

NUCLEAR POLYHEDROSIS VIRUS
DISPERSAL TRIALS
AGAINST
THE DOUGLAS-FIR TUSSOCK MOTH
1983

by
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by

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ABSTRACT

Nuclear polyhedrosis virus (NPV) was applied by a commercial ground sprayer to a small group of Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) within a Douglas-fir tussock moth (Orgyia pseudotsuga (McDunnough) infestation, approximately 20 Kilometers northwest of Cache Creek, British Columbia. The dosage of NPV used was 3.6×10^9 PIB/liter mix/tree. Larval population data and NPV smear analysis indicated the spread of NPV from the inoculum center of 15 meters south and west and 25 meters to 50 meters north of the inoculum center. Spray drift and the occurrence of a natural NPV epicenter precluded any evaluation of the spread of NPV in the east direction. There was evidence of minimal foliage protection of 1982 growth within the inoculum center.

I. INTRODUCTION

Outbreaks of the Douglas-fir tussock moth (DFTM), Orgyia pseudotsuga (McDunnough), have occurred periodically in the interior of British Columbia since 1916 (Canadian Forestry Service 1980; Wood and Woensdregt 1982) (Figure 1). These outbreaks occur mainly in dry belt Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) stands that contain a mixed component of ponderosa pine (Pinus ponderosa Law.) and Douglas-fir. In 1982 a total of 12 820 hectares were attacked by this forest pest; 9 743 hectares of this total being productive forest area (Ross and Taylor 1983). Ross and Taylor (1983) predicted that 6 514 hectares would be attacked in 1983; 4 944 hectares of this total being productive forest area.

These outbreaks are usually characterized by a rapid increase in insect numbers, 1 or 2 years of high densities and finally a collapse in the population (Mason 1981; Brookes et al 1978). Tree damage is caused through larval feeding. First- and second-instars feed on the underside of new needles turning the needles a reddish brown. Third to last instars feed on both new and old needles. The amount of tree mortality, top-kill, growth reduction and foliage recovery is directly related to the degree of defoliation (Brookes et al 1978; Wickman 1978, 1979).

Natural control agents of DFTM infestations include parasites, predators, pathogens and starvation. The nuclear polyhedrosis virus (NPV) is generally regarded as the most important natural agent which causes the population decline of a DFTM infestation

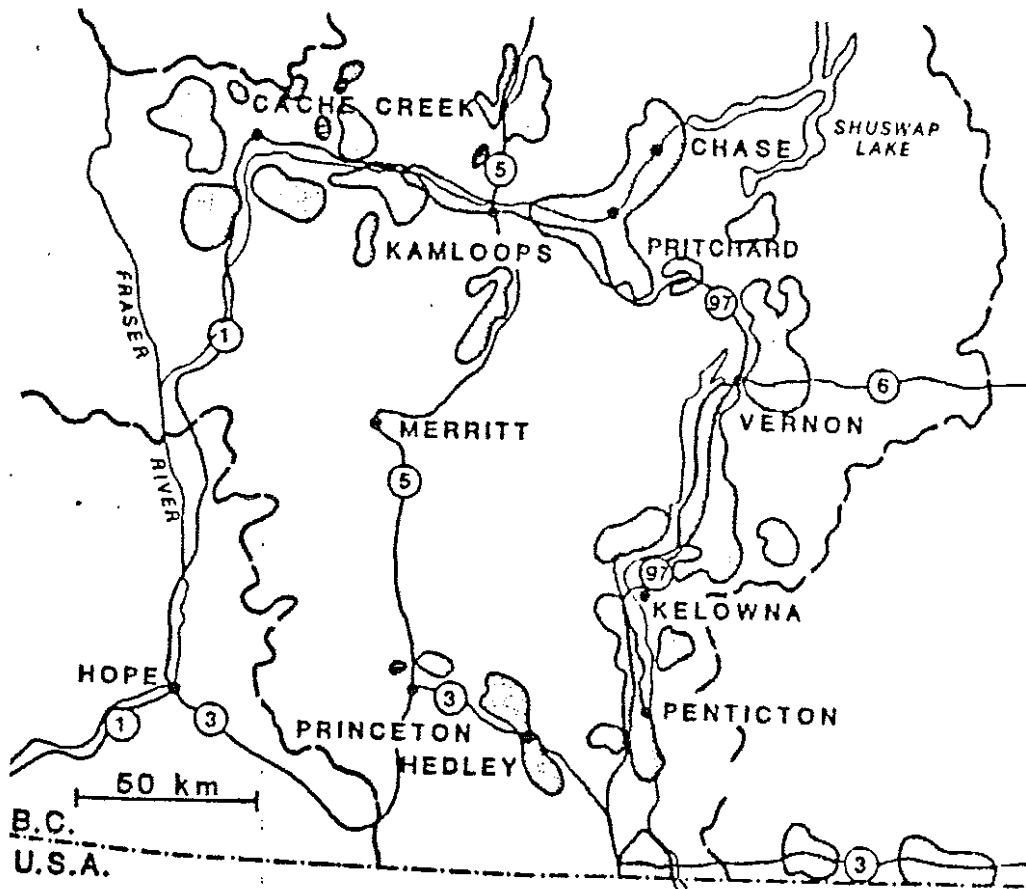


Figure 1: Areas that have sustained one or more DFIM outbreaks since 1916 (Source: Canadian Forestry Service report BC-X-201)

(Dahlsten and Thomas 1969). Under natural conditions the amount of NPV build-up within a population which would be sufficient to cause the population collapse usually only occurs after 2 to 3 years of defoliation. During this period tree growth loss, top-kill dieback and some tree mortality has already occurred.

Management systems for the DFTM include chemical (insecticides or pheromones) or microbial (viral or bacterial) agents (Brookes et al 1978). Few of these agents are registered for the control of DFTM in Canada. At present, the microbial agent NPV (VIRTUSS^R)¹ has temporary registration for the control of DFTM in Canada².

Though NPV has shown to be effective when applied aerially in controlling DFTM infestations (Shepherd, Otvos & Chorney 1984a; Friskie and Chorney 1983), there is a need to demonstrate its effectiveness when spot applications are made with ground spraying equipment. In particular, further information is needed to determine the dispersal of NPV from a spot application point within an infested area. Preliminary work in 1981 (Shepherd, Otvos & Chorney 1984a) indicated that ground treatment of individual trees, at 2.4×10^{10} PIB/tree was sufficient to cause an epizootic up to 100 meters from the treated trees. In an attempt to verify the spread of NPV within an infested area the British Columbia Ministry of Forests with the advise and assistance of the Canadian Forestry Service [Pacific Forest Research Centre (PFRC), Victoria, B.C.; Forest Pest Management Institute (FPMI), Sault Ste. Marie, Ontario] carried out a

1: Trademark, CFS, Sault Ste. Marie, Ontario.

2: Dr. J. Cunningham CFS/FPMI, Sault Ste. Marie, Ontario, pers.comm.

trial in the summer of 1983.

The objective of this trial was to determine if the application of NPV to a small group of trees within a DFTM infestation, would create an epizootic which would then spread outward from the application center.

