera**[*]	411	862	36	41	87	5
N. obliqua	398	1004	38	43	84	7
E. urnigera	593	1328	42	39	41	6
E. gunnii	521	1344	44	42	59	8
N. pumilio	378	817	38	42	77	9
N. antarctica	374	778	39	42	70	10
Q. humilis	**445**	**991**	**45**	**45**	**42**	**15**
N. betuloides	364	831	43	47	81	11
N. dombeyi	393	926	43	49	67	12
P. radiata	394	964	45	52	74	13
E. irbyi	473	1062	49	47	41	14
E. stellulata	372	1222	46	41	28	16
N. obliqua macro	337	862	49	53	90	17
*N. nervosa**	284	714	44	47	71	18
N. glauca	327	673	40	46	35	19
N. cunninghamii	233	524	40	49	68	20
E. sideroxylon	292	497	32	45	31	21
N. nitida	315	668	42	49	39	22
E.coccifera	345	749	53	50	50	23
N. solandri cliffortioides	326	723	46	52	36	24
E. aggregata	363	646	52	53	38	25
N. solandri solandri	323	573	48	55	44	26
E. grandis	199	504	33	44	7	27
P. contorta	316	1061	53	64	22	28
E. simmonsii	325	675	58	61	38	29
E. dalrympleana	257	264	52	54	41	30
E. camaldulensis	223	327	47	62	52	31
E. macarthurii	137	383	51	56	66	32
N. leonii	137	815	59	58	28	33
E. amygalina	305	715	59	68	14	34
E. pauciflora	237	628	58	55	13	35
E. linearis	228	455	58	62	22	37
N. fusca	224	468	55	65	25	36
E. melliodora	207	549	60	55	4	38
E. nitens	239	415	55	65	8	39
E. perrineana	329	-	64	-	2	40
E. delegatensis	192	494	67	83	14	41
N. truncata	269	-	70	-	4	42
N. menziesii	223	-	68	-	1	43

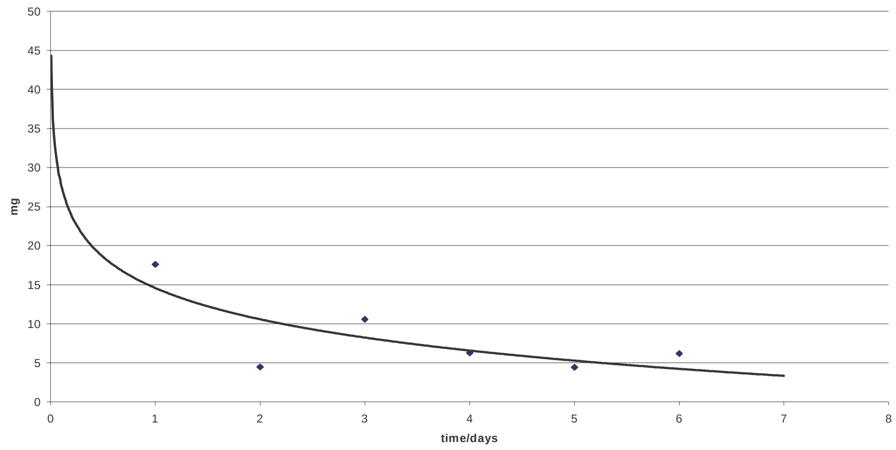
---

[*] = *Nothofagus alpina*, identities to be confirmed.

Pupal weight loss and potential fecundity

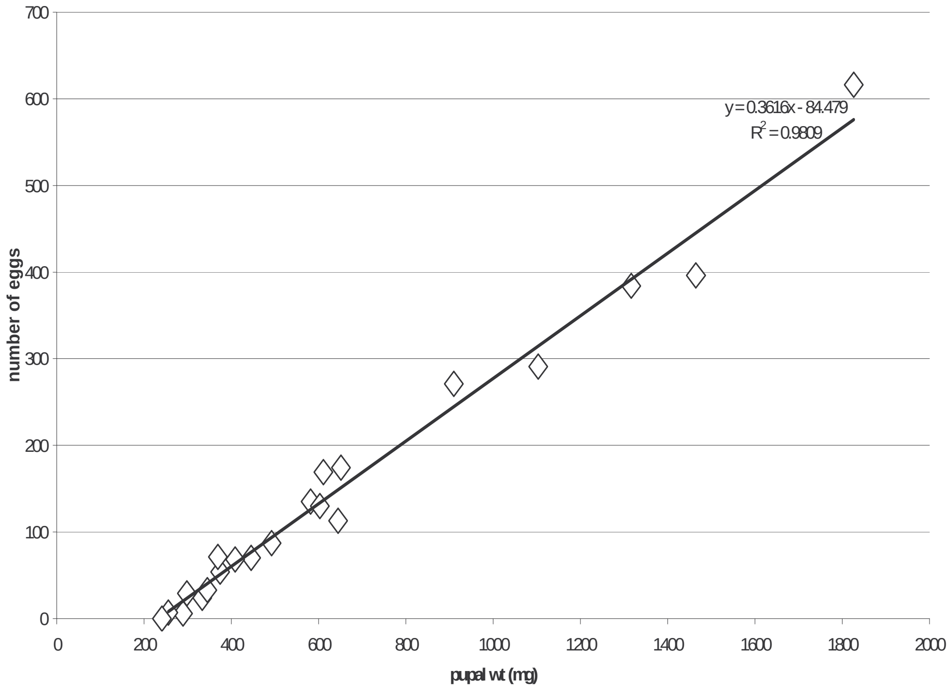
Pupal weight loss stabilised after four to five days (Fig. 3).

Fig. 3. Average pupal weight loss over time



In the wild population from Siberia and the non-diapausing strain from China, fresh pupal weight of females measured five days after pupation was linearly related the number of eggs (Fig. 4). Moreover, the relationships between fresh pupal weight and the number of eggs in the two populations were indistinguishable from each other. Extrapolation showed that no eggs should be produced from pupae below a threshold weight of 233mg. (One pupa at 241.2mg produced no eggs). There appeared to be a threshold pupal mass of about 185mg below which male emergence was less than 50% (Fig. 5). A few pupae from some *Eucalyptus*

Fig.4. Pupal wt. vs egg production (threshold wt 233.6mg)



spp. were below these thresholds. The relationship between fresh pupal weight and the number of eggs in the French population was not examined.

Temperature thresholds

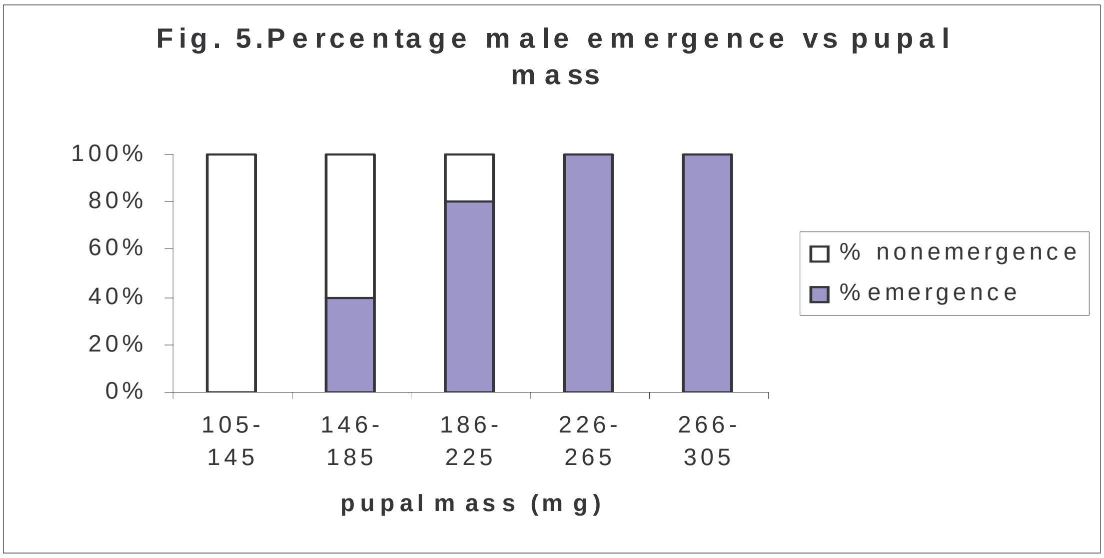


Fig. 5. Percentage male emergence vs pupal mass

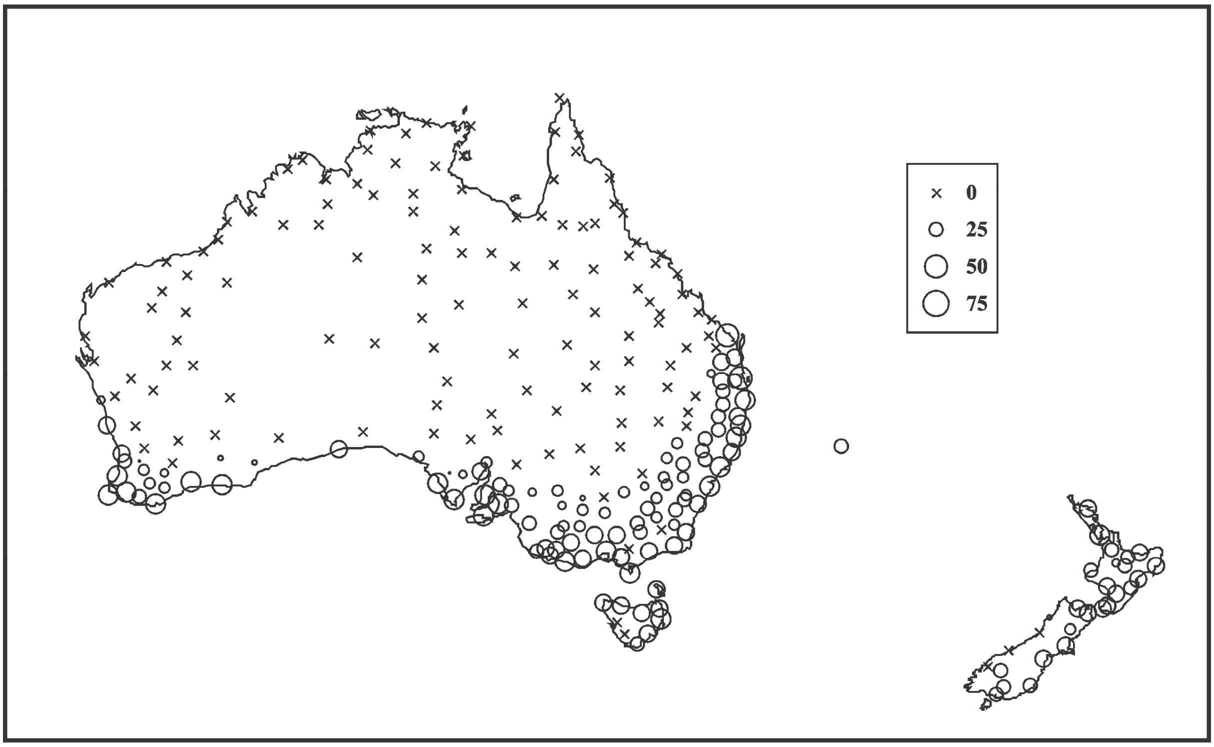
Extrapolation of the larval feeding data reveals a threshold temperature of 6°C for larval activity. This equates to degree-day values for larval development of approximately 890 and 1100, for seedling oak and *N. solandri* respectively.

At 20°C, pupation lasted approximately 4 weeks: mean ± SD, females 27.0 ± 2.1 days (n = 8) and males 28.5 ± 2.1 days (n = 2). There was no adult emergence at 10°C over the eight weeks of observation.

Prediction of the distribution of AGM in Australia and New Zealand

The CLIMEX model predicted that AGM could colonize the North Island and the east coast of the South Island of New Zealand and all southern regions of mainland Australia (Fig. 6). The constraints to range expansion appear to be the dry conditions towards inland Australia, and the high rainfall of southwest Tasmania and the west coast of the South Island of New Zealand.

Figure 6. Prediction of the distribution of AGM in Australia and New Zealand. The size of circle indicates likelihood of survival, x indicates no establishment.



Discussion

Pupal weights and growth rates reported here are similar to those reported elsewhere and the positive correlation between female pupal mass and potential fecundity has been previously documented (Barbosa and Carpinera 1977, Barbosa and Greenblatt 1979, Hough and Pimental 1978, Roden and Surgeoner 1991). The slope of the weight:fecundity curve from our trials is shallower suggesting that the greater allocation of resources to flight in AGM is at the expense of egg production.

As expected, the wild Far East AGM population seemed more robust and developed faster than Beijing AGM from the lab. colony. The non-diapausing Beijing insects have been in continuous culture for five years, with several generations per year, on artificial diet and the NE France gypsy moth have been in culture for nine generations on artificial diet. However, the three populations of gypsy moth showed similar responses to hosts with few minor differences.

The conclusion drawn from many gypsy moth host studies is that although it is an extremely polyphagous species, *L. dispar* has strong preferences for some tree species (Barbosa *et al.*1979, Liehbold *et al.*1995). Oaks (*Quercus spp)* are the primary hosts and this is reflected by the co-incident ranges of gypsy moth and *Quercus* and the extraordinary univoltine life strategy with a prolonged diapause which synchronises the very short larval period with its host phenology. The synchrony is presumably to counter the potent chemical defence of oak and to evade the build up of late season predators and parasitoids. The phenolic-based defence of oak to lepidopterous defoliators has been well documented (Feeny 1974, Hough

and Pimental 1978, Denno and McLure 1983, Rossiter 1988) and the gypsy moth life strategy probably demonstrates the level of co-evolution with this host.
The narrow phenological window open to AGM was demonstrated by the change in suitability of the oak controls over the duration of our trials. One control used the continually produced foliage from seedlings of *Q. robur* and provided consistently good results across the trials. The other control was foliage from a mature tree whose flush coincided with the start of the first trial. This also provided good results in the first trial but larval growth rates and survivorship fell dramatically with subsequent trials, four and six weeks later. Neonate larvae failed to establish on foliage of mature *Q. humilis* six weeks after the flush. The effect is further demonstrated in the use of a mature *Q. humilis* 'cut' foliage control, where foliage was cut from the same tree and stored in the cool store in the same manner as other test foliage. There may have been up to a 10-day physiological difference between this foliage and fresh cut material. This may well have been responsible for the higher growth rate of this control in trial one (Table 1). (The use of the REML, Genstat. Statistical package which included all results over all the trails, probably masks these effects and results in the poor showing of mature *Q. humilis (Table 4)*).

The use of cut host material (excised leaves), rather than whole plants, is recognised as producing a better performance in gypsy moth (Wallner and Walton 1979), possibly because defence mechanisms may be minimised (Green and Ryan 1972, Haukioja and Niemela 1976, 1977, Mattson and Scriber 1987, Schultz and Baldwin 1982). The results of the trials reported here, where the use of excised material was unavoidable, should be seen in this light. On the other hand the presumed effect of juvenility of seedlings may be responsible for the lesser performance of AGM on *Q. robur*, although previous studies of host specificity with other insects have shown that host specificity did not differ between potted plants and cut foliage (Palmer 1999).

In the absence of field trials, the extrapolation from lab. trials of host suitability for gypsy moth appears to be realistic (Peterson and Smitley 1991).The rank order of oak and other hosts determined by laboratory studies may, however, be moderated by the influence of other factors, such as larval age and population density, host nutritional status etc., while the actual levels of forest defoliation will be influenced not only by host food quality, but also host availability, host 'texture' –in providing larval and pupal refugia (Liehbold *et al.*1986)- and differential rates of predation, parasitism and pathology (Hwang *et al.*1995, Kruse and Raffa 1999) . Then again, gypsy moth outbreaks are only partly governed by forest species composition. Edaphic and tritrophic factors may also play a role in the regulation of the population, as adjacent forests of similar species composition often experience different levels of defoliation (Houston 1981).

Until recently Nothofagus was placed taxonomically within the Fagaceae, the same plant family as oak (Nixon 1982, Hill and Jordan 1993). Given the accepted dogma, that islands are inherently invasible, it seemed prudent to assess this genus for potential hosts of AGM.

The phylocentric argument for the results reported here, would reason that the genus Nothofagus is a suitable host for L. dispar. Fortunately, intra-generic variation results in the New Zealand species being less palatable than those of South America, some of which were capable of producing pupae at the same development rate and weight as the recognised favoured host, Quercus.

Individual plant species employ different life strategies for survival. Life strategies are considered (Silverton *et al.*1997) to result from a compromise in the allocation of limited available resources between reproduction, growth and defence. Climax forest species (k-strategists) are usually long-lived, slow maturing, forest dominants, which cannot rapidly escape threats through dispersal. Whereas r-strategists ('weedy' species) employ bountiful and precocious seeding, and high vagrancy, to escape threats or to invade new pest-free sites. The ineffectual dispersal capabilities of Nothofagus (Preest 1963) are legendary. Nothofagus species, as climax forest dominants, would be considered to be k-strategists but within Nothofagus the susceptibility-resistant-immunity spectrum recorded here occurs across sub-genera and species origins. Within New Zealand the taxa of N. solandri are considered the most r-selected (Ogden *et al.*1996). It is no surprise then that this is the most palatable of the New Zealand species – a result similar to that found for another lymantriid, Orgyia thyellina (Kay in prep.)

Liebhold *et al.*(1995), in a review and collation of past assessments of tree species as hosts for gypsy moth, ranked tree species into three relative ranks of 'susceptible', 'resistant' and 'immune', based on the various parameters measured.

The rankings in Table 1 contrast with those of Table 3, probably because the REML across all trials fails to recognise the phenological status of the different test plants over the duration of the trials. Mortality of neonate larvae on old evergreen foliage in the first two trials may have resulted from the inability of larvae to cope with the physical toughness of these leaves. Older foliage appears less palatable than flush, but both states of *N. menziesii* foliage appear resistant to AGM feeding. The late flushing habit of New Zealand species may be important in the epidemiology of an AGM establishment.

Naturally there was some variation between the same species from different sites however, the use of foliage of the same species from different sites is not considered to be detrimental to the assessment. Most other studies do not record whether foliage used in trials was from individual trees and some (Doane & Leonard 1975, Liebhold *et al.*1986) indicate that swapping between host species is common, even beneficial (Stoyenoff *et al.*1994, Kruse and Raffa 1999), for gypsy moth larvae. In mono-specific forests, such as those of *Nothofagus*, host tree, rather than host species, swapping would likely be normal behaviour.

Although all the trials tested only a small sub-set of the meta-population of individual plant species, the observation that *Podocarpus totara* and *P. halli* appear immune, with no progression of larvae beyond the first instar, is consistant with the *k*-selected nature of this ancient, long-lived plant family. Of the weedy conifers trialed *Pinus radiata* appears quite palatable to AGM, but *P. contorta* less so. Miller and Hanson (1989) recorded survival to pupation on *P. radiata*, with growth rates similar to those recorded here but recorded higher growth rates for *P. contorta*. In our trials newly eclosed larvae could only survive on newly flushed foliage of *P. contorta.*

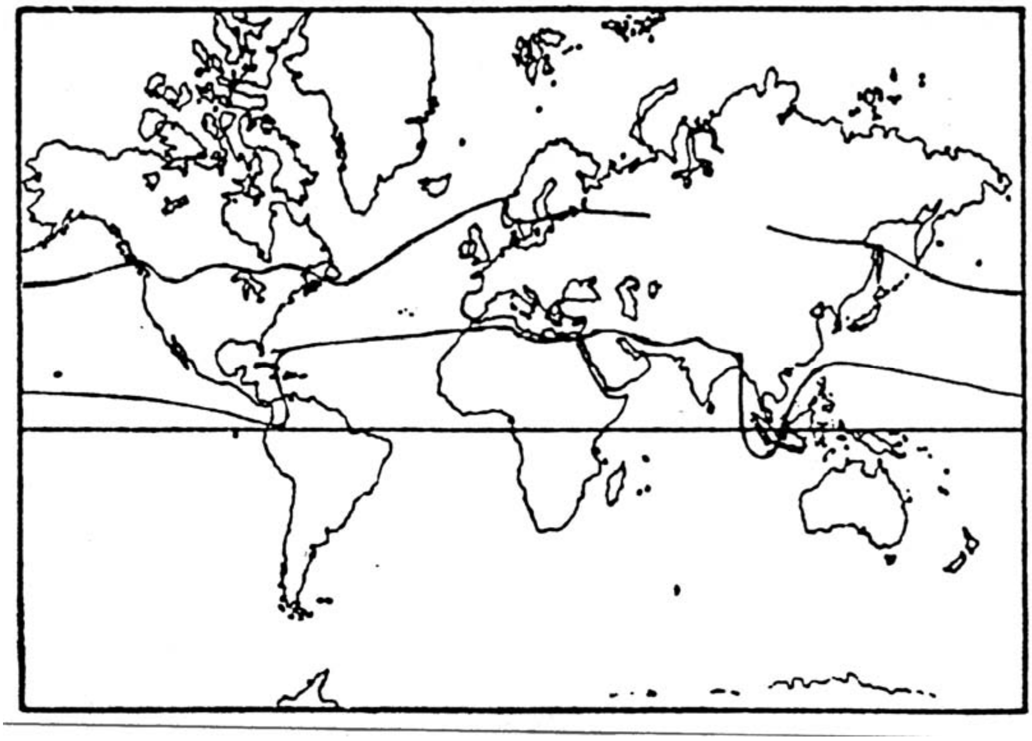
The performance of AGM on *P. radiata* suggests that plantations in New Zealand and SE Australia may be at risk, although, despite the development of EGM on *Pinus* species (Miller and Hanson 1989, Roden and Surgeoner 1991), gypsy moth populations apparently do not become established on pines in North America (Baker 1941, Leonard 1981, McManus *et al.*1979). The moderate development of AGM on 'unimproved' *P. radiata* from neonate larvae however, is cause for concern. It has been shown (Kay unpub. data) that selection for production within this commercial species leads to a decrease in resistance to lepidopterous defoliators and Barbosa *et al.*(1986) showed that EGM on diets incorporating *Pinus* had fecundities higher than those reared on single species hosts. Host switching to conifers is not uncommon. Rossiter (1981) observed a naturally occurring shift of late instar larvae from oak to pine, which resulted in a disproportionately lower mortality. She considered that this differential mortality may contribute to the maintenance of polyphagy within gypsy moth.

There has been one known record of natural encounters between gypsy moth and *Eucalyptus* and *Pinus radiata*. In Morocco, *E. camaldulensis* and *P. radiata* in plantations have been severely damaged by EGM during outbreaks (Fraval 1989). Gypsy moth moved from native oak species to more than a dozen species of exotic plants when oak leaves were exhausted.
At present, there is no strong evidence to suggest that AGM will be able to cause major damage to three species of *Eucalyptus* (*E. globulus*, *E. nitens*, and *E. grandis)* that are of commercial importance in New Zealand and Australia. GM seems to be able to develop as well on *Corymbia maculata* as two species of deciduous oaks. *Corymbia maculata* is of minor importance in New Zealand, but is becoming increasingly important in commercial plantations in NE New South Wales and Queensland. The Climex assessment however, suggests that the risk of AGM establishment in NE New South Wales and Queensland would be lower than SE Australia because of climatic conditions such as high humidity and mild winter temperatures.

The extrapolation of the purely climatic parameters, such as those of Climex, to predict the range of a biological population should not be taken too critically (Lawton 2000). For the ubiquitous gypsy moth there was difficulty in producing a Climex model which encompassed the whole range of the insect in Asia. A better prediction may be achieved by the inclusion of a parameter for the presence of oak, the primary host (Fig. 7).

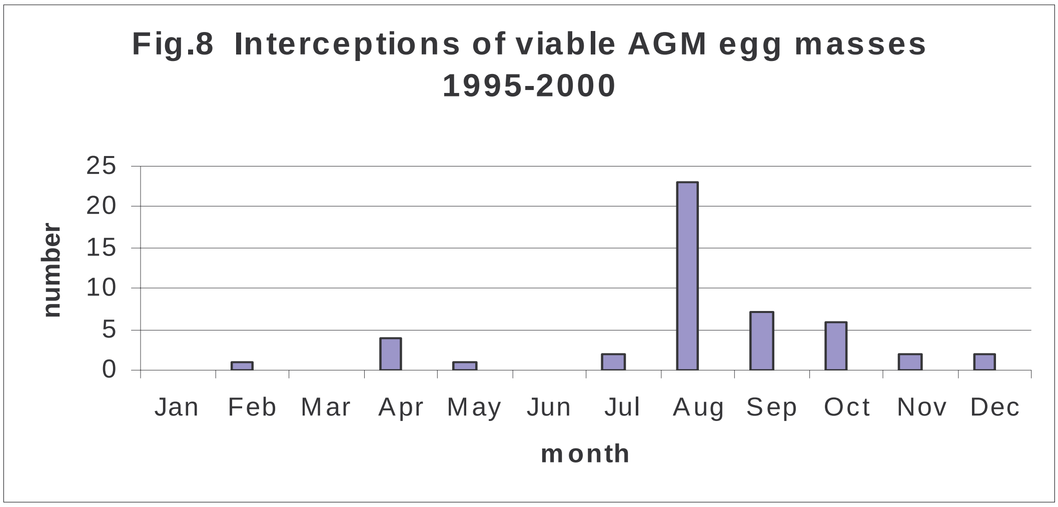
The relevance of a prediction for a non-diapausing strain stems from the incompletely understood larval eclosion of AGM and the possible development of multi-voltinism in mild climates (Walsh 1993). The establishment of such a strain in both Australia and New Zealand appears to be uninhibited by climate.

Figure 7. The Distribution of *Quercus*



Conclusion

If the establishment of an organism in a new habitat requires maximised growth, survival and fecundity, the establishment of AGM in New Zealand would probably require its arrival to coincide with the spring flush of *Quercus* species in the 'urban forest'. NZFRI records indicate that the peak interception of viable egg masses at New Zealand ports occurs in August –before the flush of oak- but interceptions continue at a low levels over the spring/summer period (Fig. 8). (The high number of viable AGM egg masses intercepted contrasts with the low number of similar intercepts of *Orgyia thyellina*, a bi-voltine lymantrid, which successfully established a population in Auckland).



From an urban forest bridgehead it is possible, but unlikely, that the distant indigenous forest could be threatened directly by dispersing insects. More accessible scrub and understory species may be at risk but indications are (Kay in prep) that they will not be suitable hosts for AGM and the mono-generic nature of the *Nothofagus* forests, would not provide a great opportunity for AGM populations to build on alternate hosts.

In the unlikely event of a serious outbreak of AGM within these forests the defoliation, death, or die-back of the most palatable species, *N. solandri*, would probably lead to its replacement by the extensive 'advanced growth' seedling population of *N. menziesii* (Ogden *et al.*1996). The immunity of the two podocarp species tested suggest that the lowland podocarp forest is also not at great risk.

The overall impression is that AGM would not establish easily in New Zealand's indigenous forest but that S. America –Chile in particular, with its predominantly susceptible *Nothofagus* indigenous forest and a large *P. radiata* plantation forest- would suffer badly.

Acknowledgements

We thank J. Vitou, C. Espiau, Y. Mas, T. Thomann, M. Jourdan, C. Ducatillon, J.-N. Marien, F. Hérard, H. Scheel, and G. Farrell for assistance. The following botanic gardens and research organizations granted permission to collect foliage: The Royal Botanic Garden

Edinburgh, (Scotland); Wakehurst Place – Kew Gardens Arboretum, Hilliers Arboretum, Nymans Garden (England); Tintern (Wales) and Bedgebury Pinetum (England), UK Forestry Commission; Mount Congreve Estate, Mount Usher Garden, Fota Estate (Ireland); Arboretum National de Barres, La Roche Fauconniere, Institut National de la Recherche Agronomique, and Association Foret Cellulose (France). Arthur Vanner for foliage collections in SE England. Gypsy moth eggs were provided by Gabriele Lobinger (Bavarian State Forest Research Institute, Germany), Galina Yurchenko (Russian Far East Forest Research Institute, Russian Republic), and F. Hérard (USDA, France). M. Steinbauer, C. McArthur, J. Elek, A. Lock, and C. Stone kindly shared their knowledge on phenology of eucalypts. Financial support was provided by Ministry of Agriculture and Forestry, New Zealand and Agriculture, Fisheries and Forestry – Australia.

References

Abdullaev, Y.N. (1967) Biological characteristics of the gypsy mopth in the forests of Samarkand Province. *In*: Voprosy zashchity rasteniy. Tashkent: 80-

Allen, J.C., Foltz, J.L., Dixon, W.N., Liebho, A.M., Colbert, J.J., Regniere, J., Gray, D.R., Wilder, J.W. and Christie, I. (1993) Will the gypsy moth become a pest in Florida? *Florida Entomologist*, 76, 102-113.

Baker, W.L. (1941) Effect of gypsy moth defoliation on certain forest trees. J. For 39: 1017-1022

Baranchikov, Y.N., Nikitenko, G.N. and Montgomery, M.E. (1998) Russian and Ukrainian literature on the gypsy moth: an annotated bibliography. USDA Forest Service General Technical Report NE 253.166 pp.

Barbosa, P. and Carpinera, J.L. (1977) The influence of food on developmental characteristics of the gypsy moth, *Lymantria dispar*. Can. J. Zool. 55: 1424-1429

Barbosa, P. and Greenblatt, J. (1979) Suitability, digestibility and assimilation of various host plants of the gypsy moth *Lymantria dispar*. Oecologia 43: 111-119

Barbosa, P., Greenblatt, J., Withers, W., Cranshaw, W. and Harrington, E.A. (1979) Host-plant preferences and their induction in larvae of the gypsy moth *Lymantria dispar*. Ent. exp.& appl. 26: 180-188

Barbosa, P., Martinat, P. and Waldvogel, M. (1986). Development, fecundity and survival of the herbivore *Lymantria dispar* and the number of plant species in the diet. Ecol. Entomol. 11:1-6

Barteneva, Y.V., Gostjunin, I.V. and Persidskaya, L.T. (1974). The effect of temperature on the feeding rate of gypsy moth larvae. *In*:Nauchnyye trudy Moskovogo lesotekhniceskogo instituta. Voprosy zashchity lesa. MLTI, Moskova: 93-96

Benkevich V.I. (1955) The role of solar radiation in ther heat balance of developing gypsy moth eggs. *In* : Uchenyye zapiski Orekhovo-Zuyevskogo pedagogicheskogo instituta. Moscow: 163-175 Abstract 173. (see Baranchikov *et al.* 1998).

Berryman, A.A. (1991) The gypsy moth in North America: A case of successful biological control? *Trends in Ecology and Evolution*, **6**, 110-111.

Campbell, R.W. (1979) Gypsy Moth: Forest Influence. USDA FS Agric. Inform. Bull. N°.423

Campbell, R.W. (1981) Population dynamics. *In*. Doane, C.C. and McManus, M.L. The gypsy moth: research toward integrated pest management. USA Forest Service Science and Education Agency Animal and Plant Health Inspection Service Technical Bulletin 1584. pp 65-86.

