A STUDY OF THE NATURAL IMMUNITY OF THE LARCH SAWFLY (PRISTIPHORA ERICHSONII (HTG.)) TO THE INTRODUCED PARASITE MESOLEIUS TENTHREDINIS MORLEY, IN MANITOBA AND SASKATCHEWAN, CANADA.

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I STATEMENT OF THE PROBLEM

The larch sawfly, Pristiphora erichsonii (Hartig), is the most important pest of tamarack (Larix laricina) in North America. Entomologists differ as to whether this species is an introduction from Europe or a native of America. The available evidence appears to indicate that the insect was introduced from Europe and that it has probably been present in North America since about the beginning of the nineteenth century (2, 17, 19, 32, 34, 41). A number of devastating outbreaks of the insect have been recorded in North America since 1882 (27, 36). The most recent and continuing outbreak began in Manitoba about 1938 and now covers extensive areas in Saskatchewan, Manitoba, Ontario and the Lake States (47).

An important phase of the problem that has been studied intensively concerns the effectiveness of Mesoleius tenthredinis Morley, an introduced parasite of the larch sawfly. It will be shown in a succeeding section that in the past this parasite must have been an effective controlling factor in western and central Canada. Studies and observations undertaken since 1940 have revealed that the larch sawfly in Manitoba and Saskatchewan has evidently developed an effective immunity to the parasite. The author conducted an investigation of the nature of this immunity from 1949 to 1951 and this paper gives an account

of the results of this study. Research has been conducted in Riding Mountain National Park, The Whiteshell Forest Reserve, and Winnipeg, Manitoba and at Prince Albert, Saskatchewan.

Since M. tenthredinis has decreased in effectiveness in Manitoba and Saskatchewan in past years it is important to understand the reasons for this decrease in order to properly appraise the possibility of remedying the situation. In British Columbia M. tenthredinis is still an effective parasite of the larch sawfly and it is important to know what to watch for when sampling the larch sawfly population in British Columbia in the future in order to determine whether or not this parasite is maintaining its effectiveness in that province.

II REVIEW OF THE LITERATURE ON MESOLEIUS TENTHREDINIS

A. Classification (86).

Order	-	Hymenoptera
Suborder	-	Apocrita (= Clistogastra)
Superfamily	-	Ichneumonoidea
Family	-	Ichneumonidae
Subfamily	-	Scolobatinae (= Mesoleiinae)
Tribe	-	Mesoleiini
Subtribe	-	Mesoleiina
Genus	-	Mesoleius
Species	-	tenthredinis Morley.

B. Literature Review

Mesoleius tenthredinis Morley was imported into Canada from England in 1910 by Dr. Gordon Hewitt (34). Previous to 1910, the parasitism of the larch sawfly by M. tenthredinis in England was 5.8 percent in 1908, 10.9 percent in 1909, and 62 percent in 1910 (34, 49). Correlated with this build-up of the parasite was a reduction of the larch sawfly population. Of the 1,260 cocoons shipped to Canada in 1910, an estimated 64.7 percent were parasitized by M. tenthredinis (34).

In the summer of 1910, over 1,000 M. tenthredinis adults reared from the English material were liberated

near Ottawa and about 150 were liberated in the Algonquin National Park, Ontario. It is reported that this attempt at colonization was unsuccessful, probably due to the fact that the liberation sites were poorly chosen (31). In 1911, imported cocoons were distributed near Quebec City, St. Agathe des Monts and Point Platon in Quebec and in the Algonquin National Park, Ontario. A small lot of 957 cocoons was sent to R. H. Pettit in Michigan and he reported that M. tenthredinis parasitized 7 percent of this collection (34). Another lot of 202 cocoons retained at Ottawa for study showed a parasitism by M. tenthredinis of 12.5 percent (34). The liberations made in Quebec are reported as having been highly successful (31).

In 1912, ten seven-pound buscuit boxes of imported parasitized cocoons were released by Dr. Swaine in two larch swamps in the Riding Mountain National Park, Manitoba. This release was apparently successful since the larch sawfly did not become a serious problem in this area until 1940 when four areas of heavy attack by this insect were discovered in the Park (73). Further importations from England in 1913 were sent to Norman Criddle at Treesbank, Manitoba. This shipment consisted of eight cans of cocoons and these were placed out in a stand of larches growing in a bog situated in a bend of

the Assiniboine river. These larches, at the time, were heavily defoliated by larch sawfly. A check lot of these cocoons kept at Ottawa yielded a parasitism by M. tenthredinis of 51 percent (17).

Criddle (17) reports that in the spring of 1914 cocoons were collected from this swamp but no parasites were recovered from them. No further efforts were made to recover parasites until the spring of 1916 when several hundred sawfly cocoons collected were found to have a parasitism by M. tenthredinis of 19 percent. In 1917 the rate of parasitism by M. tenthredinis had risen to 22 percent, in 1919 to 40 percent, and in 1920 to 66 percent. After this year the larch sawfly became scarce and no further notes on the activities of the parasite were made until 1926, when a series of sawfly cocoons were gathered about ten miles from the locality where the parasites had originally been liberated. Of these, 66 percent were parasitized by M. tenthredinis. In 1927 the parasitism, in some places, ran as high as 88 percent, while it averaged 75 percent over the whole area covered by the Spruce Woods Forest Reserve.

In 1927 and 1928 Criddle assisted Mr. A. B. Baird to introduce M. tenthredinis into Eastern Canada. Parasitized larvae and cocoons were collected in Spruce Woods

and shipped to the parasite laboratory, then located at Chatham, Ontario, for emergence, whence the adult parasites were redistributed to Frederickton, N. B., and the St. Williams Forestry Station, Ontario. The parasite became established rapidly at St. Williams in the reforestation stands of larch that were then heavily infested by sawfly. The increase of the parasite was followed by a correspondingly rapid decline in the sawfly infestation. It was reported that no further injury to the larch trees in this area occurred (31).

From this recolonization at St. Williams, it was possible to secure parasites to establish a colony near North Bay, Ontario, in 1929. In 1929 it was found that M. tenthredinis had spread eastward from Treesbank, Man., inside of the Ontario border to Bustead, a distance of over 200 miles (31). Graham (30) reported parasitism of 30 percent at Glen Murray, Quebec, in 1929.

The larch sawfly was first noted in British Columbia in 1930. It was not brought to the attention of entomologists, however, until 1933. Cocoons were collected and the larvae dissected. No evidence of parasitism was found and arrangements were made to release parasites in the infested area (87). In July, 1934, a shipment of 673 M. tenthredinis was released

near Fernie, British Columbia. As the sawfly spread north and east, 2,196 M. tenthredinis were released in 1935 and 781 in 1936 in the newer areas of infestation. The parasite became established and spread with the sawfly. At no time since its initial establishment in British Columbia has the sawfly reached outbreak proportions except in isolated areas. The population in each of the heavily infested areas for which there are records became heavily parasitized by M. tenthredinis and subsided without serious injury to the trees. Further releases of 624 M. tenthredinis in 1941 and 702 in 1942 were made in British Columbia (87).

Dowden (21) reports that in 1917 a small lot of parasitized cocoons was sent from Canada for liberation in Michigan, and that small colonies of the parasite were sent from Canada to New England in 1929 and 1931 for liberation in New Hampshire and Massachusetts. Dowden (20) noted the recovery of this parasite in Montana, Michigan, Wisconsin and Minnesota and since then it has been recovered in Connecticut, New York and Pennsylvania (21).

Some forest entomologists are of the opinion that the larch sawfly is circumpolar, and has always been with us. Dowden (21) suggests that the wide-spread occurrence

of M. tenthredinis might be considered further proof of this. The remarkable early success of this parasite, however, in contrast to the low effectiveness of native parasites, would seem to indicate that M. tenthredinis was not present in this country prior to 1910 and also that the larch sawfly itself is an introduced species. Hewitt (34) points out that the history of the larch sawfly in North America is what one would expect of a pest insect introduced into a foreign country without its native parasites, i.e., a series of rapid and devastating outbreaks.

Surveys through south eastern Quebec in 1934 and 1935 disclosed that M. tenthredinis was well established as far east as the centre of the Gaspe peninsula and several places were found where collections could be made (31). From these collections and others made in New Brunswick, colonies of the parasite were distributed in 1938 in Nova Scotia, New Brunswick, Quebec, and Ontario. In 1939 and 1940 colonies were liberated in Nova Scotia, Quebec and Ontario and in 1940 liberations were also made in Newfoundland (1). It is reported that M. tenthredinis gave control both in the Maritimes and Newfoundland after establishment in these areas.

Sweetman (80), in a study of most of the numerous

attempts to control pest insects through the use of parasites and predators up to 1936, considered that only 25 of these attempts could be termed "unusually successful". The control of the larch sawfly by M. tenthredinis was one of these 25 cases.

In 1943 it was found that a relatively high rate of parasitism by M. tenthredinis prevailed in British Columbia and cocoons were collected there. In 1944, 5,362 adult parasites obtained from these cocoons were released in Newfoundland (1). In 1946 a great many cocoons were collected in B.C. for release of parasites in Newfoundland, Ontario and Manitoba. In 1947 a large collection of cocoons was not made in British Columbia. In 1948 an area was found with a parasitism by M. tenthredinis of approximately 68 percent and 105,000 cocoons were collected for parasite stock. In 1949 the rate of parasitism was found to average about 61 percent and 130,000 cocoons were collected. In 1950 there was a great reduction in the larch sawfly population in British Columbia. The scarcity of cocoons made it impossible to make collections for recovery of M. tenthredinis for release in central and eastern Canada. In that year a collection of 441 cocoons showed a parasitism by M. tenthredinis of 55 percent (87).

Most of the cocoons collected in British Columbia

in 1946, 1948, and 1949 were sent to Belleville, Ontario, for rearing. In the years 1947 to 1950 inclusive the M. tenthredinis adults recovered were redistributed and liberated as follows:

Newfoundland	- 1,450	
Ontario	- 36,058	(prior to 1947, 10,238 were released in Ontario).
Manitoba	- 23,045	(prior to 1947, an unknown number were released in 1912 and 1913).
Saskatchewan	- 7,054	(prior to 1947, none were released in Saskatchewan).

There is only one generation of this parasite each year. The parasite overwinters in the larval stage within the cocooned larch sawfly host larva. Dowden (21) states that the parasite hibernates within the prepupal larch sawfly larva in the first instar stage. In Manitoba and Saskatchewan, however, it was found that the majority of the hibernating parasite larvae found during winter dissections are in what appear to be the third and fourth instar larval stages. However, a number of first and second instar M. tenthredinis larvae have also been found in the overwintering larch sawfly larval hosts. Hewitt (34) dissected many host larvae during the winter months and found that M. tenthredinis larvae within them were about half grown, measuring 5 to 6 mm. in length, on the average. These parasite larvae do not feed until the ground warms up in the spring and the sawfly larvae in the cocoons become active. They then consume the tissues of the host, emerge from the empty larval integument, and spin a thin parchment-like cocoon inside that of the host. They pupate within this inner cocoon. Hewitt (34) found that in England adults of M. tenthredinis emerge from early May to about the middle of July. In the Prairie Provinces of Canada, however, emergence of the adult parasites begins in late June or early July and continues

through July into the first, and sometimes the second, week of August. In both England and Canada it appears that the parasites first emerge at the time when the sawfly larvae are just beginning to appear on the larch trees. After mating, the female parasites search out the sawfly larvae and deposit eggs internally into the third-, fourth-, and fifth-instar larval stages. Although Criddle (17) states that all sizes of sawfly larvae are attacked, the author found that only in very rare cases are eggs deposited in second-instar larvae. No cases of the parasitism of first-instar larch sawfly larvae were found. The egg of M. tenthredinis normally hatches in seven to ten days and the small larva then feeds, grows and moults, until it enters the state of hibernation.

IV NATURE OF THE IMMUNITY REACTION OBSERVED IN THE LARCH SAWFLY

A. Historical

In 1938 in Manitoba, an outbreak of the larch sawfly occurred in a larch swamp near the Assiniboine River about five miles northwest of Aweme. Dr. Hanford of the Entomological Laboratory, Brandon, estimated the defoliation to be 50 percent. From this area cocoons were collected as follows to determine parasitism; 1,531 in 1938, 7,850 in 1939, and 2,040 in 1940. The over-all parasitism of these 11,462 cocoons, based on rearings, was found to be only 0.017 percent and the parasitism was wholely by the Tachinid Bessa harveyi Tns. (73). M. tenthredinis was seemingly absent from this area in which it had been so prevalent only ten years previously.

In 1940, 1,250 cocoons were collected in Riding Mountain National Park, Manitoba. These cocoons were reared and it was recorded that three parasites emerged (1.14 percent) although the records did not name the species (73). In 1941 dissections of 100 cocoons from each of ~~these~~ four areas in R.M.N.P. gave the following figures for total parasitism by M. tenthredinis; 33%, 27%, 39% and 22% respectively.* It was found, however, that most of the sawfly larvae that harbored M. tenthredinis

* Unpublished data. Annual Technical Report, Forest Insect Laboratory, Winnipeg. 1941 pp. 256-257.

contained not living M. tenthredinis larvae but unhatched eggs of the parasite. As opposed to the total parasitism by M. tenthredinis, the parasitism by M. tenthredinis larvae only was termed the "effective parasitism" and it was found to be, for each of the four areas; 6%, 5%, 13% and 6% respectively. A large number of cocoons from each of these four areas was set aside for rearing at the Belleville Parasite Laboratory and these gave the following results: - ✱

Area in R.M.N.P.	Number of Cocoons reared	Effective parasitism by M. tenthredinis
1. Wasagaming Townsite	3,258	4.0%
2. Mile 7 Norgate Road	7,261	4.4%
3. Near Jct. of Dauphin Highway and Lake Audy Road	4,325	5.7%
4. Mile 13 Lake Audy Road	8,159	5.6%

In 1941, dissections of cocoons collected at Epinette in the Spruce Woods Forest Reserve, Manitoba, showed a 5 percent total parasitism and a 4 percent effective parasitism by M. tenthredinis. Dissections of cocoons from Delta, S.W.F.R., showed a total parasitism

✱ Ibid., 1942. p. 92.

of 1 percent, which was also the effective parasitism in this case.*

In 1942, 10,000 cocoons collected in the R.M.N.P., Man., were reared at Belleville and showed an effective parasitism by M. tenthredinis of 4.3 percent.**

The poor egg hatch of M. tenthredinis was noted in succeeding years in a number of other areas in Manitoba. In 1945 it was recorded that, in all cases, the unhatched eggs were completely surrounded by "thick, translucent coatings, presumably laid down by the action of the host." It was also recorded that the majority of the unhatched encapsulated eggs that were dissected from larch sawfly larvae in November appeared to be still living, whereas the majority of the encapsulated eggs dissected out in February or later appeared to be brown and dead.*

B. Prevalence of the Immunity Factor in Manitoba and Saskatchewan.

Data collected since 1945 by the Forest Insect Laboratory at Winnipeg from dissections of cocooned larch sawfly larvae tend to show that the liberations made in Manitoba and Saskatchewan during 1947, 1948, and 1949 have had little, if any, beneficial effect to date on

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* Ibid., 1942. p. 92.

