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**Effects of long-term storage on the efficacy of TM Biocontrol-1^â, the viral insecticide of the
Douglas-fir tussock moth, *Orgyia pseudotsugata* (McDunnough)**

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Effects of long-term storage on the efficacy of TM Biocontrol-1[®]

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Abstract

TM Biocontrol-1[®] is a viral insecticide that was registered in 1976 by the US Environmental Protection Agency for the control of Douglas-fir tussock moth, *Orgyia pseudotsugata* (McDunnough). Between 1985 and 1995 the US Department of Agriculture Forest Service produced and stockpiled this virus.

This paper presents the results of 47 bioassays on samples of the stored products from the 17 different sizes of vacuum sealed packages that were stored at -10°C from 5 to 15 years. This is the first study that presents efficacy data for a virus stored for such a long time.

The bioassays have shown that TM Biocontrol-1[®] stored at -10°C for 5-15 years is still effective, although it lost about 30% of its effectiveness during storage. This loss in potency does not appear to be directly related to length of time in storage. The size of the package, in which TM Biocontrol-1[®] was stored, did not affect potency. There were no clear, significant differences in the efficacy of the various lots of the product processed by the four different organizations. The bioassays showed that there are significant differences in the susceptibility of Douglas-fir tussock moth from the different geographic regions to TM Biocontrol-1[®] infection.

Key Words: *Orgyia pseudotsugata*, nucleopolyhedrovirus, TM Biocontrol-1, long term storage, shelf-life, efficacy

TM Biocontrol-1[®] is a viral insecticide that was produced and registered in 1976 by the Environmental Protection Agency at the request of the United States Department of Agriculture (USDA) Forest Service for the control of Douglas-fir tussock moth, *Orgyia pseudotsugata* (McDunnough). TM Biocontrol-1[®] was produced in the *O. pseudotsugata* Goose Lake laboratory strain (GL-1) at the USDA Forest Service facility in Corvallis OR (Martignoni 1978, 1999, Hadfield and Magelssen 1995). The Goose Lake colony originated from field collected *O. pseudotsugata* egg masses from northern California, was maintained in the laboratory in Corvallis under controlled conditions from the mid-1960s up to 1995. Since 1995 the colony has been maintained at the Pacific Forestry Centre (Natural Resources Canada, Canadian Forest Service (NRCan-CFS)) in Victoria, BC.

The virus was produced *in vivo*. Fifth instar larvae were infected with the *O. pseudotsugata* virus, OpNPV, reared until death, and the cadavers were harvested and frozen. Over the course of 9 years, three private companies, Reuter, Espro and Crop Genetics, were contracted to process the virus-killed larvae and remove excess insect debris. The cadavers were lyophilized to remove moisture and milled to pass a 100-mesh screen resulting in a high potency, small particle size, virus-containing powder. The finished product was a wettable powder insecticide meeting the requirements of the registered product TM Biocontrol-1[®] that could be easily mixed and used with aerial spray equipment under field conditions. The powder was vacuum-sealed in various sized packages, generally containing 100 to 1000 acre-doses per package, and placed in long-term storage at -10°C at the facilities of the USDA Forest Service Forestry Sciences Laboratory in Corvallis, OR. The activity or infectivity titre of the processed product was determined by the USDA Forest Service on advanced samples provided by the processing companies. The bioassays were conducted using the diet surface contamination

technique (Martignoni and Iwai 1977). Second instar larvae of the Goose Lake colony of *O. pseudotsugata* were exposed to several different concentrations of the virus sample being tested. The virus dilutions were applied to the surface of the artificial diet (Thompson and Peterson 1978) to determine how much of the preparation was required to cause 50% mortality in the test insects (median lethal concentration or LC₅₀) (Martignoni 1978, 1999; Martignoni and Iwai 1977, 1978).

The USDA Forest Service produced and stored 10 lots of TM Biocontrol-1[®] between 1985-1995. Small amounts of the virus were used between 1985 and 1995. In 1995 there were 1,243 vacuum-sealed packages that contained the equivalent of approximately 400,000 acre-doses (Table 1) (Hadfield and Magelssen 1995). From these packages, 47 samples, representing the 10 lots, were selected and sent to Natural Resources Canada, Canadian Forest Service (NRCan-CFS), Pacific Forestry Centre, Victoria, BC, for efficacy testing. The testing was conducted under a Cooperative Agreement between the USDA Forest Service and NRCan-CFS. The selection of samples was based on lot number, package size and time in storage (Table 1). The effects of the following variables were investigated in connection with the efficacy of the stored product:

1. Potency of stored TM Biocontrol-1 samples tested against different field strains of *O. pseudotsugata* from different geographical regions and against the Goose Lake strain.
2. Package size,
3. Time in storage and,
4. Company processing the virus killed insects.

The results and discussion will be presented separately for each variable.

Materials and Methods

Bioassay Protocol. The purpose of the bioassays was to determine the current activity titre (potency) of the stored TM Biocontrol-1[®] samples against *O. pseudotsugata* larvae. The potency was measured as the amount of preparation that caused the death of 50% of the test population of the larvae after 14 days. While we ran our bioassays for 21 days, because there were no significant differences in mortality at day 14 and day 21 we chose the shorter one for comparison.

Activity standardization bioassays are the first step in the process needed for acre-dose determination (Martignoni 1978, Scott 2000). Each of the 10 production lots of TM Biocontrol-1[®] have a potency or activity titre that equates to an acre-dose treatment, based on bioassays with the Goose Lake or field strains, and is expressed as the activity units per gram (AU_{GL/g}) (Martignoni 1978, Scott 2000). After determination of the potency of the product in the laboratory, the acre-dose (the amount of virus preparation to be used to treat 1 acre of *O. pseudotsugata* infested stands) can be calculated from the second or acre-dose bioassay data for field application. Unlike the activity standardization studies, the acre-dose bioassays require *O. pseudotsugata* larvae from the target field population (the population to be treated) and TM Biocontrol-1[®] from the lot(s) to be used in that particular suppression project. These two bioassays ideally should be done each time a new field population is scheduled for treatment in a new area and whenever a new lot of the product is used.

To obtain Douglas-fir tussock moth larvae for activity standardization bioassays in the laboratory, field-collected or Goose Lake colony *O. pseudotsugata* egg masses were decontaminated in a manner similar to the methods used by Thompson and Peterson (1978). The egg masses were gently teased apart and the eggs were washed three times with 1% sodium hypochlorite (2% bleach) solution to reduce potential virus contamination, rinsed three times with distilled water, and then allowed

to air dry. The decontaminated eggs were placed in sterile petri dishes and larvae reared at $25\pm 1^{\circ}\text{C}$, 50-60% RH with a photoperiod of 16:8 (L:D). Larvae were reared in groups of 10 on artificial diet (Thompson and Peterson 1978). Newly molted third instar larvae (<24 h old) were used to standardize the age of the test larvae and were starved for 16-20 h before the bioassays.

Virus Preparation. Twenty mg of the stored TM Biocontrol-1[®] sample was weighed out and prepared as a dilute slurry with 20 ml of distilled water. Samples were stirred on a magnetic stirrer for 2 h and viral samples prepared in triplicate. Serial dilutions were made of the virus 1 week prior to inoculation, and stored at 4°C until they were used to infect the larvae in the bioassays. We chose concentrations to give approximately the LD₃₀, LD₃₅, LD₄₅, LD₇₀ and LD₇₅, as recommended by Robertson and Preisler (1992), for the accurate determination of the median lethal dose (LD₅₀). In our case the concentrations generally ranged from 16-200 polyhedral inclusion bodies (PIBs) per larva.

The USDA Forest Service determined the polyhedral inclusion bodies (PIBs) concentrations of the TM Biocontrol-1[®] samples prior to storage using the haemocytometer method (Kalmakoff 1980). These counts were available in the original bioassay data provided to us by Mr. R. Magelssen, USDA Forest Service (Hadfield and Magelssen 1995). However, as a check, we did haemocytometer counts of polyhedral inclusion bodies (PIB) on five randomly selected samples of the TM Biocontrol-1[®] samples to be tested: sample #2 from lot 2, #20 from lot 6a, #36 and #37 from lot 9b&c, respectively, and #43 from lot 10b (Table 1). The current PIBs/ml calculated at the start of this cooperative agreement were the same as the original PIB counts done at Corvallis, therefore, it was deemed unnecessary to recount the remaining 42 samples sent for bioassay. Serial dilutions were based on the original PIB counts.

A fresh OpNPV virus sample, to be used as a positive control, was prepared prior to the bioassay, by homogenizing in 5 ml of distilled water 100 Goose Lake strain Douglas-fir tussock moth larvae previously inoculated with OpNPV from lot 1 (sample #1) and reared until death. The fresh homogenate was filtered through cheesecloth, centrifuged once at 10 x g for 5 min and twice at 7100 x g for 20 min on an IEC centrifuge and resuspended in distilled water. PIB counts were done using a haemocytometer to quantify the PIB counts of the virus. The fresh OpNPV sample was produced 1-2 weeks prior to use, serial dilutions prepared and stored at 4°C until use in the bioassays. None of the serial viral dilutions were kept longer than 3 months.

Inoculation. We used a diet plug inoculation bioassay technique similar to that of Kaupp and Ebling (1990). Only those larvae that ate the whole diet plug within 24h were used in the experiment. The use of diet plug bioassay is superior to diet surface contamination because it eliminates any variation in the distribution of PIBs on the diet surface and eliminates any differential feeding rates and its consequences among the larvae. When the diet surface contamination technique is used there can be differences in larval feeding rate, number of PIBs consumed and interference among larvae reared in the same container due to crowding. In our tests, either 1 µl of each viral dilution, or distilled water in the control, was added to a small diet plug (3-4 mg) inside each well of a 24-well tissue culture plate. Previous experiments have shown that the diet plugs were large enough to fully absorb the 1µl of liquid and small enough that one, third instar larva could generally consume it in 24 hours.

