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Influence of Insect Defoliators on Seedling Establishment of Four Species of the Fagaceae Family in Northern Japan: Leaf Area Loss and Survivorship of Seedlings

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ABSTRACT Following defoliation of Siebold's beech (*Fagus crenata*) and three deciduous oak species (*Quercus crispula*, *Q. serrata*, and *Q. variabilis*), foliage at lower positions had greater leaf area loss than foliage at higher positions, and seedlings had greater leaf area loss than adult trees. Both foliage quality and gravity were probably related to these events. The percentage of leaf area loss influenced seedling mortality. However, there was a great difference between Siebold's beech and the oaks in leaf area loss of their respective seedlings. Current-year seedlings of *F. crenata* had greater leaf area loss than older seedlings. However, current-year oak seedlings had less leaf area loss than older oak seedlings. Possible causes for these differences include foliage quality related to the amount of carbohydrate supplied from cotyledons to current-year seedlings and phenological escape caused by late flushing of current-year oak seedlings.

INSECT FOLIVORY AFFECTS host plants both directly by reducing photosynthesis and indirectly by increasing chances of infection from diseases. Because young seedlings are small and have a small number of leaves, they often lose most of their leaves to insect attacks even when insect density is not very high. Seedlings living under the canopy usually experience great stress because light is limited. These facts suggest that insect folivory has a stronger impact on seedlings than on larger trees. Manual defoliation experiments involving seedlings have demonstrated that insect folivory reduces seedling growth and enhances mortality in *Quercus rubra* (Wright et al. 1989), *Q. douglasii* (Hook and Arn.) (Welker and Menke 1990), and *Liriodendron tulipifera* (Madgwick 1975). On the other hand, plants chemically and physically defend themselves against herbivores (e.g., Karban and Baldwin 1997).

Population outbreaks of folivorous insects sometimes occur in forests and almost all the trees are defoliated. However, foliage loss due to insect folivory was estimated to be around 10% at most of the total canopy in latent years (Bray 1964, Whittaker and Woodwell 1968, Gosz et al. 1972, Mattson and Addy 1975). The beech caterpillar (*Syntypistis punctatella* (Motschulsky)) (Lepidoptera: Notodontidae) and some sawfly species sometimes defoliate beech trees completely in northern Japan (Kamata 2000), but the total loss due to insect defoliation in latent years was estimated to be around 10% of the total canopy biomass (Kamata and Igarashi 1996). However, it was not rare for seedling leaves to have severe insect damage even when overhead canopy foliage was fed on minimally in latent years. This phenomenon suggests that beech seedlings are apt to have more insect folivory than canopy

Pages 56-64 in Liebhold, A.M.; McManus, M.L.; Otvos, I.S.; Fosbroke, S.L.C., eds. 2001. **Proceedings: integrated management and dynamics of forest defoliating insects**; 1999 August 15-19; Victoria, BC. Gen. Tech. Rep. NE-277. Newtown Square, PA; U.S. Department of Agriculture, Forest Service, Northeastern Research Station.

leaves. Folivorous insects often fall from canopy leaves because of such elements as wind and rainfall and thus escape from attacks by natural enemies. It is therefore easy to speculate that the leaves of seedlings, which are located near the forest floor, have more damage due to fallen insects than upper leaves. It is also possible that the foliage of current-year seedlings is more palatable to folivores than canopy leaves. However, there have been no reports that studied this phenomenon. If this phenomenon is universal, then both the phenomenon and its causes are quite interesting and lend themselves to many ecological hypotheses.

In this paper, Siebold's beech and three deciduous oak species are investigated. We compared leaf area loss of adult trees, current-year seedlings, and older seedlings. Seedling mortality was also investigated in relation to seedling age. These relationships were compared among tree species.

Materials and Methods

Study Sites. Study sites were located in natural secondary forests of Iwate (Site A: 40° N, 141° E, 700 to 800 m above sea level) and Ishikawa (Site B: 36 ° 30' N, 136 ° 40' E, 160 to 200 m above sea level) (Fig. 1). The study site in Iwate is located in cool temperate forests mainly occupied by Siebold's beech (*Fagus crenata*) and a deciduous oak (*Quercus crispula*). The study site in Ishikawa is warmer than the Iwate site and is occupied by two other deciduous oak species (*Q. serrata* and *Q. variabilis*).

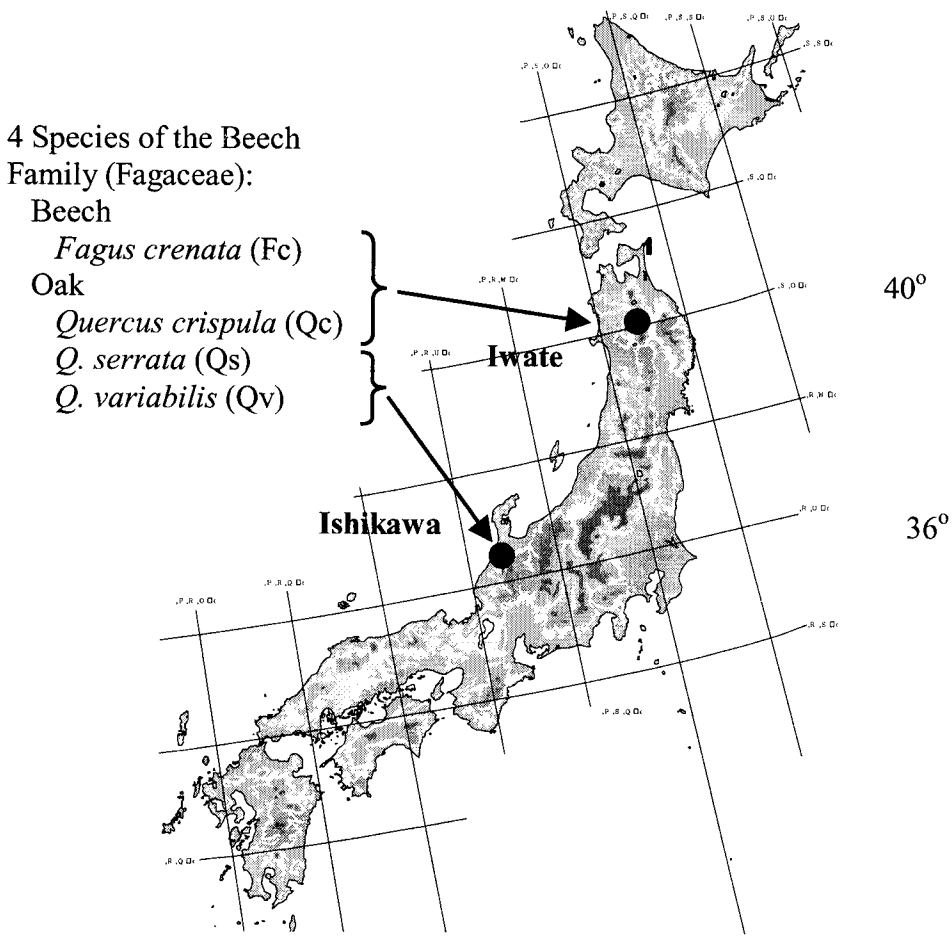

Figure 1. Study sites.

Methods. At Site A, two plots were established in 1996; four quadrants, two per plot, were established after snow melt (mid May). At site B, four quadrants for *Q. serrata* and 10 quadrants for *Q. variabilis* were established in late April of 1998. The size of each quadrant was 1 m x 1 m. Inside the quadrant, all current-year seedlings of the target species were numbered. At Site A, current-year seedlings were numbered in 1996. In 1997, these numbered seedlings were investigated to determine both folivory and mortality in the second year. At Site B, 1-year-old seedlings were also numbered in late April of 1998. Leaf area loss and mortality of both current-year seedlings and 1-year-old seedlings were investigated in the same year (1998).

Branches (ca. 1 m in length) were sampled at heights of 1 to 3 m and > 5 m. All leaves were scanned by an electronic scanner and the percentage of insect damage was calculated using the following equation:

$$\% \text{ Damage} = (\text{Area of insect damage}/\text{Area of original leaf}) \times 100$$

At site B, ca. 100 leaves were marked at each of three different heights (8, 12, and 16 m) for each of the two species. Photographs of marked leaves were taken during every site visit. The percentage of insect damage for each leaf was calculated using the same method mentioned above.

Results and Discussion

F. crenata. Figure 2 shows the survivorship curves of beech seedlings in the two plots (Plots 1 and 2) at Site A. Tendencies were similar between these two plots: most of the mortality occurred during the rainy season from mid June to mid July. No significant difference was found in the survival time of the current-year seedlings between the two plots (Cox-Mantel test, $P>0.05$).

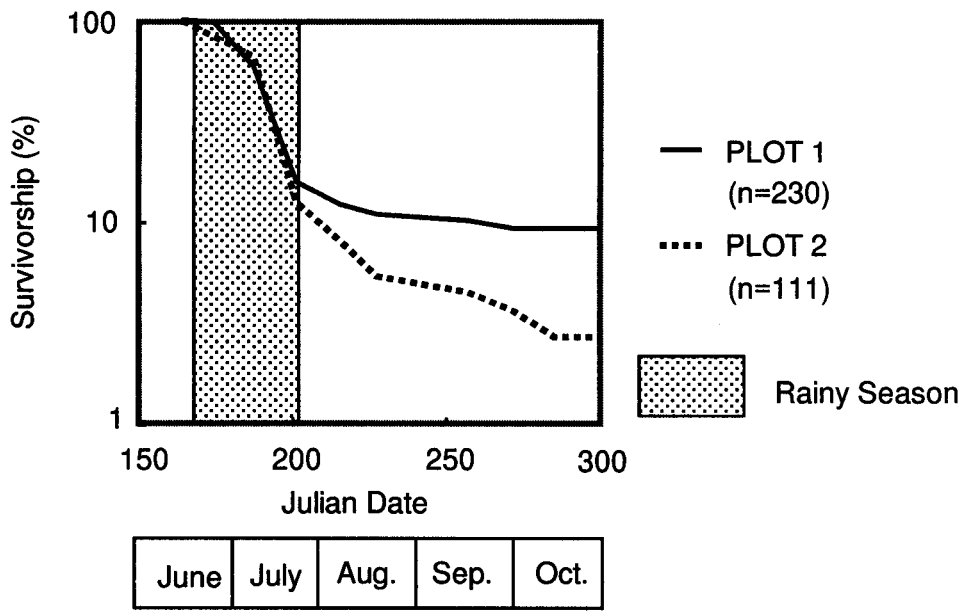

Figure 2. Survivorship curve of current-year seedlings of *Fagus crenata*.