** Ibid., 1943. p. 97.

the larch sawfly situation in these provinces. Figures 1. and 2. indicate the trend in parasitism by M. tenthredinis in five areas in Manitoba and one in Saskatchewan from 1945 to 1951.

Figure 1.

The Total Parasitism and Effective Parasitism by *M. tenthredinis* in Three Areas in Riding Mountain National Park, Manitoba, from 1945 to 1951.

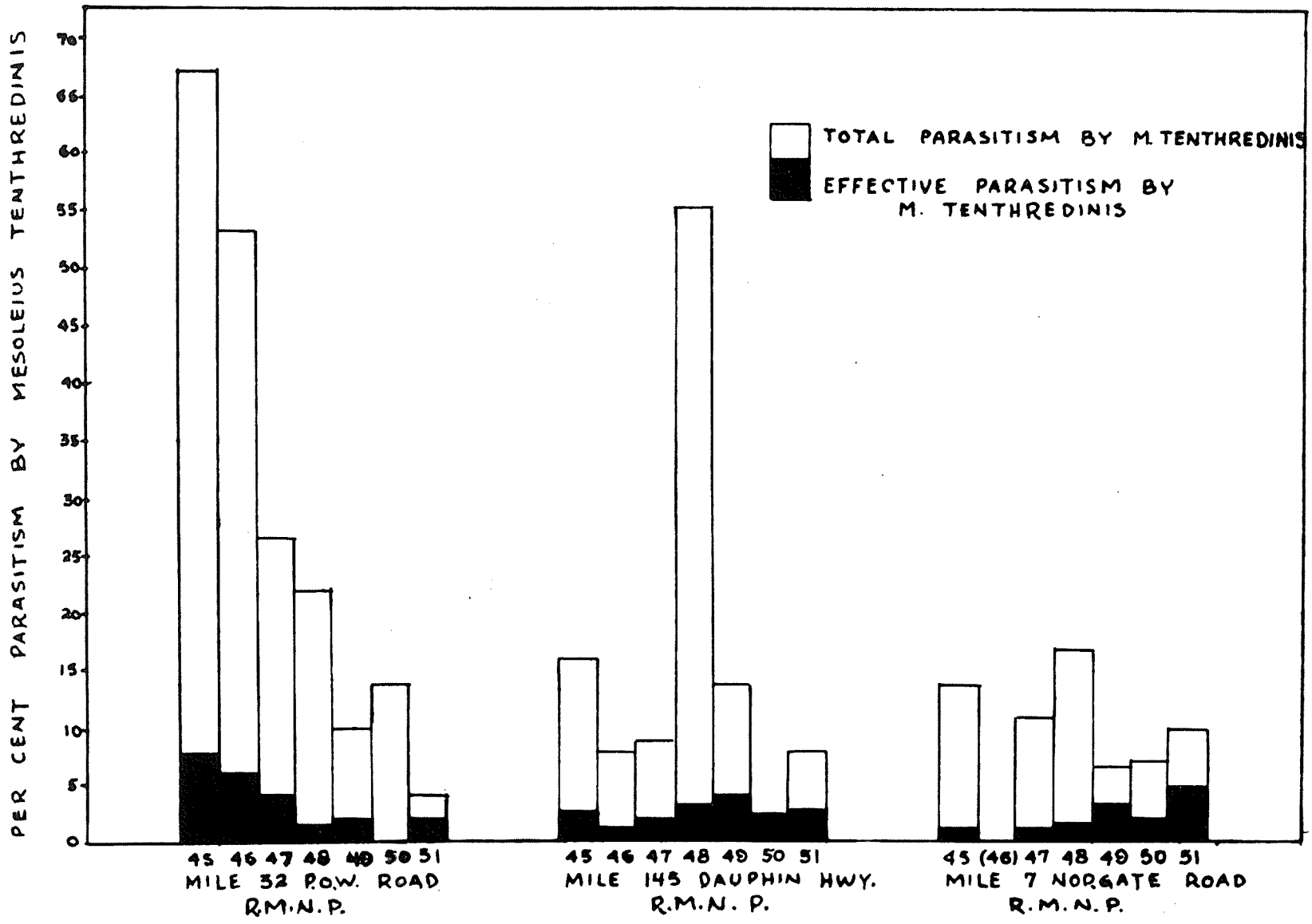
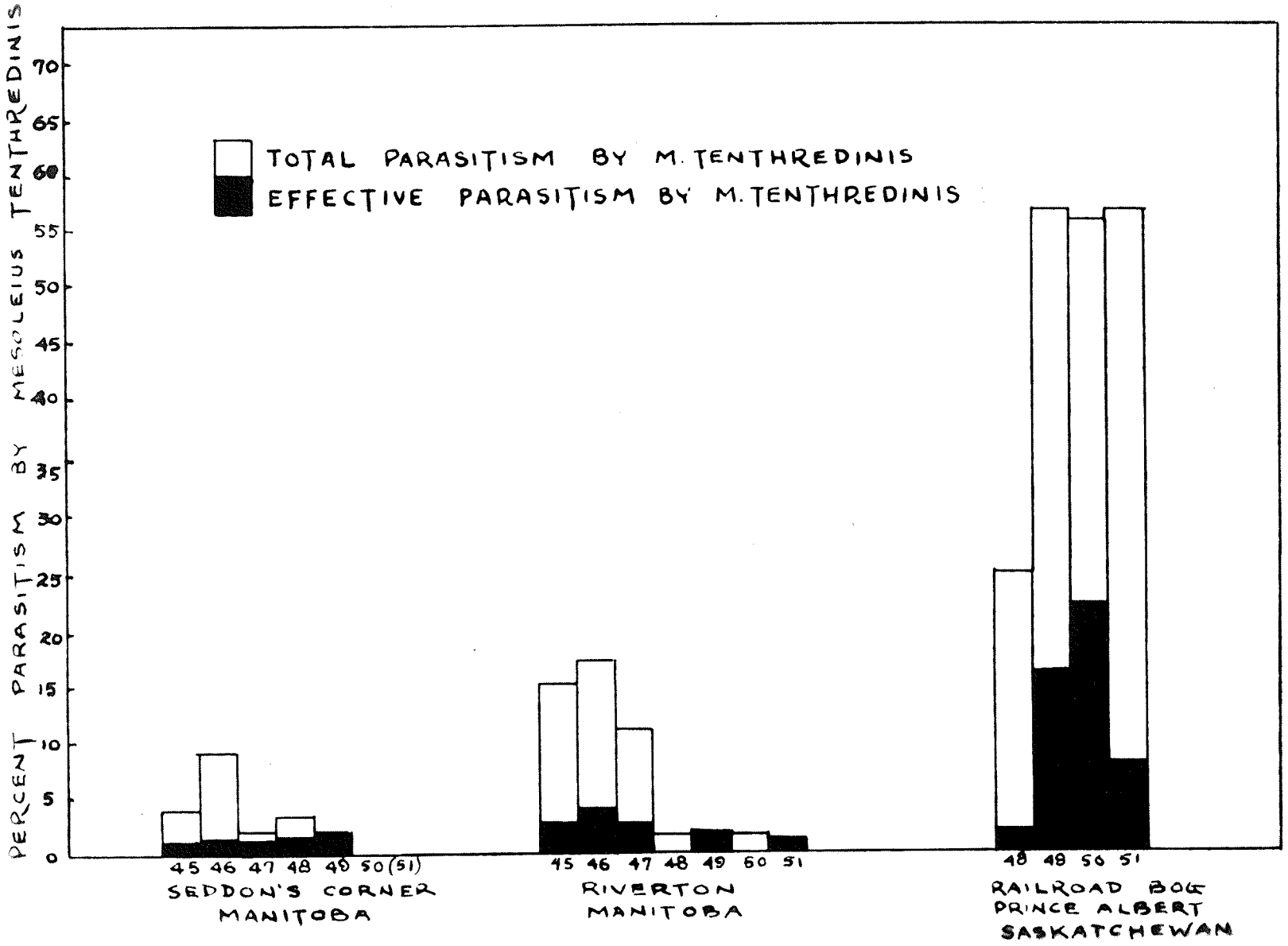

PER CENT PARASITISM BY MESOLEIUS TENTHREDINIS
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
TOTAL PARASITISM BY M. TENTHREDINIS
EFFECTIVE PARASITISM BY M. TENTHREDINIS
45 46 47 48 49 50 51
MILE 32 P.O.W. ROAD R.M.N.P.
45 46 47 48 49 50 51
MILE 145 DAUPHIN HWY. R.M.N.P.
45 (46) 47 48 49 50 51
MILE 7 NORGATE ROAD R.M.N.P.

Figure 2.

The Total Parasitism and Effective Parasitism by *M. tenthredinis* in Two Areas in Manitoba and One in Saskatchewan from 1945 to 1951.


PERCENT PARASITISM BY MESOLEIUS TENTHREDINIS
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
TOTAL PARASITISM BY M. TENTHREDINIS
EFFECTIVE PARASITISM BY M. TENTHREDINIS
45 46 47 48 49 50 (51)
SEDDON'S CORNER
MANITOBA
45 46 47 48 49 50 51
RIVERTON
MANITOBA
48 48 50 51
RAILROAD BOG
PRINCE ALBERT
SASKATCHEWAN

C. Review of the Literature on the Immunity of Insects to Insect Parasites

A number of workers have considered the problem of insect immunity to parasites. Their conclusions on the causes and the mode of operation of the immunity reaction are by no means in agreement.

Bess (5) discussed the use of the word "phagocytosis" in connection with the encapsulation of insect parasite eggs and larvae. He pointed out that the formation of agglomerations of cells around internal parasite eggs and larvae can hardly be termed a true intracellular phenomenon like that exhibited where bacteria are definitely ingested and digested by phagocytes, but he believed that the reactions are probably the same in principle.

He pointed out that there are two main viewpoints about the nature of this type of immunity. The first is that phagocytes attack and encapsulate living parasite eggs and larvae and are a primary cause of their death. Cuénot (18), an early exponent of this view, postulated that successful parasites resist the phagocytes which would otherwise destroy them. The second view is that immunity is based on some humoral phenomenon, and that phagocytes merely act as scavengers, protecting the host against injury from unhealthy and dead parasite eggs and larvae. Pantel (68), one of the first to advance this

view, concluded from his studies that generally the phagocytes do not attack free and healthy parasitic dipterous larvae but only accumulate about dead or weakened parasites and moult skins as they do about inert objects that occur in the body of the host.

The role of the phagocytes in the immunity of insects to insect parasites has been studied by several investigators. Timberlake (86) believed that unadapted parasites cannot repel phagocytes and may regularly succumb to them. A successful parasite, he suggested, either is so similar to the host in physical being that its presence is not resented or it may secrete substances into the blood that paralyze the protective reactions of the host. Thompson (82, 83) at first opposed these views, believing that the phagocytes of arthropods did not constitute a defensive mechanism against parasitic enemies, but later (84) admitted that possibly some parasite eggs normally secrete substances which immobilize the phagocytes. Bess (5) found that immunity is not necessarily accompanied by phagocytosis and concluded that phagocytosis is not a primary factor in immunity but independent of it. He found, however, that a greater proportion of dead parasites were encapsulated in the more immune host species than in the more susceptible ones and conceded that possibly

the phagocytes could increase the amount of chemical substances around a parasite and thus be responsible for its destruction. Paillot (65) in his studies on the European corn borer concluded that the phagocytic reaction undoubtedly caused the death of parasite eggs.

Strickland (77, 78) studied a number of parasites of noctuid larvae and decided that the parasites gain immunity after entering the body of the host. One parasite species developed normally except when the eggs were introduced just before the pupation of the host. Strickland postulated that the parasites could not acquire sufficient immunity to resist the increased activity of the phagocytes at pupation. Other experimental work led Strickland to believe that the larvae of all leaf-ovipositing tachinids gain immunity to the phagocytic action of the host by burying themselves in some structure such as a ganglion or muscle fiber immediately after entering the body cavity of the host. Strickland's work lends support to the "compatibility of protoplasm" theory first advanced by Timberlake (86). Chorine (12) found that pathogenic protozoa will only develop successfully in their proper insect hosts, where there is little phagocytosis. Ephrussi and Beadle (23) and Wigglesworth (91) found that implants of living tissue from the same or allied species can live and develop

without molestation by phagocytes. Ries (72) showed that implants from unrelated species may be the center of considerable hematocytic activity.

Several theories have been advanced pertaining to the "attraction" of the phagocytes to the parasite. Mellanby (52) expressed his opinion that the attraction of the phagocytes of insects to an invading body is chemotactic, dependent upon the liberation of attractive substances. He considered that the absence of phagocytosis indicates that a parasite is adapted to its host. Wigglesworth (89) commented on mammalian blood cells remaining unchanged for weeks in the hemocoele of the bed bug and Mellanby suggested that these cells do not emit chemotactic substances. Thompson (84) considered the behaviour of phagocytes to be reactions to the presence of pathological exudates resulting from degeneration of cells and tissues. Mellanby (52) suggested that probably the main normal function of the phagocytes is the removal of dead tissues. This idea is supported by Wigglesworth's work on Rhodnius (90). There is some controversy (24, 70, 52) as to whether the phagocytes actually initiate the breakdown of tissues during pupation or whether they are attracted by the first chemical products of the breakdown.

Pemberton and Willard (69), in their studies on

several parasites of the melon fly, found that the parasite Tetrastichus giffardianus was encapsulated and killed within the host except when the host was subjected to prior parasitization by Opius fletcheri. In the latter case Tetrastichus developed and Opius died. When the melon fly larvae were parasitized by O. fletcheri alone, or by O. fletcheri followed by parasites other than Tetrastichus, O. fletcheri developed normally. They concluded that the immunization of the melon fly to Tetrastichus is destroyed by the injection of toxic substances by the adult female of O. fletcheri at oviposition. Bess (5) disagreed with this theory as a result of his study of the immunity of Pseudococcus gahani to the parasite Coccophagus gurneyi.