For each of the TM Biocontrol-1[®] samples, as well as the fresh OpNPV sample (positive control), five virus concentrations were tested. Forty-eight larvae were used for each viral dilution and in the control. Bioassay of the five dilutions of each TM Biocontrol-1[®] sample, plus the fresh preparation of OpNPV, and an untreated or negative control (distilled water) were replicated three times. Each

replicate consisted of 1,488 larvae [five dilutions x 48 larvae x (5 virus samples plus 1 positive control) + 1 negative control. Each experimental run included five TM Biocontrol-1[®] samples, as well as the positive and negative controls and took 3-4 months to complete. Each experimental run required approximately 5,000 larvae. To insure that this number of larvae were available at the appropriate stage of development (i.e., 5,000 newly molted third instars over a 3-day period), 10,000 larvae had to be reared to select the 5,000 freshly molted third instars, which in turn had to be reared from about 30,000 eggs to insure sufficient numbers of insects in each replicate.

The replicates were done on three successive days. Freshly molted third instar Douglas-fir tussock moth larvae, starved for 16-20 h before the bioassay, were used in our bioassays. Immediately after inoculation of the diet plugs, one newly molted third instar (<24 h old) larva was placed into each well of a 24-well tissue culture plate to feed on the treated diet plug. Larvae were held in darkness for 24 h with the inoculated diet plug at 25±1°C, 50-60% RH. Only larvae that consumed the entire diet plug were used in the bioassays and were placed in individual cups (Solo P100, Solo Cup Co. Urbana, Illinois 61801-2895) with fresh, untreated diet and returned to the same growth chamber. Larvae, which 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. In addition, control and viral infected insects were reared in separate growth chambers, set at the same rearing conditions, to guard against viral transmission to the control insects. Diet was changed at least weekly or more often, if the diet dried out. Larvae were reared for 21 days post inoculation and the mortality recorded daily. Only larvae that died from NPV infection, as determined by gross pathology and verified by microscopic examination, were included in the analysis.

TM Biocontrol-1[®] Samples Selected for Testing. Of the 1,243 packages of TM Biocontrol-1[®] product stored at the USDA Forest Service facility in Corvallis, OR, 47 samples, each weighing 20g, were sent to the Pacific Forestry Centre in Victoria, BC for testing. The 47 samples sent to Victoria represented all 10 lots. The 47 samples were ranked in terms of time in storage, package size, and acre-dose (the amount of TM Biocontrol-1[®] in storage) available. It would have been too time-consuming, costly, and unnecessary to bioassay all 47 samples. Therefore, the 47 samples representing 17 different package types were grouped into three weight classes: small, medium and large. These were defined as: small <100 g/pkg (n=4), medium 100-999 g/pkg (n=9) and large >1000 g/pkg (n=6). Two of the package sizes were identical in both the medium and large package sizes (Table 1) and only one from each of these were bioassayed.

The bioassays were performed, initially, using larvae reared from field-collected Douglas-fir tussock moth egg masses from California (CA), Oregon (OR), Idaho (ID), and British Columbia (BC), and later exclusively with the Goose Lake laboratory colony (GL-1 strain) which has been maintained at the Pacific Forestry Centre since fall 1995.

In year 2 and year 3 of the efficacy evaluations the bioassays were done using the Goose Lake colony. Goose Lake larvae were chosen for the comparisons of package size, time in storage, and possible differences caused by processing by the different companies because the bioassays in year 1, using field collected *O. pseudotsugata* from CA, OR, ID and BC, indicated that geographic origin of the field strain of the *O. pseudotsugata* affected the LD₅₀ values (see Fig. 1 and 2). In addition, egg masses could be obtained more readily and reliably from laboratory rearing than from field collections, and the laboratory strain is also free of egg parasitism and the naturally occurring virus. The Goose Lake strain can also be “produced year round” in the laboratory with the appropriate cold storage to break

diapause, thus providing a more “flexible” larval hatching schedule, therefore, more bioassays can be done in a shorter time. Furthermore, the original efficacy data used for registration was also obtained from tests conducted with the Goose Lake strain.

Of the 47 samples of the stored TM Biocontrol-1[®], at least two of each package size were tested and more samples (up to six) were bioassayed from lots that have large acre-doses of the product in storage (Table 1). Two of the samples were tested twice in a total of 41 individual bioassays in seven large bioassay runs over 3 years.

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

Variation of LD₅₀ among TM Biocontrol-1[®] samples were also examined. There were no significant differences ($p < 0.05$) between LD₅₀ values at day 14 compared to day 21 for 39 of the viral samples tested. Therefore, we used the day 14 data in the analysis.

Experiments were designed to calculate the median lethal dose (LD₅₀). Concentrations were selected to give approximately 30%, 35%, 45%, 70% and 75% mortality, as recommended by Robertson and Preisler (1992). For each bioassay, the concentrations chosen were based on previous experiments as a best guess of what was appropriate. However, the samples were not all the same and

in rare cases the dilutions chosen did not give the desired mortality levels for the sample tested resulting in large error bars (at the $p < 0.05$ level) as in sample #15 (Fig. 2) where the upper value of the fiducial limit is 269.8. The bioassays are costly and time consuming, and since two or more samples per package size per lot were tested, not all TM Biocontrol-1[®] samples were bioassayed or certain bioassays were not always repeated when the results of the two replicates agreed or were not significantly different.

The efficacy of a fresh sample of OpNPV was compared among four different field strains of Douglas-fir tussock moth (CA, OR, ID and B.C.) and the GL-1 (Goose Lake) laboratory strain. In addition, when sufficient numbers of larvae of these field strains were available, samples of the stored TM Biocontrol-1[®] were also tested with the fresh OpNPV sample. TM Biocontrol-1[®] samples tested with the CA strain were lot 4 (samples #8 and #9), lot 7 (samples #23 and #24), and lot 8 (sample #33). Idaho strain was tested with lot 2 (sample #3), lot 6 (sample #21), and lot 10 (sample #44). The BC strain was tested with lot 3 (sample #5) and lot 5 (samples #14 and #15) (Fig. 2). The low number of larvae that emerged from the egg masses received from Oregon in the first year (1999) only permitted testing fresh OpNPV.

Results and Discussion

1) Potency of Stored TM Biocontrol-1[®] Against Different Field Strains. Results from challenging different strains of *O. pseudotsugata* with a fresh OpNPV sample indicate that there is some variation in LD₅₀ values among the different strains of *O. pseudotsugata* (Fig. 1). The three field strains from the US (CA, OR, and ID) have similar, relatively low LD₅₀ values when compared to the

BC strain. The LD₅₀ value of the BC strain is significantly higher, about three-fold higher with fresh OpNPV, even when overlap of the fiducial limits are compared (p<0.05). The LD₅₀ values for the Goose Lake strain challenged with fresh OpNPV gave intermediate values, i.e. it was higher than for the three field strains from the US but lower than the LD₅₀ value for the BC strain.

The mortality response of different strains of Douglas-fir tussock moth to TM Biocontrol-1[®] is shown in Figure 2. LD₅₀ values among TM Biocontrol-1[®] samples tested are similar to that of the fresh OpNPV sample for the CA and ID strains (fiducial limits overlap). For the ID strain, the LD₅₀ value for one TM Biocontrol-1[®] sample #21 of lot 6, was significantly higher (p<0.05) than for fresh OpNPV. Because different lots of TM Biocontrol-1[®] were used it is difficult to make comparisons and generalizations. Nevertheless, it is apparent that the three US strains (CA, OR, and ID) of *O. pseudotsugata* appear to be more susceptible to TM Biocontrol-1[®] than the Canadian strain from BC. Because the strain of *O. pseudotsugata* used in the bioassays affects LD₅₀ values, we decided to use the Goose Lake strain after year 1 to compare all TM Biocontrol-1[®] samples to assess the effects of time in storage, effects of package size and possible differences caused by processing by different companies, for the reasons mentioned earlier.

This is in agreement with what others have also reported that the strain of insects can affect the susceptibility to nucleopolyhedrovirus (NPV). Milks (1997) reported a 3.5-fold difference in susceptibility of 12 lines of cabbage looper, *Trichoplusia ni* (Hubner) (*T. ni*), to the singly embedded nucleopolyhedrovirus of *T. ni* (TnSNPV). Vail and Tebbets (1990) also reported a significantly higher LD₅₀ value (2.3 and 3.4-fold) for two of the four wild-type and two laboratory strains of the Indian meal moth, *Plodia interpunctella* (Hubner) to a granulosis virus. Similarly, Skatulla (1987) observed variations of mortalities ranging from 26.1% to 90.0% in gypsy moth, *Lymantria dispar* (L.), larvae

from different geographical regions of Europe when the larvae were given the same concentration of NPV in the bioassays. Reichelderfer and Benton (1974) have found a 5-fold difference in the LD₅₀ values of two strains of *Spodoptera frugiperda* (J.E. Smith), given the same doses of a nucleopolyhedrovirus. Similarly, Aratake (1973) found large variation in LD₅₀ values among various strains of *Bombyx mori* (L.) challenged with NPV. Most authors suggest that a genetic difference in the insect strains could account for the differences in susceptibility to viruses. However, none of the studies provided proof that this was the case. In our study, the BC strain was less susceptible to OpNPV than the strains from the US. It is possible that the US strains are more genetically similar than the BC strain (where Douglas-fir tussock moth is at the northern limit of its distribution), which could account for differences in susceptibility, however, because we did not test for this, we can only speculate.

Since there is variation in the median lethal dose values of different strains it is important and necessary to consider the target population when determining the acre-dose for suppression or control projects. The amount of TM Biocontrol-1[®] available is limited. If a target field strain of Douglas-fir tussock moth is tested to be more susceptible in new infested areas to be treated then it may be possible to use less TM Biocontrol-1[®] per acre and still achieve the desired levels of larval mortality and foliage protection. In anticipation of this, perhaps reduced amounts of the approved virus dosage could and should be tested for efficacy under field conditions in the US. Reduced dosages of TM Biocontrol-1[®] were tested successfully in BC and the results showed that the registered dose (2.5 x 10¹¹ PIBs/ha) can be reduced to about 1/3 of this and still get high larval mortality (95% at full dose, 91% at 1/3 dose) Otvos et al. (1987).

