THE MEASUREMENT OF SPRUCE BUDWORM MORTALITY CAUSED BY DISEASE

by

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1.0 INTRODUCTION

The spruce budworm is now the most important forest insect in New Brunswick from the point of view of numbers involved, area affected, and potential damage to stands of spruce and fir. In 1945 a co-operative project was set up between Fraser Companies Limited, Canada Forestry Branch, Canada Department of Agriculture, and the New Brunswick Forest Service with the object of studying the spruce budworm and its possible control by forest management. As a direct result of this arrangement a Forest Biology field laboratory was erected on the Green River Watershed in northwestern New Brunswick for the study of the biology and epidemiology of the spruce budworm.

One of the major objects of this venture has been to prepare life tables for the spruce budworm for different stand types. A requisite for life tables is that all mortality factors and their effect on spruce budworm populations be studied. This requisite had not been fully satisfied prior to 1953 since the effects of disease as a mortality factor had been more or less neglected. A preliminary survey undertaken in 1953 emphasized the need to fill this gap, with the result that in 1954 intensive studies of diseases and their role in spruce budworm epidemiology were initiated.

The objects of these studies were:

(1) To follow the present infestation through its course, determining what part diseases play in population fluctuations from year to year, and in the eventual decline of the infestation. This involves the estimation of mortality from disease for inclusion in life tables.

(2) To find what diseases are present in the field, which of them

exert measurable control, and in what stage of the host they kill.

(3) To find, if possible, a simple and rapid, yet statistically sound, method of obtaining a reliable estimate of the incidence of disease on regular sample plots.

(4) To assess unidentified mortality under identical and optimum rearing conditions each year, its relation to infestation age, and the possibility of intrinsic larval weakness in old populations.

(5) To check on the smear technique used to diagnose cause of death, especially in the case of capsule virus disease.

The following is a preliminary report on the progress made in 1954 towards satisfying the above objects.

2.0 MATERIALS AND METHODS

2.1 Study Plots

Two plots, K2 and G11, situated in northwestern New Brunswick were selected for this study. These were two out of several plots under intensive study by the staff of the Green River Project. Both plots are fairly close (10 to 12 miles) to one of the original foci of the budworm infestation in northern New Brunswick and have suffered severe defoliation for the past four years. Stand types are almost identical, the stands being composed of dense balsam fir 35 years old. They differ only in that G11 has been partially isolated by cutting, and budworm populations on K2 have always been slightly higher than on G11.

In addition a collection was made from each of three other plots at about the mid point in the feeding season to see if there might be differences in incidence of disease between different stand types and different ages of infestation. A brief description of stand type, infestation history and present level of infestation for these plots is given in Table 1.

2.2 Collecting and Rearing

Collections of 100 to 140 insects were made once a week from

Table 1

Third-Instar Population in 1954, Defoliation History, and Approximate Age of Plots G4, I1, K1, K2 and K3

Plot	Population per 10 sq. ft.	Approximate age	Past defoliation				
			1950	1951	1952	1953	1954
G4	61	105	M	L	L	L	L
G11	100	35	M-S	S	S	S	S
K1	73	105	S	S	S	S	S
K2	123	35	M	M-S	S	S	S
I1	98	35	N	N	L	M	S
K3	27	35	L	S	S	S	L

Legend: N = Nil; L = Light; M = Moderate, S = Severe

each of the two intensively studied plots, commencing with the beginning of larval activity in the spring and continuing until adult eclosion in late summer. One collection was taken at about the mid-point in larval life from three other plots, G4, K1, and I1. Insects were collected from the mid-crown branches of trees adjacent to population sampling trees. Handling was minimized by snipping off the feeding site along with the larva and placing both in an individual, sterile, screw-cap vial. Sterile technique was employed in collection as far as was practically possible.

Pupae were collected by felling a tree and then shaking it vigorously over a square canvas mat; loose pupae falling on the mat were then picked up, usually with the fingers, and placed in individual vials. This method of pupal collection will be changed in future years because of the high mortality resulting from excessive handling during collection.

All collections were shipped to Fredericton as soon after collection as possible, where they were divided into two equal portions; one to be reared and the other to be sectioned.

The former insects were reared through to the adult stage, and were examined every second day for general state of health and external symptoms of disease. Diagnosis of the cause of death was attempted for all that died. The vials containing insects were kept in a dark incubator at 72 $\pm$ 5° F. with R.H. uncontrolled. Food consisted of fresh current year's balsam fir shoots which were gathered from budworm-free areas behind the laboratory in Fredericton. Upon pupation the insect was removed to a clean vial. Individual rearing was employed so that notes could be kept on external symptoms of disease. Also it was desired to (1) know incidence of disease at the time of collection, (2) circumvent any error due to the effects of crowding and (3) avoid possible spread of disease from one individual to another.

A sample of moths emerging from each rearing lot was prepared for

sectioning; others were mated to obtain fertile eggs for experiments to be described later.

2.3 Sectioning

All insects that were to be sectioned were first killed by immersing them in hot water for 30 seconds. The integument was then pierced in several places to ensure good penetration of the fixative before fixing in Bouin's fluid. The specimens were washed and then stored in 70 per cent alcohol until the winter when they were sectioned. The insects sectioned included one-half of all weekly collections, a sample of adults emerging from each rearing lot, and a sample of freshly emerged second-instar larvae which had been collected in hibernaculae in the fall of 1953.

Sections were cut at four microns with the exceptions of pupae and adults. Difficulties were encountered in trying to cut thin sections of these two stages because of the large amounts of hardened chitin present in the exoskeletons, and also because complete penetration of the paraffin into developing ovarioles was not achieved. Pupae and adults were therefore sectioned at ten microns.

This sectioning program was undertaken as a check on diagnoses made from insects dying in the rearings, and on live smearings, especially in those cases where death was attributed to capsule virus. There is some confusion in diagnosing the capsule virus disease of the spruce budworm, using light microscope techniques, because of the very small, highly refractive capsule-like bodies often found in both healthy and dead budworm larvae. On the advice of F. T. Bird (Laboratory of Insect Pathology), it was decided that sectioning techniques should be employed. The capsule virus disease in this insect apparently causes a characteristic cellular change that cannot be detected in blood or crushed body smears. This change occurs primarily in the nuclei of the cells of the fat body and consists of a

coagulation and later alignment of the chromatin into long dark-staining ropy strands. This phenomenon has been considered as indicative of positive presence of capsule disease (1). However, since this project was initiated some doubt has been introduced as to the validity of this method of diagnosis (2). Meanwhile, diagnoses of the cause of death as capsule virus may be doubtful. However, the mortality listed in this report under this category does represent mortality due to some cause, and until this "capsule" situation has been elucidated this heading will be maintained with the reservation that mortality listed under it may later be attributed to some other cause.