Campbell, R.W. and Sloan R.J. (1977) Forest stand responses to defoliation by the gypsy moth. For. Sci. Monogr. 19: 1-34

Corbeil, R.R. & Searle, S.R. (1976) Restricted maximum likelihood (REML) estimation of variance components in the mixed model. *Technometrics*, **18**, 31-38.

Denno, R.F. and McClure, M.S. (eds) 1983 Variable plants and herbivores innatural and managed systems Ac. Press N.Y. USA

Doane, C.C and Leonard D.E. (1975) Orientation and dispersal of late-stage larvae *Porthetria dispar*. Can Entomol. 107 1333-1338

Elkinton, J.S. and Liebhold, A.M. (1990) Population dynamics of gypsy moth in North America. *Annual Review of Entomology*, 35, 571-596.

Feeny, P. (1970) Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars. Ecology 51: 565-581

Fraval, A. (1989) Degats et nuisances. Pages 73 – 76, *In* Lymantria dispar (A. Fraval, ed.). Actes Editions, Rabat, Morocco.

Garner, K.J. and Slavicek, J.M. (1996). Identification and characterisation of a RAPD-PCR marker for distinguishing Asian and North American gypsy moths. *Insect Molecular Biology*, **5**, 81-91.

Genstat 5 Committee. (1993) *Genstat 5 Release 3, Reference manual*. Oxford University Press, Oxford, UK.

Giese, R.L. and Schneider, M.L. (1979) Cartographic comparisons of Eurasian gypsy moth distribution (*Lymantria dispar* L.; Lepidoptera: Lymantriidae). *Entomological News,* 90, 1-16.

Gould, J.R., Elkinton, J.S., and Wallner, W.E. (1990) Density-dependent suppression of experimentally created gypsy moth, *Lymantria dispar* (Lepidoptera: Lymandtriidae) populations by natural enemies. *Journal of Animal Ecology*, **59**, 213-233.

Green, T.R. and Ryan C.A. (1972) Wound induced proteinase inhibitor in plant leaves: A possible defence mechanism against insects. *Science* 175: 776-777

Haukioja, E and Niemela, P. (1976) Does birch defend itself actively against herbivores? Rep. Kevo Subartic Res. Stn. 13: 44-47

Haukioja, E and Niemela, P. (1977). Retarded growth of a geometrid larva after mechanical damage to leaves of its host tree. Ann. Zool. Penn. 14: 48-52

Hérard, F. and Drea, J.J. (1981) Agricultural Research service sponsored public law 480 project in Morocco: 1972-1975. *In*. Doane, C.C. and McManus, M.L. The gypsy moth: research toward integrated pest management. USA Forest Service Science and Education Agency Animal and Plant Health Inspection Service Technical Bulletin 1584. pp 317-321.

Hérard, F., K. Chen, and E. A. Cameron. (1997) Natural enemy impact on gypsy moth in Alsace, France, during 1995, p.39. *In*: Fosbroke, S. L. C.and Gottschalk, K. W. (eds.) Proceedings, U.S. Department of Agriculture interagency gypsy moth research forum 1996; 1996 January 16-19; Annapolis, MD. Gen. Tech. Rep. NE-230. Radnor, PA: USDA, F.S., N.E. Exp. Sta. 91 p.

Hill, R.S.; Jordan, G.J. (1993): The evolutionary history of *Nothofagus* (Nothofagaceae). *Australian Systematic Botany 6*: 111-126.

Hough, J.H. and Pimentel, D. (1978) Influence of host foliage on development, survival and fecundity of the gypsy moth. Enviro. Entomol. 7: 97-107

Houston, D.R. (1981) Effects of defoliation on trees and stands. *In*. Doane, C.C. and McManus, M.L. The gypsy moth: research toward integrated pest management. USA Forest Service Science and Education Agency Animal and Plant Health Inspection Service Technical Bulletin 1584. pp 217-296.

Hoy, M.A. (1977) Rapid response to selection for a nondiapausing gypsy moth. *Science*, 196, 1462-1463.

Hwang, S.Y., Lindroth, R.l., Montgomery M.E. and Shields, K. (1995) Aspen leaf quality affects gypsy moth susceptibility to *Bacillus thuringiensis*. J. Econ. Entomol. 88(2):278-282

Idrisova, N.T. (1977). Materials on the biology and ecology of the gypsy moth in Bashkirian ASSR. *In*: Nasekomyye – vrediteli lesov Bashkirii. Ufa: 38-54 Abstract 372. (see Baranchikov *et al*. 1998).

Kruse, J.J. and Raffa, K.F. (1999) Effect of food plant switching by a herbivore on its parasitoid: *Cotesia melanoscela* development in *Lymantria dispar* exposed to reciprocal dietary crosses. Ecol. Entomol. 24: 37-45

Lawton J.H. (2000) Community Ecology in a Changing World. Ecology Institute, Nordbünte 23, Germany.

Leonard, D.E. (1981) Bioecology of the gypsy moth. *In*. Doane, C.C. and McManus, M.L. The gypsy moth: research toward integrated pest management. USA Forest Service Science and Education Agency Animal and Plant Health Inspection Service Technical Bulletin 1584. pp 9-29.

Liebhold, A.M, Elkinton, J.S. and Wallner W.E (1986). Effect of burlap bands on between-tree movement of late-instar gypsy moth, *Lymantria dispar*. Environ. Entomol. 15: 373-379

Liebhold, A.M., Gottschalk, K.G., Muzika, R., Montgomery, M.E., Young, R., O'Day, K., and Kelly, B.. (1995) Suitability of North American tree species to the gypsy moth: a summary of field and laboratory tests. USDA General Technical Report NE-211, USDA Forest Service, Delaware, Ohio, USA.

Liebhold, A.M., Halverson, H.A., and Elmes, G.A. (1992) Gypsy moth invasion of North America: a quantitative analysis. *Journal of Biogeography*, **19**, 1-8.

Lipa, J.J and Kolk, A. (1995) The recent situation of the gypsy moth (*Lymantria dispar*) and other Lymantriids in Poland. EPPO Bull 24L 4) 623-629

Lyamtsev, N.I. (1983). Impact of weather on changes in gypsy moth density. *In*: Molodyye unchenyye k yubileyu instituta. Trudy SSSR VNIILM, Pushkino: 188-194 Abstract 657. (see Baranchikov *et al*. 1998).

MAF media release *NZ Herald* 18. 09. 2000

Mason, C.J. and McManus, M.L. (1981) Larval dispersal of the gypsy moth. In. Doane, C.C. and McManus, M.L. The gypsy moth: research toward integrated pest management. USA Forest Service Science and Education Agency Animal and Plant Health Inspection Service Technical Bulletin 1584. pp 161-202.

Mattson, W.J. and Scriber, J.M. (1987) Nutritional ecology of insect folivores of woody plants: Nitrogen, water,fibre and mineral considerations. *In*: Nutritional ecology of insects, spiders and mites. Wiley N.Y

Matsuki, M., Kay, M., Serin, J., and Scott, J.. (ms) Variation in the ability of larvae of phytophagous insects to deal with evolutionarily unfamiliar plants: A study with the gyspy moth and *Eucalyptus*.

Maufette, Y., Lechowicz, M.J. and Jobin, L. (1982) Host preferences of the gypsy moth, *Lymantria dispar,* in southern Quebec. Can. J. For. Res. 13:53-60

Maufette, Y. and Lechowicz, M.J. (1984) Differences in the utilization of tree species as larval hosts and pupation sites by the gypsy moth, *Lymantria dispar*. Can. Ent. 116:685-690

McManus, M.L. Houston, D.R. and Wallner, W.E. (1979). The homeowner and the gypsy moth: Guidelines for control. USDA Comb. For. Pest Res. Dev. Prog.: Home Garden Bull. 227 34pp

McQuillan, P.B. (1993) *Nothofagus* (Fagaceae) and its invertebrate fauna- an overview and preliminary synthesis. Biol.J.Linn.Soc. 49: 317-354

Miller, J.C., and Hanson, P.E. (1989a) Laboratory studies on development of gypsy moth *Lymantria dispar*, larvae on foliage of gymnosperms. Can. Entomol. 121: (6) 425-429

Miller, J.C., and Hanson, P.E. (1989b) Laboratory feeding tests on the development of gypsy moth larvae with reference to plant taxa and allelochemicals. Stn. Bull. 674. Oregon State University, Agriculture Experiment Station, Corvallis, OR. 63p.

Montgomery, M.E. (1991) Variation in the suitability of tree species for gypsy moth. *In*: Gottschalk, Kurt W, Twery, M.J., Smith, S.I. (eds) Proceedings, USDA interagency GM research review 1990. USDA FS.Gen. Tech. Rep. NE-146

Montgomery, M.E. (1994) Comparison on several tree species of growth and survival of newly hatched larvae of gypsy moth from three continents. On file at Northeastern Forest Experiment Station, Hamden, CT. 2p.

Nixon, K.C. (1982): In support of recognition of the family Nothofagaceae Kuprianova. *Botanical Society of America, Miscellaneous Publication 162*: 102. [Abstract]

Ogden, J., Stewart, G.H. and Allen, R.B. (1996) Ecology of New Zealand *Nothofagus* Forests. *In*: Veblen, T.T, Hill, R.s. and Read, J. (Eds.) The Ecology and Biogeography of *Nothofagus* Forests. Yale Uni. Press, New Haven & London

Palmer, WA. (1999) The use of cut foliage instead of whole plants for host specificity testing of weed biocontrol insects – is this acceptable practice? In Withers, TM, Browne, L, and Stanley J. (Eds). *Host specificity testing in Australasia: towards improved assays for biological control.* pp 20-29. CRC for Tropical Pest Management, Brisbane, Australia.

Petersen, N.C. and Smitley, D.R. (1991). Susceptibility of selected shade and flowering trees to gypsy moth. J. Econ. Entomol. 84(2); 587-592

Preest, D.S. (1963). A note on the dispersal characteristics of the seed of the New Zealand podocarps and beeches. *In*: J.L. Gressitt (ed.) Pacific Basin Biogeography. Bishop Museum Press, Hawaii pp 415-424.

Roden, D. and Surgeoner, G.A. (1991) Survival, development time and pupal weights of larvae of gypsy moth reared on foliage of common trees of the Upper Great Lakes Region. North. J. Appl. For. 8(3): 126-128.

Rossiter, M.C., (1981) Factors contributing to the host range expansion in the gypsy moth, *Lymantria dispar*. PhD thesis State University of New York, Stony Brook (cited in Maufette & Lechowicz 1984)

Rossiter, M.C., Schultz, J.C. and Baldwin, I.T. (1988) Relationships among defoliation. Red oak phenolics, and gypsy moth growth and reproduction. Ecology 69 (1): 267-277.

Roy, A.S., McNamara, D.G. and Smith, I.M. (1995) Situation of *Lymantria dispar* in Europe. EPPO Bull. 25: (4) 611-612.

Rubtsov, I.A. (1938). Effect of constant and fluctuating temperatures on egg development in the gypsy moth (*Porthetria dispar* L). Zashchita rasteniy. 17: 25-38Abstract Savotikov, I.F., Smetnik, A.L and Orlinskii, A.D. 1995 Situation of the Asian form of gypsy moth (*Lymantria dispar*) in Russia and the world. EPPO Bull. 25:4) 617-622.

Schaefer, P.W. (1981) Explorations in Japan and Korea by the ARS Asian Parasite Laboratory: 1975-77. In. Doane, C.C. and McManus, M.L. The gypsy moth: research toward integrated pest management. USA Forest Service Science and Education Agency Animal and Plant Health Inspection Service Technical Bulletin 1584. pp 340-348.

Schultz, J.C. and Baldwin, I.A. (1982). Oak leaf quality declines in response to defoliation by gypsy moth larvae. Science 217: 149-151

Silverton, J., Franco, M. and Harper, J.L. (Eds)(1997). Plant Life Histories. Cambr. Uni. Press. UK

Stoyenoff, J.L., Witter, J.A. and Montgomery, M.E. (1994) Nutritional indicies in the gypsy moth (*Lymantria dispar*) under field conditions and host switching situations. Oecologia 97: 158-170

Sutherst, R.W., Maywald, G.F., Yonow, T. and Stevens, P.M. (1999) CLIMEX: predicting the effects of climate on plants and animals. CSIRO Publishing, Collingwood. 88 pp.

Wardle, J.A. (1984). "The New Zealand Beeches." New Zealand Forest Service.

Wallner, W.E. (1996) Invasion of the tree snatchers. *American Nurseryman*, March 15 issue, 28-30.

Wallner, W.E. and Walton, G.S. (1979) Host defoliation: a possible determinant of gypsy moth population quality. Ann. Ent. Soc. Amer. 72:1) 62-67

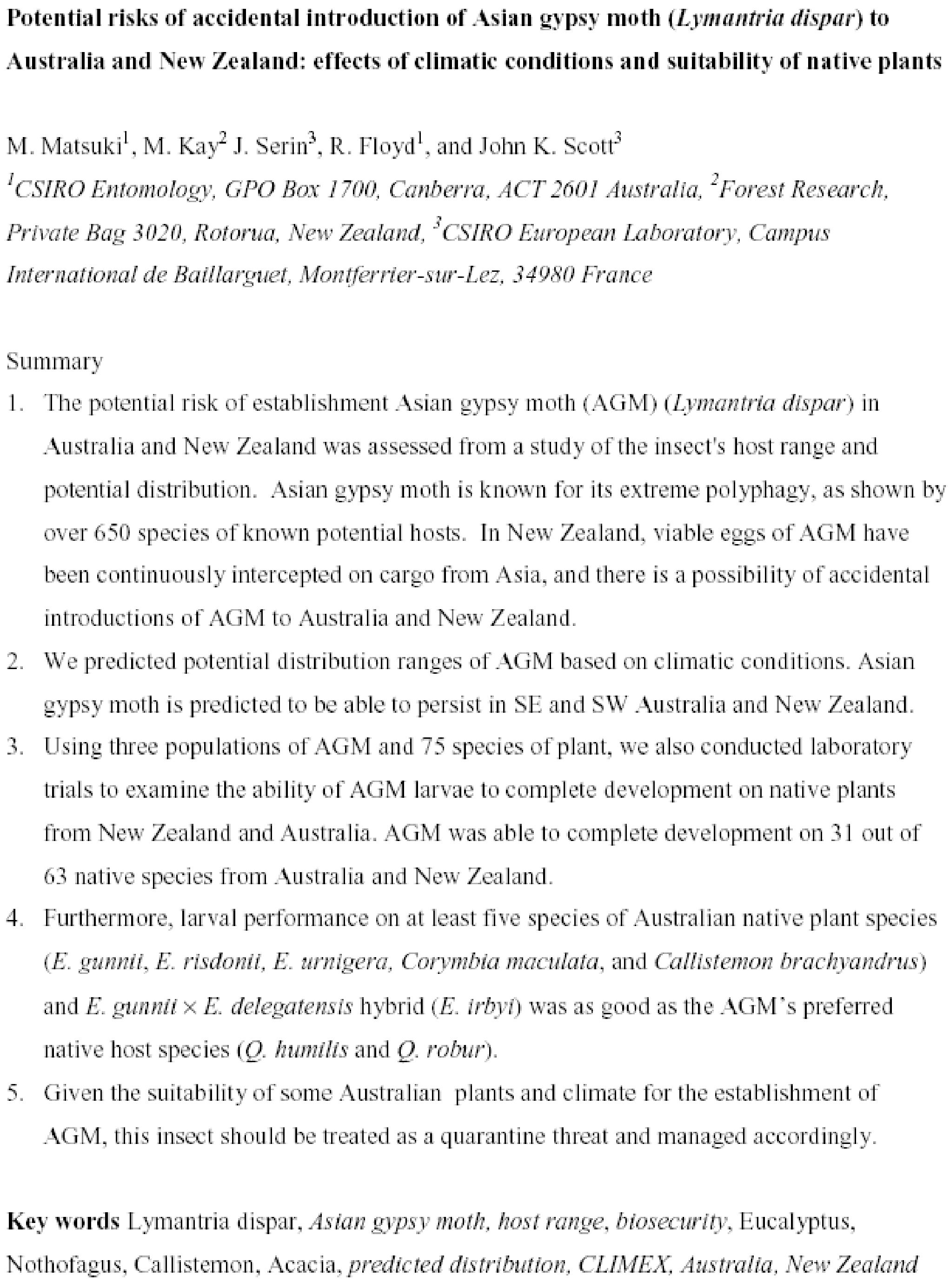
Walsh, P.J. (1993) Asian gypsy moth: the risk to New Zealand. *N. Z. Forestry*, 38 (3) 41-43.

Wapshere, A. (1974) A strategy for evaluating the safety of organisms for biological control agents for weeds. Annals App. Biol . 77 210-211

Williams, D.W., and Liebhold, A.M. (1995) Influence of weather on the synchrony of gypsy moth (Lepidoptera: Lymandtriidae) outbreaks in New England. *Environmental Entomology*, 24, 987-995.

14.2.2. Potential risks of accidental introduction of Asian gypsy moth to Australia and New Zealand.

From *Agric. & For. Ent.* (2001) 3 305-320



Potential risks of accidental introduction of Asian gypsy moth (*Lymantria dispar*) to Australia and New Zealand: effects of climatic conditions and suitability of native plants

M. Matsuki[1], M. Kay[2] J. Serin[3], R. Floyd[1], and John K. Scott[3]
[1]CSIRO Entomology, GPO Box 1700, Canberra, ACT 2601 Australia, [2]Forest Research, Private Bag 3020, Rotorua, New Zealand, [3]CSIRO European Laboratory, Campus International de Baillarguet, Montferrier-sur-Lez, 34980 France

Summary

1. The potential risk of establishment Asian gypsy moth (AGM) (*Lymantria dispar*) in Australia and New Zealand was assessed from a study of the insect's host range and potential distribution. Asian gypsy moth is known for its extreme polyphagy, as shown by over 650 species of known potential hosts. In New Zealand, viable eggs of AGM have been continuously intercepted on cargo from Asia, and there is a possibility of accidental introductions of AGM to Australia and New Zealand.
2. We predicted potential distribution ranges of AGM based on climatic conditions. Asian gypsy moth is predicted to be able to persist in SE and SW Australia and New Zealand.
3. Using three populations of AGM and 75 species of plant, we also conducted laboratory trials to examine the ability of AGM larvae to complete development on native plants from New Zealand and Australia. AGM was able to complete development on 31 out of 63 native species from Australia and New Zealand.
4. Furthermore, larval performance on at least five species of Australian native plant species (*E. gunnii*, *E. risdonii*, *E. urnigera*, *Corymbia maculata*, and *Callistemon brachyandrus*) and *E. gunnii* × *E. delegatensis* hybrid (*E. irbyi*) was as good as the AGM's preferred native host species (*Q. humilis* and *Q. robur*).
5. Given the suitability of some Australian plants and climate for the establishment of AGM, this insect should be treated as a quarantine threat and managed accordingly.

Key words Lymantria dispar, *Asian gypsy moth, host range, biosecurity*, Eucalyptus, Nothofagus, Callistemon, Acacia, *predicted distribution, CLIMEX, Australia, New Zealand*

Introduction

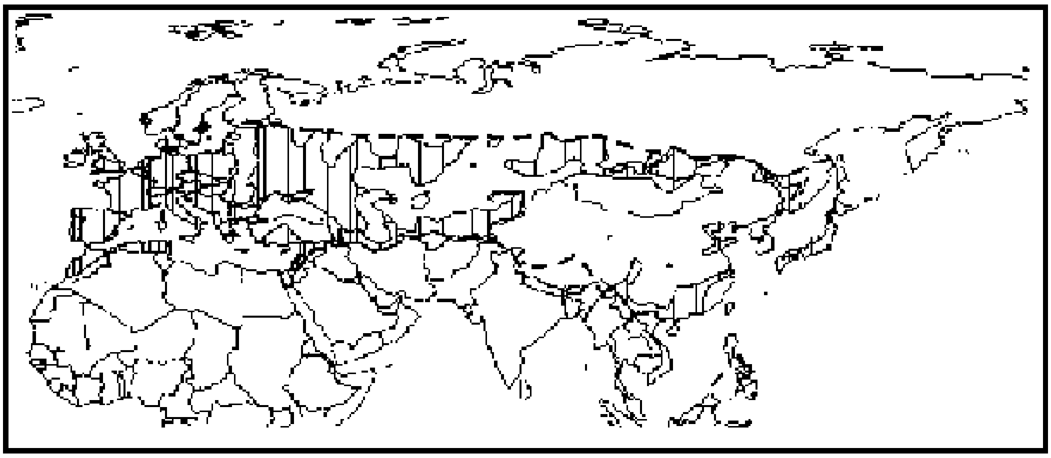
The gypsy moth (*Lymantria dispar* (L.), Lymantriidae, Lepidoptera) is native to Eurasia, and has two strains, the European gypsy moth (EGM) and Asian gypsy moth (AGM). Females of EGM are flightless, but those of AGM are capable of flight and can disperse up to 30 km (Wallner 1996). The two strains can be distinguished by genetic markers (Garner and Slavicek 1996). Asian gypsy moth is native to northeastern Asia but occurs in Europe along with European gypsy moth (Fig. 1) and has recently been reported from North America (Wallner 1996). From field and laboratory studies the gypsy moth is known to complete development on more than 650 species of trees (Liebhold *et al.* 1995), but few of the plant families of the Southern Hemisphere have been tested.

In North America, EGM has caused considerable damage to several important tree species in the 100 years since its introduction (Liebhold *et al.* 1992). Since 1980, US$30 million has been spent each year on gypsy moth control (Wallner 1996). Also, US$34 million has been so far spent on eradication of AGM introduced accidentally from Russia (Wallner 1996).

It is possible that AGM may be introduced accidentally to Australia and New Zealand. Females of AGM are attracted to light and lay egg masses on ships and light poles during outbreaks (Walsh 1993). In New Zealand, egg masses of AGM have been found on the cargo of ships from Russia (Walsh 1993). Also, 59 viable egg masses of AGM have been detected on motor vehicles imported into New Zealand since 1993 (MAF media release 28.09.2000). If introduced to Australia and New Zealand, the gypsy moth may pose a threat to trees and result in a considerable economic loss because it has been shown that it can feed on native tree species (e.g., *Eucalyptus* spp., *Acacia* spp., and *Nothofagus* spp.), commercially important species (e.g., *Pinus radiata*, apples, pears, and cherries), and amenity trees (e.g., oaks and poplars) (Liebhold *et al.* 1995). Without host information, New Zealand assumed the worst and spent NZ$8 million eradicating a closely related exotic pest, the white spotted tussock moth (*Orgyia thyellina* Btlr. Lymantriidae), with little knowledge of the potential impact this moth's introduction would have.

The objective of this study was to provide a scientific basis for predicting potential risks of accidental introduction of AGM to Australia and New Zealand. Using three populations of AGM and 75 species of plants, we conducted laboratory experiments to examine survivorship and pupal weight on the native plants of Australia and New Zealand. We also predicted potential distribution ranges of AGM based on climatic conditions.

Figure 1. Distribution of gypsy moth, *Lymantria dispar* in Eurasia (hatched area). The map is based on Giese and Schneider (1979) and is reprojected to match the CLIMEX format. The upper and lower dotted lines represent possible geographic limits.



Methods

Experimental tests of host range

AGM is a recognised biosecurity risk throughout the world and despite its occurrence in Europe, the trials were undertaken within a quarantine glasshouse with natural lighting, daylength, and minimum temperatures, but a maximum temperature of 22°C.

AGM eggs were sourced from:

- a Bavarian State Forest Research colony (a non-diapausing strain of AGM originating from the Beijng area),
- a wild population at Karbarovsk supplied by the Russian Far East Forest Research Institute (FEFRI) and
- a USDA colony at Montpellier (AGM originating from NE France). A molecular genetic study has shown that a single egg mass from this population contains individuals of AGM, EGM and their hybrids (Hérard *et al.* 1997).

We used 75 spp. of plants in this study (Table 1). Sources of foliage within Europe are as follows.

- *Nothofagus*: The Royal Botanic Garden Edinburgh, (Scotland); Wakehurst Place – Kew Gardens Arboretum, Hilliers Arboretum, Nymans Garden (England); Tintern, UK Forestry Commission (Wales); Mount Congreve Estate, Mount Usher Garden, Fota Estate (Ireland); Arboretum National de Barres, La Roche Fauconniere (France).

- *Pinus* and *Podocarpus*: Bedgebury Pinetum, UK Forestry Commission (Wales).
- *Eucalyptus*, *Callistemon*, *Melaleuca*, *Grevillea*, *Acacia*, and *Hakea*: Latte (Italy); la Nartelle; Sta. Maxine; Pons-Mûres; CSIRO European Laboratory; Jardin Botanique de la Villa Thuret, Institut National de la Recherche Agronomique; and la Mole and Cessenon, Association Foret Cellulose (France).

We also used seedlings of 5 *Eucalyptus* spp. and *Corymbia maculata*. Seeds were obtained from Australian Tree Seed Centre, Canberra, Australia.

- *Corymbia maculata*: seedlot 19308; Kioloa, NSW.
- *Eucalyptus globulus globulus*: seedlot 18886; Lorne, Vic; population know to be susceptible to at least some insect herbivores in Australia.
- *Eucalyptus globulus globulus*: seedlot 18723; Geeveston, Tas: population known to be resistant to at least some insect herbivores in Australia.
- *Eucalyptus nitens*: seedlot 16752; Thomson Valley Rd, Vic.
- *Eucalyptus nitens*: seedlot 18310; SSO APPM, Tas.
- *Eucalyptus grandis*: seedlot 13883; Urunga, NSW.
- *Eucalyptus melliodora*: seedlot 15939; 33K NE of Peak Hill, NSW.
- *Eucalyptus sideroxylon*: seedlot 15094; 33K NE of Parkes, NSW.

Host selection by female AGM is limited by its co-adapted behaviour, which favours oviposition on structures, which may eventually be covered by a thermal blanket of snow. Host selection is primarily a larval role, so the feeding trials consisted of 'no-choice' tests, where newly eclosed first instar larvae were caged with cut foliage in a two-chamber system. This kept the stem of the plant specimen in a lower water-filled container, while allowing a dry enclosed upper arena around the foliage for larvae. Ten larvae were released into each arena and five replicates were run for each plant individual or species (depending on species: Table 1) used.

Using two climate control cabinets within the quarantine area (14 hr photoperiod) we made observations on temperature limits to larval development. However, we were not able to measure larval growth at different temperature due to time constraints. The determination of threshold temperatures for pupal development was done using the two climate control cabinets (10° and 20°C, 14 hr photoperiod). Pupal development was recorded as time till adult emergence at the given temperature.

Four trials were established during 2000.

- Late April to early July: Beijng AGM with 7 *Nothofagus* spp. (3 NZ & 4 S American), 28 *Eucalyptus* spp. and 1 *Corymbia* sp.
- Early May to mid August: Siberian AGM with 14 *Nothofagus* spp. (4 NZ & 10 S American), 20 *Eucalyptus* spp., 1 *Corymbia* sp., 4 *Callistemon* spp., 1 *Grevillea* sp., and 1 *Melaleuca* sp.
- Late May to late August: Beijng AGM with, 12 *Nothofagus* spp. (5 NZ, 5 S American & 2 Australian), 2 *Pinus* spp., 2 *Podocarpus* spp., 14 *Eucalyptus* spp., 2 *Acacia* spp., and 1 *Hakea* sp.
- Late May to late August: NE France AGM with 5 *Nothofagus* spp. (4 NZ & 1 S American), 6 *Eucalyptus* spp., and 1 *Corymbia* sp.

In each trial oak foliage (seedling *Quercus robur* and mature tree *Q. humilis*) was used as a control. Trials were inspected each day and larval mortality (number out of 10), development time to pupation of each survivor (days), individual pupal weight (mg, five days after pupation) and sex were recorded for each container. Pupal weight stabilised from the initial metabolic loss after four to five days (Fig. 2). In the survivorship experiments, we used randomised block design with five blocks. Because we had unbalanced data sets, we used restricted maximum likelihood analysis (REML: Corbeil and Searle 1976) to estimate means. All statistical analyses were conducted using GENSTAT (Genstat 5 Committee 1993).

Due to logistical constraints, we were forced to use freshly cut foliage of some species and foliage that were stored at 5°C for other species. However, we found no difference in larval performance of AGM between freshly cut and stored foliage of *Q. humilis* (REML testing for the foliage type: $P = 0.2$ for pupal weight and $P = 0.3$ for larval duration. REML testing for the foliage type × sex interaction: $P = 0.2$ for pupal weight and $P = 0.1$ for larval duration).

Table 1. List of plant species used in the glasshouse experiments.