2) Effects of Package Size on Efficacy. Ten lots of TM Biocontrol-1[®] were produced between 1985 and 1995 and were stored in a total of 1,243 packages (Table 1). These 10 lots were packaged in different vacuum-sealed package sizes ranging from 31.7 g/package to 2,150 g/package. Within lots, generally one or two, and occasionally three, different package sizes were produced, resulting in a total of 19 different package “types” (Table 1). The number of packages in each of these 19 different package types varied from 1 to 483. Within the 19 different package “types”, two pairs of “types” were identical in weight (lots 4a and 5a [both 368 g/pkg], and lots 4b and 5b [both 1,840 g/pkg]); thus there were 17 different package sizes within the lots. These 17 package sizes were divided based on size into three groups for analysis: small (<100 g/pkg), medium (between 100-999 g/pkg), and large (>1,000 g/pkg). Among the packages included in the analysis, the small packages (n=4) ranged in weight from 31.7 - 58 g/pkg, the medium packages (n=8) from 124.7 - 580 g/pkg, and the large packages (n=5) 1,200 - 2,150 g/pkg (Table 1). Three additional package types, representing lot 6b, lot 8a, and lot 9a, with one package each (383, 690, and 84 acre-doses, respectively), were excluded from the analysis because of their small sizes. It was considered to be more important to test samples from lots with higher total acre-doses.

All but three of the comparisons of LD₅₀ values from the different package sizes were made with the results of the bioassays using the Goose Lake strain of *O. pseudotsugata*. In two of these three cases the comparison was done using larvae of the CA strain (parts of Fig. 3 and Fig. 6), and in the third case larvae from BC were used (part of Fig. 4). Because the comparisons of the different package sizes were done using the same strain even with the CA and BC strain all of these comparisons are valid.

LD₅₀ values of the samples bioassayed from the different package sizes but from the same lot are compared in Figures 3-7. Although two different (field and laboratory) strains of *O. pseudotsugata* were used in some of the comparisons (see Fig. 3, 4 and 7), the effects of package size were always compared between and among the bioassays using the same strain of *O. pseudotsugata*.

Medium and Large Packages. All but three of the comparisons were made with larvae of the Goose Lake strain of *O. pseudotsugata*. A comparison of LD₅₀ values between the medium and large packages in lot 4 (samples #8 and #9, respectively) showed no significant differences when the CA field strain of *O. pseudotsugata* larvae were used for the comparison (Fig. 3). Similarly, there was no significant difference in LD₅₀ for medium (#8, #10) and large packages (#9, #11) when the comparisons were made with the Goose Lake strain of *O. pseudotsugata* (Fig. 3). The LD₅₀ values for both samples 8 and 9 tested with the field strain from California were significantly lower (i.e. this strain was more susceptible) than with the Goose Lake strain. For lot 5 (Fig. 4), there was also no significant difference when a medium package (sample #14) was compared to a large (#15) using the BC strain. Similarly, there was no significant difference for a medium package (#14) compared to large packages (#15 and #17) tested with the Goose Lake strain. However, the LD₅₀ for the medium package (represented by #16) was significantly lower ($p < 0.05$) than the LD₅₀ value for the other medium package (#14) and both large packages (#15 and #17) using the Goose Lake strain (Fig. 4). We have no explanation for this difference, except that TM Biocontrol-1[®] in this package (sample #16) may have degraded faster or was already less effective at the time of packaging than in the other packages. However, there could be other explanations for this. It may be interesting to note that there was no significant difference between the medium size packages when sample #14 (bioassayed with the BC

strain *O. pseudotsugata*) is compared with sample #14 (bioassayed with GL) or sample #16 also bioassayed with GL. However, there is significant difference between samples #14 and #16 even though both were tested against GL strain (Fig. 4).

In lot 10 (Fig. 5), the LD₅₀ values obtained with the Goose Lake strain for a medium package (sample #44) was not significantly different from large packages (#45 and #47). Similarly, the LD₅₀ value for a medium package (#42) was not significantly different from the LD₅₀ values obtained from large packages (#45 and #47). However, the LD₅₀ value of medium package (#42) is significantly higher ($p < 0.05$) than medium package (#44). Again, we have no explanation for this difference.

Small and Medium Packages. In lot 7 (Fig. 6), there was no significant difference in LD₅₀ values in the small package (sample #23) compared to the medium package (#24) when bioassays were done with CA strain. Similarly, in bioassays done with the Goose Lake strain, there was no significant difference in LD₅₀ between small packages (#23 and #27) compared to medium packages (#24, #25 and #28). However, medium package (sample #26) had a significantly higher LD₅₀ value ($p < 0.05$) than small packages (#23 and #27) and was also higher than LD₅₀ values obtained for medium packages (#25 and #28) but not for medium package (#24) with the Goose Lake strain of *O. pseudotsugata* (Fig. 6).

An interesting pattern can be noted here. There were no significant differences between the LD₅₀ values of the small package (sample # 23) and the medium package (sample #24) compared with either the CA or Goose Lake strain, but the LD₅₀ was significantly lower ($p < 0.05$) in the bioassay done with the CA strain. This is expected since the CA strain appeared to be the most susceptible to TM Biocontrol-1[®] among the *O. pseudotsugata* field strains tested. This high susceptibility of the field strain

of the *O. pseudotsugata* from California could be due in part to the presence of another virus, indicated by a preliminary analysis. This is currently being investigated.

For lot 9 (Fig. 7), the LD₅₀ of the small package (sample #36) was not different from the medium package (#37) in GL0 bioassay, which was the first bioassay conducted using the Goose Lake strain. The LD₅₀ values for Goose Lake in the first experiment (GL0), done in the first year of the cooperative project, were generally lower than in the following seven large experiments (GL1-GL7). This may be due to a healthier colony and to some minor adjustment to the *O. pseudotsugata* diet after the first bioassay. There was also no significant difference between small packages (samples # 38 and #40) and medium package (sample #39) (Fig. 7). Similarly, there was no significant difference in the LD₅₀ values between the medium packages (samples #39 and #41). However, the LD₅₀ value of medium package (sample # 41) was significantly lower ($p < 0.05$) than the LD₅₀ value for the small packages (sample #38 and #40) but not different from the LD₅₀ value of medium package (sample #39). No comparison could be made between the samples of the package sizes in lots 6 and 8 because lot 8 had only one package size (medium) and samples from lot 6 were only sent from the small package size.

The results from the comparisons of LD₅₀ values of different package sizes are summarized in Table 2. Fourteen comparisons were made between LD₅₀ values obtained from testing samples from small and medium packages. Of these 14, 10 (or 71%) showed no significant difference in the LD₅₀ values while the remaining four did. Fourteen comparisons were also made between LD₅₀ values of medium and large packages. Of these 14, 12 (or 85.7%) showed no difference in the LD₅₀ values. Overall, of the 28 different comparisons of LD₅₀ values obtained for samples from small to medium and medium to large, 22 (or 78.6%) showed no difference in the LD₅₀ values.

Three samples (#16, #26 and #41, all from medium package sizes) (Table 2) were involved in comparisons of package sizes where significant differences were observed. The LD₅₀ in samples #16 and #41 was lower (Figs. 4 and 7, respectively) while in sample #26 it was higher (Fig. 6) than the LD₅₀ values of their comparison samples. Because each of these samples was involved in two comparisons where there were significant differences, these three samples are suspect, i.e. probably do not represent the whole lot, but rather only the sub-lot from which they came

There were no significant differences among the LD₅₀ values of the samples from the large package sizes.

LD₅₀ values of TM Biocontrol-1[®] samples from different package sizes were compared in 24 different bioassays using the Goose Lake strain. Of these, five came from small packages (samples 23, 27, 36, 38 and 40), 13 came from medium packages (8, 10, 14, 16, 24, 25, 26, 28, 37, 39, 41, 42 and 44), and the remaining six came from large packages (9, 11, 15, 17, 45 and 47). Samples #16, #26, and #41 were the only samples showing differences in the LD₅₀ values (Table 2). These three samples represent only 12.5% of the data with 21 of the 24 samples compared or 87.5% showing no differences when compared.

There were 28 different comparisons made with the 24 samples (Table 2). LD₅₀ values of medium packages (samples #16 [Fig. 4] and #26 [Fig. 6]) do not agree with the LD₅₀ values for other medium packages in their respective lots. Sample 16 had the lowest while sample 26 had the highest LD₅₀ value among the medium size packages, i.e. they were “outliers”. However, these values may not be accurate due to experimental error or individual package variation within the same package size in a lot. If the comparisons using these two samples (#16 and #26) are excluded from the overall

comparisons of the effect of package sizes, then of the 28 comparisons made among package sizes, 26, (or 92.9%) show no differences in LD₅₀ values (Table 2).

In summary, the results of the bioassays on the effects of package sizes on virus viability indicate that when comparisons are made with the same strain of *O. pseudotsugata*, there was no difference in LD₅₀ values among samples from different package sizes from the same lot. The GL-1 strain was used to bioassay 39 of the 47 stored TM Biocontrol-1[®] samples.

3) Effects of time in storage on virus efficacy (shelf-life). Because package size of the stored virus did not affect the efficacy within a lot, the bioassay data of each sample in a lot were combined and analyzed with SAS PROC PROBIT to determine the effects of length of storage. The number of samples bioassayed from each lot is given in Table 1. This produced one LD₅₀ value for each of the lots. All fresh virus data were also combined in the same manner for an overall LD₅₀ value for a fresh OpNPV sample (Table 3 and Fig. 8).

There is no significant difference (there is an overlap of 95% fiducial limits) in the LD₅₀ values for eight lots (lots 2, 3, 4, 5, 6, 7, 9, and 10). The LD₅₀ for lot 1 is significantly higher ($p < 0.05$) than all lots except for lot 3 and is significantly lower ($p < 0.05$) than lot 8. The LD₅₀ for lot 8 is significantly higher ($p < 0.05$) than all other lots. These results indicate that there is a loss of potency of TM Biocontrol-1[®] over time, but it does not appear to be clearly and directly related to the time in storage (Table 3 and Fig 9).

