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Aerial Application of Baculovirus on Red-Headed Pine Sawfly, Neodiprion lecontei (Fitch), in 1977

by

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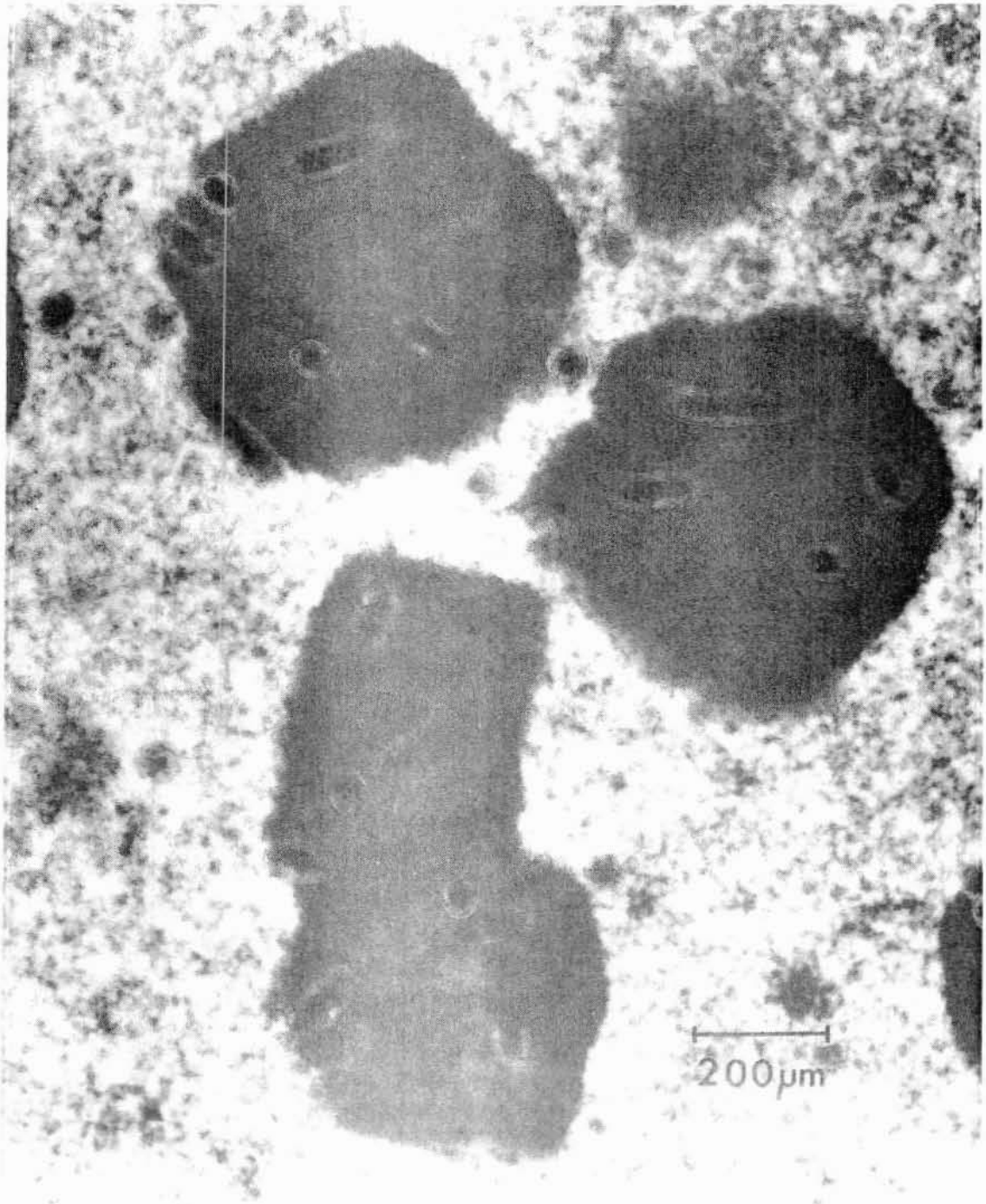

Frontispiece. An electron [illegible] ultrathin section of polyhedra in a [illegible] infected with nuclear poly[illegible] Rod-shaped virus particles are embedde[illegible] protein matrix

Abstract

Two red pine, *Pinus resinosa* Ait., plantations near Renfrew, Ontario, and one jack pine, *Pinus banksiana* Lamb., plantation south of Ottawa, Ontario, with a combined area of 48 ha and infested with red-headed pine sawfly were aerially sprayed with nuclear polyhedrosis virus. Larvae were predominantly in the second and third instar in the red pine plantations and in the fourth instar in the jack pine plantation at the time of application. A dosage of 5.5 billion polyhedra/ha was applied at 9.4 ℓ/ha in an aqueous formulation containing 250 ml/ℓ molasses and 60 g/ℓ IMC 90-001 sunlight protectant. The level of virus infection and reduction in number of sawfly colonies were monitored and excellent control was achieved. In one locality, the virus spread to an adjoining, infested 8 ha plantation and killed the sawfly larvae. Damage to the trees in all three treated areas was slight and no trees suffered terminal shoot defoliation. The use of this virus for control of red-headed pine sawfly should be promoted.

Résumé

Deux plantations de Pin rouge, *Pinus resinosa* Ait., près de Renfrew, Ontario, et une plantation de Pin gris, *Pinus banksiana* Lamb., au sud d'Ottawa, totalisant 48 ha et infestées par le Diprion de LeConte, furent arrosées par voie des airs avec un virus de la polyédrose nucléaire. Les larves étaient pour la plupart aux deuxième et du troisième stades dans les plantations de Pin rouge; elles étaient au quatrième stade dans la plantation de Pin gris au moment de l'arrosage. On appliqua une dose

de 5.5 milliards de virus polyedrès/ha, à raison de 9.4 ℓ/ha en liquide aqueux contenant 250 ml/ℓ de mélasse et 60 g/ℓ de protecteur solaire IMC 90-001. Le niveau d'infection par le virus et la diminution du nombre de colonies du Diprion furent suivis de près et la lutte s'avéra un franc succès. Dans un endroit, le virus s'étendit à une plantation adjacente de 8 ha, qui fut aussi infectée et le virus tua les larves du Diprion. Dans les trois régions traitées, les dégâts causés aux arbres furent légers et aucun arbre n'a souffert de défoliation des pousses. On devrait promouvoir l'utilisation de ce virus dans la lutte contre le Diprion de LeConte.

Acknowledgments

We wish to thank Dr. Rod Carrow, Forest Protection Branch, Ontario Ministry of Natural Resources (O.M.N.R.), Toronto, for his encouragement and support in developing red-headed pine sawfly NPV for operational use.

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We appreciate the efforts of Mr. J.C. Edwards and Mr. L. Pollock in arranging the use and making the spray application with the FPMI aircraft, Miss Betty White for microscopic examination of larvae and Mr. J.R. McPhee for assisting in the final defoliation survey.

