

AERIAL APPLICATION OF A NUCLEAR POLYHEDROSIS
VIRUS AGAINST THE RED-HEADED PINE SAWFLY,
NEODIPRION LECONTEI (FITCH).

by

W. J. Kaupp and J. C. Cunningham
Insect Pathology Research Institute
Department of Fisheries and the Environment
Canadian Forestry Service
Sault Ste. Marie, Ontario,
Canada.

Information Report IP-X-14

January, 1977

ABSTRACT

Three plots of young red pine, Pinus resinosa Ait., heavily infested with red-headed pine sawfly, Neodiprion lecontei (Fitch), were aerially sprayed with a nuclear polyhedrosis virus. The active ingredient of the aqueous spray formulation was a powder prepared from lyophilized virus-infected larvae which contained 2.5×10^9 polyhedra/g. The application rate was 9.4 l/ha and treatments of 0.5 g/ha, 1.5 g/ha and 2.5 g/ha were tested. All three treatments were successful in controlling this insect. A high level of insect mortality was obtained in 18 days in the plot receiving the heaviest dosage and there was excellent foliage protection in all three treated plots when compared to the check area. It is recommended that the use of this virus be promoted for the biological control of N. lecontei.

RESUME

Trois places-échantillons de jeune Pin rouge, Pinus resinosa Ait., gravement atteint par le Diprion de LeConte, Neodiprion lecontei (Fitch), ont été arrosées du haut des airs avec un virus de la polyhédrose nucléaire. L'ingrédient actif de la formule de pulvérisation aqueuse consistait en une poudre préparée avec des larves infectées de virus lyophilisés, contenant 2.5×10^9 polyhédres/g. Le taux d'application fut de 9.4 l/ha et des traitements à raison de 0.5 g/ha, 1.5 g/ha et 2.5 g/ha furent testés. Les trois traitements réussirent à réprimer cet insecte. On obtint un taux élevé de mortalité de l'insecte en 18 jours dans la place-échantillon qui recut la dose la plus forte et une excellente protection du feuillage dans les trois places-échantillons traitées, comparativement aux secteurs témoins. Les auteurs recommandent de promouvoir l'emploi de ce virus pour la lutte biologique livrée à N. lecontei.

INTRODUCTION

The red-headed pine sawfly, Neodiprion lecontei (Fitch), is one of the most damaging insects attacking hard pines. This sawfly is a major pest in young plantations, in nurseries and on ornamentals. Increased plantings of pure stands of red pine throughout Ontario and Quebec have provided ideal conditions for the development of periodic outbreaks of this insect. The wide range of host tree species attacked, and the severe damage inflicted upon these trees, necessitates the development of a safe and effective method of controlling this sawfly.

The spraying of chemical insecticides has provided adequate control in the past but with recent, more widespread outbreaks of this insect and the increasing concern for environmental quality, alternative methods of control have become increasingly important. Certain insect viruses may offer a substitute for chemicals. The possibility also exists that such viruses may, in the long run, prove to be cheaper because they persist and continue to have an effect on the insect population over a period of several years following application. Insect viruses have the added attraction that they have no undesirable side-effects and present no hazard to fish, wildlife or beneficial insects.

The nuclear polyhedrosis virus (NPV) which infects the red-headed pine sawfly was first discovered in Ontario in 1950 (Bird, 1961). It has been used successfully throughout Eastern

Canada and small experiments have been conducted to determine the dosage response of the insect (Bird, 1955; Anon., 1970). Occasionally private tree growers have used this virus to control small populations of sawfly in their plantations. All such applications have been made with ground-based equipment. The only recorded instance of an aerial spray against the red-headed pine sawfly in Canada occurred in 1944, when DDT was successfully applied to control a 2 ha infestation in Algonquin Park, Ontario (Stewart, 1949).

The following experiment was conducted to determine the feasibility of an aerial application of NPV against the red-headed pine sawfly, and to determine the concentration of virus required to give adequate control.