Potency Ratios. Another way to analyze the data is to calculate the potency ratios. The relative potency provides a convenient comparison of the differences among samples. The relative potency of two stimuli is defined as the ratio of equally effective doses (Finney 1971). Potency ratios were calculated, for bioassays in which we used the Goose Lake strain, by comparing the overall LD₅₀ of all

fresh OpNPV sample (potency ratio = 1) to the overall lot LD₅₀ for each of the 10 lots of the stored TM Biocontrol-1[®] samples. Comparisons of the relative potency ratios also showed that there is a loss of potency with time in storage, but again this did not appear to be directly related to the time in storage. Results indicate that each of the 10 lots is less potent than a fresh sample of OpNPV (Table 3 and Fig. 9). However, seven of the 10 lots have potency ratios of 0.7 or 0.8 (Table 3). The three other lots (lots 1, 3 and 8) had the lowest potency ratios (0.5, 0.6 and 0.3) and had been in storage for 15, 14 and 9 years, respectively (Table 3). It should be noted, however, that besides lot 3 (with a potency ratio of 0.5), lots 2, 4 and 5 have also been in storage for 14 years but their potency ratios were 0.7, 0.7 and 0.8, respectively. The low potency ratio (0.3) for lot 8 could be an anomaly.

These results suggest that the LD₅₀ values of the lots show a decrease in efficacy of the stored product, but this did not appear to be directly correlated with the length of time in storage. If length of storage had directly affected the efficacy in a linear fashion, one would have expected an increase in the LD₅₀ values with increased time in storage. However, this was not the case, but it should be noted that at the time the bioassays were conducted, all lots had been in storage for 5 years or more. Processing the virus infected dead larvae for the product by the three companies could have had additional effects that may cloud the effects that time in storage had on the efficacy of the stored product. These are only speculations based on visual impressions of the coarseness of the lots of the product processed by the different companies and whether additional grinding was necessary to extract viral DNA from the various lots, and that the number of PIBs/g in the 10 lots were not the same (Reed et al., unpublished data).

When the combined LD₅₀ values for each of the lots are compared to the combined LD₅₀ value for a fresh sample of OpNPV, there is no overlap of the 95% fiducial limits between the fresh virus and

any of the 10 lots. All 10 lots have significantly higher LD₅₀ values ($p < 0.05$) than the fresh OpNPV (Fig. 8). These results indicate that there was a loss of efficacy of TM Biocontrol-1[®] in storage for all lots when compared to a fresh OpNPV sample. Potency ratios also indicate that all lots are equally less potent now (after 5 to 15 years in storage) than a fresh sample of OpNPV. Seven of the lots have retained at least a potency of 0.7, suggesting that they have maintained most of their efficacy compared to a fresh OpNPV sample. Three of these lots (lots 5, 6, and 7) have potency ratios of 0.8 when compared to fresh OpNPV, but this difference from the fresh OpNPV was not significant. Lot 1 and lot 8 had the largest reduction in efficacy. However, this decrease in efficacy does not appear to be directly related to time in storage since lot 8 has only been stored for 9 years, yet had the greatest loss in efficacy (0.3), and lot 1 with the longest time in storage had the second lowest (0.5) loss of efficacy.

It should be noted, however, that when the TM Biocontrol-1[®] was first produced, efficacy tests with a fresh batch of OpNPV were not performed prior to storage, and only one advance sample from each lot was bioassayed. At that time the efficacy of the lots were not compared to a fresh sample of OpNPV. It is possible that the original lots were not as effective as a fresh sample of OpNPV even before the lots were vacuum-packed for storage. Our comparisons only reports the efficacy as compared to a fresh sample and do not necessarily prove that the decrease in efficacy of the different lots, i.e. lost potency from their original state, is all due to the storage, although it is very likely and expected that this happened. What is not clear is whether loss of potency is directly related to length of storage. Loss of potency of virus over time has been reported by other researchers (Cunningham 1970, Martignoni 1978, Lewis and Rollinson 1978, Kaupp and Ebling 1993, Tamez-Guerra *et al.* 2002). The lower potency ratios for some of the lots may have been partly due to processing by the different organizations or companies. Generally, there is a “learning curve” for most activities and producing viral

infected insects and their processing into a powder is not an exception. Lot 1 was the first lot to be produced on an experimental basis and was not subjected to the more refined processing procedures used for subsequent lots processed under contract. As a result, it was coarser and contained fragments of body parts and hairs and required additional grinding to extract the viral DNA.

During bioassaying of the advance samples at Corvallis, lot 6 was shown to be contaminated with CPV. Our DNA analyses of samples of the stored product samples confirmed that lot 6 indeed had CPV. In addition, we also found CPV contamination in sublots 4a and 5b. There is a synergistic effect between the NPV and CPV viruses (Tanada 1956). This synergistic effect may explain the lower LD₅₀ values (requiring fewer PIBs) in the lots contaminated with CPV (lots 4, 5 and 6). In addition to this, another confounding factor may be the coarseness of the various lots. The possibility of a reduction in LD₅₀ values in lots contaminated with CPV could be checked in the future, should it be required, by comparing the LD₅₀ values of CPV-contaminated samples against those that are not contaminated (4b versus 4a, 5b versus 5a). However, should this be the case, it does not explain why lot 7, which is free of CPV contamination, had a potency ratio as high as lots 5 and 6 (0.8), which were contaminated with CPV. Some of these confounding factors could have contributed to clouding the correlation between length of time in storage and loss of potency of the virus in TM Biocontrol-1[®].

Other studies with the same stored tussock moth virus show similar loss of efficacy with storage. Martignoni (1978), using the diet surface contamination technique, reported a shelf-life of 5 years for OpNPV when the virus was stored in a cool, dry place (the exact temperature was not specified). However, it should be noted that the virus samples used in this cooperative project were stored at -10°C. Similarly, Kaupp and Ebling (1993), using a diet plug inoculation bioassay with second instar of the closely related whitemarked tussock moth (*Orgyia leucostigma* (J.E. Smith)) larvae and Virtuss[®]

(the same virus as OpNPV, but produced in *O. leucostigma*), have also concluded that potency of the virus decreased in storage at 4°C. A 46% loss in infectivity was observed after 2 years in storage at 4 °C and Virtuss[®] stored up to 10 years at 4 °C revealed a 25-fold decrease in infectivity.

This decreasing potency of virus with storage was also reported by other authors with different insects. Cunningham (1970), using balsam fir (*Abies balsamea* [L.] Mill.) foliage dipped in virus suspensions, found that eastern hemlock looper (*Lambdina fiscellaria fiscellaria* (Guenee)) NPV, stored for 6 years at 4°C, showed a 200-fold loss of pathogenicity to third instar larvae when compared to freshly produced batch of the same virus. Lewis and Rollinson (1978) found a similar decrease with stored gypsy moth (*L. dispar* (L.)) NPV. These authors reported that in diet contamination bioassays using second instar gypsy moth larvae, suspensions of NPV retained their potency for 5 years under refrigeration (4°C), for 2 years at room temperature, 1 year as air-dried powder stored at 4°C, and 6 months as air-dried powder stored at 38°C.

Neilson and Elgee (1960), investigating the effect of storage on virulence of NPV on second and third instar larvae of European spruce sawfly (*Gilipina hercyniae* [Hartig]), reported similar results. Using foliage contaminated with virus suspension, they reported that when virus was stored at 4.5°C, loss of potency occurred after 5 years with the greatest change in virulence at 9 years in storage and total inactivation after 12 years of storage. All of the above studies reported some loss in activity of the virus with storage.

Our study examined the change in efficacy of virus that was stored for longer period of time (5 to 15 years) at the coldest temperature examined to date (-10 °C). Our results indicate that there has been a loss in potency but this loss does not appear to be directly related to the length of time in storage at -10°C. Because TM Biocontrol-1[®] was stored at much lower temperature (-10°C) than those

reported by others earlier above we would expect lower loss of potency in our samples. Perhaps storing at even lower temperature (-20°C) may further reduce the rate of loss of the efficacy of stored virus products.

4) Differences in LD₅₀ and Physical Properties of TM Biocontrol-1® Due to Company Processing the Virus Killed Insects. The first lot (lot 1) produced, was processed on an experimental basis and was not included in this comparison. Three private companies were contracted to process the virus-killed Douglas-fir tussock moth larvae in the other nine lots. Lots 2-5 were processed by Reuter, lots 6-7 by Espro and lots 8-10 by Crop Genetics. The LD₅₀ data and the potency ratios (Table 3, Fig. 8 and 9) show no obvious differences in LD₅₀ values of the samples from the various lots among the four companies processing the virus-killed larvae. Although there were differences in the physical properties of the product in the different lots, this may be partly due to the company doing the processing, or more likely it is a reflection of the learning curve and gain in experience over time by the processing companies.

The description of the coarseness of the samples from the 10 lots is based on the observation by Reed et al. (unpublished data).

Lots produced by Reuter Labs, Inc. (lots 2-5) were a more homogenous mixture. Large insect debris was not readily seen and the powdered component had a finer texture than in lot 1. Samples from lots 2-5 did not require additional grinding with mortar and pestle to facilitate extraction of the viral PIBs, although additional grinding did aid in the recovery of PIBs.

Lots 6-10, processed by Espro / Crop Genetics Inc., had the finest texture of all the lots. The texture among these lots was more consistent than the lots produced by either Reuter Labs or USDA

Agricultural Services. These lots were finely ground and insect debris was not readily observable. These lots required no extra grinding to aid in PIB recovery.

During the process of extracting viral DNA from the different samples of the various lots of TM Biocontrol-1[®], it was observed that the concentration of DNA varied, sometimes substantially, even though a consistent amount of product was weighted out at the start. The recovery of DNA is not an effective way to quantify viral concentrations in samples of TM BioControl-1 due to losses of viral DNA during the extraction and purification process. However, the differences in DNA recovered from lot to lot are, in some cases, larger than what could be accounted for as experimental loss. This suggests that the quantity of PIBs, and ultimately virions in the product processed by the various companies, is not uniform from lot to lot. This may explain, or at least contribute to explaining, the confounding effects of the various factors that caused loss of efficacy (degradation) of the stored product to not appear directly related to the length of time in storage.

