PRELIMINARY STUDIES OF NUCLEAR-POLYHEDROSIS VIRUSES INFECTING THE WHITE-MARKED TUSSOCK MOTH, *ORGYIA LEUCOSTIGMA*

BY
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INFORMATION REPORT

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ABSTRACT

Larvae of *Orgyia leucostigma* were found to be susceptible to nuclear polyhedrosis viruses isolated from *Heterocampa* (*Orgyia*) *pseudotsugata* and *O. antiqua* as well as to their homologous virus. A very marked difference in susceptibility to virus was found between first- and fourth-instar larvae, the fourth-instar being extremely resistant. It is recommended that field applications of the virus be made when the larvae are in the first-instar and an approximate dosage rate is suggested.

INTRODUCTION

The white-marked tussock moth, *Orgyia leucostigma*, is a moderately serious forest pest in Eastern Canada and the Douglas-fir tussock moth, *Heterocampa* (*Orgyia*) *pseudotsugata*, a major defoliator in the West. Nuclear-polyhedrosis viruses (NPVs) have been isolated from both these species and these viruses have been considered to be responsible for the collapse of natural populations (Neilson, unpublished; Morris, 1963). The rusty tussock moth, *O. antiqua*, is also found in Canada but is of little economic importance. An NPV has been recorded from this species both in Canada and England. Although natural virus epizootics occur in populations of white-marked and Douglas-fir tussock moths, both species can do considerable damage before the insect populations collapse, and the early initiation of virus epizootics by artificial dissemination of NPV has been considered.

This report describes preliminary semi-quantitative studies of the effect of three tussock moth NPVs on white-marked tussock moth larvae to determine if any one is more pathogenic than the others. It also describes the effect of its homologous virus on different instars. From these results recommendations are made regarding the optimal time of application and a theoretical application rate is calculated.

METHODS

A sample of white-marked tussock moth NPV was isolated from larvae collected at Espanola, Ont. in 1969, Douglas-fir tussock moth NPV was obtained from Dr. Mauro Martignoni, U.S.D.A., Corvallis, Oregon, and a sample of rusty tussock moth virus was collected in England in 1966.

Polyhedral counts were made of the three samples using a Petroff-Hausser bacteria counter and the number of polyhedra was standardized to 10^7 per ml. Serial 10 fold dilutions were prepared of 10^6, 10^5, 10^4 and 10^3 polyhedra per ml. The NPV suspensions were sprayed on artificial diet (McMorran, 1965) in plastic 1 1/2 oz. cream cups. A chromatograph sprayer was used with an air pressure of 10 lb./sq. in. and 0.04 ml was sprayed on the 8 sq. cm surface area of the diet. Six cups were sprayed with each dilution and 4- 6 third-instar larvae placed in each cup. The larvae were examined microscopically to determine if death was caused by NPV (Plate 1).

To determine the susceptibility of different instars to their homologous NPV, first- and fourth-instar white-marked tussock moth larvae were compared using the same procedure as described above. Eight cups were sprayed with each diltion and 4-6 larvae placed in each cup.

RESULTS AND DISCUSSION

White-marked tussock moth larvae were susceptible to Douglas-fir tussock moth NPV and rusty tussock moth NPV as well as to their homologous virus (Table I). There was no marked difference in virulence between the white-marked tussock moth NPV and the Douglas-fir tussock moth NPV, but the rusty tussock moth NPV had been in storage in an aqueous suspension for 5 years and its infectivity may have been reduced (Morris, 1963; Cunningham, 1970a). The viruses of the white-marked tussock moth and Douglas-fir tussock moth may be one and the same. In a reciprocal test Dr. Mauro Martignoni found that white-marked tussock moth NPV was cross-infectious to Douglas-fir tussock moth larvae and of the same order of pathogenicity as its homologous NPV. Therefore, if either of these viruses were to be mass-produced, it could be used for the biological control of either species. The same situation was found with eastern hemlock looper, *Lambdina fiscellaria fiscellaria*, western hemlock looper, *L. f. lugubrosa* and western oak looper, *L. f. somniaria*, NPVs which were all infectious to eastern hemlock looper larvae (Cunningham, 1970b).

When the susceptibility of first- and fourth-instar white-marked tussock moth larvae to NPV was tested, it was found that first-instar larvae were very susceptible and fourth-instar highly resistant (Table II). Mortality only occurred in fourth-instar larvae when they were fed diet sprayed with suspensions of 10^7 and 10^6 polyhedra/ml. From these results a rough estimate would indicate that the first-instar larvae are 1,000 times more susceptible than the fourth-instar. Results of the same test performed with third-instar larvae in another experiment are shown in Table I. Although less susceptible than first-instar larvae, third-instar are much more susceptible than fourth-instar. It is recommended that field applications be made when larvae are in the first-instar.

The phenomenon of resistance increasing to NPV with age of larvae is not uncommon. It has been found with the NPVs of the corn earworm, Heliothis zea (Tanada and Reiner, 1962), the armyworm, Pseudaletia unipuncta (Tanada, 1956), and the forest tent caterpillar (Stairs, 1965). In contrast second-, third- and fourth-instar eastern hemlock looper larvae were equally susceptible to NPV (Cunningham, 1970b).

In both experiments the mean length of time to death was calculated and is shown in Tables I and II. The time varied from 14-17 days with the highest concentration of NPV (10^7 polyhedra/ml) to 23-29 days with the lowest (10^3 polyhedra/ml).

The value of converting laboratory results to recommended field application rates is dubious but the theoretical calculations are given. If first-instar larvae are sprayed a concentration of 10^6 polyhedra/ml is recommended in the laboratory. When taking a pathogen or chemical from the laboratory to the field it is customary to increase the optimal laboratory concentration 10 fold. The application rate in the cups was 0.04 ml on 8 sq. cm. which converts to about 25 U.S. gal./acre. Hence a field application rate of 25 U.S. gal./acre of a suspension containing 10^7 polyhedra/ml. is suggested. If an aerial application is considered it would be more practical to use a suspension of 10^8 polyhedra/ml. at 2.5 U.S. gal./acre.

