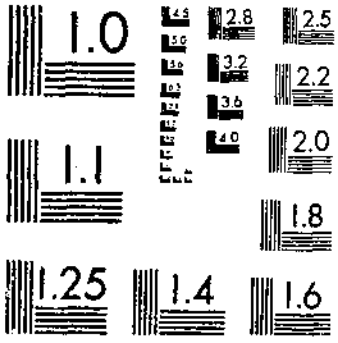
TB-1632 (1981) USDA TECHNICAL BULLETINS UPDATA

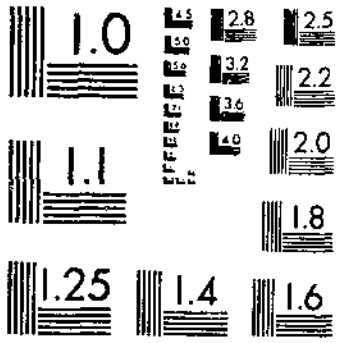
DESCRIPTIVE EPIZOOTIOLOGY OF AN APHID MYCOSIS

SOPER, R.S.; MACLEOD, D.M. 1 OF 1

START



MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A



MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

United States Department of Agriculture

Science and Education Administration

Technical Bulletin Number 1632

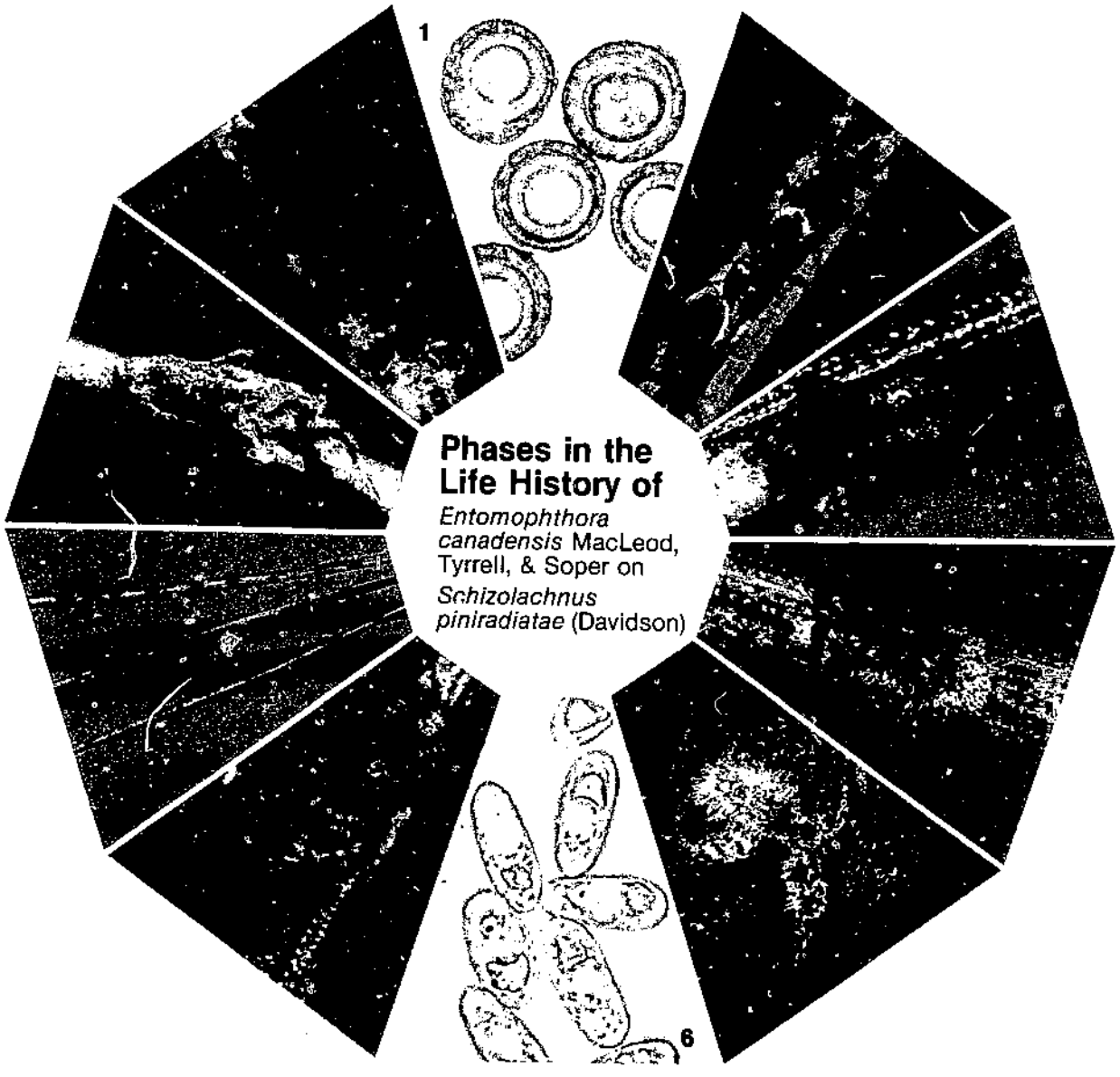
Descriptive Epizootiology of An Aphid Mycosis

Abstract

Soper, Richard S., and MacLeod, D. M. 1981. Descriptive epizootiology of an aphid mycosis. U.S. Department of Agriculture, Technical Bulletin No. 1632, 17 pp.

The epizootiology of *Entomophthora canadensis* MacLeod, Tyrrell, & Soper in populations of the woolly pine needle aphid (*Schizolachnus piniradiatae* (Davidson)) was investigated during a 5-year period in an isolated plantation of red pine (*Pinus resinosa* Ait.) in northern Ontario. Climatic factors did not limit the spread of the fungus; the major controlling factors were the density of the fungus inoculum and the density and spatial distribution of the host. An epizootic killed nearly 100 percent of the aphids in early October 1964, and in the subsequent 2 years *E. canadensis* began to spread at lower population densities and continued to spread faster than during 1961–64, probably because the inoculum density was greater after the epizootic.

KEYWORDS: Aphididae, ecology, entomopathogenic, *Entomophthora*, epizootiology, fungus, insect parasites.



1 Azygospores, the overwintering state of the pathogen.

2 Healthy aphids feeding.

3 Infected aphid shortly after death; note deep wine-red color.

4 Infected aphid covered with hyphal mat, which produces conidia.

5 Forcibly discharged conidia forming an aureole around diseased aphids.

6 Conidia.

7 Diseased aphid anchored to needle by holdfasts.

8 Dead aphids, as in 7, still adhering to red pine needles collected in midwinter.

9 Dead aphid at higher magnification, showing insect residue gradually breaking away and exposing an azygospore cluster.

10 Dead aphid with exposed azygospores.

Acknowledgments

We express our appreciation for the technical assistance of A. J. DeLyzer, Forest Pest Management Institute, Sault Ste. Marie, Ontario; the computer programing and data analysis by J. Field, Great Lakes Forest Research Center, Sault Ste. Marie; and the statistical advice of A. J. Wilson, Canadian Forest Service, Ottawa, and E. L. Cox (deceased), Beltsville Agricultural Research Center, Beltsville, Md.

Contents

United States Department of Agriculture
Agricultural Research Service
in cooperation with
Canadian Forestry Service, Environment Canada
Forest Pest Management Institute

Issued November 1981

Descriptive Epizootiology of an Aphid Mycosis

By Richard S. Soper and D. M. MacLeod[1]

Many attempts have been made to control insect populations by introducing an entomopathogenic fungus. None of these ventures were preceded by a study of the epizootiology of the pathogen. Indeed, most attempts at control consisted of scattering fungus spores in the host's environment without regard to prerequisites for germination, dispersal, or infection. In general, these attempts failed and have led most entomologists to conclude that some limiting requirements, such as abnormally high moisture levels, preclude the application of fungi in controlling insect populations. Such a view is not justified. Kenneth and Olmert (*7*)[2] reported collections of *Entomophthora fresenii* (Nowakowski) Gustafsson from very dry areas of Israel. However, a complete understanding of the distribution of the disease and the factors affecting it is necessary to manipulate a pathogen for insect control.

During a life history and ecological study of the woolly pine needle aphid (*Schizolachnus piniradiatae* (Davidson)) in Ontario, Canada, Grobler (*5*) determined the major controlling factor under certain conditions to be a fungus pathogen, which was incorrectly identified then as *Entomophthora aphidis* Hoffmann. The fungus studied was a new species described in 1979 by MacLeod et al. (*9*) as *Entomophthora canadensis* MacLeod, Tyrrell, & Soper. It was first observed attacking the woolly pine needle aphid at Kirkwood, Ontario, in the fall of 1960. A preliminary study was begun at that time on the morphology of *E. canadensis* and its role in controlling this host (*6*). In the spring of 1961, eight study plots of approximately one-half hectare in various locations near Sault Ste. Marie, Ontario, were used to determine whether this host-pathogen system would be suitable for basic studies of fungus epizootics (*18*). In the fall of 1961, a second fungus species, *E. fresenii*, occurred in aphid populations in many of the study plots (*8, 20*).

Based on information gained from these preliminary observations, we decided that this was an excellent host-pathogen system for epizootiological research.