Special thanks are given to Dr. K.M.S. Sundaram and Mr. S. Szeto of FPMI who performed gas-liquid chromatographic analysis of the tank mix when contamination with insecticide was suspected.

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Introduction

The nuclear polyhedrosis virus (NPV) of the red-headed pine sawfly, *Neodiprion lecontei* (Fitch), was discovered in Ontario in 1950. Nuclear polyhedrosis and granulosis viruses are classified in the genus "baculovirus" (Wildy, 1971). Several ground spray applications were made until 1976 when the first aerial spray trial was undertaken using this virus (Kaupp and Cunningham, 1977). In this trial dosages of 1.25 billion, 3.75 billion and 6.25 billion polyhedral inclusion bodies (PIB)/ha were tested when larvae were mainly in the second instar. All three dosages gave effective control of the sawfly. When these areas were re-examined in 1977 not a single sawfly colony could be found in the treated plots. On the other hand, the check area, which had 238 colonies per 100 trees in 1976, still had a population of 102 colonies/100 trees (unpublished data).

Currently efforts are being made to gather the data required to compile a petition for the registration of this virus under the Pest Control Products Act (Canada). In comprehensive laboratory tests, the safety of this NPV to birds (Valli and Claxton, 1976) and to mammals (Valli and Forsberg, 1978) has been demonstrated. Laboratory safety tests on fish and aquatic invertebrates have yet to be undertaken. Laboratory studies of the biochemical and biophysical properties of the virus are underway.

In order to confirm the results obtained in 1976 and to provide the data necessary for a registration petition, it was decided to replicate a treatment used in the 1976 trials and aerially spray three plots with

the same dosage of virus. On the basis of the results obtained in 1976, a dosage of 2.25 billion PIB/ha was selected. However, when it was discovered that larval development was more advanced in 1977 when the time came to apply the virus, this dosage was doubled to 5.5 billion PIB/ha.

In conjunction with the 1977 spray trials environmental monitoring studies of non-target organisms were undertaken. Bee hives were placed in one plot and studied and pre-spray and post-spray counts were made of birds and aquatic fauna. These observations are reported elsewhere (McLeod and Mortensen, 1978).

This report describes the aerial spray trials with red-headed pine sawfly NPV conducted in 1977 and gives an assessment of the results obtained.

Materials and Methods

The virus

The NPV used in the 1977 field trials was propagated in red-headed pine sawfly infesting a mature plantation of red pine on St. Joseph Island near Sault Ste. Marie, Ontario, in 1976. The plantation was sprayed with NPV when larvae were in the fourth instar. A mistblower was used and branches containing colonies of virus-infected larvae were snipped off and taken to the laboratory. Larvae were picked off, lyophilized and ground to a fine powder. The potency of the virus produced in 1976 was very high and the material contained 9.0 billion PIB/g.

The virus treated plantations

Two plantations, designated plot no. 1 and plot no. 2, with red pine, *Pinus resinosa* Ait., trees 0.9 m to 3.4 m (mean 2.2 m) and 0.7 m to 1.8 m (mean 1.2 m) high respectively and with areas of 13.2 ha and 30.8 ha were located in Admonston Twp. near Renfrew, Ontario (Fig. 1). Plot no. 2 had been treated with malathion using a hand sprayer in 1976. Another area of about 13.2 ha with 8 ha planted with red pine and infested with red-headed pine sawfly adjoined plot no. 1, but permission to spray this area was not granted in time to treat it. It is designated area "A" in Fig. 1. Some difficulty was encountered in selecting a suitable check plot in this area but one was located about 10 km from the treated plots. It was a red pine plantation of about 4 ha with trees ranging in size from 1.2 m to 3.7 m (mean 2.6 m). It was designated check plot no. 2.

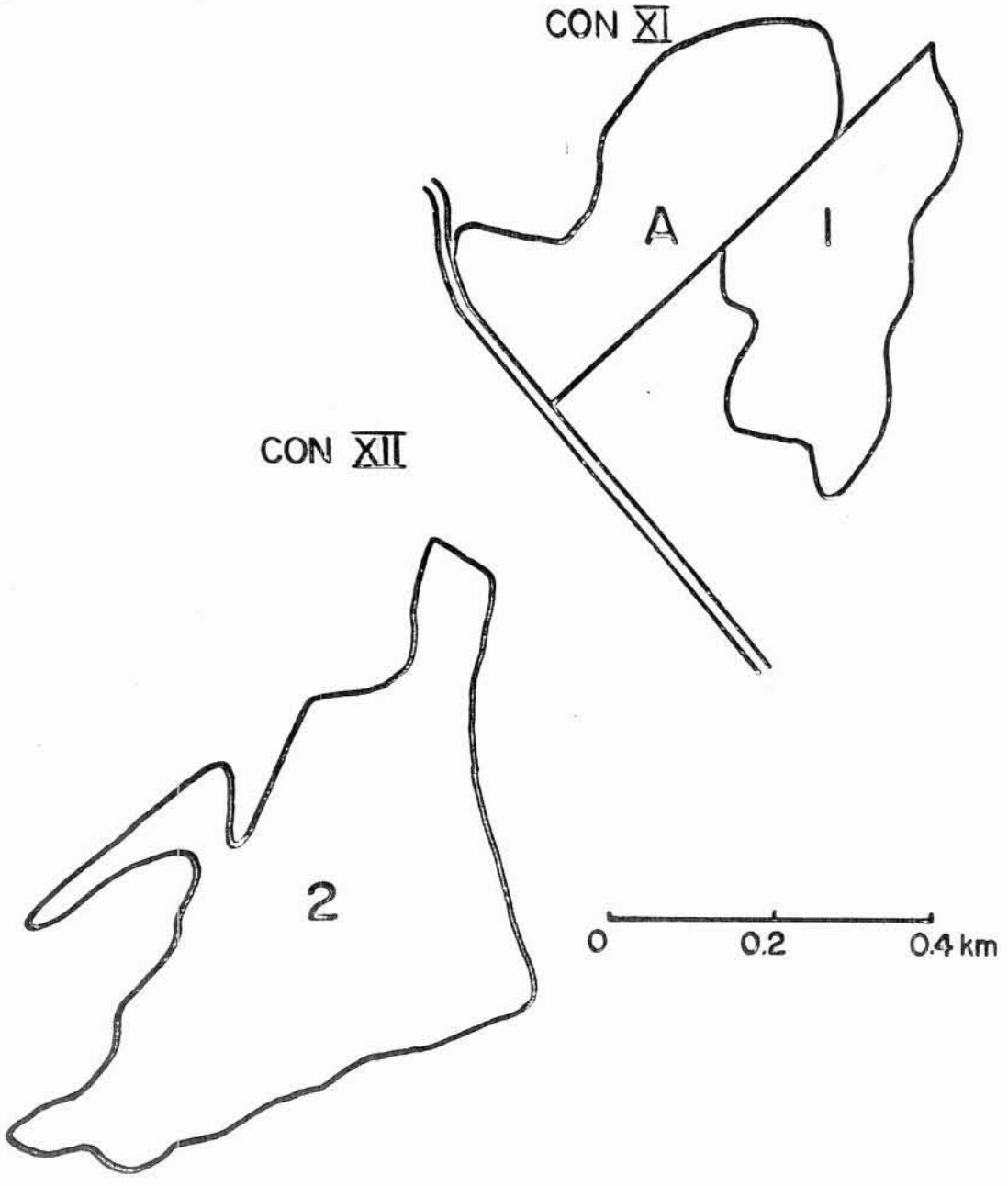

Fig. 1. The relative positions of plots no. 1 and no. 2 in Admonston Twp. and area "A" to which the NPV spread from plot no. 1.