Sectioning has also served as a check on diagnoses of other diseases. The differences observed between incidence of infection with a pathological organism as determined by examination of stained sections of living budworm, and mortality due to disease in rearings was only slight. Observation of micro-organisms multiplying within the cells of any insect was interpreted as infection with a pathological organism.

2.4 Diagnosis of Disease

Smears of crushed cadavers of insects that died in the rearings were made in sterile distilled water. These smears were examined microscopically utilizing dark field illumination at a magnification of 950x. Positive diagnosis of death due to a disease was based primarily on the observance of bodies usually associated with the disease in question. In some cases a combination of this and results obtained from observation of stained sections of living budworm was used as described below. A selection of smears was sent to the Laboratory of Insect Pathology at Sault Ste. Marie to check on diagnoses.

(1) Virus Disease:

Three virus diseases are frequently found in budworm populations - a cytoplasmic and a nuclear polyhedral disease and a capsule disease. In

this study only two of these were encountered. The two polyhedral diseases are differentiated mainly by the tissues they attack and by their size. Polyhedra of the cytoplasmic disease are found in the digestive cells of the mid-gut epithelium as colonies of uniformly dispersed crystal-like bodies enclosed by a membrane. They measure approximately 0.5 μ in diameter, with some up to 3 μ. In the nuclear disease, on the other hand, the polyhedra are formed within the nuclei of the tracheal matrix, hypodermal fat and blood cells, and measure on the average 2 μ in diameter (3). Even though there is a relatively large difference in size between the two types of polyhedra, separation was found difficult using the light microscope. Therefore, they were separated on the basis of stained sections prepared from living insects. No evidence of the nuclear polyhedral disease was noted in these sections.

Difficulties encountered in diagnosing the capsule disease of the spruce budworm were mentioned above. In diagnosing death due to capsule virus disease from observation of smears a note was made as to the abundance of capsule or capsule-like bodies present. The slides were recorded as excellent, good, fair, or poor examples. Since evidence of capsule disease from stained sections differed slightly from smear diagnosis, poor and fair examples were taken out of the capsule category and placed under unknown causes of mortality. This resulted in much better agreement between the two methods.

(2) Fungi and Bacteria:

Diagnosis of fungal diseases was based on the presence of fungal spores, hyphal bodies or mycelia in the smears, and bacterial diseases on the presence of bacteria. A number of smears and budworm cadavers were sent to the Laboratory of Insect Pathology at Sault Ste. Marie for possible identification of fungi and bacterial present. Most of these were returned

labelled as "probably secondary", so the sectioned material was used in assessing mortality from fungi and bacteria. The resulting figures do not constitute a definite assessment of mortality but rather, as mentioned above, indicate the presence of a pathological organism. However, since the numbers involved were so small this would have little effect.

(3) Microsporidia:

Positive diagnosis of death due to microsporidia was based on the presence of the characteristic spores of this protozoan. No attempt was made to separate species.

It should be pointed out here that diagnosis of disease becomes increasingly difficult because of histolysis as the larvae progress from the late 5th and 6th instars to the pupa. At this time the fat body, generally the first tissue to show the effects of histolysis, becomes filled with large dark-staining basophillic granules, accompanied by an overall breakdown. This makes observation of any possible effect of the capsule disease on the chromatin of the nuclei of the fat body almost impossible.

2.5 External Symptoms

Notes were kept throughout all rearings to see if it might be possible to distinguish between the various diseases using external symptoms as the criteria. In general this was not found possible since external symptoms varied greatly and seemed at least partially dependent upon moisture conditions within the vial at the time of death. However, distinction between diseased and non-diseased material on this basis does seem feasible. Listed below are the various diseases and the most constant external symptoms associated with them.

(1) Fungi:

Insects dying from fungus diseases were most constant as far as external symptoms were concerned. The body first became hard and mummified,

followed by the appearance of mycelial growth all over the integument.

(2) Bacteria:

Larvae dying from bacterial diseases were few in number. Those that were observed showed an overall darkening after death accompanied by by flaccid appearance resulting from liquefaction of the body contents.

(3) Microsporidia:

Changes in body appearance after death were very slight, but larvae exhibited rectal and oral discharges. On several occasions smears were made of these discharges and were found to be composed of almost pure spores of microsporidia.

(4) Virus:

Since the methods used in diagnosis of capsule disease may be invalid and also since insects dying from a polyhedral disease were so few in number no external symptoms can be given.

3.0 CALCULATION OF PERCENTAGE MORTALITY

One of the objectives of this study was to determine percentage mortality due to disease in such a manner as to permit inclusion of the figures in life tables. The advantages of this will be pointed out in a later section where this and another method of interpretation are compared.

3.1 Larvae

In order to minimize the possible effect of laboratory rearing on disease, only the first week of rearing of each collection was considered. Since collections were made one week apart, these weekly rearing figures could be combined to provide a total mortality figure for disease for the larval stages; the actual method of analysis is given below and is supported by examples in Tables 3, 4, and 5. The larval development and population figures on which many of the calculations are based were obtained from the staff of the Green River Project (Table 2).