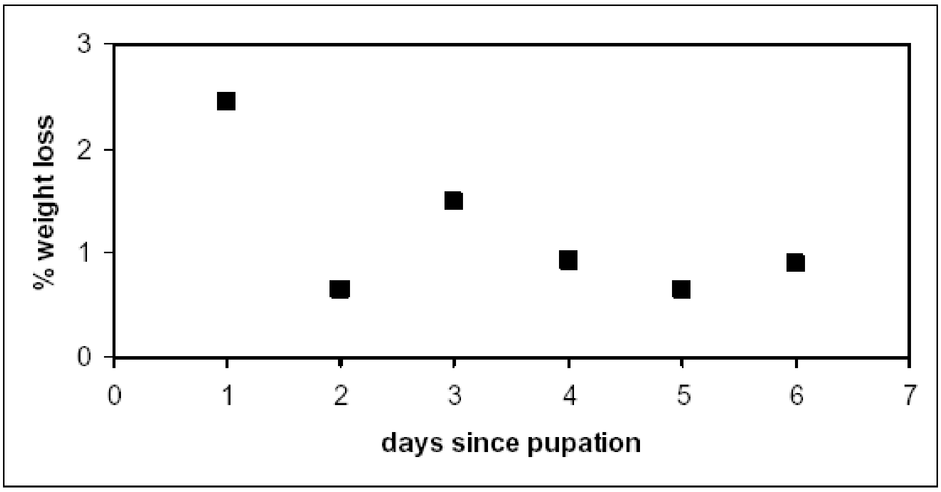
Species	**Native range**[1]	**Age of plants**	**No. populations**	**No. individuals**	**Foliage used in the four trials. n = new & o = old foliage**			
					1	**2**	**3**	**4**
Quercus humilis	Europe	mature	1	1	n	n	n	
Q. robur	Europe	seedling	1	50+	n	n		n
Corymbia maculata	NSW, Qld (coastal)	seedling	1	30+	n	n		n

Eucalyptus acaciafolia	NSW	mature	1	5	o	o		
E. archeri	Tasmania	mature	1	5	o			
E. aggregata	NSW, Vic (tableland)	mature	2	10	o			
E. amygdalina	Tasmania	mature	1	5	o	n		
E. camaldulensis	NSW, Vic, Qld, SA, NT	mature	1	1			n	
E. kartzoffiana	NSW (restricted)	mature	1	1			n	
E. cinerea	NSW, Vic	mature	2	6	o	n		
E. coccifera	Tasmania	mature	2	10	o			
E. cordata	Tasmania	mature	2	6	o	n		
E. dalrympleana	NSW, Vic, Tas	mature	1	2			n	
E. delegatensis	NSW, Vic, Tas	mature	1	5		n		
E. glaucescens	NSW, Vic	mature	1	5	o			
E. globulus globulus	Vic, Tas	seedling	2	10	n	n		n
E. globulus maidenii	NSW, Vic	mature	5	21			n	
E. globulus pseudoglobulus	Vic	mature	1	5	o/n	o/n		
E. grandis	NSW, Qld (coastal)	seedling	1	5	n	n		n
E. gunnii	Tasmania	mature	4	16	o	n	n	n
E. gunnii × E. delegatensis (E. irbyi)	Tasmania	mature	1	5	o	n		
E. laevopinea	NSW	mature	1	5	o			
E. linearis	Tasmania	mature	1	5			n	
E. macarthurii	NSW (restricted)	mature	1	1		n		
E. melliodora	NSW, Vic	seedling	1	5	n	n		n
E. melliodora	NSW, Vic	mature	1	1			n	
E. neglecta	Vic	mature	1	5	o			
E. nicholii	NSW (NE)	mature	1	5	o			
E. nitens	NSW, Vic	seedling	2	20+	n	n		n
E. nitens	NSW, Vic	mature	2	6			n	
E. obliqua	NSW, Tas, Vic	mature	3	15	o			
E. ovata	NSW, SA, Tas, Vic	mature	2	10	o			
E. parvifolia	NSW	mature	1	5		o		
E. pauciflora	NSW, Tas, Vic	mature	4	14	o		n	
E. perriniana	Tas	mature	2	10	o			
E. polyanthemos	NSW, Vic	mature	1	1			o	
E. regnans	Tas, Vic	mature	1	5			n	
E. risdonii	Tas	mature	1	5			n	
E. robertosonii	NSW, Vic	mature	1	5	o			
E. rubida	NSW, Tas, Vic	mature	1	5		o		
E. siebrii	NSW, Tas, Vic	mature	1	5	o			
E. sideroxylon	NSW, Vic	seedling	1	30+	n	n		n
E. simmondsii	Tas	mature	1	5	o			
E. stellulata	NSW, Vic (tableland)	mature	1	1			n	n
E. urnigera	Tas	mature	4	16	o	n		
E. viminalis	NSW, Tas, Vic	mature	1	2			n	
Eucalyptus sp 1		mature	1	5	o			
Eucalyptus sp 2		mature	1	5	o	o		
Callistemon brachyandrus	NSW, SA (Darling river)	mature	1	1		n		
Callistemon citrinus	NSW, Vic (coastal)	mature	1	1		n		
Callistemon viminalis	NSW, Qld	mature	1	1		n		
Callistemon macropunctatus	SA, Vic	mature	1	1		n		
Melaleuca squamea	NSW, SA Vic (coastal)	mature	1	1		n		
Grevillea juniperina	NSW (tableland)	mature	1	1		n		
Acacia melanoxilon	NSW, SA, Tas, Vic	mature	1	1			n	

Acacia dealbata	NSW, Tas, Vic	mature	1	5			n	
Hakea laurina	WA (SW)	mature	1	1			n	
Nothofagus alessandri	South America	mature	1	1		n		
N. antarctica	South America	mature	5	6		n		
N. betuloides	South America	mature	3	3	o	o		
N. dombeji	South America	mature	3	3	o	o	n	
N. fusca	New Zealand	mature	6	6	o	o/n	n	n
N. glauca	South America	mature	1	1			n	
N. leonii	South America	mature	1	1		n		
N. menzeizii	New Zealand	mature	7	8	o	o	n	
N. nervosa	South America	mature	1	1	n	n	n	
N. nitida	South America	mature	2	2		n	n	
N. obliqua	South America	mature	6	6	n	n	n	n
N. obliqua macrocarpa	South America	mature	2	2		n		
N. procera	South America	mature	3	3		n		
N. pumerii	South America	mature	3	3		n		
N. solandri	New Zealand	mature	5	5	o	n	n	n
N. solandri cliffitoides	New Zealand	mature	3	3			n	n
N. moorei	NSW, Qld (tableland)	mature	1	1			n/o	
N. cunninghamii	Vic, Tas	mature	2	2			n	
N. truncata	New Zealand	mature	1	1			n	
Podocarpus totara	New Zealand	mature	1	1			n	
Podocarpus halli	New Zealand	mature	1	1			n	
Pinus radiata	North America	mature	1	1			n	
Pinus contorta	North America	mature	1	1			n	

[1] States of Australia: NSW = New South Wales, Qld = Queensland, SA = South Australia, Vic = Victoria, Tas = Tasmania, WA = Western Australia.

Figure 2. Relationship between reduction in pupal weight of Asian gypsy moth and the number of days since pupation.



CLIMEX prediction of distribution

Incorporated in the "threat" concept that the gypsy moth represents is the important assumption that the organism will successfully establish and become widespread should it pass the quarantine barrier. This assumption should not be tested by experiment because of the dangers of escape from any experimental release sites. Hence the only way to assess the potential distribution in Australia and New Zealand is to develop predictive models based on the distribution known from elsewhere.

The computer program CLIMEX (Sutherst *et al.* 1999) enables predictions to be made of the potential growth of species at locations from climatic data and with this, their potential distribution around the globe. A fundamental assumption of the CLIMEX program is that a species distribution is determined by climate. The physical factors incorporated into the model used in the program include temperature and moisture conditions suitable for growth and various stresses, diapause, and light factors that limit growth. A full explanation of the CLIMEX program is provided in Sutherst *et al.* (1999).

The precise geographic and genetic boundaries of AGM and EGM are presently not defined. However, for the purpose of developing the CLIMEX model, as many factors as possible with relevance to AGM were used. In many cases it was not possible to determine whether or not the information on temperature or development time applied to EGM or AGM. It was considered reasonable to use all information available for both EGM and AGM as this was likely to err on the side of producing a more comprehensive model for AGM.

The procedure adopted was as follows. Firstly, we developed a model that predicts the distribution of AGM within the known distribution in Asia. The overall known distribution is reproduced in Figure 1 based on Giese and Schneider (1979) who has reconstructed to a common scale and projection historical maps to show a distribution of *Lymantria dispar* across Eurasia. Within CLIMEX, various growth and stress factors were adjusted, iteratively, to maximize the match to the known distribution and to the known phenology of the insect (both AGM and EGM depending on the region, Asia and Europe respectively). The parameter values and how they were derived are shown in Appendix 1.

Secondly, the model was tested against the known distribution of EGM in North America because AGM is not yet well established outside its native range. Further adjustment was to be considered if the model did not include all areas occupied by EGM. Thirdly, the model was used to predict the distribution of AGM in Australia and New Zealand.

Some AGM individuals develop without diapause and this has been used to develop continuous laboratory cultures (Hoy 1977). Thus, we also predicted distributions and growth index in New Zealand and Australia with and without diapause.

Results

Survivorship and pupation

Asian gypsy moth showed a wide range of responses to the native plant species from Australia and New Zealand (Table 2). Neonate larvae failed to initiate feeding and subsequently died on 14 species including the important commercial timber *E. globulus pseudoglobulus* and the northern-most Australian beech (*Nothofagus moori*). Larvae grew but failed to molt on 10 species including another timber species, *E. regnans*. On nine species such as *E. obliqua*, larvae developed to between the 3rd to the 6th instar, but failed to pupate.

On five *Nothofagus* species from New Zealand, one from Australia (*N. cunninghamii*) and 16 *Eucalyptus* spp., pupae were smaller than those on the preferred hosts of gypsy moth, *Q. humilis* and *Q. robur*. Pupae on *E. gunnii*, *E. risdonii, E. urnigera*, *Corymbia maculata*, and *Callistemon brachyandrus* were the same size as or larger than those on *Q. humilis* and *Q. robur* (Table 3).

We examined effects of leaf age on larval development of the non-diapausing strain on *E. amygdalina*, *E. gunnii*, *E. pauciflora*, and *E. irby* (*E. gunnii* × *E. delegatensis*). On all four host-species, developing leaves on young shoots supported faster growth of earlier instar larvae and consequently shorter overall larval duration and larger size of pupae (Table 4). However, later instar larvae preferred to feed on mature leaves of *Eucalyptus* spp. than on young leaves (data not shown).

Table 2. Plant species unsuitable for development of first instar AGM.

No feeding	Feeding but no molting	Molting but no pupation
E. kartzoffinana	*E. acaciafolia*	*E. neglecta*
E. cinerea	*E. glaucascens*	*E. nicholii*
E. cordata	*E. globulus globulus*	*E. obliqua*
E. globulus pseudoglobulus	*E. globulus maidenii*	*E. ovata*
E. polyanthemos	*E. laevopinea*	*E. archeri*
E. rubida	*E. regnans*	*E. parvifolia*
E. viminalis	*E. robertosonii*	*Eucalyptus* sp1
Callistemon viminalis	*E. siebrii*	*Eucalyptus* sp2
Melaleuca squamea	*Callistemon citrinus*	*Acacia dealbata*

Grevillea juniperina	*Callistemon macropunctatus*
Hakea laurina	*Acacia melanoxylon*
Nothofagus moorei	
Podocarpus totara	
Podocarpus halli	

Table 3 Test plants suitable for development of AGM. The overall rank is based on pupal weight and larval duration of both male and female and survivorship. SEs for comparing pupal weight are 50 (male) and 155 (female) and for comparing larval duration are 5 (male) and 4 (female).

	pupal mass (mg)		**larval duration (days)**		**survivorship**	**overall rank**
species	**male**	**female**	**male**	**female**		
Q. robur	**512**	**1229**	**25**	**35**	**66**	**1**
C. brachyandrus	480	1054	42	40	100	2
C. maculata	460	891	20	32	68	3
E. risdonii	507	1162	41	43	82	4
E. urnigera	593	1328	42	39	41	5
E. gunnii	521	1344	44	42	59	6
Q. humilis	**445**	**991**	**45**	**45**	**55**	**7**
P. radiata	394	964	45	52	74	8
E. irbyi	473	1062	49	47	41	9
E. stellulata	372	1222	46	41	28	10
N. cunninghamii	233	524	40	49	68	11
E. sideroxylon	292	497	32	45	31	12
E. coccifera	345	749	53	50	50	13
N. solandri cliffitoides	326	723	46	52	36	14
E. aggregata	363	646	52	53	38	15
N. solandri	323	573	48	55	44	16
E. grandis	199	504	33	44	7	17
P. contorta	316	1061	53	64	22	18
E. simmonsii	325	675	58	61	38	19
E. dalrympleana	257	264	52	54	41	20
E. camaldulensis	223	327	47	62	52	21
E. macarthrii	137	383	51	56	66	22
E. amygalina	305	715	59	68	14	23
E. pauciflora	237	628	58	55	13	24
E. linearis	228	455	58	62	22	25
N. fusca	224	468	55	65	25	26
E. melliodora	207	549	60	55	4	27
E. nitens	239	415	55	65	8	28
E. perrineana	329	-	64	-	2	29
E. delegatansis	192	494	67	83	14	30
N. truncata	269	-	70	-	4	31
N. menzeisii	223	-	68	-	1	32

Table 4. Effect of leaf age on pupal weight and larval duration. SEs are for comparing all means in each sex.

	Pupal weight (mg)		**Larval duration (days)**	
	New foliage	**Old foliage**	**New foliage**	**Old foliage**
Male				
E. amygdalina	337.5	235.1	49.8	57.5
E. gunnii	497.5	413.0	33.3	43.0
E. irbyi	588.2	409.9	40.9	44.2
E. pauciflora	250.0	186.6	41.7	53.3
SE	35.8		2.7	
Leaf age effect	$P < 0.0001$		$P < 0.0001$	
Leaf age × species effect	$P = 0.034$		$P = 0.068$	
Female				
E. amygdalina	731.2	641.1	59.5	68.5
E. gunnii	1350.1	903.6	39.5	50.0
E. irbyi	1231.9	891.7	44.5	47.9
E. pauciflora	470.2	423.5	49.8	64.0
SE	274.7		4.7	
Leaf age effect	$P = 0.01$		$P < 0.0001$	
Leaf age × species effect	$P = 0.66$		$P = 0.33$	

AGM on E. globulus

AGM larvae never developed beyond the 2nd instar on the two subspecies of *E. globulus* tested: *E. g. globulus* (seedlings) and *E. g. pseudoglobulus* (adult and juvenile foliage of mature plants). When the 3rd and 4th instar larvae, previously fed *Q. robur* and *Q. humilis* leaves throughout larval development, were placed in meshed bags on branches of potted seedlings of *E. g. globulus*, all larvae died of starvation. When the 6th instar larvae, previously fed *Q. robur* and *Q. humilis* leaves throughout larval development, were given juvenile and mature leaves from 21 mature trees of *E. g. maidenii*, some larvae ate very small amounts of leaf material (i.e., several bites), but all larvae eventually died of starvation or terminated further growth and pupated. We have repeatedly observed the premature pupation of the 6th instar larvae when food becomes scarce on other preferred host species. In a third host switch experiment, larvae were grown on *E. coccifera* until the 2nd to 4th instar and then were given mature leaves of *E. g. maidenii*. All larvae died of starvation.

Differences among AGM populations

Three populations of AGM showed similar responses with few minor differences. The Siberian population was able to complete development on *E. melliodora* and *E. grandis*, while two other populations could not complete development on these species. Larvae of the

Siberian population developed slightly faster, and pupae were larger than two other populations across different host plant species (Table 5).

Temperature limits

Larvae did not die after 24 hr exposures to 6°C or 35°C and resumed normal development when they were returned to 22°C. There was no adult emergence at 10°C up to 8 weeks. At 20°C, pupation lasted approximately 4 weeks: mean ± SD, females 27.0 ± 2.1 days (n = 8) and males 28.5 ± 2.1 days (n = 2).

Pupal weight – egg count relationship

In the wild population from Siberia and the non-diapausing strain from China, fresh pupal weight of females measured five days after pupation was linearly related to the number of eggs (Fig. 3). Moreover, the relationships between fresh pupal weight and the number of eggs in the two populations were indistinguishable from each other. Extrapolation in Figure 3 showed that no eggs should be produced from pupae below a threshold weight of 233mg. A few female pupae from some *Eucalyptus* spp. were below this threshold. The relationship between fresh pupal weight and the number of eggs in the French population was not examined.

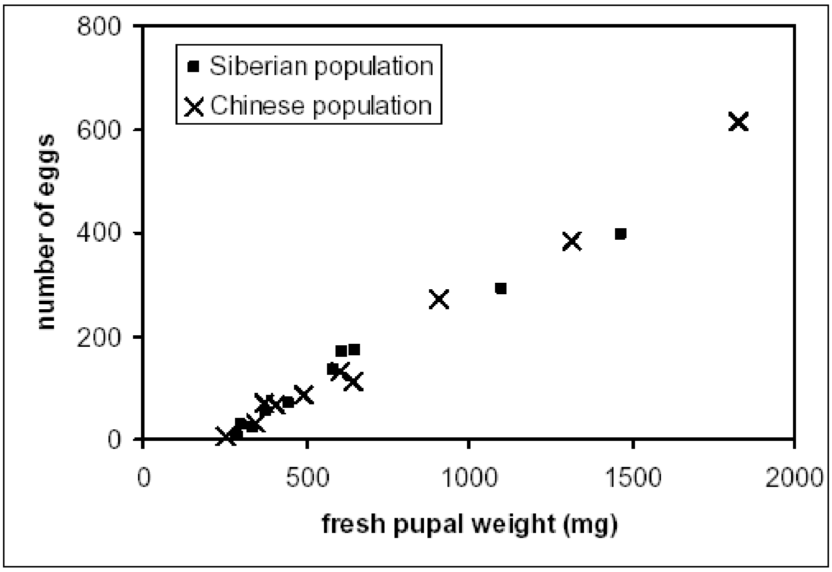
Table 5. Effect of AGM origin on pupal weight and larval duration. SEs are for comparing all means within each sex

	Pupal weight (mg)			**Larval duration (days)**		
	China	**Siberia**	**France**	**China**	**Siberia**	**France**
Male						
E. melliodora	-*	-	-	-	-	-
E. grandis	-	240.5	-	-	46.0	-
E. nitens	251.3	224.6	216.9	70.0	75.6	55.0
E. sideroxylon	294.9	309.1	-	50.8	44.3	-
C. maculata	458.0	486.9	347.3	37.2	31.5	37.6
SE	58.1			3.3		
Population effect	$P = 0.006$			$P = 0.01$		
Population × species effect	$P = 0.59$			$P < 0.0001$		
Female						
E. melliodora	-	529.4	-	-	61.0	-
E. grandis	-	530.5	-	-	50.0	-
E. nitens	-	441.0	-	-	71.9	-
E. sideroxylon	463.4	531.0	366.9	74.0	49.1	47.0
C. maculata	831.0	905.6	734.4	44.4	36.2	48.9
SE	148.2			6.4		
Population effect	$P = 0.03$			$P < 0.0001$		

Population × species effect	$P = 0.99$	$P = 0.01$

* no pupation

Figure 3. Relationship between the size of pupa and the number of eggs produced in Asian gypsy moth of Siberian (diapausing) and Chinese (non-diapausing) origin.



CLIMEX Model

Actual and predicted distribution ranges of gypsy moth are shown in Fig. 4. The upper boundary of the predicted distribution is strongly determined by the degree day value or the DV0 temperature. The effect of low temperature and cold stress is shown for Chita in eastern Russia where a positive growth index (GI) is restricted to the period from July to August (Fig. 5a). The model predicts that AGM would be able to survive in places with temperatures lower than those suitable for EGM such as the northern half of UK (Fig. 4).

The area of predicted distribution that has the poorest match to the distribution of AGM is that of southeast China, an area of subtropical climate (Fig. 4). It was not possible to assemble a set of CLIMEX parameters that were able to predict this area and at the same time, predict the major part of the gypsy moth distribution. Heat stress is an important excluding factor. The effect of heat stress is shown for the GI at Osaka in Japan (Fig. 5b). The accumulation of heat stress restricts the growth during the same period when the insect is not in diapause.

The model also predicts the presence of AGM throughout dryer inland parts of China and southern Russia where there are scarce records (Fig. 4). The effect of dry conditions is shown for Larache in Morocco (Fig. 5c). Diapause is completed by February and growth is restricted by moisture after which there follows periods of heat and dry stress. Figure 5d also shows the GI for Montpellier, which corresponded with the development of the insect during our research. The overall model for AGM corresponds best at the northern, cold limits and the southern dry limits. It is weakest for subtropical areas.

Figure 4. CLIMEX model of the distribution of AGM (circles). The recorded distribution is shown as hatched area (see Fig. 1). Crosses indicate climate stations where AGM is predicted not to survive. The size of circle indicates likelihood of survival.

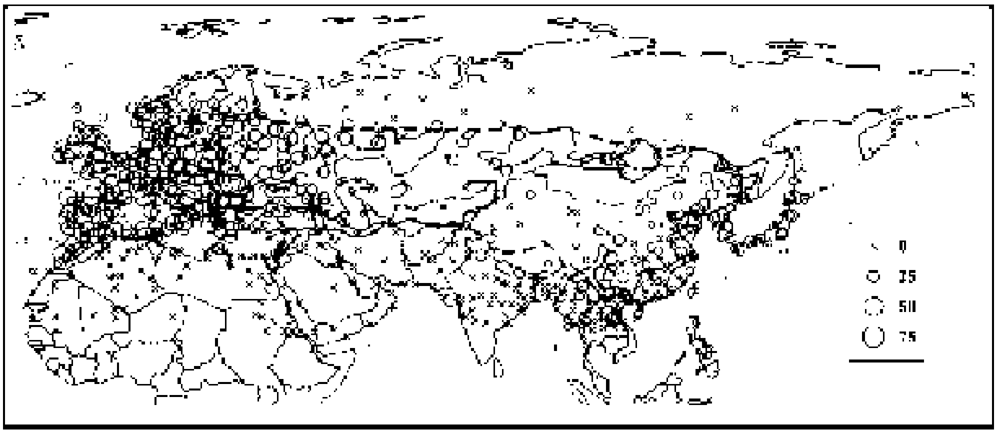
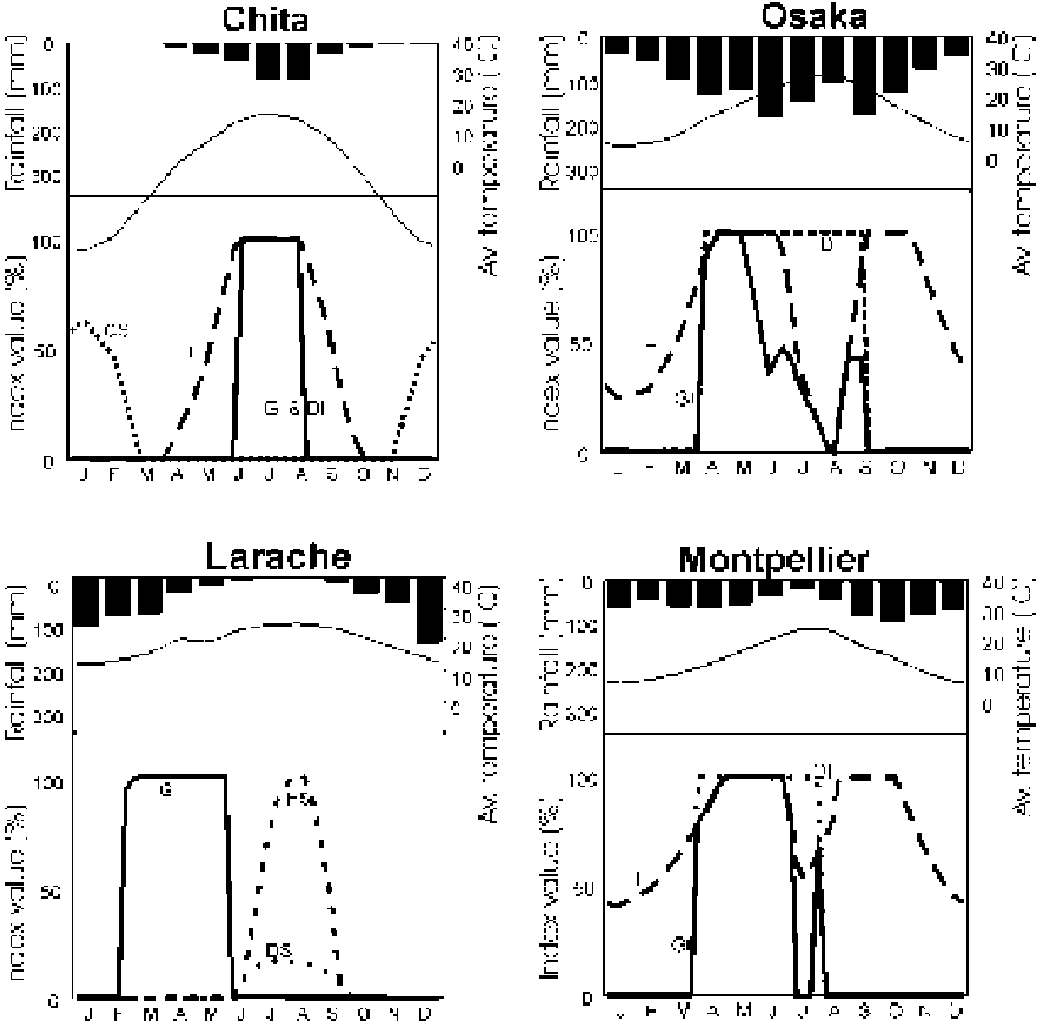


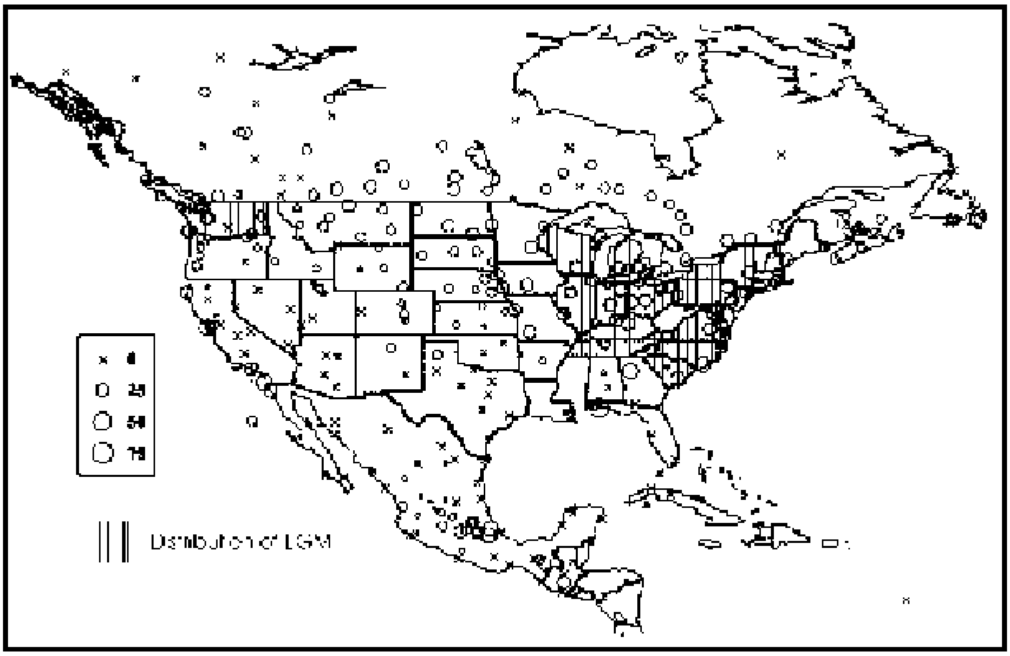
Figure 5. Monthly index values for a) Chita in eastern Russia, b) Osaka in Japan, c) Larache in Morocco and d) Montpellier in France.



Test of the model - prediction of the distribution of AGM in North America

The CLIMEX model predicts that AGM has the potential to colonize the northeastern USA, and Washington, where EGM has already become established (Fig. 6). The area including southern Canada, the west coast of USA and upland Mexico are also predicted as suitable for the establishment of AGM. As the predicted distribution includes the known EGM distribution in North America, no further adjustments were made to the model.

Figure 6. Predicted distribution of AGM in North America. The shaded areas show the States where EGM has been recorded. The size of circle indicates likelihood of survival.



Prediction of the distribution of AGM in Australia and New Zealand

The CLIMEX model predicted that AGM could colonize the east coast of New Zealand and all southern regions of mainland Australia (Fig. 7). The limits to the predicted distribution are dry conditions towards the inland and high moisture in southwest Tasmania and west coastal South Island of New Zealand.

Examples of the predicted growth index in Australia are shown in Figure 8. These indicate that at Manjimup, WA, the insect could come out of diapause in June and develop through to December (Fig. 8a). At colder sites, for example Hobart, TAS, development occurs between October to January (Fig. 8b). Bairnsdale, Vic is suitable for development between September to January (Fig. 8c) and an even longer period occurs at Brisbane, QLD (Fig. 8d). At all four sites, the period of larval development predicted by CLIMEX coincides with the period of leaf flush of *Eucalyptus* species (Fig. 8). Examples of the predicted growth index for New Zealand are shown in Fig. 9. Development can occur from July to January in the north gradually reducing to a period of November to February in the south.

Figure 7. Prediction of the distribution of AGM in Australia and New Zealand. The size of circle indicates likelihood of survival.

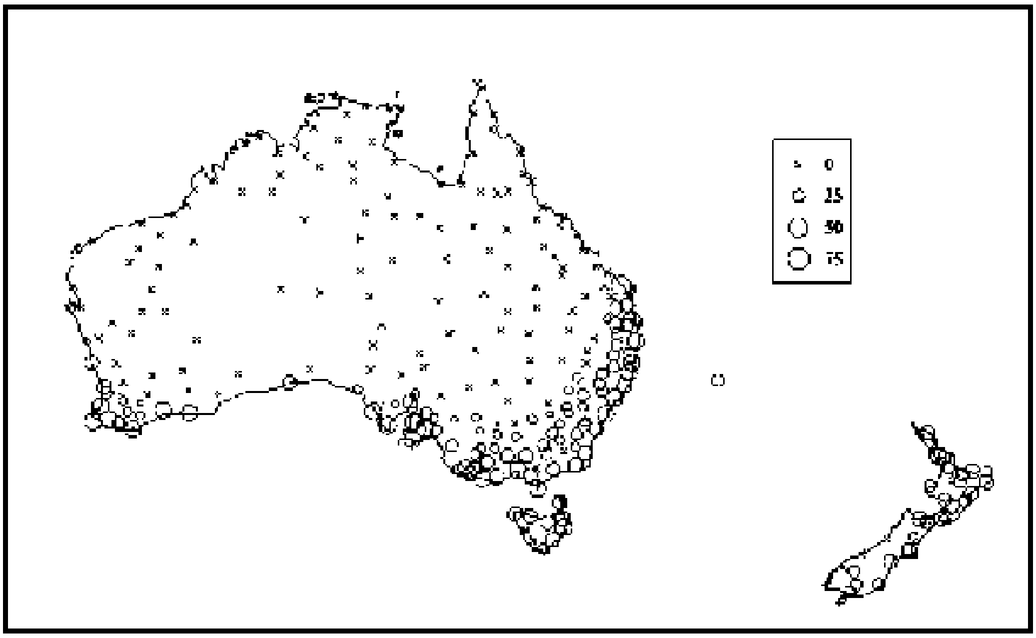


Figure 8. Monthly growth index values for AGM in Australia. a) Manjimup in Western Australia. Favourable development occurs between July to December. Leaf flush occurs between August and December. b) Hobart in Tasmania. favourable development occurs between October to mid January. Leaf flush occurs between September and January. c) Bairnsdale in Victoria. Favourable conditions for development occur between September to January. Leaf flush occurs between September to January. d) Brisbane in Queensland. Favourable conditions for development occur between July and March. Leaf flush occurs between August and May.