Another possible explanation postulates the secretion of active protective substances by the parasite egg or larva to prevent phagocytosis. Bess (5) considered this a more plausible explanation than that advanced by Pemberton and Willard. He obtained inconclusive evidence indicating that in some instances phagocytosis is initiated by substances liberated by the parasite about the time of hatching. However encapsulation does not always occur about the time of hatching (63). Thorpe (85) obtained evidence indicating that the encapsulation of Encyrtus infelix within Sassetia hemisphaerica is a response to a secretion of the segmental

dermal glands of the parasite larva.

Several workers have postulated that the immunity of a host to a parasite may be the result of an unsuitable food medium in the host which causes the parasite to succumb to malnutrition or starvation (52, 26, 5, 63).

Hollande (35) considered a combination of factors involving nutrition, secretion of phagocyte-repelling substances by the parasite, and secretion of phagocyte-attracting substances by the capsule. He worked on the formation of leucocytic agglomerations around trematode larvae encysted and fixed to the digestive tube of Dytiscus marginalis. Depending on the nutritive state of the trematode, evidently both repelling and attracting reactions took place.

"Melanization" has been considered by various workers (22, 5, 85) as a defense reaction of insects to internal parasites.*

Thorpe (85) described a case in which a parasite apparently overcame the adverse effect of the host-deposited, phagocytic capsule and used the capsule for its own purpose. In this instance the tracheal system of the host became attached to the capsule at four or six sclerotized points and the spiracles of the parasite were

* See Section V, part G, for a further discusstion of the findings of these workers.

put into functional communication with the tracheal system of the host. The fact that the parasite survived may indicate that, in general, phagocytic capsules destroy enclosed parasites by stopping or reducing the flow of gases to and from these parasites.

Parasite embryos within the egg chorions seem to resist phagocytic destruction more so than do the tissues of the parasite larva within the larval integument (86). A number of workers (60, 44, 86, 5) have found healthy parasite eggs and larvae completely encapsulated. Sometimes the embryos complete development and the small larvae escape from the capsules (44), though the incubation period of such eggs may be abnormally long (5, 44). More often, apparently, the living encapsulated parasites eventually die (5). Bess (5) found that the process of encapsulation, once begun, is completed within a few hours and intermediate stages of encystment are rarely observed. Normal health and longevity are apparently experienced by adult hosts containing encapsulated parasites (86, 69).

V STUDIES ON THE NATURAL IMMUNITY OF THE LARCH SAWFLY TO MESOLEIUS TENTHREDINIS

A. The Host Stage Attacked and the Seasonal Period of Attack in Relation to the Effective Parasitism by *M. tenthredinis*

In 1945[1] a possible explanation for the poor hatchability of *M. tenthredinis* eggs was advanced. It was suggested that possibly the parasite eggs were laid in late-stage sawfly larvae, and that they did not have time to hatch before the larvae spun their cocoons. Another possibility suggested was that the eggs were laid late in the season, and did not have time to hatch before cold weather began.

To test these hypotheses the following experiment was carried out to ascertain (1) the stadia of sawfly larvae attacked by *M. tenthredinis* and the relation between the stadium attacked and the effective parasitism by *M. tenthredinis*, and (2) the relation between the seasonal period of attack and the effective parasitism by *M. tenthredinis*.

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1. Annual Technical Report, Forest Insect Laboratory, Winnipeg. 1945. pp. 139-140.

Methods.

In 1949, two larch swamps in the Riding Mountain National Park were selected and from each of these, sawfly larvae were collected at approximately weekly intervals throughout the summer. The two areas were; (1) Mile 145 on Highway #10 (sec. 25, tp. 21, rge. 19, W.P. mer.), and (2) Swamp #1 on the "Prisoner of War Camp" Road (sec. 12, tp. 21, rge. 21, W.P. mer.).

An attempt was made to collect at least 50 larvae of each instar each week. Some of these larvae were dissected in the field during the summer. As the first-, second- and third-instar sawfly larvae are difficult to dissect, these were reared, sheltered from attack by parasites, and were dissected when they were fifth-instar larvae; some before they had cocooned and others after they had cocooned. Some of the larvae collected were reared through to the cocoon stage and were kept in storage until the winter months at which time they were dissected in Winnipeg.

A considerable number of these larvae died during rearing especially first-, second-, and third-instar larvae, and the results, therefore, were mainly obtained from the dissection of those larvae which were fourth- and fifth-instar larvae when collected.

In 1950, two larch swamps in Saskatchewan were sampled; (1) the "Railroad" swamp north of the bridge near Prince Albert (sec. 8, tp. 49, rge. 26, W. 2 mer.), and (2) the "Crutwell" swamp, north of Crutwell on Highway #3 (sec. 22, tp. 49, rge. 1, W. 3 mer.). In 1950 first-instar larch sawfly larvae were not collected.

Data.

The data obtained are presented in Figures 3, 4, 5 and 6 in histogram form.

They indicate that the effective parasitism was much lower in the Riding Mountain National Park in 1949 than it was in the Prince Albert area in 1950. This may be partly due to the fact that in 1949 a considerable number of larch sawfly larvae were dissected during the summer shortly after they were collected and the parasite eggs contained within these host larvae may not have had time enough to hatch. Some of the small first-instar parasite larvae may have been missed during the summer dissections, as they are much harder to find during dissection than are the later-stage parasite larvae found during the winter.

Discussion.

Figures 3 to 6 show that percent parasitism based on one or more larch sawfly larval collections made during

Figure 3.

Seasonal Trend of the Total and Effective Parasitism of the Larch Sawfly by M. tenthredinis in Swamp #1 "Prisoner of War Camp" Road, R.M.N.P., Manitoba, in 1949.

Note: Where no column appears above an instar number none of the larvae dissected were parasitized.

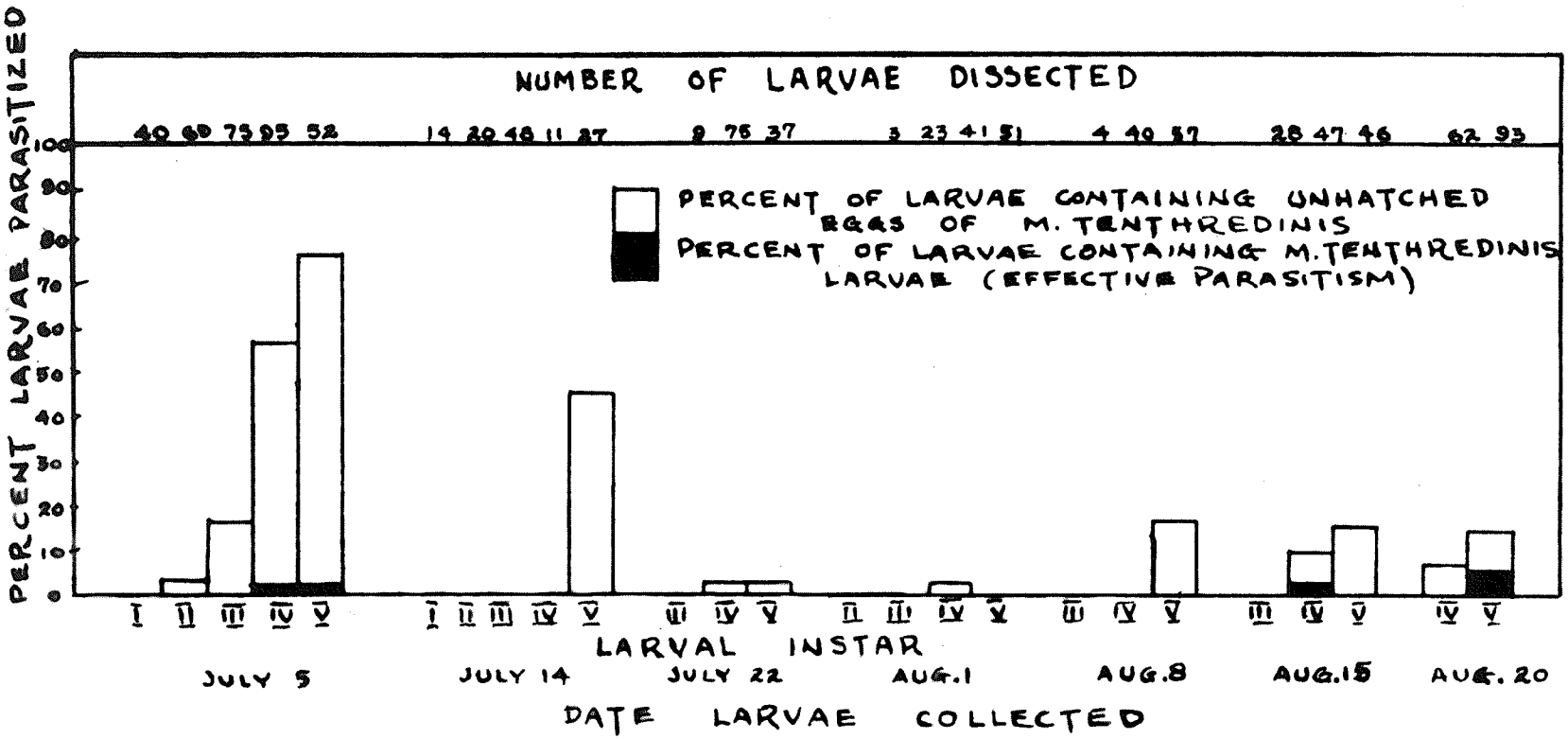

NUMBER OF LARVAE DISSECTED
40 60 73 95 52
14 20 46 11 27
8 75 37
3 23 41 51
4 40 57
28 47 46
62 93
PERCENT LARVAE PARASITIZED
0
10
20
30
40
50
60
70
80
90
100
PERCENT OF LARVAE CONTAINING UNHATCHED EGGS OF M. TENTHREDINIS
PERCENT OF LARVAE CONTAINING M. TENTHREDINIS LARVAE (EFFECTIVE PARASITISM)
I II III IV V
LARVAL INSTAR
JULY 5
JULY 14
JULY 22
AUG. 1
AUG. 8
AUG. 15
AUG. 20
DATE LARVAE COLLECTED

Figure 4.

Seasonal Trend of the Total and Effective Parasitism of the Larch Sawfly by M. tenthredinis in the Swamp at Mile 145 Dauphin Highway, R.M.N.P., Manitoba, in 1949.

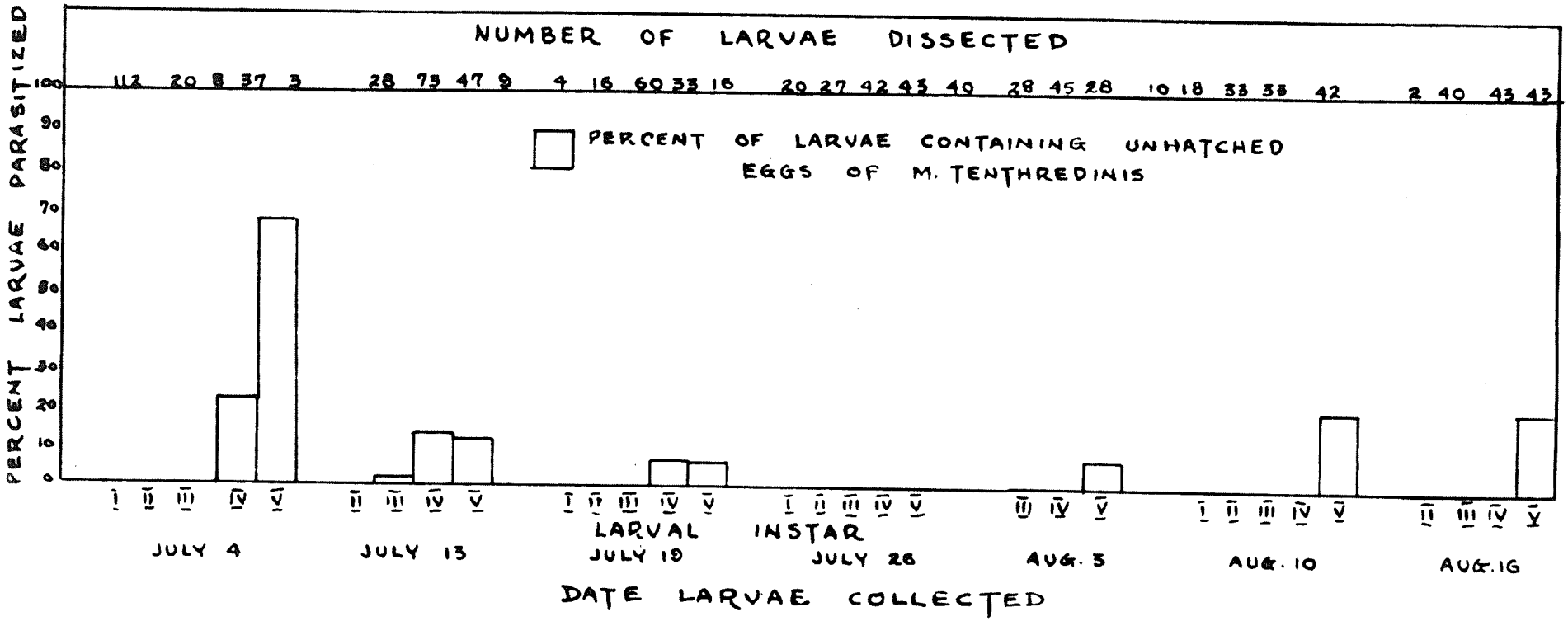

NUMBER OF LARVAE DISSECTED
112 20 8 37 3 28 73 47 9 4 16 60 33 16 20 27 42 43 40 28 45 28 10 18 33 33 42 2 40 43 43
PERCENT OF LARVAE CONTAINING UNHATCHED EGGS OF M. TENTHREDINIS
PERCENT LARVAE PARASITIZED
0 10 20 30 40 50 60 70 80 90 100
I II III IV V
LARVAL INSTAR
JULY 4
JULY 13
JULY 19
JULY 26
AUG. 3
AUG. 10
AUG. 16
DATE LARVAE COLLECTED

Figure 5.

Seasonal Trend of the Total and Effective Parasitism of the Larch Sawfly by M. tenthredinis in the "Railroad" Swamp North of Prince Albert, Saskatchewan, in 1950.