Aphid Life History

The woolly pine needle aphid spends its entire life cycle on red pine (*Pinus resinosa* Ait.). Fundatrices emerge from the overwintering eggs in mid-May in northern Ontario. They mature in 2 to 3 weeks and parthenogenetically produce an average of eight nymphs per female. These nymphs, as well as the progeny of later generations, remain in family groups to form colonies. Under conditions of low density, the colonies remain distinct and, unless disturbed, the nymphs remain in place until mature. Newly born and recently molted aphids are dark, shiny olive green, but this appearance soon changes with the production of wax. Healthy aphids produce a flocculent whitish wax, which covers about a third of the body and readily adheres to the needles. This wax persists on the needles until the first heavy rain and aids greatly in locating colonies from a distance. From the time the fundatrices are produced in early spring until late summer, only apterous and alate ovoviviparous females are present. DeLyzer (*4*) reported that the number of young produced per female averaged from 5.2 ± 2.9[3] to 7.7 ± 3.3, with a life cycle of 14–17 days depending on environmental conditions.

Grobler (*5*) observed seven generations, the last producing the sexual stage resulting in the overwintering eggs. The cylindrically shaped eggs are brick red when first laid but gradually become shiny black as they age. They are laid on the outer part of the tree crown on new needles. The greatest concentration is in the upper third of the crown, at least in young trees.

[1]Respectively, Insect Pathology Research Unit, USDA, Boyce Thompson Institute, Cornell University, Ithaca, N.Y. 14853; and Forest Management Institute, Canadian Forestry Service, Environment Canada, Sault Ste. Marie, Ontario, Canada P6A 5M7.
Forest Pest Management Institute Contribution No. 336.

Since the manuscript was submitted for publication, several nomenclatural changes have occurred to fungi mentioned in this bulletin. The currently acceptable scientific names are *Conidiobolus obscurus* (Hall & Bell) Remaudière & Keller (syn. *Entomophthora thaxteriana*), *Erynia canadensis* (MacLeod, Tyrrell, & Soper) Humber & Ben Ze'ev (syn. *Entomophthora canadensis*), *Erynia neoaphidis* Remaudière & Hennebert (syn. *Entomophthora aphidis*), and *Triplosporium fresenii* (Nowakowski) Batko (syn. *Entomophthora fresenii*).

[2]Italic numbers in parentheses refer to Literature Cited at the end of this bulletin.

[3]Mean ± standard deviation.

Etiology

Although the mycosis caused by *E. canadensis* is the primary subject of this investigation, a second entomopathogenic fungus, *E. fresenii,* also attacks the woolly pine needle aphid. Generally it is similar to *E. canadensis* in its life history, but it differs in many respects. Soper and MacLeod (*20*) suggested that the production of capillary tubes under certain environmental conditions by conidia, or primary spores, of *E. fresenii* may be associated with the infection process. Almond-shaped secondary conidia, produced on the tips of these upright capillary conidiophores, or spore-bearing structures, are in a better position to contact a susceptible host. Resting spores produced by *E. fresenii* are black, broadly elliptical zygospores and are, therefore, easily distinguished from those of *E. canadensis.*

Infection is assumed to start through the integument or cuticle of the aphid because entry through the digestive tract is unlikely for a sucking insect. Studies (*3, 4*) of *E. aphidis* showed that infection originated through the integument. Once the fungus is inside the aphid, the mycelium grows until it completely fills the haemocoel. The mycelium is apparently continuous in its early stages and eventually fragments into short segments called hyphal bodies. The spore-bearing conidiophores arise from these free floating cells and emerge through the insect integument.

Conidia are formed at the apices of the conidiophores and then are ejected sometimes to considerable distances. They are produced in great numbers, and although short lived, they account for the rapid spread of disease during an epizootic.

Resting spores are formed inside the host body and are characterized by thick walls. They are resistant to adverse conditions, and in this state the fungus survives the winter. For *E. fresenii,* infection results in the production of either conidia or resting spores. Also double infections involving both *E. fresenii* and *E. canadensis* are not uncommon (*20*). A small percentage of resting spores of the latter species can be induced to germinate when newly formed, but most of them immediately become dormant. Germination normally begins the following year commensurate with high host populations and in response to increasing day length (*23*). On germination, *E. canadensis* resting spores produce germ conidia similar to those reported for other *Entomophthora* species (*19, 21*). Germ conidia are probably responsible for establishing initial infections each season. Wilding (*24*) observed that germinating resting spores of *E. fresenii* also produced germ conidia, which were morphologically similar to the almond-shaped secondary conidia produced by primary conidia (cf. *20*).

Materials and Methods

The field study was made 5 miles north of Sault Ste. Marie, Ontario, Canada, at a plantation with approximately 5,600 red pines 1 to 2 m high planted 122 by 122 cm apart. Data were collected between September 1961 and September 1966.[4]

Overwintering Host and Pathogen Populations

The distribution of the host insect population and pathogen inoculum at the beginning of each season was investigated to determine the subsequent development of disease. The autumn distribution, the overwintering mortality, and the spring distribution of host eggs and fungus inoculum were determined by making sample counts in the fall after all the aphids had laid their eggs and in the spring after the snow had melted but before the eggs began to hatch.

The plantation was divided into 14 equal blocks, each containing approximately 400 trees. Five trees were chosen at random within each block. Each tree was divided into three levels, and the branches within each level were assigned a number. Two branches were selected at random from the top, three from the middle, and five from the bottom. The first 30 cm of outer crown foliage of each branch were sampled, since this was where the host population was confined (*5*).

We observed that the pathogen overwintered in the resting spore state within dead aphids, which were attached to the needles by fungus rhizoids. The quantity of *E. canadensis* was estimated by counting dead aphids containing spores defined here as clusters. We found that the resting spores within a cluster averaged 2,100 for adult aphids and 860 for late nymphs. The aphid eggs were counted on the same 30-cm samples. This gave an estimated distribution of both host and pathogen density within the tree and throughout the field.

After data for five seasons were accumulated from the permanent study area, a more efficient sampling scheme was devised based on sequential sampling of overwintering aphid eggs and disease inoculum. This technique was needed for general surveys apart from the detailed epizootiological study. The object was to classify plantations into light, moderate, or severe with respect to the presence of aphid egg masses and disease spore clusters. The technique was similar to that used by Morris (*12*) to classify infestations of the spruce budworm (*Choristoneura fumiferana* (Clemens)).

[4]Although the data on which this publication is based were collected in 1961-66, the findings are still valid and useful as guidelines for developing research on fungus diseases to control insects.

Based on data from the detailed permanent study, aphid egg masses (groups of 4) per 30 cm of branch were defined for the top level as light = less than 1, moderate = 2-5, and severe = greater than 10. Disease spore clusters per 30 cm were defined as light = less than 0.5, moderate = 1-1.5, and severe = greater than 5.0. Although the original data were collected from three tree levels, only the top level was used in the sequential scheme for convenience.

Sampling tables after Morris (*12*) were applied to the red pine plantation where the trees were between 1 and 4 m high. The observer walked 20 m into the plantation and then across the contours sampling every fifth tree. Two branches were selected from each sample tree from the top level, and cumulative egg masses and disease spore clusters were tabulated by branch. Sampling was continued until both aphid and disease levels fell within the decision range (α and β errors = 10 percent). At this point, a decision was reached as to the level of the host infestation and of the inoculum, provided a minimum of 10 branches had been sampled. Table 1 can be used by field workers to determine aphid infestation levels and *Entomophthora canadensis* inoculum density in red pine plantations.

Table 1.—*Cumulative overwintering aphid egg masses and* Entomophthora canadensis *disease spore clusters per 30 cm of branch in red pine plantation, with decision ranges for infestation and inoculum levels*[1]

Branch	Decision ranges for indicated infestation and inoculum levels			Branch	Decision ranges for indicated infestation and inoculum levels		
	Light	Moderate	Severe		Light	Moderate	Severe
	Number	*Number*	*Number*		*Number*	*Number*	*Number*
	APHID EGG MASSES[2]				DISEASE SPORE CLUSTERS[3]		
10	0-7	19-29	112 +	10	0-6	11-15	37 +
11	0-8	20-36	119 +	11	0-7	12-17	39 +
12	0-10	22-43	126 +	12	0-8	13-20	42 +
13	0-11	23-50	133 +	13	0-9	14-22	45 +
14	0-13	24-57	140 +	14	0-10	15-25	47 +
15	0-14	26-64	147 +	15	0-11	16-27	50 +
16	0-15	27-71	154 +	16	0-12	16-30	52 +
17	0-17	28-78	161 +	17	0-13	17-32	55 +
18	0-18	30-85	168 +	18	0-14	18-35	57 +
19	0-19	31-92	175 +	19	0-15	19-37	60 +
20	0-21	33-99	182 +	20	0-16	20-40	62 +
21	0-22	34-105	189 +	21	0-17	21-42	65 +
22	0-23	35-112	196 +	22	0-17	22-45	67 +
23	0-25	37-119	203 +	23	0-18	23-48	70 +
24	0-26	38-126	210 +	24	0-19	24-50	72 +
25	0-27	39-133	216 +	25	0-20	25-53	75 +
26	0-29	41-140	223 +	26	0-21	26-55	77 +
27	0-30	42-147	230 +	27	0-22	27-58	80 +
28	0-32	43-154	237 +	28	0-23	38-60	82 +
29	0-33	45-161	244 +	29	0-24	38-63	85 +
30	0-34	46-168	251 +	30	0-25	29-65	88 +
31	0-36	47-175	258 +	31	0-26	30-68	90 +
32	0-37	49-182	265 +	32	0-27	31-70	93 +
33	0-38	50-189	272 +	33	0-28	32-73	95 +
34	0-40	51-196	279 +	34	0-28	33-75	98 +
35	0-41	53-203	286 +	35	0-29	34-78	100 +
36	0-42	54-210	293 +	36	0-30	35-80	103 +
37	0-44	56-217	300 +	37	0-31	36-83	105 +
38	0-45	57-224	307 +	38	0-32	37-85	108 +
39	0-46	58-231	314 +	39	0-33	38-88	110 +
40	0-48	60-238	321 +	40	0-34	39-91	113 +

[1]Based on 90 percent confident characterization (*12*).