Figure 3 shows the cumulative distribution curve of seedlings in relation to leaf area loss. Curves biased in the upper left indicate that leaf area loss was small. Leaf area loss was compared between dead and live seedlings. Significant differences in leaf area loss were found on July 5th, July 19th, and August 2nd, when mortality was great. This result indicates that dead seedlings had greater leaf area loss than live seedlings and suggests that leaf area loss promoted seedling mortality.

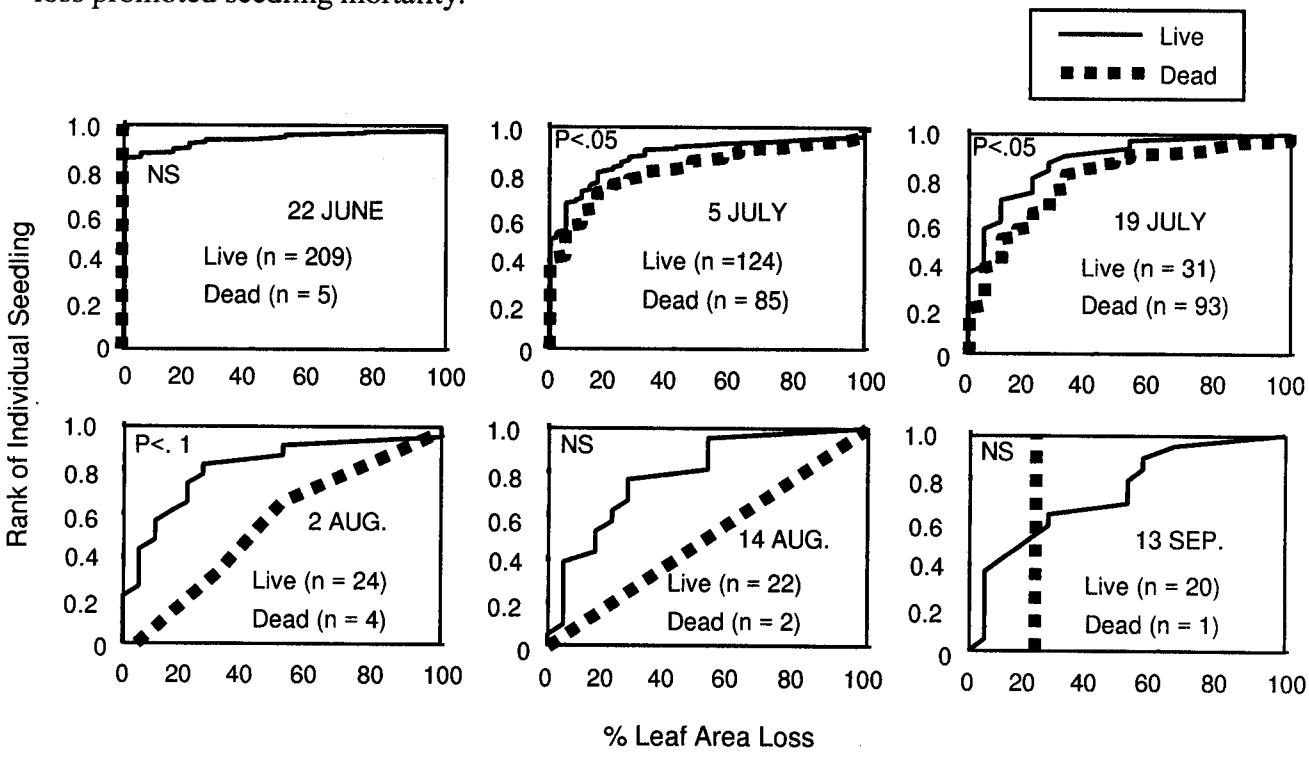

Figure 3. Percent leaf area loss due to insect defoliators and seedling mortality of current-year seedlings of *Fagus crenata* in the two plots of Iwate. The Kolmogorov-Smirnov 2-sample test and Mann-Whitney's U test were applied for detecting the difference in leaf area loss between the live and dead seedling groups.

Leaf area losses were compared among current-year seedlings, older seedlings, and adult trees. In order to study the effect of height on leaf area loss in adult trees, leaves were sampled at heights of 1 to 3 m above the ground and above 5 m. In order to exclude the effect of height on leaf area loss in seedlings, seedlings shorter than 20 cm were selected for this investigation. Figure 4 shows the cumulative distribution curve of individual leaves in relation to leaf area loss. With respect to leaf height, leaves in lower positions had greater leaf area losses; seedlings had greater leaf area losses than adult trees, and between the two groups of leaves sampled at different heights in adult trees, leaves in lower positions also had greater leaf area losses. These results indicate that leaf position is one of the factors that determine leaf area loss by insect folivory. However, current-year seedlings had greater leaf area losses than older seedlings, even though their heights were almost the same. This indicates that in addition to height, foliage quality influences insect folivory.

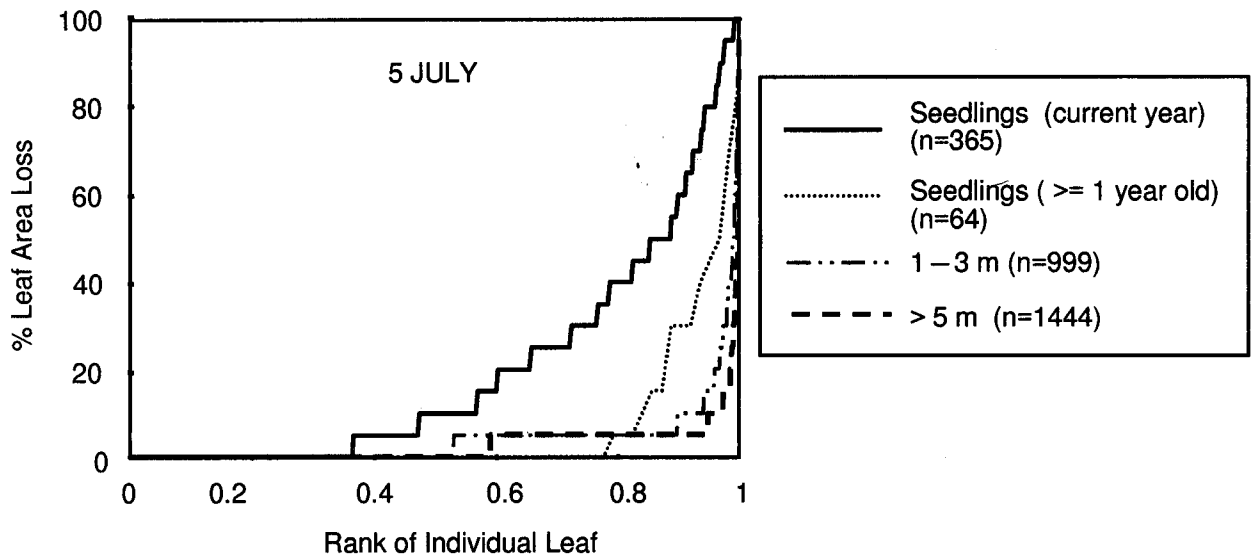

Figure 4. Percent leaf area loss of *Fagus crenata* due to insect folivory for both seedlings and adult trees. Leaf area losses due to insects were compared among leaves at different heights and among seedlings of different ages. Data on 5 July 1996 are shown.

The results for *F. crenata* are summarized below:

(1) Beech foliage located at lower heights had greater leaf area losses due to insect defoliators, suggesting the possibility that (1) more defoliators are distributed at lower heights because they fall by means of gravity and (2) foliage at lower heights is more palatable to insect defoliators than foliage at higher heights.

(2) Seedlings had greater leaf area losses than adult trees.

(3) Current-year seedlings had more severe leaf area losses than older seedlings even though their heights were almost the same, suggesting that in addition to height, foliage quality was related to the event.

(4) Leaf area loss influenced the survival of current-year seedlings.

Three *Quercus* Species. Figure 5 shows the leaf area loss of two deciduous oak species (*Q. variabilis* and *Q. serrata*) at Site B with respect to foliage height. It is clear for both species that foliage in lower positions was heavily defoliated. Seedlings of these two oak species had more severe leaf area losses than their respective adult trees. Figure 6 shows leaf area loss in relation to seedling age and mortality. The effects of seedling age and mortality on leaf area loss were tested by 2-way ANOVA. Both variables were statistically significant, but their interaction was not. These results were the same for *Q. crispula* (Fig. 7). These results suggest that current-year oak seedlings of all three species had less leaf area loss than older seedlings, which was completely the opposite of the results for *F. crenata*. Dead oak seedlings had more leaf area loss than live seedlings, which corresponds with the result for *F. crenata*. The percentage leaf area loss significantly influenced seedling mortality (logistic regression, $P<0.001$).