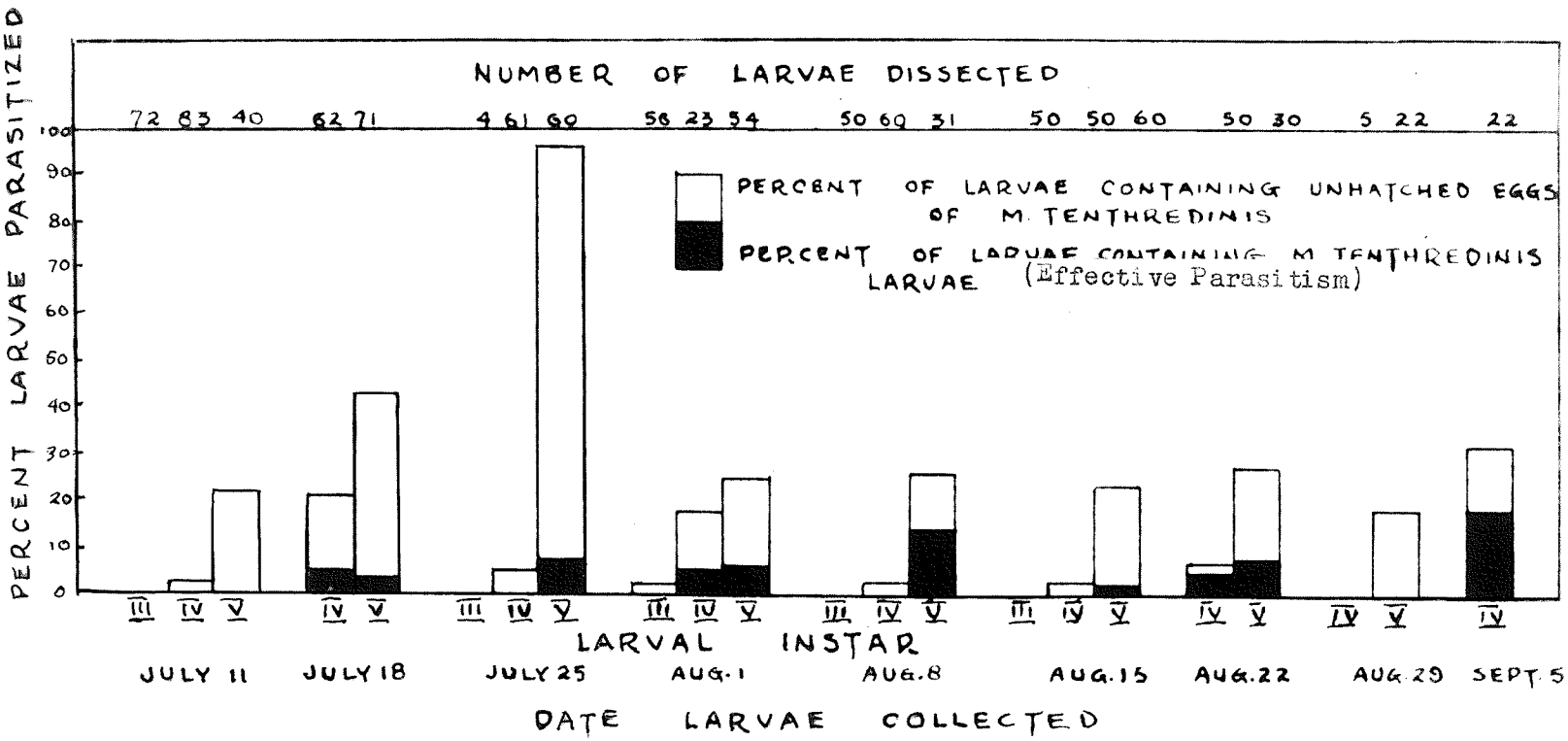
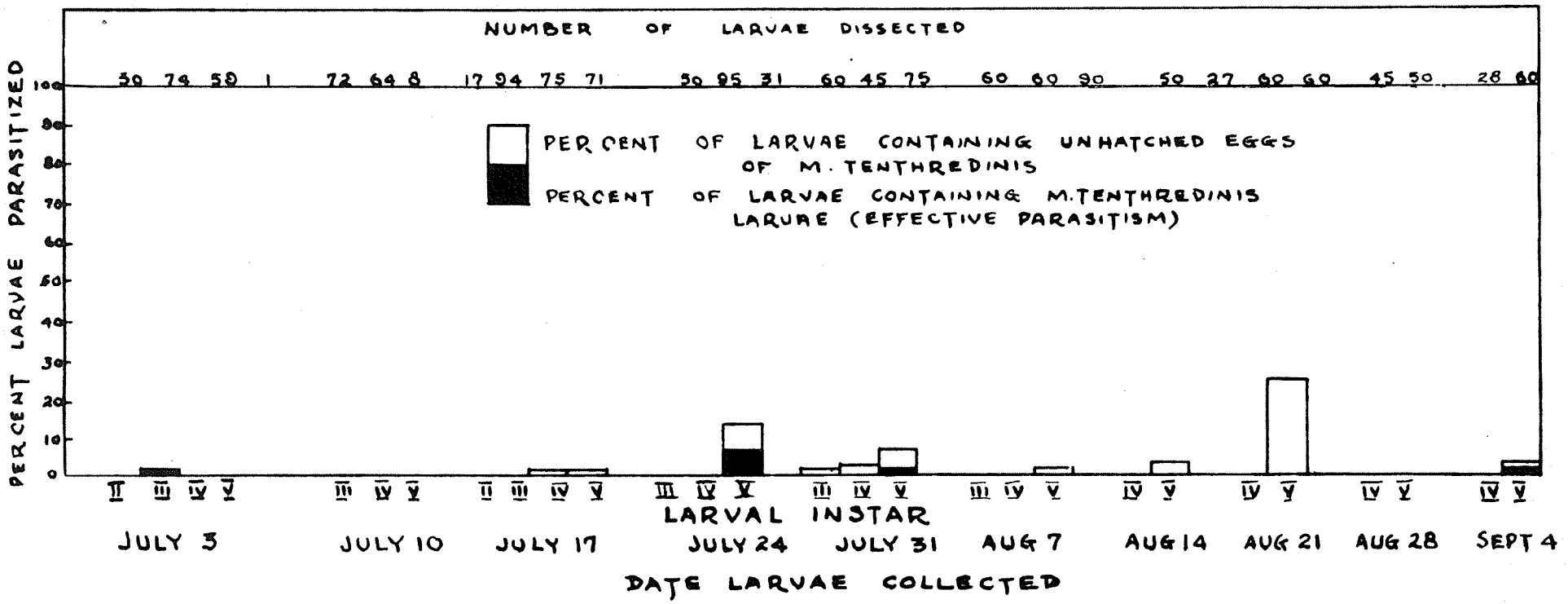

NUMBER OF LARVAE DISSECTED
72 83 40 82 71 4 61 99 58 23 54 50 60 31 50 50 60 50 30 5 22 22
PERCENT LARVAE PARASITIZED
100
90
80
70
60
50
40
30
20
10
0
PERCENT OF LARVAE CONTAINING UNHATCHED EGGS OF M. TENTHREDINIS
PERCENT OF LARVAE CONTAINING M. TENTHREDINIS LARVAE (Effective Parasitism)
III IV V
IV V
III IV V
III IV V
III IV V
III IV V
IV V
IV V
IV
LARVAL INSTAR
JULY 11
JULY 18
JULY 25
AUG. 1
AUG. 8
AUG. 15
AUG. 22
AUG. 29
SEPT. 5
DATE LARVAE COLLECTED

Figure 6.

Seasonal Trend of the Total and Effective Parasitism of the Larch Sawfly by M. tenthredinis in the "Crutwell" Swamp, Saskatchewan, in 1950.


NUMBER OF LARVAE DISSECTED
50 74 58 1 72 64 8 17 94 75 71 30 95 31 60 45 75 60 60 90 50 27 60 60 45 50 28 60
PERCENT OF LARVAE CONTAINING UNHATCHED EGGS OF M. TENTHREDINIS
PERCENT OF LARVAE CONTAINING M. TENTHREDINIS LARVAE (EFFECTIVE PARASITISM)
PERCENT LARVAE PARASITIZED
0
10
20
30
40
50
60
70
80
90
100
II III IV V
III IV V
II III IV V
III IV V
III IV V
III IV V
IV V
IV V
IV V
LARVAL INSTAR
JULY 3
JULY 10
JULY 17
JULY 24
JULY 31
AUG 7
AUG 14
AUG 21
AUG 28
SEPT 4
DATE LARVAE COLLECTED

the summer does not give a true indication of the degree of control exerted by M. tenthredinis. To give such data significance ~~in this respect~~, fairly accurate population estimations of the larch sawfly larvae in the field at the time each larval collection was made would have to be carried out in conjunction with the dissections. Because an accurate method of estimating populations was not available such estimations were not attempted.

Estimations of percentage parasitism by M. tenthredinis from cocoons collected in the fall, after the sawfly has disappeared from the trees, give a better idea of the control being exerted by M. tenthredinis. In this connection, however, it should be noted that many of the cocoons formed during July and early August are either eaten or stored in caches by small mammals and therefore a higher proportion of the cocoons formed in late August and September appear in the cocoons collected in the fall.

The following Table was constructed from the data presented in Figures 3, 4, 5 and 6, and shows the percent parasitism of the total number of larvae dissected in each instar-group.

TABLE I

Total Percent Parasitism by M. tenthredinis Found in Each Sawfly Larval Instar.

Larval instar	Total number dissected	Total number parasitized	Percent parasitized
I	130	0	0.0
II	250	2	0.8
III	1,080	16	1.4
IV	1,627	108	6.6
V	1,347	275	20.4

The figures in the fourth column do not indicate the relative extent to which the various larch sawfly larval instars were attacked by M. tenthredinis since parasite eggs and larvae found in fifth-instar sawfly larvae were deposited into a number of them when they were fourth-, third-, and second-instar larvae. The same applies to the parasite eggs found in fourth- and third-instar sawfly larvae. Table II gives a better indication of the relative extent to which the various sawfly instars were attacked by M. tenthredinis. In this table calculated values were derived by subtracting, from the parasitism of each instar, the parasitism of the preceeding instar.

TABLE II

Estimated Percent of Each Sawfly Instar in which *M. tenthredinis* Eggs were Deposited.

Larval instar	Calculated percent of each instar attacked by *M. tenthredinis*		
I			0.0
II			0.8
III	1.4 - 0.8	=	0.6
IV	6.6 - 1.4	=	5.2
V	20.4 - 6.6	=	13.8

It is of interest to note that if the parasitism of the second-instar larvae is arbitrarily changed to 0.35 percent instead of 0.8 percent and if the corrected values 0.0, 0.35, 1.05, 5.2, and 13.8 are plotted on semi-log paper, a close approximation to a straight line is obtained.

Conclusions.

Parasite larvae were found in third-, fourth-, and fifth-instar larch sawfly larvae. There appears to be no obvious relation between the instar attacked and the effective parasitism by *M. tenthredinis*.

Parasite larvae were found in host sawfly larvae

throughout the season from the first week in July to the first week in September. There appears to be no obvious relation between the seasonal period of attack and the effective parasitism by M. tenthredinis.

The evidence obtained in this study does not offer support to either of the two possible explanations of the immunity of the larch sawfly proposed in 1945.

B. Immunity of Host Material from British Columbia

In the review of the literature on M. tenthredinis it was pointed out that this parasite is still very effective in British Columbia. During the winter of 1950-51 a collection of 250 cocoons of the larch sawfly were supplied for experimental purposes through the kindness of Mr. J. H. McLeod, Insect Parasite Laboratory, Vancouver, British Columbia. These cocoons were taken to the Red Rock Lake Field Station in the Whiteshell Forest Reserve, Manitoba. They were refrigerated until June, and then placed in individual shell vials for emergence. During the summer of 1951 the adults from these cocoons were caged for oviposition and the larval progeny obtained were reared under insectary conditions for controlled parasitism experiments. Groups of these larvae were parasitized by M. tenthredinis

obtained from Saskatchewan cocoons and M. tenthredinis obtained from British Columbia cocoons. Concurrently, larch sawfly larvae collected near the Red Rock Lake Field Station, Manitoba, were also parasitized by these same parasites.

Results.

During the summer 20 sawfly larvae from British Columbia that had been parasitized as fifth-instar larvae, by M. tenthredinis from Saskatchewan, were dissected. Parasite eggs removed from larvae dissected eight days after parasitization had not hatched but embryonic development was well advanced. Fifteen of these larvae dissected ten days after parasitization each contained at least one living parasite larva and many also contained a number of well-developed parasite eggs. In no case was there any evidence of capsule formation around the parasite eggs in these larvae from British Columbia. Sawfly larvae from Manitoba parasitized by these same M. tenthredinis from Saskatchewan in the same cage, shortly after removing the British Columbia host larvae, were dissected two weeks after parasitization. They showed the usual capsules formed around the parasite eggs and low hatchability of these eggs.

Several groups of larvae from British Columbia parasitized on July 16th and 18th, 1950, by M. tenthredinis from different sources were stored in moss after they had cocooned. These larvae were dissected in January, 1952. The results of these dissections are shown in Table III. The capsules around the two phagocytized eggs found were more transparent than those formed by sawfly larvae from Manitoba and Saskatchewan around M. tenthredinis eggs. The parasite embryos could be seen within the capsules. The two capsules were relatively dark brown in color. These two eggs were the only ones found to be encapsulated by host larvae from British Columbia although in some cases foreign bodies in the unparasitized host larvae from British Columbia were sometimes encapsulated.

In the 38 parasitized sawfly larvae dissected during January, 37 living M. tenthredinis larvae were found in 36 of the hosts. In one host two living parasite larvae were found. One was considerably larger than the other and it is doubtful whether both would have survived to the adult stage. Besides the 37 living M. tenthredinis larvae, 23 dead, unencapsulated first-instar larvae and 11 dead, unencapsulated eggs were found. The small dead first-instar larvae appeared to have died shortly after they hatched. Localized portions of most of the dead first-instar larvae

TABLE III

Results of Dissections of Larch Sawfly Larvae from British Columbia Parasitized by *M. tenthredinis* obtained from Different Sources.

No. of sawfly larvae parasitized	Instar parasitized	Source of *M. tenthredinis*	No. of hosts containing living larvae of *M. tenthredinis*	Percent of hosts containing a living larva of *M. tenthredinis* (effective parasitism)	Hosts containing only unhatched eggs of *M. tenthredinis*	
					with capsules	without capsules
7	IV	B.C.	7	100	0	0
5	V	B.C.	5	100	0	0
7	IV	Sask.	7	100	0	0
19	V	Sask.	17	89	2	0

and embryos appeared to be melanized, i.e., dark brown spots had formed in places while the remainder of the bodies of these larvae and embryos had remained creamy-white in color.