II. METHODS

In the Spring of 1983 representatives of the BCMF conducted a sequential egg mass survey (Shepherd, Otvos & Chorney 1984b) in the study area located approximately 20 Kilometers North-west of Cache Creek, British Columbia (Figure 2). This survey indicated that sever defoliation could be expected in this area in 1983. Overwintering egg masses were collected and sent to PFRC for NPV analysis. The results indicated that virtually no NPV was present in the study area.

Egg hatch and larval dispersal were monitored prior to NPV treatment. The area was spot treated with NPV when there was 100 percent larval dispersal from the egg masses and larval development was predominately second-instar.

Spot treatment consisted of treating a group of 18 Douglas-fir trees with NPV using a commercial ground spray system (B & W Agricultural Services). The system components were: two mixing tanks having a total capacity of 1134 liters, a mechanical agitator and a spray gun system (Jet 2A: nozzle type AYHSS 60).

Each tree was sprayed to 'drip point'. The formulation

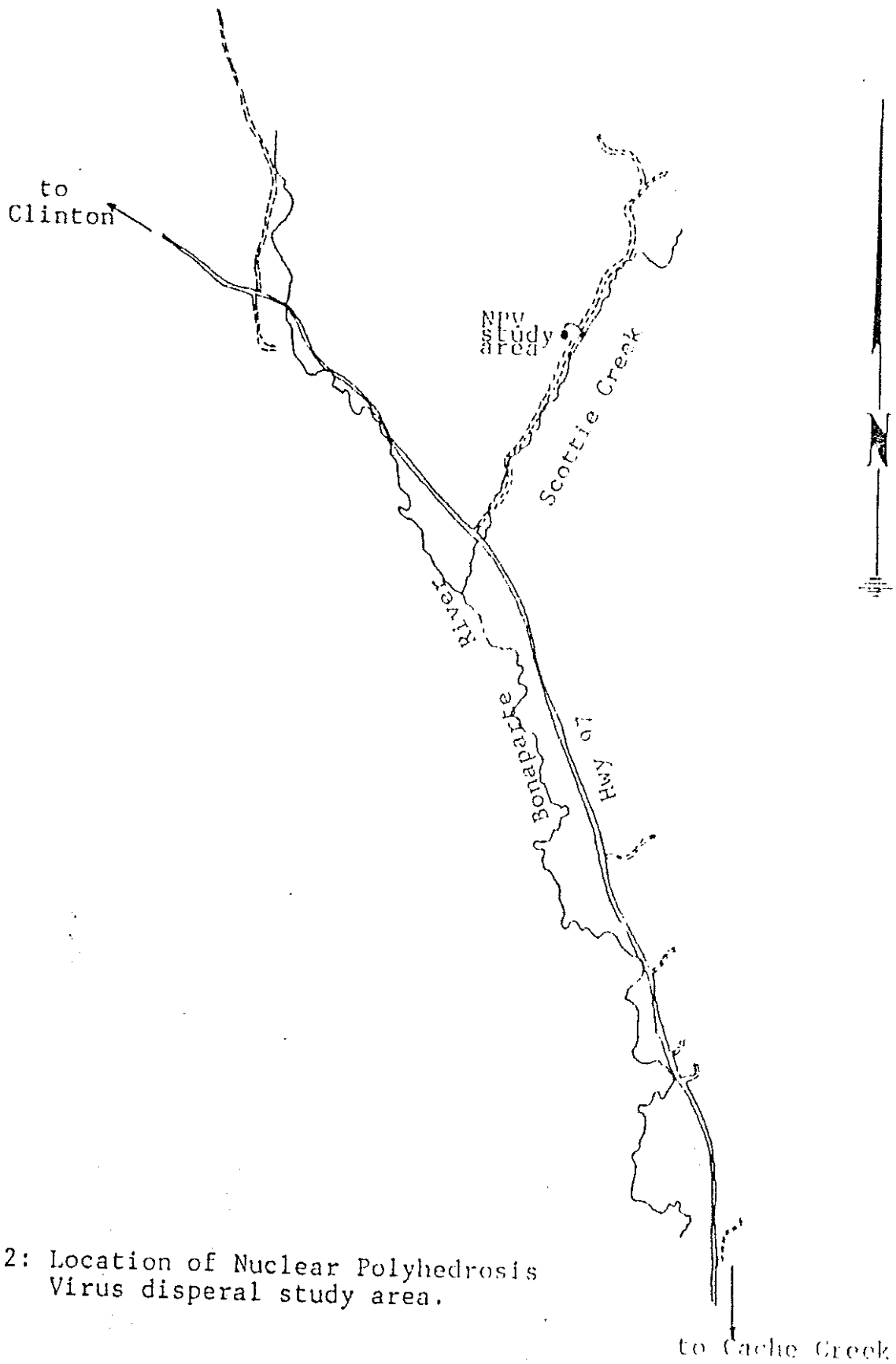


Figure 2: Location of Nuclear Polyhedrosis Virus dispersal study area.

of the virus was 80 liters of water plus 20 liters of molasses plus 1 liter of NPV (1 liter NPV = 1×10^{11} PIB)¹. The dosage of NPV used was 3.6×10^9 PIB/liter mix/tree.

Kromekote^R cards were placed in the cardinal directions and 45 degrees to the cardinal directions, 10 meters from the perimeter of the virus inoculum center to monitor spray drift during treatment. NPV application occurred at dawn (temperature = 9 degrees Centigrade; wind was calm; precipitation was nil).

The cardinal directions were used to locate the larval sampling sites. These sites were positioned 10 meters, 25 meters, 75 meters and 100 meters from the virus inoculum center on the North and South lines. Only three sample sites, located 10 meters, 25 meters and 40 meters from the center were established on the East and West lines (Figure 3). Natural boundaries on the East and West lines precluded additional sampling sites.

DFTM larval population density and NPV-infection of larvae were monitored at the pre-spray time (4 days prior to treatment) and at weekly intervals from week 2 post-spray until pupation (week 9 post-spray). Monitoring of larval population density consisted of clipping two 45 centimeter branch tips from the mid-crown section of 2 pre-selected sample trees, at each of the cardinal sampling sites; 4 pre-selected trees were sampled within the NPV inoculum center. A basket equipped pruning pole was used to reach and clip these branches. The foliated surface area was measured and the number of larvae counted and recorded for each branch.

¹: Dr. J. Cunningham, CFS/FPMI, Pers. Commun.

Mean larval density per square meter was calculated at the pre-spray sample time and each of the post-spray sample times (Table I). This data was transformed into a survival ratio¹ (Table III; Figure 4) to allow a comparison of larval survival between each sample site, through time.

Pre-spray larval NPV-infection was assessed by collecting 100 larvae (25 larvae/sample tree) at the virus center and 50 larvae (25 larvae/sample tree) at each of the cardinal sample sites. These larvae were grouped by sample site, placed in bags containing foliage and sent to FPPI for rearing and subsequent determination of the incidence of natural virus within the study area. The diagnosis of viral infection was through the staining and microscopic examination of the viral particles (Wigley 1980).

Post-spray larval NPV infection was assessed as per the pre-spray larval NPV infection assessment.

Defoliation was assessed by visually estimating the percentage of defoliation of new growth (1983) and old growth (1982) for each of the sample branches. This assessment was made at each of the post-spray sample times at each sample site.