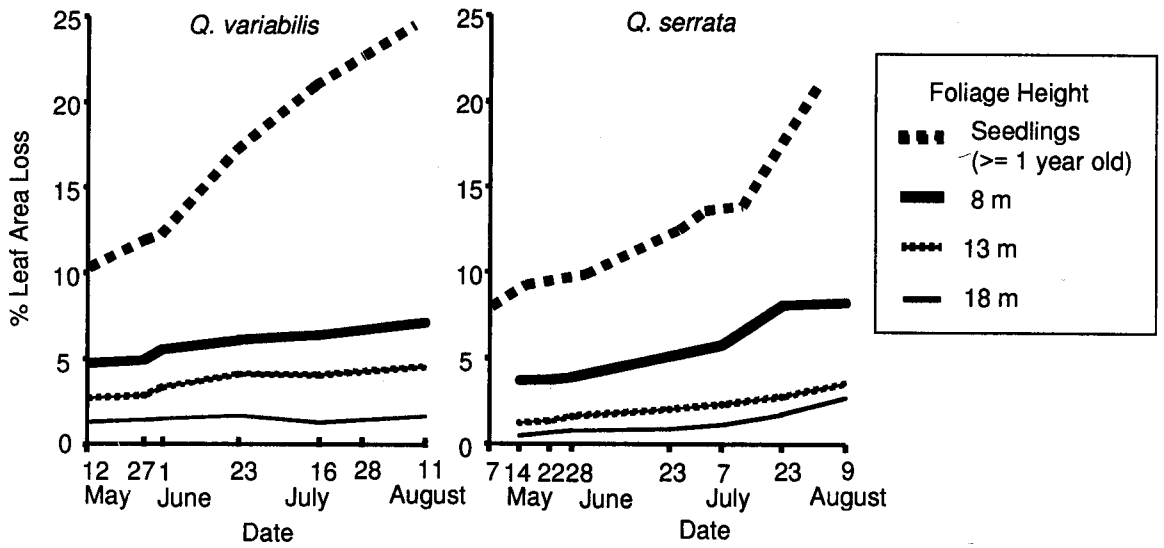

Figure 5. Seasonal changes in percent leaf area loss due to insect folivory of two oak species (*Q. variabilis* and *Q. serrata*) in relation to foliage height.

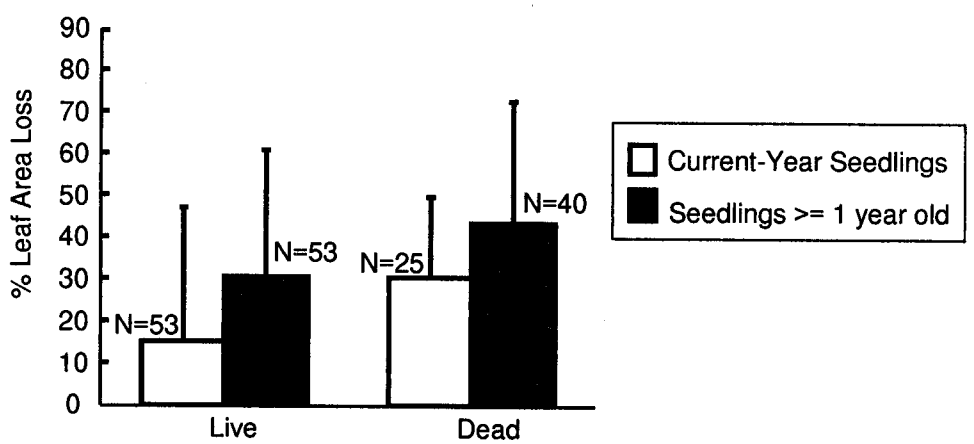

Figure 6. Influence of seedling age and mortality on leaf area loss due to insect folivory of *Quercus variabilis*. Both mortality and seedling age significantly influenced leaf area loss (ANOVA, $p<0.05$), but their interaction did not ($p>0.05$). Leaf area loss significantly influenced seedling mortality (logistic analysis, $p<0.05$), but seedling age did not ($p>0.05$).

Carbon and Defense. For both the three deciduous oak species and Siebold's beech, foliage at lower heights had greater leaf area losses, and leaf area loss influenced seedling mortality. Among the oak species and Siebold's beech, however, there was a difference in insect folivory between current-year seedlings and older seedlings. Current-year seedlings of oaks had less leaf area loss than older seedlings, and the mortality of current-year seedlings was lower. In contrast, current-year seedlings of Siebold's beech had greater leaf area losses, and mortality at age 0 was higher than that of older seedlings.

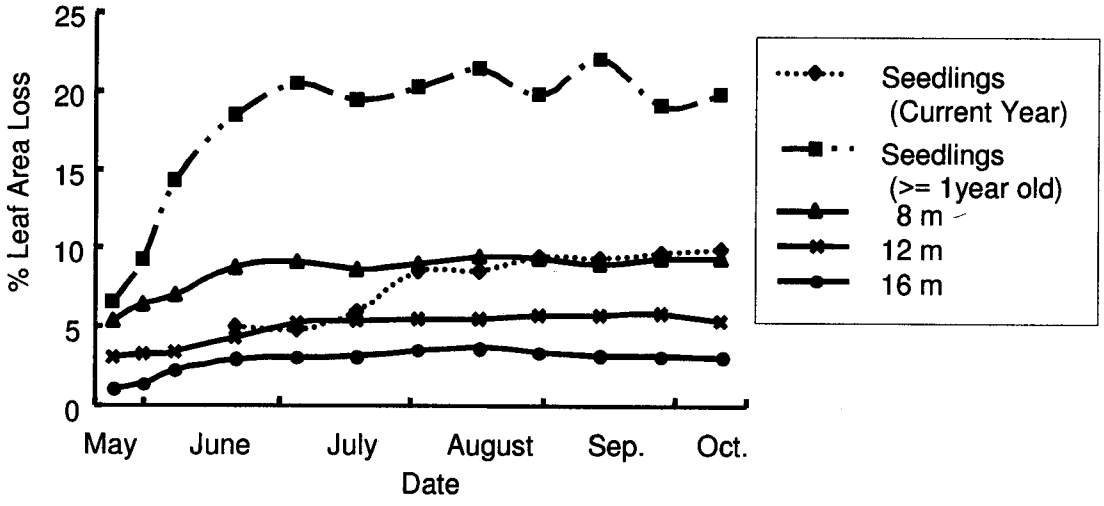

Figure 7. Seasonal changes in percent leaf area loss due to insect folivory of *Quercus crispula* in relation to foliage height and seedling age.

One of the plausible causes to explain this difference between Siebold's beech and oaks is foliage quality (Fig. 8). For Siebold's beech, nitrogen concentration was higher but tannin concentration was lower in the foliage of current-year seedlings compared to the foliage of older seedlings. The results were completely different for the oaks. No significant difference was found in nitrogen concentration between current-year and older seedlings, but current-year seedlings contained more soluble tannin. We speculate that this is related to the amount of carbohydrate supplied from cotyledons. Cotyledons of Siebold's beech seedlings drop almost two weeks after germination whereas those of oaks remain attached to seedlings for several years. Great amounts of carbohydrates are supposed to be supplied from an acorn to a current-year oak seedling. However, older seedlings must synthesize carbohydrates by themselves, or they can use a stock of carbohydrates from the previous year. The photosynthetic rate is low under the light-limited conditions found on the forest floor beneath tree canopies. The C/N ratio of the foliage is likely to become low under such circumstances, so that the foliage of older oak seedlings becomes a more palatable food for defoliators than the foliage of current-year seedlings.

Phenological Escape of Current-Year Seedlings of *Q. crispula*. Another plausible cause for low leaf area losses in current-year oak seedlings is phenological escape. For *Q. crispula*, a concurrent species of Siebold's beech, current-year seedlings had much less leaf area loss than older seedlings (Fig. 7). The mortality of current-year *Q. crispula* seedlings was also significantly lower than for older seedlings (Fig. 9).

We compared the phenology of canopy flush and seed germination between Siebold's beech and *Q. crispula* with respect to seasonal changes in the abundance of insect defoliators in the canopy (Fig. 10). Defoliator abundance was estimated using dry weight of fallen frass. In 1997, canopy flushing of Siebold's beech started around May 5th, and the first peak of canopy defoliators occurred in early June. This first peak was caused by flush feeders. The second peak was caused mainly by the outbreak species *Syntypistis punctatella* (Lepidoptera: Notodontidae). Because this graph shows these events during an endemic year, the second peak was not so high. This second peak has been known to vary on an order magnitude of 4, corresponding to the density cycles of the outbreak species (Kamata and Igarashi 1996, Kamata 2000). Most Siebold's beech seeds germinated simultaneously at almost the same time as the first peak of canopy defoliators. In contrast to Siebold's beech, *Q. crispula*

canopies flushed almost 10 days later, so the first peak of defoliators (mainly flush feeders) were a bit behind the beech. There was a great difference in the timing of seed germination. Strictly speaking, these oaks had germinated their root before winter. The timing of leaf flush of current-year oak seedlings was late and varied greatly among individuals; leaf flush started in late June and ended in late August, with a peak in early July. Because the timing of leaf flush of current-year oak seedlings was late and greatly behind the peak of flush feeders, these seedlings could phenologically escape from defoliators for the first year.

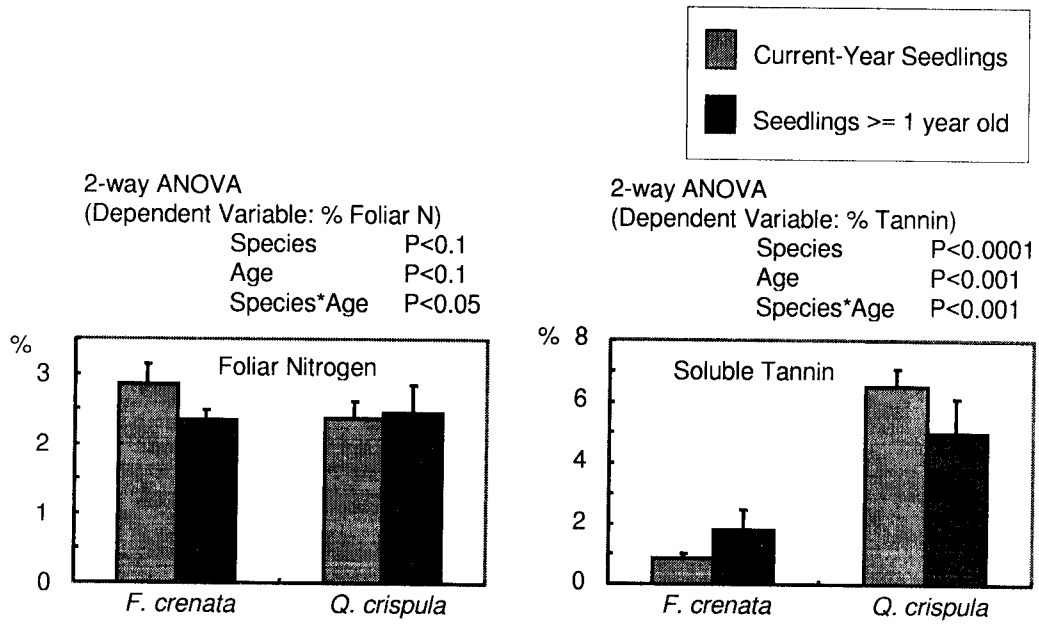

Figure 8. Foliage chemical properties of *Fagus crenata* and *Quercus crispula* in relation to seedling age.