Table 2

Population and Larval Development on Plots G11 and K2 for 1954

A. Population

Date	Population per 10 sq. ft. branch area	
	G11	K2
June 2	122	
" 15	93	119
" 23	74	102
" 30	57	87
July 7	40	73
" 13	28	60
" 21	20	44

B. Larval Development

Date	% of population in each instar						
	II	III	IV	V	VI	Pupae	Adults
June 2	90	10					
" 15		60	40				
" 23			60	40			
" 30			1	30	69		
July 7				5	94	1	
" 13					80	20	
" 21					20	72	8
" 28						50	50

Table 3

Per Cent Mortality from Disease on Plots G11 and K2 Based on Rearing for One Week

Plot	Coll. date	No. in sample	Micro-sporidia	Cap-sules	Fungi	Poly-hedra	Bac-teria	Un-known	Total disease
K2	6/15	57	5.3	7.0	0	0	0	18.0	12.3
	6/23	63	3.2	0	3.2	0	0	0	6.4
	6/30	66	6.1	3.0	0	0	0	5.0	9.1
	7/7	65	0	0	1.5	0	0	3.1	1.5
	7/13	40	5.0	2.5	5.0	0	0	0	12.5
	7/21	6	0	0	0	0	0	16.7	0
G11	6/2	70	1.4	2.9	0	0	0	7.5	4.3
	6/15	64	0	4.7	0	1.6	0	18.3	6.3
	6/23	61	4.9	8.2	0	-	0	11.3	13.1
	6/30	65	1.5	1.5	1.5	1.5	0	1.7	6.0
	7/7	57	0	1.7	0	0	0	3.6	1.7
	7/13	44	0	4.5	0	0	0	11.9	4.5
	7/21	15	26.7	0	0	0	6.7	30.0	33.4

In life tables prepared for the spruce budworm by Morris and Miller (10) the larval period is broken down into intervals corresponding to periods when certain important parasites are causing mortality. There appears to be no particular point in the larval life of this insect when the effect of disease is much more pronounced than another (Table 3); therefore just about any breakdown would be feasible as far as inclusion of disease in life tables is concerned. For convenience the existing divisions are employed.

Potential rather than actual mortality is used throughout the following calculations because of the difficulties involved in determining actual mortality in the larval stage when two or more mortality factors are acting on the same insect. Potential mortality may be defined as that mortality that would be expected if there were no other mortality factors present which might cause interference. The assumption is made when using potential mortality that all mortality factors act independently of one another. It is calculated by the following formulae:

$$(1)\ \text{Disease} = \frac{(\text{No. of insects in sample dying from disease}) \times 100}{(\text{No. of insects in sample}) - (\text{No. insects in sample killed by parasites})}$$

$$(2)\ \text{Unknown mortality} = \frac{(\text{No. insects in sample dying from unknown causes}) \times 100}{(\text{No. insects in sample}) - (\text{No. insects in sample dying from disease and parasites})}$$

Formula 1 may appear to be incorrect on first inspection because the number of insects dying from unknown causes has not been subtracted from the sample size in the denominator. This was not done because the numerator contains both insects that died from disease alone and those few insects that died from one or a combination of unknown causes and/or disease. It is impossible to separate these two types of mortality by smearing, because

Table 4

Dx* Values for Potential Mortality
from Disease on Plots G11 and K2 Based on Rearing for One Week

Plot	Coll. date	Popu. per 10 sq. ft. foliage	Micro-sporidia	Cap-sules	Fungi	Poly-hedra	Bac-teria	Un-known	Total diseases	Total d_x
K2	June 15	119	6.3	7.0	0	0	0	21.4	14.6	36.0
	" 23	102	3.3	0	3.3	0	0	0	6.6	6.6
	" 30	87	5.3	3.0	0	0	0	4.3	7.9	12.2
	July 7	73	0	0	1.1	0	0	2.3	1.1	3.4
	" 13	48	2.4	2.5	2.4	0	0	0	6.0	6.0
	" 21	12	0	0	0	0	0	2.0	0	2.0
Totals			17.3	12.1	6.8	0	0	30.0	36.2	66.2
G11	June 2	122	1.7	3.5	0	0	0	7.5	5.2	14.3
	" 15	93	0	4.4	0	1.5	0	18.3	5.9	22.9
	" 23	74	3.6	6.1	0	0	0	11.3	9.7	18.1
	" 30	57	0.8	0.9	0.8	0.9	0	1.7	3.4	4.4
	July 7	40	0	0.7	0	0	0	3.6	0.7	2.1
	" 13	22	0	1.0	0	0	0	11.9	1.0	3.6
	" 21	6	1.6	0	0	0	0.4	30.0	2.0	3.8
Total excl. 6/2			6.0	13.1	0.8	2.4	0.4	32.2	22.7	54.9

*Where $d_x = (A/B \times 100) \times C$

A = The No. of larvae dying in one week rearing

B = " " " " in the sample

C = The larval population per 10 sq. ft. at the beginning of the week

diagnosis as diseased is based upon the presence of disease-causing bodies observed in the smear. In short, some larvae infected with disease may actually have died from unknown factors.

A figure for per cent potential mortality was derived from weekly rearing data for each week of rearing. These per cent mortality figures were then applied to the larval population (l_x) on the particular plot for the data on which the collection was made to give a d_x figure for each collection data. The population figures used were obtained from the Green River Project, and are expressed as the number of insects per ten square feet of branch area (branch area being the sample unit) (Green River Annual Technical Report 1954). For later collections a deduction for that per cent of the population that had pupated had to be made from these figures to give the larval population on which the determination of d_x is based. (D_x denotes the number of insects dying during a specified interval, x.) Summation of these d_x's provided a total d_x for the larval period. Subdivision of mortality into larval periods was based on this total d_x (Table 5).

Period I (10) falls at the peak of the third instar, so all collections containing third-instar larvae were checked for larvae dying as thirds. The percentage of third-instar larvae in each collection was determined (using larval development figures) and these percentages were then applied to the collection sizes to find the number of thirds in each collection. Since larval instar was noted at death it was possible to calculate a figure for the potential per cent of third-instar larvae dying in rearings. This procedure was repeated for each of the three remaining periods, and a potential percentage dying worked out for each. These potential mortality figures for each period were totalled. By using this total it was possible to apportion, by means of ratios, the total d_x figure worked out from weekly rearings to each one of the periods (Table 5). This does not change total

Table 5

Potential d_x* by Period and Diseases for Plots K2 and G11 for the Larval Stages

Period	K2 Disease					G11 Disease				
	Cap.	Micro.	Fungi	Other diseases	Un-known	Cap.	Micro.	Fungi	Other diseases	Un-known
I	0	0	0	0	19.5	0	0	0	0	5.6
II	3.1	4.7	0	0	3.1	3.4	1.2	1.2	1.2	6.8
III	1.8	3.5	1.7	0	7.8	1.4	0.7	0	0	4.1
IV	2.1	4.1	6.3	2.1	6.3	3.7	9.2	1.8	1.8	12.8

*Where d_x for any 1 period is calculated by the following formula:

$$d_x = \frac{(A/B \times 100) \times C}{D}$$

where: A = The number of insects in those instars covered by the period in question that die from a disease.