Environmental impact studies were conducted in plots no. 1 and no. 2 with beehives located in plot no. 2. Bird populations were monitored in both plots and aquatic invertebrates studied in a stream located in plot no. 1.

A third plot which contained mainly jack pine, *Pinus banksiana* Lamb., 1.1 m to 3.4 m (mean 2.0 m) high and a few red pine had an area of 8 ha and was located in lot 17, Con VIII, Rideau Twp., south of Ottawa. A check area, designated check no. 1 was located about 8 km from the treated plot. It contained only jack pine which ranged in size from 0.6 m to 2.8 m (mean 1.8 m) and its area was about 0.5 ha.

Treatments and formulations

All three plots were treated with the same dosage of virus, 5.5 billion PIB/ha at a rate of 9.4 ℓ/ha. A stock suspension of virus was made up by mixing the lyophilized virus-infected larval powder in water using a Kalish® turbo homogeniser. This stock suspension contained 3.6×10^8 PIB/ml. Just prior to the aerial spray, aqueous formulations were prepared by adding 250 ml/ℓ molasses and 60 g/ℓ IMC 90-001[1/] sunlight protectant to the correct volumes of water and concentrated virus suspension.

1/ Sandoz Inc., 480 Camino del Rio South, San Diego, California 92108

Spray application and larval development

Spraying commenced at 5:55 a.m. on the morning of July 14th on plot no. 1 in the Renfrew area and was completed on plot no. 2 at 7:10 a.m. The aircraft used was a Balanca Scout[2/] fitted with 20 no. 8 nozzles and calibrated to deliver 9.4 ℓ/ha. The spray tank was a Sorrensen spring unit type holding a maximum of 300 ℓ and two loads were required to spray both plots. Larvae were predominantly in the second and third instars in plot no. 1 and were mainly in the second instar in plot no. 2 when sprayed.

Plot no. 3 in Rideau Twp. was sprayed on July 15th. The operation commenced at 6:55 a.m. and was finished by 7:05 a.m. The aircraft used was the FPMI Cessna 185E fitted with 4 Micronair AU3000 units and calibrated to deliver 9.4 ℓ/ha. It was also fitted with a Sorrensen spring unit tank. Larval development was more advanced in this area than in plots no. 1 and no. 2 near Renfrew and they were predominantly in the fourth instar.

Monitoring spray deposit

The spray deposit was monitored on Kromekote® spray cards mounted on 100 x 150 mm aluminum backings. The cards were placed at 15 m intervals across each plot at right angles to the flight lines and for 60 m on each side of the plots to monitor spray drift. Spray droplet density per cm^2 and size were determined from these cards.

2/ Lammen's Spraying Service, R.R. #5, Langton, Ontario.

Meteorological observations

A weather station was located in plot no. 1 in Admonston Twp. Temperature and relative humidity were recorded using a hydrothermograph.

Efficacy Assessment

To record the impact of the NPV on the sawfly population, 100 trees were selected in each treated and check plot with no regard to the insect population and were tagged. The insect population was low in plot 2 with only 12 colonies recorded on 100 trees. It had been sprayed with malathion in 1976. Hence a further 50 trees (101 - 150 in Appendix 1) were selected which contained sawfly colonies. Tree height and the number of healthy colonies were recorded before the spray application. This information is listed in Appendix 1. Following the spray application, colonies were counted on the tagged trees periodically until pupation occurred.

In order to check that larval mortality was, in fact, due to NPV, random collections of insects from untagged trees were taken to the laboratory where they were dissected and their guts examined microscopically for the presence of polyhedra.

At the termination of the experiment, after all remaining larvae had pupated, a defoliation survey of the tagged trees was made. Percent current defoliation was estimated by examining the trees and permanent tree damage was measured by recording terminal shoot damage.

Results

Measurements of the height of the trees selected for the assessment are given in Appendix 1 together with the number of sawfly colonies per tree. An analysis of the spray cards showed that the Micronair spray equipment used on plot no. 3 gave considerably more droplets per cm^2 than the boom and nozzle equipment used on plot no. 1 and no. 2 (Table 1). An analysis of the droplet size, as measured on the Kromekote^R cards shows no major difference in size range between the three plots with most of the droplets falling in the classes between 50µ and 200µ (Figs. 2 and 3).

For the 26 days following the spray application, during which period the records were taken, the mean day-time temperature was 25.5°C and the mean night-time temperature was 15.1°C.

Counts of colonies were made 5, 11, 13, 20 and 26 days post-spray. Significant virus mortality was noted 11 days post-spray in plots no. 1 and no. 2 (Table 2), but 5 days post-spray a large number of colonies disappeared (132 reduced to 47) in plot no. 3 (Table 3). This could not be attributed to NPV and other reasons for this sudden population decline were sought. An analysis of the tank mix from the aircraft by Dr. K.M.S. Sundaram of FPMI revealed that the virus formulation was contaminated with phosphamidon which was sprayed from the aircraft on its previous mission. The analysis, by gas-liquid chromatography, revealed 156 µg/ml of the *trans*-isomer of phosphamidon and 366 µg/ml of the *cis*-isomer giving a total of 522 µg/ml.

After 26 days, colony counts in plots no. 1 and no. 2 revealed population reductions from 163 colonies/100 trees to 7/100 trees and 81 colonies/150 trees to 0 colonies/150 trees respectively compared to a

reduction in the check of 217 colonies/100 trees to 200 colonies/100 trees (Table 2).

In plot 3, 132 colonies/100 trees were reduced to 47 colonies/100 trees five days post-spray due to the accidental phosphamidon application. After 20 days these 47 colonies/100 trees were further reduced to 25 colonies due to NPV with the count on the corresponding check plot dropping from 248 colonies/100 trees to 226 colonies/100 trees (Table 3). On day 26 there was a marked decline in both the treated and check plot with the 25 colonies recorded on day 20 declining to 2 and the 226 in the check declining to 114. It appears that between day 20 and day 26 larvae dropped to the ground and commenced pupation in the duff layer. Hence, after day 20, reduction in the number of colonies cannot be attributed solely to mortality from NPV.