III. RESULTS AND DISCUSSION

1. General

Throughout this section the sampling site locations are coded by letters (Figure 3). The first letter identifies the cardinal direction; the second letter identifies the distance from the NPV inoculum center (A=10m, B=25m, C=50m, D=75m and

1: survival ratio = mean larval density post-spray/mean larval density pre-spray.

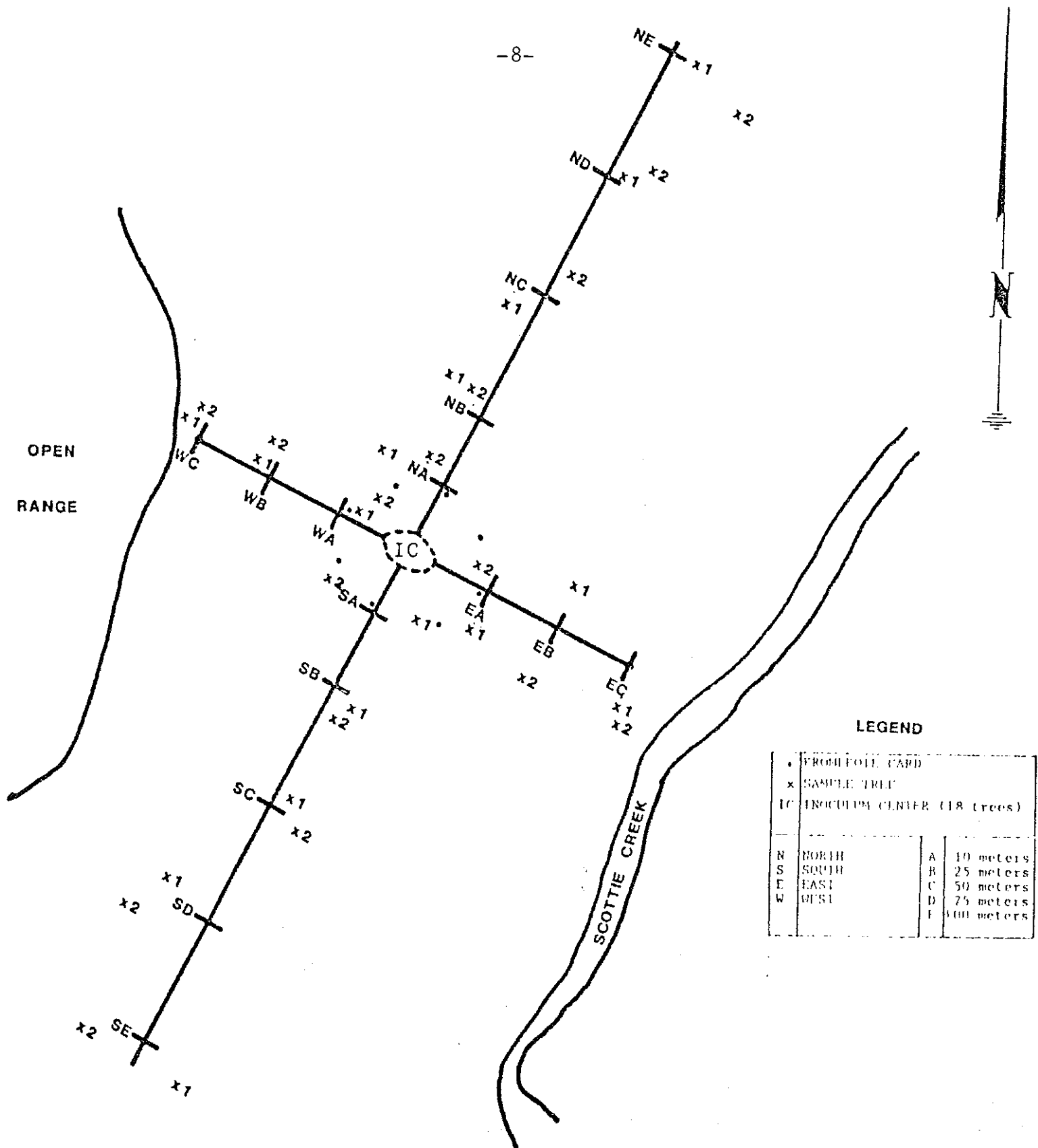


Figure 3: Location of sample trees and virus center.

100m). The exceptions to this coding distance is site 'C' in the East and West directions (C=40m).

Kromekote^R cards placed on the ground 10 meters from the perimeter of the NPV inoculum center showed that some spray drift had occurred in the West, South-west, South and East directions. This would account for the 11.46% NPV-infection at EA and 17.2% NPV-infection at WA two weeks after treatment (Table III).

Site SE, 100m from the inoculum center, had 10.75% NPV-infection at two weeks post-spray. It is likely that this site had a naturally occurring NPV epicenter which was not detected from the overwintering egg mass NPV diagnosis.

All other sites had low levels of NPV ranging from 0% to 5.3% (Table III; Figure 5b-e) indicating that naturally occurring NPV was present throughout the area. Naturally occurring virus usually becomes evident when larvae are in their final instars¹. This would explain the absence of NPV in this infestation from either the overwintering egg mass or pre-spray larval samples. Consequently, the assessment of the spread of NPV from the inoculum center was not as straightforward as expected.

2. NPV inoculum center.

Larval survival in the inoculum center decreased from a pre-spray ratio of 1.0 to 0.24 by post week 2; thereafter the decrease was much slower until it reached 0.01 at post week 7 (Table III; Figure 4).

1: Dr. J. Cunningham, CFS/FPMI, Pers. Commun.

Percent NPV-infected larvae increased from 72.3% (post week 2) to 82.0% (post week 7). Larval survival therefore appears to be inversely correlated with percent NPV-infected larvae. The exception to this was at post week 6 when the percent infection decreased to 22.9% (Table III; Figure 5a).

At post week 6 there was slightly less defoliation of the trees in the inoculum center than at the 10m and 25m sites surrounding this center (Table IV). Possibly there was an immigration of larvae to these center trees. There is also a phenomenon, 'tree top disease', with other insects where virus infected larvae migrate to the top of the tree. Though no evidence of this occurs with the DFTM, it has been noted with the Gypsy moth in Europe and the Eastern hemlock looper in Newfoundland ¹.

Larval survival (Table II; Figure IV) and NPV-infected larvae data (Table III; Figure 5) indicate that there was a spread of NPV from the inoculum center. All cardinal sites at the 10m interval, with the exception of EA were reduced to a larval survival between 0.08 and 0.19 by post week 2. EA increased to 1.29 by post week 2; then decreased to 0.07 by post week 4.

3. East line

Spray drift occurred at EA during NPV application to the center. Natural virus appeared to be present at the other sample 1: Dr. J. Cunningham, CFS/FPMI, pers. commun.

sites at post week 2. The data was therefore inconclusive as to the spread of NPV in the East direction from the inoculum center.

4. West line.

Reduction in larval survival was evident by post week 2 at WA and WB. WC had a substantial reduction (0.47) by post week 4. It is believed that a natural virus present at WC accounted for the 4.16% NPV larval infection by post week 2 (Table III). Spray drift was the cause of NPV at WA (post week 2) therefore NPV spread did not account for the presence of NPV at WA.