[2]Egg masses (groups of 4) per 30 cm of branch: Light = less than 1, moderate = 2-5, and severe = greater than 10.

[3]Spore clusters per 30 cm of branch: Light = less than 0.5, moderate = 1-1.5, and severe = greater than 5.0.

Host and Pathogen Population Dynamics

The same trees for determining density of overwintering host and pathogen populations were used as permanent sample trees during the growing season. Counts were made every 7 days of the apterous and alate adult, nymph, diseased, and parasitized aphids, the predators, and the "stripped needles." This last observation gave an index of predator activity. Needles were counted on which fragments and waxy secretions of aphids occurred.

Sampling commenced as soon as the aphid eggs began to hatch and continued until living aphids could no longer be found in the field. Counts were made at the top, middle, and bottom of the tree. During the first year (1962), counts were also made on the north and south sides of the tree. Two branches were selected from each level and this sample size was maintained throughout the observation period. During the following year (1963), the sampling was modified to concentrate on a smaller part of the plantation to reduce sampling variation. This method was of no avail because the variation was inherent in the population distribution and not as a result of the sampling method. For the remainder of the study, the 1962 sampling scheme was used with the following modifications: Since at both the beginning and the end of the season the population densities were very low, a minimum sample size was employed so that sampling was completed in a single day with the field crew available. During these periods, two branches were sampled at the top, three in the middle, and five at the bottom. As the population increased, branches were eliminated at random from each level in order that sampling could be completed in 1 day.

A weather station with a hygrothermograph, barograph, and a rain gage was located in the center of the plantation. Temperature and relative humidity were recorded at 2-hour intervals. These instruments were checked weekly using a sling psychrometer and mercury thermometer.

The Pathogens

Entomophthora canadensis—Unlike some species, resting spores and conidia of *E. canadensis* may occur simultaneously in the same individual host. Resting spores are not restricted to fall infection but are present early in the epizootic. Data were collected from an epizootic that occurred between July 19 and August 7, 1963.

The results of diagnosing diseased aphids sampled at the end of the epizootic showed that *E. canadensis* infections generally terminated in the production of both resting spores and conidia within the host, although nearly as many infected aphids contained only resting spores. Only 8.4 percent of the infected specimens produced conidia but no resting spores. Within 2 or 3 days prior to death, the previously olive-green (freshly molted) or slate-gray aphids became distinctly wine red. This color change was more pronounced because the infected insect lost its ability to produce wax (see frontispiece). Rhizoids developed shortly after death of the aphid and prior to the beginning of sporulation. These structures, which are analogous to the holdfast of seaweeds, firmly anchored the aphid to the needle. Attachment was so firm that most of the dead aphids remained on the needles until midsummer or early fall of the following year.

Entomophthora fresenii—Aphids affected by *E. fresenii* that produced conidia neither increased in volume nor lost their shape as did aphids affected by *E. canadensis*. In comparison to the red color of *E. canadensis*-infected aphids, *E. fresenii*-infected dead aphids were a light smoky color. Infection resulting in resting spore production was characterized by the liquefaction of the host not unlike that associated with a bacteria infection. This is a very unusual condition for a fungus infection. It also occurs in forest tent caterpillars (*Malacosoma disstria* Hübner) killed by *Entomophthora* sp. and has been reported for *Entomophthora dissolvens* Vosseler (9). This characteristic enables one to easily distinguish an aphid killed by *E. fresenii* from one killed by *E. canadensis*.

A result of this mode of action led to a second important difference: Since the host was liquefied, the resting spores of this species did not remain in the cadavers but eventually were washed away and distributed on needles and branches of the trees. Estimating the overwintering inoculum of *E. fresenii* has not been possible, whereas *E. canadensis* density can be estimated by counting the dead aphids attached to needles.

Predators and Parasitoids

Coccinellids were the most active group of predators. Seven species were collected. The syrphids were also predaceous. Figure 1 shows the abundance of these groups in relation to the tree level. Other predators collected were species of Neuroptera and one Cleridae species.

Only one parasitoid was observed attacking the woolly pine needle aphid. It was identified as a braconid, *Pauesia californica* (Ashmead). Its effect on host density was inconsequential.

Overwintering Host and Pathogen Populations

After the egg counts and overwintering inoculum data were collected, they were checked for normality as to skewness and kurtosis. The asymmetry of a frequency distribution can be determined by calculating the parameter g_1 of Fisher (cf. *17*).

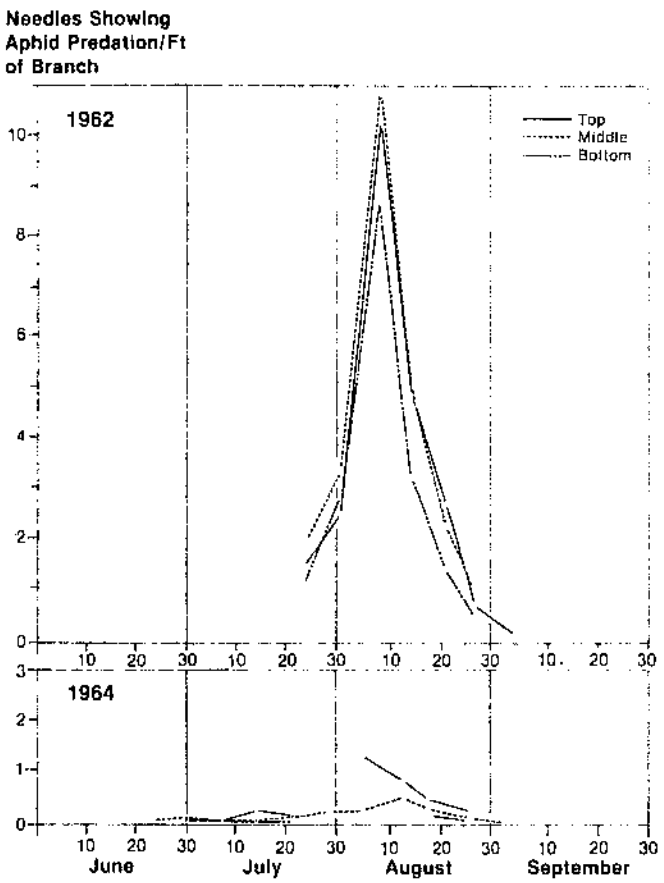


Figure 1.—Coccinellid and syrphid predator activity relative to tree level during 2 years of high host density of the woolly pine needle aphid.

Those data failing to fit a normal distribution were compared to a negative binomial model. The methods for deriving the theoretical distribution for the negative binomial are given by Bliss and Fisher (*2*). To determine whether negative binomial distributions did, in fact, fit the data, it was necessary to compare observed frequencies with frequencies calculated from the distribution, using the chi-square goodness-of-fit test. A common value for the k statistic applicable to the observed distributions was estimated using the formula developed by Anscombe (*1*).

The fall survey of aphid eggs and fungus resting spores formed the basis for determining the year-to-year spread of host and pathogen. The shifting distribution of these populations can be described as change in (1) density, (2) location, and (3) dispersal pattern.

Change in Density—The relationship of $\bar{x}$ to s^2 and coefficients of skewness (g_1) and kurtosis (g_2) indicated that aphid egg and fungus inoculum followed a contagious distribution. To employ an analysis of variance to determine whether a change in density occurred within tree location, it was necessary to normalize the data. Several transformations were applied including—

$$y = \log (x + 1)$$
$$y = \log (x + \tfrac{1}{2}k)$$
$$y = \sin h^{-1} (x + \tfrac{3}{8}) (k - \tfrac{3}{4})$$

Most of the aphid egg and fungus inoculum data failed to fit a normal distribution before transformation. The overwintering aphid eggs were deposited in groups of four, because four eggs matured simultaneously within the aphid and were deposited before a new batch was formed. When the fall egg counts were divided into groups of four and a new frequency distribution was calculated for the groups, a negative binomial distribution generally closely approximated the data. Fall distributions of fungus inoculum also usually did fit the negative binomial model. Consequently, the sequential sampling scheme described previously could be developed to classify density levels of both host and pathogen.

Data from 5 years of fall egg and inoculum surveys were used to compute the common k_c as follows:

Counts per 30 cm of branch

	Aphid eggs (groups of 4)		*Fungus inoculum (spore clusters)*	
Tree level	*Range of x's*	k_c	*Range of x's*	k_c
Top	0.08–26.89	0.50	0.07–11.94	0.37
Middle	.06–19.65	.49	.36–11.35	.34
Bottom	.06– 8.53	.43	.29– 4.68	.33

A sequential sampling scheme was devised to determine the density level of aphid infestation and overwintering inoculum. All the fall egg and fungus inoculum counts adequately did fit a log normal distribution for all the counts until the 1964 epizootic. Data on overwintering aphid populations in 1964 and 1965 did not fit any known distribution, nor were aphid or pathogen estimates normalized for those counts by the usual transformations. Therefore, an analysis of variance was undertaken only on the metameters of the transformed data for 1961, 1962, and 1963. No strong trends could be detected except that the density of eggs and inocula generally differed between the top and the bottom of the tree.