Samples of 100 larvae per plot for microscopic examination were collected 7 days post-spray from each plot and from the check areas. The levels of visible virus infection were recorded as 35%, 22% and 13% in plots no. 1, no. 2 and no. 3 respectively indicating that the NPV was well established in the sawfly population. No virus was found in larvae from either of the check areas.

The final defoliation survey revealed that damage to trees in plots no. 1 and no. 2 was negligible. Damage was heavier in plot no. 3 but could still be regarded as slight with a mean defoliation estimate of less than 5% for the 100 tagged trees. There was no terminal shoot defoliation in any of the treated plots. In contrast, in Renfrew check plot no. 2 there was a mean defoliation estimate of 30%, and 16% of the trees suffered terminal shoot defoliation. In Rideau Twp. check plot no. 1, the mean defoliation estimate was 32%, and 29% of the trees suffered

terminal shoot defoliation (Appendix 1).

During the final defoliation survey an interesting observation was made. When the plantation adjoining plot no. 1, designated A in Fig. 1, was examined, dead sawflies were seen adhering to the needles and twigs. Samples were taken to the laboratory and it was confirmed that they were killed with NPV. Considerable damage to the trees was noted in this area but not recorded. Since the location of this plantation was upwind during the spray application, it is presumed that the virus spread from plot no. 1 late in the season after the sawflies had consumed a lot of foliage and it exerted some control throughout this area. The number of surviving healthy pupae in this plantation is unknown and it will require a survey in 1978 to determine the full impact of the horizontal transmission of this NPV which occurred in the year of application.

Table 1

Mean number of droplets per cm^2 on spray cards

Plot	Application	Mean Number Drops/cm^2	Standard Deviation
1	Boom and Nozzle	10	4
2	Boom and Nozzle	11	6
3	Micronair	60	35

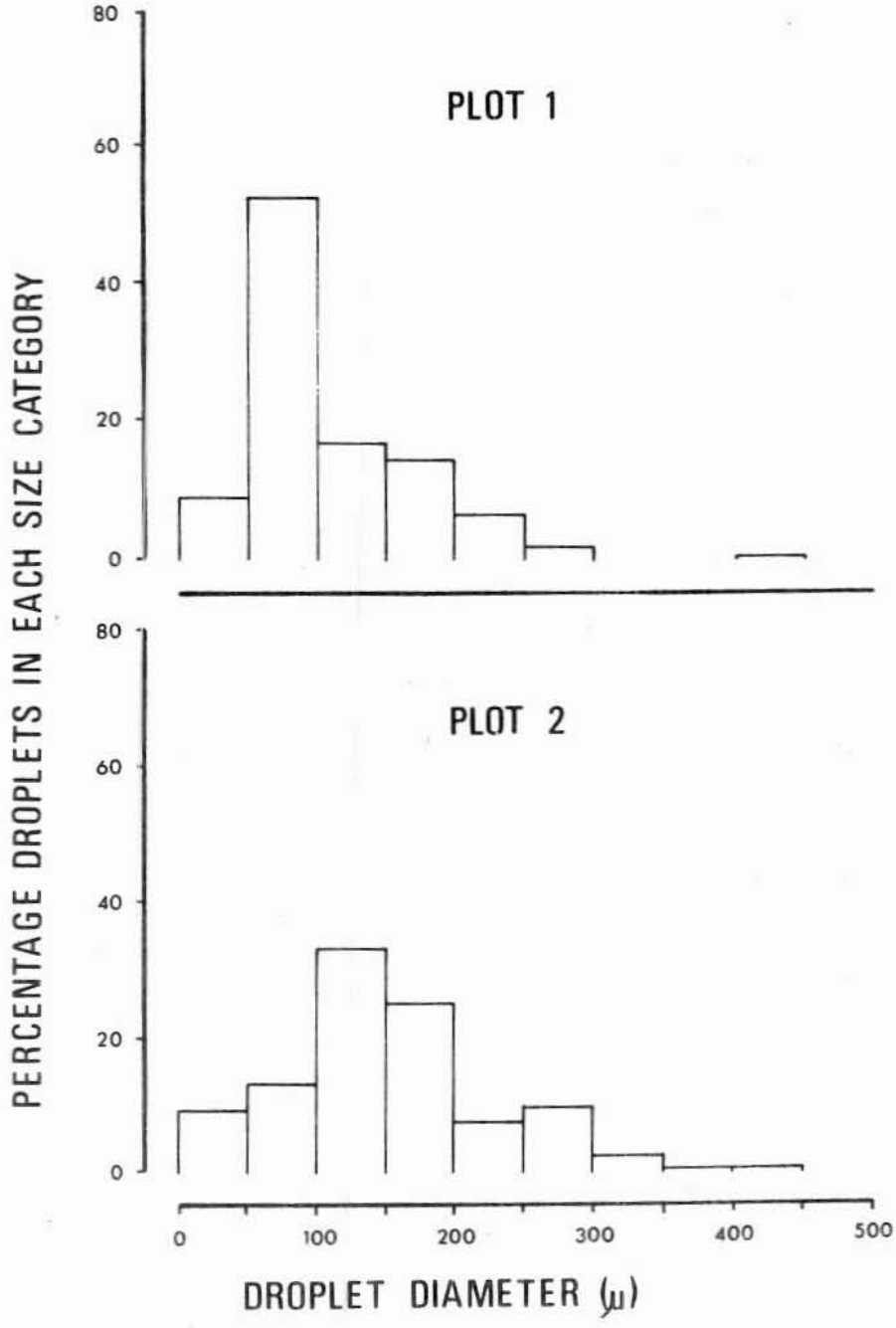

Fig. 2. Analysis of spray droplets on Kromekote® cards from plots no. 1 and no. 2 near Renfrew, Ontario.