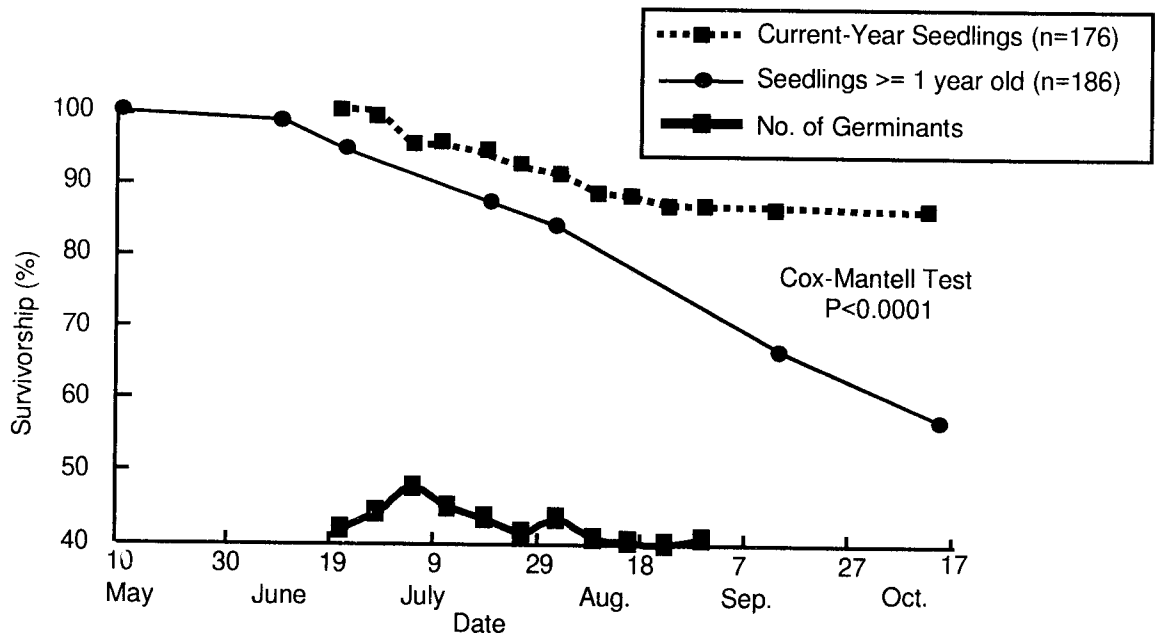

Figure 9. Survivorship curve of *Quercus crispula* seedlings in relation to their age.

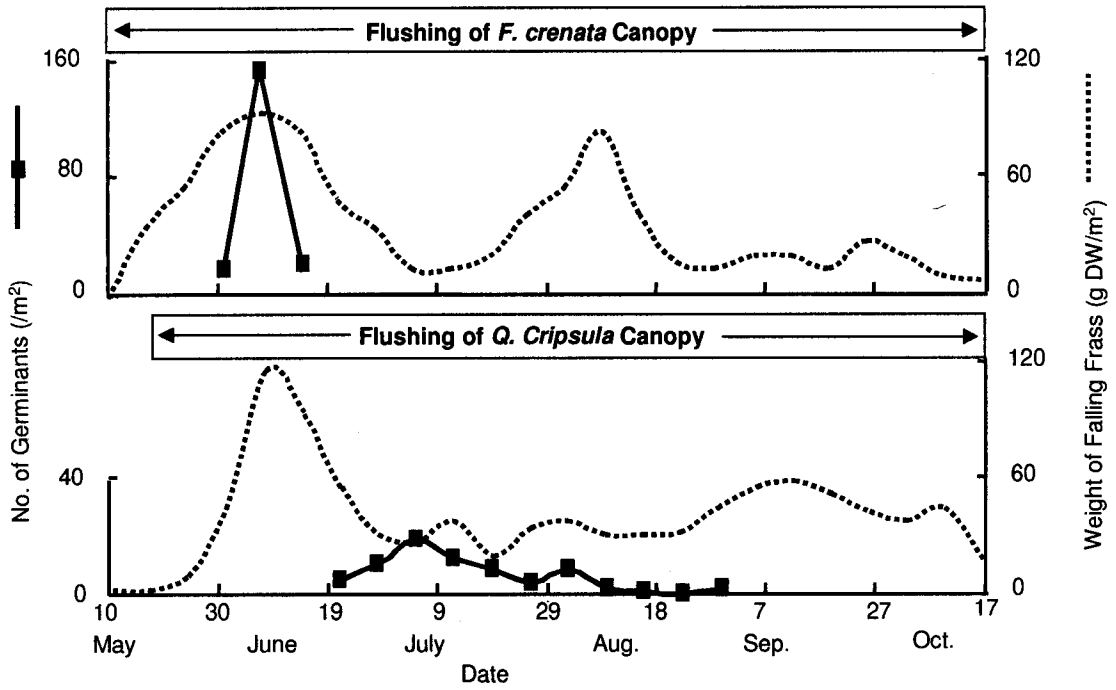

Figure 10. Seasonal changes in falling frass under *Fagus crenata* and the concurrent oak *Quercus crispula* and seasonal changes in the number of germinants of both species.

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European Parasitoids of the Pine False Webworm (*Acantholyda erythrocephala* (L.)) and Their Potential for Biological Control in North America

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ABSTRACT The pine false webworm (*Acantholyda erythrocephala*) is a pine defoliator of Palearctic origin introduced into North America early in the 20th century. A literature search and field surveys showed that the parasitoid complex of the pine false webworm is much richer in Europe than in North America. The potential for introducing European parasitoids into North America is evaluated here. The most promising biological control agent is the tachinid *Myxexoristops hertingi*, a larval parasitoid that lays microtype eggs on pine needles. The release of *M. hertingi* is proposed. Other larval parasitoids, particularly the ichneumonid *Xenoschesis* sp. and the egg parasitoid, *Trichogramma* sp., also show some potential but further studies are required before considering them for release into North America.

THE PINE FALSE webworm (PFW) (*Acantholyda erythrocephala* (L.)) (Hym.: Pamphiliidae) is a Palearctic insect, distributed from Japan to Britain and from Lapland to Italy. It was first observed in the United States in 1925 (Wells 1926) and in Canada in 1961 (Eidt and McPhee 1963). It reached pest status in the late 1970s when heavy damage was observed in young red pine (*Pinus resinosa*) plantations in Ontario (Syme 1981). In 1993, the first outbreaks occurred in mature red pine stands. In New York State, the first severe defoliation was noticed in the early 1980s; since then, the PFW has become a persistent and increasing problem in white pine (*Pinus strobus*) plantations in the Adirondack Mountains (Asaro and Allen 1999, Allen 2000). Although most defoliation reports have come from Ontario and New York, the PFW is in several states in the northeastern United States, Quebec, Alberta, and Newfoundland (Howse 2000). In addition to white and red pine, nearly all pine species are occasionally attacked. Persistent outbreaks, as observed in North America, cause severe growth loss (Allen 2000). Tree mortality often occurs after 5 to 7 years of heavy defoliation due to secondary pests such as bark beetles. Even low populations of the PFW can be particularly destructive in Christmas tree plantations, reducing the market value of the trees.

In contrast, the PFW is considered a minor pest in its area of origin. Outbreaks are rare and usually of short duration (2 to 3 years). Notable exceptions include an 8-year outbreak in the 1960s in eastern Austria (Schmutzenhofer 1974) and populations in southern Poland that remained at semi-outbreak levels for a decade in the 1980-1990's (M. Kenis, unpublished). In the late 1990s, unusually heavy outbreaks started in northwestern Italy in 40-year-old white pine plantations situated far from the natural distribution of pine species. As in North America, *A. erythrocephala* is found on most pine species, with the exception of Mediterranean species. The heaviest outbreaks were observed in plantations of *P. strobus*, *P. sylvestris*, and *P. nigra*. In addition, defoliation is often observed on isolated *P. mugho* and *P. cembra* in gardens, particularly in the Southern Alps (Hellrigl 1996).

Pages 65-73 in Liebhold, A.M.; McManus, M.L.; Otvos, I.S.; Fosbroke, S.L.C., eds. 2001. **Proceedings: integrated management and dynamics of forest defoliating insects**; 1999 August 15-19; Victoria, BC. Gen. Tech. Rep. NE-277. Newtown Square, PA; U.S. Department of Agriculture, Forest Service, Northeastern Research Station.

The biology and ecology of *A. erythrocephala* has been studied by several authors (e.g. Schwerdtfeger 1941, 1944; Schmutzenhofer 1974; Lyons 1994, 1995, 1996; Asaro and Allen 1999). Its life history can be summarized at follows. Adults emerge from the soil in spring, from March (Italy) to May (Canada). Mating and oviposition start soon after emergence. Eggs are laid in rows of one to ten on 1-year-old pine needles. Hatching occurs 3 to 4 weeks later and newly hatched larvae start feeding at the base of the needles, then construct silken webs within which they feed. On average, a larva destroys about 50 needles of *P. sylvestris*, most of them being only partly consumed. Males go through five instars and females go through six instars. From May to July, mature larvae (eonymphs) drop to the soil and build a pupal cell below the humus layer, usually at depths of 3 to 12 cm. After a summer diapause, most eonymphs transform to pronymphs, but some go into prolonged diapause for 2 to 3 years. The rate of prolonged diapause varies among years and with latitude. Pronymphs pupate in spring and adults emerge a few weeks later.