There was little indication of a difference between the spring counts of viable eggs for 1962 and 1964, yet 1964 produced an epizootic. A similar conclusion was reached when considering the spring density estimates of fungus inocula. Therefore, mean density had little value by itself as a predictive parameter of an epizootic.

Change in Location—The second measure of distribution is the change in location. This was assessed by calculating the center for the aphid infestation and the epicenter of the fungus infection and determining whether these locations changed. The coordinates of this center and epicenter computed from the coordinates of the block center were weighted by estimated density counts, viz:

$$cgn = \frac{\sum_{i=1}^{14} (c_i \cdot n_i)}{\sum_{i=1}^{14} c_i}$$

$$cge = \frac{\sum_{i=1}^{14} (c_i \cdot e_i)}{\sum_{i=1}^{14} c_i}$$

where

cgn = north coordinate for the infestation center or infection epicenter
cge = east coordinate
c_i = density estimate for plot i
n_i = northerly coordinate for center of plot i
e_i = easterly coordinate for center of plot i

Figure 2 shows that the center of aphid infestation and the epicenter of fungus pathogen infection moved easterly across the plantation from 1961 until 1964 and then shifted back toward the west. Figures 3 and 4 show that there was a general spread of the aphid population throughout the plantation resulting in a complete distribution of the host by the spring of 1964. The disease inoculum was also more widely spread in the spring of 1964 than in the previous enzootic periods. This set the stage for the epizootic that occurred in the fall of 1964. In the following postepizootic year, there was a discontinuous distribution of the host and a more prevalent distribution of the overwintering disease inoculum. The second postepizootic year resulted in an almost complete elimination of the aphid population with the disease inoculum still abundant.

Change in Dispersal Pattern—The third measure of distribution of the host and pathogen populations is the degree of aggregation. Theoretically, the distribution of a population in the environment can be (1) uniform, (2) random, or (3) clumped. Simply measuring the density in terms of the means gives no indication of dispersion of individual variates. Several indices were calculated in search of a trend in the changes in

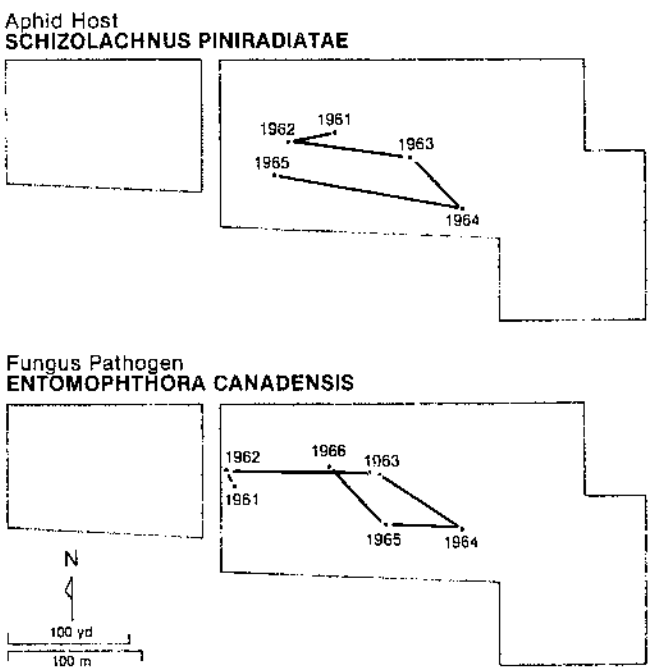


Figure 2.—Diagrams showing distribution changes in center of woolly pine needle aphid infestation (above) and in epicenter of fungus pathogen infection (below) based on fall egg and inoculum surveys during 5-year study. (1966 egg count was insufficient to calculate center of gravity.)

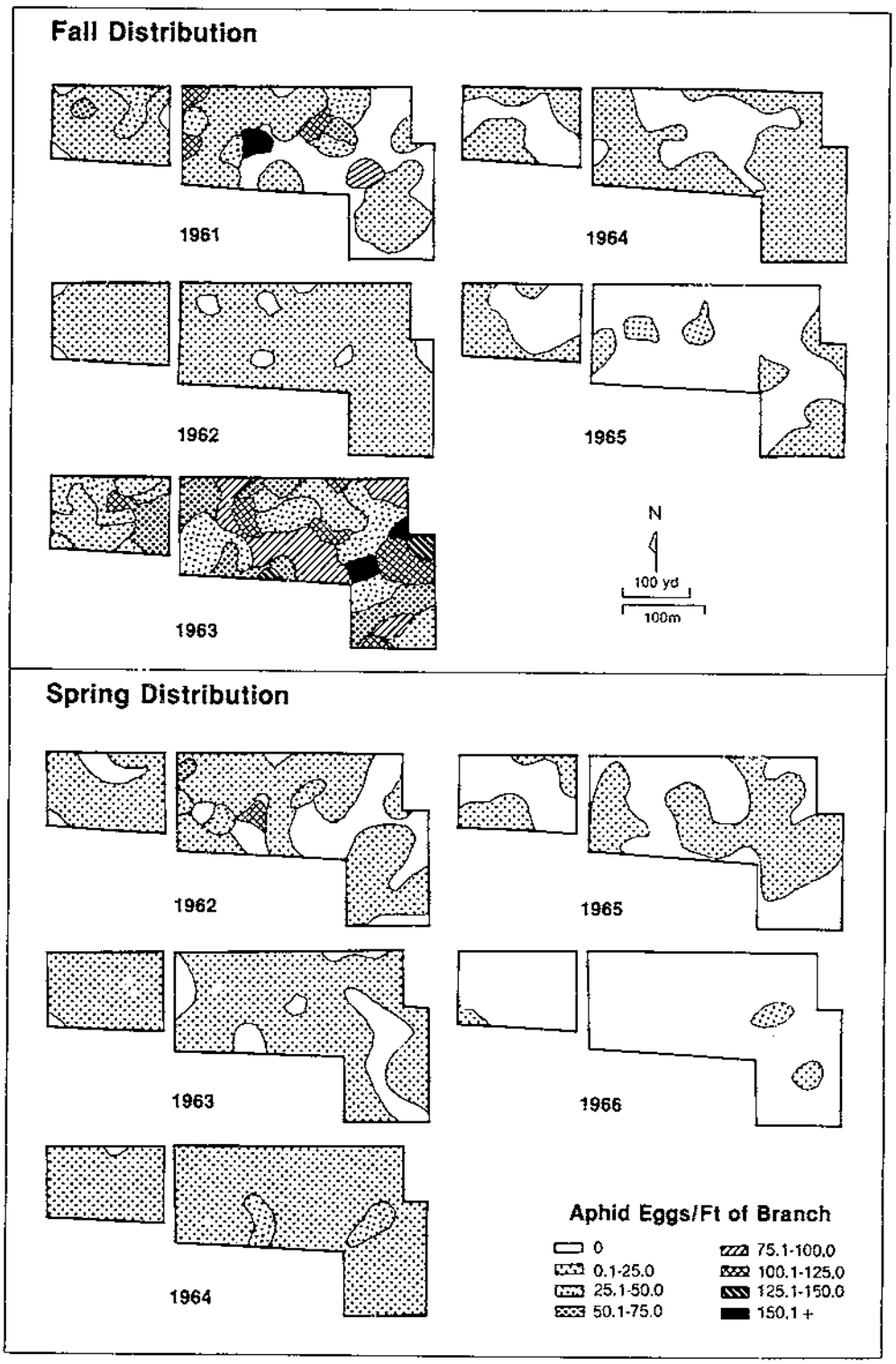


Figure 3.—Diagrams showing distribution changes in woolly pine needle aphid density in red pine plantation between fall and spring, 1961-66.