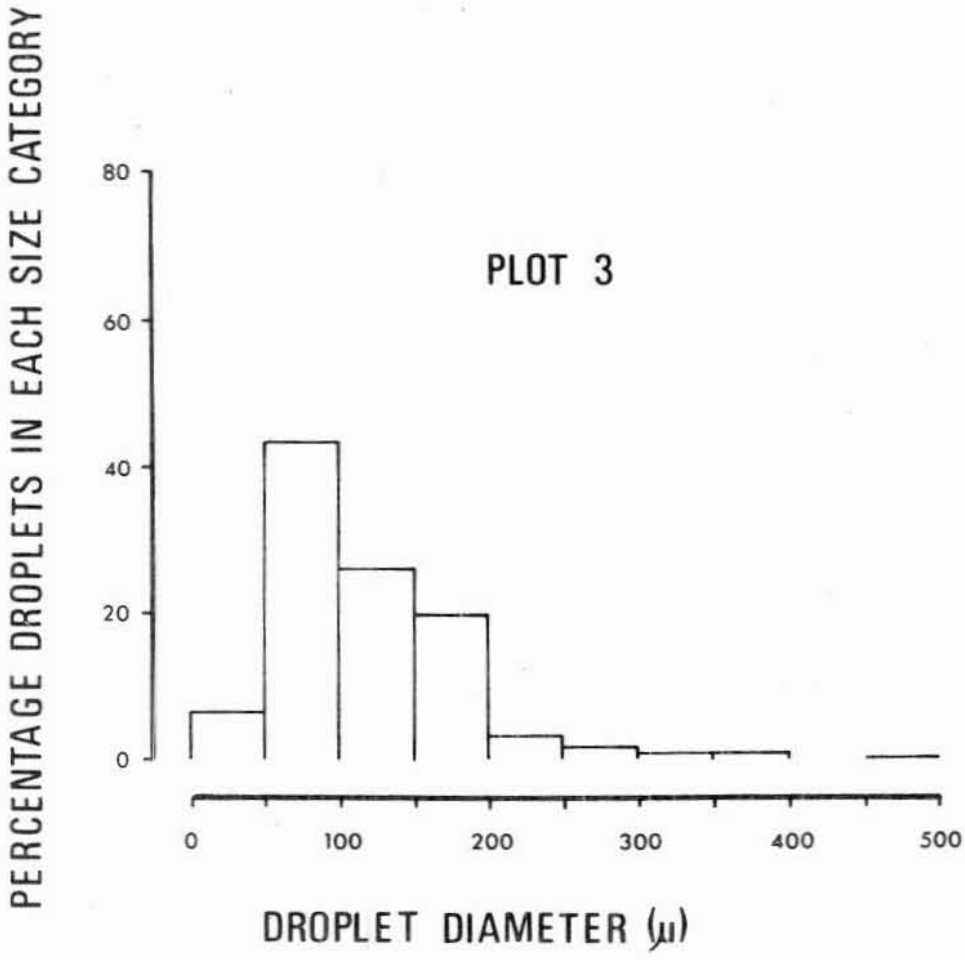

Fig. 3. Analysis of spray droplets on Kromekote® cards from plot no. 3 in Rideau Twp., south of Ottawa, Ontario.

Table 2

Reduction in the number of sawfly colonies per 100 trees following application of NPV on second and third instar larvae in Admonston Twp. near Renfrew in 1977.

	Plot No. 1	Plot No. 2	Check No. 2
Pre-spray	163	81*	217
Days Post-spray			
5	154	79	---
11	90	46	234
13	94	49	---
20	31	11	212
26	7	0	200

*Number of colonies per 150 tagged trees.

Table 3

Reduction in the number of sawfly colonies per 100 trees following application of NPV on fourth instar larvae in Rideau Twp. in 1977.

	Number of colonies/100 trees	
	Plot No. 3	Check No. 1
Pre-spray	132	248
Days Post-spray		
5	47*	291**
11	47	264
13	41	---
20	25	226
26	2	114***

* Decline in sawfly population due to phosphamidon contamination of NPV formulation.

** Increase due to migration and division of some colonies.

*** Decline in sawfly population in check indicates onset of pupation.

Discussion

This is the second season of aerial spray trials with NPV on red-headed pine sawfly. The aim of these trials which was to generate efficacy data from 3 replicated plots, was not entirely successful. Larvae were further developed this year than in the 1976 operation when they were predominantly in the second instar with some still hatching at the time of application. In plots no. 1 and no. 2 in the Renfrew area they were in the second and third instars but a dosage of 5.5 billion PIB/ha gave outstanding control. After 26 days no larvae could be found in plot no. 2 and the 7 colonies recorded in plot no. 1 were along an edge of that plantation which received poor coverage due to spray drift.

In plot no. 3, the larvae were even larger, being mainly in the fourth instar, so the results from this plot are not comparable with those from plots no. 1 and no. 2. This problem was compounded by the contamination of the aircraft with the chemical insecticide phosphamidon. The concentration of phosphamidon was 522 µg/ml and the amount applied was calculated to be 4.91 g active ingredient/ha. Sawflies are particularly susceptible to phosphamidon (DeBoo, pers. comm.) and this accident may have demonstrated an interesting concept in integrated control. Given a heavy population of red-headed pine sawfly in the fourth instar, which is causing a high level of damage, a mixture of a very low dosage of phosphamidon and NPV could be applied. The phosphamidon would give a quick knock-down of a sawfly population but leave sufficient larvae to become infected with NPV and initiate a virus epizootic which would eradicate the pest.

Discussions with several pest managers in Ontario lead one to form the opinion that the use of chemicals alone does not give satisfactory control of red-headed pine sawfly and applications have to be repeated year after year to avoid severe defoliation and tree mortality. This situation was observed in plot no. 2 which was treated with malathion in 1976 but was still infested in 1977.

The dosage of 5.5 billion PIB/ha, although adequate on the second and third instar larvae in plots no. 1 and no. 2, was probably too low to kill all the larvae in the year of application in plot no. 3. On examining the population decline compared to the untreated check plot (Table 3), it is evident that some of the loss of colonies between day 20 post-spray and day 26 can be attributed to pupation and not to mortality caused by NPV. However, sub-lethally infected adults will emerge from a proportion of these pupae and initiate a virus epizootic in 1978 which will almost certainly terminate the infestation.

The horizontal transmission of the NPV to an unsprayed 8 ha plantation in the year of application is most encouraging and so is the observation that there were no colonies of sawflies in any of the 3 plots sprayed in 1976 when they were examined in 1977 (unpublished data). Total spray coverage of a plantation with NPV is probably not required if some damage to trees can be tolerated in the year of application. The cost of the virus material, at the concentration applied in these tests, has been estimated at about $2.40/ha and this is considered inexpensive. This cost can be reduced even further by applying a lower dosage on earlier instars. However, the supply of material is a limiting factor when contemplating large scale operational

spraying. In future tests more emphasis will be placed on assessing the impact of virus introductions into infested plantations as opposed to total coverage bio-insecticide spraying which has been employed to date.

Both field and laboratory testing of this virus will continue and it is hoped that the documentation for registration will be ready sometime in 1979.

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Appendix I

Height, number of healthy sawfly colonies (pre-spray and 26 days post-spray), final defoliation estimates and terminal shoot damage for sample trees in each treated and check plot.