MATERIALS AND METHODS

The Virus

The NPV used in this operation was propagated in larvae infesting a red pine plantation near Palmer Rapids, Ontario in 1975. This method of virus production has been used frequently and is reported by Cunningham et al (1975a). Colonies of NPV-infected larvae were collected in the field, larvae were picked off, lyophilized and ground to a fine powder using a domestic blender. This virus-infected material contained 2.5 billion polyhedral inclusion bodies (PIBs) per gram.

The Experimental Plots

Three experimental plots and one check plot were located in two red pine plantations managed by the Ontario Ministry of Natural Resources under the Woodland Improvement Act (WIA). Plots 1 and 3 were selected in the largest plantation located on Lots 11 and 12, Concession XVII in Harvey Twp. near Lakefield, Ontario. These plots were 16 ha and 19.2 ha in area respectively, and contained red pine trees ranging in age from 4-6 years. Plot 2 and the check plot were located in a plantation on Lot 16, Concession I of Duro Twp., Ontario. These plots were 8 ha and 4 ha in area respectively and contained 8 year old red pine. All plots were heavily infested with red-headed pine sawfly.

Treatments and Formulations

Three different virus dosages were tested in this experiment, with 0.5 g/ha, 1.5 g/ha and 2.5 g/ha of freeze dried virus-infected

material applied to plots 1, 2, and 3 respectively (Table 1).

The virus material used in each plot was suspended in 20 l of distilled water using a Pyrex tissue grinder and stored at 4°C for three days prior to the spray. IMC 90-001¹, an ultraviolet protectant, was added at a rate of 60 g/l to help prevent sunlight inactivation of the NPV. This protectant has a dark colouration and also marks spray cards used in deposit assessment. Final mixing, using water from a nearby river, was done just prior to spraying. The virus suspensions were mixed, filtered through a double layer of nylon fabric and loaded into the aircraft.

Spray Application and Larval Development

The spray was applied at 6 a.m. on the morning of July 5, 1976 under ideal weather conditions. A Piper PA18 Super Cub², equipped with a boom and nozzle spray system (26 D7 disc nozzles), applied the formulations. The aircraft was guided from the ground by moving a red helium-filled meteorological balloon at 30 m intervals across each plot. The aircraft sprayed from an approximate height of 15 m above the trees and, flying a 30 m swath width, the rate of application was 9.4 l/ha.

Plot 1 was sprayed first with the lowest dosage, followed by plot 2 and then plot 3 to avoid contamination of the spray equipment with the higher virus concentrations. The sawflies were predominantly in the second larval instar when sprayed.

- 1) Sandoz-Wander Inc., Homestead, Florida.
- 2) Sandham Air Service, Port Hope, Ontario.

Table 1

Location, size and virus dosage applied
to the experimental plots

	Location	Area (ha)	Virus Dosage PIB/ha
Plot 1	Lot 11&12; Con. XVII Harvey Twp.	16	1.25×10^9
Plot 2	Lot 16; Con. I Duro Twp.	8	3.75×10^9
Plot 3	Lot 11&12; Con. XVII Harvey Twp.	19.2	6.25×10^9
Check Plot	Lot 16; Con. I Duro Twp.	4	

Plot 2 showed more variation in larval development since 27% of the colonies were still unhatched at the time of spray application.

Monitoring Spray Deposit

The spray deposit was monitored on Kromekote^R spray cards mounted on 100 x 150 mm aluminum backings. Millipore gridded filters, Type HA 0.45 μ , also attached to the backings were used to directly monitor the deposit of active ingredient (PIBs). The cards and filters 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 and size were determined from the Kromekote^R cards. The millipore filters were stained, mounted on a slide and examined under a Leitz Ortholux microscope to determine the numbers of PIBs per droplet (Morris, 1973).

Weather data were recorded daily. Temperature, relative humidity and precipitation were monitored in each plot using a hygrothermograph and standard rain gauge.

Assessment

To record the impact of NPV on the sawfly population, 50 trees were selected with no regard to insect population and tagged. Each plot was transected twice by the sample lines. Tree height, number of healthy sawfly colonies (hatched and unhatched), and the degree of defoliation were then recorded for each tree. These trees were checked repeatedly both for reduction in the sawfly population density and for defoliation.