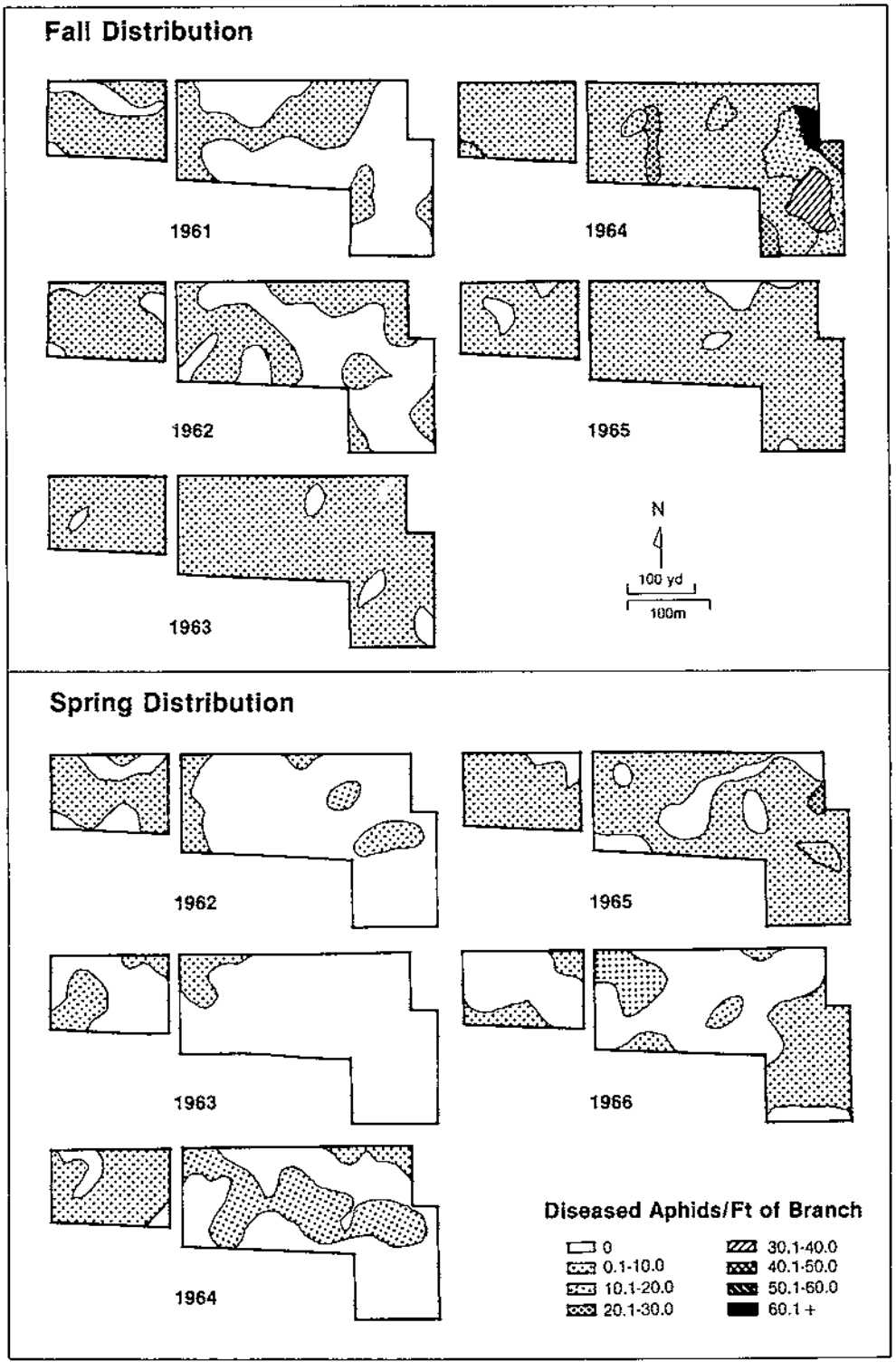


Figure 4.—Diagrams showing distribution changes in fungus inoculum density in red pine plantation between fall and spring, 1961–66.

both host and pathogen distributions. Morisita's $I\delta$-index of dispersion is calculated by Morisita (*11*).

$$I\delta = \frac{n\Sigma x_i\,(x_i - 1)}{\Sigma x_i\,(\Sigma x_i - 1)}$$

When $I\delta = 1$, the distribution is random, whereas a value greater than 1 indicates contagion and less than 1 regularity. The index of expansion $e = \bar{x} - s_{\bar{x}}$ and the index of aggregation $\lambda = s_{\bar{x}}$ were also calculated.

When Morisita's $I\delta$-index of dispersion was used, the fall egg counts showed a trend toward a random distribution from 1961 to 1964, when the trend reversed toward contagion. The pathogen overwintering populations did not show any trend with the $I\delta$-index except that all were contagiously distributed. Of the two other indices calculated, the index of expansion seemed to give predictive results. The most useful index, however, was simply the proportion of branches with eggs or fungus spore clusters (fig. 5).

Proportions of branches with inoculum in the fall seemed to be more significant than proportions based on spring counts when predicting epizootics. Estimates of aphid egg distribution in the fall and spring were of equal value in predicting aphid population trends. These indices were judged on their value in forecasting the epizootic that occurred during the 1964 and 1965 seasons. In general, it can be concluded from these

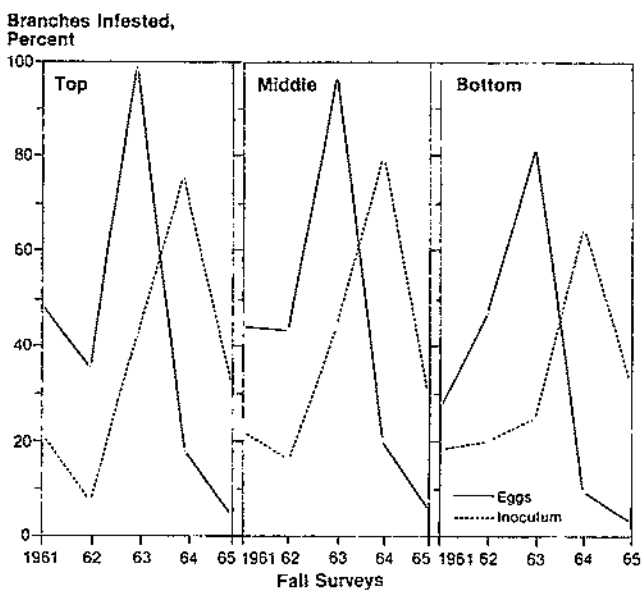


Figure 5.—Proportion of top, middle, and bottom branches with woolly pine needle aphid eggs and fungus spore clusters in fall surveys, 1961–65.

observations that average host and pathogen density in the spring of 1962 was similar to that in the spring of 1964. However, the degree of aggregation differed greatly, with the size of the aggregations decreasing in 1964. Consequently, the physical distance between aggregations also decreased. It is tempting to speculate that closer proximity of the aphids allowed the 1964 epizootic to develop, since the host and pathogen mean densities were the same in 1962, an enzootic year.

Overwintering Mortality—The degree of mortality was determined by comparing the amount of loss within the populations from fall to spring. This was accomplished by analyzing the paired observations for fall and spring on the permanent sample branches. The mortality trends of the overwintering host population were stratified according to location within the tree. The mortality was greatest in the top of the tree and least at the bottom. Loss of spores was generally higher in the middle of the tree than the bottom. The loss in the top of the tree could not be related to either of the other locations.

The aphid eggs were apparently subjected to two kinds of mortality: One when the eggs were removed from branches and one when the eggs shriveled. Both types of loss seemed equally important. The first type was related to snow depth and to late winter or early spring thaws, and the second was primarily mite predation. Those eggs below the snowline were not subjected to as low a temperature. For example, the lowest temperature recorded in 1965 was $-40°$ C, while at the same time the temperature at the base of the trees under 1.5 m of snow was $-12°$. Also, when the branches were laden with snow and a thaw occurred, both the aphid eggs and the fungus spore clusters were loosened and dislodged.

When germination tests were conducted on the spores remaining until spring and early summer, only about 0.5 percent of the spores in the top of the tree were viable. Similar tests of spores on the bottom branches showed an average of about 10 percent viability, a difference which, again, was related to snow depth. The tests were conducted by breaking up the spore cluster, spreading the spores on a glass slide in a water suspension, and incubating at several constant temperatures ranging from 14° to 28° C. During these tests, 94+ percent of all the viable spores germinated within the first 18 hours.

In the sequential sampling technique, it is possible to specify the level of the α and β error rates. Although

they were set at 10 percent, the exact levels cannot be theoretically derived when making a three-level decision. The resulting sampling schemes are presented in table 1. They were tested by sampling a red pine plantation in the original manner and then repeating the sampling by using the sequential technique. In one test using the former technique, four people required 3 hours to complete the sampling, whereas in the latter, two people took 15 minutes to reach the same conclusion. Although it was not possible to set a probability confidence on theoretical grounds, the sequential technique compared favorably with the standard sampling scheme.

Summer Host and Pathogen Distribution Within Trees

Distribution Analyses—In 1961, 1962, and 1963, *E. canadensis* was enzootic, and in 1964, an epizootic of *E. canadensis* occurred; population data were collected for 2 postepizootic years (fig. 6). The aphid population density tended to differ among the tree levels. There was little significant difference in the number of aphids recorded from the top and middle of the tree crown. Both levels usually had significantly higher populations than the bottom. In all years except the second postepizootic (1966), the aphid population reached its highest level in late July. Prior to the epizootic in 1964, the median level was approximately 50–80 aphids per 30 cm of branch. In the years following the epizootic, the population declined to 10–20 aphids per 30 cm, approximately a 75-percent sustained reduction caused by the disease.

It is interesting to note that during the preepizootic and epizootic seasons (1962–64), distinct epizootic waves occurred at approximately 20-day intervals, which are about three incubation periods from infection to sporulation. Four such cycles were required during the epizootic to attain 100 percent mortality of the aphid population. During the postepizootic phase (1965, 1966), aphid mortality from disease was exponential (fig. 6) and showed no distinct waves of infection. The disease first occurred each year at the bottom of the tree. During the preepizootic years (1962, 1963), the median population of aphids on the bottom branches was 40–50 per 30 cm. Following the epizootic, the median level fell to 5 per 30 cm. This lower population threshold of activity was related to the widespread inoculum.

The second-year postepizootic (1966) was characterized by a very slow population buildup caused by the very heavy preovipositional mortality the previous fall. The first occurrence of disease was delayed 1 month until August 30 and coincided with a median population peak of five aphids per 30 cm at the bottom level.