Very little is known about the population dynamics of the PFW in Europe, but natural enemies, particularly parasitoids and pathogens, have been commonly observed and have sometimes been associated with the collapse of outbreaks (e.g. Schwerdtfeger 1941, Rumphorst and Goossen 1960, Jahn 1967, Schmutzenhofer 1974). In North America, however, natural enemies are rare and of low incidence (Lyons 1995, Asaro and Allen 1999). Considering the differences between Europe and North America in terms of both natural enemy impact and outbreak frequency and duration, a biological control program was set up with the aim of introducing European natural enemies into North America. This paper presents information on PFW parasitism in North America and Europe as well as data on the main parasitoid species and an evaluation of their potential as biological control agents. More precise data on parasitoid collection and rearing will be presented elsewhere (Kenis and Kloosterman, in preparation).

Parasitoid Complex of *A. erythrocephala* in North America

The parasitoid complex of the PFW in North America is shown in Table 1. Egg parasitoids are nearly absent. The polyphagous species *Trichogramma minutum* Riley has occasionally been reared from PFW eggs in Ontario (Lyons 1995, Bourchier et al. 2000). Strains of *T. minutum* and *T. platneri* Nagarkatti have been used for inundative releases against the sawfly with some success (Bourchier et al. 2000).

Table 1. Parasitoids of *Acantholyda erythrocephala* in Canada and the USA

	Canada	USA
Egg Parasitoid		
Trichogramma minutum Riley (Trichogrammatidae)	X	
Larval Parasitoids		
Ctenopelma erythrocephalae Barron (Ichneumonidae)		X
Homaspis interruptus (Provancher) (Ichneumonidae)		X
Olesicampe sp. (Ichneumonidae)	X	
Sinophorus megalodontis Sanborne (Ichneumonidae)	X	

Sources: Canada: Bourchier et al. 2000; Lyons 1995, 1999
USA: Asaro and Allen 1999; Barron 1981

Four ichnemonid larval parasitoids were observed on the PFW in Canada and the USA. *Sinophorus megalodontis* Sanborne and *Olesicampe* sp. were reared from eonymphs in Ontario (Lyons 1995, 1999; Bourchier et al. 2000) and *Homaspis interruptus* (Provancher) was found parasitizing the PFW in New York (Asaro and Allen 1999). In addition, *Ctenopelma erythrocephalae* Barron was observed ovipositing in eggs of the PFW in New Jersey (Barron 1981). All these species are apparently of Nearctic origin and none of them seem to have an important impact on sawfly populations, probably because they are poorly adapted to their novel host. Larval parasitism has not exceeded 10%, even after 15 years of continuous outbreaks. *H. interruptus* is also known from another *Acantholyda* sp. and a *Cephalcia* sp. (Barron 1990), whereas the regular hosts of the three other parasitoid species are not known.

Parasitoid Complex of *A. erythrocephala* and Congeneric Species in Europe

Table 2 presents the parasitoid species found in the literature or during our own surveys in Poland, Italy, and Switzerland. The parasitoid complexes of the two congeneric species *A. posticalis* Matsumura (= *nemoralis* Thomson) and *A. hieroglyphica* (Christ) are added since they could represent alternative hosts for parasitoid collections should *A. erythrocephala* populations not be available in Europe. Comparing the parasitoid complexes of closely related hosts might also give important information on parasitoid specificity. Although the literature data on parasitoids of *Acantholyda* spp. were checked for the most obvious mistakes, the list presented in Table 2 has to be considered with caution because it probably contains taxonomic errors, such as different names referring to the same species or a single name referring to two species.

As expected, the parasitoid complex of the PFW is much richer in Europe than in North America. At least four to five egg parasitoids and 8 to 10 larval parasitoids attack *A. erythrocephala* in Europe. *A. posticalis* has a parasitoid complex that is similarly rich, with several species overlapping. Only three species were reared from *A. hieroglyphica*, probably because few studies focused on this species.

Egg Parasitism. Several gregarious *Trichogramma* species are reported from the PFW and other *Acantholyda* spp. in Europe. Schwerdtfeger (1944) mentions high parasitism by *T. evanescens* Westwood on *A. erythrocephala* in Germany in the 1940s and Burzynski (1961) reports *T. embryophagum* (Hartig) as a parasitoid of *A. erythrocephala* in Poland. Both species are also known to attack *A. posticalis* (Herting 1977). Recently, an undescribed *Trichogramma* sp. of the group *fasciatum* was found attacking outbreak populations of *A. posticalis* and low-density populations of *A. erythrocephala* in the Valle d'Aosta, northeastern Italy. This species is univoltine, overwinters in host eggs, and is more specific than the majority of its congeneric species. Screening tests were made on four Lepidopteran species frequently used as rearing hosts for *Trichogramma* spp. (Bourchier et al. 2000). The *Trichogramma* sp. refused to oviposit in *Anagastes kuehniella* (Zeller), *Actebia fennica* (Tauscher), and *Choristoneura fumiferana* (Clemens), whereas oviposition was observed on *Lambdina fiscellaria* (Guenée) but no development occurred. In additional tests (Kenis and Kloosterman, unpublished), females rejected eggs of two diprionid pine sawflies, *Diprion pini* L. and *Gilpinia frutetorum* F. The species most closely related to the undescribed *Trichogramma* sp., both ecologically and morphologically, is *T. cephalciae* Hochmut & Martinek, a parasitoid mainly associated with *Cephalcia* spp., another pamphiliid genus

Table 2. Parasitoids of *Acantholyda erythrocephala*, *A. posticalis*, and *A. hieroglyphica* in Europe; data is from the literature (L) and own surveys (S)

	A. erythr.	*A. postic.*	*A. hierogl.*
Egg Parasitoids			
Trichogramma sp. (Trichogrammatidae)	S	L,S	
Trichogramma cephalciae Hochmut & Martinek (Trichogr.)		L	
Trichogramma embryophagum (Hartig) (Trichogrammatidae)	L	L	
Trichogramma evanescens Westwood (Trichogrammatidae)	L	L	
Trichogramma semblidis (Aurivillius) (Trichogrammatidae)	S	S	
Aprostocetus sp. (Eulophidae)		S	
Neochrysocharis formosa (Westwood) (Eulophidae)	L,S	L,S	
Mesopolobus subfumatus (Ratzeburg) (Eulophidae)		L,S	
Larval Parasitoids			
Ctenopelma lucifer (Gravenhorst) (Ichneumonidae)		L	
Ctenopelma nigrum Holmgren (Ichneumonidae)	L	L	
Holocremnus heterogaster Thomson (Ichneumonidae)	L		
Homaspis rufinus (Gravenhorst) (Ichneumonidae)	L	L	
Notopygus sp. (Ichneumonidae)	S	S	
Olesicampe monticola (Hedwig) (Ichneumonidae)	L		
Netelia oscellaris (Thomson) (Ichneumonidae)	L		
Sinophorus sp. (Ichneumonidae)	S		
Sinophorus crassifemur (Thomson) (Ichneumonidae)	L	L	
Xenoschesis sp. (Ichneumonidae)	S		
Xenoschesis fulvipes (Gravenhorst) (Ichneumonidae)	L	L	L
Euexorista obumbrata (Pandellé) (Tachinidae)		L	
Exorista larvarum (L.) (Tachinidae)		L	
Myxexoristops bonsdorffi (Zetterstedt) (Tachinidae)		L,S	
Myxexoristops hertingi Mesnil (Tachinidae)	L,S		
Nemorilla maculosa (Meigen) (Tachinidae)			L
Pseudopachystylum gonioides (Zetterstedt) (Tachinidae)	L	L	
Undetermined Tachinidae			L

Sources: *A. erythrocephala*: Kenis and Kloosterman (unpublished surveys in Poland, Italy and Switzerland); Burzynski 1961; Eichhorn 1988 (review); Hellrigl 1996; Herting 1964, 1977 (review); Jahn 1967; Joakimov 1921; Pschorn-Walcher 1982 (review); Rumphorst and Goossen 1960; Schmutzenhofer 1974; Schwerdtfeger 1941, 1944; Thompson 1944 (review)
A. posticalis: Kenis and Kloosterman (unpublished surveys in Italy); Casale and Campò 1977; Eichhorn 1988 (review); Herting 1977 (review); Hochmut and Martinek 1963; Pschorn-Walcher 1982 (review); Thompson 1944 (review)
A. hieroglyphica: Herting 1977 (review); Pschorn-Walcher (1982 (review)

that feeds mainly on spruce. *T. cephalciae* has also been occasionally reared from *A. posticalis* (Hochmut and Martinek 1963). This species is partly univoltine and apparently specific to Pamphiliidae, but is morphologically distinguishable from undescribed *Trichogramma* sp. (Pintureau, Stefanescu, and Kenis, in preparation). Another species, the multivoltine, polyphagous *T. semblidis* (Aurivillius) was reared occasionally from the same

populations of *A. posticalis* and *A. erythrocephala* in Italy (Kenis and Kloosterman, in preparation).

Burzynski (1961) and Kenis and Kloosterman (in preparation) reared the solitary eulophid *Neochrysocharis formosa* Westwood (=*Achrysocharella ovulorum* Ratzeburg) from *A. erythrocephala* in Poland. *N. formosa* is a polyphagous parasitoid also known from *A. posticalis* (Casale and Sampò 1977; Herting 1977; Kenis and Kloosterman, in preparation) and pine diprionid sawflies (e.g. Pschorn-Walcher and Eichhorn 1973).

Larval Parasitism – Ichneumonidae. Several ichneumonid species are reported from *A. erythrocephala* and congeneric species (Table 2), but usually only one or two species are mentioned per host population. The ichneumonids most commonly associated with *A. erythrocephala* belong to the genera *Xenoschesis* and *Sinophorus*. In previous studies, they were usually identified as *X. fulvipes* (Gravenhorst) and *S. crassifemur* (Thomson) (e.g. Schwerdtfeger 1944, Schmutzenhofer 1974), two species better known as parasitoids of *Cephalcia* spp. However, a closer examination of *Xenoschesis* sp. from Poland and Italy and *Sinophorus* sp. from Poland (Kenis and Kloosterman, in preparation) revealed morphological differences when compared with specimens reared from *Cephalcia* spp. Since the phenology of *Cephalcia* spp. is at least one month later than that of *A. erythrocephala* and *A. posticalis*, it is likely that the two host genera support different, closely related parasitoids. A similar taxonomic problem arises with *Ctenopelma lucifer* (Gravenhorst), the main parasitoid found in *A. posticalis* outbreaks in the Valle d'Aosta, Italy (Casale and Sampò 1977). Recent observations in the same area suggest that it is not *C. lucifer*, but *Notopygus* sp., a species also reared from *A. erythrocephala* in Poland (Kenis and Kloosterman, in preparation).