Tree No.	Height (m)	Number of healthy colonies pre-spray	Number of healthy colonies 26 days post-spray	Final defoliation estimate
1.	2.35	2	1	0
2.	2.18	2	1	Trace
3.	2.26	1	1	0
4.	2.22	6	2	0
5.	2.46	2	0	0
6.	2.26	1	0	0
7.	2.31	2	0	0
8.	1.47	0	0	0
9.	1.69	0	0	0
10.	1.77	0	0	0
11.	2.20	5	0	0
12.	2.54	1	0	0
13.	2.67	3	0	0
14.	2.79	0	0	0
15.	2.08	3	0	0
16.	0.88	1	0	0
17.	1.80	0	0	0
18.	2.60	2	0	0
19.	1.53	0	0	0
20.	2.62	6	0	0
21.	2.54	5	0	0
22.	2.61	2	0	0
23.	2.96	0	0	0
24.	2.73	0	0	0
25.	2.19	0	0	0
26.	1.97	0	0	0
27.	1.84	0	0	0
28.	2.28	1	0	0
29.	2.34	0	0	0
30.	2.14	0	0	0
31.	3.03	1	0	0
32.	2.72	5	0	0
33.	3.05	4	0	0
34.	3.12	1	0	0
35.	3.30	1	0	0
36.	2.46	4	0	0
37.	3.36	2	0	0
38.	2.73	0	0	0
39.	2.23	2	0	0
40.	2.01	3	0	Trace
41.	2.23	0	0	0
42.	1.75	1	0	0
43.	2.17	0	0	0
44.	1.78	1	0	Trace
45.	2.00	1	0	0
46.	2.36	5	0	Trace
47.	2.45	1	0	0
48.	2.01	1	0	0
49.	1.86	1	0	0
50.	1.97	1	0	Trace

Tree No.	Height (m)	Number of healthy colonies pre-spray	Number of healthy colonies 26 days post-spray	Final defoliation estimate
51.	2.23	1	0	0
52.	2.20	1	1	Trace
53.	2.55	0	0	Trace
54.	2.35	1	0	10
55.	2.21	3	1	10
56.	2.07	2	0	0
57.	1.93	2	0	0
58.	2.66	0	0	0
59.	2.35	2	0	0
60.	2.79	3	0	0
61.	1.86	3	0	0
62.	1.84	5	0	0
63.	2.61	4	0	Trace
64.	2.47	2	0	0
65.	1.06	3	0	0
66.	2.32	4	0	0
67.	3.03	0	0	0
68.	2.33	0	0	0
69.	1.35	1	0	0
70.	2.11	1	0	0
71.	1.84	0	0	0
72.	2.56	0	0	0
73.	2.64	1	0	0
74.	2.05	1	0	0
75.	1.55	0	0	0
76.	1.77	2	0	0
77.	3.03	0	0	0
78.	2.98	0	0	0
79.	2.13	1	0	0
80.	2.41	1	0	0
81.	2.50	1	0	0
82.	1.92	0	0	0
83.	2.86	3	0	Trace
84.	2.43	0	0	0
85.	2.01	1	0	0
86.	2.44	6	0	Trace
87.	1.87	5	0	Trace
88.	1.76	3	0	Trace
89.	1.63	3	0	0
90.	1.57	6	0	0
91.	1.35	0	0	0
92.	1.36	2	0	Trace
93.	1.62	1	0	0
94.	3.16	2	0	0
95.	2.18	0	0	0
96.	1.93	0	0	0
97.	1.36	0	0	0
98.	1.83	1	0	0
99.	2.18	2	0	0
100.	2.33	3	0	Trace

No trees suffered terminal shoot defoliation

PLOT 2

Tree No.	Height (m)	Number of healthy colonies pre-spray	Number of healthy colonies 26 days post-spray	Final defoliation estimate
1.	1.76	0	0	0
2.	1.47	0	0	0
3.	0.81	0	0	0
4.	1.00	0	0	0
5.	1.53	0	0	0
6.	0.86	0	0	0
7.	1.07	0	0	0
8.	1.01	0	0	0
9.	1.41	0	0	0
10.	1.19	0	0	0
11.	1.31	1	0	0
12.	1.71	1	0	0
13.	1.12	0	0	0
14.	0.80	4	0	0
15.	1.43	1	0	0
16.	1.26	0	0	0
17.	1.52	0	0	0
18.	1.44	0	0	0
19.	1.23	0	0	0
20.	1.06	0	0	0
21.	1.11	0	0	0
22.	1.13	0	0	0
23.	1.25	0	0	0
24.	1.18	2	0	0
25.	0.92	0	0	0
26.	1.16	1	0	0
27.	1.17	0	0	0
28.	1.23	0	0	0
29.	1.63	0	0	0
30.	1.12	0	0	0
31.	1.41	0	0	0
32.	0.93	0	0	0
33.	1.19	0	0	0
34.	1.38	0	0	0
35.	1.22	0	0	0
36.	1.01	0	0	0
37.	1.58	0	0	0
38.	1.42	0	0	0
39.	1.08	0	0	0
40.	0.93	0	0	0
41.	1.46	0	0	0
42.	1.32	1	0	0
43.	0.70	0	0	0
44.	0.77	0	0	0
45.	1.19	0	0	0
46.	0.99	0	0	0
47.	1.19	0	0	0
48.	1.24	0	0	0
49.	0.92	0	0	0
50.	1.37	0	0	0

No trees suffered terminal shoot defoliation

Tree No.	Height (m)	Number of healthy colonies pre-spray	Number of healthy colonies 26 days post-spray	Final defoliation estimate
51.	1.31	0	0	0
52.	1.26	0	0	0
53.	1.35	0	0	0
54.	1.25	0	0	0
55.	1.21	0	0	0
56.	1.35	0	0	0
57.	1.18	0	0	0
58.	1.10	0	0	0
59.	1.03	0	0	0
60.	1.14	0	0	0
61.	1.11	0	0	0
62.	1.08	0	0	0
63.	1.30	0	0	0
64.	0.94	0	0	0
65.	1.14	0	0	0
66.	1.04	0	0	0
67.	1.41	0	0	0
68.	1.36	0	0	0
69.	1.53	0	0	0
70.	1.13	0	0	0
71.	1.19	0	0	0
72.	1.50	0	0	0
73.	1.22	0	0	0
74.	1.16	0	0	0
75.	0.83	0	0	0
76.	1.27	0	0	0
77.	1.05	0	0	0
78.	1.27	0	0	0
79.	1.29	0	0	0
80.	1.00	0	0	0
81.	1.07	0	0	0
82.	1.42	0	0	0
83.	1.29	0	0	0
84.	1.00	1	0	0
85.	1.19	0	0	0
86.	1.05	0	0	0
87.	1.20	0	0	0
88.	0.90	0	0	0
89.	1.22	0	0	0
90.	1.03	0	0	0
91.	1.30	0	0	0
92.	1.27	0	0	0
93.	1.02	0	0	0
94.	1.04	0	0	0
95.	1.38	0	0	0
96.	0.81	0	0	0
97.	1.43	0	0	0
98.	1.22	0	0	0
99.	1.27	0	0	0
100.	1.38	0	0	0