Discussion and Conclusions.

Fifty three sawfly larvae from British Columbia that had been parasitized by M. tenthredinis were dissected ten or more days after being parasitized. It was found that only two of these did not contain at least one living larva of M. tenthredinis. In other words 96 percent of the larch sawfly larvae from British Columbia were found to be susceptible to M. tenthredinis. This is in sharp contrast to the situation in Manitoba and Saskatchewan where a relatively low percentage of the larch sawfly are susceptible to this parasite.

The two sawfly larvae that formed capsules about the parasite eggs within them may have been larvae from Manitoba that contaminated the group of larvae from British Columbia either during rearing or parasitization, although strict precautions were taken to see that such contamination did not occur. If, however, they were larvae from British Columbia, this indicates that a few sawfly larvae from British Columbia were able to encapsulate

M. tenthredinis eggs in the same way that the larch sawfly larvae from Manitoba and Saskatchewan do. It should be noted that the dead supernumerary M. tenthredinis eggs found in the other larvae from British Columbia dissected during this study were not encapsulated, and this may perhaps be considered as evidence against the view that the capsules around the two eggs mentioned above were formed after these eggs had died from some other cause.

In the majority of cases it appeared that the first parasite larva to emerge was, in some way, able to kill the rest of the M. tenthredinis that occurred within the same host. In most cases the supernumerary parasites were killed as first-instar larvae shortly after they emerged from the chorion. In a number of instances, dead first-instar larvae were found with the anterior end of the larva projecting out of the chorion. A number of M. tenthredinis eggs and well developed embryos were apparently killed while still within the chorion.

C. Viability of Encapsulated Eggs of M. tenthredinis

This study was carried out at Prince Albert, Saskatchewan during the summer of 1950. Prior to 1950 the author and others had observed that many of the

encapsulated parasite eggs removed from sawfly larvae one to eight weeks, or even more, after parasitization contained embryos that were fairly well-advanced in development. All of these embryos appeared to be at approximately the same stage of development and they were seemingly alive and healthy. During July, 1950, an attempt was made to determine whether or not these embryos were still viable. Viability could be demonstrated if the eggs could be treated in such a manner that they would resume embryonic growth and develop through to the first-instar larval stage. The attempt made to do this was based on the assumption that it was the phagocytic capsules that prevented the embryos from completing their development within the sawfly hosts. A few encapsulated eggs were removed from some parasitized sawfly larvae and the capsules were carefully dissected off. The resulting capsule-free eggs were placed in Ringer's invertebrate physiological solution. The eggs were examined periodically and further embryonic development was observed. Approximately five days after the eggs had been removed from their hosts and placed in the Ringer's solution hatching of the majority of these eggs had taken place. The small first-instar larvae died about two days after eclosion, presumably from starvation.

Further small-scale studies revealed that the embryos

would resume development and hatch in the Ringer's solution whether or not the capsules were removed before placing them in the solution. On the basis of these small-scale experiments more extensive studies were conducted. These are described in the following pages.

1. Relation Between the Time Spent by M. tenthredinis Eggs in Sawfly Larvae and the Viability of These Eggs.

Method.

Larch sawfly larvae were parasitized by placing the larvae for approximately 24 hours in cages containing about 50 male and 50 female adults of M. tenthredinis. The majority of the M. tenthredinis adults used in this experiment came from sawfly cocoons collected in British Columbia in 1949. These cocoons were reared at Belleville, Ontario, and the adults were shipped to Prince Albert by the Belleville Laboratory.

The parasitized sawfly larvae were dissected at intervals following parasitization. The parasite eggs found were placed in small glass dishes each containing about 10 cc. of Ringer's invertebrate physiological solution. These were covered by inverted jelly jars to prevent excessive evaporation of the saline, and tin cans to shade the eggs from bright light.

Data.

Tables IV to VIII inclusive show the relationship between the time spent by M. tenthredinis eggs in sawfly larvae and the viability of these eggs. The time spent by the parasite eggs in sawfly larvae is taken as being the time between parasitization of these larvae and dissection and transfer of the eggs to Ringer's solution. The time that the sawfly larvae spent in the parasitization cage, however, extended over a considerable period and since it was impossible to know when the individual parasite eggs were laid during this period, the time spent by the parasite eggs in sawfly larvae was taken to start at the median of the period spent by the sawfly larvae in the parasitization cage. These periods for the five groups of sawfly larvae parasitized were as follows:
Group #1, parasitized July 30th to 31st within 24 hours.
Group #2, parasitized August 1st to 2nd within 19 hours.
Group #3, parasitized August 8th to 10th within 44 hours.
Group #4, parasitized August 8th to 10th within 44 hours.
Group #5, parasitized July 31st to August 1st within 20 hours.

Because of this factor each of the figures listed in Tables IV to VIII inclusive, for the times spent by the various lots of parasite eggs in the host larvae (the second column in each Table), is subject to a possible error

which is half of the period spent by the host larvae in the parasitization cage. These possible errors are given in each Table in the box heading over the second column.

TABLE IV

Time Spent in Host in Relation to Viability for M. tenthredinis Eggs Deposited in Fourth-Instar Sawfly Larvae (Group #1).

Dish No.	Time (in days) spent by parasite eggs in sawfly larvae (possible error=± 0.5)	No. of parasite larvae found in the sawfly larvae	No. of eggs placed in Ringer's Invertebrate solution	Percent hatch of eggs before dissection	Percent of eggs having a visible capsule	No. of eggs that hatched in the Ringer's solution	Percent hatch of the treated eggs
1	1.3	0	15	0	0	15	100
2	1.5	0	20	0	0	19	95
3	1.6	0	17	0	0	17	100
4	2.3	0	27	0	18	25	93
5	2.5	0	29	0	17	28	96
6	3.5	0	25	0	32	19	76
7	3.6	0	25	0	28	25	100
8	4.3	0	24	0	62	20	83
9	4.4	0	28	0	46	23	82
10	6.3	2	26	7.1	88	19	73
11	7.4	1	30	3.2	87	28	93
12	9.4	0	25	0	80	22	88
13	11.6	0	25	0	100	19	76
14	12.4	3	34	8.1	91	30	88
15	15.5	1	25	1.8	84	19	76
16	15.5		30			21	70
17	17.5	2	13	13.3	100	7	54
18	20.5	1	30	3.2	100	15	50
19	58.7	1	19	3.4	100	6	32
20	58.7		19			2	5
21	76.7	0	20	0	100	6	30

TABLE V

Time Spent in Host in Relation to Viability for *M. tenthredinis* Eggs Deposited in Fourth-Instar Sawfly Larvae (Group #2).

Dish No.	Time (in days) spent by parasite eggs in sawfly larvae (possible error=± 0.4)	No. of parasite larvae found in the sawfly larvae	No. of eggs placed in Ringer's Invertebrate solution	Percent hatch of eggs before dissection	Percent of eggs having a visible capsule	No. of eggs that hatched in the Ringer's solution	Percent hatch of the treated eggs
51	0.7	0	25	0	8	19	76
52	0.8	0	25	0	0	18	72
53	2.5	0	15	0	53	15	100
54	6.2	0	25	0	100	18	72
55	22.2	0	12	0	100	5	42
56	22.2	0	11	0	100	7	64

TABLE VI

Time Spent in Host in Relation to Viability for *M. tenthredinis* Eggs Deposited in Fifth-Instar Sawfly Larvae (Group #3).

Dish No.	Time (in days) spent by parasite eggs in sawfly larvae (possible error-$\pm$ 0.9)	No. of parasite larvae found in the sawfly larvae	No. of eggs placed in Ringer's Invertebrate solution	Percent hatch of eggs before dissection	Percent of eggs having a visible capsule	No. of eggs that hatched in the Ringer's solution	Percent hatch of the treated eggs
101	1.1	0	44	0	0	34	77
102	1.1	0	57	0	0	43	75
103	6.4	0	50	0	44	38	76
104	9.1	2	38	5.0	21	26	68
105	11.9	5	85	5.5	45	50	59
106	15.9	0	14	0	100	11	79
107	15.9	0	14	0	100	8	57
108	29.0	0	21	0	100	4	19
109	29.0	0	23	0	100	4	17
110	50.7	0	21	0	100	3	14
111	50.7	0	21	0	100	5	24
112	81.1	0	18	0	100	4	22

TABLE VII

Time Spent in Host in Relation to Viability for M. tenthredinis Eggs Deposited in Third-Instar Sawfly Larvae (Group #4).

Dish No.	Time (in days) spent by parasite eggs in sawfly larvae (possible error=± 0.9)	No. of parasite larvae found in the sawfly larvae	No. of eggs placed in Ringer's Invertebrate solution	Percent hatch of eggs before dissection	Percent of eggs having a visible capsule	No. of eggs that hatched in the Ringer's solution	Percent hatch of the treated eggs
151	1.4	0	64	0	0	48	75
152	6.5	0	36	0	64	29	80
153	8.9	3	69	2.1	75	47	68
154	8.9		74			57	77
155	11.3	19	59	24.4	25	39	66
156	13.2	17	46	11.3	63	27	59
157	13.2		42			31	74
158	13.2		45			32	71
159	29.2	2	10	8.3	100	5	50
160	29.2		12			4	33

TABLE VIII

Time Spent in Host in Relation to Viability for M. tenthredinis Eggs Deposited in Fifth-Instar Sawfly Larvae (Group #5).

Dish No.	Time (in days) spent by parasite eggs in sawfly larvae (possible error=≤ 0.4)	No. of parasite larvae found in the sawfly larvae	No. of eggs placed in Ringer's Invertebrate solution	Percent hatch of eggs before dissection	Percent of eggs having a visible capsule	No. of eggs that hatched in the Ringer's solution	Percent hatch of the treated eggs
201	156	1	40	2.4	100	4	10.0
202	217	0	35	0.0	100	1	2.9

The data presented in these five tables are summarized below in Table IX. The total number of M. tenthredinis eggs treated during the experiment are combined into ten units based on the time spent by these eggs in the sawfly larvae.

Discussion.

The hatch of those parasite eggs which spent four or less days in the sawfly larvae ranged from 72 percent to 100 percent. A considerable proportion of the mortality that occurred in those dishes in which a lower percentage of the eggs hatched was probably due to the eggs being damaged during the removal of the capsules. It was also found that contaminating fungous organisms grew to a greater extent in some dishes than in others and this may have been partly responsible for the difference in mortality among the various dishes.

Only two out of 267 M. tenthredinis eggs removed from sawfly larvae two days or less after parasitization were recorded as being encapsulated. The maximum possible amount of time these two eggs could have spent in the host larvae was 27 hours.

The proportion of encapsulated eggs increased as the time spent by these eggs in the host larvae increased

TABLE IX

The Relationship Between the Time Spent by M. tenthredinis Eggs in Sawfly Larvae and the Viability of These Eggs. (Summary of data in Tables IV - VIII inclusive)

Time (in days) spent by parasite eggs in sawfly larvae	Total No. of eggs treated	Total No. of parasite larvae found on dissection	Percent hatch of parasite eggs within the sawfly larvae	Percent of eggs having a visible capsule	Percent hatch of eggs in Ringer's solution
0 to 3	338	0	0.0	3.5	82.5
3 to 5	102	0	0.0	43.0	85.3
5 to 10	373	8	2.1	73.7	76.1
10 to 15	336	44	11.6	72.9	67.9
15 to 20	96	3	3.0	90.6	68.7
20 to 30	119	3	2.5	100.	37.0
50 to 60	80	1	1.2	100.	20.0
75 to 80	38	0	0.0	100.	26.3
156	40	1	2.4	100.	10.0
217	35	0	0.0	100.	2.9

until a peak of approximately 100 percent encapsulation was reached after the eggs had spent about ten days in healthy sawfly hosts. Table IX is misleading in this respect as it indicates that the peak of 100 percent encapsulation was not reached until after the eggs had spent twenty days in the sawfly hosts. It was found that a lower proportion of eggswere encapsulated in "unhealthy" host sawfly larvae than in healthy sawfly larvae. This phenomenon is discussed in more detail in a later section. Table IX considers the eggs removed from both "unhealthy" and healthy host larvae. The following Table considers only the eggs removed from healthy larvae.

TABLE X

Encapsulation of M. tenthredinis Eggs Removed from Healthy Larch Sawfly Larvae Ten or More Days after Parasitization.

Host larval instar	Total number of eggs removed	Percent of these eggs that were encapsulated
III	22	100.
IV	267	92.
V	207	100.

The capsules found around eggs that had spent two or more days in the sawfly hosts were differentiated visually into thin capsules and thick capsules. Both thin and thick capsules were found throughout the study, around eggs that had spent only two or three days in sawfly larvae and also around those that had spent over two months in sawfly larvae. The data did not indicate any regular increase in the proportion of thick to thin capsules as the length of time spent by the eggs in the host larvae increased.

The hemocytes of insect larvae that float free in the blood stream are known to increase greatly during moulting. One of the possibilities contemplated was the occurrence of a significant increase in the rate of deposition of the capsule during ecdysis. It was postulated that the fact that a parasite egg deposited in a third-instar larva is present while the host moults twice; and that a parasite egg deposited in a fourth-instar larva is never present in the host larva during a moulting period, might have a bearing on the rapidity of formation of the capsule and consequently on the effective parasitism in relation to the larval instar attacked. The evidence obtained from this study was not conclusive, but it indicated that, proportionately speaking, eggs were encapsulated

more rapidly in fourth-instar larvae than in third-instar larvae and more rapidly in third-instar larvae than in fifth-instar larvae. This evidence does not support the theory that the rate of phagocytosis increases during moulting.

Conclusions.

This experiment showed (a) that a proportion of the encapsulated M. tenthredinis eggs will hatch, after their removal from sawfly larvae, in Ringer's invertebrate solution (this is true whether or not the capsules are removed from these eggs before they are placed in the solution), and (b) that the longer the eggs remain in the host larvae the lower is the proportion of them that hatch after they are placed in Ringer's invertebrate solution.