The build-up of NPV at WB could have been due to the spread of NPV from WA or the migration of larvae from WC. It is therefore possible that NPV spread from WA to WB, a distance of 15m in the West direction.

5. South line.

SA showed signs of reduction in larval survival 2 weeks post treatment; SB at 4 weeks post treatment, and SC to SE at 2 weeks post treatment. There was evidence of a substantial increase of larval NPV infection at all sites by post week 3 (Table III; Figure 5). The increase of NPV at SA was likely due to spray drift at the time of NPV application. The presence of NPV at SC to SE was likely due to natural virus. It is possible that the presence of NPV at SB was due to the NPV inoculum spreading from SA. The data therefore suggests a spread of

NPV inoculum from SA to SB, a distance of 15m.

6. North line.

A substantial reduction in larval survival occurred 2 weeks post treatment at NA and NB; 4 weeks post treatment at NC and ND, and 6 weeks post treatment at NE (Table II; Figure 4). A increase in the presence of NPV occurred at NA by post week 4; at NB by post week 5, and at NC to NE by post week 4 (Table III; Figure 5). It is possible that a natural NPV epicenter was present at ND influencing the occurrence of NPV at NC and NE. This would account for the NPV increase at these three sites at the post week 4 sample period. It therefore appears that NPV from the inoculum center spread to a minimum distance of between 25m to 50m on the North line.

7. Defoliation assessment.

The sequential egg mass survey indicated that severe defoliation would occur in the study area. All sites surrounding the NPV inoculum center, with the exception of SB, had 100% defoliation of new growth (1983) by post week 2; SB had 100% defoliation of new growth by post week 3 (Table IV). Old growth (1982) defoliation ranged from 53% to 100% throughout the site by post week 2. By post week 4 virtually all new growth was defoliated at all sample sites and over 75% of old growth was defoliated at all sample sites with the exception of WC (46.2%).

The inoculum center had virtually 100% defoliation of new growth by post week 2. The defoliation of old growth fluctuated

between 61.2% and 73.1% throughout the post sample periods (Table IV).

IV. CONCLUSIONS AND RECOMMENDATION.

It was difficult to assess the spread of NPV from the inoculum center due to the apparent presence of naturally occurring NPV throughout the study area. Nevertheless, it appears that the minimal distance NPV spread was 15 m to the south and west and 25 m to 50 m to the north.

The study area was located in a valley which ran in the northeast/southwest direction. The wind direction was predominately from the south. This was probably the major factor which caused the concentric NPV spread pattern to the north.

There was evidence of minimal foliage protection within the inoculum center; a result of the initial high density of DFTM larvae within the study area and the slow mode of action of the virus.

It is recommended that further studies be undertaken to determine if NPV will spread from an NPV inoculation site. Consideration should be made to use strip, aerial inoculation rather than spot, ground inoculation.

TABLE I: Mean Douglas-fir tussock moth larval density per square meter at each sample period.¹

Sample Site	Pre-spray.	Post week. ² 2	Post week. ² 3	Post week. ² 4	Post week. ² 5	Post week. ² 6	Post week. ² 7	Post week. ² 8
Center	547.59	132.21	152.76	63.70	111.58	42.67	10.15	7A/30D
EA (10m)	258.52	335.00	546.95	45.53	19.13	7.78	0.00	0A/0D
EB (25m)	725.83	138.83	139.85	40.82	48.16	7.78	0.00	0A/0D
EC (40m)	396.41	362.05	846.44	19.05	39.25	11.80	0.00	0A/1D
WA (10m)	797.26	67.00	51.83	37.96	75.95	54.69	12.20	4A/23D
WB (25m)	627.45	151.69	178.69	84.10	44.37	58.09	22.13	16A/5D
WC (40m)	168.05	143.44	215.87	80.39	198.95	120.36	90.08	30A/2D
SA (10m)	1021.98	158.05	94.39	23.16	65.24	53.33	20.72	1A/7D
SB (25m)	520.42	290.76	260.65	132.43	85.07	5.93	10.14	10A/40D
SC (50m)	536.07	197.73	244.55	261.35	224.87	22.16	26.43	9A/63D
SD (75m)	624.11	83.47	284.34	74.74	75.03	19.81	10.81	3A/4D
SE (100m)	330.32	109.02	281.94	147.65	386.08	79.54	4.73	0A/24D
NA (10m)	771.72	144.23	134.45	66.97	25.72	3.92	0.00	0A/3D
NB (25m)	862.43	97.33	292.52	26.09	10.51	5.06	4.39	0A/0D
NC (50m)	546.01	211.77	226.23	25.29	12.08	1.79	0.00	0A/0D
ND (75m)	389.79	323.25	403.97	86.19	24.38	7.49	3.12	1A/2D
NE (100m)	197.17	108.21	114.64	118.10	263.88	17.90	4.28	0A/1D

1: mean larval density = $\frac{\text{number DFTM larvae}}{\text{area (sq. cm.)}} \times 10000 \text{ cm}^2 = \frac{\text{larvae}}{\text{sq. m.}}$

2: Beating sample; A=live larvae; D=dead larvae

TABLE II: Mean Douglas-fir tussock moth survival ratio at each sample period.

Sample Site	Pre-spray.	SURVIVAL RATIO ¹					
		2	3	4	5	6	7
Center	1.0	0.2414	0.2789	0.1163	0.2038	0.0779	0.0185
EA (10m)	1.0	1.2958	2.1157	0.1761	0.0739	0.0301	0.000
EB (25m)	1.0	0.1913	0.1927	0.0562	0.0663	0.0107	0.000
EC (40m)	1.0	0.9133	2.1352	0.0480	0.0990	0.0298	0.000
WA (10m)	1.0	0.0840	0.0650	0.0476	0.0953	0.0686	0.0153
WB (25m)	1.0	0.2417	0.2848	0.1340	0.0707	0.0926	0.0353
WC (40m)	1.0	0.8535	1.2845	0.4784	1.1839	0.7162	0.5360
SA (10m)	1.0	0.1546	0.0923	0.0226	0.0638	0.0522	0.0203
SB (25m)	1.0	0.5587	0.5008	0.2545	0.1635	0.0114	0.0195
SC (50m)	1.0	0.3688	0.4562	0.4875	0.4195	0.0413	0.0493
SD (75m)	1.0	0.1337	0.4556	0.1197	0.1202	0.0317	0.0173
SE (100m)	1.0	0.3300	0.8535	0.4469	1.1688	0.2408	0.0143
NA (10m)	1.0	0.1869	0.1742	0.0868	0.0333	0.0051	0.000
NB (25m)	1.0	0.1128	0.3392	0.0302	0.0122	0.0059	0.0051
NC (50m)	1.0	0.4062	0.4143	0.0463	0.0221	0.0033	0.0000
ND (75m)	1.0	0.8295	1.0366	0.2212	0.0626	0.0192	0.0080
NE (100m)	1.0	0.5488	0.5814	0.5989	1.3383	0.0908	0.0217

ND= No data collected.

1: Survival ratio = Mean larval density post-spray/mean larval density pre-spray.