Two other ichneumonid species worth mentioning are *Olesicampe monticola* (Hedwig), a parasitoid usually associated with *Cephalcia* spp. that was found parasitizing 44% of the larvae of an isolated population of *A. erythrocephala* in South Tyrol, Italy (Hellrigl 1996), and *Holocremnus heterogaster* Thomson, the dominant parasitoid during an *A. erythrocephala* outbreak from 1915 to 1917 in Bulgaria (Joakimov 1921).

Ichneumonid parasitoids of *Acantholyda* spp. attack eggs or larvae and kill their host in the pupal cell, either before or after the winter. In most cases, ichneumonids are able to follow their host into prolonged diapause (Kenis and Kloosterman, unpublished). Eggs and young larvae are frequently encapsulated in the host haemolymph.

Larval Parasitism – Tachinidae. Only two tachinid species are reported from *A. erythrocephala* (Table 2). *Pseudopachystylum gonioides* (Zetterstedt), a species frequently reared from *A. posticalis* in Central Europe, has also been cited as a parasitoid of *A. erythrocephala* (Herting 1977). However, the most regularly encountered tachinid parasitoid of *A. erythrocephala* is *Myxexoristops hertingi* Mesnil. It is found throughout the entire European distribution of its host and has often been mentioned as the main parasitoid of the PFW; Rumphorst and Goossen (1960) suggested that the fly was the main cause of the collapse of an outbreak in Germany. *A. posticalis* and the closely related *Cephalcia* spp. are attacked by the congeneric species *M. bonsdorffi* (Zetterstedt) and *M. abietis* Herting, respectively (Herting 1964, Eichhorn 1988), suggesting high host specificity in this genus. *M. hertingi* was found in low density populations on isolated *Pinus cembra* in the Swiss and Italian Alps. Specimens were also reared from the closely related host *Acantholyda pumilionis* (Giraud) in the same areas (Kenis and Kloosterman, in preparation). However, these had a much more rapid development, with large larvae found in mature host larvae dropping to the soil, which suggests the possible existence of sibling species.

M. hertingi lays over 1,000 microtype eggs on pine needles that are ingested by sawfly larvae. Parasitoid development occurs when the eonymph is in the soil. The winter is passed as a mature maggot in the dead host skin. In spring, the maggot climbs to the soil surface to build a puparium. Adults emerge after about a month and mate soon after emergence. *M. hertingi* puparia are sometimes heavily attacked by hyperparasitoids such as a gregarious diaprid wasp *Trichopria* sp. that killed over 20% of the puparia in an outbreak in Italy in 1999 (Kenis and Kloosterman, in preparation).

In recent years, we have put much emphasis on the development of a rearing method for *M. hertingi*. Although possible, rearing *M. hertingi* in the laboratory proved to be very difficult for two reasons: (1) the low rate of mating success and (2) the poor development of tachinids after egg ingestion (Kenis and Kloosterman, in preparation).

Biological Control Potential

There is general agreement on the most important attributes of a good biological control agent to introduce against an exotic pest (e.g. Cock 1986). In particular, the natural enemy should (1) have an important impact on the host in the region of origin, (2) be host specific, (3) be well synchronized with the host, (4) have a high searching capacity, (5) be well adapted to a wide range of environmental conditions, and (6) occupy an empty ecological niche in the natural enemy community of the pest in the region of introduction. The tachinid *M. hertingi* combines all these attributes. Literature and field surveys showed that it is the most abundant and frequently encountered parasitoid of the PFW in Europe. Its broad distribution indicates that it has a wide climatic range and the observation of isolated PFW populations being attacked in the Alps suggest that the fly has a good search capacity. There is no tachinid or other late larval parasitoid reported from the PFW in North America. *M. hertingi* is a univoltine parasitoid and is well synchronized with its host. Finally, *M. hertingi* is apparently very specific and is probably restricted to one or two *Acantholyda* spp. Therefore, we recommend *M. hertingi* for introduction into North America.

The difficulty in rearing *M. hertingi* suggests that releases in North America may have to be made with field collected material. Since outbreaks in Europe are scarce, every opportunity should be taken to collect *M. hertingi* when sizeable populations are available. However, efforts should be made to improve laboratory mating techniques because establishment in North America would more likely be achieved by releasing mated females than unmated flies.

Ichneumonids, particularly the most common species *Xenoschesis* sp., may represent additional candidates. Ichneumonids have long been considered less specific than *M. hertingi* because the same species were reported from *Acantholyda* spp. and *Cephalcia* spp. However, recent observations showed that a complex of sister species was involved that was probably more specific than previously thought. Further studies, however, are needed on the taxonomy, specificity, biology, and ecology of the main species before considering them for introduction. In particular, the high rates of encapsulation often observed in Europe may hamper their establishment or efficiency in North America.

Finally, the undescribed *Trichogramma* sp. would merit further studies. In contrast to most other *Trichogramma* spp., it is univoltine and would not require an alternate host to become established in North America. Host specificity would need to be further evaluated but preliminary observations suggest that this species is more host specific than most of its

congeneric species. However, its real impact and occurrence in Europe as well as its taxonomic status need to be further assessed.

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Preliminary Results on the Efficacy of Stored TM BioControl-1®

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ABSTRACT Douglas-fir tussock moth nucleopolyhedrovirus (OpNPV) was produced and stored in various sized packages during the 1980s under the registered name of TM BioControl-1®. This virus is an important component of the pest management system for this tussock moth. In this paper, preliminary results of a collaborative project between the USDA Forest Service and the Canadian Forest Service to test the efficacy, or shelf life, of stored TM BioControl-1® are presented. Bioassays were done using 1-day-old third instar Douglas-fir tussock moth larvae inoculated with different concentrations of virus preparation. Comparisons of LD50 and LD90 values indicated a trend of higher values for samples stored for 6, 10, and 13 years than for virus that was stored for 4 years or was freshly produced. Potency ratios suggested a loss in potency with longer storage. There appeared to be no difference in LD values or potency in samples from different package sizes from the same lot.

FROM THE EARLY 1980s to 1995, the USDA Forest Service undertook an ambitious project to produce, register, and store Douglas-fir tussock moth nucleopolyhedrovirus (OpNPV) for use against the Douglas-fir tussock moth (DFTM). Numerous lots of OpNPV were produced during this time and stored at -10 °C at USDA Forest Service facilities in Corvallis, OR. The multicapsid isolate of OpNPV was registered in the United States in 1976 under the name TM BioControl-1® (Martignoni 1978, 1999). The same product received registration in Canada in 1983 along with the same virus produced in *Orgyia leucostigma* under the name Virtuss® (Otvos et al. 1998). This virus is now an important component of the pest management system that was developed for the Douglas-fir tussock moth (Shepherd and Otvos 1986, Otvos and Shepherd 1991). Considerable time has elapsed since the original production and storage of TM BioControl-1® lots. A collaborative project between the USDA Forest Service and the Canadian Forest Service was initiated in the summer of 1998 to test the efficacy, or shelf life, of the stored TM BioControl-1® product. This is a preliminary report of early results of these efficacy tests in this on-going project.

Douglas-fir Tussock Moth. The Douglas-fir tussock moth (*Orgyia pseudotsugata* (McDunnough)) (Lepidoptera: Lymantriidae) is a native defoliator and causes periodic damage in the interior dry-belt coniferous forests of British Columbia, Canada and the western United States. Outbreaks occur every 7 to 11 years and persist up to 4 years (Mason and Luck 1978, Harris et al. 1985). Although DFTM larvae feed on the foliage of several tree species, the primary hosts in British Columbia and the U.S. are Douglas-fir (*Pseudotsuga menziesii* variety *glauca* Biessen (Franco)) and *Abies* spp. (Beckwith 1978, Shepherd 1985). Severe economic damage may occur during outbreaks. There may be significant tree mortality due to defoliation as well as top kill, growth reduction, and secondary attacks by insects and fungi (Wickman 1978, Alfaro et al. 1987).

Pages 74-84 in Liebhold, A.M.; McManus, M.L.; Otvos, I.S.; Fosbroke, S.L.C., eds. 2001. **Proceedings: integrated management and dynamics of forest defoliating insects**; 1999 August 15-19; Victoria, BC. Gen. Tech. Rep. NE-277. Newtown Square, PA; U.S. Department of Agriculture, Forest Service, Northeastern Research Station.

History of TM BioControl-1® Samples. Production of TM BioControl-1® occurred at the USDA Forest Service facility in Corvallis, OR. Fifth instar DFTM larvae were infected with OpNPV, reared until death, and frozen. Over the course of 9 years, three private companies were contracted to process the virus-killed larvae and remove excess insect parts. The cadavers were lyophilized to remove moisture, resulting in a high potency, small particle size, virus-containing powder capable of being mixed as an insecticide and sprayed through ground and aerial spray equipment. The finished product was a wettable powder meeting the requirements of the registered product TM BioControl-1®. The packaging was to be suitable for long-term storage at -10°C and ease of use in field locations where control measures were required. Packages of TM BioControl-1® are kept in cold storage at the Forestry Sciences Laboratory in Corvallis.

The infectivity titre (LC50) of the processed product was determined by the USDA Forest Service on advance samples provided by the contractors. This was done at Corvallis using the diet surface contamination technique. Second instar Goose Lake colony tussock moth larvae were exposed to several different concentrations of the virus sample. The Goose Lake colony, which originated from northern California field-collected DFTM, has been maintained in the laboratory under controlled conditions since the mid 1960s. The virus dilutions were applied to the surface of the artificial diet to determine how much of the preparation was required to cause 50% of the test insects to die (LC50) (Martignoni and Iwai 1977, Martignoni 1999).