The fact that encapsulated M. tenthredinis eggs removed from larch sawfly larvae will hatch in Ringer's invertebrate solution even if the capsules are left on these eggs does not necessarily indicate that the capsules are not the main factor responsible for inhibiting the development of these eggs in the host larvae. The cells composing the capsule may be alive when in the host larvae but may die after the transfer of the encapsulated eggs to the Ringer's solution and it may be necessary for the cells

composing the capsule to remain alive in order to effectively inhibit the growth of the M. tenthredinis eggs. Another factor that may be of importance in this respect was noted during the study. It was found in many cases that after the encapsulated eggs were transferred from the sawfly larvae to the Ringer's solution the chorions of the eggs expanded considerably, probably because the Ringer's invertebrate solution was hypotonic, and often split the capsule open at one end and protruded from the opening. The parasite embryos were often found adjacent to that part of the chorion that protruded from the capsule. Some embryos, however, appeared to develop and hatch even though the capsule did not split open during their development.

Most of the encapsulated eggs that were removed two or more months after oviposition were found to be dead although a small proportion of a group of eggs that had spent over seven months in the host larvae hatched out. None of the groups of parasite eggs removed from sawfly larvae during this study showed complete mortality, but evidence obtained from other studies indicates that none of the encapsulated eggs removed eight or more months after parasitization are viable.

As the length of time that the M. tenthredinis eggs spend in the host larvae increases the proportion of the eggs found to be encapsulated increases and the viability of the eggs decreases. The decreasing viability of the eggs may be due to a detrimental effect caused by the phagocytic capsules but this was not definitely proved by this study. The detrimental effect of the phagocytic capsules may be due to a permeability phenomenon affecting the availability of oxygen and/or the elimination of toxic materials.

This study showed that the factor responsible for preventing the M. tenthredinis eggs from developing and hatching in the sawfly hosts does not kill the parasite eggs outright but only inhibits growth and differentiation of the parasite embryos. The factor responsible may be the phagocytic capsule or it may perhaps be an inhibitory chemical substance produced either by the phagocytic cells of the capsule or by some other cells of the larval host.

Since the normal incubation period of M. tenthredinis is about seven to ten days, the fact that viable M. tenthredinis embryos are still present in the sawfly larvae seven months after the eggs were deposited suggests that the mechanism inhibiting the development of the M. tenthredinis embryos is similar to that which inhibits

the development of insects in diapause. The quiescent state of the M. tenthredinis embryo, however, probably cannot be considered to be a true state of diapause. As soon as favorable conditions replace the unfavorable ones, growth and development of the M. tenthredinis embryo resumes. Diapause is considered to be a spontaneous arrest of activity not quickly terminated, even though environmental conditions become optimum for development (93).

Miscellaneous observations.

Some evidence was obtained showing that in some instances encapsulated M. tenthredinis embryos develop and hatch out within host larvae. In one host larva dissected 29 days after parasitization, two living first-instar parasite larvae were found and beside them were two empty chorions from which parasite larvae had emerged, both of which were surrounded by thick capsules. In the vast majority of cases where first-instar larvae hatched within host larvae, however, the empty chorions were found to be unencapsulated.

Some evidence was found which indicated that possibly the small first-instar parasite larvae were less resistant to phagocytic digestion than were the parasite embryos within the chorions. In four cases empty unencapsulated chorions were found from which parasite larvae

had apparently emerged. The parasite larvae themselves, however, were nowhere to be found within these host larvae, even though a painstaking search of the internal contents of each host was made. The small parasite larvae may have been completely devoured by phagocytes. No empty phagocytic cysts or empty first-instar larval integuments were found in these hosts. This phenomenon probably occurred more frequently than was observed since empty chorions of M. tenthredinis eggs were very difficult to find.

Where first-instar parasite larvae were found in larch sawfly larvae during this study, in no case were they found to be encapsulated and this was true for both living and dead first-instar parasite larvae.

2. Relation Between the Time Required for the Eggs of M. tenthredinis to Hatch in Ringer's Solution and the Presence or Absence of Capsules Around These Eggs.

In a number of cases during the experiment discussed above the M. tenthredinis eggs removed from groups of parasitized sawfly larvae were split up into two or three approximately equal lots and these lots were subsequently treated in different ways. Half of the encapsulated eggs found were transferred to a separate dish of Ringer's invertebrate solution without prior treatment.

The capsules around the other half of these encapsulated eggs were carefully removed and the resulting capsule-free eggs were placed in Ringer's solution. Unencapsulated eggs found in the sawfly hosts on dissection were sometimes kept separate and placed in a separate dish of Ringer's solution. At other times the unencapsulated eggs found were included with the eggs from which the capsules had been removed. The results of part of this study are shown graphically in Figures 7, 8, 9, 10, and 11.

In a number of cases, insufficient data were obtained to plot the results graphically. These data are presented in Table XI.

In most cases a higher percentage of the eggs from which the capsules were not removed hatched out eventually than of the eggs from which the capsules were removed. This was probably due to injuries sustained by the latter groups of eggs during removal of the capsules.

Conclusions.

(a) M. tenthredinis eggs from which the capsules are removed develop and hatch out more rapidly after transfer to Ringer's invertebrate solution than do eggs from which the capsules are not removed.

(b) Unencapsulated M. tenthredinis eggs found within

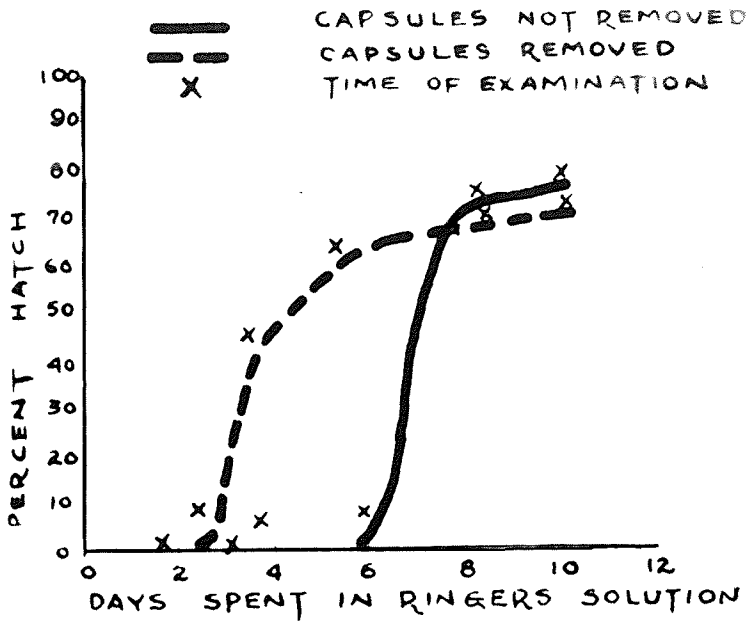
Figure 7.

The Relation Between the Rate of Hatch of 25 Encapsulated M. tenthredinis Eggs from which the Capsules were not Removed (Dish #15 in Table IV) and 30 Eggs from some of which the Capsules were Removed (Dish #16 in Table IV).

Note: The latter group of 30 eggs included 9 eggs which were found to be unencapsulated at dissection.

Figure 8.

The Relation Between the Rate of Hatch of 11 Encapsulated M. tenthredinis Eggs from which the Capsules were not Removed (Dish #56 in Table V) and 12 Encapsulated Eggs from which the Capsules were Removed (Dish #55 in Table V).


CAPSULES NOT REMOVED
CAPSULES REMOVED
TIME OF EXAMINATION
PERCENT HATCH
0
10
20
30
40
50
60
70
80
90
100
0
2
4
6
8
10
12
DAYS SPENT IN RINGERS SOLUTION

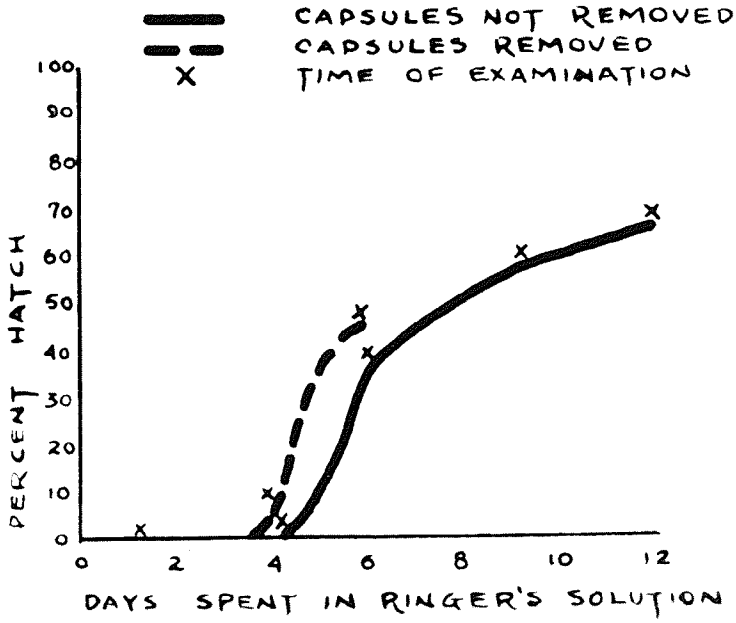

CAPSULES NOT REMOVED
CAPSULES REMOVED
TIME OF EXAMINATION
PERCENT HATCH
0
10
20
30
40
50
60
70
80
90
100
0
2
4
6
8
10
12
DAYS SPENT IN RINGER'S SOLUTION

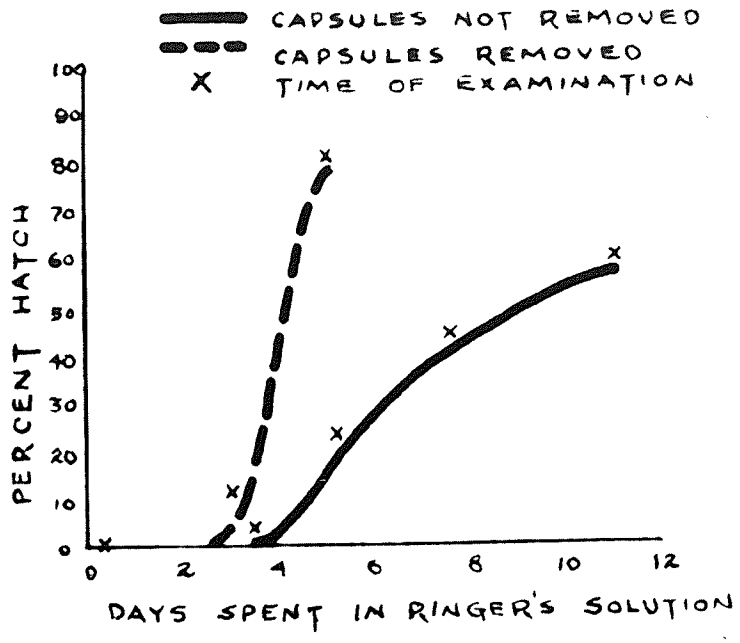
Figure 9.

The Relation Between the Rate of Hatch of 14 Encapsulated M. tenthredinis Eggs from which the Capsules were not Removed (Dish #106 in Table VI) and 14 Encapsulated Eggs from which the Capsules were Removed (Dish #107 in Table VI).

Figure 10.

The Relation Between the Rate of Hatch of 69 Encapsulated M. tenthredinis Eggs from which the Capsules were not Removed (Dish #153 in Table VII) and 74 Eggs from some of which the Capsules were Removed (Dish #154 in Table VII).

Note: The latter group of 74 eggs included 25 eggs which were found to be unencapsulated at dissection.


CAPSULES NOT REMOVED
CAPSULES REMOVED
X TIME OF EXAMINATION
PERCENT HATCH
0
10
20
30
40
50
60
70
80
90
100
0
2
4
6
8
10
12
DAYS SPENT IN RINGER'S SOLUTION

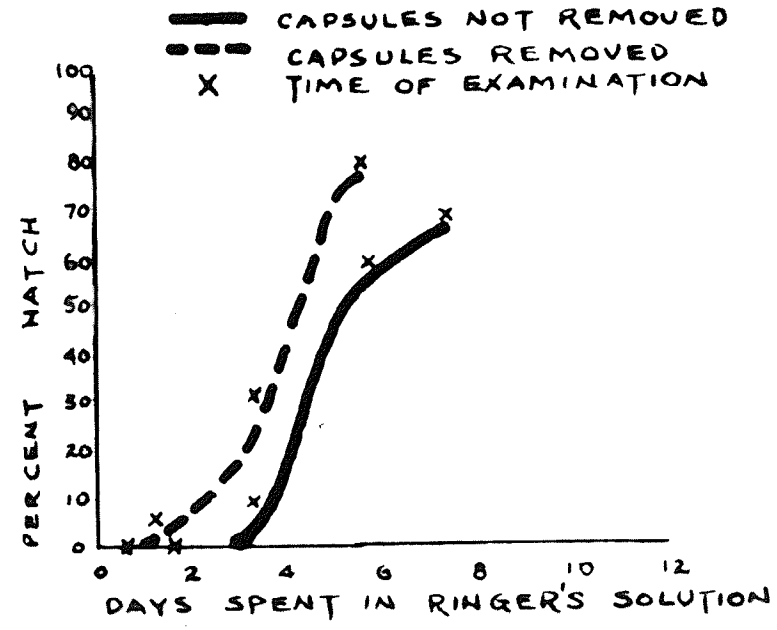

CAPSULES NOT REMOVED
CAPSULES REMOVED
X TIME OF EXAMINATION
PERCENT HATCH
0
10
20
30
40
50
60
70
80
90
100
0
2
4
6
8
10
12
DAYS SPENT IN RINGER'S SOLUTION

Figure 11.

The Relation Between the Rate of Hatch of 42 Encapsulated M. tenthredinis Eggs from which the Capsules were not Removed (Dish #157 in Table VII), 45 Encapsulated Eggs from which the Capsules were Removed (Dish #158 in Table VII) and 46 Eggs which were Found to be Unencapsulated at Dissection (Dish #156 in Table VII).