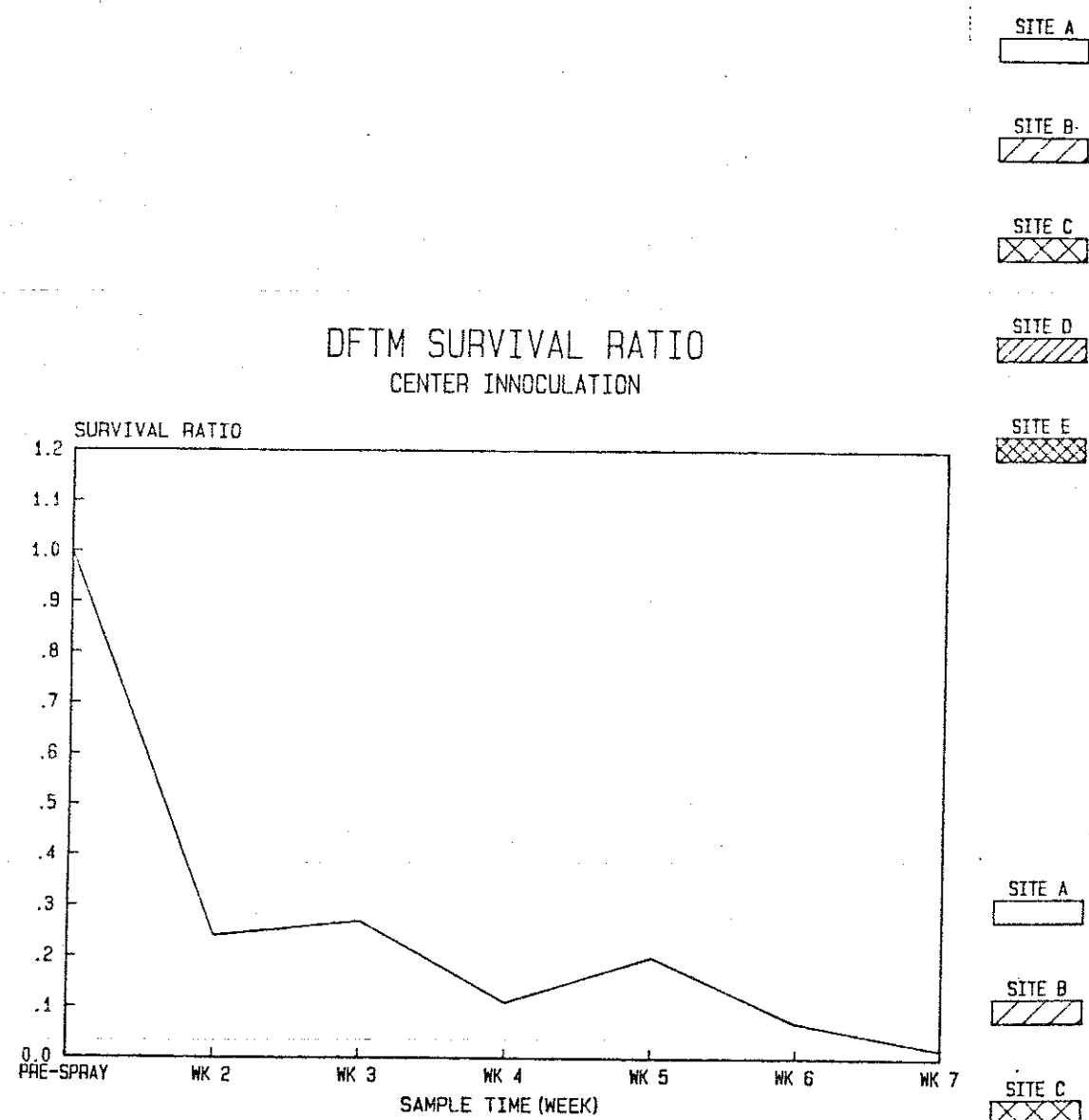
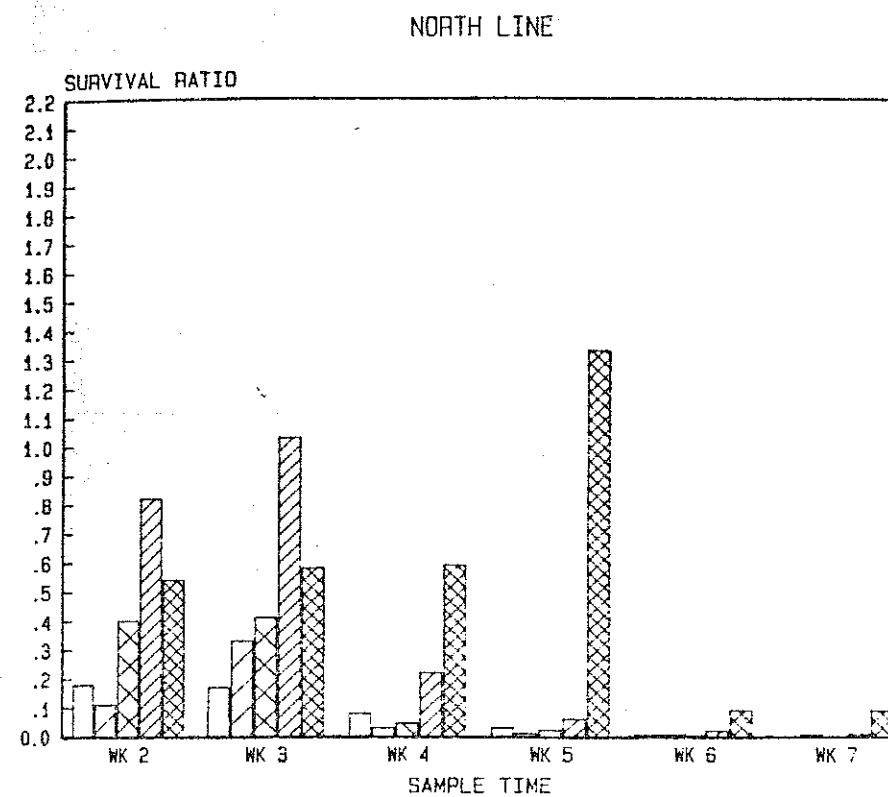
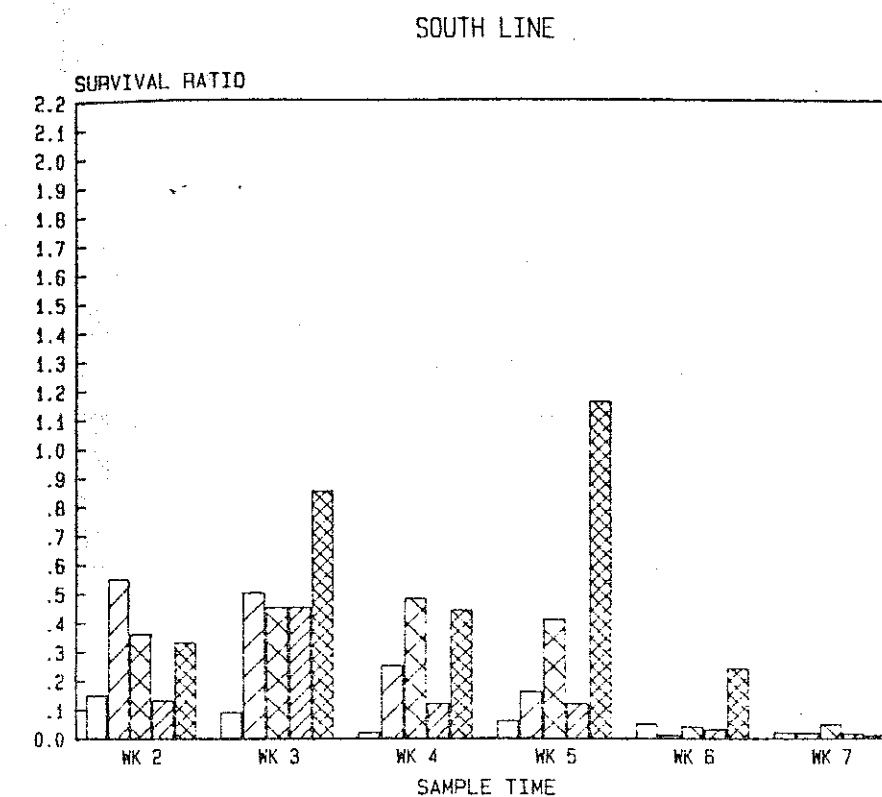