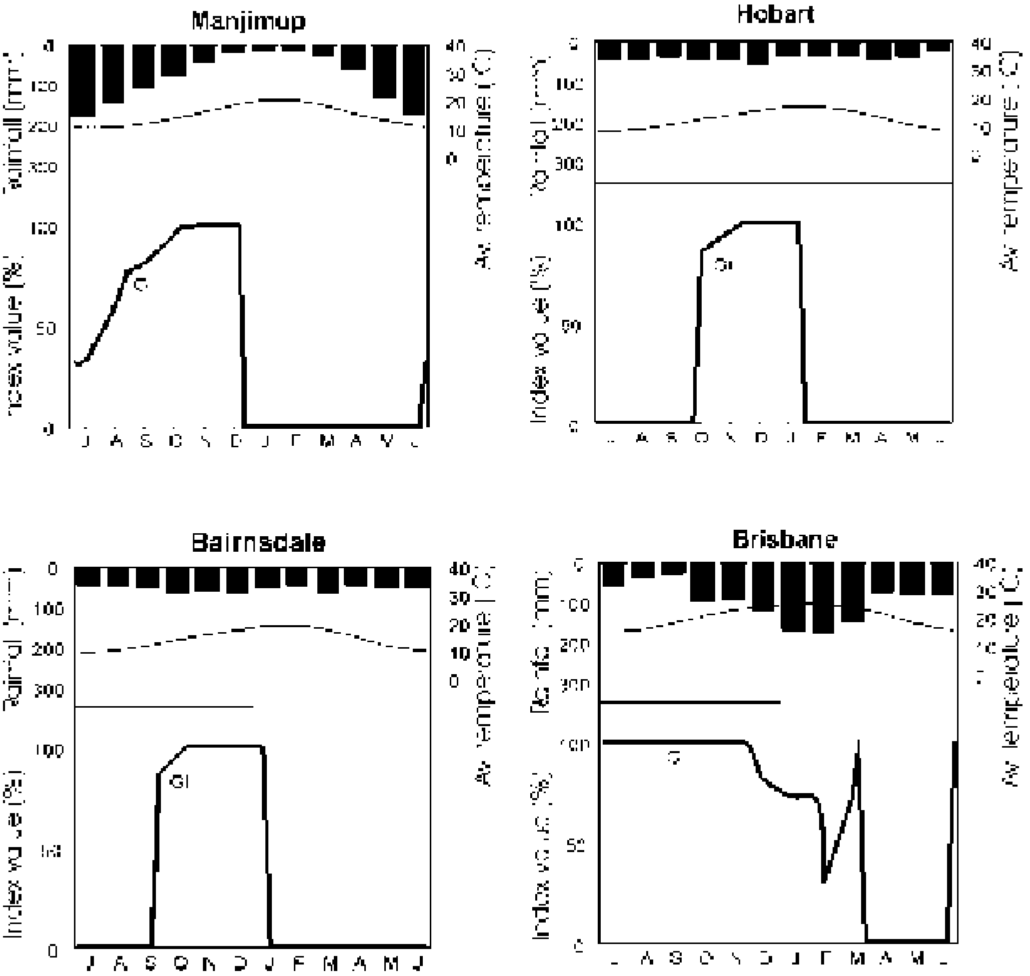
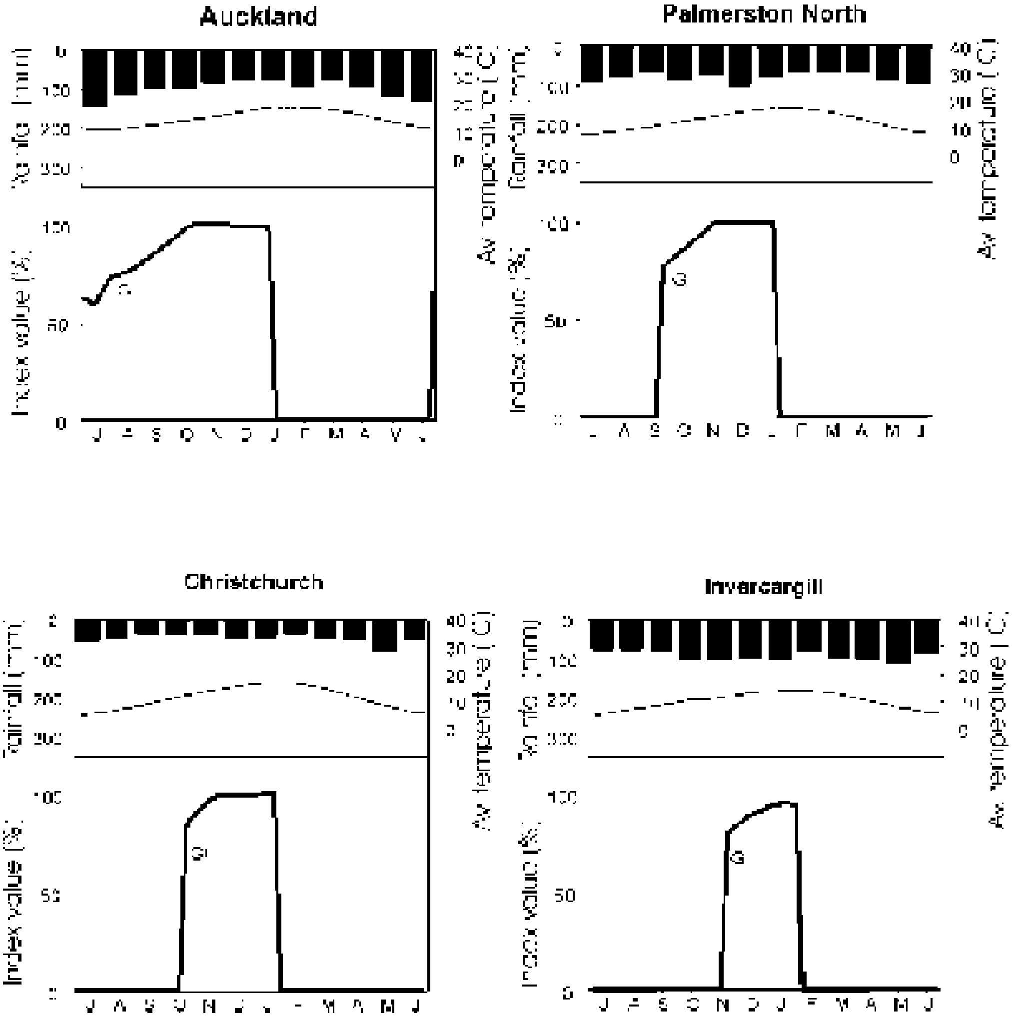


Figure 9. Monthly Growth (GI) index values for sites in New Zealand a) Auckland, b) Palmerstone North, c) Christchurch, d) Invercargill.



Effect of lack of diapause

Removing the diapause condition did not have any effect on the overall predicted distribution in Australia and New Zealand (as shown in Fig. 7). However, a significant change occurred to the growth index. At Manjimup, dry conditions limit growth during summer (Fig. 10a). At Hobart, Bairnsdale and Brisbane, however, there appears to be no physical limit to population growth (Fig. 10b, c, and d). Throughout New Zealand a similar pattern occurs where the insect will not be limited by climatic conditions in the absence of diapause (Fig. 11).

Figure 10. Australian sites showing monthly Growth Index (GI) values for an AGM model without diapause.

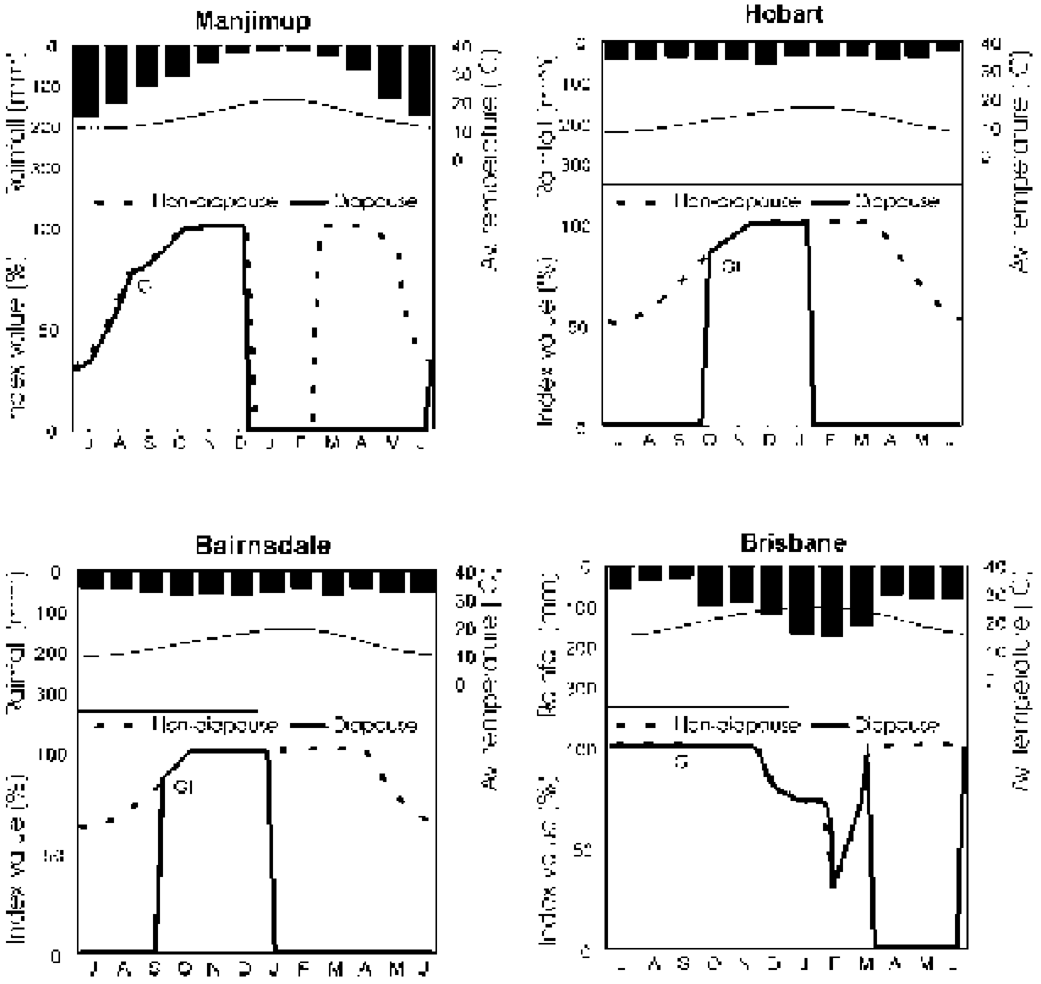
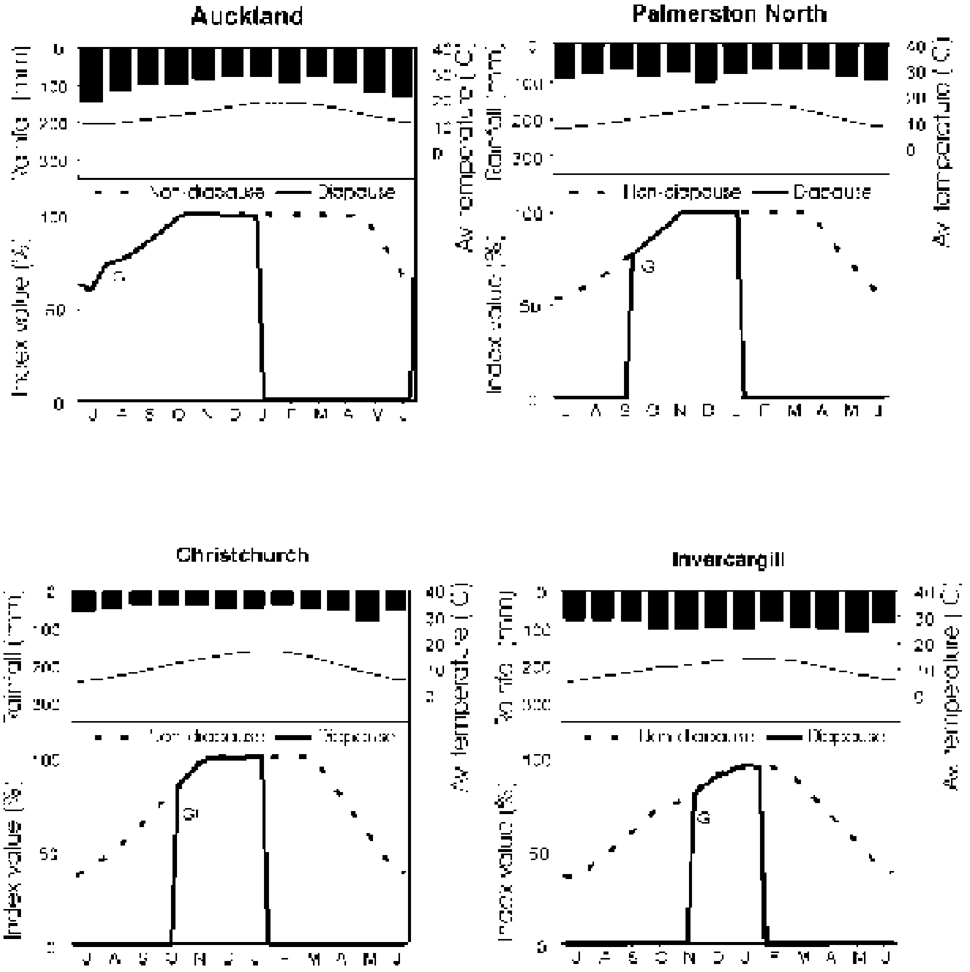


Figure 11. New Zealand sites showing monthly Growth Index (GI) values for an AGM model without diapause.



Discussion

Based on climatic conditions, AGM is predicted to be able to persist in SE and SW Australia and New Zealand (see also Allen *et al.* 1993). Moreover, experimental results showed that AGM are able to complete development on 31 out of 63 native species from Australia and New Zealand tested. Furthermore, larval performance on at least five species of Australian native plant species (*E. gunnii*, *E. risdonii*, *E. urnigera*, *Corymbia maculata*, and *Callistemon* *brachyandrus*) and *E. gunnii* × *E. delegatensis* hybrid (*E. irbyi*) was as good as the AGM's preferred native host species (*Q. humilis* and *Q. robur*).

At least fourteen species of *Eucalyptus* had been examined for suitability to development of the gypsy moth larvae (reviewed in Liebhold *et al.* 1995). Of the 14 species previously studied, seven species (*E. camaldulensis*, *E. camphora*, *E. cinerea*, *E. globulus*, *E. gunnii*, *E. polyanthemos*, and *E. sideroxylon*) were included in this study. *Eucalyptus gunnii* supported 100% survivorship from the 1st instar to pupation and pupal mass of greater than 1000 mg (Miller and Hansen 1989 cited in Liebhold *et al.* 1995). Larval performance on *E. cinerea* was reported to be as well as that on *E. gunnii* (Miller and Hansen 1989 and Montgomery 1994 cited in Liebhold *et al.* 1995). In the present study, however, neonate larvae failed to feed on *E. cinerea*. This difference may be due to misidentification of *Eucalyptus* species in the earlier study (M. Montogomery, *personal communication*). Larvae also failed feed on *E. polyanthemos* in the present study, but at least some larvae on *E. polyanthemos* had pupated in an earlier study (Miller and Hansen 1989 cited in Liebhold *et al.* 1995). Larval performance on *E. camaldulensis*, *E. camphora*, and *E. sideroxylon* in the earlier study appear slightly better than that in this study. At least seven other species of *Eucalyptus* (*E. botryoides*, *E. diversifolia*, *E. leucoxylon*, *E. pulchella*, *E. rudis*, *E. tereticornis*, and *Eucalyptus* spp.) and six species of *Acacia* (*A. baileyana*, *A. fernesiana*, *A. greggii*, *A. koa*, *A. tortuosa*, and *A. wrightii*), which were not included in the present study, had also been shown to support development of gypsy moth larvae (Miller and Hansen 1989 cited in Liebhold *et al.* 1995). Therefore, it is likely that more species of native Australian plants will be shown to support development of gypsy moth larvae as further studies are carried out.

In this study, we observed that *C. maculata* seedlings supported better larval performance than seedlings of *E. sideroxylon*, *E. melliodora*, *E. grandis*, or *E. nitens*. Moreover, old leaves from mature trees of *E. grandis* and *E. irbyi* supported better larval performance than seedling of *E. sideroxylon*, *E. melliodora*, *E. grandis*, or *E. nitens*. These differences in larval performance are more likely to be due to differences in secondary metabolites than those in leaf physical characteristics.

There has been one known record of natural encounters between gypsy moth and *Eucalyptus* and *Pinus radiata*. In Morocco, *E. camaldulensis* and *P. radiata* in plantations has been severely damaged by European gypsy moth during outbreaks (Fraval 1989). Gypsy moth moved from native oak forests to more than a dozen species of exotic plants when oak leaves were exhausted, but it is not known whether larvae have been able to complete development on *E. camaldulensis* in Morocco.

Potential threat by AGM to plantations in New Zealand and Australia

Pinus radiata plantations in New Zealand and SE Australia may be at risk. The moderate development of AGM on 'unimproved' *P. radiata* from neonate larvae is cause for concern. It has been shown (M. Kay *unpub. data*) that selection for production within this commercial species leads to a decrease in resistance to lepidopterous defoliators. Barbosa *et al.* (1986) showed that EGM on diets incorporating *Pinus* had fecundity higher than those reared on single species hosts and host switching to conifers is not uncommon. Rossiter (1981) observed a naturally occurring shift of late instar larvae from oak to pine, which resulted in a disproportionately lower mortality, and considered this may contribute to the maintenance of polyphagy within this insect.

Asian gypsy moth seems to be able to develop as well on *Corymbia maculata* as two species of deciduous oaks. *Corymbia maculata* is becoming increasingly important in commercial plantations in NE NSW and Qld. However, the risk of AGM establishment in NE NSW and Qld is lower than SE Australia because of climatic conditions such as high humidity and mild winter temperatures. At present, there is no strong evidence to suggest that AGM will be able to cause major damage to three species of *Eucalyptus* that are important in commercial plantations in SE NSW, Victoria, Tasmania, SA, and SW Western Australia (*E. globulus*, *E. nitens*, and *E. grandis*), but further studies are needed to confirm results of this study.

Potential threat to native vegetation of Australia

AGM larvae were able to grow at least as well on *E. gunnii*, *E. urnigera*, *E. risdonii*, *Corymbia maculata*, and *Callistemon brachyandrus* as their preferred hosts (two species of deciduous oaks). *Eucalyptus gunnii*, *E. risdonii*, and *E. urnigera* occur in Tasmania, and *C. brachyandrus* occurs along Darling River. These areas are within the predicted distribution ranges of AGM based on climatic conditions. Some other *Eucalyptus* species endemic to Tasmania (*E. coccifera*, *E. simmondii, E. perrineana, E. linearis*, and *E. amygdalina*) are also shown to be susceptible to AGM.

Some wide spread species in SE Australia such as *E. camaldulensis*, *E. pauciflora*, *E. dalrympleana*, and *Corymbia maculata* are susceptible to AGM. Distribution ranges of these *Eucalyptus* species collectively covers from the sea level to tree lines on mountain peaks and from coast to inland along rivers. Therefore, if accidentally introduced, AGM is likely to be able to persist in native forests and woodlands in much of SE (and SW) Australia at low population densities and cause damage to exotic trees in populated areas (e.g., oaks, poplars, and apples). Because we were able to use only a small subset of native plant species of Australia, there remains possibilities of other untested species being susceptible to AGM, and therefore, the predicted risk of accidental introduction of AGM to native vegetation from this study should be considered as the minimum potential risk.

Furthermore, AGM showed considerable variation in the ability to develop on *Eucalyptus* species (Matsuki *et al.* ms). If AGM establishes in native forests and woodlands of Australia, we are likely to see differential survival and reproduction among individuals of AGM. Thus, *Eucalyptus* species would act as a constant selection agent and might facilitate evolution of AGM that is better able to develop on *Eucalyptus* species.

Future research directions

Extensive studies conducted in North American and Europe identified two potentially important factors contributing to outbreaks of the gypsy moth: availability of young foliage to earlier instar larvae and nonlinear interactions between the gypsy moth and its natural enemies (Berryman 1991, Gould *et al.* 1990, Elkinton and Liebhold 1990, Williams and Liebhold 1995, Leonard 1974, Hwang *et al.* 1995). Our results on *Nothofagus* and *Eucalyptus* are in agreement with earlier studies in North American and Europe that larval performance is increased on young foliage compared with old foliage. Predicted periods of larval development in Australia and New Zealand coincide with periods of leaf flush, thus increasing risks of outbreaks. There is very little data on effects of temperature on larval development when larvae feed on *Nothofagus* or *Eucalyptus*. Further studies in this area will enable more precise prediction of the gypsy moth distribution and the periods of larval development in Australia and New Zealand.

Acknowledgements

We thank J. Vitou, C. Espiau, Y. Mas, T. Thomann, J.-L. Gornes, M. Jourdan, C. Ducatillon, J.-N. Marien, F. Hérard, H. Scheel, and G. Farrell for assistance. The following botanic gardens and research organizations granted permission to collect foliage: The Royal Botanic Garden Edinburgh, (Scotland); Wakehurst Place – Kew Gardens Arboretum, Hilliers Arboretum, Nymans Garden (England); Tintern and Bedgebury Pinetum, UK Forestry Commission (Wales); Mount Congreve Estate, Mount Usher Garden, Fota Estate (Ireland);

Arboretum National de Barres, La Roche Fauconniere, Institut National de la Recherche Agronomique, and Association Foret Cellulose (France). Gypsy moth eggs were provided by Gabriele Lobinger (Bavarian State Forest Research Institute, Germany), Galina Yurchenko (Russian Far East Forest Research Institute, Russian Republic), and F. Hérard (USDA, France). M. Steinbauer, C. McArthur, J. Elek, A. Lock, and C. Stone kindly shared their knowledge on phenology of eucalypts. Financial support was provided by Ministry of Agriculture and Forestry, New Zealand and Agriculture, Fisheries and Forestry – Australia.

References

Abdullaev, Y.N. (1967) Biological characteristics of the gypsy moth in the forests of Samarkand Province. In: Voprosy zashchity rasteniy. Tashkent: 80-82. (Abstract 9 in Baranchikov *et al.* 1998).

Allen, J.C., Foltz, J.L., Dixon, W.N., Liebho, A.M., Colbert, J.J., Regniere, J., Gray, D.R., Wilder, J.W. and Christie, I. (1993) Will the gypsy moth become a pest in Florida? *Florida Entomologist*, **76**, 102-113.

Baranchikov, Y.N., Nikitenko, G.N. and Montgomery, M.E. (1998) Russian and Ukrainian literature on the gypsy moth: an annotated bibliography. USDA Forest Service General Technical Report NE 253.166 pp.

Barbosa, P., Martinat, P., and Valdvogel, M. (1986) Development, fecundity and survival of the herbivore *Lymantria dispar* and the number of plant species in its diet. *Ecological Entomology*, **11**, 1-6.

Barteneva, Y.V., Gostjunin, I.V. and Persidskaya, L.T. (1974) The effect of temperature on the feeding rate of gypsy moth larvae. In:Nauchnyye trudy Moskovogo lesotekhniceskogo instituta. Voprosy zashchity lesa. MLTI, Moskova: 93-96. (Abstract 115 in Baranchikov *et al.* 1998).

Benkevich V.I. (1955) The role of solar radiation in the heat balance of developing gypsy moth eggs. In : Uchenyye zapiski Orekhovo-Zuyevskogo pedagogicheskogo instituta. Moscow: 163-175. (Abstract 173 in Baranchikov *et al.* 1998).

Berryman, A.A. (1991) The gypsy moth in North America: A case of successful biological control? *Trends in Ecology and Evolution*, **6**, 110-111.

Campbell, R.W. (1981) Population dynamics. In. Doane, C.C. and McManus, M.L. The gypsy moth: research toward integrated pest management. USA Forest Service Science and Education Agency Animal and Plant Health Inspection Service Technical Bulletin 1584. pp 65-86.

Corbeil, R.R. and Searle, S.R. (1976) Restricted maximum likelihood (REML) estimation of variance components in the mixed model. *Technometrics*, **18**, 31-38.

Elkinton, J.S. and Liebhold, A.M. (1990) Population dynamics of gypsy moth in North America. *Annual Review of Entomology*, **35**, 571-596.

Fraval, A. (1989) Degats et nuisances. Pages 73 – 76, in Lymantria dispar (A. Fraval, ed.). Actes Editions, Rabat, Morocco.

Garner, K.J. and Slavicek, J.M. (1996) Identification and characterisation of a RAPD-PCR marker for distinguishing Asian and North American gypsy moths. *Insect Molecular Biology*, **5**, 81-91.

Genstat 5 Committee. (1993) *Genstat 5 Release 3, Reference manual*. Oxford University Press, Oxford, UK.

Giese, R.L. and Schneider, M.L. (1979) Cartographic comparisons of Eurasian gypsy moth distribution (*Lymantria dispar* L.; Lepidoptera: Lymantriidae). *Entomological News*, **90**, 1-16.

Gould, J.R., Elkinton, J.S., and Wallner, W.E. (1990) Density-dependent suppression of experimentally created gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantriidae) populations by natural enemies. *Journal of Animal Ecology*, **59**, 213-233.

Hérard, F. and Drea, J.J. (1981) Agricultural Research service sponsored public law 480 project in Morocco: 1972-1975. In. Doane, C.C. and McManus, M.L. The gypsy moth: research toward integrated pest management. USA Forest Service Science and Education Agency Animal and Plant Health Inspection Service Technical Bulletin 1584. pp 317-321.

Hérard, F., Chen, K., and Cameron, E.A. (1997) Natural enemy impact on gypsy moth in Alsace, France, during 1995. In: Fosbroke, S.L.C. and Gottschalk, K.W. (eds.) Proceedings, U.S. Department of Agriculture interagency gypsy moth research forum 1996; 1996 January 16-19; Annapolis, MD. Gen. Tech. Rep. NE-230. Radnor, PA: USDA, F.S., N.E. Exp. Sta., p.39.

Hoy, M.A. (1977) Rapid response to selection for a nondiapausing gypsy moth. *Science*, **196**, 1462-1463.

Hwang, S.Y., Lindroth, R.L., Montogomery, M.E., and Sheilds, K.S. (1995) Aspen leaf quality affects gypsy moth (Lepidoptera: Lymantriidae) susceptibility to Bacillus thuringiensis. *Journal of Economic Entomology*, **88**, 278-282.

Idrisova, N.T. (1977) Materials on the biology and ecology of the gypsy moth in Bashkirian ASSR. In: Nasekomyye – vrediteli lesov Bashkirii. Ufa: 38-54. (Abstract 372 in Baranchikov *et al.* 1998).

Liebhold, A.M., Gottschalk, K.G., Muzika, R., Montgomery, M.E., Young, R., O'Day, K., and Kelly, B. (1995) Suitability of North American tree species to the gypsy moth: a summary of field and laboratory tests. USDA General Technical Report NE-211, USDA Forest Service, Delaware, Ohio, USA.

Liebhold, A.M., Halverson, H.A., and Elmes, G.A. (1992) Gypsy moth invasion of North America: a quantitative analysis. *Journal of Biogeography*, **19**, 1-8.

Leonard, D.E. (1974) Recent development in ecology and control of the gypsy moth. *Annual Review of Entomology*, **19**, 197-299.

Leonard, D.E. (1981) Bioecology of the gypsy moth. In. Doane, C.C. and McManus, M.L. The gypsy moth: research toward integrated pest management. USA Forest Service Science and Education Agency Animal and Plant Health Inspection Service Technical Bulletin 1584. pp 9-29.

Lyamtsev, N.I. (1983) Impact of weather on changes in gypsy moth density. In: Molodyye unchenyye k yubileyu instituta. Trudy SSSR VNIILM, Pushkino: 188-194. (Abstract 657 in Baranchikov *et al.* 1998).

Mason, C.J. and McManus, M.L. (1981) Larval dispersal of the gypsy moth. In. Doane, C.C. and McManus, M.L. The gypsy moth: research toward integrated pest management. USA Forest Service Science and Education Agency Animal and Plant Health Inspection Service Technical Bulletin 1584. pp 161-202.

Matsuki, M., Kay, M., Serin, J., and Scott, J.K. (ms) Variation in the ability of larvae of phytophagous insects to deal with evolutionarily unfamiliar plants: A study with the gypsy moth and *Eucalyptus*.

Miller, J.C., and Hanson, P.E. (1989) Laboratory feeding tests on the development of gypsy moth larvae with reference to plant taxa and allelochemicals. Stn. Bull. 674. Oregon State University, Agriculture Experiment Station, Corvallis, OR. 63p.

Montgomery, M.E. (1994) Comparison on several tree species of growth and survival of newly hatched larvae of gypsy moth from three continents. On file at Northeastern Forest Experiment Station, Hamden, CT. 2p.

Rossiter, M.C., (1981) Factors contributing to the host range expansion in the gypsy moth, *Lymantria dispar*. PhD thesis State University of New York, Stony Brook.

Rubtsov, I.A. (1938) Effect of constant and fluctuating temperatures on egg development in the gypsy moth (*Porthetria dispar* L). Zashchita rasteniy. 17: 25-38. (Abstract 855 in Baranchikov *et al*. 1998).

Schaefer, P.W. (1981) Explorations in Japan and Korea by the ARS Asian Parasite Laboratory: 1975-77. In. Doane, C.C. and McManus, M.L. The gypsy moth: research toward integrated pest management. USA Forest Service Science and Education Agency Animal and Plant Health Inspection Service Technical Bulletin 1584. pp 340-348.

Sutherst, R.W., Maywald, G.F., Yonow, T. and Stevens, P.M. (1999) CLIMEX: predicting the effects of climate on plants and animals. CSIRO Publishing, Collingwood. 88 pp.

Wallner, W.E. (1996) Invasion of the tree snatchers. *American Nurseryman*, **March 15 issue**, 28-30.

Walsh, P.J. (1993) Asian gypsy moth: the risk to New Zealand. *N. Z. Forestry*, **August issue.**

Williams, D.W., and Liebhold, A.M. (1995) Influence of weather on the synchrony of gypsy moth (Lepidoptera: Lymantriidae) outbreaks in New England. *Environmental Entomology*, **24**, 987-995.

Appendix 1. Information sources used to develop the CLIMEX model for AGM, *Lymantria dispar*.

Parameter	**Information used to establish parameter values**
Temperature	
DV0 = 1°C	A theoretical threshold for egg development is 3-4°C (Rubtsov 1938). Mason and McManus (1981) concluded that EGM has a base threshold temperature near 4°C for the accumulation of degree-days during egg development in spring. Starting at 5°C, successively lower temperatures were tried in the model until sites where gypsy moth is present in north east Asia were included.
DV1 = 14.5°C	This temperature represents the lower value in the ideal range of development. At 15°C larvae feed and gain weight (Barteneva *et al.* 1974). In Samarkand Province, Tashkent, larvae hatch at 14.1°C (Abdullaev 1967).
DV2 = 28°C	This temperature represents the upper value in the ideal range of development and is based on the observation that maximum food consumption occurs at 27-28°C (Barteneva *et al.* 1974).
DV3 = 32°C	Larvae and pupae reared at 32°C produce females that are infertile (Leonard 1981) consequently this temperature was chosen as the upper limit for development.
PDD = 1700	The value of 1700 degree-days (DD) for the development of AGM was derived from the sum of development periods for embryos, larvae, pupae and adults and adjusted so that the predicted distribution corresponded with the northern distribution limit. The DD are accumulated above the value DV0. Benkevich (1955) records 121 DD in spring for embryonic development of larvae before hatching. Idrisova (1977) records 168.7 - 210 DD for embryonic development, 922.5 DD for larval development and 279.8 DD for pupal development for insects from Bashkirian ASSR. A value of 240 DD was assumed for adults. Lyamtsev (1983) indicates for larvae in the Saratov Province, a development time of 1176 and 1202.5 DD at 14.7°C and 18.5°C respectively. Our laboratory calculation of DD for AGM indicated that at about 24°C a minimum of 700 DD is required for pupal development.
Moisture	
SM0 = 0.09	Information on the limit to the distribution of AGM due to dry desert conditions is difficult to model because of 1) a lack of climate stations in the CLIMEX database for dry areas in Asia details and 2) the distribution map (Figure 1) does not indicate a definite boundary reflecting a declining rainfall gradient. Consequently, the information in Hérard and Drea (1981) showing the distribution of EGM in Morocco was used and the value SM0 adjusted to exclude Moroccan sites where EGM was not recorded.
SM1 = 0.1	Both SM1 and SM2 were adjusted to maximize the moisture component of the index throughout the distribution of AGM.
SM2 = 1.3	Both SM2 and SM3 were chosen to ensure that Japanese sites with AGM (Schaefer 1981) were included.
SM3 = 1.5	GM are adversely affected by high rainfall which causes high mortality of first instars which are washed off the plant (Leonard 1981). High rainfall, particularly in June, probably brings with it a biotic limiting factor, high incidence of disease (Campbell 1981). The upper moisture value was also adjusted so that high rainfall locations were excluding along with those typical of the tropics .were excluded.