B = The total number of insects in the sample which are in those instars covered by the period in question.

C = Total d_x for all periods and all diseases (from Table 4).

D = $\sum A/B$ for all periods and diseases.

d_x for larvae, of course; it is merely a method of sub-dividing it into the periods commonly shown in the supplementary life tables.

3.2 Pupae

A very large difference in overall mortality was noticed between field-collected and laboratory-reared pupae. Literature available on rearing the spruce budworm shows that other workers (12, 13) have experienced high mortality in the pupal stage, and have attributed it to the result of excessive handling. Stehr(13) recommends that budworm pupae never be touched directly during rearing. Since pupae in this study, as mentioned above, were subjected to rigorous handling, it was decided that a different approach from that used for larvae should be used in calculating pupal mortality due to disease.

All dead intact pupae from field collections, and those dead intact pupae resulting from larvae that did not exist as larvae for more than three days in the laboratory were examined for the presence of disease. The resulting figures for plots G11 and K2 were pooled to provide an estimate of potential percentage diseased. These pooled figures, when applied to data on pupal mortality supplied by the Green River staff, would yield an estimate of 100 q_x (100 q_x is the number dying in any period, x, expressed as a percentage of the numbers alive, l_x, at the beginning of the period). The data obtained from the Green River studies were derived in the following manner. Branches were collected from sample trees at the time when 80 per cent of the adults had emerged from pupae. Pupal population per 10 square feet of branch area was obtained by counting both emerged and sound intact pupae on these branches. All sound intact pupae were reared; of these some emerged, some were parasitized, and the remainder died of unknown causes. The figure for potential per cent mortality from disease was applied to this number dying

from unknown causes, giving the number dying from disease. Application of this figure to the population size at the time of sampling (l_x) gave the desired d_x figure for pupae. The remaining pupae dying from other causes provided the figure for d_x for death due to unknown or "intrinsic" causes. Using pupal mortality figures derived from G11 and K2, pupal 100 q_x figures for disease and unknown causes of mortality were calculated for all plots (Table 6).

3.3 Adults

Only a very few adults were sectioned so d_x figures were not calculated (Table 7).

4.0 INTRINSIC MORTALITY

During the rearing of spruce budworm from year to year in connection with parasite studies at Green River a significant percentage mortality from what has been termed "death due to other causes" has invariably been encountered. Other workers having occasion to rear the spruce budworm have also run into this type of mortality (12, 13). During the past two years disease studies have been carried out on Green River material with the result that mortality due to other causes has been reduced because it no longer includes mortality due to disease. The remaining figure, consisting of mortality outside the range of diagnosis using light microscope techniques, nevertheless remains a fairly substantial figure. Even though rearing techniques were selected with the aim of supplying conditions close to the optimum, interpretation of the figures listed under "unknown" mortality as representing mortality due to intrinsic causes may be questionable. Problems arise when considering data of this sort as to how much of this figure may be attributed to the effects of rearing, handling, intrinsic causes, and most important how these factors may be separated. A solution to this problem may lie in the determination of the intrinsic rate of natural increase for the spruce budworm under optimal conditions. Such an approach might provide a most conservative estimate for

Table 6

Pupal Mortality (100 q_x) for All Green River Project Sample Plots Using Pooled Estimate of 17.3 Per Cent for Disease (from Rearings) in the Calculation.

Plot	G2	G4	G5	G8	G9	G10	G11	G12	G13	K1	K2	K3	M1	I1
100 q_x for disease	1.3	2.9	3.6	4.6	1.0	1.1	2.6	2.9	2.0	1.6	3.5	1.3	4.9	3.6
100 q_x for unknown	6.2	13.8	17.3	22.1	4.6	5.3	12.2	14.0	9.8	7.9	16.6	6.0	23.4	17.1

Example - G9

Intact = 59 Dead = 25% Collection size = 266 % diseased = 17.3

25% of 59 = 14.7 17.3% of 14.7 = 2.5

100 q_x = 2.3/266 x 100 = 1.0

Table 7

Results of Examination of Stained Slides of Budworm Adults

Plot	Coll. date	No. insects in sample	Micro-sporidia		Poly. hedra		Diseased	
			No.	%	No.	%	No.	%
K2	June 15	6	2	33.3	-	-	2	33.3
K2	" 23	9	2	22.2	-	-	2	22.2
K2	" 30	8	1	12.5	-	-	1	12.5
K2	July 7	9	3	33.3	-	-	3	33.3
G11	June 15	5	2	40.0	-	-	2	40.0
G11	" 23	8	0	0	-	-	0	-
I1	" 30	5	0	0	-	-	0	-
K2M	" 30	5	1	20.0	-	-	1	20.0
G4	" 30	8	2	25.0	-	-	2	25.0
GSM	" 30	9	2	22.2	1	11.1	3	33.3

Total 72

	Male	Female
	37	35
Total diseased	8	8

% Diseased using total figures = 22.2

this type of mortality. However, the important thing in this study is whether it changes from year to year and, if so, by how much? If rearing techniques are stabilized rearing records from year to year should provide a reasonable answer, because deaths due to faulty rearing would be expected to remain constant.

Because of the similarity of techniques employed in this and disease studies, a preliminary effort towards measuring this type of mortality is included here. For want of a better term this mortality will be labelled as "intrinsic" mortality. Rearing records on the spruce budworm for the past five years by C. A. Miller were summarized for death due to other causes (including disease) to see if there might be a difference between years and between plots (Table 8). Records on death due to other causes (excluding disease) were kept during the present study and the results are tabulated under "unknown" causes in all tables concerned with mortality. There appears to be very little difference between years and plots from records kept for five years, but rearing records for 1954 do indicate that this type of mortality may be highest in the earlier instars.

Experiments were set up on a small scale to measure possible mortality resulting from some first-instar larvae failing to enter diapause. This type of mortality would be classified under intrinsic causes. The method employed was almost identical with that described by Stehr in rearing spruce budworm for getting freshly-hatched larvae to spin hibernaculae (13). The main difference was that food was supplied for those few larvae that did not enter diapause. The figure calculated for this mortality of 3.7 per cent is comparable to that obtained by other workers (5, 7, 13).