In helicopter spray trials in Oregon, using Douglas-fir tussock moth NPV, 50 x 10^9 polyhedra were disseminated per acre in volumes of 0.2 U.S. gal./acre, 1.0 U.S. gal./acre and 2.0 U.S. gal./acre. No marked difference was found between the 3 treatments and 80-92% mortality was reported (Maksymiuk _et al_, 1968). This is a considerably lower application rate than recommended above; when converted to number of polyhedra/acre, 10^8 polyhedra/ml at 2.5 U.S. gal./acre gives a deposit of 10^{12} polyhedra/acre (20 times the quantity used in Oregon).

Although these dosages seem very heavy in numbers of polyhedra, it must be pointed out that white-marked tussock moth polyhedra are very small. They vary in size from 1.0μ to 1.5μ (Plate 2) and the yield from virus-killed final instar larvae is very high. Production of virus to spray at the application rate suggested above is well within the realms of practicality.

CONCLUSIONS

1. White-marked tussock moth larvae are susceptible to the NPVs of Douglas-fir tussock moth and rusty tussock moth as well as their homologous NPV. Dr. Mauro Martignoni has shown that Douglas-fir tussock moth larvae are susceptible to white-marked tussock moth NPV.

2. White-marked tussock moth NPV and Douglas-fir tussock moth NPV are equally pathogenic to both hosts. Either virus could be mass-produced and used in the control of both species.
3. It is recommended that field applications be applied when larvae are in the first-instar; it is of little value to apply virus after they have reached the fourth-instar.
4. The theoretical application rate has been calculated at 2.5 U.S. gal./acre of a suspension containing 10^8 polyhedra/ml.

ACKNOWLEDGMENTS

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Table I

Pathogenicity tests of 3 viruses on third-instar white-marked tussock moth larvae

Original host of virus	Number of polyhedra/ml	Number of larvae	% larval death from virus	Mean time to death (days)
White-marked tussock	10^7	30	73.3	16.0 ± 5.7*
	10^6	31	41.9	20.7 ± 6.9
	10^5	35	25.7	20.8 ± 8.2
	10^4	32	21.8	22.0 ± 4.9
	10^3	33	12.1	24.1 ± 7.0
Douglas-fir tussock	10^7	32	81.2	13.8 ± 3.2
	10^6	34	58.8	15.7 ± 4.3
	10^5	35	31.2	14.6 ± 5.8
	10^4	32	14.2	21.1 ± 3.9
	10^3	32	9.3	23.0
Rusty tussock	10^7	28	50.0	16.5 ± 7.5
	10^6	30	40.0	23.0 ± 8.9
	10^5	26	38.4	24.9 ± 7.5
	10^4	28	29.4	20.3 ± 8.1
	10^3	32	18.7	27.3 ± 5.6
Control		50	0	-

*Standard deviation

Table II

Pathogenicity tests of white-marked tussock moth NPV on first- and fourth-instar larvae

Instar	Number of polyhedra/ml	No. of larvae	% Virus infection	Mean time to death(days)
I	10^7	38	100.0	14.1 ± 3.3*
	10^6	33	100.0	16.7 ± 7.9
	10^5	34	88.2	21.4 ± 7.6
	10^4	31	41.9	32.7 ± 6.6
	10^3	45	6.7	29.7 ± 3.1
	Control	42	0	-
IV	10^7	38	42.1	17.0 ± 4.6
	10^6	41	14.6	17.8 ± 2.7
	10^5	46	0	-
	Control	46	0	-

*Standard deviation

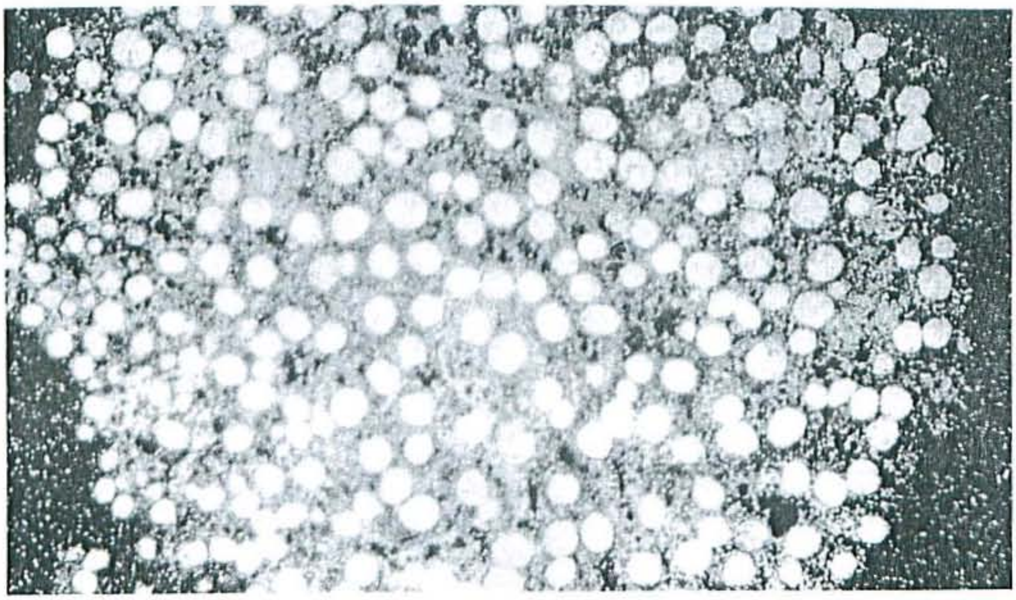


Plate 1. A smear preparation of fat-body from a white-marked tussock moth larva infected with NPV observed microscopically under phase contrast. The highly refractive white spots are nuclei packed with polyhedra. X170.

