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STARVATION OF FIFTH INSTAR LARCH SAWFLY LARVAE

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STUDIES ON THE STARVATION OF FIFTH INSTAR LARCH SAWFLY LARVAE

1. INTRODUCTION

Preliminary investigations on the effects of starvation on fifth instar larch sawfly larvae were reported in the Annual Technical Report for 1951. Data were presented at that time on the mortality caused by periods of starvation of varying duration. Fifth instar larvae were subjected to six types of treatment as follows:-

Group I -- Controls - larvae were fed throughout development.

Group II -- Larvae were fed for three days and then starved.

Group III -- Larvae were fed for five days and then starved.

Group IV -- Larvae were starved for three days and then fed.

Group V -- Larvae were starved for five days and then fed.

Group VI -- Larvae were starved throughout the fifth instar.

Larval mortality was 100% in Groups V and VI. Partial mortality occurred in Groups II and III and in Groups I and IV there was no mortality.

The mean duration of the fifth stadium under optimal nutritional conditions is seven days. Under the conditions in which the larvae in the above-mentioned treatments were reared, starvation for a period in excess of one-half the normal duration of the stadium resulted in complete or near complete mortality.

During the field season of 1952 larvae were reared to determine the effects of sub-lethal starvation on the larvae. Larvae were reared in the insectary according to the treatments of Groups I, II, III and IV as outlined above. An additional treatment, Group VII, was added in which the larvae were fed for four days and then starved.

In addition to these insectary rearings field col-

lections of cocoons were made in two areas of Manitoba:

1) the Whiteshell Forest Reserve, Manitoba,
2) South-eastern Manitoba in the vicinity of Middleboro and South Junction.

These are two areas where large populations of the larch sawfly were present in 1952 and where defoliation conditions were conducive to starvation. Collections were made under selected trees some of which were completely defoliated and others which were only partially defoliated.

The object in undertaking these rearings and collections was to obtain data concerning sub-lethal influences of starvation as reflected in larval weights, moisture content, fat content and adult fecundity.

The larvae used in the experimental rearings were collected in the vicinity of Telford, in the Whiteshell Forest Reserve and all rearings were carried out at the field station in the Whiteshell Forest Reserve.

2. METHODS AND MATERIALS

2.1. Insectary Rearings

In order to obtain larval material subjected to known degrees of starvation of sub-lethal duration, controlled insectary rearings of field collected larvae were undertaken. Fourth instar larvae were collected in the field and brought to the insectary. There they were reared in mass lots which were examined twice daily. Twice each day the newly-molted fifth instar larvae were removed. There larvae were then divided into groups for experimental treatment.

As indicated in the Introduction, during the initial studies reported in 1951, larvae were subjected to six different types of treatments. In the studies made in 1952 the treatments represented by Groups V and VI were omitted as these resulted in 100% mortality and therefore were not pertinent. An additional treatment, designated Group VII, was added in 1952 in which the larvae were fed for four days and then starved.

Larvae were reared in a simple type of cage, which

had proven satisfactory in earlier rearings. Wilting of the foliage was minimized by inserting the twigs in vials of water.

During the starvation period the larvae were kept in closed containers with a moist base of sphagnum moss. This minimized mortality due to desiccation and provided a suitable cocooning medium.

All successfully cocooned larvae were layered in moss and wrapped in cotton packets. In late September these were stored in the cold temperature room at the laboratory in Winnipeg where they remained until removal for analyses.

2.2. Field Collections.

In September collections of cocoons were made under selected trees in a heavily defoliated stand in the Whiteshell Forest Reserve and in two similarly heavily defoliated areas in south-eastern Manitoba near South Junction and Middleboro.

Collections were made both under completely defoliated trees and partially defoliated trees.

2.3. Determination of the Sub-Lethal Effects of Starvation.

Partial starvation of larvae should result in decreased weight and storage of reserves when compared to fully fed larvae and in a reduction in the fecundity of adults developing from these partially starved individuals.

One objective of the present study was to determine the differences in weight and reserves of larvae starved to varying degrees when compared to fully-fed larvae. Quantitative determination of lipids was taken as a measure of the reserves as these are easily measured and also form the principal energy storage. As weight and lipid content are relatively easily measured, if they were to show a high degree of correlation with fecundity this would provide a readily determinable index to this important population factor.

2.3.1. Wet Weight Determinations.

Overwintering larvae were removed from cocoons immediately on being taken from cold storage and weighed individually on a Roller-Smith Torsion Balance. Using this balance the larvae could be weighed quickly and with an accuracy of $\pm$ 0.2 mg.

2.3.2 Dry Weight Determinations.

Immediately following wet weight determination the larvae were dried to constant weight in a vacuum oven at 100° C.

2.3.3. Lipid Determinations.

The total lipid content of each larva was determined by extraction with a solvent mixture (1/3 ethyl ether and 2/3 absolute methyl alcohol) in a Soxhlet extractor. Each extraction was carried out for a period of 24 hours. Following extraction the adhering solvent mixture was removed and the larvae dried over $CaCl_2$ and reweighed. The difference in weight between this determination and the dry weight was taken as the total ether extractives or total lipid content.