5.0 REQUIRED SAMPLE SIZE

One objective of this study was to try to find a simple and rapid, yet statistically sound, method of obtaining an estimate of the incidence of

Table 8

Per Cent Mortality from Unknown Causes
(Incl. Disease) in Rearings for Various Plots for the Years 1949 to 1954

Year	K1			K2			G2			G4		
	June 1 June 15	June 15 June 30	July 1 July 15	June 1 June 15	June 16 June 30	July 1 July 15	June 1 June 15	June 16 June 30	July 1 July 15	June 1 June 15	June 16 June 30	July 1 July 15
1954	-	12.3	9.7	-	19.5	10.5	-	-	19.8	18.9	-	15.1
1953	27.7	12.4	13.3	64.3	21.0	14.8	9.3	-	21.0	-	15.2	18.2
1952	-	12.0	12.5	-	-	22.2	11.5	25.0	30.0	32.4	32.7	13.8
1951	-	-	-	-	-	-	6.5	27.9	34.4	16.2	19.1	18.9
1950	-	-	-	-	-	-	-	-	-	-	17.4	33.3
1949	-	-	-	-	-	-	-	-	-	-	14.8	-
		G5			G8			G9			Q1	
1954	-	-	5.9	17.3	-	-	-	-	-	-	-	-
1953	16.1	13.9	13.0	-	11.2	27.8	17.2	29.1	9.8	9.1	8.6	-
1952	-	27.8	32.1	17.0	30.5	14.4	-	55.8	15.9	5.3	31.1	-
1951	9.6	6.9	16.7	10.2	23.8	11.4	-	-	-	6.8	9.2	-
1950	-	6.8	11.7	-	21.0	10.0	-	-	-	-	8.3	-
1949	-	16.4	-	-	13.3	-	-	-	-	-	-	-

mortality due to disease on permanent sample plots. A portion of this object could be fulfilled by finding the average sample size necessary to establish this estimate. The data accumulated in the present study have not yet been subjected to a complete statistical analysis. Application of a sequential analysis, however, has given an indication of the sample size necessary to establish the percentage incidence of disease within fairly broad classifications. Outlined below is a derivation of the average sample size required for determining incidence of disease for several classifications. The classifications selected in the following example are arbitrary and can be altered to conform to future demands of the work.

The sequential analysis applied was that outlined by Oakland for the binomial background (11). A binomial distribution was assumed for this work (i.e., either diseased or not diseased). The two sets of classification, chosen on the basis of experience gained from previous work, were 5 to 15 per cent and 25 to 35 per cent. The two types of error that may be encountered (denoted by α and β) were accepted as 10 per cent. Using the above data it is possible to construct the two pairs of lines shown in Figure 1. The average sample size required to place the percentage disease within these categories may also be derived from these data by constructing the average sample number curve for each set of lines (Fig. 2).

6.0 COMPARISON BETWEEN PLOTS

From casusal inspection of the data (Table 3) it appeared that incidence of disease on plots G11 and K2 was the same. They were subjected to a test for similarity using X^2 and no significant difference was found at the 5 per cent level of significance. This was to be expected because of the similarities in the two plots in stand composition and infestation history. At the same time, the five plots from which one collection was made on June 30 also appeared to show about the same percentage incidence of disease

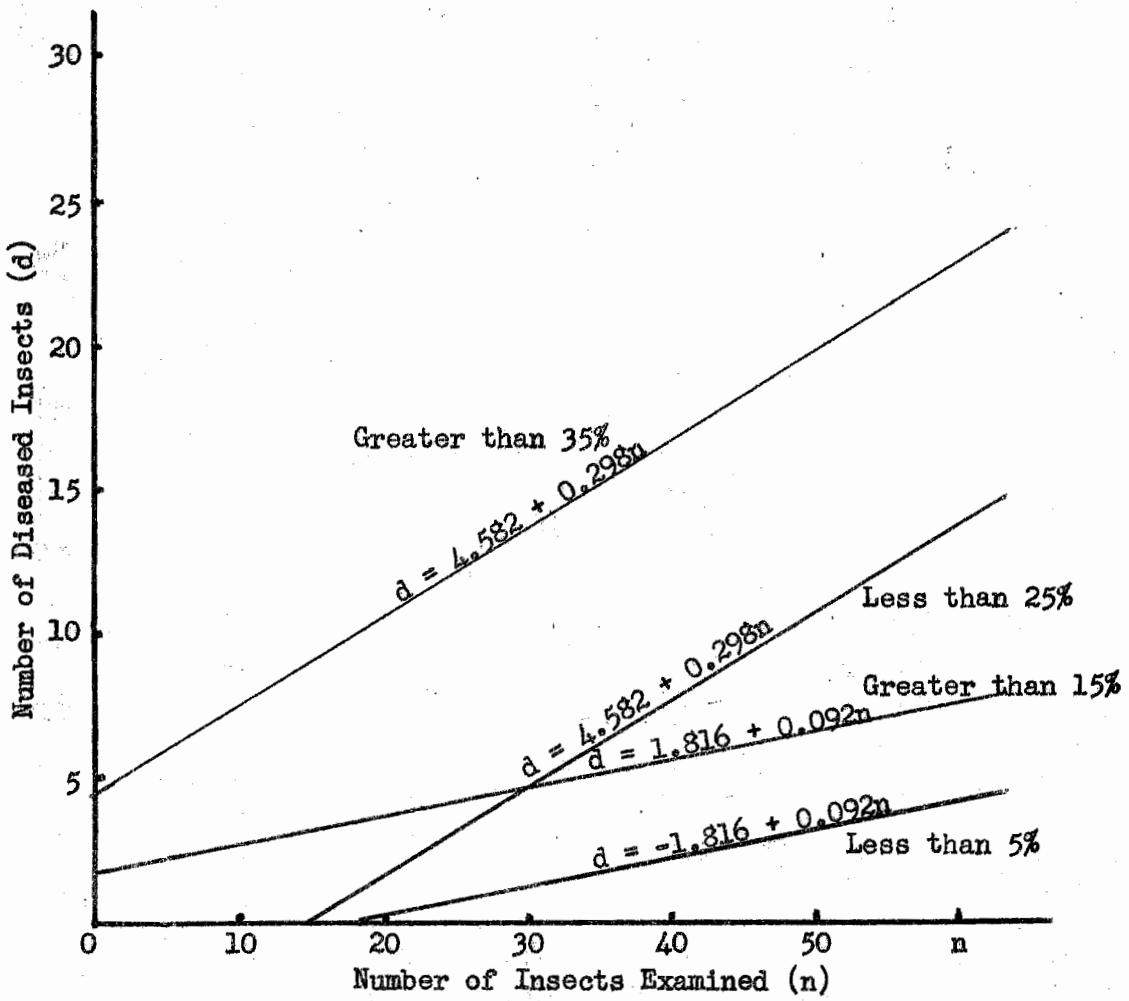

Fig. 1. Graph of sequential sampling plan for finding incidence of disease.