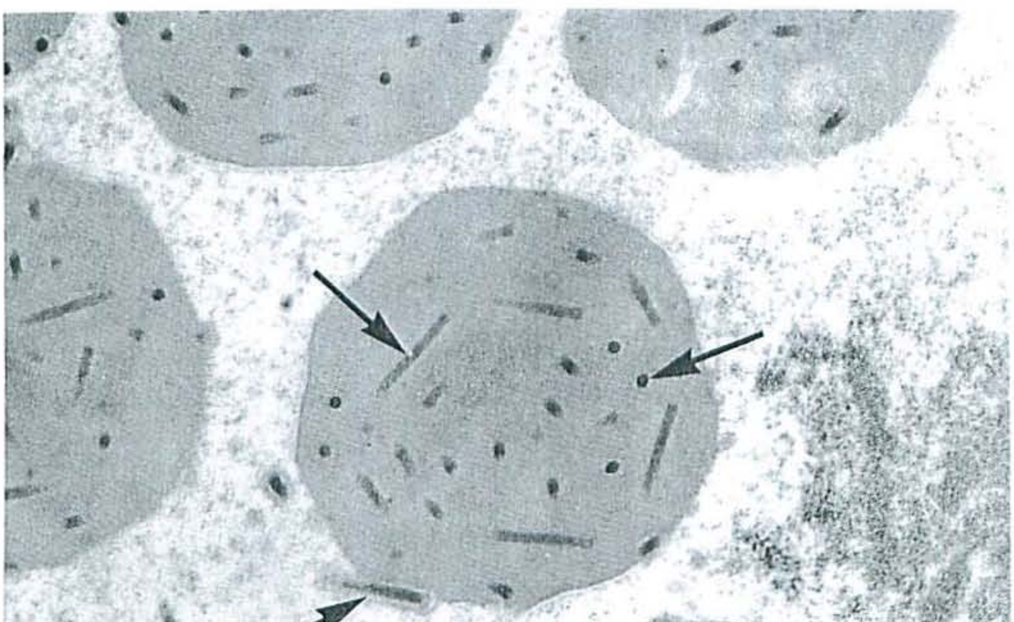


Plate 2. An electron micrograph of sectioned polyhedra in tissue of a white-marked tussock moth larva infected with NPV. Single virus rods (arrows) are embedded in the polyhedron protein matrix. X42,000.

The provenance tests in Kirkwood Township and at Randolph Lake have essentially the same disease history. They were planted in 1953 and 1954 respectively, became infected with disease in 1958, suffered rather heavy mortality the first two years (1958-59), and a continuing lesser mortality each year since that time. The major difference in the two tests was that the Randolph Lake test was located in the area where red pine mortality was first reported in 1954, whereas the Kirkwood Township test was planted in an area which at the time of planting did not show any noticeable degree of mortality in surrounding established stands. This difference may be the reason for the heavier mortality in the Randolph Lake test (Tables IV and V).

The third test, north of Rose Lake, was planted in 1956 in an area somewhat isolated from extensive red pine plantings. Unlike the other tests, it was unaffected by disease until 1962, so that the mortality experienced before that time (Table VI) can be assumed to be largely the result of planting failure. The variation in mortality between trees of various seed sources is not great, but it does suggest that there may be some relationships. However, since all the surviving trees show signs of infection with disease at present, a more meaningful picture may be forthcoming from future records.

7. ENVIRONMENTAL CONDITIONS

7.1 Soils and Climate

The distribution of heavy mortality (Fig. 1) in the Kirkwood Unit coincides closely with the occurrence of the Petawawa sand soil type. However, since most of the red pine in the Unit was planted on the sand plains, there is little opportunity to compare growth and survival on different soil types. Mortality is lower in the southern part of Kirkwood Township, and some of this soil has a higher than average silt content, but if the disease is spreading south and east, as has been suspected, and the southern border of Kirkwood Township constitutes the present margin of the infected area, the lower mortality there could be explained without reference to soils.

A relationship between nutrient deficiency and the disease condition has been suggested many times in the past. Indicator experiments have suggested that certain types of fertilizer applications may increase tree mortality (Sec. 6), increase growth but exert no appreciable effects on disease resistance (Sec. 8.2), or retard growth without noticeably affecting mortality from disease (Sec. 9). Mr. Ray Leach of the Research Branch, Ontario Department of Lands and Forests has established a number of carefully planned fertilization experiments in the Kirkwood Unit, and he may be able to provide considerable valuable information in the future.

A theory has been put forward in the past that the large stem cankers originate as cracks in the bark caused by rapidly dropping night-time temperatures following extremely hot days. Supposedly these cracks are then infected with pathological organisms and cankers result. Such weather conditions do occur in Kirkwood, particularly on the sand plains, and daytime temperatures of 80° to 90° F. are sometimes followed by night frosts in mid-summer. Bleeding of the main stem from what appear to be cracks in the bark of young trees five to 10 feet in height is common as well. However, the connections, if any, between summer frosts, bark cracks, and stem cankers have yet to be proven.

The effects of underplanting are illustrated by two red pine stands planted in Kirkwood Township in 1950. The planting sites consisted of old fields partially covered with young aspen-white birch stands. Mortality in the openings ranged from 65 to 85 per cent, whereas under the hardwoods, losses dropped to between 40 and 50 per cent.

An indicator experiment designed in 1960 has shown some very interesting results. A four-feet wide strip of old-field sod was thoroughly rototilled in May, 1960, and three pairs of plots, four by four feet, were staked out with eight feet between each pair. One additional pair of plots was marked out in adjacent undisturbed sod. On the tototilled strip, one pair of plots were covered with two bushels of 15-year-old stable manure, one pair with 8-12-6 fertilizer at the rate of 1000 lbs. per acre, and one pair were left untreated. Twenty 2-2 red pine seedlings were planted on each plot; and one of each pair of plots was covered with an aluminum-screened cage four by four feet by two feet high.