Ten lots of TM BioControl-1® were produced and stored between 1985 and 1995. These lots were placed in 1,243 packages and contained the equivalent of approximately 400,000 acre doses (Hadfield and Magelssen 1995). From these packages, 47 samples were selected for efficacy testing. The selection was based on lot number, time in storage, and package size (Table 1). We are testing the effects of the following variables on the efficacy of the stored products: (1) time in storage, (2) package size, (3) company processing the virus-infected insects, and (4) potency against strains of DFTM from different geographical regions. This paper presents preliminary results of the bioassays conducted in the first year.

Materials and Methods

Selection of Samples for Testing. Of the 1,243 packages stored, 17 different package sizes, representing small, medium, and large packages, were sampled for bioassays. Twenty-gram samples were weighed out and sent to the Pacific Forestry Centre in Victoria, B.C., for testing. Fifteen of the package sizes had three samples each and two had one sample each for a total of 47 samples. The 47 samples were ranked in terms of time in storage, package size, and acre dose available.

To date, three large bioassay runs, each using approximately 4,500 larvae (for a total of approximately 14,000 larvae) have been completed employing larvae from the following sources: (1) Goose Lake DFTM colony, (2) wild DFTM reared from egg masses collected in California, and (3) wild DFTM reared from egg masses collected in British Columbia.

Egg masses of DFTM have to be stored at cold temperatures (5°C) to break diapause. Storage from 4 to 7 months is ideal to give maximum hatching rate while storage for longer periods results in a decrease in larval hatch (Beckwith 1978). Each large bioassay run takes approximately 2 to 3 months to complete. We are reporting here on some preliminary data from the first large bioassay run using the Goose Lake strain.

Table 1. List of the 20-gram samples removed for efficacy determination from TM BioControl-1® packages in storage at Corvallis

Lot #[a]	g/Pkg[b]	# Pkgs	Total Acre Doses	# 20-g Samples	Date Stored
1	1,200	1	480	1	1985
2	2,150	18	18,000	3	1986
3	1,830	13	13,000	3	1986
4a	368	124	24,800	3	1986
4b	1,840	33	33,000	3	
5a	368	54	10,800	3	1986
5b	1,840	26	26,000	3	
6	180	170	17,000	3	1989
7a	31.7	485	48,300	3	1990
7b	158.5	100	50,000	3	
7c	317	50	50,000	3	
8a	202	1	690	1	1991
8b	293	68	68,000	3	
9b	58	57	5,700	3	1993
9c	580	9	9,000	3	
10a	124.7	10	1,000	3	1995
10b	1,247	24	24,000	3	

[a] Lot 1 processed by USDA Agricultural Research Service
Lots 2-5 processed by Reuter
Lots 6-7 processed by Espro
Lots 8-10 processed by Crop Genetics

[b] Package size: small = <100g/pkg, medium = 100-999g/pkg, large = >1,000g/pkg

Bioassays with Goose Lake Strain DFTM. Five samples, representing four different time periods in storage and three package sizes (small, medium, and large) were selected for the first large bioassay run. These samples were: lot 2 (TM2) stored 13 years, lot 6 (TM20) stored 10 years, lot 9 (TM36 and TM37) stored 6 years, and lot 10 (TM43) stored 4 years (Table 2). All of these samples represented lots with medium to high acre doses in storage. In addition, TM36 and TM37 were from the same lot and represented small and medium package sizes.

Table 2. History of TM BioControl-1® samples used in the 1999 bioassay reported here

Lot #	Sample #	Year Stored	Time in Storage (yr)	Package Size (g/pkg)
2	2	1986	13	2,150
6a	20	1989	10	47
9b	36	1993	6	58
9c	37	1993	6	580
10b	43	1995	4	1,247

Bioassay Protocol. To obtain DFTM larvae, Goose Lake colony (maintained at the Pacific Forestry Centre since March 1996) eggs were washed three times with a 0.1% sodium hypochlorite (2% bleach) solution to reduce potential virus contamination, rinsed three times with distilled water, and allowed to air dry. The eggs were hatched and larvae reared at 25°C, 50 to 60% RH with a photoperiod of 16:8 [L:D]. Larvae were reared in groups of 10 on artificial diet in petri dishes (Thompson and Peterson 1978). Newly molted third instar larvae were selected from the colony and starved for 16 to 20 hr before the bioassay.

Virus Preparation. Twenty mg of each of the five TM BioControl-1® samples to be tested in the first bioassay were weighed out and 20 ml of distilled water were added. Samples were stirred for 2 hr and viral samples prepared in triplicate. One week prior to inoculation, serial dilutions were made and stored at 4°C. The USDA Forest Service had already counted the polyhedral inclusion body (PIB) concentrations of these samples and these counts were available in the original bioassay data provided to us. However, as a check, haemacytometer counts of PIBs were done on five of the TM BioControl-1® samples. The PIBs/ml calculated were the same as the original PIB counts done at Corvallis. A fresh OpNPV virus sample, used as a positive control, was prepared prior to the bioassay by homogenizing Goose Lake DFTM larvae previously inoculated with OpNPV and reared until death. The fresh OpNPV sample was prepared 2 weeks prior to use and stored at 4°C; dilutions were discarded after 3 months. The homogenate was filtered through cheesecloth, centrifuged several times, and resuspended in distilled water. PIB counts were done using a haemacytometer to quantify the virus.

Inoculation. A diet plug inoculation bioassay technique, similar to that of Kaupp and Ebling (1990), was used. Diet plug bioassay is superior to diet surface contamination because it eliminates any variation in the distribution of PIBs on the diet surface and negates differences in larval feeding rates and interactions among larvae reared in the same container.

Serial viral dilutions ranging from 2.5 to 300 PIBs/µl were used in our bioassay. One µl of each viral dilution or control (distilled water) was added to a small diet plug (3 to 4 mg) inside each well of a 24-well tissue culture plate. The diet plugs were large enough to fully absorb the 1 µl of liquid. Immediately after inoculation, one third instar larva was placed into each well to feed on the treated diet plug. Five virus concentrations and one control were tested for each of the TM BioControl-1® samples as well as the fresh OpNPV sample (positive control). Bioassay of the five dilutions of each TM BioControl-1® sample, the fresh preparation of OpNPV, and an untreated control were replicated three times. For each viral dilution or control, 48 larvae were tested.

Larvae were held in darkness for 24 hr with the inoculated diet plug at 25°C, 50 to 60% RH. Larvae that consumed the entire diet plug were placed in individual cups (Solo P100, Solo Cup Co., Urbana, IL 61801-2895) with a fresh cube of diet and returned to the same growth chamber. Larvae that did not consume the entire diet plug were discarded. Because of the high virulence of OpNPV, it was necessary to rear the larvae individually after inoculation to avoid cross infection. Separate growth chambers, set at the same rearing conditions, were maintained for the control and viral-infected insects to guard against viral transmission to the control insects. Diet was changed at least weekly or more often if required. Larvae were reared until adult emergence or death and were examined daily and had their mortality recorded. Only those larvae that died from NPV infection, as determined by gross pathology or microscopic examination, were included in the analysis.

Data Analysis: Preliminary Data. The equations of the dosage mortality curves and LD values with associated 95% fiducial limits were calculated using PROC PROBIT analysis (SAS Institute 1989-1996). Each replicate at day 14 post inoculation (or day 21) was tested as a separate preparation to verify that there was no difference in the replicates. If there was no difference, data for the three replicates were combined. Estimates of LD50 and LD90 for each TM BioControl-1® sample and the fresh OpNPV sample were calculated. LD values at day 14 and day 21 were compared to see if lethal doses changed over time. LD50 and LD90 values were examined for significant differences (no overlap of the 95% fiducial limits).

Potency ratios comparing each TM BioControl-1® sample to the fresh OpNPV preparation were calculated. Also, potency ratios were calculated comparing each TM BioControl-1® sample to Lot 10 (TM43).

Preliminary Results

Within Sample Variation of LD50 and LD90. There were no significant differences between LD50 values at day 14 compared to day 21 for any of the TM BioControl-1® samples tested or for the fresh OpNPV sample. There were no significant differences between LD90 values at day 14 compared to day 21 for any of the TM BioControl-1® samples tested or for the fresh OpNPV sample. Therefore, we decided to continue the analysis using day 14 data only.

Between Sample Comparisons of LD50 and LD90. There were no significant differences in LD50 values when the fresh OpNPV sample was compared to any of the TM BioControl-1® samples (Fig. 1). However, there was a clear trend of higher LD50 values for lots stored longer than 4 years. When the LD50 value of lot 10 (TM43), which was stored for 4 years, was compared to the other TM BioControl-1® samples, it was significantly lower than lot 2 (TM2) stored 13 years; the LD50 values for the other three samples were between these two values.

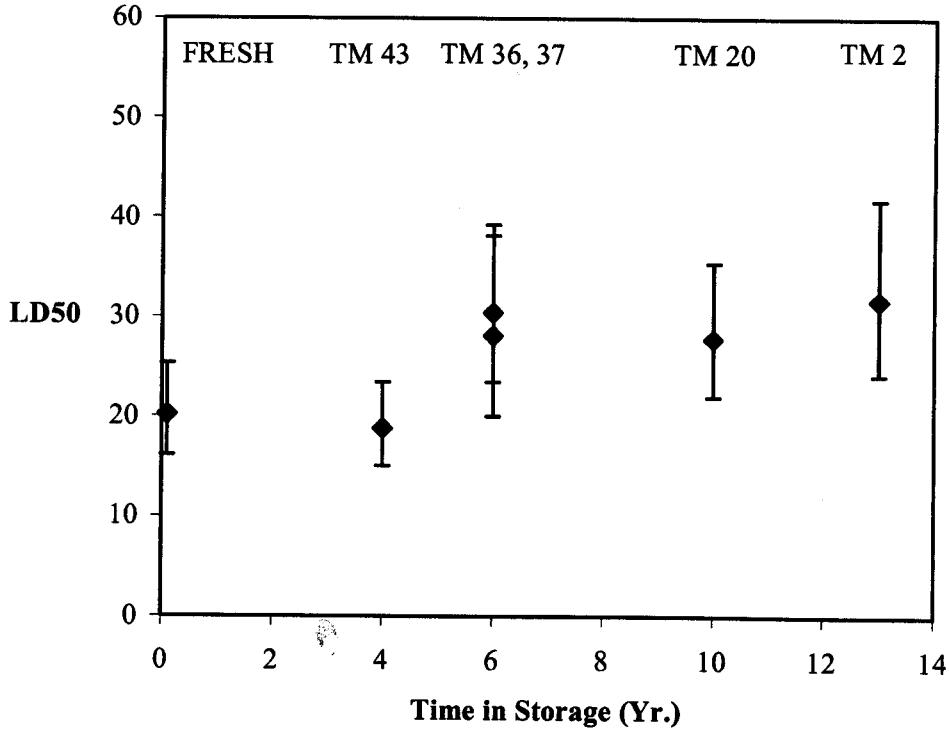

Figure 1. LD50 values with 95% fiducial limits at day 14 of the bioassay with the Goose Lake strain of Douglas-fir tussock moth larvae plotted against time in storage. Data from each bioassay fit the probit model (p>0.05).