No trees suffered terminal shoot defoliation

Tree No.	Height (m)	Number of healthy colonies pre-spray	Number of healthy colonies 26 days post-spray	Final defoliation estimate
101.	0.81	2	0	30
102.	1.11	1	0	0
103.	0.96	2	0	0
104.	0.98	1	0	Trace
105.	0.99	2	0	0
106.	0.86	1	0	0
107.	0.84	2	0	10
108.	0.93	1	0	0
109.	1.07	1	0	10
110.	1.20	1	0	0
111.	1.79	3	0	0
112.	0.92	2	0	0
113.	1.04	1	0	0
114.	1.42	1	0	0
115.	1.22	1	0	0
116.	1.47	3	0	0
117.	1.31	1	0	0
118.	1.05	1	0	Trace
119.	0.97	2	0	Trace
120.	1.38	1	0	0
121.	1.36	1	0	0
122.	1.27	1	0	0
123.	1.24	2	0	Trace
124.	1.53	1	0	0
125.	0.99	1	0	0
126.	1.38	1	0	10
127.	1.22	2	0	0
128.	1.39	2	0	Trace
129.	1.02	1	0	0
130.	1.03	2	0	Trace
131.	1.35	1	0	0
132.	1.17	4	0	0
133.	1.37	1	0	Trace
134.	0.64	1	0	Trace
135.	1.61	1	0	0
136.	1.06	1	0	0
137.	1.36	2	0	0
138.	1.29	1	0	0
139.	1.18	1	0	0
140.	0.89	1	0	0
141.	0.92	1	0	Trace
142.	1.42	1	0	0
143.	1.97	1	0	0
144.	1.48	2	0	0
145.	1.88	1	0	0
146.	0.99	1	0	0
147.	1.38	1	0	0
148.	1.17	1	0	0
149.	1.38	1	0	Trace
150.	1.07	1	0	10

No trees suffered terminal shoot defoliation

Tree No.	Height (m)	Number of healthy colonies pre-spray	Number of healthy colonies 26 days post-spray	Final defoliation estimate
1.	2.10	0	0	0
2.	2.29	3	0	0
3.	1.85	0	0	0
4.	1.53	1	0	0
5.	1.63	0	0	0
6.	1.50	0	0	0
7.	1.42	1	0	Trace
8.	2.53	0	0	Trace
9.	2.66	0	0	0
10.	2.17	1	0	0
11.	2.13	0	0	0
12.	1.99	0	0	0
13.	1.69	6	0	Trace
14.	1.81	1	0	0
15.	1.22	3	0	0
16.	1.28	0	0	0
17.	1.41	1	0	Trace
18.	1.30	1	0	0
19.	1.09	1	0	0
20.	1.26	1	0	Trace
21.	1.61	0	0	0
22.	1.94	0	0	0
23.	1.60	0	0	0
24.	2.12	1	0	0
25.	2.77	0	0	0
26.	3.15	0	0	0
27.	2.57	0	0	0
28.	2.68	1	0	0
29.	2.63	0	0	0
30.	2.73	0	0	0
31.	2.30	1	0	0
32.	1.86	0	0	0
33.	2.11	0	0	0
34.	1.73	0	0	0
35.	1.58	3	0	10
36.	2.38	0	0	0
37.	1.97	0	0	0
38.	2.21	0	0	0
39.	2.25	0	0	0
40.	1.38	2	0	20
41.	2.03	0	0	0
42.	1.69	3	0	Trace
43.	2.13	0	0	0
44.	1.76	0	0	0
45.	2.51	0	0	0
46.	2.27	0	0	0
47.	1.72	2	0	0
48.	2.06	0	0	0
49.	2.56	0	0	0
50.	2.05	0	0	0

No trees suffered terminal shoot defoliation

Tree No.	Height (m)	Number of healthy colonies pre-spray	Number of healthy colonies 26 days post-spray	Final defoliation estimate
51.	2.11	3	0	10
52.	2.19	0	0	0
53.	2.23	1	0	0
54.	2.24	0	0	0
55.	2.61	0	0	0
56.	1.76	1	0	Trace
57.	2.22	2	0	0
58.	3.41	4	0	10
59.	2.32	2	0	10
60.	2.91	4	1	10
61.	2.54	0	0	0
62.	1.83	2	0	0
63.	1.95	0	0	0
64.	2.37	0	0	0
65.	1.44	1	0	0
66.	2.89	5	0	20
67.	2.42	2	0	0
68.	1.58	2	0	0
69.	3.24	1	0	Trace
70.	1.39	2	0	Trace
71.	2.36	0	0	0
72.	2.00	0	0	0
73.	1.72	2	0	0
74.	2.16	2	0	Trace
75.	1.37	1	0	10
76.	1.80	1	0	Trace
77.	2.15	1	0	Trace
78.	2.28	0	0	0
79.	2.43	2	0	Trace
80.	2.53	0	0	0
81.	1.63	0	0	0
82.	2.12	2	0	Trace
83.	2.61	2	0	10
84.	1.27	1	0	0
85.	1.82	2	0	Trace
86.	2.06	2	0	Trace
87.	1.91	1	0	0
88.	1.83	2	0	0
89.	1.89	6	0	20
90.	2.67	6	0	20
91.	2.19	4	1	20
92.	1.26	4	0	30
93.	1.66	2	0	Trace
94.	1.95	2	0	10
95.	2.30	3	0	20
96.	1.37	8	0	20
97.	2.56	5	0	10
98.	2.29	2	0	10
99.	1.34	2	0	0
100.	2.27	2	0	Trace

No trees suffered terminal shoot defoliation

Tree No.	Height (m)	Number of healthy colonies pre-spray	Number of healthy colonies 26 days post-spray	Final defoliation estimate	Terminal defoliation
1.	2.37	9	4	50	
2.	2.06	2	1	20	
3.	1.76	0	1	10	
4.	1.31	0	4	Trace	
5.	1.96	4	0	30	
6.	1.06	0	5	10	
7.	2.16	2	0	60	
8.	2.11	2	0	90	
9.	1.70	0	0	0	
10.	2.06	4	3	80	
11.	1.41	3	1	10	
12.	0.58	0	0	0	
13.	0.58	0	0	0	
14.	1.42	0	0	0	
15.	1.94	4	2	60	
16.	2.37	9	0	20	
17.	1.27	0	0	0	
18.	2.18	8	0	80	
19.	0.87	0	0	0	
20.	1.34	0	1	10	
21.	1.42	1	0	80	
22.	1.43	4	0	20	
23.	1.01	1	1	20	
24.	1.21	1	0	10	
25.	1.83	2	0	50	yes
26.	2.07	9	4	40	
27.	2.27	4	2	60	yes
28.	1.60	2	0	80	yes
29.	0.84	0	0	0	
30.	1.82	4	2	20	yes
31.	2.53	5	1	10	
32.	2.31	2	0	30	
33.	1.02	0	2	10	
34.	2.22	4	0	Trace	
35.	1.61	3	0	50	yes
36.	1.69	0	1	Trace	
37.	2.04	5	0	Trace	
38.	1.12	0	0	0	
39.	1.35	0	0	0	
40.	1.35	0	0	0	
41.	1.43	0	0	0	
42.	2.22	3	0	40	yes
43.	0.70	0	0	0	
44.	2.20	5	0	30	
45.	1.21	1	2	90	yes
46.	2.03	1	1	70	
47.	1.39	2	1	Trace	
48.	1.60	4	0	50	
49.	0.88	0	0	0	
50.	1.39	0	2	50	yes