The manured plots rapidly became covered with a luxuriant growth of alsike clover, and all the trees died. Growth measurements of the remaining plots showed that the trees planted in sod averaged 4.5 inches increase in height each year from 1960 to 1963, those in rototilled and unfertilized ground averaged 5.5 inches, and the trees on rototilled and fertilized soil averaged 7.4 inches per year during the four-year period.

The trees became infected with disease in 1962, and Table VII shows that there was extremely high mortality in all the uncaged plots, whereas those in trees in the cages suffered only very light losses.

Table VII

Red pine mortality in cage experiment, Kirkwood Twp., 1963

Treatment	No. Trees in cage	Mortality No. of trees	No. Trees uncaged	Mortality No. of trees
Sod	20	3	20	19
Rototilled Unfertilized	20	0	20	16
Rototilled Fertilized	20	1	20	20

Although the cages were originally placed over the trees to prevent insect feeding, the results of the experiment cannot be safely attributed to this factor, since the cages brought about other important modifications. It is a well-known fact that screened cages modify the climate within by levelling temperature extremes, increasing soil moisture and air humidity by decreasing wind velocity and evaporation, and by decreasing light intensity.

In addition, the screen on these cages effectively prevented accumulation of snow around the trees. During the winter of 1961-62, immediately preceding infection, the trees outside the cages were completely snow covered, but only about three inches of snow sifted into the cages, and the trees inside were left almost completely exposed. The preceding winter (1960-61), following which no infection occurred, was one of unusually light snowfall, and the trees both in and out of the cages remained exposed all winter.

Periodic examinations of the trees each summer showed that light weevil feeding had occurred on some of the trees. However, most of the trees bore no obvious signs of insect attack.

This experiment is particularly interesting because it appears to hold important keys to an understanding of the red pine mortality problem. The author feels that insect feeding was not an important factor, and if this is true, then climatic factors must be considered. It has already been pointed out that the disease infection pattern on the trees duplicates the feeding habits of the weevil, P. approximatus. However, since most mortality occurs within 3 feet of the ground, it might equally well be associated with snow depth.

8. A STAND HISTORY

In 1960, a stand of red pine was planted specifically for studies being carried out by the author in Kirkwood Township. The seedlings were 3 - 0 stock from the Kirkwood nursery, and they were slit-planted by machine at a four by seven foot spacing in old sod.

A five-acre block was divided into 10 one-half acre plots and marked with corner stakes immediately after planting. In 1962, four of the 10 plots were fertilized with 10 - 5-10 fertilizer at the rate of about five ounces per tree applied in a circular band about six inches from the stem. Detailed observations on insect populations and disease were made several times each year, and records of tree growth and mortality were maintained from 1960 to 1963.

Nineteen sixty was a good year for tree growth with rainfall well distributed throughout the growing season (Fig. 4). Records showed that 6.9 per cent of the trees died from planting shock, and 2.0 per cent from rodent and weevil attack, making a total of 8.9 per cent for 1960. Insect feeding was negligible with 3.0 per cent of the trees infested with the pine tip moth, Rhyacionia adana Hein., and 4.0 per cent with the