Lot 1 may only have a high LD₅₀ value and a low potency, of 0.5, because it was the first lot produced and contained a larger amount of insect debris. Also, there could have been some initial problems in working out the protocols for the processing of the larvae and cleaning up the dead insect debris in the samples after homogenization. There is a learning curve with developing and perfecting all new products. An examination of this lot indicated that it contained more debris than other lots, which may have resulted from cruder processing. This could also have affected the shelf life.

Lot 1 had a high LD₅₀ value (77.1 PIBs) and a potency ratio of 0.5 while lot 8 had the highest LD₅₀ value (113.8 PIBs) and a potency ratio of 0.3. Lot 3 also had a relatively high LD₅₀ value (60.7 PIBs) and a potency ratio of 0.6. The other seven lots were all in the same range of potency ratio values (0.7-0.8).

However, there seems to be no obvious reason why lot 8, stored for only 9 years, failed so badly. It was the first lot processed by Crop Genetics, so this may have had an effect. The first lots or production by a new agency usually have problem(s), and it may take a while to “work out the bugs”. The Government contracts with the processors set out standards that had to be met and we can only assume that this was done. Not enough is known about the handling of the frozen dead larvae and the processing to reach a definite conclusion concerning how the different processors and their processing techniques affected the final efficacy of the TM Biocontrol-1[®] lots.

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Table 1. Samples of the 10 lots of stored TM Biocontrol-1[®] bioassayed using third instar Douglas-fir tussock moth larvae (GL-1 strain) in diet plug experiments at Pacific Forestry Centre in Victoria, BC

Lot #	Processed by ^a	Package size ^b types	g/pkg	# pkgs	Total acre-dose (determined at storage)	TM Biocontrol-1 [®] sample # bioassayed ^c	Date stored
1	A	Large	1200	1	480	1(2X)	1985
2	B	Large	2150	18	18000	2(2X) 3 4	1986
3	B	Large	1830	13	13000	5 6 7	1986
4a ^d	B	Medium	368	124	24800	8 10 12	1986
4b ^d	B	Large	1840	33	33000	9 11 13	1986
5a ^d	B	Medium	368	54	10800	14 16 18	1986
5b ^d	B	Large	1840	26	26000	15 17 19	1986
6a	C	Small	47	170	17000	20 21 22	1989
6b ^e	C	Medium	180	1	383	None was sent	1989
7a	C	Small	31.7	483	48300	23 27 29	1990
7b	C	Medium	158.5	100	50000	24 26 30	1990
7c	C	Medium	317	50	50000	25 28 31	1990
8a ^f	D	Medium	202	1	690	32 ^f	1991
8b	D	Medium	293	68	68000	33 34 35	1991
9a ^e	D	Small	49	1	84	None was sent	1993
9b	D	Small	58	57	5700	36 38 40	1993
9c	D	Medium	580	9	9000	37 39 41	1993
10a	D	Medium	124.7	10	1000	42 44 46	1995
10b	D	Large	1247	24	24000	43 45 47	1995
Total		17		1243	400,237 ^g	39	

^a A-USDA Agricultural Research Service; B-Reuter; C-Espro; D-Crop Genetics

^b Package sizes were defined as: small <100g/pkg (n=4, each of different sizes), medium 100-999g/pkg (8 of 9 are of different sizes), large >1000g/pkg (5 of 6 are of different sizes).

^c Highlighted samples were not bioassayed because the LD₅₀ of the other two samples from the same lot were not significantly different. Therefore, bioassaying the third sample was deemed unnecessary.

^d Note that lots 4a and 5a were stored in identical sized packages (368 g/pkg), as were lots 4b and 5b (1,840 g/pkg).

^e Lots 6b and 9a had only one package each (383 and 84 acre-doses, respectively); therefore, no sample was taken from these for efficacy evaluation after storage.

^f Sample #32 came from lot 8a and represented only 690 acre-doses; therefore, it was not included in the analysis even though it was bioassayed.

^g The total acre-dose in 1995 was 400,237.

Table 2. Summary of the bioassay results comparing LD₅₀ values obtained by testing samples from the various package sizes in the same lot of stored TM Biocontrol-1[®]

Lot #	Douglas-fir tussock moth strain	Samples compared	Significant difference	Comments
4	CA	8 M and 9 L	No	See Fig. 3
	GL	8 M and 9 L	No	See Fig. 3
	GL	8 M and 11 L	No	See Fig. 3
	GL	10 M and 9 L	No	See Fig. 3
	GL	10 M and 11 L	No	See Fig. 3
5	BC	14 M and 15 L	No	But high 95% F.I. for BC 15 (Fig. 4)
	GL	14 M and 15 L	No	See Fig. 4
	GL	14 M and 17 L	No	See Fig. 4
	GL	16 M and 15 L	Yes	16 M is lower than 15 L; see Fig. 4
	GL	16 M and 17 L	Yes	16 M is lower than 17 L; see Fig. 4
7	CA	23 S and 24 M	No	See Fig. 6
	GL	23 S and 24 M	No	See Fig. 6
	GL	23 S and 25 M	No	See Fig. 6
	GL	23 S and 28 M	No	See Fig. 6
	GL	23 S and 26 M	Yes	26 M is higher than 23 S; see Fig. 6
	GL	27 S and 24 M	No	See Fig. 6
	GL	27 S and 25 M	No	See Fig. 6
	GL	27 S and 28 M	No	See Fig. 6
	GL	27 S and 26 M	Yes	26 M is higher than 23 S and 27S; see Fig. 6
9	GL0	36 S and 37 M	No	See Fig. 6
	GL	38 S and 39 M	No	See Fig. 6
	GL	38 S and 41 M	Yes	41M is lower than 38S; see Fig. 6
	GL	40 S and 39 M	No	See Fig. 6
	GL	40 S and 41 M	Yes	41 M is lower than 40S; see Fig. 6
10	GL	42 M and 45 L	No	See Fig. 5
	GL	42 M and 47 L	No	See Fig. 5
	GL	44 M and 45 L	No	See Fig. 5
	GL	44 M and 47 L	No	See Fig. 5

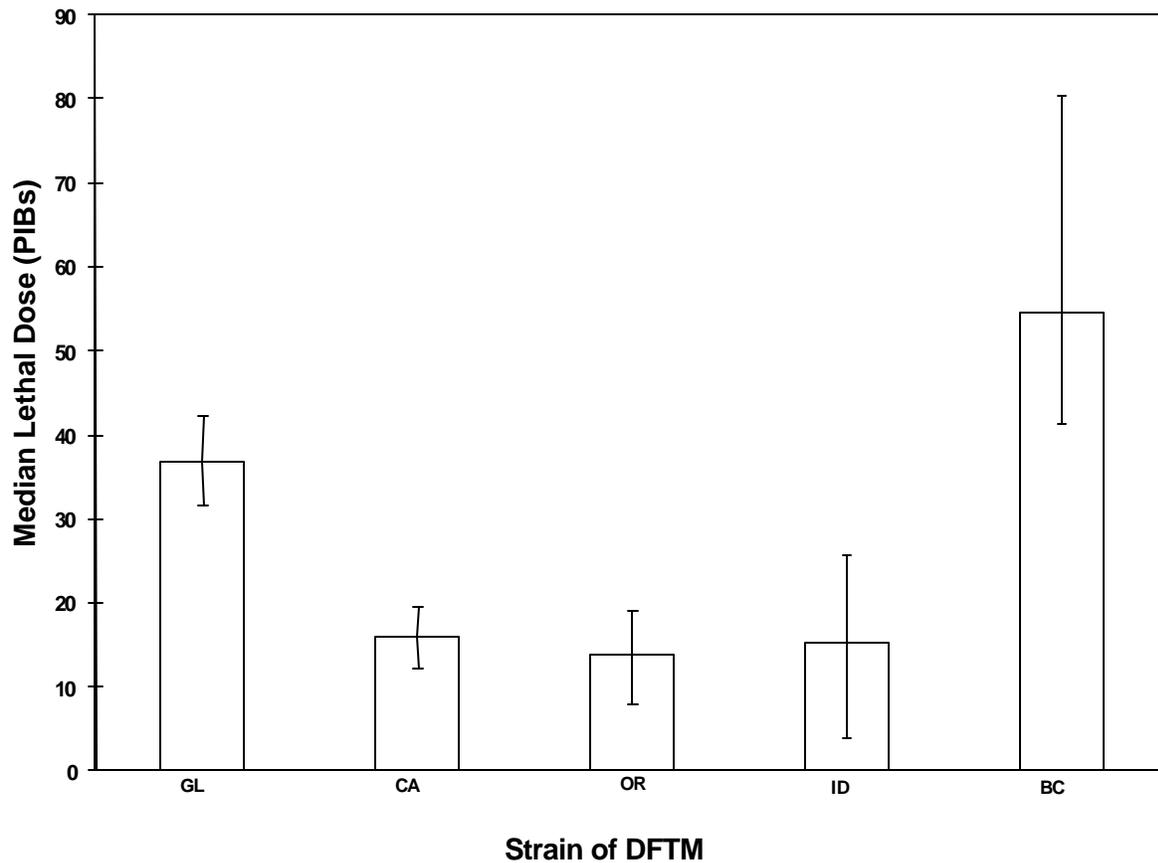
Table 3. Comparison of LD₅₀ values, and potency ratios to fresh OpNPV, of the different lots of TM Biocontrol-1[®] samples stored for various lengths of time at -10°C^a at Corvallis, OR

Sample	Years in storage (to 2000)	LD ₅₀ (PIBs)	Potency ratio ^b
Lot 2	14	49.6	0.74
Lot 3	14	60.7	0.61
Lot 4	14	52.1	0.71
Lot 5	14	45.7	0.81
Lot 6	11	44.5	0.83
Lot 7	10	46.1	0.80
Lot 8	9	113.8	0.32
Lot 9	7	55.9	0.66
Lot 10	5	54.0	0.68
Fresh OpNPV	0	36.9	1.00

^a Only data from bioassays done with the Goose Lake strain were used to calculate potency ratios.