2.3.4. Adult Fecundity.

This aspect was not dealt with in 1952.

3. RESULTS AND DISCUSSION

3.1. Insectary Reared Material.

3.1.1. Wet Weights.

In Table I are recorded the summarized data on the wet weight determinations of representative overwintering larvae from each of the experimental series I, II, III, IV and VII.

The mean wet weights for each of the groups fed for three, four and five days respectively and then starved were significantly less than the mean wet weight

of the control group. The larvae that were starved for an initial period of three days and then allowed to feed until they formed cocoons (Series IV) were of identical mean wet weight to the control group.

It will be noted that the mean wet weight of the group fed for four days and then starved (Series VII) is actually less than that of the group fed for three days and then starved (Series II) but this difference between the two is not significant (at the 5% level).

It is apparent from these results then that relatively short periods of starvation are sufficient to cause significant weight differences between such partially starved larvae as compared to fully fed larvae.

3.1.2. Dry Weights.

These data are summarized in Table II. The determinations are based on the same material as that used in the wet weight determinations.

The results here are similar to those reported in (a) for wet weights. The dry weights however exhibit somewhat less intra-group variability than the wet weights as indicated by the smaller standard deviations. The differences between the dry weights of the various groups, when compared to the control group on a percentage basis, show a greater difference than was evident for the wet weights. It would appear from this that the dry weight would be a better criterion for comparison than wet weight.

3.1.3. Per Cent Moisture.

Data for per cent moisture as calculated from wet and dry weight determinations are presented in summarized form in Table III. These figures show an interesting relationship which appears to be indicative of the metabolic changes occurring under conditions of partial inanition. The mean values for per cent moisture of all the groups of specially treated larvae are significantly greater than for the control group of fully fed larvae. The groups having experienced the greater degrees of starvation (Series II and VII) also had the highest percentage moisture content. This may be due to the accumulation of metabolic water formed during the metabolism of fat. As fat is the principle reserve material drawn upon during starvation

it would be most actively metabolized in the groups experiencing the greatest degree of starvation.

TABLE I

Wet Weights - Insectary Reared Material

Series		No. of Meas.	Mean Wet Wt. (mg.)	S. D.	% Diff. from Controls	"t" (values for difference from control group)
I	Controls	11	45.9	4.37	--	--
III	Fed 5 days and then starved	19	39.7	4.56	13.5	3.52 *
VII	Fed 4 days and then starved	6	29.6	6.15	35.5	5.95 *
II	Fed 3 days and then starved	5	33.4	3.42	27.2	5.32 *
IV	Starved 3 days and then fed	10	45.9	6.93	0.0	0.00

* Difference significant at the 1% level.

TABLE II

Dry Weights - Insectary Reared Material

Series		No. of Meas.	Mean Dry Wt. (mg.)	S. D. (mg.)	% Diff. from Controls	"t" (values for difference from control group)
I	Controls	11	18.7	4.5	--	--
III	Fed 5 days and then starved	19	14.9	2.2	20.3	3.14*
VII	Fed 4 days and then starved	6	8.3	3.9	55.6	5.33*
II	Fed 3 days and then starved	5	9.0	1.2	51.9	4.66*
IV	Starved 3 days and then fed	10	16.4	2.2	12.3	1.46

* Difference significant at the 1% level.

TABLE III

% Moisture - Insectary Reared Material

Series		No. of Meas.	Mean % Moisture	S.D. (%)	"t" (values for difference from control group)
I	Controls	11	59.1	2.7	--
III	Fed 5 days and then starved	19	62.6	2.2	3.86 *
VII	Fed 4 days and then starved	6	71.6	5.2	7.94 *
II	Fed 3 days and then starved	5	73.1	1.1	10.94 *
IV	Starved 3 days and then fed	10	64.0	3.2	3.79 *

* Difference significant at the 1% level.

3.2. Field Collection Material.

3.2.1. Wet Weights.

The data on wet weight determinations for the field collected material from South-eastern Manitoba and the Whiteshell Forest Reserve are summarized in Tables IV (a) and IV (b) respectively.

It will be noted that there is considerable intra-group and inter-group variability in wet weights. The standard deviations of some of these field collections are however smaller than those of some of the insectary reared series. Variability is not greater in the collections made under the heavily defoliated trees than in the trees with only 50% defoliation. It was expected that the larvae from the heavily defoliated trees where starvation was liable to occur would show a greater intra-group variability in weights than is indicated here.

3.2.2. Dry Weights.

The data for dry weights of the field collected material is presented in summarized form in Tables V (a) and V (b).

In these tables the dry weight data for the five collections from heavily defoliated trees are compared the collections from the trees with 50% defoliation. Three of the five collections from south-eastern Manitoba had significantly lower dry weights (at the 5% level or less) and all four of the collections from the Whiteshell area had mean dry weights significantly less (at the 5% level or less) than the collection from the lightly defoliated tree. A discrepancy is apparent in collections No. 4 and No. 5 from south-eastern Manitoba where the mean dry weights are actually greater than that of collection No. 2. The differences are not statistically significant however.