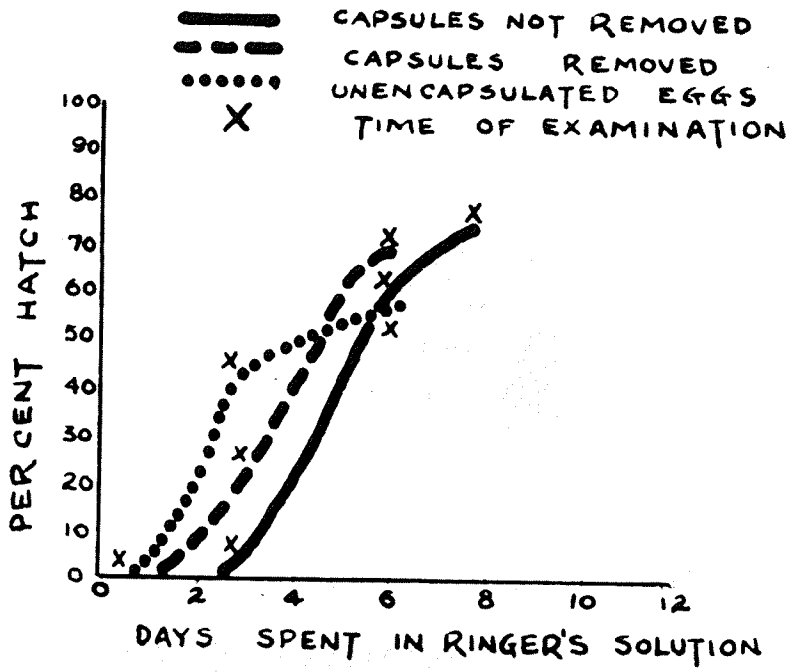

CAPSULES NOT REMOVED
CAPSULES REMOVED
UNENCAPSULATED EGGS
TIME OF EXAMINATION
PER CENT HATCH
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
DAYS SPENT IN RINGER'S SOLUTION

TABLE XI

Relation Between the Time Required for the Eggs of *M. tenthredinis* to Hatch in Ringer's Solution and the Presence or Absence of Capsules Around These Eggs.

				Percent Hatch		
Days spent by eggs in Ringer's solution =				4.0	5.6	12.6
Dish #	No. of eggs treated	Condition of eggs when dissected out	Treatment of eggs			
19	19	all encapsulated	None	0	0	32
20	19	all encapsulated	Capsules removed	0	5	5
Days spent by eggs in Ringer's solution =				0.9	7.3	
Dish #	No. of eggs treated	Condition of eggs when dissected out	Treatment of eggs			
108	23	all encapsulated	None	0	17	
109	21	all encapsulated	Capsules removed	0	19	
Days spent by eggs in Ringer's solution =				4.6	11.6	
Dish #	No. of eggs treated	Condition of eggs when dissected out	Treatment of eggs			
110	21	all encapsulated	None	0	24	
111	21	all encapsulated	Capsules removed	0	14	
Days spent by eggs in Ringer's solution =				0.7	7.1	
Dish #	No. of eggs treated	Condition of eggs when dissected out	Treatment of eggs			
160	12	all encapsulated	None	0	33	
159	10	all encapsulated	Capsules removed	0	50	

the sawfly larvae develop and hatch out more rapidly after transfer to Ringer's invertebrate solution than do eggs from which the capsules are removed before placing them in the solution.

3. Unencapsulated Eggs.

Discussion.

In Section 1 it was stated that more unencapsulated eggs were found in "unhealthy" host larvae than in healthy host larvae. Most of the unencapsulated eggs were found either in host larvae dissected less than ten days after parasitization or in host larvae that contained living M. tenthredinis larvae. However, unencapsulated eggs were found in apparently healthy host larvae, in which no living M. tenthredinis larvae were present, 15.5 days after parasitization. In one case a group of these unencapsulated eggs removed from sawfly larvae 13.2 days after parasitization was placed in a separate dish (#156) of Ringer's solution and 59 percent of them hatched out. This showed that unencapsulated eggs found in host larvae are not all dead.

Since the normal incubation period of M. tenthredinis eggs is approximately seven to ten days it is difficult to explain why these unencapsulated eggs had not yet

hatched 15 days after parasitization. It was the presence of just such unencapsulated, unhatched parasite eggs discovered by Bess (5) during his studies on the immunity of mealybugs to insect parasites that caused him to conclude that "phagocytosis is not a primary factor in immunity but independent of it."

The presence of these unencapsulated, unhatched parasite eggs in larch sawfly larvae appears to discredit the theory that the phagocytic capsules inhibit the development of M. tenthredinis embryos and seems to add support to the hypothesis that a chemical substance produced by the host inhibits the development of M. tenthredinis embryos in larch sawfly larvae.

It may be, however, that capsules had formed about these unencapsulated eggs and had inhibited development, and then the cells forming the capsule may have dispersed shortly before the larvae were dissected, possibly because they were attracted by a stronger stimulus elsewhere in the host. The possibility remains, nevertheless, that encapsulation may not be the only, or even the most important, factor in the immunity of the larch sawfly to M. tenthredinis.

4. Relation Between the Time Spent by M. tenthredinis Eggs in Larch Sawfly Larvae and the Time Required for These Eggs to Hatch in Ringer's Solution.

After M. tenthredinis eggs were placed in the Ringer's invertebrate solution, as described in Section 1 of this study, each dish was examined at intervals and the numbers of hatched eggs found were recorded. This was continued until all the parasite eggs and larvae in each dish were dead. The average time required for each group of eggs to hatch was then determined.

Results and Discussion.

The data obtained are presented in summary form in Table XII. It is seen that the average time required for the eggs to hatch in Ringer's solution at first decreased as the time spent by the eggs in sawfly larvae increased. This is because the minimum time required for a M. tenthredinis egg to hatch is approximately seven days and, therefore, the total time spent by a parasite egg in both the host larva and the Ringer's solution must be at least seven days. The shortest average time of hatching in Ringer's solution was required by those eggs that spent ten to fourteen days in the sawfly larvae. As the time spent by the eggs in the sawfly larvae increased

TABLE XII

Relation Between Time Spent by *M. tenthredinis* Eggs in Larch Sawfly Larvae and Time Required for These Eggs to Hatch in Ringer's Solution.

Time (in days) spent by *M. tenthredinis* eggs in sawfly larvae from parasitization to dissection	Average time (in days) required for eggs to hatch in Ringer's invertebrate solution
0 to 1	6.1
1 to 2	5.2
2 to 3	4.6
3 to 5	4.3
6 to 10	4.0
10 to 14	3.8
14 to 18	4.6
18 to 24	5.9
29	7.
156	13.
217	17.

over fourteen days, the average period required for these eggs to hatch in Ringer's solution became longer.

Embryonic development of M. tenthredinis eggs apparently began shortly after the eggs were deposited into the sawfly larvae and continued for a period of three to four days. Then embryonic development was seemingly abruptly inhibited by some factor, possibly the phagocytic capsules, and no further growth and differentiation occurred until the inhibiting factor was removed or ceased to function.

Visual observations indicated that growth in all of the M. tenthredinis embryos in the host larvae was inhibited at the same stage of embryonic development. Further evidence to support this is the fact that those eggs which had remained in the host from three to eighteen days all took approximately four days to hatch.

The data indicate that as the parasite embryos spend an increasingly longer time over eighteen days in the state of quiescence caused by the inhibitory factor, their ability to develop rapidly after the removal of the inhibitory factor decreases proportionately. Those eggs which had spent over eighteen days in the host larvae required from six to seventeen days to hatch in the Ringer's solution depending on the age of the embryos.

D. Health of the Larch Sawfly Larvae in Relation to Their Immunity to *M. tenthredinis*.

While carrying out the study described in Section C, it was found that a few of the groups of parasitized larvae dissected were "unhealthy". This "unhealthiness" or "sickliness" was often caused by failure to provide suitable cocooning media, such as moist moss, for the full-grown larvae. After about two to three days in the absence of moist moss the material in the silk glands of these larvae began to harden and turn a purplish-brown color. Sometimes the "unhealthiness" of the host larvae was due to partial starvation. In other cases the "unhealthiness" may have been due to excessive parasitization by *M. tenthredinis* or disease.

The eggs placed in dish #105 (see Table IV) were all removed from "unhealthy" sawfly larvae and a considerable number of the eggs placed in dishes #104, 155, 156, 157 and 158 (see Tables VI and VII) were removed from "unhealthy" sawfly larvae.

Results and Discussion.

Forty two of these "unhealthy" sawfly larvae were dissected between nine and thirteen days after parasiti-

zation. It was found that at least one egg hatched in 15 or 36 percent of these "unhealthy" host larvae. This percentage is high compared to only 6 percent of the healthy larvae that were found to contain at least one parasite larva.

The 15 "sickly" host larvae that contained at least one parasite larva contained a total of 71 M. tenthredinis eggs and 51 M. tenthredinis larvae. Thus of the original 122 eggs deposited in these host larvae, 42 percent hatched out. This percentage would probably have been higher were it not for the fact that the first parasite larvae to hatch apparently kill in some way, other parasite larvae shortly after the latter emerge from the egg chorions and also, seemingly, parasite embryos within egg chorions.

All 71 of the M. tenthredinis eggs found in the 15 "unhealthy" sawfly larvae in which at least one parasite larva was found were unencapsulated. Within the other 27 of the 42 "unhealthy" host larvae in which no parasite larvae were found, 103 M. tenthredinis eggs were found. Of these, 65 were unencapsulated, 32 were enclosed in relatively thin capsules and 6 were enclosed in capsules of normal thickness. In other words, 37 percent of these 103 eggs were encapsulated as compared with 80 to 100

percent encapsulated eggs in a group of 32 healthy larvae dissected at the same time after parasitization. Thus the encapsulation of 37 percent of the parasite eggs found in the "unhealthy" host larvae appears to be considerably lower than would be expected had these larvae remained healthy.

Conclusions.

Experimental evidence obtained shows (a) that fewer M. tenthredinis eggs hatch in healthy host larvae, and (b) that in "unhealthy" larvae fewer M. tenthredinis eggs are encapsulated and where capsules are formed they are usually thinner than in healthy larvae. This indicates that the health of the larch sawfly is a factor in its immunity to M. tenthredinis.

E. Relation Between the Number of Eggs Deposited in Individual Sawfly Larvae and the Hatch of These Eggs.

The data collected in this experiment were analyzed to test the hypothesis that those host larvae which were excessively parasitized became "unhealthy" due to excessive parasitization. It was postulated that this "unhealthiness" would be reflected in a greater hatch of

M. tenthredinis eggs in these host larvae. This analysis is presented in Table XIII. These observations were on a relatively small scale.

Discussion and Conclusions.

There was no definite indication that host larvae which were excessively parasitized were less effective in inhibiting the development of M. tenthredinis than were the lightly parasitized ones. More extensive observations, however, especially on host larvae containing six or more parasite eggs, might show a significant trend in this respect.

The data showed that in heavily-parasitized sawfly larvae in which at least one parasite egg hatched, there were more larvae per host in unhealthy hosts than in healthy ones.

F. Relation Between Temperature and the Immunity of the Larch Sawfly to M. tenthredinis

1. Field Studies

Dissections of larch sawfly cocoons collected from a number of areas in Manitoba since 1945 reveal that the percent hatch of parasite eggs has followed a fairly

TABLE XIII

Hatch of M. tenthredinis Eggs in Relation to the Number of Eggs Deposited in Individual Sawfly Larvae.

No. of eggs deposited per sawfly larva	Healthy host larvae			Unhealthy host larvae		
	No. of sawfly larvae	Percent of hosts containing at least one M. tenthredinis larva	Average No. of M. tenthredinis larvae per host containing at least one M. tenthredinis larva	No. of sawfly larvae	Percent of hosts containing at least one M. tenthredinis larva	Average No. of M. tenthredinis larvae per host containing at least one M. tenthredinis larva
1	56	1.8	1.0	9	33.3	1.0
2	50	4.0	1.0	7	0.0	-
3	47	4.3	1.0	7	14.3	1.0
4	27	18.5	1.25	5	20.0	1.0
5	22	9.1	1.0	6	33.3	1.0
6	13	0.0	-	8	0.0	-
7	8	12.5	1.0	5	60.0	4.7
8	8	12.5	1.0	5	40.0	2.5
9	5	20.0	1.0	3	33.3	3.0
10	5	0.0	-			
11	3	0.0	-	3	33.3	3.0
12	1	100.0	1.0	2	50.0	5.0

consistent trend in each area. It was relatively higher in the odd-numbered years and lower in the even-numbered years. A cursory study of the July and August mean temperatures for these years in a few areas in Manitoba showed that they fluctuated in the same way as the percent hatch of the M. tenthredinis eggs. An attempt was made to correlate high temperatures (arbitrarily chosen as the sum of those portions of the daily maximum temperatures that were over 80° F. from July 16th to Aug. 20th inclusive), and low temperatures (the sum of those portions of the daily minimum temperatures under 50°F. for the same period), with the percent hatch of M. tenthredinis eggs from 1945 to 1951 inclusive. A positive correlation was obtained using the high temperatures and this is shown in Figure 12.

The temperature values for "total degrees" plotted for each year in each of the six graphs were computed from weather station records closest to the locations at which the percent hatch of M. tenthredinis eggs was determined. Since complete records from 1945 to 1951 were not available for all of the closest stations, in some cases data from a number of stations were used.

The six locations used, at which the percent hatch of M. tenthredinis eggs was determined, are the same as

those for which the total parasitism and effective parasitism by the parasite were compared in Figures 1 and 2.

The first three locations, A, B, and C, are in the Riding Mountain National Park, Manitoba. They are:

A - Mile 7 Norgate Road

B - Mile 145 Dauphin Highway

C - Mile 32 "Prisoner of War Camp" Road.

The temperatures correlated with the percent hatch for each of these three locations were taken at Minnedosa from 1945 to 1948 and at Neepawa from 1949 to 1951. The other areas are:

D - Seddon's Corner, Manitoba; temperatures taken at Kenora from 1945 to 1947, at Rennie in 1948 and 1949 and at Seven Sisters in 1950 and 1951.

E - Riverton, Manitoba; temperatures taken at Gimli, Manitoba.

F - Prince Albert, Saskatchewan; temperatures taken at Prince Albert, Saskatchewan.

Discussion and Conclusions.

The correlation in trend between the fluctuations in percent hatch and high temperatures is apparently

Figure 12.

The Relation Between the Percent Hatch of *M. tenthredinis* Eggs and High Temperatures (arbitrarily chosen as the sum of those portions of the daily maximum temperatures that were over 80°F. from July 16 to Aug. 20 inclusive) at Five Locations in Manitoba and One in Saskatchewan from 1945 to 1951 inclusive.

Location A - Mile 7 Norgate Road, R.M.N.P.