The initiation of disease did not seem to depend on photoperiod as suggested by the data of Wallace et al. (*23*). The aphid density was characteristic of the postepizootic disease initiation.

Multiple Regression Analysis of Disease Distribution—In a complex relationship, such as that of the woolly pine needle aphid and its fungus pathogens, it is very difficult to determine which factors are important in the disease distribution. Multiple regression was employed to unravel such relationships.

Generally multiple regression has been used to predict the value of the dependent variable by regression of it on a number of independent variables. It can also be used, not to predict, but to discover which variables are related to the dependent variable; in this instance, the number of diseased aphids. The independent variables tested for their effect on disease transmission were density of inoculum, apterous aphids, alate aphids, adults, nymphs, and total aphids; mean, maximum, and minimum temperatures; number of hours of saturation deficit between 0–0.1, 0.1–1, 1–2, 2–3, 3–4, 4–5, and 5 + mm; amount of rainfall and sunshine; and the following transformations of both inoculum and host density, x, x^2, $1nx$, e^x, *and* $1/x$.

The values of the dependent variable were given by the weekly observations. The disease prevalence was high enough during 1964, 1965, and 1966 to produce sufficient data for a multiple regression analysis. Based on field observations, the incubation period was between 8 and 9 days. Dead diseased aphids, the dependent variable, counted during one observation period could have died during the week since the last observation. This means that infection could have occurred from a minimum of 8 days to a maximum of 16 days prior to the current observation. Since all the weather variables were recorded daily, they were easily summarized for each infection period. Although population data were obtained at weekly intervals, the density estimates for the 8-day infection intervals had to be interpolated. In order to accomplish this, the change in host and in inoculum density was assumed to be linear between observations. The population levels were then determined for each of the days within the infection period and all values were averaged over those days.

The computer was programed to analyze the data with multiple regression by either forward selection or backward elimination. In the forward selection method, the most significant independent variable was chosen first by the computer and placed in the multiple regression equation. The next most important independent variable was selected second, and the regression equation was recalculated together with all the parameters

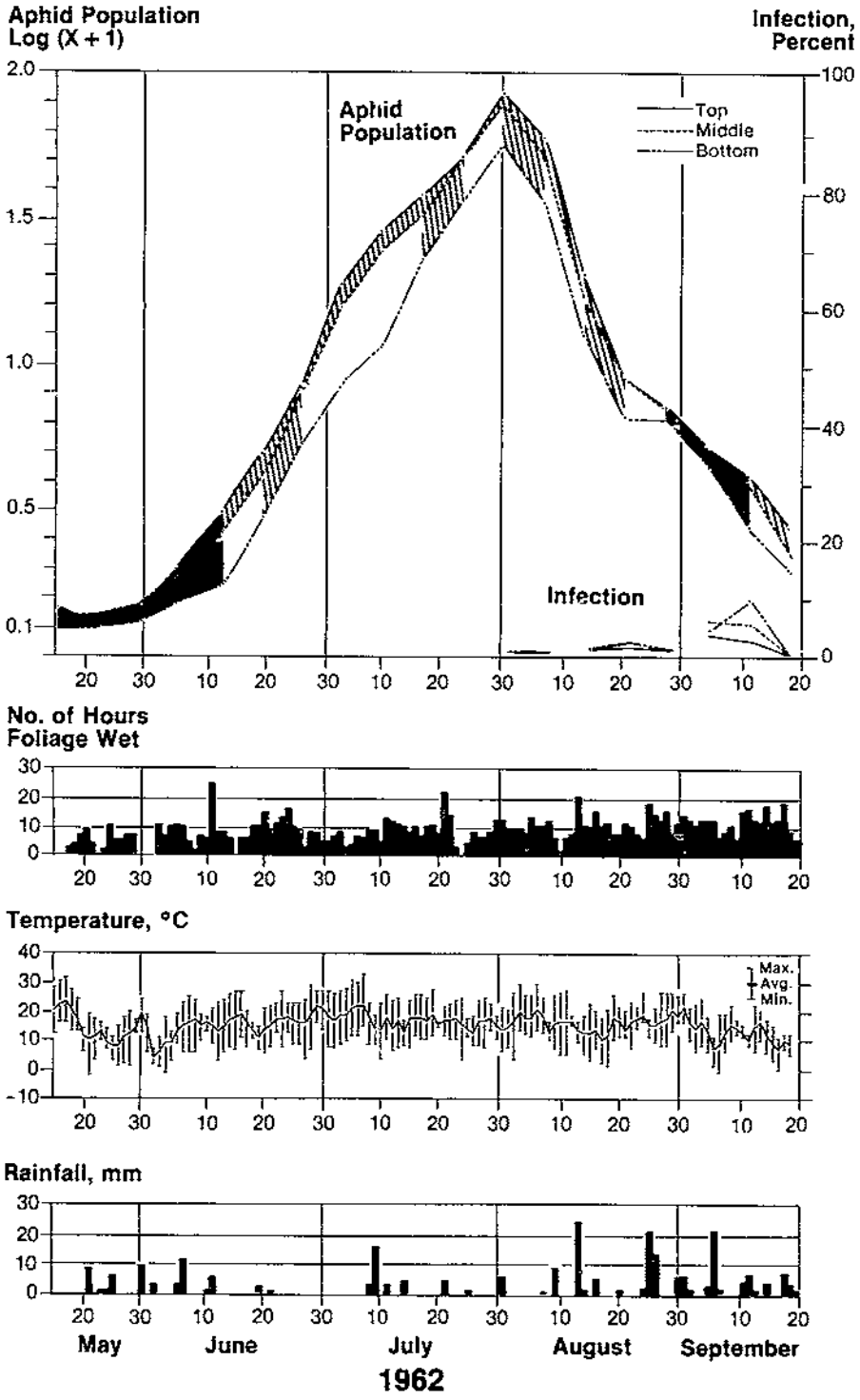


Figure 6.—Population trends of the woolly pine needle aphid per 30-cm branch and infection by *Entomophthora canadensis* at three tree levels, with temperature and rainfall data: 1962, preepizootic; 1964, epizootic; 1965, first-year postepizootic; 1966, second-year postepizootic. (Black areas indicate no significant difference among tree levels, and shaded areas show no significant difference between adjacent levels at 95 percent confidence interval.)

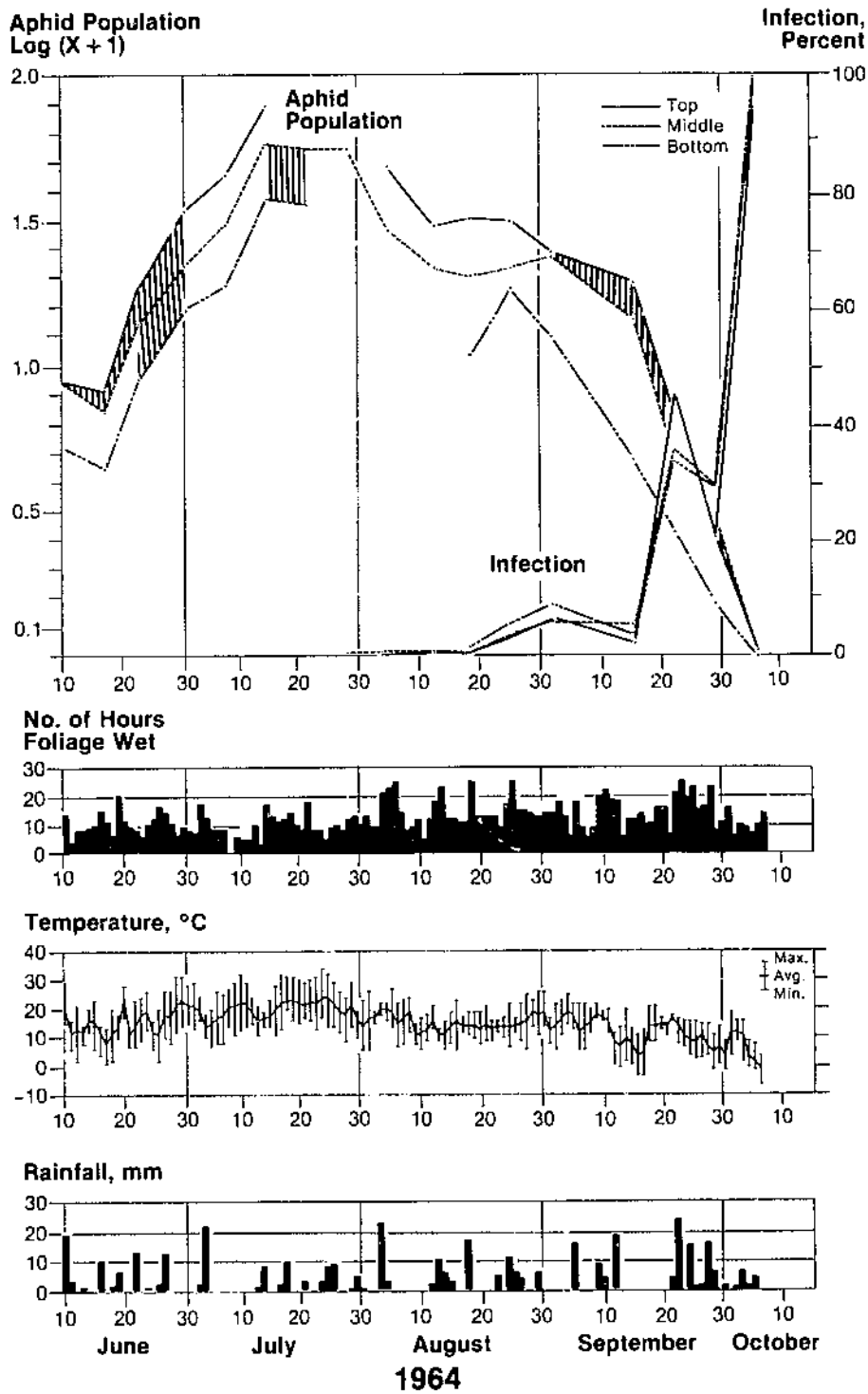


Figure 6.—Population trends of the woolly pine needle aphid per 30-cm branch and infection by *Entomophthora canadensis* at three tree levels, with temperature and rainfall data: 1962, preepizootic; 1964, epizootic; 1965, first-year postepizootic; 1966, second-year postepizootic. (Black areas indicate no significant difference among tree levels, and shaded areas show no significant difference between adjacent levels at 95 percent confidence interval.)