Appendix 1 continued.

Diapause	
DPD0 = 14.5	GM starts diapause as an egg, consequently the daylength was set to correspond to the period of egg laying.
DPT0 = 19°C	Diapause is induced following summer. Iterations were made around this temperature so that diapause corresponded to the phenology of the insect.
DPT1 = 7°C	This represents the average weekly temperature that terminates diapause. Again this value was adjusted to match known phenology.
DPD = 80	The minimum number of days to complete diapause.
Co stress	
TTCS = -20.0 THCS = 0.0005	GM is unable to sustain long periods of co, however the insect often lays eggs in micro-climates, that once under snow, are sufficiently warm to enable egg survival. The values for co stress were established to exclude the most north eastern climate stations in Russia. Note these are also excluded by the day-degree calculation.
Heat stress	
TTHS = 30.00 THHS = 0.005	GM larvae are unable to develop above 32°C. The values chosen for heat stress were used to exclude tropical regions.
Dry stress	
SMDS = 0.09 HDS = 0.02	Dry stress values were used to adjust for the phenology and distribution of EGM in Morocco as reported in Hérard and Drea (1981).
Wet stress	
SMWS = 1.2 HWS = 0.001	GM larvae are adversely affected by high rainfall. Wet stress was adjusted to ensure the inclusion of Japanese sites (Schaefer 1981) while excluding as many tropical sites as possible.

Appendix 14.3: Contingency plan for the detection and, if necessary, eradication of Asian Gypsy moth in New Zealand.

by Patrick Walsh (previous of FR and FRASS, New Zealand) (Report prepared by FR and MAF, 1996).

Trap Locations

Pheromone traps that attract male gypsy moths (*Lymantria dispar*) have been placed around the following ports in the second and third weeks of November 1993.

TABLE 1 - Location and number of traps (1996)

Port	No. of Traps
Auckland	290
Tauranga	100
New Plymouth	135
Napier	100
Wellington	315
Nelson	150
Lyttelton	210
Timaru	160
Port Chalmers	190
Dunedin	240
Bluff	100

These ports have been selected because they have been visited by Russian ‘at risk’ vessels in the past four years.

Trap Density

The density of traps at each port is 2/km^2 in a 7 km radius from the centre of the port. As far as practicable, an even distance is maintained between traps. There are also traps where vessels would have approached with ca. 4 km of land as they approach the port. This applies only at the ports of Auckland and Wellington.

The decision to trap within a 7 km radius is based on recommendations made by the United States Asian Gypsy Moth Science Advisory Panel. A trap density of 2 per km^2 is the minimum. Estimated probabilities for **NOT** detecting a population of 10, 100 and 1000 gypsy moth males/km^2 is given in the table below. This is based on work done in the United States. Note that <5% of males fly more than 800 metres after emergence.

TABLE 2 - Probabilities of not detecting a population

Traps/km^2	**Asian gypsy moth (male) numbers/km^2**		
	10	**100**	**1000**
2	91%	41%	0.01%
10	51%	0.12%	6.48 x 10^{-28}%

Trap Inspection

The traps will be in place for 6 months and will be inspected weekly. Trap inspectors have been appointed for all ports. If a moth(s) is found in a trap it will immediately be sent to the Forest Health group at NZ FR for identification.

Action to be taken on detection of Asian Gypsy Moth

If an Asian Gypsy moth is trapped the Secretary of Forestry will be advised and he will immediately:

1. Declare a 50 km radius “infected” under the Forest Disease Control Regulations.
2. Take action outlined below, through the North and South Island Protection Managers and the Forest Research Institute.
3. Form an Advisory Committee.
4. Make application to obtain the required funding.
5. Appoint a public relations person.

A. If the moth(s) is found near the **centre of the trapping zone** (i.e. 0-5 km of the 7 km radius) more traps will immediately be deployed to delineate the infested area as follows:

16 traps/km^2 in 2 km radius of find
8 traps/km^2 in 2-4 km
2 traps/km^2 in 4-7 km

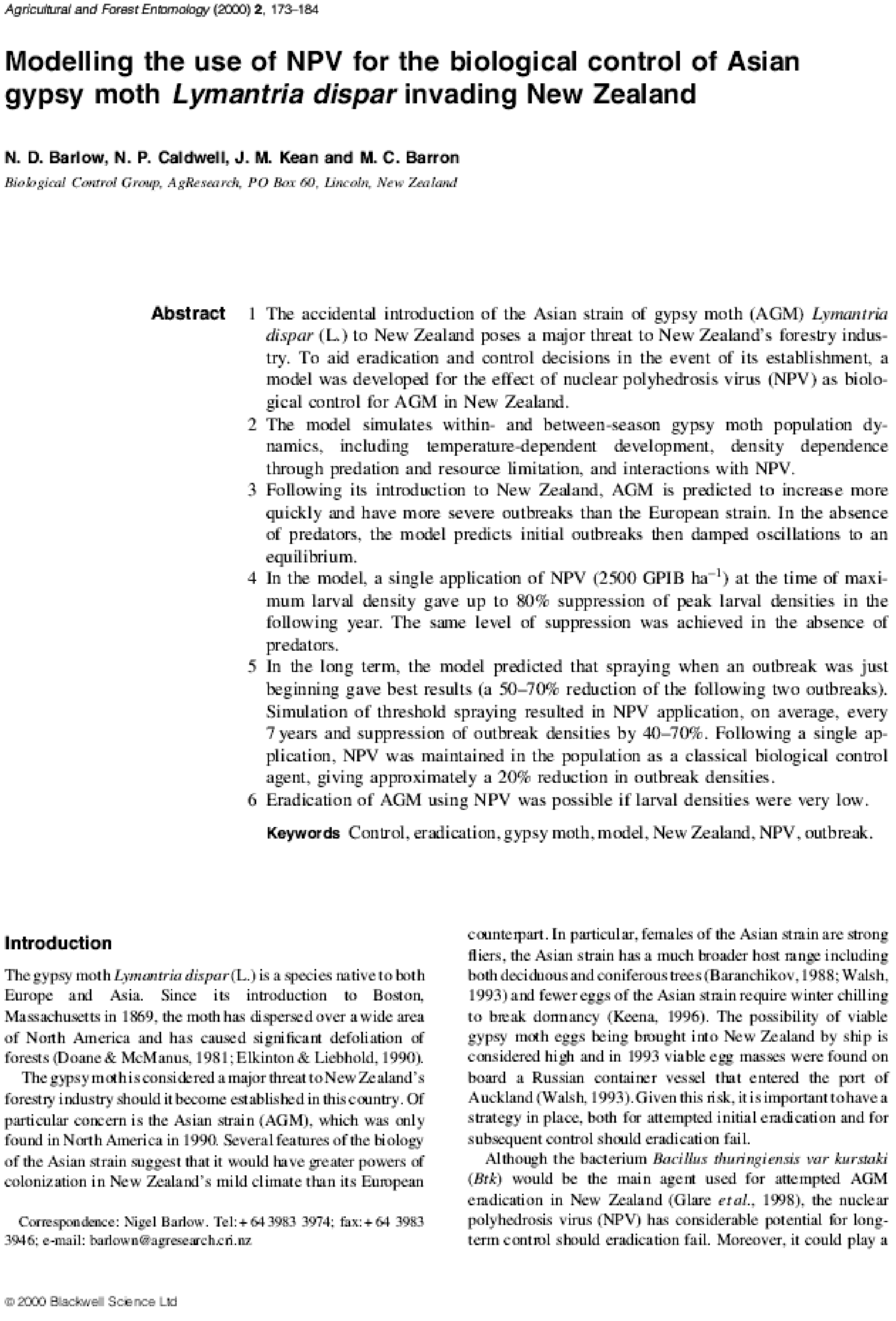
If the moth(s) is found on the **edge of the trapping zone** (i.e. 5-7 km of the 7 km radius) then traps will be deployed as above **plus**:

2 traps/km^2 in a 14 km radius around the find

Appendix 14.4: Summary of the Vancouver gypsy moth programmes

For an in-depth review of gypsy moth infestations and treatment in Vancouver, see http://www.for.gov.bc.ca/hfp/gypsymoth/history.htm

Appendix 14.5: Use of NPV for eradication or follow-up control: results from a predictive model



Agricultural and Forest Entomology (2000) **2**, 173–184

Modelling the use of NPV for the biological control of Asian gypsy moth *Lymantria dispar* invading New Zealand

N. D. Barlow, N. P. Caldwell, J. M. Kean and M. C. Barron
Biological Control Group, AgResearch, PO Box 60, Lincoln, New Zealand

Abstract

1 The accidental introduction of the Asian strain of gypsy moth (AGM) *Lymantria dispar* (L.) to New Zealand poses a major threat to New Zealand's forestry industry. To aid eradication and control decisions in the event of its establishment, a model was developed for the effect of nuclear polyhedrosis virus (NPV) as biological control for AGM in New Zealand.

2 The model simulates within- and between-season gypsy moth population dynamics, including temperature-dependent development, density dependence through predation and resource limitation, and interactions with NPV.

3 Following its introduction to New Zealand, AGM is predicted to increase more quickly and have more severe outbreaks than the European strain. In the absence of predators, the model predicts initial outbreaks then damped oscillations to an equilibrium.

4 In the model, a single application of NPV (2500 GPIB ha^{-1}) at the time of maximum larval density gave up to 80% suppression of peak larval densities in the following year. The same level of suppression was achieved in the absence of predators.

5 In the long term, the model predicted that spraying when an outbreak was just beginning gave best results (a 50–70% reduction of the following two outbreaks). Simulation of threshold spraying resulted in NPV application, on average, every 7 years and suppression of outbreak densities by 40–70%. Following a single application, NPV was maintained in the population as a classical biological control agent, giving approximately a 20% reduction in outbreak densities.

6 Eradication of AGM using NPV was possible if larval densities were very low.

Keywords Control, eradication, gypsy moth, model, New Zealand, NPV, outbreak.

Introduction

The gypsy moth *Lymantria dispar* (L.) is a species native to both Europe and Asia. Since its introduction to Boston, Massachusetts in 1869, the moth has dispersed over a wide area of North America and has caused significant defoliation of forests (Doane & McManus, 1981; Elkinton & Liebhold, 1990).

The gypsy moth is considered a major threat to New Zealand's forestry industry should it become established in this country. Of particular concern is the Asian strain (AGM), which was only found in North America in 1990. Several features of the biology of the Asian strain suggest that it would have greater powers of colonization in New Zealand's mild climate than its European counterpart. In particular, females of the Asian strain are strong fliers, the Asian strain has a much broader host range including both deciduous and coniferous trees (Baranchikov, 1988; Walsh, 1993) and fewer eggs of the Asian strain require winter chilling to break dormancy (Keena, 1996). The possibility of viable gypsy moth eggs being brought into New Zealand by ship is considered high and in 1993 viable egg masses were found on board a Russian container vessel that entered the port of Auckland (Walsh, 1993). Given this risk, it is important to have a strategy in place, both for attempted initial eradication and for subsequent control should eradication fail.

Although the bacterium *Bacillus thuringiensis var kurstaki* (*Btk*) would be the main agent used for attempted AGM eradication in New Zealand (Glare *et al.*, 1998), the nuclear polyhedrosis virus (NPV) has considerable potential for long-term control should eradication fail. Moreover, it could play a

Correspondence: Nigel Barlow. Tel: + 64 3983 3974; fax: + 64 3983 3946; e-mail: barlown@agresearch.cri.nz

role in eradication in ecologically sensitive areas. This is because *Btk* is lethal to a variety of Lepidoptera, whereas NPV is specific to *Lymantria* spp. and therefore more suitable for use in areas where native Lepidoptera occur. NPV is present in most gypsy moth populations and is an important regulator in high density populations (Podgwaite, 1981). It has been developed as a pesticide for control of gypsy moth and was first registered with the US Environmental Protection Agency in 1978 under the name Gypchek. Several field trials have been conducted since then to determine the effectiveness of this product in reducing larval densities (Webb *et al.*, 1989; Cunningham & Kaup, 1991; Podgwaite *et al.*, 1992). NPV aerially applied at a rate of 2500 giga (1×10^9) polyhedral inclusion bodies (GPIB) per hectare has resulted in 80–90% mortality of gypsy moth larvae (Webb *et al.*, 1989). Following the discovery of AGM and AGM/EGM hybrids in North Carolina in 1993, Gypchek was used in local eradication attempts as part of the 1994 AGM project. Gypchek was used in areas thought to have beneficial or endangered insects and approximately 6000 acres were sprayed with Gypchek (Glare *et al.*, 1999). To help meet the potential threat of AGM in New Zealand, Gypchek was acquired and safety testing conducted on native Lepidoptera (Glare *et al.*, 1995) so that the product is available for registration and use (Glare *et al.*, 1999).

The transmission of NPV in gypsy moth has been modelled by several workers, either separately (Valentine & Podgwaite, 1982; Dwyer & Elkinton, 1993; Elkinton *et al.*, 1995; D'Amico *et al.*, 1996) or as part of a larger model concerned with other interactions (Colbert & Racin, 1991; Foster *et al.*, 1992). Some models (e.g. Valentine & Podgwaite, 1982) have attempted to simulate NPV transmission in considerable detail but these models tend to be complex and the many parameters involved are difficult to estimate. Other models, such as Dwyer & Elkinton's (1993), are considerably simpler and focus on disease transmission within a season. All the above models were for the European strain of gypsy moth and none have considered AGM.

This paper describes a mechanistic model of intermediate complexity for AGM invading New Zealand. The model is used to compare the behaviour of European and Asian strains, and to predict and optimize the impact of NPV for eradication and control. Together with safety testing of Gypchek, the model forms part of a proactive response to the threat of a potentially devastating invasive pest.

Model structure

The model is based as closely as possible on the known biology of the gypsy moth and includes the seasonal biology of the insect as well as its interactions with predators and host plants. The gypsy moth is univoltine and overwinters in the egg stage; eggs hatch in spring and develop over summer through the larval, pupal and adult stages (Montgomery & Wallner, 1988). In the model, development rate through each of the stages was temperature-dependent, and immature stages (larvae and pupae) were subject to a temperature-dependent mortality. Density dependence was assumed to act through predation of late-instar larvae, resource limitation and female fecundity. Predation is most important in low-density populations (Campbell *et al.*, 1977), whereas starvation and reduced fecundity are more important in dense populations (Campbell, 1967; Campbell & Sloan, 1978). This system of density dependence is similar to that in the model of Ludwig *et al.* (1978) for spruce budworm *Choristoneura fumiferana*.

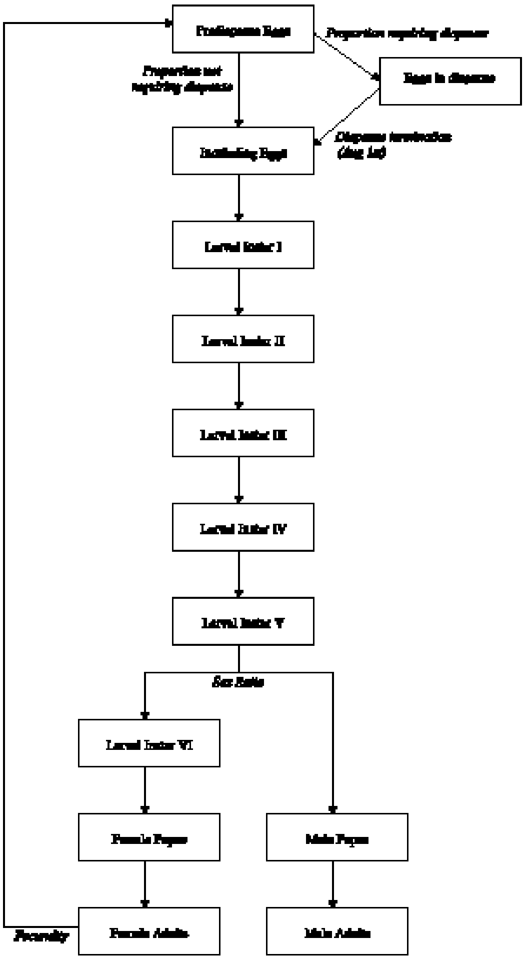


Figure 1 Representation of gypsy moth life stages in the model.

The gypsy moth model is broadly described by the following differential equations, which represent a limiting case, with no subdivision of the three egg classes or larvae into instars. In practice and in all the modelling that follows, the pre-diapause and post-diapuase incubation egg stages (Fig. 1) are subdivided into four classes and larvae into five or six instars. This effectively generates a distributed delay for the residence time in each egg developmental stage and in the total larval stage. In addition, male and female moths were differentiated at the end of the fifth larval instar, after which the females underwent an additional larval stage, and the males pupated (Fig. 1). This is consistent with findings that development time is 15% shorter for males than for females (Casagrande *et al.*, 1987). Tables 1–3 list

Table 1 Description of model parameters and their default values (where appropriate).

Symbol	Description	Value
E	density of eggs in pre-diapause embryonic development	
D	density of eggs in diapause	
H	density of eggs in post-diapause incubation	
S	density of susceptible uninfected larva	
I	density of infected larvae	
P	density of pupae	
A	density of adults	
W	density of NPV in the environment	
Q	foliage relative to maximum in the absence of defoliation	$(0 \leq Q \leq 1)$
b	adult reproductive rate (depends on adult density)	250–450 eggs female^{-1}
m_E	mortality rate of eggs	
m_L	mortality rate of larvae (includes temperature-related mortality and predation)	
m_P	mortality rate of pupae (includes temperature-related mortality)	
m_A	mortality rate of adults	
f_1	temperature-related pre-diapause egg development rate	
f_1'	temperature-related post-diapause egg development rate	
f_2	temperature-related larval development rate	
f_3	temperature-related pupal development rate	
k	proportion of eggs requiring diapause	0.75 (AGM), 0.95 (EGM)
λ	diapause termination switch	1 (on Aug 1), 0 (rest of year)
σ	starvation-related mortality factor	0.10 larva ha^{-1} day^{-1}
r	intrinsic rate of resource renewal, i.e. foliage growth	0.65 ha^{-1} day^{-1}
δ	defoliation rate parameter	1.0E^{-8} ha larva^{-1} day^{-1}
β	NPV transmission coefficient	1.7E^{-7} ha GPIB^{-1} day^{-1}
Λ	NPV released by an infected larva at death	2 GPIB larva^{-1}
μ	decay rate of NPV in the environment	0.003 day^{-1}
τ	time between infection and death	14 days
t	time	

the model parameters and the default values used, together with the monthly air temperatures at the site. Note that the only relevant differences between the Asian and European strains are in the proportion of eggs requiring diapause (Table 1) and the development rate parameters of the eggs (Table 3).

$$dE/dt = bA - m_E E - f_1 E \tag{1}$$

$$dD/dt = kf_1 E - m_E D - \lambda D \tag{2}$$

$$dH/dt = (k-1)f_1 E + \lambda D - m_E H - f_1{}' H \tag{3}$$

$$dS/st = f_1 H - \beta WS - \sigma S(1-Q) - m_L S - f_2 S \tag{4}$$

$$dI/dt = \beta WS - \beta W_{(t-\tau)} S_{(t-\tau)} - \sigma(\beta W_{(t-\tau)} S_{(t-\tau)})$$

$$(1-Q) - mL(\beta W_{(t-\tau)} S_{(t\tau)}) \tag{5}$$

$$dP/dt = f_2 S - m_P P - f_3 P \tag{6}$$

$$dA/dt - f_3 P - m_A A \tag{7}$$

$$dW/dt = \Lambda \beta W_{(t-\tau)} S_{(t-\tau)} - \mu W \tag{8}$$

$$dQ/dt = rQ(1-Q) - \delta(S+I) \tag{9}$$

Modelling temperature

Temperature was modelled by fitting the mean monthly maximum and minimum temperatures (see Table 2) to a sine curve. The best-fit equation was:

$$T = sin(a+x)b + c \tag{10}$$

where T is temperature, x is the time of year (1 Jan = 1 and 30 Dec = 360) and a, b, c are constants fitted to the local temperature data.

Egg development

Egg development in the gypsy moth involves three steps: pre-diapause embryonic development, diapause, and then post-diapause incubation (Johnson *et al.*, 1983). Egg incubation was assumed to follow the simple day-degree accumulation model proposed by Johnson *et al.* (1983):

$$f_1 = (T-a)/b \tag{11}$$

where f_1 is the proportion of total development time completed this day (day^{-1}), T is the daily temperature (°C), a is the base temperature below which no development occurs (°C) and b is the total day-degrees required for egg development (day °C).

Each day, values of f_1 for the maximum, minimum, and mean daily temperature were obtained and the overall development rate for the day was assumed to be:

Table 2 Monthly air temperatures (Lincoln, New Zealand).

Month	Mean daily maximum (°C)	Mean daily minimum (°C)
January	22.1	10.7
February	21.9	10.8
March	20.0	9.3
April	17.3	6.8
May	13.7	4.0
June	11.1	1.7
July	10.3	1.2
August	11.8	2.2
September	14.4	4.2
October	17.1	6.2
November	19.0	7.6
December	20.9	9.6

Table 3 Development rate parameters for gypsy moth (from Casagrande *et al.*, 1987).

Stage	c	a	b
Eggs (Asian)	–	8.0000	88.0000
Eggs (European)	–	3.0000	282.0000
Instar 1	0.3169	4.4040	0.2132
Instar 2	0.1712	3.7830	0.1895
Instar 3	0.1205	4.0700	0.1988
Instar 4	0.0763	3.9710	0.2065
Male larvae	0.0415	4.1310	0.2202
Female larvae	0.0329	5.0430	0.2753
Male pupae	0.0298	3.6110	0.1865
Female pupae	0.0250	4.9620	0.2725

$$f_1 = (f_{max} + f_{min} + (2 * f_{mean}))/4$$

where f_{max}, f_{min} and f_{mean} are the proportions of development achieved per day at the maximum, minimum and mean temperatures, respectively (Barlow & Dixon, 1980). Values for a and b were taken from Johnson *et al.* (1983) for the European strain and Pantyukhov (1962) for the AGM, the latter representing the model defaults (Table 3). Little work has been done on the duration of the pre-diapause embryonic development stage. Pantyukhov's (1962) data suggested that this stage is approximately 3.17 times as long as the post-diapause incubation stage. This assumption was made in the model, but has little effect on its behaviour, as most eggs must remain dormant until diapause cessation (assumed to be on 1 August), and so are not limited by the requirement for embryonic development. The proportion of eggs requiring diapause is approximately 95% for North American gypsy moths, but only 75% for the Asian strain (Walsh, 1993).

Larval and pupal development

Larval and pupal development (f_2 and f_3) were modelled by determining the expected cumulative development from a sigmoid function (Casagrande *et al.*, 1987):

$$f = c/(1 + \exp(a - bT)) \tag{12}$$

where f is the proportion of development, from egg hatch to the end of the current stage, completed in one day (day^{-1}), T is the daily temperature (°C) and a, b, c are constants (see Table 3).

Sex ratio and fecundity

The sex ratio (females/total) of larvae at the end of instar 5 was assumed to be constant, with a default value of 0.65 (Campbell, 1967). Each female was assumed to lay a single egg mass one day after maturation, and then die. It was assumed that there were always sufficient males to ensure fertilization of eggs. This is reasonable for established populations because males develop faster than females, and survival to adulthood is higher for males than for females (Casagrande, 1981).

Female fecundity was assumed to be density-dependent and varied from a maximum of 450 eggs female^{-1} at adult densities of less than 100 females ha^{-1}, down to 250 eggs female^{-1} at densities higher than 2500 females ha^{-1} (Morse & Simmons, 1979). Between these values the fecundity was scaled linearly, hence:

$$b = (458.3 – 0.083(\text{adults} \times 0.65))/0.65;\ 250 \leq \text{females} \leq 450 \tag{13}$$

where b is the reproductive rate per adult.

Temperature-dependent mortality

The four sources of mortality in the model were from low temperatures, predation and starvation, and a background level of mortality independent of environment. Larval starvation appears separately in Eqs (4) and (5), but the remaining mortalities were combined in the terms m_E, m_L, m_P and m_A. In constant-temperature conditions in the laboratory, Casagrande *et al.* (1987) found that none of up to 40 hatched eggs survived to adulthood below a temperature of 17.8 °C. This is quite high in comparison to ambient air temperature in New Zealand, and therefore temperature-dependent mortality is likely to be an important factor in the determination of New Zealand gypsy moth populations. In the model, larval and pupal stages were assumed to be subject to temperature-related mortality whenever the mean air temperature was below 18 °C. Based on the data of Casagrande *et al.* (1987), the daily temperature-dependent survival rate for each stage was calculated as:

$$s = ((T - 13)/5^f; \ldots 0 \leq s \leq 1 \tag{14}$$

where s is the temperature-dependent survival rate, T the daily mean temperature (°C) and f the proportion of development, from egg hatch to adulthood, completed in one day.

Separate values for f were calculated for males and females, and the resulting survival rates were applied to the appropriate stages (instar 6 and pupae). Instars 1–5 (for which gender was unknown) were subject to a temperature-dependent survival rate consistent with a mean development rate.

Predation

Campbell *et al.* (1977) identified predation of instar 4–6 larvae by birds as the primary density regulation factor in consistently sparse gypsy moth populations. Following Ludwig *et al.* (1978), the predation rate was modelled as a sigmoid functional response:

$$P = gL^2/(h^2 + L^2) \qquad (15)$$

where P is the daily predation rate (larvae eaten ha^{-1} day^{-1}), L is the density of instars 4–6 (larvae ha^{-1}), g is the maximum possible predation rate (larvae ha^{-1} day^{-1}) and h is the larval density (instars 4–6) at which $P = g/2$.

Because the predation rate reaches an upper asymptote (g), predation is most effective when moth populations are sparse. Default parameters ($g = 60$, $h = 800$) were chosen to give similar mortality rates to those in fixed predator mortality submodel of the Gypsy Moth Life System Model (GMLSM; Colbert, 1998) and which gave the outbreak behaviour observed in northern temperate forests (Myers, 1988; Elkinton & Liebhold, 1990). Because of the uncertainty about predation in New Zealand, control strategies in the model were evaluated with and without the outbreak-inducing predation component.

Resource-related mortality

Gypsy moth larvae were assumed to be limited by their resources in a density-dependent manner. The model used a 'forest quality' index, Q, ranging from 0 to 1, to model the state of the gypsy moth's resources. All larvae experienced a resource-related mortality thus:

$$s = \sigma(1 - Q) \qquad (16)$$

where s is the daily mortality from starvation (larvae dying ha^{-1} day^{-1}), σ is the maximum mortality rate from starvation (day^{-1}) (when resources completely depleted and $Q = 0$) and Q is the forest quality index, or amount of susceptible foliage relative to that in the absence of defoliation ($0 \leqslant Q \leqslant 1$). This expression is a simplification of what is more likely to be an asymptotic numerical and functional response relating s to Q. The data are not available to parameterize such a response, but the simplification means that the model may behave unrealistically at low values of Q: for example, the population can go extinct through resource depletion.

An arbitrary default value for σ of 0.1 day^{-1} was used in the model, this is similar to the maximum starvation mortality rate of 0.2 day^{-1} in GMLSM (Colbert, 1998) and was chosen to generate realistic outbreak patterns over time given the presence of predators.

Resource renewal

Each larva was assumed to reduce the forest quality index at a rate δ, while the resource was assumed to renew logistically at a specific rate r (day^{-1}):

$$dQ/dt = rQ(1 - Q) - \delta L \qquad (17)$$

where L is the total larval density (ha^{-1}).

In the field, gypsy moth densities are often assessed qualitatively using estimates of percent defoliation, which corresponds to the resource quality index ($1 - Q$) in the model. Parameters governing the moth/foliage interaction were similar to those in Wilder *et al.* (1994). Thus, the recovery rate of foliage (r) of 0.65 years^{-1} is close to their value of 1.0 years^{-1}, while the defoliation rate (δ) of 10^{-8} larva^{-1} day^{-1} resembles these authors' 1.65×10^{-8} larva^{-1} day^{-1}. Again, the values used in the present model were consistent with realistic outbreak behaviour.

Mortality from NPV

NPV was modelled using a modified version of Dwyer & Elkinton's (1993) delayed differential equation model for the within-season population dynamics of a single cohort of gypsy moth larvae. In the present model (Eqs 4, 5 and 8), larvae become infected with NPV by consuming PIBs on contaminated foliage at a rate βWS. Fourteen days (τ) later, the infected larvae die ($\beta W_{(\tau-t)}S_{(\tau-t)}$), releasing PIBs into the environment ($\Lambda\beta W_{(\tau-t)}S_{(\tau-t)}$) to decay ($\mu W$) or infect more hosts ($\beta WS$). The model ignores PIBs lost from the environment due to ingestion by larvae, because this is small compared to NPV production and decay.

Dwyer & Elkinton (1993) used the following parameter estimates: $\beta = 1.45 \times 10^{-7}$ ha GPIB^{-1} day^{-1}, $\Lambda = 2$ GPIB larva^{-1}, $\mu = 0.003$ day^{-1} and $\tau = 14$ days. We used the last three of these parameters but found a slightly higher β value of 1.7×10^{-7} ha GPIB^{-1} day^{-1} gave a better fit to data from Woods & Elkinton's (1987) field study. The model gave a good fit to the field data for plots that had both high initial larval density and high disease incidence, but the fit was poor for plots that had low initial larval density and disease incidence. At low larval densities and a low level of disease infection, the model predicts a much smaller epizootic than observed. This suggests that disease transmission is non-linearly related to density, as was shown to be the case experimentally by D'Amico *et al.* (1996). An attempt was made to improve the model fit to Woods & Elkinton's (1987) field data by incorporating the non-linear transmission term of Valentine & Podgwaite (1982). Using this term in the model gave a slightly better fit to the field data. However, this improvement was not considered great enough to justify adding more parameters. A variable transmission coefficient for high and low larval densities was also tested. This improved the model fit, but because of difficulties in estimating the parameter values and the increased complexity they added to the model, a variable β term was not incorporated into the model. Finally, an attempt was made to simplify the model by replacing the explicit time lag (τ) between infection and death in the Dwyer & Elkinton (1993) model with a constant daily mortality from disease. Without the lag the model did not follow the bimodal pattern of mortality observed in the field data, so the explicit lag time of 2 weeks was retained in the model.