Defoliation was determined by estimating the current percent of foliage consumed on each sample tree in each of the plots. These defoliation estimates were recorded periodically throughout the course of the experiment, with the final defoliation figures being taken three months after sawfly pupation. Permanent tree damage was assessed by recording the number of sample trees in each plot having terminal shoots which were completely defoliated.

The rate of development of the NPV infection within the sawfly population was studied by selecting ten colonies in each plot and recording the first signs of mortality. Kromekote^R cards placed beneath these colonies showed that spray had been deposited on them. Five living insects were also selected at three day intervals from each of these colonies for microscopic diagnosis. This entailed examining insect gut squash preparations for the presence of nuclei filled with polyhedra using a Wild M5 dissecting microscope.

RESULTS

Measurements of the height of the trees selected for the assessment and the number of sawfly colonies found on them are shown in Appendix 1. Prior to the spray operation and throughout the post spray population monitoring, hot and dry weather conditions prevailed with a mean day temperature of 23°C and a mean night temperature of 16°C. The lowest temperature recorded was 5°C, twenty days post-spray. The total rainfall recorded was 1.1 cm and 1.7 cm on plots 2 and 3 respectively.

The results of the spray card analysis to determine droplet density are shown in Table 2. Deposit was recorded on all sample cards within the plot perimeters. The variations in droplet density between the plots reflect the changing meteorological conditions during the spray operation. The droplet size spectrum (Figure 1) shows that in each plot over 50% of the droplets were under 200 μ in diameter.

Determination of the PIB deposit from the stained millipore filters was unsuccessful due to the difficulty in measuring the diameters of small droplets. Without this information, it was impossible to establish any relationship between droplet size and PIB content. Since this technique has been used successfully with aqueous formulations containing molasses, it is assumed that the failure of this experiment was due to the lack of molasses in this formulation. Nevertheless, stained PIBs were found in all droplets examined.

The first virus killed larvae were recorded 16, 14 and 11 days post-spray for plots 1, 2 and 3 respectively. The sawfly