^b Potency ratio is the ratio of the LD₅₀ value for the fresh sample compared to the LD₅₀ value of the TM Biocontrol-1[®] samples of that particular lot.

Fig. 1. Median lethal dose (LD₅₀) values for field and laboratory strains of third instar Douglas-fir tussock moth larvae challenged with fresh OpNPV.

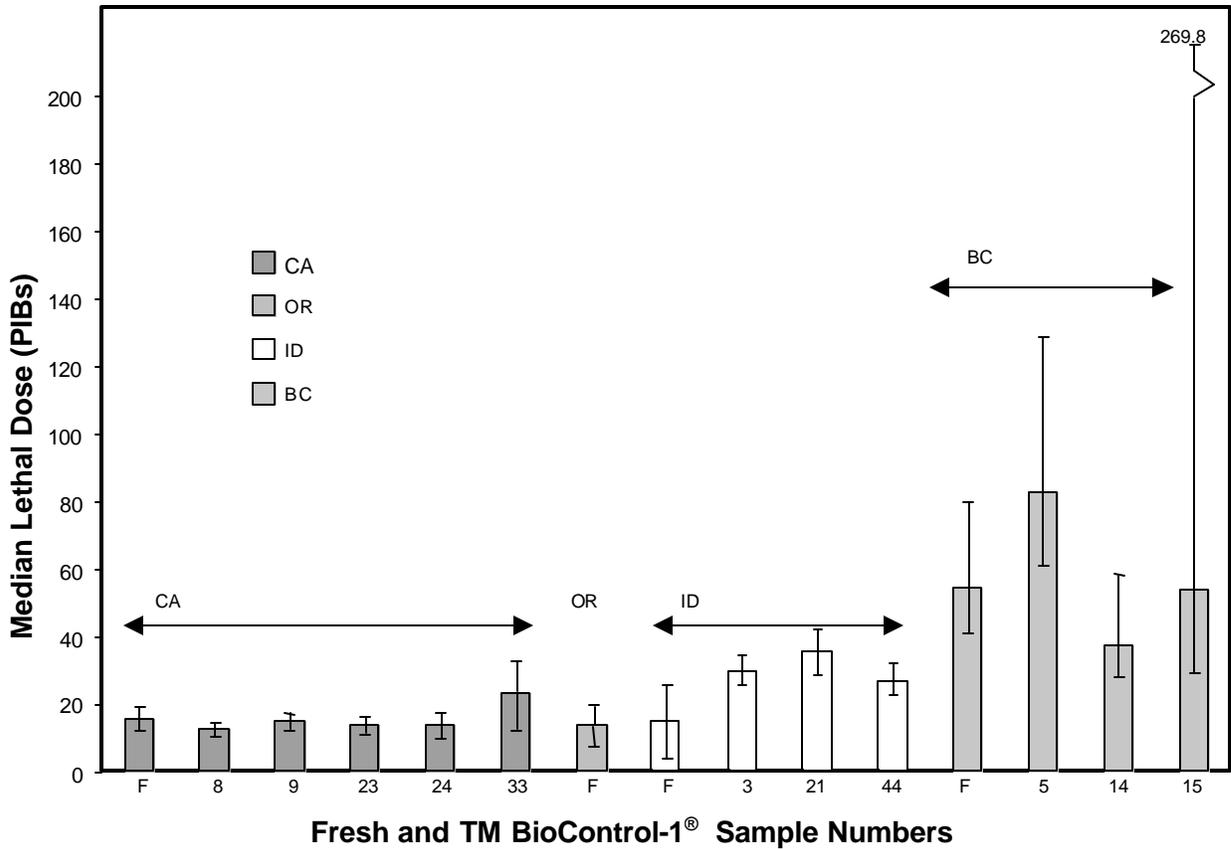


^a GL = Goose Lake laboratory colony.

Field strains: CA = California, OR = Oregon, ID = Idaho, BC = British Columbia.

Bars indicate 95% Fiducial Limits

Fig. 2. Comparison of the median lethal dose (LD₅₀) values, in PIBs per third instar larvae of the different field strains of Douglas-fir tussock moth challenged with a fresh sample of OpNPV (F) or with stored TM Biocontrol-1[®].^a



^a Douglas-fir tussock moth strains tested: CA = California, OR = Oregon, ID = Idaho, BC = British Columbia

CA with stored TM Biocontrol-1[®] samples 8 and 9 (lot 4), 23 and 24 (lot 7), and 33 (lot 8).

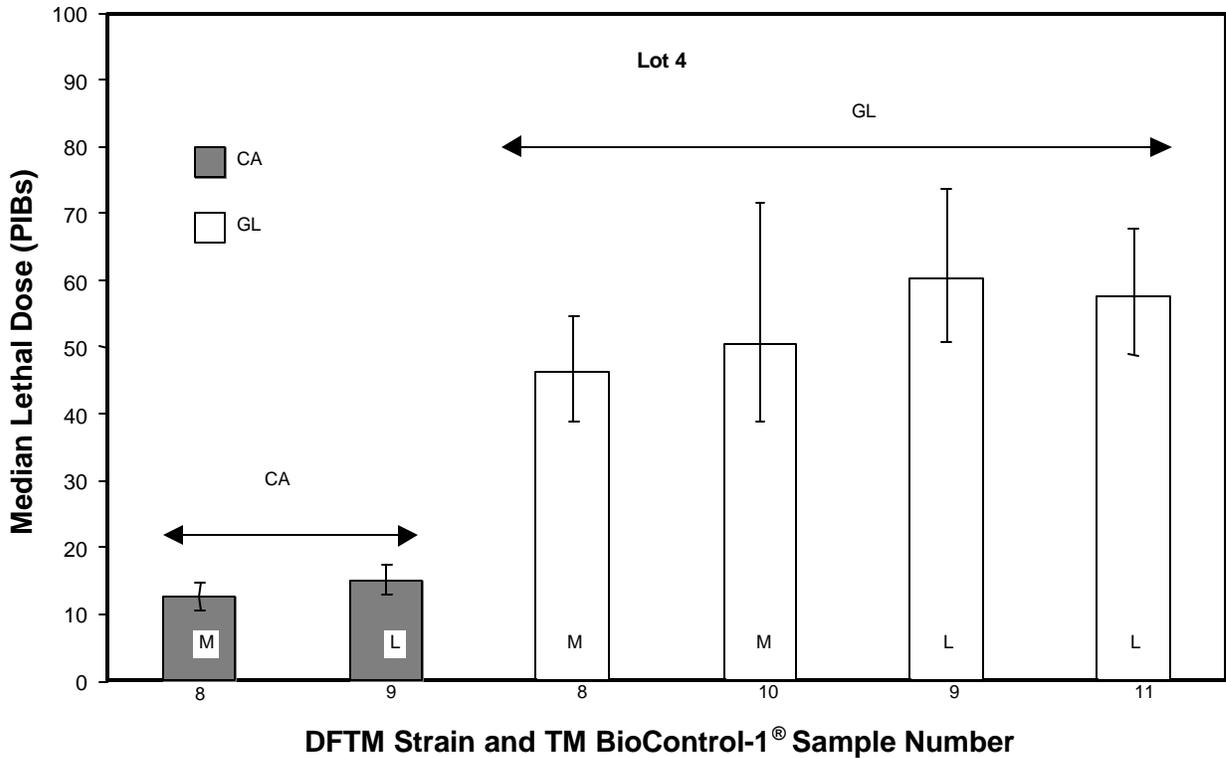
ID with TM Biocontrol-1[®] sample 3 (lot 2), 21 (lot 6), and 44 (lot 10).

BC with TM Biocontrol-1[®] samples 5 (lot 3), 14, and 15 (lot 5).

All three of these strains were also challenged with freshly produced OpNPV.

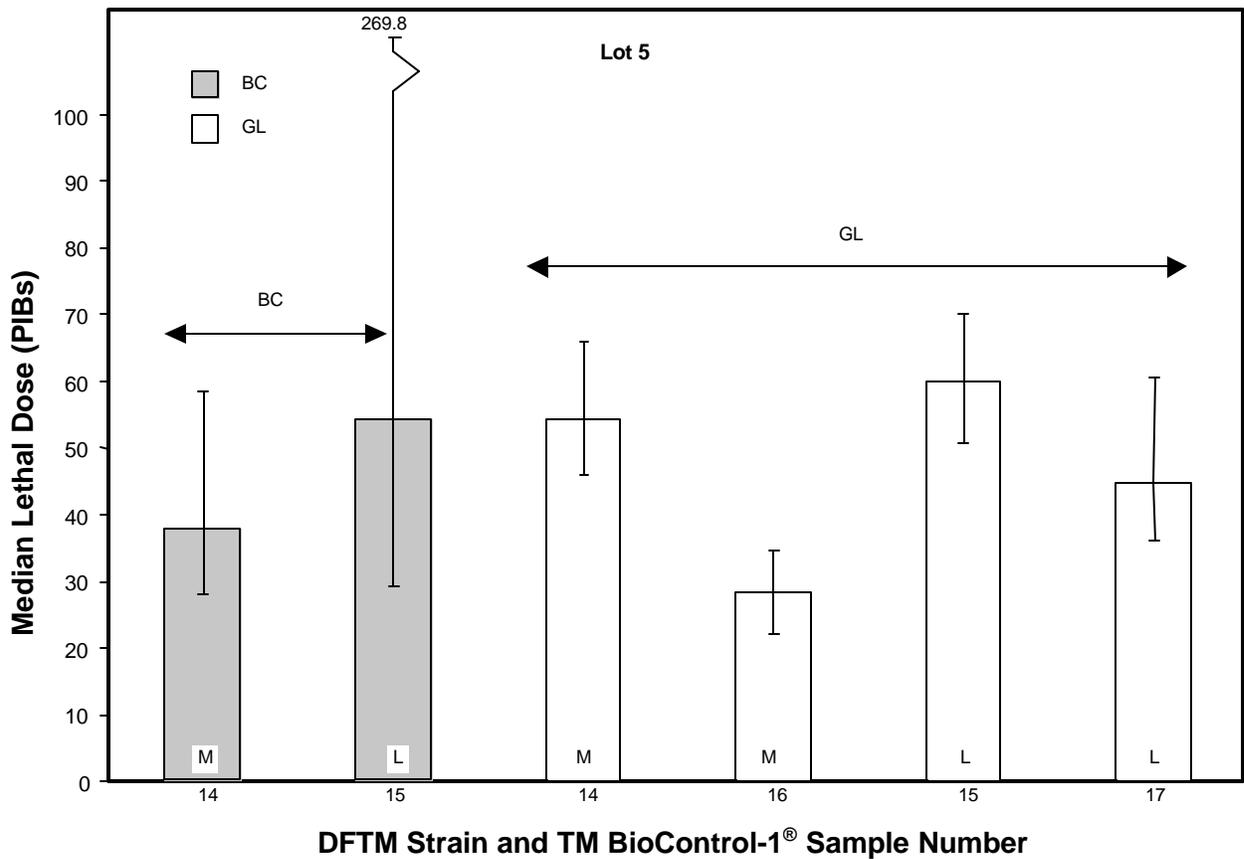
OR was tested only with fresh OpNPV (F).