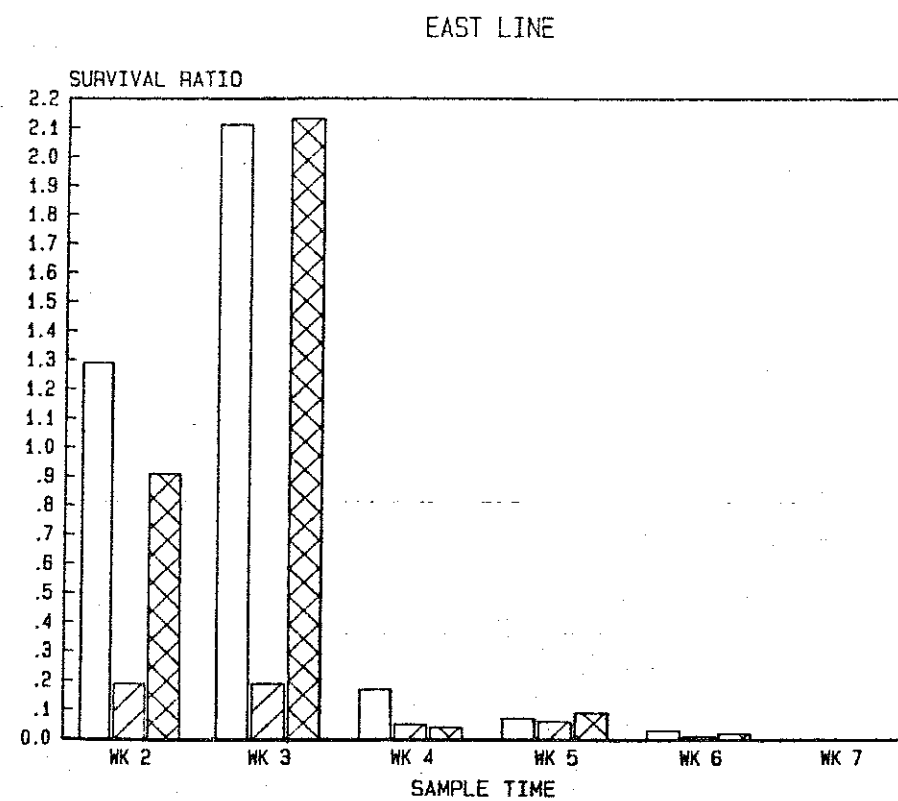
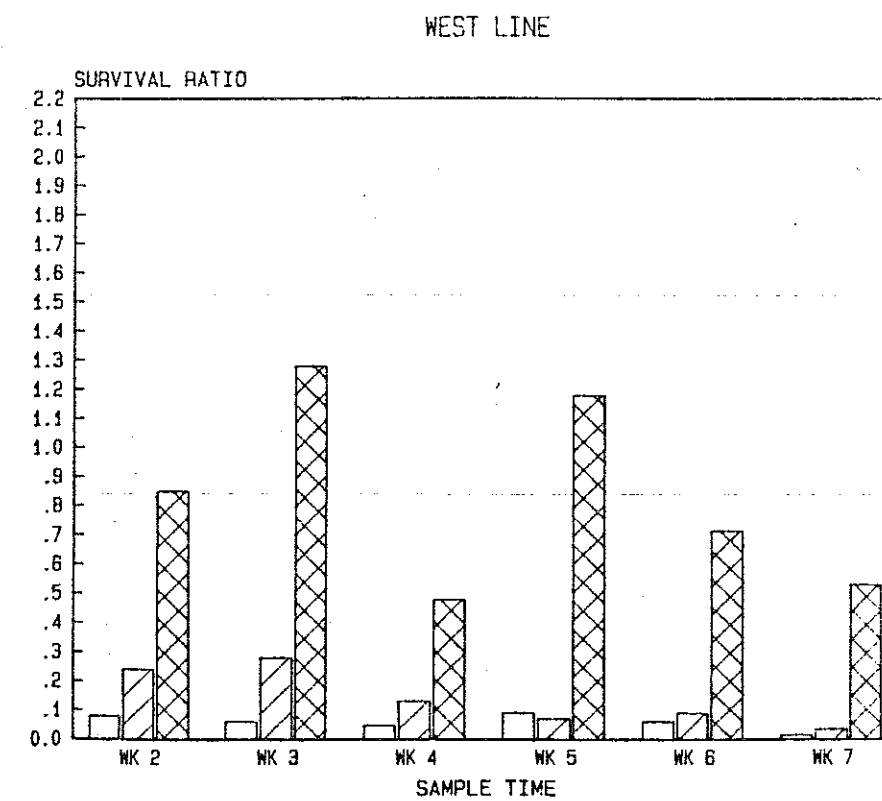