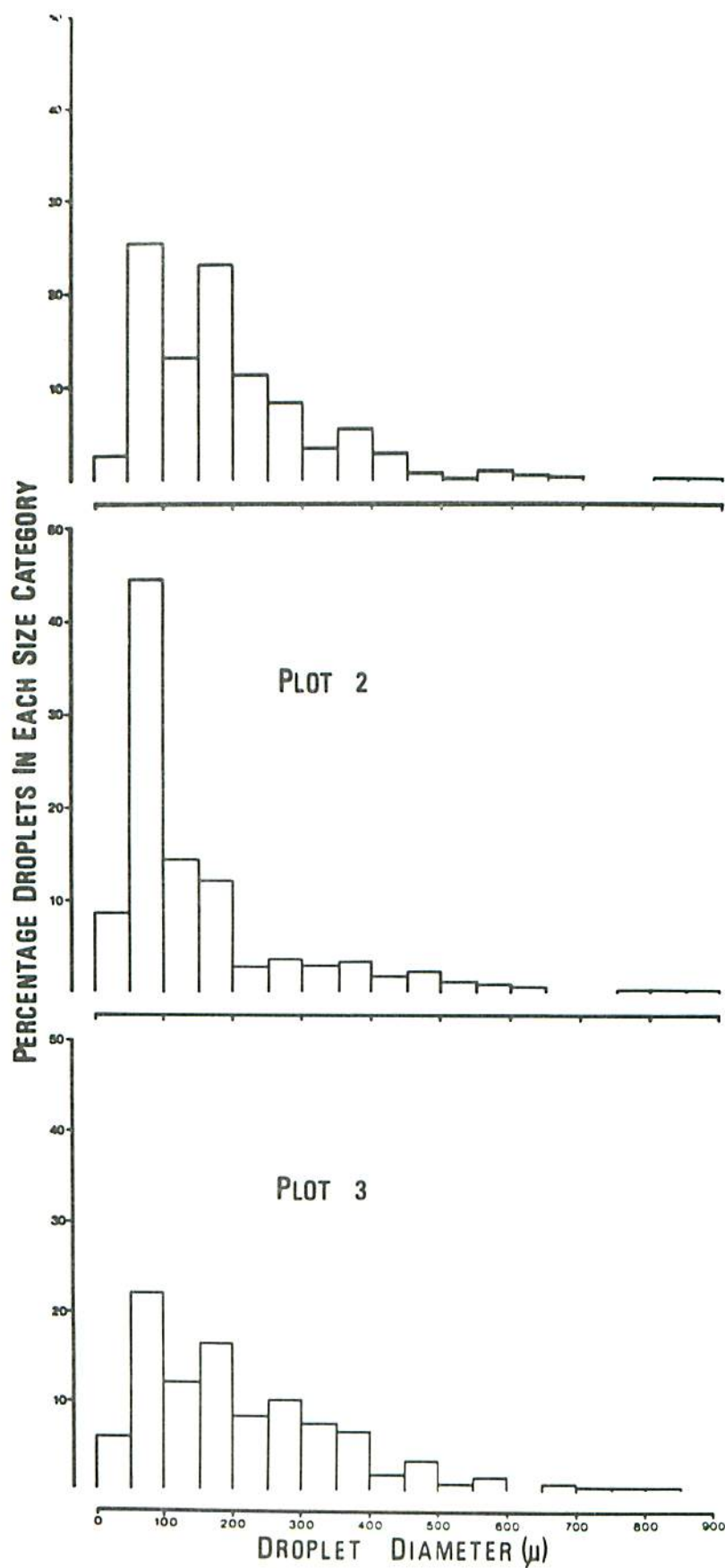


Fig. 1. Spectrum of Droplet Size.

larvae had advanced to the fifth instar before mortality occurred in plot 1, and were predominantly in the fourth instar in plots 2 and 3. Results of microscopic diagnosis to determine the levels of NPV infection in the sawfly populations in each spray plot are shown in Table 3. Infection in plot 3, which received the heaviest dosage, was always more advanced than in the other two plots and plot 1 had the slowest rate of infection. Low levels of NPV infection, 7%, 4% and 4%, were recorded in the check plot 11, 14 and 17 days post-spray.

Population reduction in the three sprayed plots was directly related to the rate of development of the NPV infection, which in turn was related to the dosage applied. Reduction in the number of sawfly colonies following NPV treatment for each plot is shown in Table 4. The 46 colonies recorded at 18 days post-spray for plot 2 contained many recently hatched larvae and microscopic diagnosis indicated that 50% of these colonies, which had emerged after the spray application, were infected with NPV. The number of colonies in the check area remained the same throughout the experiment. The sawfly population was decimated by the virus in all three spray plots 22 days after the spray application, the rate of population decline depending upon the NPV dosage.

Defoliation estimates recorded while the experiment was in progress gave indications that trees in the sprayed plots suffered less defoliation than those in the check plot. A final defoliation estimate, taken three months after pupation of the sawfly, supported this observation (Appendix 1). Plots 1, 2 and 3 had

average defoliation estimates of 3.4%, 5.2% and 3.2% respectively as compared to 21% for the check plot. Examination of the terminal shoots of the sample trees for defoliation (indicating permanent tree damage) revealed that only the check plot suffered such defoliation. Here 30% of the sample trees had the terminal shoots completely defoliated and will probably develop into deformed trees.

Table 2

Mean number of droplets per cm^2 on spray cards

Plot	Mean number Drops/ cm^2	Standard Deviation
1	33.0	14.7
2	45.4	15.8
3	27.8	19.6

Table 3

Results of microscopic examination of samples
of 50 larvae per plot to determine the level
of virus infection

Days Post Spray	Percent Virus Infection			
	Plot 1	Plot 2	Plot 3	Check
8	36	54	74	0
11	32	60	74	7
14	74	76	95	4
17	92	96	98	4

Table 4

Reduction of number of sawfly colonies
following NPV treatment

Number of colonies per 50 trees				
	Plot 1	Plot 2	Plot 3	Check
Pre-spray	59	138	173	119
Days Post spray				
12	45	116	109	110
18	17	47	6	116
22	7	46*	1	115

*This figure is misleading since 27% of the colonies were unhatched at the time of application (see text).