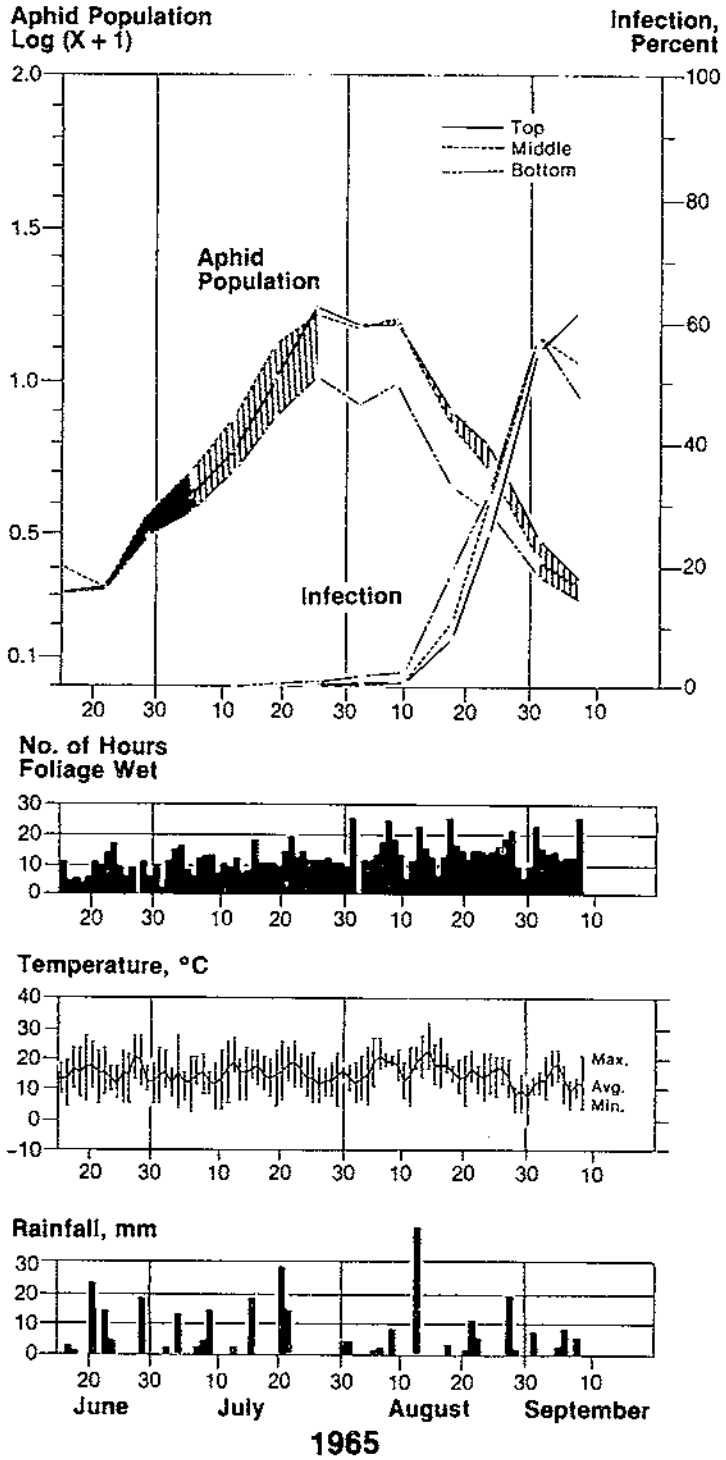


Figure 6.—Population trends of the woolly pine needle aphid per 30-cm branch and infection by *Entomophthora canadensis* at three tree levels, with temperature and rainfall data: 1962, preepizootic; 1964, epizootic; 1965, first-year postepizootic; 1966, second-year postepizootic. (Black areas indicate no significant difference among tree levels, and shaded areas show no significant difference between adjacent levels at 95 percent confidence interval.)

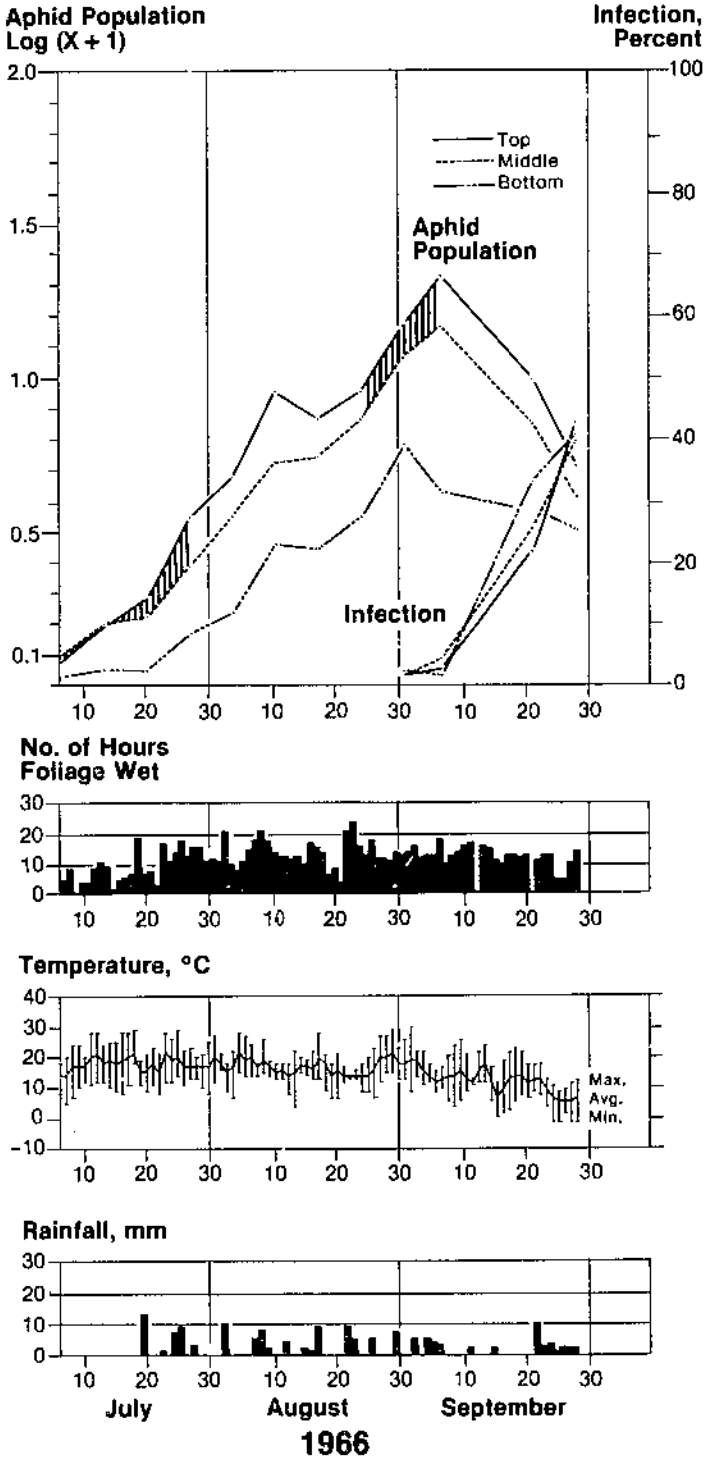


Figure 6.—Population trends of the woolly pine needle aphid per 30-cm branch and infection by *Entomophthora canadensis* at three tree levels, with temperature and rainfall data: 1962, preepizootic; 1964, epizootic; 1965, first-year postepizootic; 1966, second-year postepizootic. (Black areas indicate no significant difference among tree levels, and shaded areas show no significant difference between adjacent levels at 95 percent confidence interval.)

associated with this type of analysis including an analysis of variance. This process was repeated until all the variables that would significantly affect the regression (95 percent confidence level) had been selected and placed in the equation. In the backward elimination method, all the independent variables that were to be tested were placed in the regression equation and a complete analysis was made. The second step was to eliminate the least important independent variable and recalculate all the parameters and statistics associated with the new equation. This was repeated until all the nonsignificant variables had been eliminated.