The disease part of the model also assumes that infected larvae dying from causes other than disease do not release PIBs into the environment. This assumption seems valid, as NPV grows exponentially inside the larval host, so that an infected larva dying before the infected period is completed would contain few PIBs. Infected larvae do not develop pupae, and all larval instars are assumed to be equally susceptible to NPV and to release the

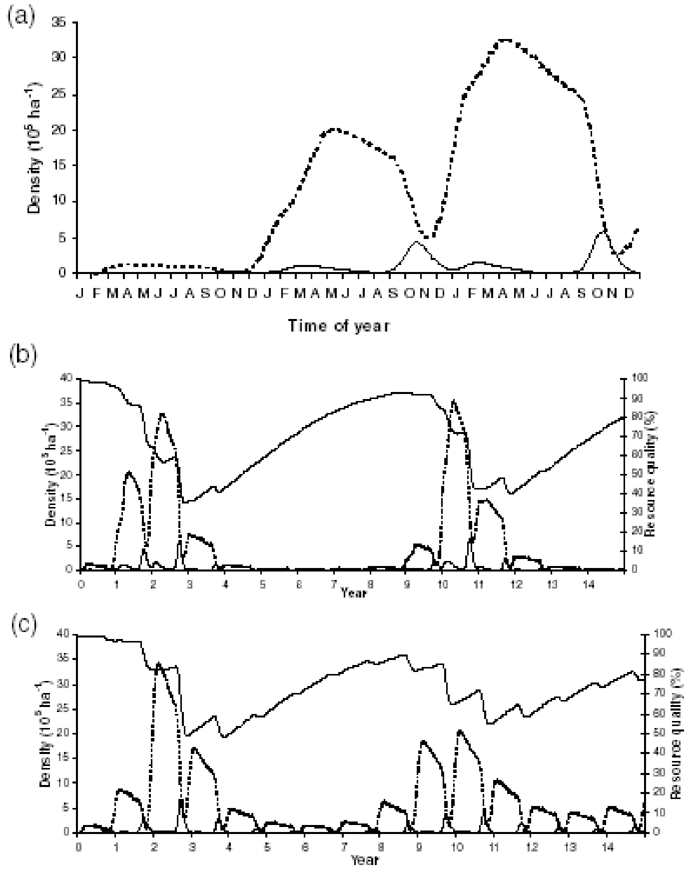


Figure 2 (a) Model prediction of short-term Asian gypsy moth population dynamics starting with an initial population of 10000 first-instar larvae per ha on 1 Jan. (b) Predicted long-term outbreak behaviour of the Asian gypsy moth in New Zealand, in interaction with predation and foliage. (c) Predicted long-term outbreak behaviour of the European gypsy moth in New Zealand, in interaction with predation and foliage. Solid line = larvae; dashed line = eggs. Resource quality (thick line) is expressed as a percentage of the maximum, undefoliated density.

same number of PIBs ($\Lambda = 2$ GPIB larvae^{-1}; Dwyer & Elkinton, 1993) into the environment when the infected larvae die.

Results

Model predictions for Asian gypsy moth dynamics in New Zealand

Figure 2a shows the predicted short-term population dynamics of Asian gypsy moth eggs and larvae under New Zealand temperatures. Eggs reach a peak at the start of April and larvae reach their main peak in mid-October. In the longer term, the model re-creates the typical pattern for gypsy moth in Europe, with outbreaks occurring approximately every 8 years, driven by predation and an interaction with the foliage resource (Fig. 2b).

The model predicts differences in the behaviour of Asian and European strains of gypsy moth under New Zealand temperatures. The Asian strain increases more rapidly at first (compare heights of egg and larval peaks in the second year in Fig. 2b and c), partly because it has an additional generation, shown by the two peaks in larval density each year in Fig. 2a and b (compare European strain in Fig. 2c). Thereafter, AGM maintains more widely fluctuating densities and more pronounced outbreaks than the European strain, given the presence of predators functionally equivalent to those elsewhere in the moth's range (compare Fig. 2b and c).

Sensitivity analysis (without disease)

Some gypsy moth populations do not undergo regular outbreak cycles (Campbell, 1981) and these populations are thought to be constrained by high predation rates or poor-quality habitats. A high predation rate was modelled by increasing the value for *g*, the maximum predation rate, from 60 to 160, which prevented outbreaks in the modelled population. Poor habitat quality was mimicked by setting the maximum attainable forest quality (*Q*) value to 0.90 (default = 1.00). Under this scenario the population also failed to undergo outbreaks. The existence or otherwise of outbreak behaviour was also highly sensitive to several of the model parameters, in particular those related to the vegetation. A reduction from 0.65 year^{-1} to 0.3 year^{-1} in the recovery rate of foliage following defoliation (*r*) reduced the frequency of

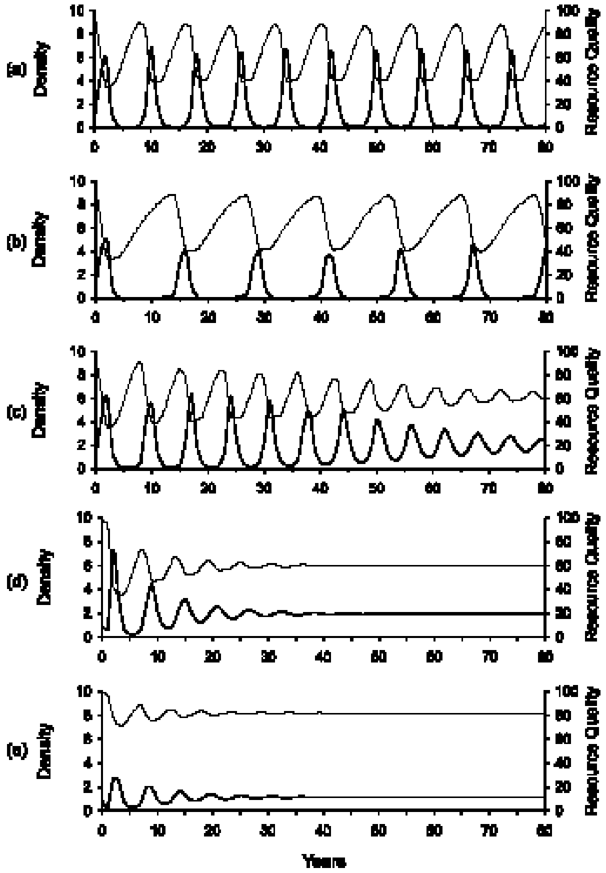


Figure 3 Effect of varying predation and resource parameters. Thick line = larval density (10^5 larvae ha^{-1}), thin line = resource quality. (a) Default model, (b) intrinsic rate of increase of foliage (r) reduced from 0.65 to 0.3 $years^{-1}$, (c) r increased to 0.7 $years^{-1}$, (d) predation removed, (e) maximum starvation mortality (s) increased from 0.1 to 0.2 day^{-1}.

outbreaks from 8-yearly (Fig. 3a) to 13-yearly (Fig. 3b). Increasing the foliage recovery rate to 0.7 $year^{-1}$ caused the outbreaks initially to occur more frequently (7-yearly) but eventually to dampen to a steady state (Fig. 3c). Similarly, removing predation (Fig. 3d) or increasing the starvation mortality factor (σ) from its default value of 0.10 day^{-1} to 0.20 day^{-1} (Fig. 3e) also prevented outbreak behaviour. In the absence of predators the model predicted a more stable gypsy moth population, with no outbreak behaviour and densities roughly equal to the average densities of outbreak populations in the presence of predation and about 30% of those at the peaks of the outbreaks (Fig. 3d compared with Fig. 3a). Interestingly, apparently small changes to the model's structure also affected outbreak behaviour. For example, the outbreaks disappeared when the four classes within each egg stage were combined into one.

Effects of NPV: timing of spraying

The timing of larval and egg peaks is important in determining the optimum time of year in which to spray. As NPV causes mortality to gypsy moth only in the larval stage, one would expect it to be most effective when applied at the time of peak larval density, i.e. mid-October. The model suggests that this is the case. A single spray of NPV at the highest recommended application rate of 2500 GPIB ha^{-1} (GPIB = virus polyhedral inclusion bodies $\times 10^9$ per hectare) applied in mid-October reduced the peak larval density in the following year by nearly twice as much as a spray applied at the start of July, when larval density was at its minimum. Therefore, in the model experiments described below, it can be assumed that the spray was applied in mid-October at an application rate of 2500 GPIB ha^{-1}, unless stated otherwise.

Effects of NPV: short-term suppression

Short-term suppression is defined as the decrease in peak larval density one year after spraying compared to the peak density in that year for an unsprayed population. The level of suppression varied depending on the presence or absence of predation, the initial larval density at the time of spraying and the spray application rate. The degree of suppression (curves for virus application compared with the curve with no virus applied) increased steadily as the application rate increased (Fig. 4) and

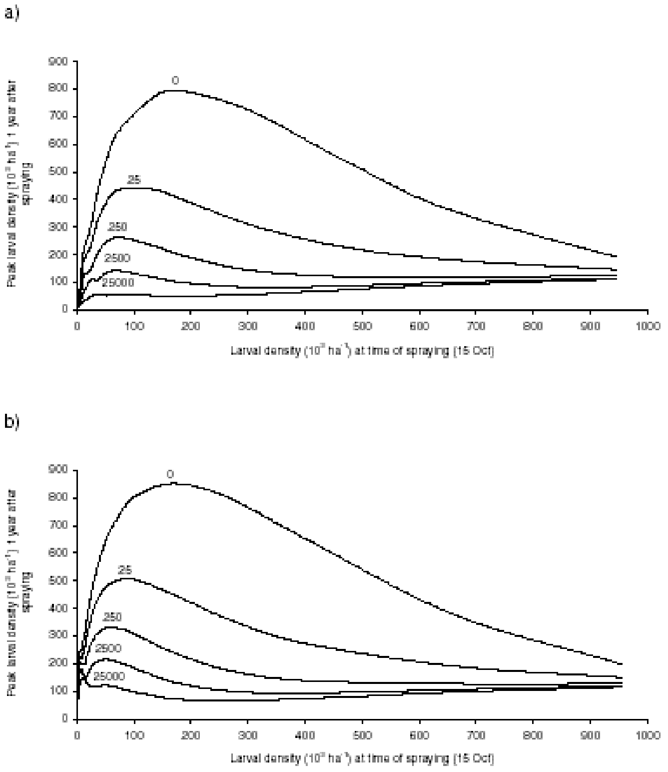


Figure 4 Effect of larval density at the time of spraying (mid-October) on the peak larval density one year after spraying, in the absence of spraying (0) and for four spray application rates (25, 250, 2500 or 25 000 GPIB ha^{-1}). (a) Default model, (b) with predation removed.

the response was fairly density-independent up to an initial larval density of 50 000 ha^{-1} but became overcompensating at higher densities. This response is inherent in the moth/resource interaction but the addition of disease, and the secondary cycling of infection that occurs as a result, reduced the threshold at which overcompensation occurred. In the absence of virus this was around 150 000–200 000 larvae ha^{-1} but decreased to 50 000 larvae ha^{-1} when virus was applied at 250 GPIB ha^{-1} or more (Fig. 4a). Removing predation from the model gave lower suppression levels but gave a similar shaped response to NPV application rates and initial larval densities (compare Fig. 4a,b),

Figure 5 shows the effect of initial larval density on the proportional suppression achieved at two application rates. Best results, with or without predation, come from treating a very low density, at which eradication is also possible (see below), or a very high density of around 10^5 larvae ha^{-1} or greater (Fig. 5a and b). Under these circumstances, 80% suppression is possible, compared with the 30% obtainable for low–medium larval densities of around 100 ha^{-1} (Fig. 5a).

Effects of NPV: prospects for local eradication

Using a single spray, eradication occurred only if the larval density at the time of spraying was below 1.2 larvae ha^{-1} (2.2 larvae ha^{-1} for the higher application rate of 25 000 GPIB ha^{-1}). Eradication is defined here as a peak larval density of less than 0.7 larvae ha^{-1} in the year following spraying, which gives a 50% chance of local eradication from a single hectare (from the Poisson distribution the probability of zero larvae in any one hectare = exp(– expected mean density) = 0.5, so mean density = –ln(0.5) = 0.69). If three sprays were applied, one week apart starting from 15 October, eradication was achieved with a slightly higher larval population of 5.3 ha^{-1} (7.4 ha^{-1} at the higher application rate of 25 000 GPIB ha^{-1}).

Given the normal application rate, eradication of much larger populations occurred in the model when the disease transmission coefficient, β was increased. If β is doubled to 3.4×10^{-7} ha GPIB^{-1} day^{-1}, the model predicts that after an initial NPV application to a larval population of 90 000 ha^{-1}, the disease accentuates the outbreak behaviour, causing oscillations which gradually increase in amplitude until the population becomes extinct after 64 years. If β is doubled again to 6.8×10^{-7} ha GPIB^{-1} day^{-1}, the population goes extinct just 3 years after spraying.

Effects of NPV: long-term control

Here we consider first the effect of a single application of NPV, carried out at the optimum time of year (15 October) at the standard rate of 2500 GPIB ha^{-1}. Figure 6 shows that long-term suppression depended on the timing of the spray relative to the outbreak cycle. Not surprisingly, there was little point in treating

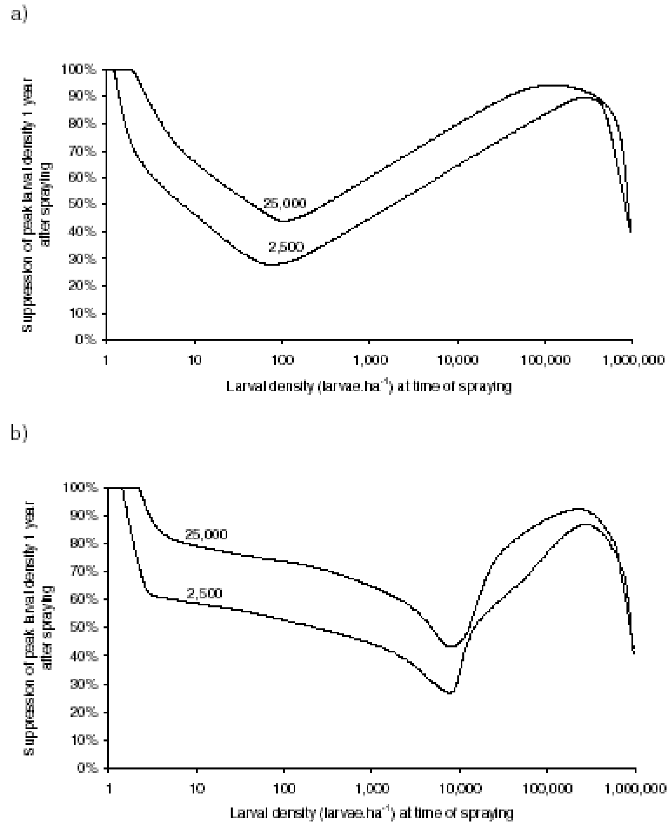


Figure 5 Variation in the percentage suppression with respect to larval density at the time of spraying. Suppression is defined as the decrease in peak larval density one year after spraying compared to the peak density in that year for an unsprayed population. Spraying consisted of a single spray with NPV in mid-October at an application rate of either 2500 or 25 000 GPIB ha^{-1}. (a) Default model, (b) with predation removed.

populations between outbreaks (Fig. 6a) and the best results occurred with NPV added early in the build-up phase of an outbreak (Fig. 6b). Such a treatment gave 12–16 years of substantial control, reducing the following two outbreaks by around 50–70%. Poor results (approximately 15% suppression) were obtained by treating an outbreak too late, when it had reached its peak (Fig. 6c). The difference between the initial control in Fig. 6(b) and the significantly poorer control in Fig. 6(c) came from a delay in NPV spraying of only one year. In the long term, the effect of any initial timing of application became insignificant and NPV maintained itself in the population as a classical biological control, giving about a 20% reduction in outbreak densities (Fig. 6a).

In the possible New Zealand scenario with no predators and a stable, mid-density gypsy moth population, adding NPV gave a greater long-term level of suppression of around 50% (Fig. 6d compared with Fig. 6b).

For repeated applications of NPV, the most usual basis for a control decision is a threshold rule, involving monitoring the population and spraying only when an 'action threshold' density is reached. Threshold spraying in the model was based on egg masses, as these are the easiest stage to sample in the field. Using the action threshold of 1250 egg masses ha^{-1} recommended by the Ontario Ministry of Natural Resources (Cunningham & Kaupp, 1991), and assuming each egg mass has 450 eggs, spray was applied in the model (in mid-October) if the density of eggs had reached 5.6×10^5 ha^{-1} at any time during the preceding year. This resulted in approximately one spray every 7 years over a 55-year period (Fig. 7a), and this long-term frequency was unaffected by either a halving (Fig. 7b) or a doubling (Fig. 7c) of the threshold. In all cases the average suppression level was similar, with peak densities reduced by 40–70% (Fig. 7).

Effects of NPV: sensitivity analysis

In view of the uncertainty over the value of β, the virus transmission coefficient, and as a simple indication of the sensitivity of the results to this value, two of the control scenarios were examined with the value of β doubled. The first was eradication and doubling the value of β allowed eradication at considerably higher density populations. The second scenario was long-term classical biological control. Doubling β in the presence of predation gave sustained suppression of outbreaks for a considerable period (Fig. 6e), but led to more irregular behaviour which eventually culminated in increasing amplitude oscillations and extinction of the population.

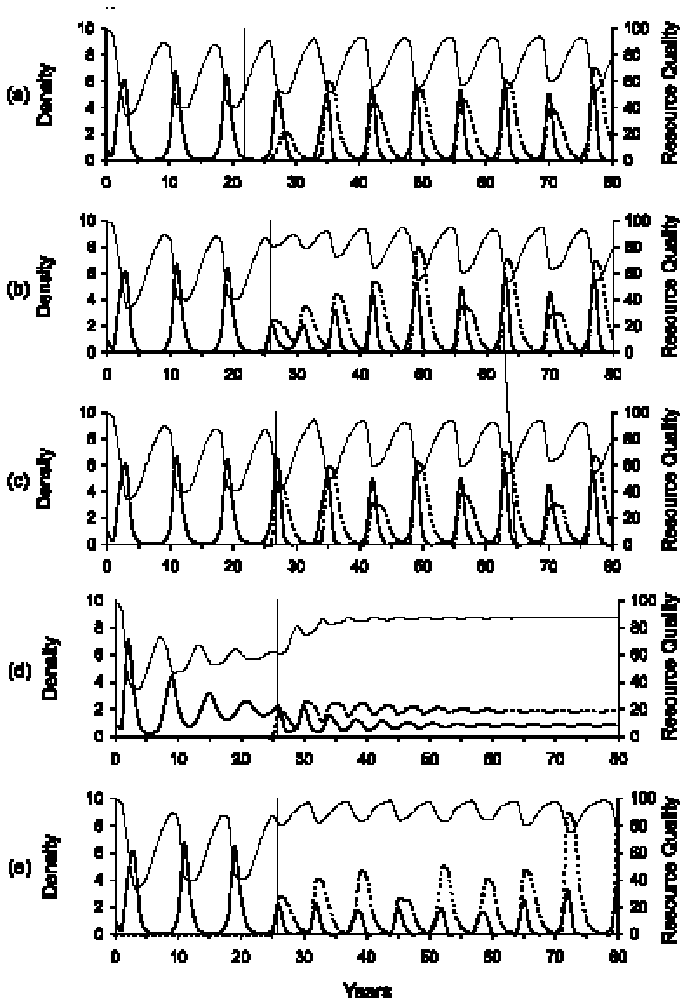


Figure 6 Effect of timing of NPV application relative to the outbreak cycle, and effect of model parameters on predicted long-term control. Thick line = larval density (10^5 larvae ha^{-1}), dashed line = disease density (10^5 GPIB ha^{-1}), thin line = resource quality. (a) NPV introduced (thin vertical line) in year 22 between population outbreaks, (b) NPV introduced 4 years later, just before an outbreak, (c) NPV introduced one year later during an outbreak, (d) NPV introduced in year 26 but no predation, (e) NPV introduced in year 26 and disease transmission coefficient doubled.

Discussion

The presence or absence of sustained outbreaks in the model is sensitive to several of the parameter values and to the model's age-class structure. This suggests that simple models that ignore the effects of age-classes, time lags, egg diapause and temperature may give quite misleading results. On the other hand, it is impossible to consider all components of the highly complex life system in detail because the data do not exist to parameterize them properly. The present model seeks a middle course. Because gypsy moth has not established in New Zealand, there are no independent data available to test the model predictions. However, the disease component of the model gave a good fit to the field data of Woods & Elkinton (1987) and the same bimodal pattern of disease mortality. The parameters for development of gypsy moth are well established, while those relating to predation and resource interactions were chosen to give the long-term behaviour of outbreaks and innocuous (low density) periods resembling those actually observed in many gypsy moth populations. For example, in south-eastern Europe outbreaks occur on average (but not regularly) every 7–9 years (Campbell, 1981; Montgomery & Wallner, 1988). The mechanistic basis for this outbreak behaviour in the model is similar to that in Ludwig *et al*. (1978) for the spruce budworm.

The model predicts rather different dynamics for the Asian and European strains, given the same parameters except for the different egg diapause requirements and temperature-dependent development rates. The Asian strain in the model seemed prone to more severe outbreaks under conditions typical of New Zealand's South Island, with peak egg and larval numbers 70–80% higher than for the European strain, but also lower densities in the inter-outbreak years (Fig. 2b). In the warmer North Island,

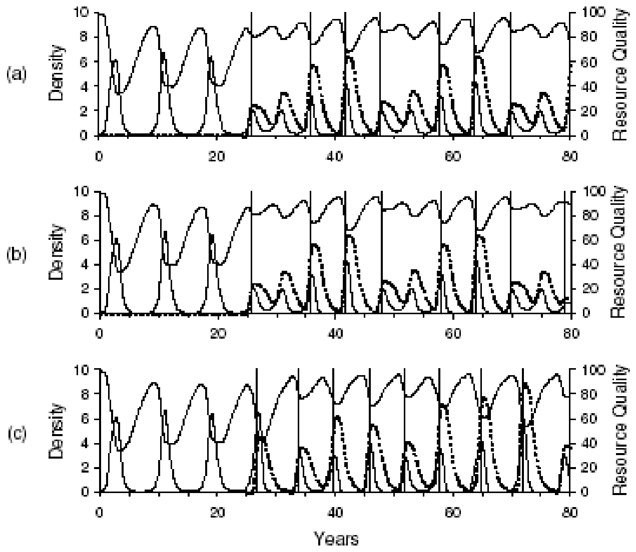


Figure 7 Effect of spray threshold level on the degree of suppression achieved with repeated spraying. NPV spray applied in mid-October (thin vertical line) at an application rate of 2500 GPIB ha^{-1} if the total egg density during the previous year exceeded (a) 5.6×10^5 eggs ha^{-1}, (b) 2.8×10^5 eggs ha^{-1} and (c) 1.12×10^6 eggs ha^{-1}. Thick line = larval density (10^5 larvae ha^{-1}), dashed line = disease density (10^5 GPIB ha^{-1}) and thin line = resource quality.

the difference between strains is likely to be even more marked. The crucial difference is that because fewer eggs must undergo winter diapause, the Asian strain can produce an extra larval generation each season (as shown in Fig. 2b). This contrasts with the citations of Schaefer *et al.* (1984) and Goldschmidt (1934, cited in Keena, 1996), who state that gypsy moth is univoltine throughout its range. However, model predictions of the long-term dynamics of AGM and EGM must be interpreted with caution because they are sensitive to resource parameters and the values for these are uncertain. For shorter-term predictions, the uncertainty is less important.

It is possible that the small mammal and avian predators which affect gypsy moth in North America and Europe may be absent in New Zealand. If AGM establishes in New Zealand and in the absence of the predators which characterize it elsewhere, then the model predicts that outbreak behaviour will not occur. There may be initial outbreaks over the first few years due to an interaction with the resource, and significant damage is likely in the absence of control. If unchecked, however, the model predicts that these oscillations would rapidly damp to a more stable population, whose mean density may be similar to that in the presence of predators but in which damaging outbreak densities will no longer be realized. Theoretically, permanent outbreak cycles with extended intermediate periods of low density cannot be generated through an interaction involving only a herbivore and its resource; a third agent is required, such as predators, parasitoids or disease (Kean & Barlow, 1995). In practice, should weather-induced fluctuations disturb the predicted equilibrium in the absence of predators, to the extent that densities reach damaging levels, then the model predicts that the short-term effect of a spray is similar to that in the presence of predation.

The model indicates that a local gypsy moth population can only be eradicated by application of NPV if the larval density at the time of spraying is very low. Eradication of higher densities was possible if the disease transmission coefficient (β) was doubled, but there are no data to support such inflated β values. NPV did provide good control, reducing outbreak peaks by up to 80% depending upon spray concentration, timing of spraying and the presence or absence of predation. The result also depended on the larval density at the time of spraying, unlike that from pesticide or *Btk*, which would generally be density-independent, giving the same proportional kill irrespective of density.

Despite the complexity of the gypsy moth life system, the unknown status of potentially important influences like predation in New Zealand, and the world-wide absence of data and models on the Asian strain of gypsy moth in the field, this modelling exercise has proved a valuable compliment to other proactive measures being taken to prepare for a potentially devastating invading species. It has shown that AGM is likely to have local dynamics that are different to and potentially more damaging than those of the European strain in the short term, but that NPV can give good control, even of the Asian strain and even to the extent of likely eradication under favourable circumstances. It remains to consider combinations of controls in the model, including *Btk*, NPV and the fungus *Entomophaga maimaiga*, and if AGM was ever found, then it is vital that detailed monitoring accompanies any control. In this way the model could be tuned more accurately to local conditions and used in a more tactical role as part of an adaptive approach to eradication or containment.

References

Baranchikov, Y.N. (1988) Ecological basis of the evolution of host relationships in Eurasian gypsy moth populations. *Proceedings, Lymantriidae: a Comparison of Features of New and Old World*

Tussock Moths (ed. by W. E. Wallner and K. A. McManus), pp. 319–338. US Department of Agriculture General Technical Report, NE-123. USDA, Washington DC.

Barlow, N.D. & Dixon, A.F.G. (1980) *Simulation of Lime Aphid Population Dynamics*. Pudoc, Wageningen, The Netherlands.

Campbell, R.W. (1967) Studies on the sex ratio of the gypsy moth. *Forestry Science*, **13**, 19–22.

Campbell, R.W. (1981) Population dynamics. *The Gypsy Moth: Research Toward Integrated Pest Management* (ed. by C. C. Doane and M. L. McManus), pp. 161–202. US Department of Agriculture Technical Bulletin 1584. USDA, Washington DC.

Campbell, R.W. & Sloan, R.J. (1978) Numerical bimodality among North American gypsy moth populations. *Environmental Entomology*, **7**, 641–646.

Campbell, R.W., Sloan, R.J. & Biazak, C.E. (1977) Sources of mortality among late instar gypsy moth larvae in sparse populations. *Environmental Entomology*, **6**, 865–871.

Casagrande, R.A. (1981) Larval and pupal development. *The Gypsy Moth: Research Toward Integrated Pest Management* (ed. by C. C. Doane and M. L. McManus), pp. 154–160. US Department of Agriculture Technical Bulletin 1584. USDA, Washington DC.

Casagrande, R.A., Logan, P.A. & Wallner, W.E. (1987) Phenological model for the gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantriidae), larvae and pupae. *Environmental Entomology*, **16**, 556–562.

Colbert, J.J. (1998) Gypsy Moth Life Simulation Model installation disks. ftp: //www.fsl.wvu.edu/pub/gyspymth/lsm/disk1.exe (16 June 1999).

Colbert, J.J. & Racin, G.E. (1991) Gypsy moth life system model. *Proceedings, 1990 Third International Conference on Computers in Agricultural Extension Programs, Lake Buena Vista (Orlando) FL* (ed. by F. S. Zazueta, D. G. Watson and A. B. Botcher), pp. 115–120. University of Florida, Gainsville, FL.

Cunningham, J.C. & Kaupp, W.J. (1991) Development of nuclear polyhedrosis virus for control of gypsy moth (Lepidoptera: Lymantriidae) in Ontario. I. Aerial spray trials in 1988. *Canadian Entomologist*, **123**, 601–609.

D'Amico, V., Elkinton, J.S., Dwyer, G. & Burand, J.P. (1996) Virus transmission in gypsy moths is not a simple mass action process. *Ecology*, **77**, 201–206.

Doane, C.C. & McManus, M.L. (1981) *The Gypsy Moth: Research Towards Integrated Pest Management*. Forest Service Technical Bulletin 1584. USDA, Washington D.C.

Dwyer, G. & Elkinton, J.S. (1993) Using simple models to predict virus epizootics in gypsy moth populations. *Journal of Animal Ecology*, **62**, 1–11.

Elkinton, J.S., Dwyer, G. & Sharov, A. (1995) Modelling the epizootiology of gypsy moth nuclear polyhedrosis virus. *Computers and Electronics in Agriculture*, **13**, 91–102.

Elkinton, J.S. & Liebhold, A.M. (1990) Population dynamics of gypsy moth in N. America. *Annual Review of Entomology*, **35**, 571–596.

Foster, M.A., Schultz, J.C. & Hunter, D. (1992) Modelling gypsy moth-virus–leaf chemistry interactions: implications of plant quality for pest and pathogen dynamics. *Journal of Animal Ecology*, **61**, 509–520.

Glare, T.R., Barlow, N.D. & Walsh, P.J. (1998) Potential agents for eradication or control of gypsy moth in New Zealand. *Proceedings of the 51st New Zealand Plant Protection Conference*, pp. 224–229.

Glare, T.R., Newby, E.M. & Nelson, T.L. (1995) Safety testing of a nuclear polyhedrosis virus for use against gypsy moth, Lymantria dispar, in New Zealand. *Proceedings of the 48th New Zealand Plant Protection Conference*, pp. 264–269.

Glare, T.R., Walsh, P.J. & Barlow, N.D. (1999) *Strategies for the Eradication or Control of Gypsy Moth in New Zealand.* FRST report, AgResearch and Forest Research Associates, New Zealand, p. 108.

Johnson, P.C., Mason, D.P., Radke, S.L. & Tracewski, K.T. (1983) Gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae), egg eclosion: degree-day accumulation. *Environmental Entomology*, **12**, 929–932.

Kean, J. & Barlow, N.D. (1995) *Simple Models That Give Realistic Forest Insect Outbreak Patterns.* Internal report, AgResearch, Lincoln, New Zealand, p. 17.

Keena, M.A. (1996) Comparison of the hatch of *Lymantria dispar* (Lepidoptera: Lymantriidae) eggs from Russia and the United States after exposure to different temperatures and durations of low temperature. *Annals of the Entomological Society of America*, **89**, 564–572.

Ludwig, D., Jones, D.D. & Holling, C.S. (1978) Qualitative analysis of insect outbreak systems: the spruce budworm and forest. *Journal of Animal Ecology*, **47**, 315–332.

Montgomery, M.E. & Wallner, W.E. (1988) The gypsy moth, a westward migrant. *Dynamics of Forest Insect Populations* (ed. by A. A. Berryman), pp. 353–375. Plenum, New York.

Morse, J.G. & Simmons, G.A. (1979) Simulation model of gypsy moth introduced into Michigan forests. *Environmental Entomology*, **8**, 293–299.