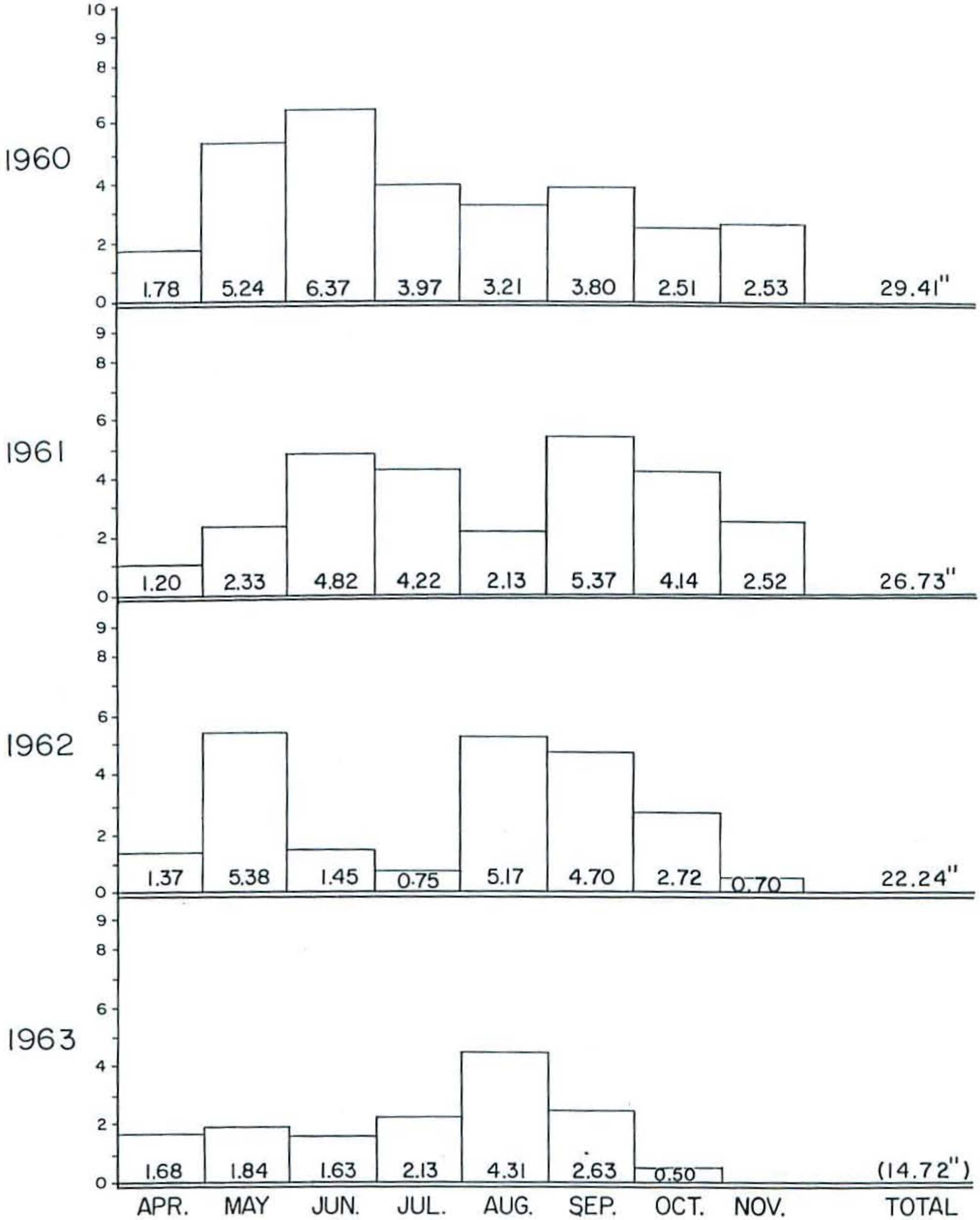


Fig. 4

RAINFALL-KIRKWOOD TWP., ONTARIO

1960-1963

sulphur leaf roller, Sparganothis sulfureana Clem.

Following a winter of deep, hard frost, and an average snow depth of only six inches from December until April, rain was scarce in the spring of 1961 until the last week of May. Shoot growth was retarded, but the rains came before serious injury occurred, and no serious shortage of water took place during the remainder of the summer.

Mortality was light in 1961, with only 1.2 per cent of the trees killed by root-feeding insects. As in 1960, 3.4 per cent of the trees were infested with the pine tip moth, but the number of trees infested with sulphur leaf roller increased to 79.0 per cent. Feeding scars of the weevil, P. approximatus, were found on 4.0 per cent of the trees, and 6.9 per cent were infested with the pine bark aphid, Pineus strobi (Htg.). Disease made its appearance in 1961, with 0.2 per cent of the trees bearing stem cankers.

The snow came early in the fall of 1961, and frost penetrated only the top two inches of soil. The snow averaged 16.8 inches in depth from December until April, and the trees were completely covered from January 10 until the end of March, 1962. Rain was abundant in May when growth began, but after the first week in June a severe drought began and continued until the middle of August.

When the snow disappeared in April, the trees appeared to be in excellent condition. However, shortly thereafter trees began to turn brown, and by mid - August counts showed that 13.3 per cent of the trees had died of disease, and 49.7 per cent of the survivors bore diseased leaders or side branches. Many of the trees which had been severely weakened by disease were killed by weevils and bark beetles.

The tip moth infestation doubled in 1962 (6.0 per cent of the trees infested), but remained rather insignificant, and the sulphur leaf roller infestation decreased to 43 per cent, probably because its alternative groundcover hosts dried up due to the drought. The pine bark aphid occurred on 10.7 per cent of the trees, and several newcomers appeared; another tip moth, Eucosma gloriola Heinr. (2.0 per cent), two adelgids, Cinara spp., (15 per cent), and the wooly pine needle aphid, Schizolachnus pini-radiatae Davidson (2.7 per cent). The new shoots of 3.0 per cent of the trees wilted from lack of water and failed to recover.

The snow came early again in 1962, and soil-frost penetration was only one inch. Snow depth from December until April averaged 13 inches, with a maximum of 24 inches occurring in February and March. The snow disappeared the first week of April, and rainfall was very light until the middle of July, 1963.

Counts in mid-June showed that 33 per cent of the trees had died of disease, and another 30 per cent had partially dead crowns. Weevils and bark beetles were abundant in the dead and severely diseased trees so that mortality could be expected to increase before the end of the summer. With the exception of the sulphur leaf roller which infested only 12 per cent of the trees, the percentage of trees infested by various insects had increased to the following values: wooly pine needle aphid - 16.8 per cent, Cinara spp. - 14.8 per cent, pine bark aphid - 21.2 per cent, and E. gloriola - 3.4 per cent. Predators were noticeably more abundant, with chrysopids on 30.9 per cent, coccinellids on 4.4 per cent, and spiders on 8.0 per cent of the trees.

By the end of 1963, total mortality in this stand had reached 58.7 per cent, and all but 10.1 per cent was primarily due to disease (Table VIII). With at least 30 per cent of the surviving trees infected, and bearing either dead leaders or side shoots, further mortality in the future is to be expected.

The pattern of mortality throughout the stand is extremely interesting (Fig. 5). The plantation is located between a natural mixed stand, made up of aspen, white pine, white spruce, and white birch, on the west, and a red pine stand planted in 1955 on the east. The latter has suffered 80 per cent mortality from disease since 1958. The young plantation is bounded on the south by a white pine stand planted in 1929, and on the north by old field sloping rather sharply downward to an outcropping of bedrock and a tamarack swamp.

Mortality was lowest at the southern end of the plantation and increased steadily towards the north. Mortality was considerably lower on the west side next to the natural mixed stand than it was on the east beside the 1955 red pine planting. The progressive mortality from south to north, and the substantial difference between east and west, suggest that certain very important factors related to the spread of the disease are operating here.

The 1955 stand on the east may have served as a source of infection, since 80 per cent of the trees in that stand were killed and practically all the survivors showed signs of infection with the disease. However, it should be noted that the bulk of the mortality occurred there in 1958 and 1959, and the remains of the dead trees have now disappeared. Some tree mortality and lower branch mortality has occurred each year since 1959, but these infected trees are relatively scarce and scattered.