Fig. 3. Comparison of median lethal dose (LD₅₀) values, in PIBs per third instar Douglas-fir tussock moth larvae, challenged by samples from the different package sizes in lot 4 of the stored TM Biocontrol-1[®].^a



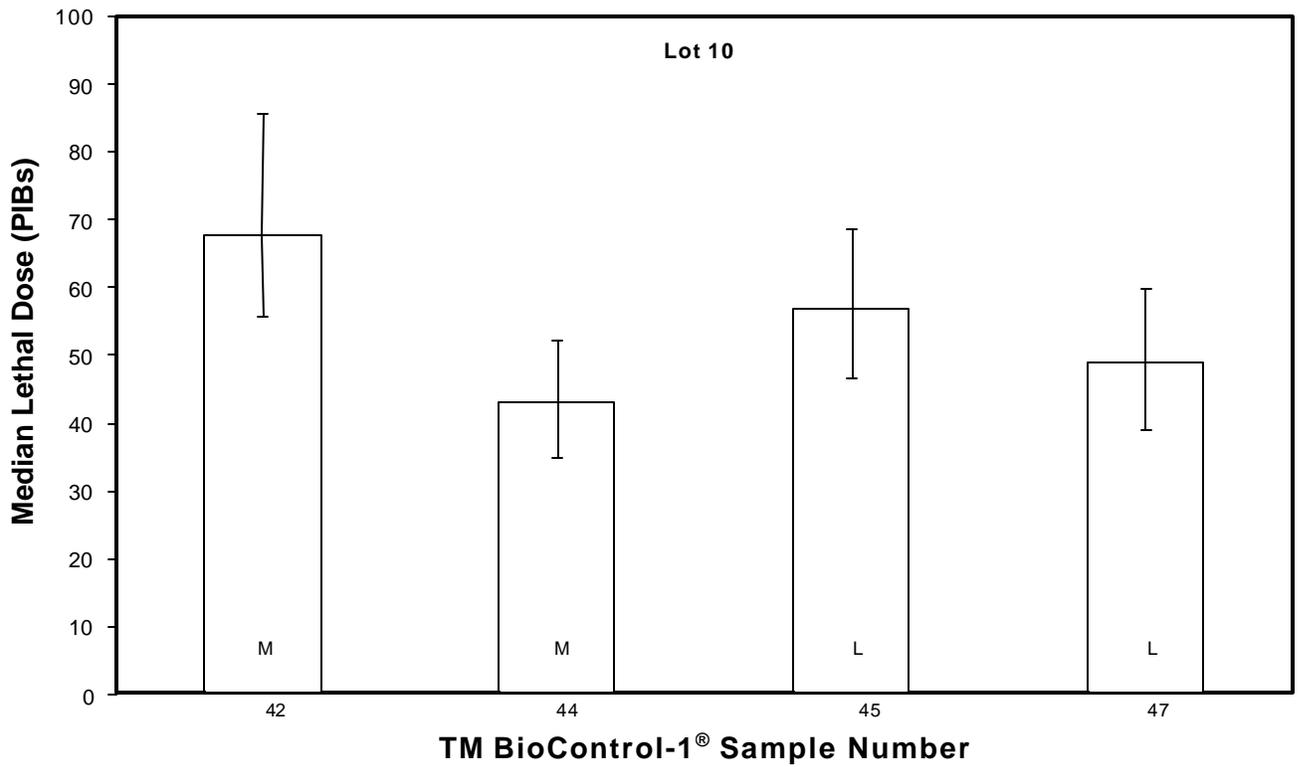
^a Strains used: CA = California field strain, and GL = Goose Lake laboratory strain. Package sizes were M = medium 100-999 g/pkg., and L = large > 1000 g/pkg. Numbers under the bar graphs denote sample numbers of the stored TM Biocontrol-1[®] virus product.

Fig. 4. Comparison of median lethal dose (LD₅₀) values, in PIBs per third instar Douglas-fir tussock moth larvae, challenged by samples from the different package sizes in lot 5 of the stored TM Biocontrol-1®.^a



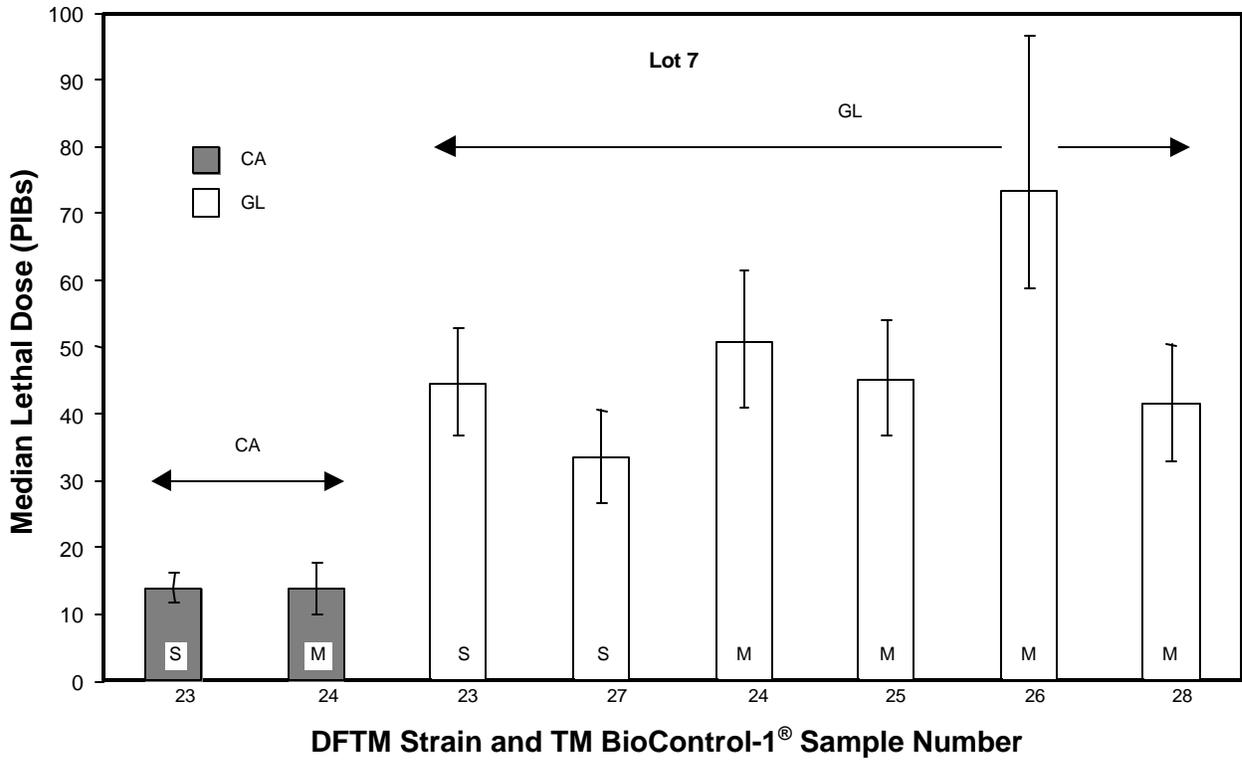
^a Strains used: BC = British Columbia field strain, and GL = Goose Lake laboratory strain. Package sizes were M = medium 100-999 g/pkg., and L = large > 1000 g/pkg. Numbers under the bar graphs denote sample numbers.