Figure 4: Douglas-fir tussock moth survival ratio at the pre-spray time and weekly post-spray intervals.

TABLE III: Percentage NPV-infected larvae at each sample period.

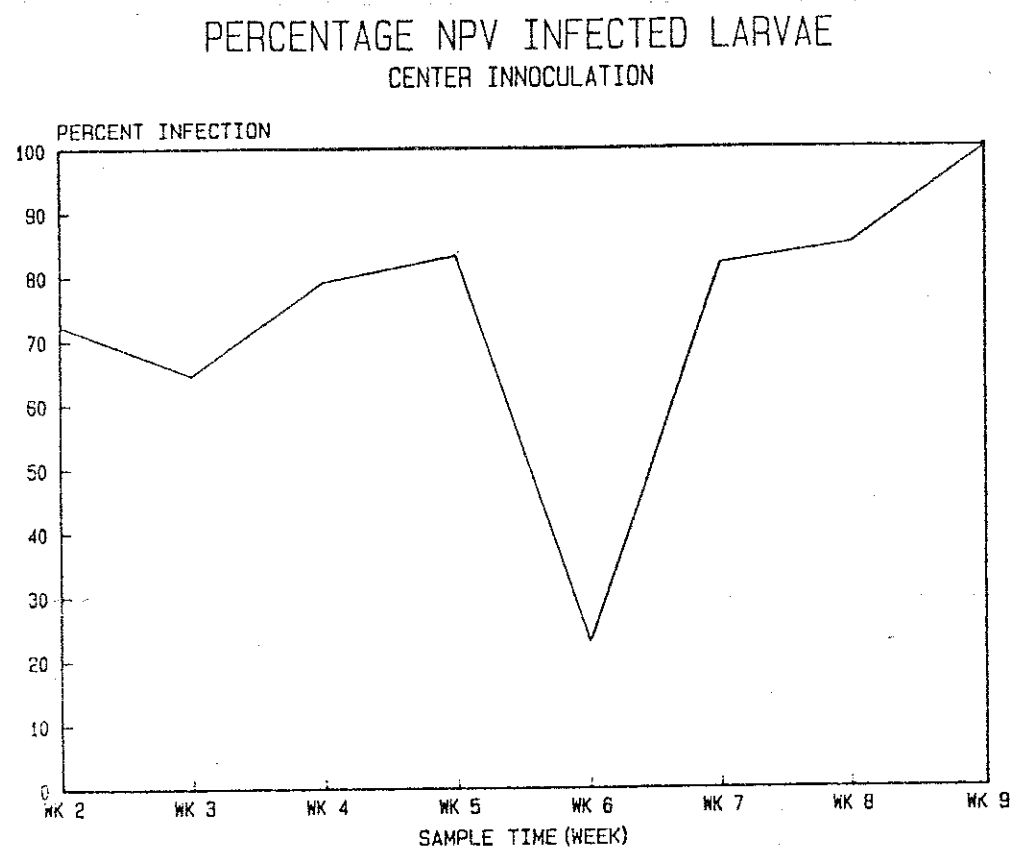
PERCENTAGE NPV-INFECTED LARVAE									
Sample Site	Pre-spray	Post week 2	Post week 3	Post week 4	Post week 5	Post week 6	Post week 7***	Post week 8**	Post week 9**
Center	0.0	72.30	64.58	79.20	83.34	22.90	82.00	85.00	100
EA	0.0	11.46	4.17	25.00	70.83	73.90	NL*	NL	NL
EB	0.0	3.13	0.00	25.00	25.00	25.00	100**	NL	100
EC	0.0	1.04	0.00	6.25	16.67	22.80	37.00	100	50.00
WA	0.01	17.02	12.50	71.11	38.50	69.00	100	100	100
WB	0.0	0.00	4.17	10.42	38.78	10.30	72.00	83.00	88.00
WC	0.0	4.16	2.08	2.33	12.50	0.0	66.00	100	87.50
SA	0.0	4.71	0.0	35.42	50.00	32.00	86.00	100	100
SB	0.0	5.32	2.08	12.50	22.22	61.40	66.00	91.60	100
SC	0.0	4.40	0.0	18.75	35.42	20.60	13.00	83.33	100
SD	0.0	1.08	2.08	12.77	45.83	17.90	20.00	66.70	100
SE	0.01	10.75	3.13	29.55	72.50	45.50	100**	100	100
NA	0.0	4.16	6.25	21.15	69.23	40.00	69.00	66.70	NL
NB	0.0	4.35	1.61	6.25	34.88	20.90	50.00**	NL	NL
NC	0.0	2.22	0.00	38.89	38.89	33.80	50.00**	NL	NL
ND	0.0	0.0	0.0	25.00	29.41	25.00	67.00**	66.70	100
NE	0.0	2.17	2.08	16.67	34.88	40.70	39.00	100	100

* = NL= No larvae in sample.

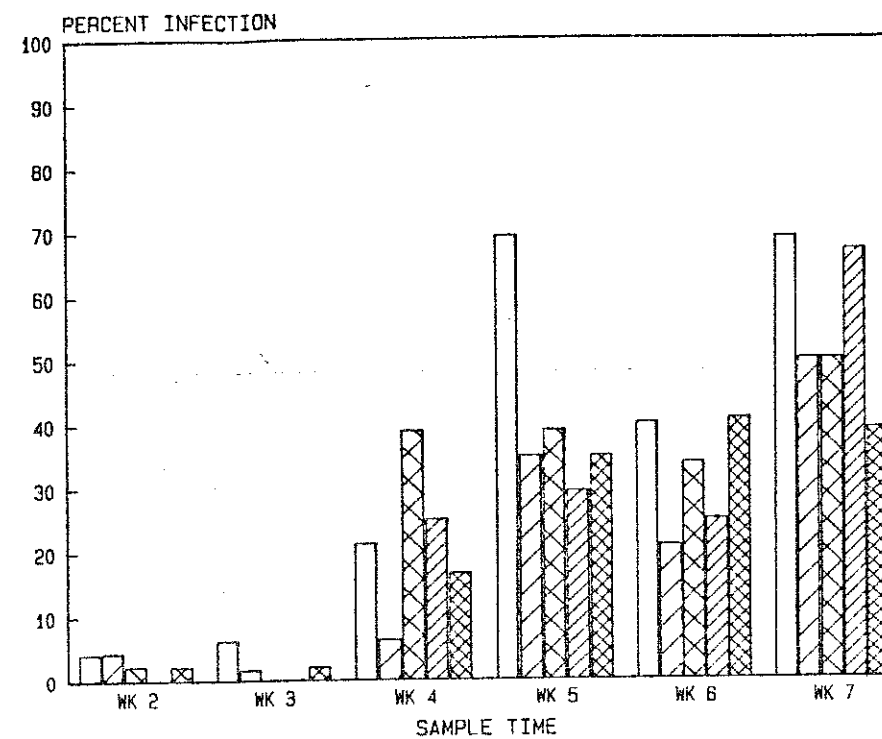
** = Non-significant number of larvae diagnosed.

*** = Not including beating samples.

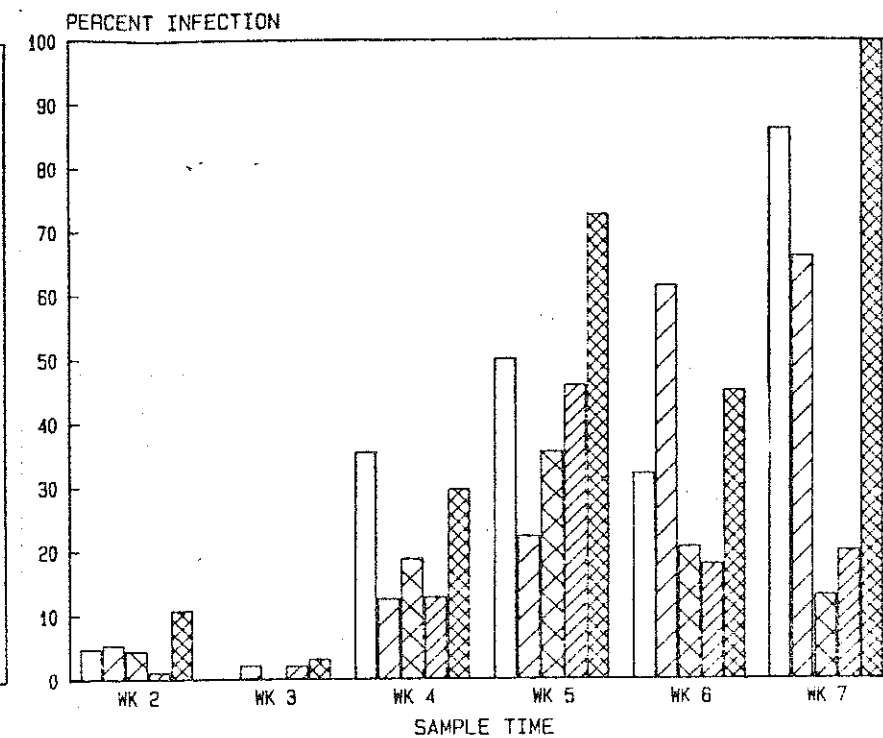
1 = Beating sample.