DISCUSSION

The results of this experiment prove that NPV can be successfully applied from an aircraft to control outbreaks of the red-headed pine sawfly. Under epidemic conditions, dosages of 6.5 billion PIBs/ha, 3.25 billion PIBs/ha and 1.25 billion PIBs/ha all give good control which resulted in excellent foliage protection. One of the goals of the experiment was not achieved in that the lowest dosage of virus giving acceptable control was not established. Differences in the degree of defoliation among the sprayed plots are due mainly to population variations in each plot. The average defoliation estimates for plots 1, 2 and 3 compared to the check plot, along with the respective population reduction figures, clearly demonstrate the potential usefulness of NPV for the control of this sawfly. The defoliation estimate for plot 2 which is higher than plots 1 and 3 is due to the late hatch and subsequent late infection of the larvae by NPV.

The excellent spray application, as reflected by the deposit analysis, contributed to the success of the experiment. The colonial habits of the insect and the virulence of the NPV ensure that death of a colony will result if only one insect becomes infected, because infected larvae spread the NPV to other members of the colony. This gives the virus a considerable advantage over chemical control methods.

The diagnosis of low levels of NPV in the check plot, 11 days after the spray, is an important point to consider (Table 3).

Although mortality was never found in the check plot, the presence of the virus may be an indication of natural spread from the sprayed areas by birds or predators.

It is anticipated that the virus will be found in the sawfly population in these plantations in 1977 and will control any insects which survived the 1976 operation. This phenomenon has been observed and documented for NPVs infecting Neodiprion sertifer (Geoff.) and Diprion hercyniae (Hartig) (Bird, 1961, Entwistle, personal communication).

The virus is also expected to spread to other nearby red-headed pine sawfly infestations in 1977. This occurrence has been noted in previous ground spray trials but has never been documented. It is postulated that this spread is due to predators such as Syrphids which are commonly found in association with sawfly colonies and by sub-lethally infected sawfly adults. Birds have been found to be important vectors of D. hercyniae NPV in Wales, U.K. (Entwistle, personal communication) by spreading virus in their faeces.

In Canada, viable N. sertifer NPV was recovered from birds' stomachs (Bird, 1955). No information is available regarding the role of birds in the transmission of N. lecontei NPV but, based on observations on other sawfly viruses, it is highly probable that they are involved.

A rough estimate puts the cost of the freeze-dried virus-infected larval material at \$3.00 to \$4.00 per hectare for the

heaviest concentration used in this experiment. This estimate has been made from the cost of the labour required to infect, collect and process sawfly larvae in the field. Cost of laboratory-produced virus would be considerably higher.

Further field testing using even lower dosages, along with safety testing required for registration under the Pest Control Products Act, are recommended to establish this NPV as an effective, readily available bio-insecticide.

ACKNOWLEDGMENTS

We gratefully acknowledge the assistance and co-operation of Mr. J.H. Broderick, Management Forester, Ministry of Natural Resources, Lindsay, Ontario. The excellent technical assistance of Mr. H. Weir, Forest Insect Technician, Great Lakes Forest Research Centre, and Mr. P. de Groot and Mr. J.R. McPhee, Insect Pathology Research Institute was greatly appreciated. We also wish to thank the individual land owners for granting us permission to use their plantations for this research.

REFERENCES

- Anonymous. 1970. Virus trials to control red-headed pine sawfly in Quebec plantations. Can. For. Ser. Inf. Rept. DPC-X-1.
- Bird, F. T. 1955. Virus diseases of sawflies. Can. Ent. 87, 124-127.
- Bird, F. T. 1961. Transmission of some insect viruses with particular reference to ovarian transmission and its importance in the developments of epizootics. J. Insect Pathol., 3, 352-380.
- Cunningham, J. C., W. J. Kaupp, J. R. McPhee, W. L. Sippell and C. A. Barnes. 1975a. Aerial application of a nuclear polyhedrosis virus to control European pine sawfly, Neodiprion sertifer (Geoff.) at Sandbanks Provincial Park, Quinte Island, Ontario in 1975. Can. For. Ser. Sault Ste. Marie, Inf. Rept. IP-X-7.
- Morris, O. N. 1973. A method of visualizing and assessing deposits of aerially sprayed insect microbes. J. Invertebr. Pathol., 22, 115-121.
- Stewart, K. E. 1949. Application of DDT sprays by aircraft in Canada for the control of the spruce budworm, Archips fumiferana (Clem.) pp. 93-140. In Forest Spraying and some effects of DDT. Ont. Dept. Lands and Forests. Biol. Bull. No. 2.