Myers, J.H. (1988) Can a general hypothesis explain population cycles of forest Lepidoptera? *Advances in Ecological Research*, **18**, 179–242.

Pantyukhov, G.A. (1962) The effect of positive temperatures on different geographic populations of the European gold tail (*Euproctis chrysorrhea* L.) and the gypsy moth (*Lymantria dispar* L. – Lepidoptera, Orgyidae). *Entomological Review*, **41**, 169–175.

Podgwaite, J.D. (1981) Natural disease within dense gypsy moth populations. *The Gypsy Moth: Research Toward Integrated Pest Management* (ed. by C. C. Doane and M. L. McManus), pp. 125–134. US Department of Agriculture Technical Bulletin 1584. USDA, Washington DC.

Podgwaite, J.D., Reardon, R.C., Walton, G.S., Venables, L. & Kolodny-Hirsch, D.M. (1992) Effects of aerially applied Gypchek on gypsy moth (Lepidoptera: Lymantriidae) populations in Maryland woodlots. *Journal of Economic Entomology*, **85**, 1136–1139.

Schaefer, P.W., Weseloh, R.M., Sun, X., Wallner, W.E. & Yan, J. (1984) Gypsy Moth, *Lymantria (=Ocneria) dispar* (L) (Lepidoptera: Lymantriidae), in the People's Republic of China. *Environmental Entomology*, **13**, 1535–1541.

Valentine, H.T. & Podgwaite, J.D. (1982) Modeling the role of NPV in gypsy moth population dynamics. *Invertebrate Pathology and Microbial Control. Proceedings of the III International Colloquium on Invertebrate Pathology*, pp. 353–356. University of Sussex, Brighton, U.K.

Walsh, P.J. (1993) Asian gypsy moth: the risk to New Zealand. *NZ Forestry*, **38**, 41–43.

Webb, R.E., Shapiro, M., Podgwaite, J.D., Reardon, R.C., Tatman, K.M., Venables, L. & Kolodny-Hirsch, D.M. (1989) Effect of aerial spraying with Dimilin, Dipel, or Gypchek on two natural enemies of the gypsy moth (Lepidoptera: Lymantriidae). *Journal of Economic Entomology*, **82**, 1695–1701.

Wilder, J.W., Voorhis, N., Colbert, J.J. & Sharov, A. (1994) A three variable differential equation model for gypsy moth population dynamics. *Ecological Modelling*, **72**, 229–250.

Woods, S.A. & Elkinton, J.S. (1987) Bimodal patterns of mortality from nuclear polyhedrosis virus in gypsy moth (*Lymantria dispar*) populations. *Journal of Invertebrate Pathology*, **50**, 151–157.

Accepted 13 May 2000

Appendix 14.6: Molecular identification of gypsy moth strains

Travis Glare, Duglad Hall and Nicky Richards
AgResearch, PO Box 60, Lincoln
March, 1997

Methods

Gypsy moth larvae were collected from the Czech Republic and the Slovak Republic in May 1996, and stored in ethanol, originally at room temperature, and then at 4°C. The dried adult moth cadavers were from a third laboratory generation, supplied by the Northeastern Center for Forest Health Research, Connecticut, USA. The Asian specimens were from Mineralni in Far Eastern Russia, and the North American specimens were from Bethany, Connecticut. The tussock moth samples were from a lab culture at the Forest Research Institute at Rotorua. These samples were originally kept alive in the insect quarantine buildings on the AgResearch Lincoln campus, but were later killed and stored in ethanol at 4°C. The tussock moth samples were included to act as an out-group to the gypsy moth.

TABLE 1: Gypsy moth samples for DNA analysis.

Sample Code	Region	Date Collected	Sample State
AM	Mineralni, Far Eastern Russia	14/8/96	Dried adults
CB	Bethany, Connecticut	14/8/96	Dried adults
11	Pennsylvania lab culture	5/96	Ethanol stored larvae
CLB	Cifare, Czech Republic	15/5/96	Ethanol stored larvae
TLA	Trebisov, Slovakia	14/5/96	Ethanol stored larvae
18 (Tussock Moth)	FRI, Rotorua	10/96	Fresh larvae (originally)

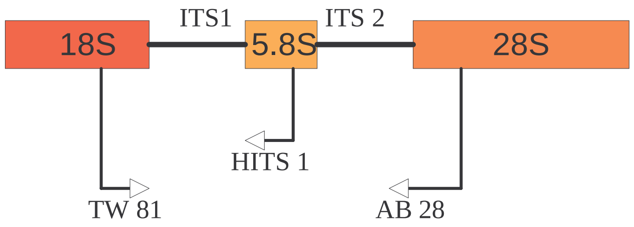
DNA isolation methods

Three different methods were used for the extraction of DNA from the Gypsy moth samples. The first of these methods was from Pfeifer *et al.* (1995). The second method used involved the use of Bio-Rad biotechnology grade Chelex-100 resin (Walsh *et al.*, 1991). The third method used was a variation of the method used by Henry *et al.* (1990).

PCR Using Specific ITS Region Primers

Primers TW81 and AB28 were both designed as fungal primers to specifically amplify rDNA from the 18S to 28S region. The primer locations and direction of amplification are shown in Figure 1.

FIGURE 1: Location and amplification direction of rDNA primers.



These primers amplify the internal transcribed spacer regions, ITS 1 and ITS 2, which are highly variable within a species as well as the more conserved 5.8s region, which has a high degree of homology between species.

In the insects used in this study the AB28 (5'...ATATGCTTAAGTTCAGCGGGT...3') and TW81 (5'...GTTTCCGTAGGTGAACCTGC...3') primers produce a large fragment (about 1200 bp), and often multiple bands were produced in the gypsy moth samples, so another primer was used. This primer was a variation of that designed by Pfeifer *et al.* (1995) that amplified a region starting in the 5.8S region of the rDNA and was called HITS1 (Richards *et al.*, 1997). The sequence of this primer was 5' ... AATGTGCGTTCGAAATGTCG .. 3'. This primer was designed specifically for gypsy moth DNA and was used in conjunction with the fungal primer TW81 to amplify the ITS1 region of rDNA (Fig. 1).

PCR was performed in 25 µl volumes. RAPD reaction mixes contained 2.5 µl buffer (10x, with Mg), 0.2 µl dNTP's (10 mM), 2.0 µl primer (10 mM, Operon Technologies OPF series) and 0.16 µl Taq DNA polymerase (5 units/µl, Boehringer Mannheim). ITS PCR reaction mixes contained 2.5 µl buffer, 0.5 µl dNTPs (10 mM), 1.0 µl of each primer (10 mM) and 0.125 µl Taq DNA polymerase. Two drops of mineral oil were added to each reaction tube. RAPDs were cycled in a Perkin Elmer thermal cycler with cycles consisting of 40 repetitions of the following: 94°C for 1 minute, 36°C for 2 minutes and then 72°C for 2 minutes. ITS region amplification was performed in an Ericomp thermal cycler with cycles consisting of 30 repetitions of the following: 94°C for 1 minute, 55°C for 1 minute and then 72°C for 2 minutes. Every reaction contained a negative control (no DNA).

Analysis of PCR Products

PCR products (12.5 µl of total 25 µl volume for RAPD; 5 µl of total 25 µl volume for ITS) were resolved by horizontal electrophoresis through 2% agarose in 0.5% TBE [50 mM Tris, 50 mM Boric acid, 1 mM EDTA] electrophoresis buffer. Two µl of 100 bp ladder (Gibco BRL) was included as a size standard in each gel. Gels were stained in ethidium bromide, viewed on a transilluminator and photographed using a Kodak instant system. Samples of the amplified ITS region were sent to Otago University for sequencing.

Restriction endonucleases were used to identify differences in amplified rDNA bands. They included: Hae III (Gibco BRL) [GG / CC], Alu I (Gibco BRL) [AG / CT], and Tru 91 (Boehringer Mannheim) [T / TAA]. All digests were performed using standard methods (Sambrook *et al.*, 1989), and resolved on 2% agarose gels in 0.5% TBE buffer.

Bands produced by the RAPD primers were scored as either present or absent (1,0). A binomial comparison using Genstat (Genstat, 1987) was then made between all bands. This enabled us to express the similarity of the samples by the number of shared bands. Segregation analysis was performed with three methods: analysis of shared fragments (Gower, 1985), Jacquard similarity (Gower, 1985) and Nei-Lei genetic distance (Nei and Lei, 1979).

PCR Using RAPD Primers from the OPF Series

Amplifications were performed in 25 µl containing 100 µM dNTP, 0.1 µM primer, 0.55 units of *Taq* DNA polymerase (Boehringer Mannheim) in the reaction buffer supplied (containing 0.038 mM Mg). Each tube was overlayed with a drop of mineral oil. PCR reactions were executed in a Perkin Elmer 480 thermal cycler using 40 cycles of one minute at 94^{o}C, one minute at 36^{o}C, and two minutes at 72^{o}C. A negative (water) control was included in every experiment. Ten-base randomly generated primers pair produced by Operon Technologies Ltd were used.

RAPD products were resolved on 1.4-2% agarose in 0.5 x TBE (50mM Tris, 50mM Boric acid and 1mM EDTA). Standards (100bp DNA Ladder, Gibco, BRL) were included on each gel. Banding patterns from three separate DNA isolations were compared and only recurring bands included in the assessment.

A binomial comparison using GENSTAT 5 (1987) was made between all scored bands from RAPD analyses. Segregation analysis was performed using three statistics: analysis of shared fragments (Gower, 1985), Jacquard similarity (Gower, 1985), Nei-Lei genetic distance (Nei & Lei, 1979).

Results

ITS1 Primers

We were able to amplify rDNA ITS1 region satisfactorily using the fungal primer, TW81, and the gypsy moth primer, HITS1. This produced regions of various size in all species tried. The sizes of the fragments produced in each species is shown in Table 2.

TABLE 2: ITS1 region fragment sizes for various insect species.

Sample Species	ITS 1 Region Size
Gypsy Moth (*L. dispar*)	700 bp
Tussock Moth (*Orgyia thyellina*)	575 bp

The analysis of this ITS1 region with various restriction enzymes gave various sized fragments. We only used three restriction enzymes to digest this fragment as this same fragment was being sequenced, and using a large number of restriction enzymes would be a waste. With the gypsy moth samples, the restriction enzymes that showed the most differences between the strains were *Hae*III and *Tru*91.

RAPD Analysis

Five random primers were used for gypsy moth. However three were dropped for the gypsy moth, as they did not produce scorable bands in all samples. This resulted in only 16 bands for the gypsy moth. This confirmed the differences in the gypsy moth strains found using rDNA analysis.

References

Genstat 5 committee (1987). *Genstat 5 reference manual*. Oxford University Press, Oxford.

Gower, J.C. (1985). Measures of similarity, dissimilarity and distance. In *Encyclopaedia of Statistical Sciences*, *Volume 5*, (eds. S. Kotz, N.L. Johnston & C.B. Read), pp. 397-405. Wiley, New York

Henry, J.M., Raina, A.K. and Ridgway, R.L. (1990). Isolation of high-molecular-weight DNA from insects. Analytical Biochemistry, 185: 147-150.

Nei, M. & Lei, W.H., (1979). Mathematical model for studying genetic variation in terms of restriction endonycleases. *Proceedings of the National Academy of Science USA* 76, 5269-5273.

Pfeifer, T.A., Humble, L.M., Ring, M., Grigliatti, T.A. (1995). *Characterisation of Gypsy Moth Populations and Related Species Using a Nuclear DNA Marker*. *Canadian Entomologist*, 127, 49 - 58

Richards, N.K., Glare, T.R. and Hall, D.C.A. (1997). Genetic variation in grass grub, *Costelytra zealandica*, from several regions. *Proceedings of the 50th New Zealand Plant Protection Conference* 338-343.

Sambrook, J., Fritsch, E.F. and Maniatis, T., (1989). Molecular Cloning. 2nd edition. Cold Springs Harbour Laboratory Press, Cold Spring Harbour.

Walsh, S. P., Metzger, D. A and Higuchi., R. (1991). Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material *Biotechniques* 10: 506-513.

Appendix 14.7: Publications from the programme

14.7.1 from Proceedings of the 48th NZ Plant Protection Conference (1995): 264-269.

SAFETY TESTING OF A NUCLEAR POLYHEDROSIS VIRUS FOR USE AGAINST GYPSY MOTH, *LYMANTRIA DISPAR*, IN NEW ZEALAND

TRAVIS R. GLARE, ELLY M. NEWBY and TRACEY L. NELSON

AgResearch, PO Box 60, Lincoln, New Zealand

ABSTRACT

Gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantriidae), is a serious pest of forests and horticulture in the northern hemisphere. Viable egg masses have been found on ships in New Zealand ports. If gypsy moth does reach onshore, it is important that measures for its eradication have minimal negative environmental impact. A microbial pathogen used to control gypsy moth with success is a nuclear polyhedrosis virus (LdNPV). This virus appears to be relatively specific, attacking only *L. dispar*. in the field. It was imported to New Zealand to determine its safety against Lepidoptera, especially endemic species. Twenty species of endemic and introduced Lepidoptera were exposed to LdNPV, without visual evidence of infection. This was confirmed by probing any dead treated caterpillars using labelled LdNPV DNA to detect latent infections.

Keywords: gypsy moth, *Lymantria dispar*, nuclear polyhedrosis virus, environmental safety, host range

INTRODUCTION

Gypsy moth (GM) is a major lepidopteran pest of native and plantation forests and horticultural tree crops throughout the northern hemisphere. GM was accidentally released around Boston, USA, in 1869 and has since spread throughout the northeast and into Canada (Montgomery and Wallner, 1988). Females of this strain of GM are flightless, which has limited its spread. Recently, an "Asian" strain of gypsy moth (AGM), thought to have originated in Siberia, has been spreading throughout northern Asia and Europe. This strain has females which fly before egg laying, as well as having other characteristics (such as increased host range) which increase the potential pest status. The finding of viable AGM egg masses on ships in New Zealand ports in the last few years has raised awareness of the danger of this pest reaching our shores. Risk assessments by the Ministries of Forestry (MOF) and Agriculture (MAF) and the Forest Research Institute (FRI) concluded that it is almost inevitable that gypsy moth will reach New Zealand and that it would be a devastating pest (Cowley *et al.* 1993; Horgan 1994).

Biological control is a well established method of GM control in the USA, utilising several pathogens. The lepidopteran-active bacterium, *Bacillus thuringiensis* var. *kurstaki*, is used in several biological insecticides and is lethal to the GM larval stage (Reardon *et al.* 1994). It is also registered as a control agent in New Zealand, mainly for use against leafrollers. Although its specificity to Lepidoptera is preferable to a broad spectrum insecticide, the death of non-target larvae, especially rare species, makes its use in ecologically-sensitive areas unacceptable. Resistance to *B. thuringiensis* (Bt) has also been reported for some insects (eg. McGaughey 1985). Therefore additional, specific control agents would greatly assist in eradication or control of GM in this country.

A pathogen which has been successful in controlling pest outbreaks in the northern hemisphere forests is the gypsy moth nuclear polyhedrosis virus, (LdNPV). The virus is a member of the genus *Baculovirus*, and was first registered as a biological pesticide in 1978 in the USA (Reardon and Podgwaite 1992; 1994). Isolates of this virus have been produced for gypsy moth control in the USA, Canada, Czech and Slovak Republics and the "USSR". In China, a NPV routinely causes 50% natural mortality among GM populations (Yan *et al.* 1994). As the virus is produced commercially and applied to large areas of forest, it appears a good candidate for use in

New Zealand. The main benefit of the virus over *Bt* and other control measures is its specificity. Overseas, extensive research has shown that the virus attacks only *L. dispar* and possibly another Lymantriidae species (Barber *et al.* 1993), indicating that any danger of use in New Zealand is likely to be restricted to Lepidoptera. New Zealand has no Lymantriidae species (Dugdale 1988), the closest families taxonomically are Noctuidae or Hepialidae. The objective of this study was to collect, rear, and dose species of New Zealand Lepidoptera with LdNPV to determine any susceptibility.

MATERIALS AND METHODS

Collection, maintenance and identification of larvae

Lepidoptera were collected directly from the field (by the authors or those listed in the acknowledgments), or obtained commercially (Table 1). All larvae were kept at 20°C under a 18:6 L:D photoperiod. Eggs collected from moths were placed in a container with damp filter paper and kept at 20°C for hatching. Larvae were used in all pathogen treatments. Where possible, larvae were reared through to adults for identification. Pupae were kept in containers at ambient temperature, with damp filter paper helping to maintain humidity until emergence. Larvae were identified according to Hudson (1928), Scott (1984) and Dugdale (1988). Where insects could not be identified, adults were sent to John Dugdale (Landcare, Auckland).

Inoculation of larvae with virus

Larvae were dosed in a 24 well Falconer tissue culture dish. A 5 mm bore of the leaf material was contaminated with 2 μl of NPV (1.5 x 10^5 polyhedral inclusion bodies [PIB]/2 μl) in 100 mM Phosphate Buffer Solution, pH 7.2 (PBS), or 2 μl of PBS only as a control. Food samples were left to absorb the droplets and then larvae were placed in the wells. Virus and untreated controls were alternated between rows of four wells. Larger larvae, such as *B. mori*, that were too big to fit in the wells were placed in individual specimen pottles (4.5 x 5 mm) containing the diet and treated in the same manner. Where the food was a granular diet (*G. mellonella*, *P. interpunctella*, *T. bisselliella* and *H. pseudospretella*), 2 μl of virus or PBS was added to approximately 0.041 g of diet. Larvae were left at 20°C until they had consumed the food sample entirely. Generally, 7-10 days after inoculation, larvae from each treatment were transferred to communal containers, maintained at 20°C and monitored for the next 3-4 weeks. The proportions of larvae dying in the treated and control groups were compared using Chi-squared tests.

At the conclusion of the bioassays a sample of virus was sent to Dr John Podgwaite (US Forest Service, Hamden, CT, USA) for bioassay against *L. dispar* larvae (New Jersey laboratory strain F35), to confirm virulence of the virus. The sample from New Zealand was run at the same time as a standard virus strain, MR1-8. Two hundred and fifty newly moulted second instar larvae were treated with 10^2 to 10^6 PIB/ml incorporated in a high wheat germ diet (Bell *et al.* 1981) at 52°C, as described by Lewis and Rollinson (1978). Inoculated larvae were maintained at 24°C under a 16:8 L:D photoperiod, and checked daily for mortality. Lethal concentrations (LC) was calculated by probit analysis using POLO-PC program (LeOra Software, Berkeley, CA).

Table 1: Insect species used for testing with *Lymantria dispar* nuclear polyhedrosis virus.

Taxa	Life stage collected and source	Site	Diet for laboratory maintenance
BOMBYCIDAE			
Bombyx mori L	Commercially obtained[1]	Auckland	Mulberry leaves
GEOMETRIDAE			
Epiphryne verriculata (Felder & Rogenhofer)	Larvae on growing shoots of Cabbage Tree (*Cordyline australis*)	Lincoln	Growing tips of *C. australis*
Pasiphila dryas Meyrick	Larvae on native hebe (*Hebe parviflora* var. *arborea*)	Christchurch	*H. parviflora* var. *arborea*
HEPIALIDAE			
Wiseana cervinata Walker	Eggs collected from moths	Templeton	Modified soya bean diet[4]
Wiseana sp.	Eggs collected from moths	Lincoln	Modified soya bean diet
HYPONOMEUTIDAE			
Nyctemera annulata (Boisduval)	Larvae on groundsel	Christchurch	Groundsel
NOCTUIDAE			
Chrysodeixis eriosoma (Doubleday)	Larvae on Kowhai *(Sophora tetraptera*)	Lincoln, North Canterbury Christchurch	Lettuce, *S. tetraptera*
Trichoplusia ni (Hubner)	Larvae on Geranium (*Pelargonium quercifolium*)	Halswell	*P. quercifolium*
Aletia cucullina (Guenee)	Eggs collected from moths	Castle Hill	Lettuce, Plantain
NYMPHALIDAE			
Danaus plexippus L.	Larvae	Otago	Swan plants
OECOPHORIDAE			
Hofmannophila pseudospretella (Stainton)	Laboratory culture [3]	Ruakura	Supplied diet.
Proteodes carnifex Butler	Larvae on mountain beech (*Nothofagus solandri* var. c*liffortioides*)	Castle Hill	*N. solandri* var. *cliffortioides* leaves.
PIERIDAE			
Artogeia rapae (L.)	Larvae on cauliflower	Lincoln	Cabbage, cauliflower, broccoli leaves
PYRALIDAE			
Galleria mellonella L.	Commercially obtained[2]	Auckland	Farex (Boot Company, Wellington, New Zealand)
Plodia interpunctella (Hubner)	Commercially obtained[1]	Auckland	Supplied diet
TINEIDAE			
Tineola bisselliella (Hummel)	Laboratory culture[3]	Ruakura	Supplied diet
TORTRICIDAE			
Epiphyas postvittana (Walker)	Commercially obtained[1]	Auckland	Supplied diet
Cnephasia jactatana Walker	Commercially obtained[1]	Auckland	Supplied diet
Planotortrix excessana[5] (Walker)	Commercially obtained[1]	Auckland	Supplied diet
Ctenopseutis obliquana[5] (Walker)	Commercially obtained[1]	Auckland	Supplied diet

[1] Obtained from HortResearch, Auckland, laboratory culture; [2] Obtained from Biosuppliers, Auckland, commercial culture; [3] Obtained from Dr P. Gerard, AgResearch, Ruakura, laboratory culture; [4] L. Burgess, HortResearch, Auckland; [5] Kept non-communally due to their cannibalistic nature.

Probe detection of virus

All larvae which died after exposure to virus and some untreated larvae were examined for the presence of virus using ^{32}P-labelled LdNPV and a “slot blot” procedure. Insect cadavers were homogenised in a volume of PBS similar to that of the cadaver and 20-150 µl loaded onto pre wet (10 x SSC: 3M NaCl/0.3M tri-Sodium Citrate) nylon membrane (Hybond N, Amersham, NZ) using a Minifold II Slot-Blot apparatus (Scheicher and Schuell, Keene, N.H) linked to vacuum. Twenty µl containing 100, 10^3, 10^4, 10^5, 10^6 and 10^7 PIBs of LdNPV were included as positive controls. PBS was loaded in at least 10 slots as negative controls in addition to untreated (control) cadaver macerates. After samples were loaded, the membranes were air dried, denatured with 0.5M NaOH/ 1.5M NaCl for 30 min. at 65°C, then neutralised for 5 min. at RT with 0.5M Tris pH 7.0/ 1.5M NaCl, treated for 5 min. in 10 x SSC and air dried for 30 min and baked for 20 min. at approximately 120°C. These membranes were stored for several days in a desiccator at 4°C. Membranes were subsequently prehybridised, hybridised and washed according to standard methods (Sambrook *et al.* 1989). DNA for labelling was isolated from purified virons of LdNPV using the method of Bourner (1993), from 500 µl of approximately 10^8 PIBs/ml. DNA was resuspended in 30 µl of TE (10 mM Tris, 1 mM EDTA pH 8.0) and a sample labelled with ^{32}P by standard methods (Sambrook *et al.* 1989).

RESULTS

LdNPV no effect on the 20 species of Lepidoptera, except in one experiment involving *P. dryas* (Table 2). A second experiment with *P. dryas* showed no effect of the virus. In no cases was virus detected in cadavers, either through visual examination or direct DNA probing (results not shown). For the slot-blot probing, serial dilutions of “Gypchek” could be detected to approximately 10,000 PIB. The virus sample sent to the US Forest Service (Hamden, USA) at the conclusion of the host experiments had an LC_{50} of 4.6 x 10^3 PIB/ml diet (LC_{95} 6 x 10^4 PIB/ml) against *L. dispar* larvae, compared to 9.0 x 10^3 PIB/ml diet for MR1-8 (John Podgwaite, pers. comm.). Thirteen of the twenty species undergoing viral bioassay as larvae completed their life cycle by emerging as healthy adults, which could then be identified.

DISCUSSION

The object of exposing Lepidoptera to this pathogen was to determine whether LdNPV could infect New Zealand insects. Our original aim was to test against endemic Lepidoptera, but collecting and maintaining these proved difficult. Several specimens were collected but died before bioassays could be conducted. We collected larvae mainly in summer, as this is the period GM are larvae and the stage control measures would be required. To increase the number of species tested, we used any Lepidoptera available, including pest species. Other species were represented in the present study by less than 10 individuals (Table 2), so the test for infection lacked statistical power. There was no evidence in the bioassays that LdNPV could infect any of the tested Lepidoptera. These tests were conducted with greater than a LC_{95} dose of virus for GM and under constant laboratory conditions which could be expected to favour viral infection. The slot-blot examination also failed to detect any latent virus. This study supports previous findings that LdNPV does not attack hosts other than Lymantriidae (Barber *et al.* 1993 and references within). LdNPV has the advantage over the bacterial insecticide, Bt, of being specific to GM. For example, we treated nine *N. annulata* larvae with an equivalent of a field treatment of Delfin (Sandoz, Basle, Switzerland) and all died within 2 days. The lack of lymantriids in New Zealand also improves the chances that alternative hosts will not be found by the virus. Research has been conducted using both LdNPV and *Bt* for GM control in Germany, with promising results (J. Huber, pers. comm.), an approach which may have merit in New Zealand. However, further host range testing with combinations of pathogens would then be required. The research conducted both in this study and overseas suggests the virus would be a candidate for use in both an eradication campaign and against established populations of GM.

TABLE 2 The effect of feeding 1.5 x 10^5 PIBs of *Lymantria dispar* NPV to Lepidoptera (inoculated larvae maintained at 20°C).

Taxa	Date dosed	Number tested (and deaths)		Significance of difference[1]
		Treated	Controls	
BOMBYCIDAE				
Bombyx mori	13 .12. 94[2]	10 (7)	10 (5)	ns
GEOMETRIDAE				
Epiphryne verriculata	7.12. 94	26 (16)	25 (22)	ns
Pasiphila dryas	13.12.94	14 (8)	14 (0)	**
	6.1.95	7 (2)	9 (3)	ns
HEPIALIDAE				
Wiseana cervinata	9.1.95	2 (0)	1 (0)	ns
Wiseana sp.	24.1.95	48 (48)	48 (48)	-
HYPONOMEUTIDAE				
Nyctemera annulata	19.1.95	12 (0)	13 (0)	ns
NOCTUIDAE				
Chrysodeixis eriosoma	7.12.94[2]	1 (1)	1 (1)	-
	28.1.95	17 (1)	16 (0)	ns
Aletia cucullina	12.12.94	16 (5)	16(4)	ns
	17.12.94	12(0)	12(0)	ns
Trichoplusia ni	6.1.95	7 (7)	9 (9)	-
NYMPHALIDAE				
Danaus plexippus	17.3.95	9(1)	8(0)	ns
PIERIDAE				
Artogeia rapae	11.1.95	17 (17)	20(20)	-
	20.1.95	9 (3)	9 (2)	ns
PYRALIDAE				
Plodia interpunctella	13.12.94	24 (0)	24 (0)	ns
Galleria mellonella	12.1.95	10 (0)	9 (1)	ns
OECOPHORIDAE				
Proteodes carnifex	13.12.95	3 (2)	3 (3)	-
Hofmannophila pseudospretella	19.12.94	12 (0)	12 (0)	ns
TINEIDAE				
Tineola bisselliella	19.12.94	12 (4)	12 (3)	ns
TORTRICIDAE				
Epiphyas postvittana	18.1.95	23 (3)	24 (3)	ns
Cnephasia jactatana	18.1.95	22 (0)	20 (1)	ns
Planotortrix excessana	7.12.94	18 (3)	13 (5)	ns
Ctenopseutis obliquana	7.12.95	8 (2)	11 (6)	ns
	18.1.95	21 (2)	20 (1)	ns

[1] - indicates sample size too low or all died in both treated and control groups, invalidating the test; ns indicates not significant ($P<0.5$); ** significant ($P<0.1$).

[2] Inoculated with 1.5 x 10^4 PIBs twice due to taste aversion.

ACKNOWLEDGMENTS

Virus was kindly supplied by Dr John Podgwaite (US Forest Service) and Dr Dwight Lynn (USDA). *L. dispar* bioassay was conducted by Dr Podgwaite. We thank T. Jackson, A. McAra, A. Sunckell, D. Turpin, T. Sadler, H. McNab, A. Mitchell, E. Chapman, R. Townsend, T. Bourner, F. Fuller and A. Laurie for collections of Lepidoptera. Alison Inwood assisted with slot-blotting, Dave Saville with the statistics, Maureen O'Callaghan commented on the manuscript and John Dugdale (Landcare, Auckland) provided identification of Lepidoptera. This work was supported by the New Zealand Foundation for Research, Science and Technology.

REFERENCES

Barber, K.N., Kaupp, W.J. and Holmes, S.B., 1993. Specificity testing of the nuclear polyhedrosis virus of the gypsy moth, *Lymantria dispar* (L.)(Lepidoptera: Lymantriidae). *Can. Entomol. 125*: 1055-1066.

Bell, R.A., Owens, C.D., Shapiro, M. and Tardif J.R., 1981. Development of mass-rearing technology. Pp 599-632. *In:* The Gypsy Moth : Research Towards Integrated Pest Management, C.C. Doane and M.L. McManus (eds.); Forest Service Technical Bulletin 1584.

Bourner, T.C., 1993. The control of the common cutworm, *Agrotis segetum* (Lepidoptera: Noctuidae) using Baculoviruses. Ph.D Thesis, University of London

Cowley, J., Bain, J., Walsh, P. , Harete, D.S., Barker, R.T., Hill, C.F., Whyte, C.F. and Barber, C.J., 1993. Pest risk assessment for Asian Gypsy Moth, *Lymantria dispar* L. (Lepidoptera: Lymantriidae). MAF internal document, 29 pp.

Dugdale, J., 1988. Lepidoptera- annotated catalogue, and keys to family-group taxa. *Fauna of New Zealand 14*: 264 pp.

Horgan, G., 1994. Economic impact of Asian gypsy moth. Report prepared for the N.Z. Ministry of Forestry, August 1994, 20 pp.

Hudson, G.V., 1928. The butterflies and moths of New Zealand. Ferguson and Osborne Limited, Printers and Publishers.

Lewis, F.B. and Rollinson, D., 1978. Effect of storage on the virulence of gypsy moth nucleopolyhedrosis inclusion bodies. *J. Econ Entomol. 71*: 719-722.

McGaughey, W.H., 1985. Insect resistance to the biological insecticide *Bacillus thuringiensis*. *Science 229*, 193-194.

Reardon R.C., Dubois, N. and McLane, W., 1994. *Bacillus thuringiensis* for managing gypsy moth: a review. USDA, Tech. Trans. FHM-NC-01-94, 32 pp.

Reardon, R. and Podgwaite, J.D., 1992. The gypsy moth nuclear polyhedrosis virus product. USDA Tech. Trans. NA-TP-15-02-92, 9 pp.

Reardon R.C. and Podgwaite, J.D., 1994. Summary of efficacy evaluations using aerially applied Gypchek against gypsy moth in the USA. *J. Environ. Sci. Health*, *B29(4)*: 739-756.