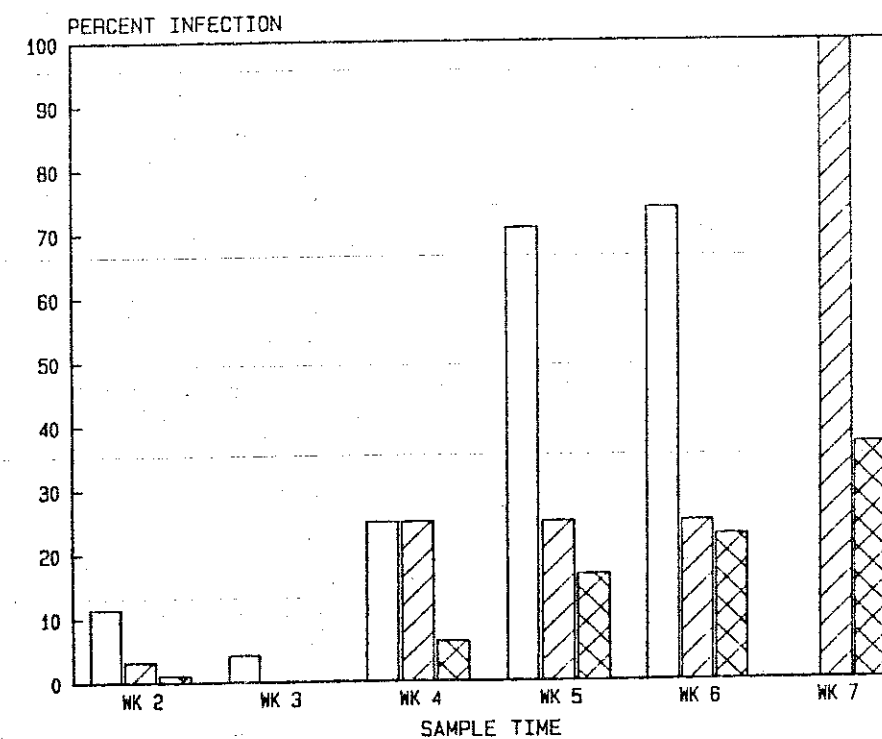
NORTH LINE



SOUTH LINE



EAST LINE



WEST LINE

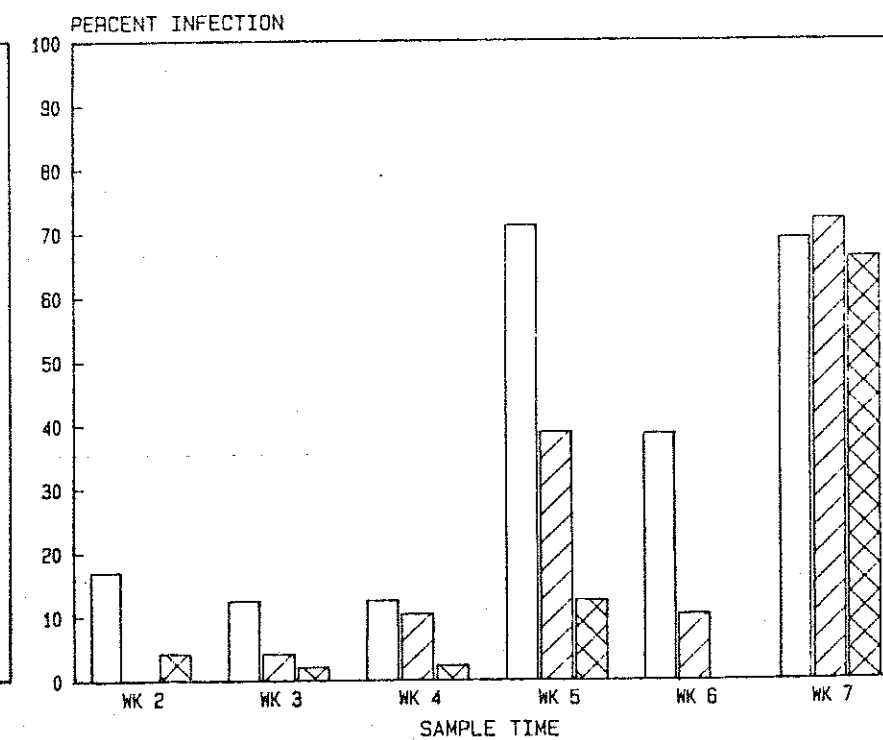


Figure 5: Percentage NPV-infected larvae at weekly post-spray intervals.

TABLE IV: Percent defoliation of Douglas-fir.

Sample Site	Pre-spray	PERCENT DEFOLIATION													
		2		3		4		5		6		7		8	
Center	Total Branch	1983	1982	1983	1982	1983	1982	1983	1982	1983	1982	1983	1982	1983	1982
	18.75	99.3	73.1	100	70.6	100	73.1	99.4	61.2	100	71.2	99.4	72.5	ND	ND
EA	8.7	90.0	100	100	97.5	100	100	100	97.5	100	97.5	100	98.7	ND	ND
EB	21.2	100	92.5	100	100	100	100	100	100	100	100	100	100	ND	ND
EC	12.5	100	100	100	100	100	100	100	100	100	100	100	100	ND	ND
														ND	ND
WA	ND	100	53.7	100	62.5	100	77.5	100	77.5	100	91.2	100	98.7	ND	ND
WB	ND	100	68.7	100	80.0	100	95.0	100	97.5	100	97.5	100	99.5	ND	ND
WC	ND	100	55.0	100	63.7	100	46.2	100	56.2	100	65.0	98.7	97.0	ND	ND
														ND	ND
SA	23.7	100	91.2	100	100	100	100	100	100	97.5	100	100	100	ND	ND
SB	12.5	80.0	100	100	100	97.5	100	93.7	100	100	100	100	100	ND	ND
SC	15.0	100	72.5	100	95.0	100	77.5	100	100	100	100	100	100	ND	ND
SD	15.0	100	97.5	100	100	100	95.0	100	100	100	100	100	100	ND	ND
SE	16.2	100	88.7	100	77.5	100	77.5	100	93.7	100	100	100	100	ND	ND
														ND	ND
NA	50.0	100	86.2	100	96.2	100	97.5	100	100	100	100	100	99.5	ND	ND
NB	37.5	100	97.5	100	86.2	100	100	100	100	100	100	100	100	ND	ND
NC	52.5	100	97.5	100	100	100	100	100	100	100	100	100	100	ND	ND
ND	16.2	100	80	100	100	100	100	100	100	100	100	100	100	ND	ND
NE	40.0	100	73.7	100	76.2	100	93.7	100	82.0	100	100	100	100	ND	ND

* = No data collected

* = No data collected.

V. APPENDIX

VI. LITERATURE CITED

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