APPENDIX 1

Height, number of healthy sawfly colonies (prespray and 22 days post-spray), final defoliation estimates and terminal shoot damage for sample trees in each plot.

PLOT 1

Tree No.	Height (m)	Number of healthy colonies pre-spray	Number of healthy colonies 22 days post-spray	Final defoliation estimate
1.	.885	4	0	30
2.	.719	0	0	0
3.	.528	2	2	0
4.	1.134	10	0	10
5.	.605	0	0	0
6.	1.040	0	0	0
7.	.643	0	0	0
8.	1.019	1	1	5
9.	.744	0	0	0
10.	.979	1	0	0
11.	.637	0	0	0
12.	1.083	3	0	0
13.	.968	1	0	0
14.	.562	2	0	10
15.	.600	1	0	5
16.	.424	0	0	0
17.	.837	0	0	0
18.	.853	1	0	0
19.	.851	1	0	20
20.	.677	0	0	0
21.	.692	0	0	0
22.	.632	0	0	0
23.	.865	0	0	0
24.	.984	1	0	5
25.	.875	0	0	0
26.	.952	5	0	25
27.	.914	3	0	0
28.	.581	0	0	0
29.	.906	2	1	20
30.	.792	0	0	0
31.	.938	1	0	0
32.	.736	0	0	0
33.	.809	1	0	0
34.	.761	0	0	0
35.	1.033	4	0	25
36.	.629	1	1	0
37.	.784	0	0	0
38.	.859	0	0	0
39.	.732	1	0	0
40.	1.047	4	1	0
41.	.674	0	0	0
42.	.648	0	0	0
43.	.931	0	0	0
44.	.953	2	0	0
45.	.901	2	0	0
46.	.568	0	0	0
47.	.706	0	0	0
48.	.636	0	0	0
49.	1.192	3	1	10
50.	1.025	2	0	5

$\bar{X} = 3.4$

No trees suffered terminal shoot defoliation

PLOT 2

Tree No.	Height(m)	Number of healthy colonies pre-spray	Number of healthy colonies 22 days post-spray	Final defoliation estimate
1.	1.316	1	0	0
2.	.912	0	0	0
3.	1.498	0	0	5
4.	1.408	2	0	35
5.	1.275	0	0	0
6.	1.018	0	0	0
7.	1.814	3	0	30
8.	1.586	5	0	5
9.	1.382	4	1	5
10.	1.844	1	0	0
11.	1.752	0	0	0
12.	1.397	0	0	0
13.	2.449	2	0	0
14.	2.214	2	4	0
15.	2.043	0	1	5
16.	1.921	2	0	0
17.	1.890	0	0	0
18.	2.276	8	0	0
19.	2.471	7	0	0
20.	2.228	10	1	-
21.	2.512	12	0	10
22.	2.284	2	2	40
23.	1.491	1	0	5
24.	2.464	14	9	5
25.	1.975	7	0	5
26.	2.493	6	9	15
27.	2.176	4	1	5
28.	1.236	4	0	0
29.	1.469	0	0	0
30.	1.386	1	0	5
31.	2.226	8	0	10
32.	2.270	6	0	0
33.	1.039	0	0	0
34.	1.889	1	3	0
35.	1.621	1	2	5
36.	1.395	0	0	15
37.	1.976	8	3	5
38.	1.869	3	0	20
39.	2.111	5	1	20
40.	1.428	1	1	0
41.	2.071	2	4	0
42.	1.506	0	0	0
43.	1.866	0	1	0
44.	1.904	0	0	5
45.	1.994	0	0	0
46.	1.941	0	0	0
47.	1.946	0	0	0
48.	1.794	1	1	0
49.	2.285	1	1	0
50.	1.981	3	1	0