Tree No.	Height (m)	Number of healthy colonies pre-spray	Number of healthy colonies 26 days post-spray	Final defoliation estimate	Terminal defoliation
51.	2.37	0	0	0	
52.	2.11	4	5	40	yes
53.	2.52	3	1	40	yes
54.	1.56	2	2	40	yes
55.	2.52	4	1	80	yes
56.	1.67	3	2	30	
57.	1.61	4	1	Trace	
58.	2.41	4	1	90	yes
59.	0.65	0	1	20	
60.	0.73	0	0	0	
61.	1.54	4	1	40	
62.	1.62	2	0	Trace	
63.	2.08	0	0	0	
64.	1.76	1	0	60	yes
65.	2.28	3	2	90	
66.	2.25	3	2	40	yes
67.	1.81	1	3	Trace	
68.	1.77	2	0	0	
69.	1.45	0	0	0	
70.	0.63	0	1	50	
71.	1.43	5	3	60	yes
72.	2.17	5	1	60	yes
73.	2.00	5	2	40	yes
74.	2.17	2	0	30	yes
75.	0.84	0	0	0	
76.	1.94	5	2	20	
77.	2.22	4	0	30	yes
78.	1.76	1	1	30	
79.	1.77	0	2	40	yes
80.	1.62	4	0	60	yes
81.	1.99	0	0	0	
82.	2.45	6	2	20	yes
83.	2.44	10	2	10	
84.	2.23	3	1	30	
85.	1.60	2	0	30	yes
86.	1.53	0	2	30	
87.	1.71	4	0	40	
88.	1.66	0	0	0	
89.	2.24	0	3	30	yes
90.	2.65	4	2	95	yes
91.	2.77	12	2	70	yes
92.	2.40	6	3	70	
93.	1.82	1	0	10	
94.	0.81	3	1	40	
95.	2.19	6	4	10	
96.	2.37	4	1	30	
97.	1.87	1	2	70	yes
98.	2.42	3	1	90	
99.	1.37	1	3	90	
100.	1.81	1	10	90	yes

CHECK PLOT 2

Tree No.	Height (m)	Number of healthy colonies pre-spray	Number of healthy colonies 26 days post-spray	Final defoliation estimate	Terminal defoliation
1.	1.92	0	0	Trace	
2.	1.91	0	0	0	
3.	2.69	0	1	0	
4.	3.06	3	3	10	
5.	1.73	0	0	0	
6.	2.86	0	0	0	
7.	1.73	0	0	0	
8.	2.34	5	4	50	
9.	1.72	9	5	50	
10.	2.40	9	5	60	
11.	2.69	7	11	90	
12.	2.10	2	0	90	
13.	2.15	4	1	90	
14.	1.18	3	0	70	
15.	2.46	0	1	80	yes
16.	2.61	5	6	50	yes
17.	2.57	1	3	90	yes
18.	2.78	3	2	70	yes
19.	2.39	1	1	90	
20.	2.72	1	1	50	
21.	2.27	1	1	50	
22.	3.12	1	3	60	yes
23.	3.08	0	0	20	
24.	3.72	0	0	20	
25.	3.62	2	4	10	
26.	2.98	3	2	10	
27.	2.94	2	7	10	
28.	3.70	1	1	20	yes
29.	2.30	0	0	0	
30.	3.01	0	0	0	
31.	3.62	2	1	10	
32.	3.71	2	4	10	
33.	3.00	0	0	0	
34.	3.61	0	0	0	
35.	2.76	0	0	0	
36.	2.76	1	0	Trace	
37.	3.32	1	0	Trace	
38.	2.20	1	1	10	
39.	2.79	2	2	10	yes
40.	2.58	0	0	0	
41.	2.90	1	1	10	
42.	2.41	1	1	20	yes
43.	2.49	2	1	Trace	yes
44.	2.62	0	0	0	
45.	3.01	3	4	30	
46.	2.64	4	2	30	yes
47.	3.08	5	6	70	yes
48.	2.13	3	2	30	
49.	2.40	11	13	80	yes
50.	1.87	1	3	90	yes

Tree No.	Height (m)	Number of healthy colonies pre-spray	Number of healthy colonies 26 days post-spray	Final defoliation estimate	Terminal defoliation
51.	1.55	2	4	40	
52.	1.62	2	0	90	
53.	2.67	6	4	90	yes
54.	2.54	0	8	40	
55.	2.64	0	0	0	
56.	2.70	3	0	10	
57.	2.43	0	2	30	
58.	2.28	0	3	Trace	
59.	2.77	3	3	30	
60.	1.79	1	0	Trace	
61.	2.22	0	0	0	
62.	2.28	1	1	10	
63.	2.62	3	4	30	
64.	1.99	0	0	0	
65.	2.98	2	2	30	
66.	2.33	0	0	0	
67.	2.51	5	3	50	
68.	2.09	3	2	60	
69.	2.71	10	6	70	
70.	1.74	3	2	80	
71.	2.12	7	6	20	
72.	2.72	3	3	10	
73.	3.00	1	1	80	
74.	2.96	6	6	10	
75.	2.46	5	2	70	yes
76.	2.10	0	0	0	
77.	3.01	5	3	30	
78.	3.23	5	2	70	
79.	3.51	6	4	40	yes
80.	2.02	5	0	90	
81.	1.97	3	1	90	
82.	3.04	7	7	50	
83.	2.11	1	0	30	
84.	2.06	0	0	0	
85.	2.65	1	2	Trace	
86.	3.12	0	0	0	
87.	2.91	0	1	20	
88.	2.27	1	0	20	
89.	3.03	4	4	30	
90.	2.59	0	0	0	
91.	2.43	1	1	20	
92.	2.26	1	0	10	
93.	2.21	1	1	30	
94.	2.21	0	0	0	
95.	2.92	2	3	20	
96.	2.04	0	0	0	
97.	2.93	3	2	20	
98.	3.39	2	3	30	
99.	2.23	0	0	0	
100.	2.58	4	4	30	