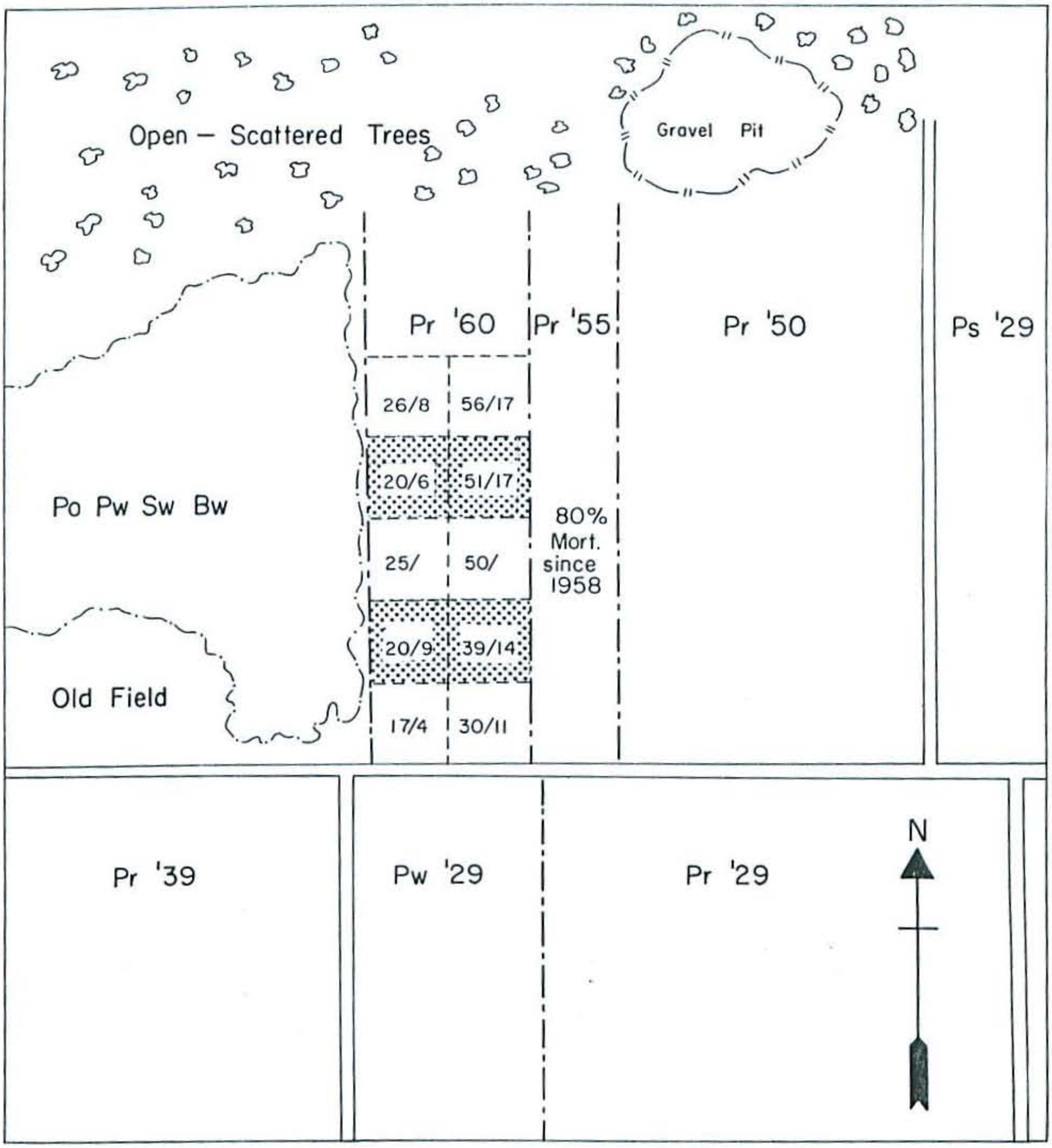


Fig. 5
The 1960 stand, showing mortality by plots and nature of stands or surrounding area. Plots with stippled margins were fertilized. Numbers above the line= Mortality 1963. Numbers below the line= Mortality 1962.

Table VIII

Annual mortality in red pine stand planted in 1960,

Kirkwood Township

Year	Per cent of trees killed by Planting Shock	Insects	Disease	Per cent survivors infected with disease	Total Mortality
1960	6.9	2.0	0.0	0.0	8.9
1961	0.0	1.2	0.0	0.2	10.1
1962	0.0	0.0	13.3	49.7	23.4
1963	0.0	0.0	35.3	30.0	58.7

Table IX

Growth Measurements*, Red Pine Planted 1960, Kirkwood Twp.

Plot Treatment		Unfert.		Fertilized				Unfert.		Unfert.	Fert.	Total
Plot No.		A1	A2	B1	B2	D1	D2	E1	E2	Mean	Mean	Mean
Overall Height (inches)	1963	20.3	19.6	19.2	18.9	19.4	18.5	19.2	18.4	19.3	19.0	19.16
Diameter Ground Level (inches)	1963	0.53	0.50	0.52	0.52	0.52	0.50	0.52	0.49	0.51	0.51	0.51
	1962	0.42	0.37	0.39	0.39	0.42	0.38	0.43	0.37	0.39	0.39	0.38
Leader Length (inches)	1963	4.70	4.4	3.9	3.7	4.1	3.5	3.7	4.0	4.2	4.0	3.8
	1962	6.4	5.6	5.8	4.9	5.6	4.9	5.8	4.6	5.6	5.4	5.3
	1961	2.5	2.3	2.2	1.8	2.3	2.4	2.5	2.3	2.4	2.3	2.2
	1960	2.5	2.5	2.3	2.4	2.6	2.7	3.0	2.6	2.6	2.6	2.5
Needle Length (inches)	1963	3.6	3.7	3.2	3.4	3.2	3.5	3.6	3.7	3.6	3.5	3.3
	1962	2.5	2.4	2.3	2.4	2.4	2.6	2.3	2.3	2.3	2.4	2.4
	1961	4.7	4.5	5.0	4.5	4.4	4.3	4.7	4.3	4.5	4.5	4.5
	1960	2.4	2.1	2.3	2.0	2.4	1.9	2.2	2.0	2.1	2.1	2.1
Needle Retention (% of trees) 1963	1963	100	100	100	100	100	100	100	100	100	100	100
	1962	100	100	100	100	100	100	100	100	100	100	100
	1961	100	100	100	100	100	100	100	100	100	100	100
	1960	79	86	71	77	76	44	84	85	83	77	67
	Prior	44	39	14	5	13	4	30	19	33	23	9

* 10 per cent sample (every 10th tree)

Other factors which might be considered are: the prevailing winds are westerly, the dominant trees of the natural mixed stand on the west reach a height of about 50 feet, and could exert considerable effect on the mesoclimate of the adjoining area, and the presence of the gravel pit (Fig. 5) suggests the possibility that the soil may be coarser and dryer towards the north end.