As can be seen from table 2, the independent variable most frequently selected by multiple regression analysis as controlling the incidence of disease was the inoculum. In seven of the eight equations, inoculum was included and in all but one it was the most important. This was followed in frequency of selection by a measure of host density (five out of eight) and then by a measure of the wetness of the environment, i.e., saturation deficit or rainfall (three out of eight). Temperature was selected only once as being important. The coefficient of multiple determination, which measures the amount of variability removed by regression, did not exceed 0.30 in any of the analyses. The fact that the analysis chose those variables previously believed to be important to the spread of a fungus disease, i.e., inoculum, host density, and "wetness," suggests that this method may have value as a screening tool. A predictive model, however, must be based on a more sophisticated approach than multiple regression.

Table 2.—*Significant independent variables selected by multiple regression as affecting fungus disease prevalence*

Year and tree level	N	Independent variables[1]	Coefficient of multiple determination
1964:			
Top	37	Inoculum	0.209
Middle	61	Average temperature + alate aphids + inoculum.	.196
Bottom	113	Inoculum	.067
1965:			
Top	116	Inoculum + total aphids + SD 0.1 to 1 – SD 1 to 2.	.289
Middle	189	Inoculum + rain + total aphids.	.296
Bottom	275	Inoculum + total aphids – SD 2 to 3.	.194
1966:			
Top	59	Inoculum	.284
Middle	73	Total aphids	.153
Bottom	53	None	-----

[1]SD = saturation deficit.

Discussion

The results of this study on the occurrence and distribution of *E. canadensis* in infestations of the woolly pine needle aphid from 1961 to 1966 show that—

(1) Disease incidence was not limited by climatic factors.

(2) Inoculum levels and host population density were the major factors controlling epizootics.

(3) The preepizootic phase was characterized by a wider distribution of the susceptible host population than the inoculum; conversely, the postepizootic phase had a greater distribution of the pathogen than the host.

(4) Population threshold for disease initiation became lower following an epizootic.

(5) Mean host density by itself was a poor indicator of population levels for the purpose of predicting epizootics. The degree of host aggregation must be considered because a less aggregated population with the same mean host density has the greater probability of sustaining an epizootic.

(6) The velocity of infection, defined as the rate of change in disease with time, became greater with increased inoculum density evidenced by the more rapid kill during the postepizootic phase.

Extremely few detailed epizootiological studies have been made of *Entomophthora* in aphid populations. Robert et al. (*14, 15*) and Rabasse and Robert (*13*) investigated the relationship of *E. aphidis, E. fresenii,* and *E. planchoniana* Cornu to their host *Aphis fabae* Scopoli in France. They concluded that climatic factors controlled the incidence of the disease and that a well-distributed inoculum was essential. They also found that the spatial distribution of the host was important. Wilding (*25*) undertook a quantitative 5-year study of *E. thaxteriana* (Petch) Hall & Bell, *E. aphidis,* and *E. planchoniana* infecting *Acyrthosiphon pisum* (Harris). He found that among climatic factors, incidence of infection could only be associated with rainfall. Voronina (*22*) also stressed the duration of rainfall as important to *Entomophthora* epizootics in aphid populations. Wilding reported that inoculum, as measured by airborne conidia, had a high correlation with aphid infection. Also, he found host density only slightly important in controlling spread of infection. Similarly, Shands et al. (*16*) studying *E. thaxteriana* in the potato aphid (*Macrosiphum euphorbiae* (Thomas)) concluded that fungus infection was regulated by host density independent factors. They postulated that weather factors were of major importance in initiation and subsequent spread of *Entomophthora*. Missionnier et al. (*10*) reported that an evenly and densely distributed inoculum was necessary for an epizootic disease followed by a mean maximum temperature of 20° C. They likewise concluded that epizootics were independent of host density. An important difference between these findings and those of both the present study and studies by Robert et al. (*15*) and Rabasse and Robert (*13*) was that host density is important if spatial distribution is considered.

The conclusions drawn from the observations made in this study need to be verified with epizootiological experimentation. Only then will predictions of the action of disease under various environmental conditions be possible. In this way, a strategy of control can be logically derived in which *Entomophthora* can be utilized as a biocontrol agent.

Literature Cited

(1) Anscombe, F. J. 1949. The statistical analysis of insect counts based on the negative binomial distribution. Biometrics 5: 165–173.

(2) Bliss, C. I., and Fisher, R. A. 1953. Fitting the negative binomial distribution to biological data. Biometrics 9: 176–200.

(3) Carner, G. R. 1976. A description of the life cycle of *Entomophthora* sp. in the two-spotted spider mite. Jour. Invert. Path. 28: 245–254.

(4) DeLyzer, A. J. 1965. Rearing of woolly pine needle aphid, *Schizolachnus piniradiatae*, viviparae. Jour. Econ. Ent. 58: 1021–1022.

(5) Grobler, J. H. 1962. The life history and ecology of the woolly pine needle aphid, *Schizolachnus piniradiatae* (Davidson) (Homoptera: Aphididae). Canad. Ent. 94: 35–45.

(6) Grobler, J. H., MacLeod, D. M., and DeLyzer, A. J. 1962. The fungus *Empusa aphidis* Hoffmann parasitic on the woolly pine needle aphid, *Schizolachnus piniradiatae* (Davidson). Canad. Ent. 94: 46–49.

(7) Kenneth, R., and Olmert, I. 1975. Entomopathogenic fungi and their insect hosts in Israel: Additions. Israel Jour. Ent. 10: 105–112.

(8) MacLeod, D. M., and Müller-Kögler, E. 1970. Insect pathogens: Species originally described from their resting spores mostly as *Tarichium* species (Entomophthorales: Entomophthoraceae). Mycologia 62: 33–66.

(9) MacLeod, D. M., Tyrrell, D., and Soper, R. S. 1981. *Entomophthora canadensis* sp. nov., a fungal pathogen of the woolly pine needle aphid, *Schizolachnus piniradiatae*. Canad. Jour. Bot. [In press.]

(10) Missonnier, J., Robert, Y., and Thoizon, G. 1970. Circonstances épidémiologiques semblant favoriser le développement des mycoses à Entomophthorales chez trois aphides, *Aphis fabae* Scop., *Capitophorus horni* Börner et *Myzus persicae* (Sulz.). Entomophaga 15: 169–190.

(11) Morisita, M. 1964. Application of Iδ-index to sampling techniques. Res. Population Ecol. 6: 43–53.

(12) Morris, R. F. 1954. A sequential sampling technique for spruce budworm egg surveys. Canad. Jour. Zool. 32: 302–313.

(13) Rabasse, J.-M., and Robert, Y. 1975. Facteurs de limitation des populations d'*Aphis fabae* dans l'ouest de la France. II – Incidence des mycoses à *Entomophthora* sur les populations des hôtes primaires et de la féverole de printemps. Entomophaga 20: 49–63.

(14) Robert, Y., Rabasse, J.-M., and Rouze-Jouran, J. 1972. Régulation naturelle des populations de *Capitophorus horni* Börner [Hom: Aphididae] par Hyménoptères Aphidiidae et Entomophthorales. Entomophaga 17: 59–69.

(15) Robert, Y., Rabasse, J.-M., and Scheltes, P. 1973. Facteurs de limitation des populations d'*Aphis fabae* Scop. dans l'ouest de la France. Entomophaga 18: 61–75.

(16) Shands, W. A., Simpson, G. W., and Hall, I. M. 1963. Importance of entomogenous fungi in controlling aphids on potatoes in northeastern Maine. Maine Agr. Expt. Sta. Tech. Bul. T6, 42 pp.

(17) Snedecor, G. W. 1956. Statistical methods applied to experiments in agriculture and biology. Ed. 5, 534 pp. Iowa State Col. Press, Ames.

(18) Soper, R. S. 1962. The woolly pine needle aphid, *Schizolachnus piniradiatae* (Davidson), attacked by the fungus *Entomophthora aphidis* (Hoffmann). Canada Dept. Forestry Bimo. Prog. Rpt. 18: 2.

(19) Soper, R. S., Holbrook, F. R., Majchrowicz, I., and Gordon, C. C. 1975. Production of *Entomophthora* resting spores for aphid control. Maine Agr. Expt. Sta. Tech. Bul. 76, 15 pp.

(20) Soper, R. S., and MacLeod, D. M. 1963. Spore morphology of *Entomophthora fresenii* Nowakowski. Jour. Insect Path. 5: 478–482.

(21) Tyrrell, D., and MacLeod, D. M. 1975. In vitro germination of *Entomophthora aphidis* resting spores. Canad. Jour. Bot. 53: 1188–1191.

(22) Voronina, E. G. 1971. [Epizootics entomophthorosis of the pea aphid, *Acyrthosiphum pisum* Harris (Homoptera, Aphidoidea).] Ent. Obozr. 50 (4): 780–799. [In Russian. English transl. in Ent. Rev. (Wash.) (1971) 50: 444–453.]

(23) Wallace, D. R., MacLeod, D. M., Sullivan, C., and others. 1976. Induction of resting spore germination in *Entomophthora aphidis* fungi by long-daylight conditions. Canad. Jour. Bot. 54: 1410–1418.

(24) Wilding, N. 1970. Entomophthoraceae. Rothamsted Expt. Sta. Rpt. 1970, pt. 1, p. 207.

(25) Wilding, N. 1975. *Entomophthora* species infection of pea aphids. Roy. Ent. Soc., London, Trans. 127: 171–183.

☆U.S. GOVERNMENT PRINTING OFFICE. 1981—349-083

U.S. DEPARTMENT OF AGRICULTURE
SCIENCE AND EDUCATION ADMINISTRATION
WASHINGTON, D.C. 20250

END