No trees suffered terminal shoot defoliation $\bar{X} = 5.2$

PLOT 3

Tree No.	Height(m)	Number of healthy colonies pre-spray	Number of healthy colonies 22 days post-spray	Final defoliation estimate
1.	.982	2	0	0
2.	.639	0	0	-
3.	.799	0	0	0
4.	.819	2	0	0
5.	.692	0	0	0
6.	.713	0	0	0
7.	.629	0	0	0
8.	.950	4	0	0
9.	1.619	13	0	5
10.	1.597	0	0	0
11.	2.005	5	0	0
12.	1.168	0	0	0
13.	1.382	2	0	0
14.	1.467	5	0	5
15.	1.926	10	0	0
16.	1.314	4	0	10
17.	1.841	13	0	25
18.	1.629	5	0	15
19.	1.192	11	0	20
20.	1.388	3	0	0
21.	1.894	8	0	5
22.	2.263	7	0	10
23.	1.193	6	0	10
24.	1.337	2	0	0
25.	2.299	11	0	5
26.	1.641	2	0	5
27.	.980	0	0	0
28.	1.035	0	0	0
29.	1.065	0	0	0
30.	1.778	2	0	0
31.	1.962	2	0	0
32.	1.734	7	0	5
33.	1.352	3	0	0
34.	1.586	1	0	0
35.	.825	0	0	0
36.	1.975	3	0	0
37.	1.556	2	0	0
38.	1.110	2	0	0
39.	1.861	6	0	5
40.	2.009	4	0	5
41.	1.362	2	0	0
42.	1.631	3	0	5
43.	1.532	6	0	0
44.	1.165	0	0	20
45.	.902	0	0	0
46.	1.431	7	0	5
47.	1.178	1	0	0
48.	.643	0	0	0
49.	.813	1	0	0
50.	1.110	6	1	0

$\bar{x} = 3.2$

No trees suffered terminal shoot defoliation

CHECK PLOT

Tree No.	Height(m)	Number of healthy colonies pre-spray	Number of healthy colonies 22 days post-spray	Final defoliation estimate	Terminal Defoliation
1.	2.125	2	1	10	Yes
2.	2.033	10	8	80	Yes
3.	2.228	5	3	60	
4.	2.375	11	11	85	Yes
5.	2.105	3	2	50	Yes
6.	1.321	0	1	25	
7.	2.628	3	4	35	Yes
8.	1.892	3	4	60	
9.	1.849	1	0	10	
10.	2.015	2	2	15	
11.	1.645	0	0	0	
12.	1.875	2	2	20	Yes
13.	1.965	2	4	25	Yes
14.	2.234	0	0	5	
15.	1.836	0	0	0	
16.	1.685	1	0	0	
17.	2.521	1	1	5	
18.	1.779	2	2	15	
19.	2.279	0	0	0	
20.	2.115	2	2	35	Yes
21.	2.072	2	3	30	Yes
22.	2.261	2	0	0	
23.	2.200	5	6	55	
24.	2.125	8	7	25	Yes
25.	1.855	3	3	80	Yes
26.	1.815	1	1	0	
27.	1.805	4	2	50	
28.	1.985	2	4	40	
29.	2.345	2	3	10	Yes
30.	1.682	1	1	5	
31.	2.152	0	0	0	
32.	1.845	3	2	10	
33.	1.719	1	1	5	
34.	2.005	0	0	0	
35.	2.638	3	3	15	
36.	1.518	1	0	0	
37.	2.200	3	2	20	Yes
38.	1.818	2	3	20	
39.	1.692	1	1	5	
40.	2.115	3	4	20	Yes
41.	1.601	1	1	0	
42.	2.135	4	2	15	Yes
43.	2.103	1	2	10	
44.	1.941	0	1	5	
45.	2.485	1	1	10	
46.	1.852	2	1	5	
47.	1.452	4	6	10	
48.	1.418	3	2	60	
49.	2.062	4	5	10	
50.	2.084	2	1	0	