Fig. 5. Comparison of LD₅₀ values, in PIBs per third instar Douglas-fir tussock moth larvae, challenged by samples from the different package sizes in lot 10 of the stored TM Biocontrol-1[®].^a



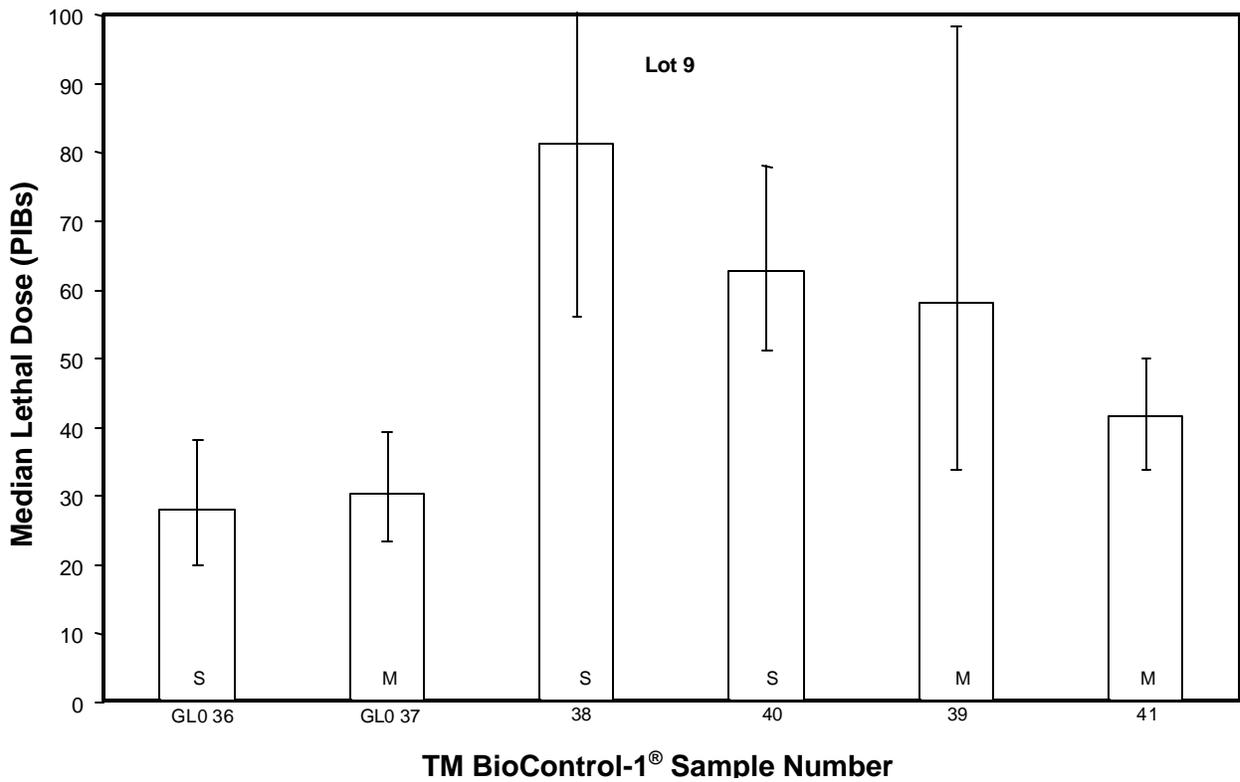
^a Strain used: GL = Goose Lake laboratory strain. Package sizes were M = medium 100-999 g/pkg., and L = large > 1000 g/pkg. Numbers under the bar graphs denote sample numbers.

Fig. 6. Comparison of median lethal dose (LD₅₀) values, in PIBs per third instar Douglas-fir tussock moth larvae, challenged by samples from the different package sizes in lot 7 of the stored TM Biocontrol-1®.^a



^a Strains used: CA = California field strain, and GL = Goose Lake Laboratory strain. Package sizes were S= small < 100 g/pkg., and M = medium 100-999 g/pkg. Numbers under the bar graphs denote sample numbers.

Fig. 7. Comparison of median lethal dose (LD₅₀) values, in PIBs per third instar Douglas-fir tussock moth larvae, challenged by samples from the different package sizes in lot 9 of the stored TM Biocontrol-1[®].^a



^a Strain used: GL = Goose Lake laboratory strain. GL0 was the first bioassay using the Goose Lake strain. Package sizes were S = small <100g/pkg., and M = medium 100-999 g/pkg. Numbers under the bar graphs denote sample numbers.

Fig. 8. Comparison of the median lethal dose (LD₅₀) values, in PIBs per third instar Douglas-fir tussock moth larvae, for all samples bioassayed, of stored TM Biocontrol-1[®] of each lot as well as a combined fresh OpNPV LD₅₀ value (F).

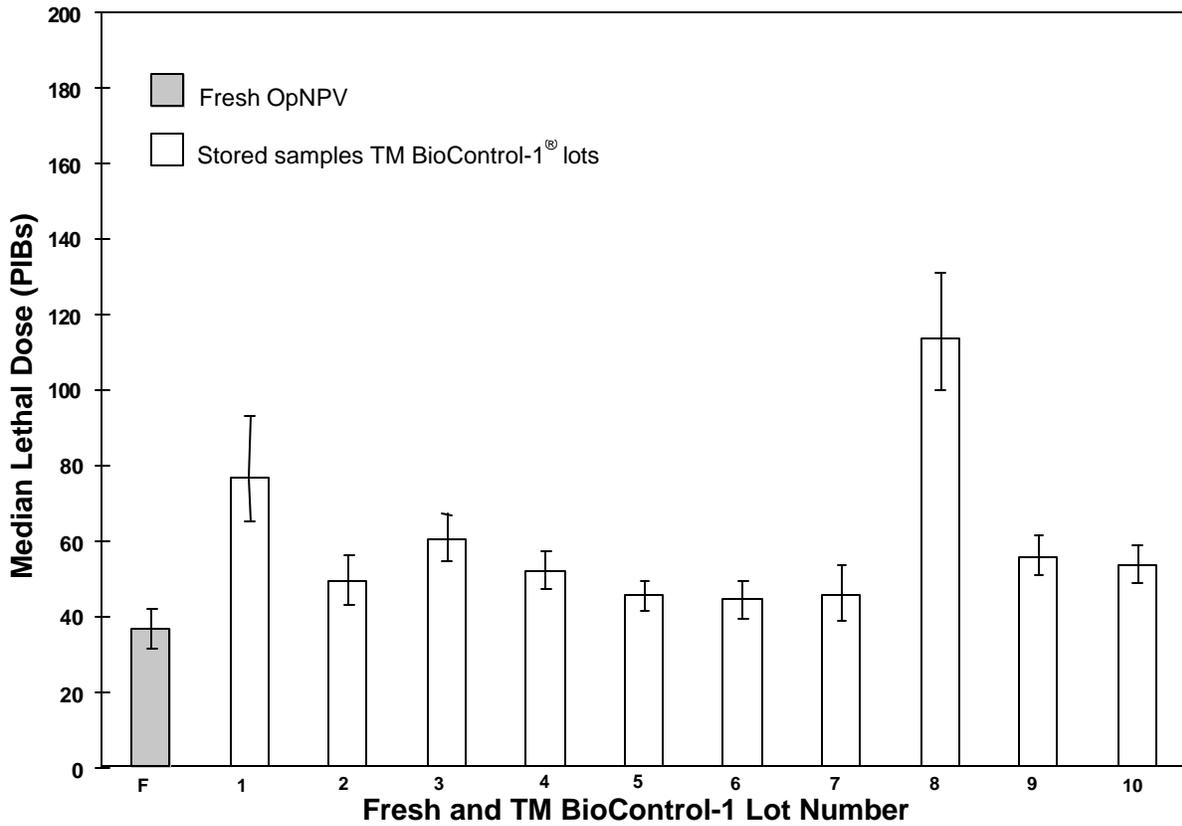
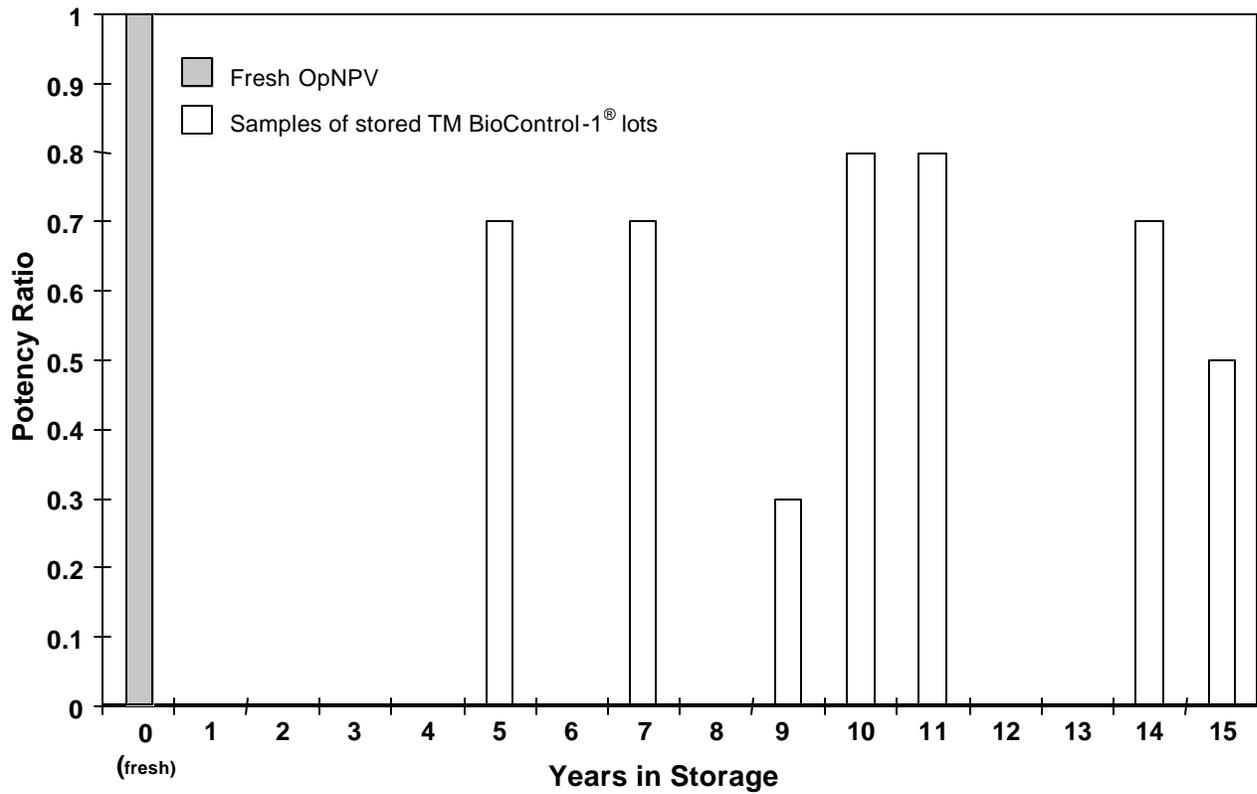


Fig. 9. Comparison of potency ratios calculated for each lot of stored TM Biocontrol-1[®] against time in storage.^a



^a Lots 2, 3, 4, and 5 were all stored for 14 years and are combined in this graph (i.e. the potency ratio for 14 years in storage is the average potency ratio calculated from the ratios of lots 2, 3, 4 and 5).