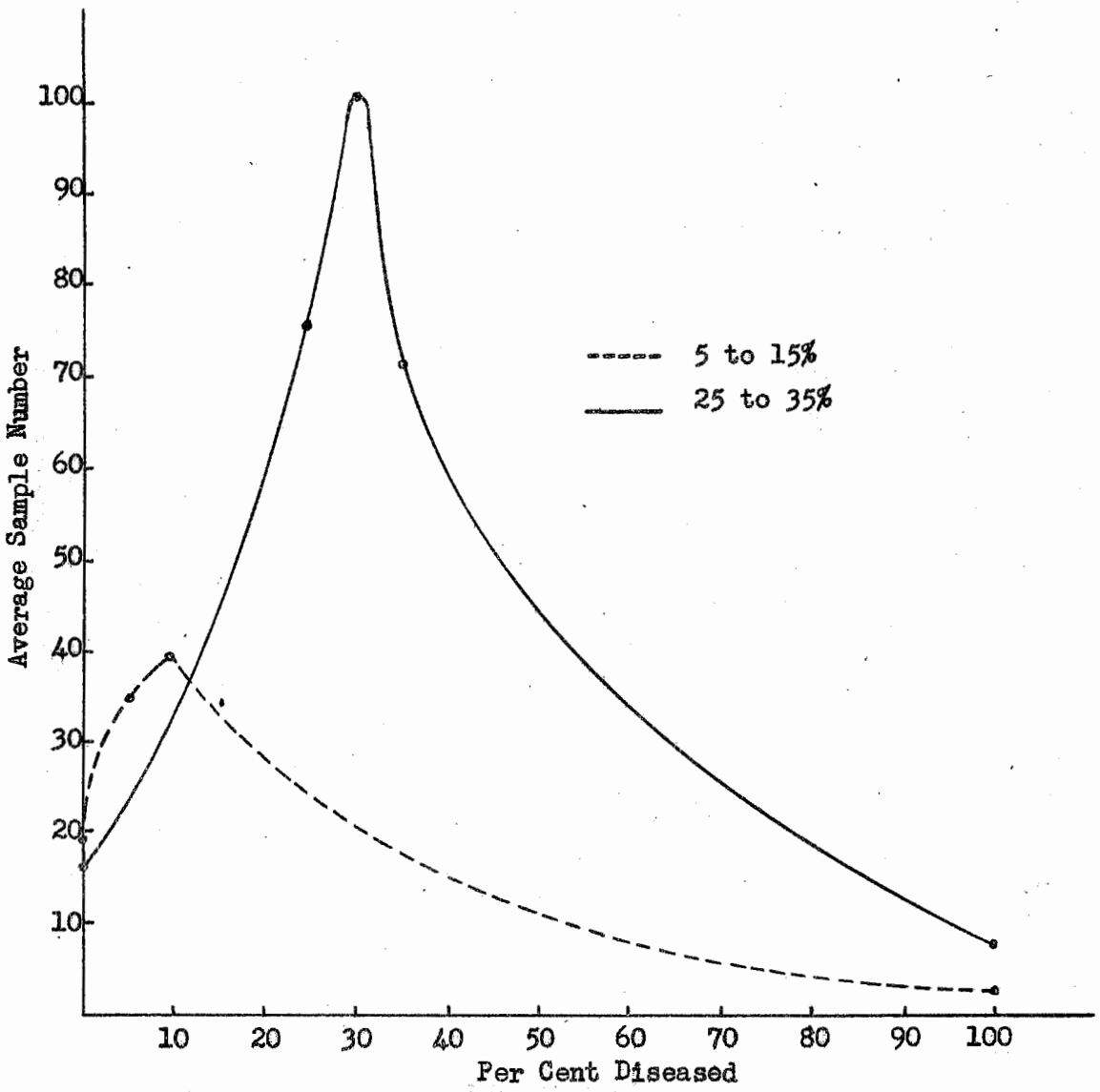

Fig. 2. Average sample number curves for 5 to 15% and 25 to 35% acceptance and rejection line.

(Table 9). These were subjected to a X^2 homogeneity test and were also found to be similar in rate of disease at the 5 per cent level. The result here is a little more surprising because of the differences between these plots in both stand composition and infestation history (Table 1).

These findings are in agreement with those of Thomson (17) and it appears that budworm diseases are not acting as density-dependent factors. Steinhaus (15) states that insect diseases are certainly density-dependent, but in this case it would appear that they are not. It may well be that in the levels of population studied the lowest population may have been high enough for the diseases present to have reached their maximum effectiveness in control. This, however, seems doubtful because third-instar populations per 10 square feet of branch area in the five plots concerned varied from 27 to 123 while at the same time the highest rate of disease was 15 per cent. Also, on plots such as K2 where defoliation has been severe for the past few years, and where resulting conditions appear to be getting more unfavourable each year for the budworm, one would certainly expect a higher incidence of disease than on a plot in a relatively new infestation where numbers are less and conditions more favourable. A more likely postulation would be that a certain percentage of budworm populations on all plots had become resistant to the more important diseases present, and that this percentage remains more or less constant.

This similarity, in any case, does suggest that results may be pooled from several plots to obtain an estimate for disease for other plots in the same general area that were not studied. In the next year or so it is hoped that by including a wider range of plots in the study this similarity in percentage infected and dying from disease may be either substantiated or refuted. If the former is the case it may well mean that only a few plots may have to be sampled and the results pooled to derive a figure for incidence

Table 9

Per Cent Larval Mortality from Disease on Plots G11, K1, K2, I1 and G4 Based on Rearing Records from the Date of Collection to the Date of Pupation.