Table IX shows the growth of the 1960 stand until 1963. Growth has been comparatively slow , probably due to the drought conditions of the past three years. The fertilizer application appears to have reduced height and diameter growth and needle retention. This is probably due to the lack of rain from the time the fertilizer was applied (May 18-21) until the middle of August in 1962. However, there does not appear to be any relationship between mortality and presence or absence of fertilizer.

There is some indication from the figures in Table IX that slightly better growth occurred towards the southern end of the stand, but it might be dangerous to attempt to relate this to degree of mortality. The indicator experiment (Table VII) showed no such relationship, and Harnden and MacDonald (1959) showed that in two stands which suffered heavy mortality in 1958 and 1959, the height growth averaged 6.6 and 7.0 inches annually in the three years prior to infection. This would be considered excellent growth for trees in the establishment stage in Kirkwood.

Observations throughout the Kirkwood Management Unit indicate that the insect and disease history of the 1960 stand during this first four-year period is typical of most young stands planted on similar sites. Although much of the information on climate and other factors may appear unrelated to insects and disease at present, future work may provide some definite correlations.

9. DISCUSSION

With our present limited knowledge of the disease organism or organisms involved in the red pine mortality problem, any discussion of the means of infection and dissemination is largely speculative. However, it may be valuable, at this time, to examine the facts we have and attempt to determine what they indicate.

As a result of past experience, insects are usually considered primary suspects in the spread of disease, and in the Kirkwood situation, there is considerable superficial evidence to support this suspicion:

1. The mortality pattern on the trees duplicates the feeding pattern of the weevil, P. approximatus.

2. The rapid increase and spread of the disease in recent years coincides with the beginning of thinning operations in Kirkwood, and thus with a considerable increase in weevil populations.

3. The feeding pits of adult weevils appear to be perfect entrance courts and incubation chambers for a fungus disease, and they are often common in the vicinity of bark lesions.

4. Mortality of trees caged so that insect feeding was prevented was practically nil, whereas uncaged trees suffered very heavy mortality (Sec. 8).

However, there is, at the same time, some evidence indicating that weevils do not play an important part in the dissemination of the disease:

1. In the 1960 stand discussed above, only 4.0 per cent of the trees bore traces of adult weevil feeding in the fall of 1961, but in the spring of 1962, 13.3 per cent of the trees died and 49.7 per cent were infected with disease.

2. The Rose Lake Provenance Test probably duplicates this situation. This plantation was isolated from other extensive red pine plantings, and several miles away from the nearest thinned stand. Mortality was negligible in the stand during the years immediately preceding infection, so that it is safe to assume that the weevil population was low and weevil feeding light. However, in 1962 and 1963, nine to 16 per cent of the trees were killed by disease, and the remainder all showed signs of infection.

3. In the cage experiment mentioned above, the trees outside the cages which died of disease showed very little weevil feeding the previous year.

4. In 1961, an old field adjacent to a newly-thinned Scots pine stand was planted with red pine. Red pine were underplanted in the Scots pine stand as well. Weevil emergence in the thinned stand was very heavy in 1961 and 1962, and the young trees were subjected to severe feeding by adult weevils. In 1963, only 3.0 per cent of the trees had died, and their death appeared to be due to causes other than disease.

5. In the summer of 1962, diseased trees infested with P. approximatus larvae were collected and banked against rows of red pine planted in 1960. The trees were caged, and when the adult weevils emerged in late July and August, they fed heavily on the young trees. Just before freeze-up, one of the cages was removed to allow the trees to become snow covered, while another cage was left in place to prevent snow accumulation. This experiment thus provided heavy weevil feeding, a source of infection from the diseased trees, and both snow cover and lack of snow cover during the winter. None of the trees showed any symptoms of disease in 1963.

It would be futile, at this time, to attempt any discussion of climatic and site factors in relation to disease infection and dissemination. However, observations indicate that future disease studies should consider the possible effects of soil moisture (and moisture content of trees), depth of soil freezing, depth of snow cover, and degree of shading.

10. APPENDIX

Mortality Survey of red pine plantations established since 1950

in the Kirkwood Forest Management Unit, 1963.

Township	Map ref. number	Year planted	Per cent mortality to 1962	Per cent mortality 1962-63	Per cent survivors disease infected
Kirkwood	AA	1955	78.4	1.0	18.2
	AB	1950	62.8	0.4	35.4
	AO	1950	55.2	0.0	44.6
	AP	1950	9.8	0.2	84.8
	AQ	1950	4.0	0.2	95.6
	AR	1950	11.0	0.2	88.4
	AY	1959	63.4	10.6	21.6
	AZ	1959	71.4	4.8	18.2
	BE	1959	70.0	9.8	15.6
	BF	1959	36.6	10.4	24.2
	BL	1959	61.2	5.0	14.0
	BR	1956	9.2	0.4	71.8
	BS	1960	69.2	3.4	15.0
	BU	1950	48.6	0.0	51.4
	BV	1959	69.6	4.4	18.0
	BW	1959	6.8	0.0	41.0
	BZ	1958	7.2	.7	7.2
	CB	1958	74.0	2.8	22.2
	CC	1958	87.0	0.4	10.0
	CD	1960	26.2	3.0	70.8