3.2.3. Per Cent Moisture.

The data for the per cent moisture determinations is summarized in Tables VI (a) and VI (b).

These values are relatively uniform in contrast to the differences shown in the different experimental

groups. The significance of this uniformity with respect to the comments regarding fat mobilization made in the discussion of the experimental material will not be evident until the data on lipid determinations has been compiled and analyzed.

TABLE IV (a)

Wet Weights - Field Collections: South-Eastern, Manitoba.

Collection No.	Defoliation %	No. of Individuals.	Mean Wet Wt. (mg.)	S. D.
No. 1	100%	20	56.8	8.3
No. 2	50%	19	62.8	7.3
No. 3	90-100%	20	54.5	8.7
No. 4	90%	18	67.9	6.1
No. 5	90%	20	65.4	5.1
No. 8	90-100%	13	43.2	6.9

TABLE IV (b)

Wet Weights - Field Collections:
Whiteshell Forest Reserve, Manitoba.

Collection No.	Defoliation %	No. of Individuals.	Mean Wet Wt. (mg.)	S. D.
No. 1	100%	28	50.7	3.3
No. 3	100%	18	50.6	7.1
No. 5	100%	10	44.3	6.8
No. 7	100%	12	47.5	10.4
No. 9	50%	20	59.7	8.3

TABLE V (a)

Dry Weights - Field Collections:
South-Eastern, Manitoba.

Collection No.	Defoliation %	No. of Individuals	Mean Dry Wt.(mg.)	S.D.	"t" (Values for difference from collection No. 2 - 50% defoliation)
No. 1	100%	20	21.2	3.4	2.57 **
No. 2	50%	19	23.9	3.1	--
No. 3	90-100%	20	20.2	3.9	3.22 *
No. 4	90%	18	25.5	3.4	1.48
No. 5	90%	20	24.7	2.7	0.86
No. 8	90-100%	13	16.0	3.3	6.87 *

* Difference significant at the 1% level.

** Difference significant at the 5% level.

TABLE V (b)

Dry Weights - Field Collections:
Whiteshell Forest Reserve, Manitoba.

Collection No.	Defoliation %	No. of Individuals	Mean Dry Wt.(mg.)	S.D.	"t" (Values for difference from Collection No.9 - 50% defoliation)
No. 1	100%	28	20.9	4.8	2.09 **
No. 3	100%	18	20.5	2.8	2.76 *
No. 5	100%	10	17.2	3.6	4.92 *
No. 7	100%	12	18.9	4.7	3.02 *
No. 9	50%	20	23.7	4.2	--

* Difference significant at 1% level.

** Difference significant at 5% level.

TABLE VI (a)

Per Cent Moisture - Field Collections:
South-Eastern, Manitoba.

Collection No.	Defoliation %	No. of Individuals	Mean % Moisture	S. D.
No. 1	100%	20	62.7	2.1
No. 2	50%	19	61.9	2.3
No. 3	90-100%	20	63.1	3.7
No. 4	90%	18	62.5	3.7
No. 5	90%	20	62.2	2.1
No. 8	90-100%	13	65.5	2.8

TABLE VI (b)

Per Cent Moisture - Field Collections:
Whiteshell Forest Reserve, Manitoba.

Collection No.	Defoliation %	No. of Individuals	Mean % Moisture	S. D.
No. 1	100%	28	58.9	2.3
No. 3	100%	18	59.5	1.9
No. 5	100%	10	61.2	3.1
No. 7	100%	12	60.5	2.2
No. 9	50%	20	60.5	2.3

4. CONCLUSIONS

As certain data, notably that from the lipid analyses, are not available for inclusion in this report no generalized conclusions can be made. Further information, especially from the analyses of field collected material, is also desirable.

A tentative conclusion, that can be drawn from the data presented, is that relatively short periods of starvation produce detectable and statistically significant differences in larval weights. This is particularly evident when dry weights are considered. Such weight differences are detectable in field collected samples obtained beneath trees exhibiting complete or partial defoliation. It is possible therefore by the analysis of relatively small cocoon samples, from individual trees, to determine the presence, or absence, of starvation conditions in field populations.

5. SUMMARY

Fifth instar larch sawfly larvae have been reared under insectary conditions and subjected to varying degrees of partial starvation. Sub-lethal effects of inanition as reflected by larval weights, moisture content and lipid content of diapausing larvae have been measured.

Relatively brief periods of starvation were found to result in statistically significant differences in larval weights. The differences in dry weights between the partially starved larvae and fully fed larvae were more pronounced than the differences in wet weights. The moisture content of starved larvae when compared to fully-fed larvae, seemed to indicate a possible accumulation of metabolic water in the starved individuals.

Cocoons were collected, beneath selected trees of known defoliation, in south-eastern Manitoba and in the Whiteshell Forest Reserve, Manitoba. Diapausing larvae from these collections were subjected to the same analyses as the insectary reared material.

The mean dry weights of the larvae collected be-

neath completely defoliated trees differed significantly from the mean dry weights of the larvae collected beneath partially defoliated trees.