Plot	Coll. date	Coll. size	Micro-sporidia	Cap-sules	Fungi	Poly-hedra	Bac-teria	Un-known	Total disease
K2	June 15	55	12.7	10.9	0	0	0	30.9	23.6
	" 23	62	11.3	0	3.2	0	0	1.9	14.5
	" 30	65	6.1	3.1	0	0	0	6.8	9.2
	July 7	65	0	0	3.1	0	0	7.9	3.1
	" 13	40	5.0	2.5	7.5	0	0	5.9	15.0
	" 21	6	0	0	0	0	16.7	20.0	16.7
G11	June 2	60	8.3	5.0	3.3	0	0	18.0	16.6
	" 15	60	1.7	8.3	1.7	1.7	0	28.8	13.3
	" 23	60	8.3	8.3	1.7	0	0	14.3	18.3
	" 30	65	1.5	1.5	1.5	1.5	0	3.3	6.0
	July 7	57	0	1.7	1.7	0	0	3.6	3.4
	" 13	43	0	4.6	2.3	0	0	12.5	6.9
	" 21	15	26.7	0	0	0	6.7	30.0	33.4
K1	June 30	45	2.2	6.7	2.2	0	4.4	10.5	15.6
I1	" 30	54	3.7	3.7	1.8	0	0	12.2	9.2
G4	" 30	37	5.4	5.4	0	0	0	15.1	10.8

of disease for all plots. Since no other estimate of the incidence of disease on other plots was available 100 q_x figures were calculated from the pooled data obtained from plots G11 and K2 (Tables 10 and 11). These 100 q_x figures were then applied to the population figures on each of the other plots thereby providing an estimate of mortality due to disease for each period.

7.0 COMPARISON BETWEEN TECHNIGUES

The incidence of disease as calculated from measurement of mortality in rearings and by noting incidence of infection in stained sections of living insects differed only slightly. Included in this difference is a discrepancy between slide and rearing figures for infection with fungi and bacteria; those from insects dying in rearings being higher. This discrepancy may be explained, at least in part, by the fact that many of the bacteria and fungi observed in dead insects may have been either fortuitous or secondary in nature.

Stained slides of the abdomens of living adult budworm indicate a high rate of infection with microsporidia (Table 7). This suggests that some larvae may be infected and yet be resistant to this disease (i.e., assuming the insect contracted the disease while in the larval stage). This observation is substantiated by the fact that the rate of infection as determined from stained slides of living budworm larvae was consistently higher than the rate of mortality from rearing records. If confidence may be expressed in the rearing techniques employed (i.e., that the larvae did not become infected with the disease after they had been brought into the laboratory) it may be stated that young as well as older larvae may survive infection with microsporidia, since some adults originating from young larvae from early collections were infected. A cytoplasmic polyhedral disease was also found in an adult budworm, indicating larval resistance to mortality from this disease also. Since at least the protozoan disease may be transmitted via the egg plans for future investigations include a study of the effect of these and

Table 10

Pooled d_x Values for G11 and K2 Based on Weekly Rearing and Using G11 Population Figures

Date	Deduction for pupae	Population per 10 sq. ft.	Microsporidia	Capsules	Fungi	Polyhedra	Bacteria	Unknown	Total diseased
June 15	0	93	2.32	5.39	0	0.74	0	16.93	8.46
" 23	0	74	2.96	2.96	1.18	0	0	4.00	7.18
" 30	0	57	2.17	1.31	0.46	0.46	0	1.88	4.33
July 7	0	40	0	0.32	0.32	0	0	1.32	0.64
" 13	6	22	0.53	0.79	0.53	0	0	1.43	1.83
" 21	14	6	1.14	0	0	0	0.29	1.50	1.43

Table 11

Pooled Estimate of 100 Q_x* by Period
and Disease from Plots G11 and K2, Using Population Figures from G11

Period	Capsules	Microsporidia	Fungi	Other diseases	Unknown
I	0	0	0	0	14.40
II	4.69	3.80	0.89	0.89	7.48
III	4.98	6.20	2.48	0	13.38
IV	13.63	31.63	18.07	9.19	45.26

*Where 100 q_x for any period may be calculated by:

$$\frac{A}{D_x} \times 100$$

Where A = Population per 10 sq. ft. branch area at the beginning of the period

D_x taken from Table 5.

Table 12

Per Cent Mortality in Rearings Based on Rearing Records from the Time of Collection to Pupation for Plots K2, G11, K1, I1 and G4

Plot	Deduction for parasites	No. in sample	Microsporidia	Capsules	Fungus	Polyhedra	Bacteria	Unknown	Total diseased
K2 June 15	2	55	12.7	10.9	0	0	0	30.4	23.6
" 23	5	62	11.3	0	3.2	0	0	1.9	14.5
" 30	7	65	6.1	3.1	0	0	0	6.8	9.2
July 7	3	65	0	0	3.1	0	0	7.9	3.1
" 13	13	40	5.0	2.5	7.5	0	0	5.9	15.0
" 21	2	6	0	0	0	0	16.7	20.0	16.7
G11 June 2	11	60	8.3	5.0	3.3	0	0	18.0	16.6
" 15	4	60	1.7	8.3	1.7	1.7	0	28.8	13.3
" 23	9	60	8.3	8.3	1.7	0	0	14.3	18.3
" 30	8	65	1.5	1.5	1.5	1.5	0	3.3	6.0
July 7	12	57	0	1.7	1.7	0	0	3.6	3.4
" 13	22	43	0	4.6	2.3	0	0	12.5	6.9
" 21	21	15	26.7	0	0	0	6.7	30.0	33.4
K1 June 30	13	45	2.2	6.7	2.2	0	4.4	10.5	15.6
I1 " 30	16	54	3.7	3.7	1.8	0	0	12.2	9.2
G4 " 30	31	37	5.4	5.4	0	0	0	15.1	10.8

other diseases found in adults on mating, fecundity, and resulting progeny.

The total d_x figure as calculated from consideration of each collection for one week differed only slightly from that derived from one early collection that was reared through to the adult stage (Tables 4 and 13). It would appear from this comparison that (1) laboratory rearing has very little, if any, effect on mortality due to disease, and (2) that most of the disease is contracted in the very early stages or is passed down via the egg. As with most other observations in this report, more data are required to either substantiate or refute this.