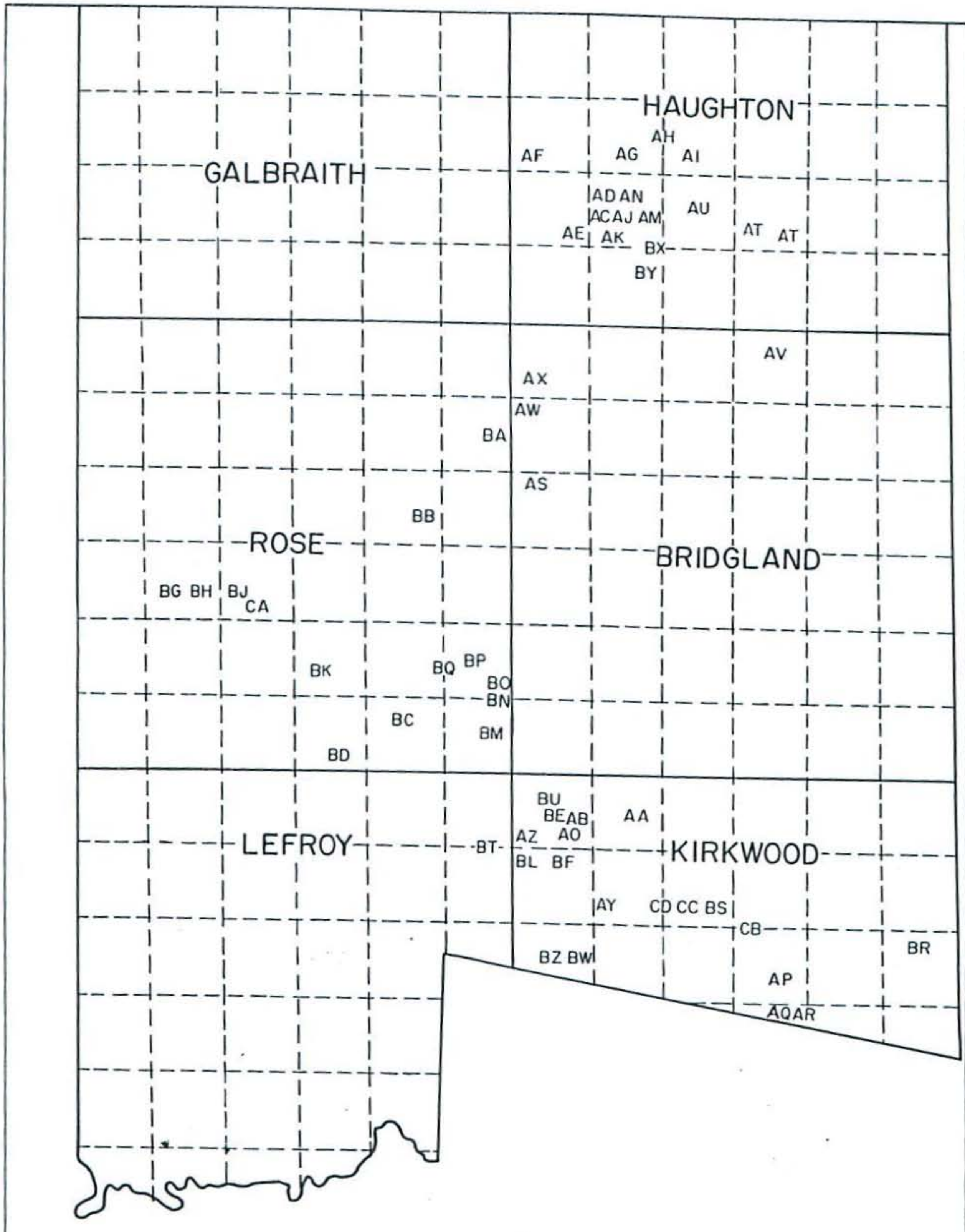
Mortality survey (Cont'd)

Township	Map ref. number	Year planted	Per cent mortality to 1962	Per cent mortality 1962-63	Per cent survivors disease infected
Haughton	AC	1958	30.4	20.1	29.2
	AD	1951	96.4	1.2	2.4
	AE	1951-54	69.4	2.4	26.6
	AF	1951-54	54.0	9.8	37.8
	AG	1951	99.2	0.0	0.8
	AH	1951	73.8	0.8	23.4
	AI	1954	92.6	1.8	5.6
	AJ	1951	98.8	0.2	1.0
	AK	1954	78.0	3.4	18.2
	AM	1951-54	69.0	9.6	21.4
	AN	1954	84.6	1.8	13.2
	AT	1954	77.8	2.0	19.6
	AU	1954	93.6	0.6	5.8
	BX	1951-54	47.2	14.8	38.0
	BY	1951-54	68.0	12.8	19.2
Bridgland	AS	1951	31.8	.3	67.6
	AV	1953	35.2	2.2	59.8
	AW	1952	0.0	1.6	97.6
	AX	1952	0.0	0.0	100.0
Rose	BA	1951	0.0	1.0	99.0
	BB	1952	86.2	0.8	13.0
	BC	1957	61.4	1.8	34.2
	BD	1957	53.8	17.4	28.8
	BG	1951	40.4	2.0	57.6

Mortality survey (Cont'd)

Township	Map ref. number	Year Planted	Per cent mortality to 1962	Per cent mortality 1962-63	Per cent survivors disease infected
Rose	BH	1951	41.0	0.6	58.4
	BJ	1951	60.0	0.0	40.0
	BK	1950	43.2	0.0	56.8
	BM	1958	69.2	20.0	10.8
	BN	1958	44.6	9.0	40.0
	BP	1958	20.8	9.0	70.2
	CA	1955	65.2	7.6	27.2
Lefroy	BT	1959	60.8	10.8	28.2

Note: Refer to map on following page for location of the above stands.



Portion of Kirkwood Management Unit
showing locations of red pine stands referred to in mortality survey.

11. REFERENCES

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