Sambrook, J., Fritsch, E.F. and Maniatis, T., 1989. Molecular Cloning. 2nd edition. Cold Springs Harbour Laboratory Press, Cold Spring Harbour.

Scott, R.R., 1984. New Zealand Pest and Beneficial Insects. Lincoln University, College of Agriculture.

Yan J., Pemberton, R.W., Yao D., Liu H. and Li G., 1994. Studies on the natural enemies of gypsy moth, *Lymantria dispar* (Lep: Lymantriidae) in Dayi, Sichuan Province. *For. Res.*, *7*: 269-276.

14.7.2 from Proceedings of the 51st NZ Plant Protection Conference (1998): 224-229

POTENTIAL AGENTS FOR ERADICATION OR CONTROL OF GYPSY MOTH IN NEW ZEALAND

T.R. GLARE[1], N.D. BARLOW[1] and P.J. WALSH[2]

[1]*AgResearch, PO Box 60, Lincoln, New Zealand*
[2]*Forest Research Associates, PO Box 1031 Rotorua*

ABSTRACT

Gypsy moth, *Lymantria dispar*, poses a major threat to New Zealand forests, especially the “Asian” strain, which has characteristics such as female flight and reduced diapause requirements for egghatching. To combat the threat, we have reviewed control options, particularly biological agents, used overseas and how they might be used in New Zealand for eradication and population suppression of gypsy moth. Our research has included molecular typing, safety testing of biologicals, and the development of tactical and strategic models. The most promising agent for eradication is *Bacillus thuringiensis* var. *kurstaki* and, for suppression, a nucleopolyhedrovirus and the fungus *Entomophaga maimaiga*.

Keywords: *Lymantria dispar*, gypsy moth, biological control, insectpathogens, insect growth regulator.

INTRODUCTION

Gypsy moth (GM), *Lymantria dispar* L. (Lepidoptera: Lymantriidae), comprises a number of “strains” which are native to much of Eurasia and North Africa and a serious pest of forests throughout the northern hemisphere. GM attacks over 500 species, including many significant forest trees (USDA 1995). While the preferred hosts are oak, apple, sweetgum, speckled alder, basswood, birch, poplar and willow, the larvae will feed on almost any plant when the larval population is sufficiently high. The moth is also an urban pest because it attacks ornamental trees and the hairs of the larvae cause allergic responses in humans. An ‘Asian’ strain of gypsy moth (AGM) has recently spread across Asia and Europe; this strain is potentially even more damaging than the European (EGM) and North American (NAGM) strains. Females of AGM can fly (EGM and NAGM strains cannot), are attracted to light and have a wider host range. A proportion of AGM eggs do not undergo obligatory diapause and they hatch over a longer period, increasing the chance of introduction to new environments. Some of these characteristics have enabled AGM to survive transport across the equator, threatening southern hemisphere countries, while the recent discovery of viable AGM egg masses on ships in New Zealand ports has raised awareness of the danger. Risk assessment (Cowley *et al.* 1993) concluded that it is almost inevitable that GM will reach New Zealand and that it would be a serious pest of forests.

In 1993, a programme was initiated to investigate biological control options of GM strains for New Zealand, funded by the Foundation for Research, Science and Technology. Herein, we briefly summarise methods and agents for use against GM, suitable for New Zealand. Efforts to control a GM outbreak could take two forms: eradication, where a population contained in small area is treated with agent(s) to kill every individual; or introduction of agent(s) for long term suppression. Population suppression would be used if eradication failed or the original incursion had spread too far for eradication. The two approaches are not exclusive and agents suitable for suppression could be introduced during an eradication campaign.

IDENTIFICATION OF GYPSY MOTH STRAINS

Much of the risk assessment and subsequent planning in New Zealand for response to GM has been based on the Asian strain. The requirements for control of an incursion of AGM would be different to that of the non-flighted strains of GM, as some agents (eg pheromones) would not be as effective against widely spreading moths. However rapid identification of the strains is difficult, as the larvae and male moths cannot be readily separated morphologically.

Molecular biology techniques have been used to show species and subspecies variation in many insects and have the advantage of applicability to larvae, pupae or adults. Several approaches have been tried with limited success, but, building on work by Pfeiffer *et al.* (1995), we have used amplification of ribosomal DNA and subsequent restriction enzyme digestion or sequencing to successfully distinguish between strains and hybrids of GM (Glare, Richards and Hall, unpubl. data). The study was conducted with insects from only four locations and needs to be confirmed with a larger sample group, but offers a potential method (tested in New Zealand) that should separate Asian and non-flight strains at all life stages.

AGENTS FOR ERADICATION AND CONTROL

In the case of GM establishing in New Zealand, an environmentally sound method of eradication and/or control will be required. As the pest has been in the USA and Canada for over 100 years, detailed information on agents and effectiveness is often available. The agents are summarised in Table 1.

Diflubenzuron

Better known under the trade name Dimilin, this insect growth regulator was used in 40% of GM treated area in the USA between 1990-1994. Although safer than broad spectrum chemical controls, there are some non-target effects on invertebrates and frequent applications (not recommended in the USA) would increase the residue risk.

Bacillus thuringiensis var. *kurstaki* (*Btk*)

The bacterium *Btk* produces a toxin active against many Lepidoptera, including GM. Unlike most insect pathogens, it does not recycle in the insect and is used as an inundatively applied agent. Between 1980 and 1994, approximately 1.7 million ha were treated with *Bt* in the eastern USA for GM control (Reardon *et al.* 1994). *Btk* was also used in the eradication campaign against the tussock moth in Auckland. Nontarget effects are limited to Lepidoptera, but the development of host resistance can be a problem during long-term control efforts.

Nucleopolyhedrovirus

Lymantria dispar nucleopolyhedrovirus (LdNPV) is a specific virus which attacks only *Lymantria* spp. (Barber *et al.* 1993; Glare *et al.* 1995), making it one of the most specific agents available. It occurs as a natural control agent in most GM populations, but is also formulated as a product in several countries (Table 1). First registered in the USA in 1978, LdNPV is aerially applied on around 20,000 acres in the USA annually (Reardon and Podgwaite 1994). The virus is not registered for use in New Zealand; sufficient information has been obtained to apply for such registration. Currently, however, the supply of any “product” of suitable quality is limited.

Entomophaga maimaiga (Entomophthorales: Zygomycetes)

The fungus, *E. maimaiga*, is a GM pathogen originally from Japan; it has recently become a very effective classical control agent of GM in the USA (Hajek *et al.* 1995). In the field it is only recorded attacking GM (Reardon and Hajek 1993). As the fungus is difficult to culture *in vitro*, its use has been restricted to natural spread and inoculative releases by moving the resting spore stage to new areas in soil. All information gathered indicates the fungus would not be suitable for eradication procedures, but would have potential for release as a long-term control agent, should GM establish in New Zealand.

Protozoa

There is some potential for microsporidia as biocontrol agents for GM (Novotn´y 1988; Jeffords *et al.* 1989), but only as introduced agents as they cannot be mass-produced. Given the lack of complete specificity, protozoa would require host range testing before recommendation for release in established GM populations in New Zealand.

Predators and parasites

In some places, natural population regulation is thought to be due to the action of a group of parasites and/or predators. However, introductions into the USA have not resulted in significant population suppression (USDA 1995), and consideration of the literature suggests that parasites would be unsuitable for use in New Zealand because of cost, the requirement for alternate hosts and lack of specificity.

Pheromone use

A synthetic sex pheromone has been commercially produced for GM under the name “disparlure” and is highly specific for GM. It is used in monitoring but also offers two methods of population suppression: mass trapping and mating disruption. Mass trapping is usually used in conjunction with other methods of control and was the primary treatment in 20% of eradication efforts in the USA between 1967 and 1993 (USDA 1995). Mating disruption was used in only 6 eradication attempts (out of 550) up to 1993 in the USA (USDA 1995), and is likely to be most effective against restricted populations where high dose levels can be maintained.

TABLE 1: Summary of control agents used for gypsy moth and evaluation of their potential for use in New Zealand.

Agent	Nature of agent	Example of product	Present use against GM	Appropriate use	Effectiveness	Comments
Diflubenzuron	Insect growth regulator	Dimilin	Widely used	Inundative application	Good as a inundative agent	Not specific amonginsects; some non-target effects
Bacillus thuringiensis var. *kurstaki*	Bacterium producing an insecticidal toxin	Dipel, Foray	Widely used	Inundative application	Good as a inundative agent	Specific to Lepidoptera; environmental and mammalian safety good
LdNPV	Specific nucleopoly-hedrovirus	Gypchek (USA) Disparvirus (Canada) Biola (Czech Republic) Virin-Ensh (Russia)	Used mainly in limited areas as inundative or classical agent	Inundative application and introduction as self-sustaining biocontrol	Good for both inundative and introductions	Highly specific to *Lymantria;* little risk of non-target effects
Entomophaga maimaiga	Fungus (Zygomycete : Entomophthorales)	No commercially available formualtion	Has spread naturally after enhanced introduction throughout northern America	Introduction as self-sustaining biocontrol only	Good for introduction only	Specific to *Lymantria* in the field; some other Lepidoptera affected in the laboratory
Pheromones	Synthetic chemical attractant	Disparlure	In some control and eradication efforts	Used for population monitoring, mass trapping and mating disruption	Can be used at edge of infestation Mass trapping used on small areas with low density populations	Specific to *Lymantria;* environmentally safe
Other	Predators, parasites and other pathogens	None	Many intro's some effectiveness	Introduction as self-sustaining biocontrol only	Sporadic	Most lack specificity; difficult to introduce to NZ

MODELLING GYPSY MOTH IN NEW ZEALAND

Following review of overseas models and population dynamics data, models were developed to predict the population dynamics of both EGM (equivalent to NAGM in all characteristics) and AGM in New Zealand in order to help in the development of control strategies (Barlow, Caldwell and Kean, unpublished). According to Barlow *et al.*, AGM is predicted to increase more rapidly than EGM at first, and then to maintain more widely fluctuating densities and more pronounced outbreaks, given the presence of predators functionally equivalent to those elsewhere in the moth's range. Under the model, predator pressures cause population fluctuations, therefore in the absence of predators in New Zealand, long-term population fluctuations are unlikely for AGM although populations would be high, especially in the first few years after introduction.

After a model of GM in New Zealand had been developed, Barlow, Caldwell and Kean examined the likely effects of control, using the LdNPV as the main control agent. In the short term (the year following application), suppression depends on the application rate of the spray and, because of the recycling of infection within the population, on the larval density at the time of application. The latter effect distinguishes LdNPV from *Btk* or other pesticides, whose proportional effect is usually densityindependent. Thus, 80% suppression of peak larval densities in the year following control can be obtained from an application of NPV at the standard rate (2.5 x 1012 virus particles/ha), if the larval density is extremely high (105/ha) or extremely low (10/ha). This drops dramatically to a 30% reduction at a pest density of 100/ha. Timing of application within a year is also important. The model suggested a spray during maximum larval abundance gives twice the level of suppression in the following year as a spray when densities are lowest.

Assuming that the default parameters in the model are correct, eradication of a gypsy moth population with LdNPV is only possible if the population is very low (1- 5 larvae/ha) and several sprays are used in rapid succession or the spray concentration is very high (2.5 x 1013 virus particles/ha). Therefore, virus alone is unlikely to be a suitable choice for eradication. The model can be used to evaluate other potential eradication and control agents.

STRATEGIES FOR ERADICATION

If GM is found in New Zealand in a limited area, eradication is feasible. In the USA and Canada, "eradication" has been performed over 550 times, mainly in California, and is considered successful against small (under 40.5 ha), well-defined populations (USDA 1995). As with the white spotted tussock moth, decisions will need to be made between available control options. While these decisions require political and public input, it is possible to summarise strategies which can then be modified to suit the public/political climate. In our opinion:

- *Btk* would be the main eradication insecticide, especially for aerial application. *Btk* has the benefits of being widely available, has been used previously in many eradication procedures and kills GM effectively. The tussock moth programme has already established the safety and method of use over urban areas in New Zealand. Drawbacks are that it could decimate exposed Lepidoptera, which may in turn temporarily affect some bird life in the spray zone.
- The insect growth regulator, Dimilin, would be useful for ground application to control GM in densely infested areas. This chemical has more potential non-target effects, so use should be limited to specific ground applications.
- LdNPV, such as Gypchek (USDA), could be used early in an eradication programme, both as a knockdown agent and to establish virus in the population. If the infested area is an important lepidopteran conservation area, it may need to be the primary method for eradication. There appear to be no non-target problems associated with its use.
- Mass trapping may be a useful addition, especially around the edges of infestations as both a monitoring tool and to trap males to reduce mating opportunities.
- Other control measures, such as mating disruption, *E. maimaiga* and parasites are not suitable for eradication in most situations.

Data are available on *Btk* and viral spray densities and timing of sprays required for eradication. In general in the USA, 2-3 applications, 2-7 days apart have been used in eradication procedures. However, control of AGM could be more difficult than NAGM or EGM, as egg hatch will occur over a longer period than non-flight GM, making the targeting of sprays against selected instars more difficult.

Both virus and *Btk* are more effective against younger instars which makes spray timing important. The modelling work has given some indication of optimal virus release times under New Zealand conditions, but this needs to be extended to *Btk* and other agents.

STRATEGIES FOR CONTROL

If GM establishes over a wide area or eradication is unsuccessful, then ongoing population suppression will be required and early introduction of a range of agents is desirable. We suggest that:

- § LdNPV be introduced as soon as GM is found in New Zealand. Modelling of the effect of virus on AGM in New Zealand suggests that it would be an effective suppression agent (Barlow, Caldwell and Kean, unpubl.).
- § The introduction of the fungus, *E. maimaiga,* should be investigated and, if it poses no threat to native insects, released as soon as is practical, as it has proved to be a very successful suppression agent in the USA.
- § *Btk* is a single action insecticide in most cases and would not maintain itself in a GM population. It could be useful to protect specific areas such as stands of susceptible trees.
- § Mass trapping and mating disruption using synthetic pheromone may be useful in slowing the spread at the front of a non-flight population of GM but would be of little use for flighted AGM established over a wide area.
- § Parasites and predators from other countries are unlikely to be imported due to potential non-target effects, such as broad host range and the requirement for alternate hosts.

CONCLUSION

There are a number of agents which could be used to combat GM should it be found in New Zealand. Decisions on which of the agents should be used in particular circumstances will require some public discussion, but our view is that there are a number of agents which would be suited for various situations. Proactive, New Zealand-oriented research such as the full testing of molecular techniques to distinguish between GM strains, further development and use of models to investigate combinations of control agents and their optimum timing, assessment of the specificity of *E. maimaiga* and the effectiveness of pheromone, would expedite the use of controls should an introduction occur.

ACKNOWLEDGEMENTS

Funding was provide by the Foundation for Research, Science and Technology under programmes C10 434 and C10 540. Discussions and access to unpublished data was provided by many GM researchers. In particular we thank Drs John Podgwaite, Jane Carter, Bill Wallner, Melody Keena, Norman DuBois and Mike Montomergy (US Forest Service, Hamden CT), Dr Ann Hajek (Cornell University, Ithaca, NY, USA), Drs Gisbert Zimmermann, Jurg Huber (director), Horst Baton and Gustav Langenbruch (BBA, Darmstadt, Germany), Dr Julius Novotny (Forest Research Institute, Banska Staivnica, Slovakia), Dr Furher, Dr Axel Schof and Gernot Hoch (Institut fur Forstentomologie, Forstpathologie und Forstschultz, Universtat fur Bodenkultur, Wien, Austria), Drs Martin Shapiro, Ed Doughety, Jean Adams, Dwight Lynn (Insect Pathology Laboratories, USDA/ARS, BARC-West, Beltsville MD, USA), Dr John Cunningham, Dr William Kaupp, Peter Ebling, Stephen Holmes, Dr Dave Tyrrell Dr G.M. Howses (Forest Pest Management Institute, Forestry Canada, Queen Street, Sault Ste. Marie, Ontario, Canada).

REFERENCES

Barber, K.N., Kaupp, W.J. and Holmes, S.B., 1993. Specificity testing of the nuclear polyhedrosis virus of the gypsy moth, *Lymantria dispar* (L.)(Lepidoptera: Lymantriidae). *Can. Entomol. 125*: 1055-1066.

Cowley, J.M., Bain, J., Walsh, P.J., Harte, R.T., Baker, R.T., Hill, C.F., Whyte, C.F. and Barber, C.J., 1993. Pest Risk Assessment for Asian Gypsy Moth, *Lymantria dispar* L. (Lepidoptera: Lymantriidae). New Zealand Forest Research Institute. Report prepared for MAF, NZ. 39pp

Glare, T.R., Newby, E.M. and Nelson, T.L., 1995. Safety testing of a nuclear polyhedrosis virus for use against gypsy moth, *Lymantria dispar*, in New Zealand. *Proc. 48th N.Z. Plant Prot. Conf.*: 264-269.

Hajek, A.E., Humber, R.A. and Elkinton, J.S., 1995. Mysterious origin of *Entomophaga maimaiga* in North America. *Amer. Entomol. 41:* 31-42.

Jeffords, M.R., Maddox, J.V., McManus, M.L., Webb, R.E. and Wieber, A., 1989. Evaluation of the overwintering success of two European microsporidia inoculatively released into gypsy moth populations in Maryland. *J. Invertebr. Pathol. 53:* 235-240.

Novotn´y, J., 1988. The use of nucleopolyhedrosis virus (NPV) and microsporidia in the control of the gypsy moth (*Lymantria dispar*, Linne). *Folia Parasitol. 35:* 199- 208.

Pfeifer, T.A., Humble, L.M., Ring, M. and Grigliatti, T.A., 1995. Characterisation of gypsy moth populations and related species using a nuclear DNA marker. *Can. Ent. 127:* 49-58.

Reardon R.C., Dubois, N. and McLane, W., 1994. *Bacillus thuringiensis* for managing gypsy moth: a review. USDA, Tech. Trans. FHM-NC-01-94, 32 pp.

Reardon, R. and Hajek, A., 1993. *Entomophaga maimaiga* in North America: a review. AIPM, Technology transfer. USDA For. Ser., Northeastern Area, NA-TP- 15-93. 22 pp.

Reardon R.C. and Podgwaite, J.D., 1994. Summary of efficacy evaluations using aerially applied Gypchek against gypsy moth in the USA. *J. Environ. Sci. Health*, *B29(4)*: 739-756.

USDA 1995. *Gypsy Moth Mangement in the United States: a Cooperative Approach.* (draft environmental impact statement). USDA Forest Service, Washington DC.

Appendix 14.8: Comparison of Biola and Gypchek for virulence against Gypsy moth

Travis R Glare and Patrick J. Walsh
AgResearch and FRASS

Introduction

Gychek is the gypsy moth virus-based product produced by the US Forest Service. Biola is a product based on the same virus produced in the Czech Republic (see section 9.4). In May 1996 we tested both products against gypsy moth larvae in the laboratories of Dr Julius Novotony, Slovakian Forest Service, to compare virulence directly.

Methods

Bioassay of Gypchek and Biola LdNPV against L3 (L2-L4) larvae (laboratory culture)

Virus samples:

A: Biola, produced by the Biola Agricultural Cooperative, Chelcice, Czech Republic (label concentration stated 4.82 x 10^9 PIBs/g), obtained in 1996.
B: Gypchek, obtained in 1992 from US Forest Service, Hamden, CT and used in host range studies in New Zealand since. Kept at 4°C (label stated 5 x 10^{10}PIBs/g).
C: Gypchek, obtained in May, 1996 (supplied by Dr J. Podgwaite, US Forest Service), (label stated 3 x 10^{10}PIBs/g).

Inoculation of gypsy moth larvae:
All solutions were adjusted to 10^9 PIBs/ml (working from the original concentrations according to the label), then diluted in 100 mls. 1 ml used to contaminate the surface of an artificial diet. 20 L2-L4 larvae were added to each treatment and left at room temperature. Dose was equivalent to a field application rate of 10^{12}/ha.

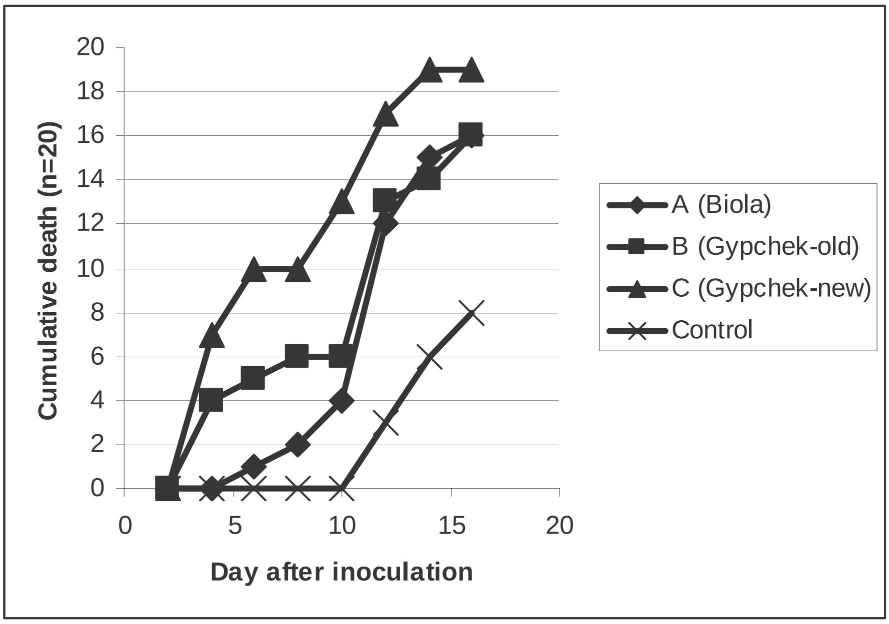
After application to larvae, the concentrations were checked using haemocytometer counts. This was not possible prior to application. After dilution in 100 mls, estimated concentrations were:
A:2.28 x 10^7PIBs/ml (according to label after adjustment this should have been 10x more concentrated)
B:3.52 x 10^8PIBs/ml
C: 2.94 x 10^8PIBs/ml

Results

Table 1: Cumulative larval death after inoculation with LdNPV from Czech Republic (Biola) and USA (Gypchek). (Out of 20 larvae per treatment, maintained at room temperature).

Treatment	Day after inoculation							
	2	4	6	8	10	12	14	16
A (Biola)	0	0	1	2	4	12	15	16
B (Gypchek-old)	0	4	5	6	6	13	14	16
C (Gypchek-new)	0	7	10	10	13	17	19	19
Control	0	0	0	0	0	3	6	8

Figure 1: Of data in Table 1.



Conclusion

Due to a lack of larvae, this test was conducted at a single dose. Unfortunately, the Biola product was not up to label specifications, therefore the dose used was about 10x more dilute than that of the Gypchek treatments. Allowing for this, the new Gypchek and Biola products could be about the same potency (Table 1). The old Gypchek, which was collected from the USA in 1992, travelled for several weeks before refrigeration and kept at 4°C for 4 years, retained virulence, and was equivalent to the Biola product (about 10 fold decrease on the new Gypchek).

On day 6, several dead larvae from each treatment were examined under the light microscope. PIBs were seen in all dead larvae examined. The highest density of infecting PIBs was in larvae from the new Gypchek treatment.

Appendix 14.9: MAF Response to finding of a single male gypsy moth in Hamilton, NZ, in 2003

Ministry of Agriculture and Forestry
Te Manatu Ahuwhenua, Ngaherehere

Gypsy Moth Incursion Hamilton
Update 1 April 2003

Background Information

Identity

Name:	*Lymantria dispar*
Taxonomic Position:	Insecta: Lepidoptera
Common Name:	Gypsy Moth

Trapping Programme

- Commenced in 1993 due to risks identified with imported containers and ships arriving from the Russian Far East.
- Trapping initiated for early detection to enhance the chances of eradication.
- Traps inspected from 1 November to 30 April each season.
- Inspected at least once every 10 ± 1 working days.
- For 2002/03 season 1066 traps have been placed around ports and high risk sites throughout New Zealand.
- 17 traps placed around Hamilton City.
- This is the first positive catch since the programme started.

Events to Date

Wednesday 27 March
Trap inspector notices unusual moth in Hamilton trap. Trap and sample sent to Forest Research.

Friday 28 March
Forest Research suspect *Lymantria* spp. and inform MAF, Forest Biosecurity.
Sample forwarded to Landcare Research for further validation.

Saturday 29/Sunday 30 March
Operational response team alerted.
Specification for intense trapping grid developed as per contingency plan.
Other traps in area rechecked.

Monday 31 March -
Confirmation received that specimen is gypsy moth, most likely Asian strain.
Molecular testing arranged at Lincoln University for final validation.
Notification to industry and affected territorial authorities commenced.
Ground searching out to 750 metres around find requested.

Tuesday 1 April
Incursion investigators begin ground inspections around immediate site.
AgriQuality (lead contractor) begin establishing trapping grid out to 7km radius around epicentre.
Statement released to media.

Main Damage

- Larval defoliation severely weakens and reduces tree growth.
- Mortality of preferred hosts shifts species composition.
- Main impacts overseas are in urban forest areas where nuisance, aesthetic, and recreational effects are most evident.

Hosts

- Has broad range of hosts (> 250 species).
- Mainly hardwoods, such as oak, poplar, willow, birch and apple.
- Conifers, including *Pinus* spp. can be defoliated during high insect densities. Conifers in mixed hardwood/conifer forest are more susceptible.
- New research information commissioned by MAF suggests New Zealand indigenous species are at lesser risk.

Geographical Distribution

- In temperate forests within latitudes 20^0-60^0 N in Europe, Asia, North Africa, United States and Canada.

Biology

- Gypsy moth is an episodic, univoltine (one life cycle/year) defoliator.
- It erupts for 1-7 years and then subsides for an indeterminate number of years.
- Masses of eggs (500 to 1000) pass the winter under tree bark, rocks, fallen branches or man-made objects.
- Egg stage lasts for about 9 months providing a pathway for long distance dispersal.
- Larvae hatch from eggs, balloon on silk threads and disperse until they encounter a suitable host.
- Over 1-1.5 months larvae pass through 5 to 6 instars, feeding during the evening.
- Adults emerge about 2 weeks after pupation, mate and lay their eggs.

Means of Movement and Dispersal

- Egg masses are a resilient easily transported life stage and at times are affixed to imported cars and shipping containers.
- Larvae hatching from eggs rarely disperse more than several hundred metres.
- Adult moths are readily attracted to artificial lights and on average are capable of sustained flight of around 20-25km.
- Egg masses are frequently intercepted on used imported cars.

Pest Significance

- Has a good capacity to establish in new environments.
- Negative impacts upon tree growth and mortality can be expected.
- Causing wide spread damage and severe economic impacts in countries where it has been introduced.

New Zealand Impact Assessment

- Economic Impact Assessment carried out by Forest Research in 1994 identified potential impacts as being:
  - reduction in value of commercial forest estimated at between $60 and $370 million.
  - Estimated reduction in foreign exchange earnings between $60 and $234 million once moth is fully established.
  - Reduction in forests to act as ‘carbon sink’.
  - Possible trade restrictions in horticulture and forest products.
  - Unquantified effects on New Zealand’s indigenous vegetation.
  - Cost of on-going spray programmes.
  - Impact on amenity trees around New Zealand.

Studies have shown New Zealand climate is no barrier to establishment.

Gypsy Moth Incursion Hamilton

UPDATE REPORT NO. 2
7 April 2003

On 31 March 2003 a male gyspy moth (*Lymantria dispar*) was confirmed from a trap placed in Hamilton City as part of MAF's national surveillance programme. The trap was located in a row of mature oak trees. This report summarises response activities from 7 to 13 April 2003.

MILESTONES

- **AgriQuality implement operational headquarters at Ruakura, Hamilton.**
- **Intense trapping grid established to 7km radius.**
- **Ground searches of immediate vicinity and identified risk sites completed.**
- **Additional traps placed in outer 30 km radius.**
- **Media release issued.**

As of 7 April 2003 ground searching and daily inspections of synthetic pheromone traps has resulted in no further evidence of gypsy moth.

Trapping Programme

Traps have been deployed at the following densities within three specified zones:
Zone 1: 0 to 2km radius: 16 traps per square km
Zone 2: 2 to 4km radius: 8 traps per square km
Zone 3: 4 to 7km radius: 2 traps per square km

Traps where possible have been attached to host vegetation.
Daily inspections are being carried out on all traps within the 0 to 7km zone.

As a precaution additional traps have been placed alongside main arterial routes in and out of Hamilton to a 30km radius. A total of 75 traps have been placed.

Ground Surveys

No gypsy moth life stages were found during a ground search around the epicentre. Host trees and high-risk sites out to a 1.5km radius were targeted during the survey.

Controlled Area

Formalisation of the 7km radius as a controlled area continues. Movement restrictions will be placed on plant matter within the Hamilton City district.

Diagnostics

19 suspect moths have been sent for identification. All have been negative.

Public Feedback

General feedback has been positive towards current response actions. Hamilton City Council and Environment Waikato are working with MAF on communicating information to the Hamilton public.

Gypsy Moth Incursion Hamilton

UPDATE REPORT NO. 3
14 April 2003

On 31 March 2003 a male gypsy moth (*Lymantria dispar*) was confirmed from a trap placed in Hamilton City as part of MAF's national surveillance programme. The trap was located in a row of mature oak trees. This report summarises response activities from 7 to 13 April 2003.

MILESTONES

- **Trapping grid enhanced - additional traps placed at satellite dumpsites.**
- **Direct publicity to local community underway.**
- **Interim flyers on gypsy moth movement controls distributed at dump sites.**
- **Meetings held with Tainui and Hamilton City Council.**
- **TAG scheduled for 30 April 2003.**

As of 14 April 2003 initial response action has resulted in no further evidence of the gypsy moth.

Trapping Programme

Total number of traps deployed in each zone:
Zone 1: 0 to 2km radius: (202)
Zone 2: 2 to 4km radius: (183)
Zone 3: 4 to 7km radius: (126)

Note that to the west (Raglan direction) of zones 2 and 3 there is mainly peat farmland with little in the way of host material or structures, hence making it difficult to place traps and maintain a complete grid. In some instance cows have interfered with traps. As an additional precaution traps have also been placed at satellite dumpsites in Morrinsville, Te Awamutu, Cambridge, Perry's dumpsite – north of Hamilton, and along main arterial routes to 30kms.

Traps are being inspected on a daily basis and will remain in the field for a further five weeks.

Technical Advisory Group

A TAG has been scheduled in Hamilton for 30 April 2003. A selection of New Zealand scientists with gypsy moth expertise and two United States experts have been invited to the meeting.

To keep TAG numbers down a meeting with key stakeholders (e.g. Forest Owner's Association, Farm Foresters, Local government) will also be scheduled to discuss and update on the gypsy moth incursion.

Controlled Area

Publicity around movement controls is being increased. Road boundaries that enclose a 7km radius have been drafted. All dumpsites are enclosed in the controlled area.

Diagnostics

47 suspect moths have been sent for identification. All have been negative.

Community Support

Initial meetings with Tainui, Hamilton City Council and Environment Waikato highlight the initial community support towards MAF's response to the gypsy moth incursion.