8.0 INTERPRETATION OF RESULTS

A comparison of two methods of measurement is presented below. The reason for the choice of Thompson's method (16) is that another worker has used this procedure for assessing mortality due to disease. Below are figures for T as derived by the formula $T = a + (1-a)b + (1-a)(1-b)c + (1-a)(1-b)(1-c)d, +$ etc. and 100 q_x as derived from the method outlined in this report for the two plots intensively studied.

	K2		G11	
	Disease	Unknown	Disease	Unknown
T	35.7	37.1	52.2	57.6
100 q_x	30.4	25.2	18.5	26.4

It is obvious that when data such as population figures, larval development, and other mortality factors are taken into consideration in the measurement of any one mortality factor, the probability of reaching a correct evaluation is much greater than when they are not available. If in the absence of these data a method of evaluation such as that outlined by Thompson in 1928 (16) is used then the assumption must be made that only the one factor is reducing population. Caution must therefore be employed in using an aggregate percentage mortality as represented by "T" for comparisons between

Table 13

D_x Values Based on Rearing Records from
Time of Collection to Pupation for Plots K2, G11, K1, I1, and G4

Plot		Deduction for pupae	Larval population per 10 sq. ft.	Microsporidia	Capsules	Fungus	Polyhedra	Bacteria	Unknown	Total disease
K2	June 15	0	119	15.1	13.0	0	0	0	36.8	28.1
	" 23	0	102	11.5	0	3.3	0	0	1.9	14.8
	" 30	0	87	5.3	2.7	0	0	0	5.9	8.0
	July 7	1	73	0	0	2.3	0	0	5.8	2.3
	" 13	12	48	2.4	1.2	3.6	0	0	2.8	7.2
	" 21	32	12	0	0	0	0	2.0	2.4	2.0
G11	June 2	0	122	10.1	6.1	4.0	0	0	22.0	20.2
	" 15	0	93	1.6	7.7	1.6	1.6	0	26.8	12.4
	" 23	0	74	6.1	6.1	1.3	0	0	10.6	13.5
	" 30	0	57	0.8	0.9	0.8	0.9	0	1.9	3.4
	July 7	0	40	0	0.7	0.7	0	0	1.4	1.4
	" 13	6	22	0	1.0	0.5	0	0	2.7	1.5
	" 14	14	6	1.6	0	0	0	0.4	1.8	2.0
K1	June 30	0	63	1.4	4.2	1.4	0	2.8	6.6	15.6
I1	" 30	0	60	2.2	2.2	1.1	0	0	7.3	9.2
G4	" 30	0	48	2.6	2.6	0	0	0	7.2	10.8

d_x at any interval = % potential mortality resulting from disease x larval population at beginning of interval.

plots and between years. This is especially important in those cases where samples are not taken from populations at the same point in the life cycle of the insect in each successive year and on each plot.

For the sake of comparison the aggregate T for each disease is listed for the Port Arthur and Green River areas for 1954 (Tables 14 and 15). A portion of the apparently large difference between the two sets of data may be explained by dissimilarities in technique and in time and frequency of sampling. Even so some part of this difference could undoubtedly be demonstrated as due to differences in the two areas under consideration.

These studies of diseases in the spruce budworm will be continued until at least the decline of the present infestation. Changes are anticipated in techniques and will be made from year to year as experience is gained. In 1955 most of the sectioning of larvae will be dropped. In the place of this studies on the effects of disease on adults will be intensified. Other minor changes will also be made in methods and times of collection.

9.0 SUMMARY

(1) A survey of disease was made in budworm populations on two plots in northern New Brunswick to determine what diseases are present, which of these exert measurable control, and in what stage of the host they kill. Two methods were used in diagnosis; stained sections of living insects, and body smears of insects dying in rearings.

(2) A method for analyzing data for inclusion in life tables is presented. Using life tables as the method of evaluation, disease was found to have a potential of reducing the population by 24.5 per cent.

(3) At least one sample was taken from each of five plots to see if there was a difference in incidence of disease under different stand conditions and infestation history. Using X^2 tests no difference was found.

(4) Disease was found in adults, indicating possible resistance

Table 14

Aggregate % Mortality from Disease as Represented by "T" for Green River and Port Arthur Districts for 1954

	Green River		Port Arthur	
Plot	K2	G11	Joe Lake	Lake Marie Louise
Protozoa	19.2	31.3	5.2	7.2
Fungi	9.4	1.5	0.6	2.1
Bacteria	0	6.7	1.6	0.5
Polyhedra	0	3.1	0	0
Capsules	12.0	19.1	0	0

Table 15

Real % Mortality from Disease Using Thompson's Method - Plots G11 and K2

Plot	Coll. date	Microsporidia	Capsules	Fungus	Polyhedra	Bacteria	Unknown	Total disease	Total mortality
K2	June 15	5.3	7.0	0	0	0	18.0	12.3	30.3
	" 23	3.0	0	3.2	0	0	0	5.6	4.5
	" 30	5.6	2.8	0	0	0	4.1	7.5	9.2
	July 7	0	0	1.4	0	0	2.4	1.1	2.6
	" 13	4.3	2.2	4.8	0	0	0	9.2	6.7
	" 21	0	0	0	0	0	12.6	0	7.8
	Total	19.2	12.0	9.4	0	0	37.1	35.7	61.1
G11	June 15	0	4.7	0	1.6	0	18.3	6.3	24.6
	" 23	4.9	7.8	0	0	0	9.2	12.3	18.4
	" 30	1.4	1.3	1.5	1.5	0	1.2	4.9	4.4
	July 7	0	1.5	0	0	0	2.6	1.3	2.8
	" 13	0	3.8	0	0	0	8.2	3.4	8.2
	" 21	25.0	0	0	0	6.7	18.1	24.0	27.6
	Total	31.3	19.1	1.5	3.1	6.7	57.6	52.2	86.0

Real % mortalities from formula

$T = A + B + C$ where a, b, c, and d are apparent mortalities in % ages

where $A = a$
$B = (1-a)b$
$C = (1-a)(1-b)c$
$D = (1-a)(1-b)(1-c)d$

of larvae to a protozoan and polyhedral disease after they had become infected.

(5) Preliminary studies of mortality due to intrinsic weakness were initiated.

(6) Data were subjected to a sequential analysis and an average sample size that would permit the definition of disease within certain classifications was determined.

(7) A comparison of the life-table method of measuring mortality due to disease and another method is presented.

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APPENDIX