Comparisons of LD90 values (Fig. 2) showed that the fresh OpNPV sample was significantly different from one sample (TM36) stored for 6 years. There was a trend of higher LD90 values for lots stored for 6 and 13 years compared to freshly produced OpNPV. When the LD90 value of lot 10 (TM43) was compared to the other TM BioControl-1® samples, it was significantly lower than lots stored for 6 and 13 years but not the lot stored for 10 years.

When looking at the effect of package size within a lot stored for 6 years, no significant differences were found in LD50 and LD90 values for a small package (TM36) compared to a medium package (TM37).

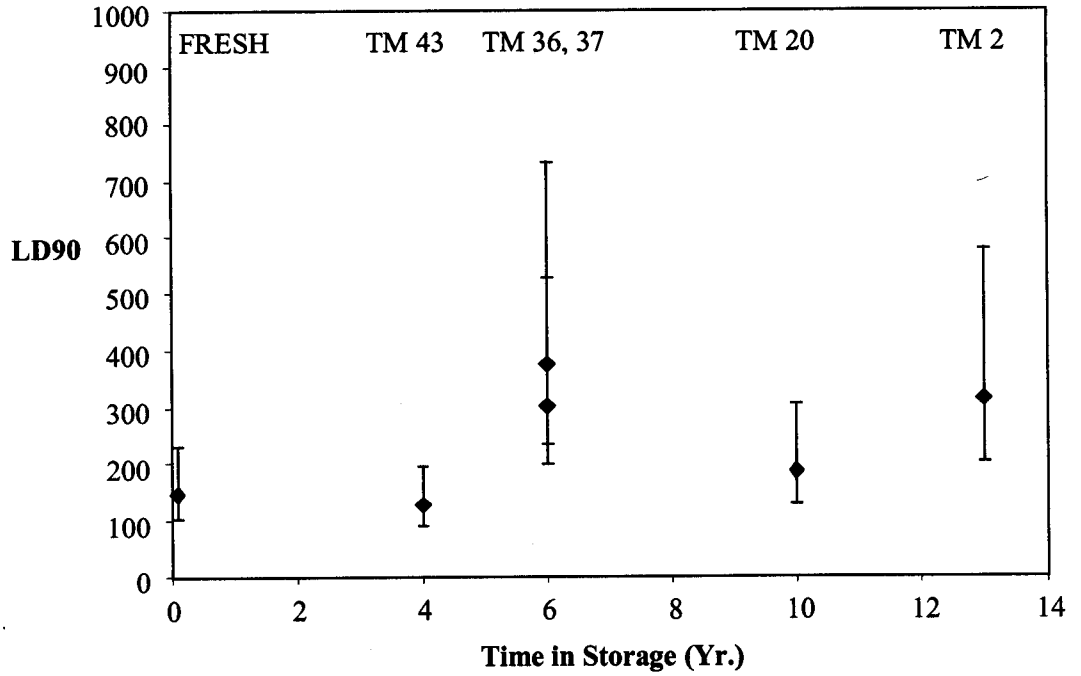

Figure 2. LD90 values with 95% fiducial limits at day 14 of the bioassays with the Goose Lake strain of Douglas-fir tussock moth larvae plotted against time in storage. Data from each bioassay fit the probit model (p>0.05).

Potency Ratios. Copies of the original bioassay data, using the advance samples from the processors, were obtained from Roy Magelssen[1]. Direct comparisons of the LC50 values from the original bioassays done in Corvallis on the advance samples from the processors and our LD50 data cannot be done because two different bioassay techniques (diet surface contamination and diet plug) and different instars (second and third) were used in the two bioassays. However, comparisons of potency ratios within the two data sets obtained by the two bioassay techniques can be done. The relative potency of two stimuli is defined as the ratio of equally effective doses (Finney 1952). The relative potency provides a convenient description of the differences among samples.

Potency Comparisons and Rankings. In our current Goose Lake bioassays, potency ratios were calculated by comparing each TM BioControl-1® sample to the freshly prepared OpNPV sample (Table 3). Potency ratios were used to rank the effectiveness of the different lots tested. One sample tested from lot 10 stored for 4 years was greater in potency than the freshly prepared sample of OpNPV. All other samples were less potent than fresh OpNPV.

Using our Goose Lake bioassay results, potency ratios were obtained by comparing TM BioControl-1® samples without the fresh OpNPV sample (Table 4). Lot 10 stored for 4 years was used as the basis for the comparison since the LD values were not significantly different from those of the fresh OpNPV sample and lot 10 was in storage for the least amount of time. In addition, original LC50 data from surface contamination bioassays done at the time of processing of TM BioControl-1® were re-analyzed to produce potency data and a ranking of the lots was done (Table 4). Again, potency comparisons were done using lot 10 as the basis for the comparison.

[1]Roy Magelssen, USDA Forest Service, Pacific Northwest Region, Forest Insect and Disease Service Centre, Wanatchee, WA.

Table 3. Comparison of potency data among lots calculated from day 14 LD50 values using the fresh OpNPV as the basis of comparison with TM BioControl-1® samples

Years Stored	Lot #	TM BioControl-1 Sample®	Potency Ratio
13	2	2	0.6
10	6	20	0.7
6	9	36	0.7
6	9	37	0.7
4	10	43	1.1
0		FRESH OpNPV	1.0

Table 4. Comparison of potency data among the different lots calculated using the original bioassay data from the processor's advance sample and potency data calculated from the current Goose Lake bioassays; Lot 10 was used as the basis for the comparisons

Lot #	Original Potency Ratio	Current Potency Ratio
10	1.0	1.0
6	1.2	0.7
9	0.8	0.7
2	0.5	0.6

Rankings obtained from data when the samples were first processed indicate that lot 10, lot 9, and lot 6 were quite potent but that lot 2 was considerably less potent than the other lots. This indicates that even at the time of processing, there was a difference in potency among the different lots. This fact is very important when interpreting current bioassay data with respect to length of storage of the samples. The comparison of potency ratios, calculated using data from the current bioassays, indicated that lot 9, lot 6, and lot 2 are less potent that lot 10.

Lot 6 was originally more potent than lot 10. In the advance bioassay data from Corvallis, lot 6 was contaminated with CPV. There may be synergistic effects between these two viruses (Tanada 1956).

Discussion

Our preliminary results indicate that storage affects efficacy directly. When LD50 values from the current bioassay were compared, the sample of fresh OpNPV and the sample from lot 10 (stored since 1995) were not significantly different. However, for samples stored longer than 4 years, there was a trend of higher LD50 values. Higher LD50 values mean that more product (a higher dose) is required to achieve the same level of larval mortality.

Comparing LD90 values, there was also a trend of higher LD90 values in lots stored longer than 4 years when compared to fresh OpNPV and lot 10. Comparisons of both LD50 and LD90 values suggested a trend of higher values for samples stored longer than 4 years. Additional bioassay data of stored samples will be used to determine if the trend shown by these preliminary results can be confirmed.

These preliminary results indicated no difference in potency among the viral samples from different package sizes within a lot and this is encouraging. The different virus samples bioassayed and their data analyzed to date are not large enough to determine conclusively if processing by different companies had an effect on potency. Preliminary results on lot 2 suggested that it was never as potent as lot 10, 9, or 6 and we offer no explanation for this difference.

Other studies with stored tussock moth virus show similar results. Martignoni (1978), using the diet surface contamination technique, reported a shelf life of 5 years for Douglas-fir tussock moth NPV when the virus was stored in a cool, dry place. Kaupp and Ebling (1993), using a diet plug inoculation bioassay with second instar whitemarked tussock moth larvae and Virtuss®, have similarly concluded that virus potency decreased in storage at 4°C. A 46% loss in infectivity was observed after 2 years in storage and Virtuss® stored up to 10 years showed up to a 25-fold decrease in infectivity. This decreasing potency with storage was also reported by other authors for different insects using different bioassays. Cunningham (1970), using balsam foliage dipped in virus suspensions, found that eastern hemlock looper NPV, stored for 6 years at 4°C, showed a 200-fold loss of pathogenicity to third instar larvae compared to freshly produced virus. Lewis and Rollinson (1978) found similar results with stored gypsy moth NPV. These authors reported that suspensions of NPV retained their potency for 5 years under refrigeration, for 2 years at room temperature, for 1 year as air-dried powder stored at 4°C, and for 6 months as air-dried powder stored at 38°C in diet contamination bioassays using second instar gypsy moth larvae. Neilson and Elgee (1960), investigating the effect of storage on NPV virulence on second and third instar larvae of the European spruce sawfly, reported similar results. Using foliage contaminated with virus suspension, they reported that when virus was stored at 4.5°C, loss of potency occurred beyond 5 years with the greatest change in virulence after 9 years in storage and total inactivation after 12 years.

All the above studies reported some loss of activity of the virus with storage. Our preliminary results suggest that storage has affected the efficacy of stored TM BioControl-1®. There seemed to be a trend of viral potency loss with storage. Additional bioassays will be conducted to confirm our results to date regarding how length of storage, package size, and processing affect viral efficacy. In addition, we will also conduct experiments on the effectiveness of TM BioControl-1® against DFTM from different geographical regions.

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