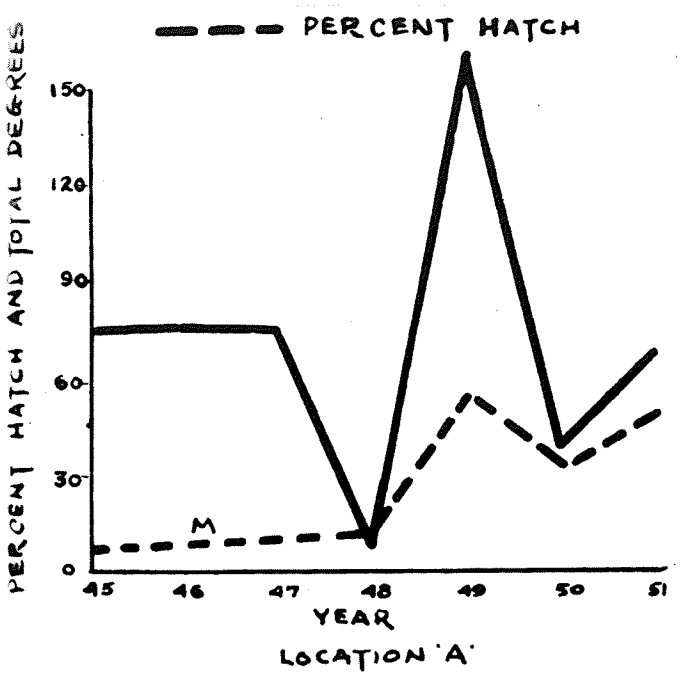
Location B - Mile 145 Dauphin Highway, R.M.N.P.

Location C - Mile 32 "Prisoner of War Camp" Road, R.M.N.P.

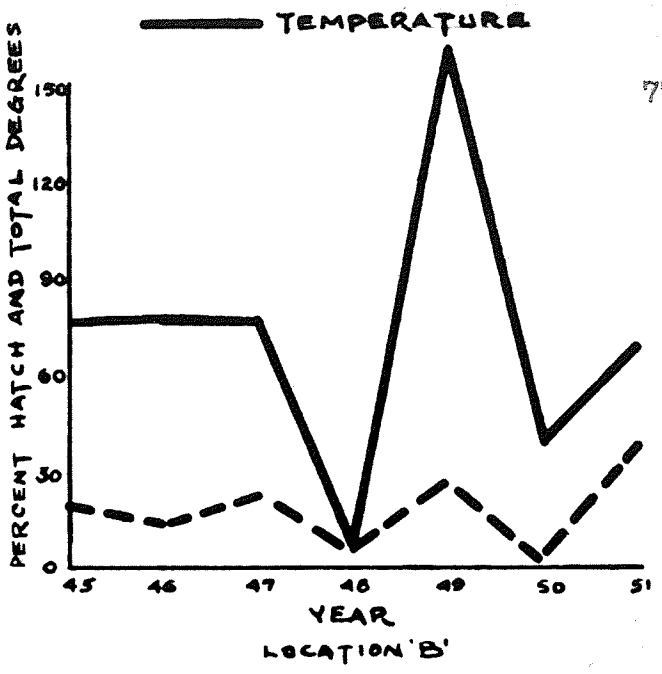
Location D - Seddon's Corner, Manitoba.

Location E - Riverton, Manitoba.

Location F - Prince Albert, Saskatchewan.



LOCATION 'A'



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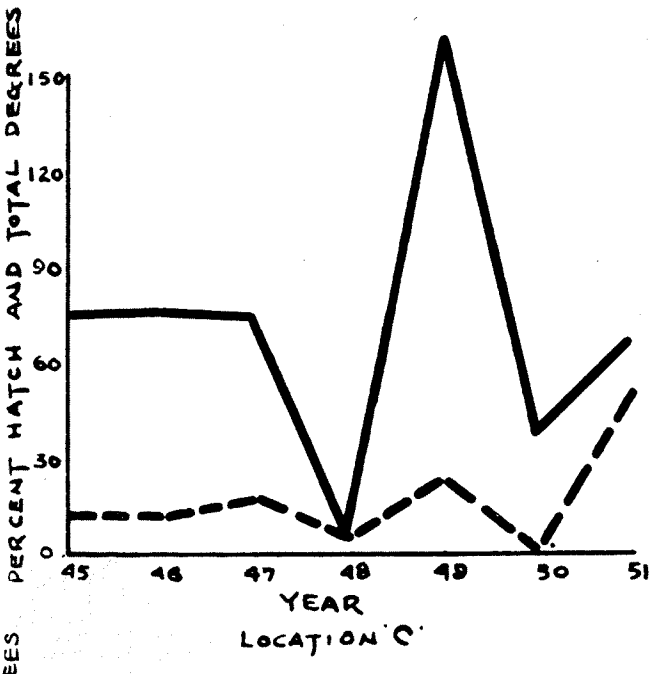
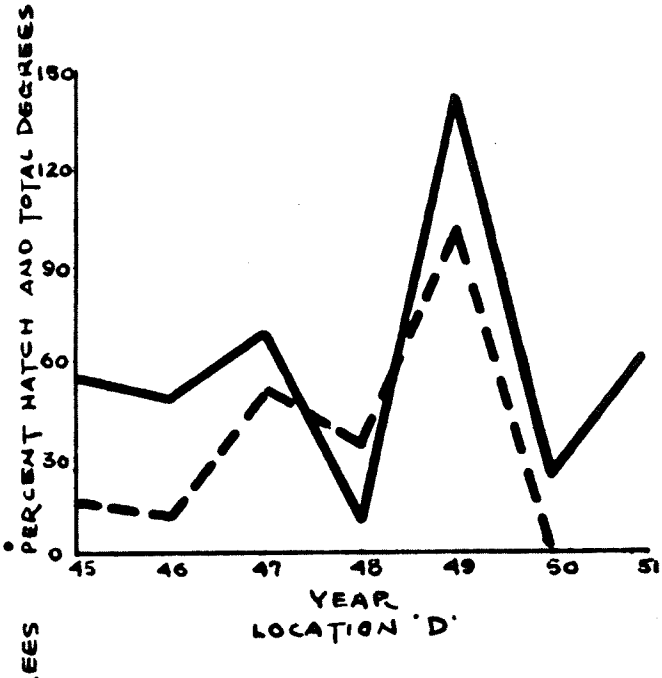
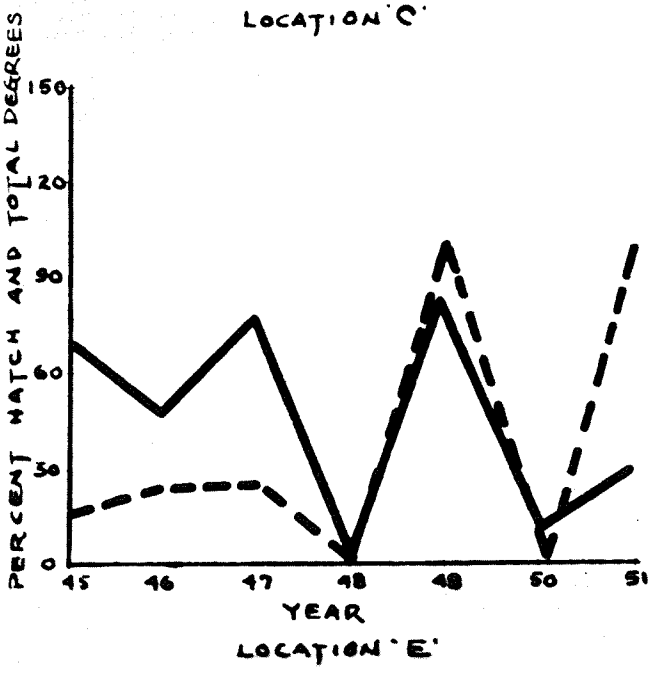


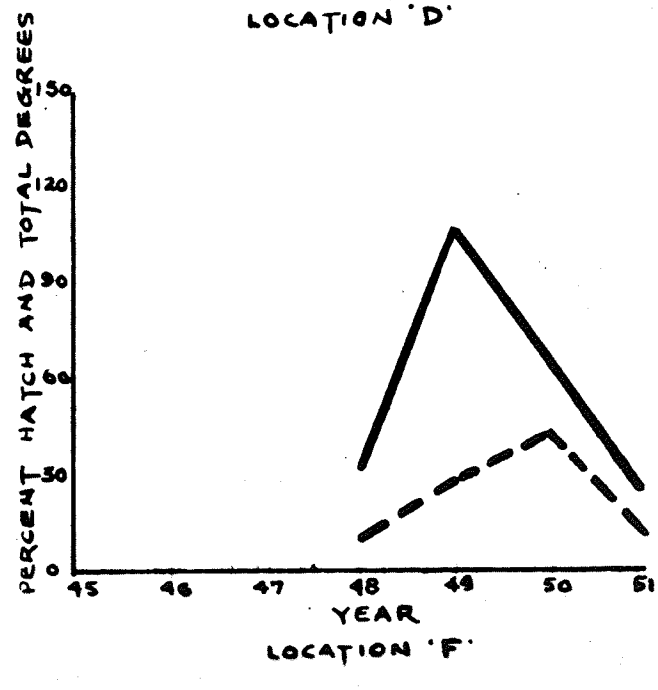
LOCATION 'C'



LOCATION 'D'



LOCATION 'E'



LOCATION 'F'

fairly consistent. The correlation coefficients and the significance of these were computed for each location. These are, however, not as impressive as are the graphs. They are:

Location.....	A	B	C	D	E	F
Correlation coefficient...	0.56	0.57	0.37	0.85	0.40	0.68
"t" value.....	1.51	1.70	0.98	3.61	1.07	1.61
"t" value at the five percent level.....	2.57	2.45	2.45	2.57	2.45	3.18

The correlation for location D is the only one that is highly significant. It is perhaps noteworthy, nevertheless, that even this one high correlation was obtained since there are a number of factors which enter the picture that could possibly throw the correlation out considerably. These are: (a) the sawfly cocoons may not have been collected at random, (b) the figures obtained for the percent hatch of the parasite eggs would probably have been more accurate than those graphed if a larger number of host larvae had been dissected (approximately 100 were dissected each year from each location). This is true especially of those cases where the total parasitism by M. tenthredinis was less than five percent (e.g., at Riverton from 1948 to 1951), (c) there may have been an experimental error in dissections, (d) very low

temperatures and other factors may also have affected the percent hatch, and (e) the actual temperatures of the parasitized larch sawfly larvae may have differed considerably, depending on cloud conditions, etc., from the air temperatures used, and, possibly, part of the effect of high temperature may act after the host larvae have cocooned (the difference between ground and air temperatures may enter the picture).

2. Laboratory Studies

In 1951 parasitized larch sawfly larvae collected in Manitoba were split into different lots shortly after they were removed from the parasitization cage, and these lots were reared at different temperatures. Two lots of larvae were reared in an incubator kept at approximately 70°F.,; these gave percent hatches of 33 and 40. These figures represent the percent of the total number of larvae parasitized that contained a living M. tenthredinis larva and not the actual percent of the total number of parasite eggs deposited that hatched. Two lots of larvae were reared in a refrigerator at a temperature of approximately 40°F., and these gave percent hatches of 69 and 78. These valves are considerably higher than those obtained from the dissection of field-collected larvae.

However, a control lot of larvae reared in the insectary during this study gave a hatch of 35 percent. The mean temperature in this insectary during the three week period following parasitization was 62° F. Another control lot of larvae was attacked by a fungous disease and destroyed.

Conclusion.

It is felt that further work on this problem should be carried out before any definite conclusions can be made concerning the effect of the rearing temperature following parasitization in relation to the immunity of the larch sawfly to M. tenthredinis.

Parasitized larvae should be reared at five or six different constant temperatures following parasitization, e.g. 35°F., 40°F., 50°F., 65°F., 75°F., and 85°F. Fluctuating temperatures and three- to four-day periods of exposure to high temperatures (85°F. to 90°F.) following parasitization might yield interesting results.

G. The Possible Significance of the Melanin Formation in the Inner Layer of the Capsule Formed Around the M. tenthredinis Egg.

Review of the Literature.

Eckstein (22) studied the encapsulation of tachinids and ichneumonids in larvae of the sawfly Lyda stellata. He stated that his investigations proved that the melanin formation around the parasites of Lyda is a defense reaction.

He believed that melanin substances in insects originate through the action of tyrosinase on tyrosine. Tyrosine, he stated, is a separation product of proteins and therefore the formation of melanin substances occurs when protein is broken down to tyrosine in the presence of tyrosinase. Thorpe (85) who studied the sheath formed around Encyrtus infelix within Sassetia hemisphaerica postulated a similar mechanism for the production of melanin in the sheath.

Bess (5) used the term "melanization" to refer to the darkening and hardening of the cells or material, or both, immediately surrounding the parasite eggs, and the larvae themselves. Parasites surrounded by a thin melanized sheath were considered by Bess to be phagocytized, although cells were not always identified in the sheaths. He stated that such sheaths may possibly be composed largely of melanin formed in the absence of cells, but it appeared more likely to him that they are composed of

melanized cells attracted to the parasite, as well as of chemical substances present.

Bess found that the capsules formed around the same species of parasite in different host were melanized more rapidly in some hosts than in others. This seemed, in some cases, to be connected with the resistance of the hosts to the parasites for where the capsules were melanized most rapidly no parasite eggs were observed to hatch. The encapsulated active parasite larvae lived longer within those capsules that were melanized slowly. Bess suggests that this may be because the melanized layer is more impervious to nutritives and certain gases essential to the life of the parasite than is the cyst of living cells. Bess concluded as follows:

> Superficially, it appears that the rapidity of melanization may be a factor in causing the death of parasites in certain hosts. However, we are not certain whether melanization is a primary defense reaction or not.

Experimental studies.

Object.

To determine (a) whether or not M. tenthredinis embryos found encapsulated in brown (melanized) capsules

would develop and hatch out after transfer from larch sawfly larvae to Ringer's solution and, if they did so, (b) to compare the percentage hatch of those embryos enveloped by white capsules with those enveloped by brown capsules.

Method.

During the period December 6th, 1951 to January 11th, 1952, M. tenthredinis eggs removed by the Forest Biology Rangers, Winnipeg, during dissection of larch sawfly larvae collected in various areas in Manitoba and Saskatchewan were, each day, split up into groups depending on whether the capsules around these eggs were white, light brown or dark brown. These groups were placed in separate dishes of Ringer's solution and observed at intervals until hatching was completed or they were all dead.

Results.

The data obtained are presented in Table XIV.

Discussion and Conclusions.

It is felt that this experiment was carried out on too small a scale to permit any definite conclusions but the indications are that a greater percentage of

TABLE XIV

Comparison of the Hatch of M. tenthredinis Eggs Enveloped by White Capsules with the Hatch of M. tenthredinis Eggs Enveloped by Brown Capsules.

Color of Capsule	No. of encapsulated embryos placed in Ringer's solution	No. that hatched out	Percent Hatch
White	195	36	18.5
Light brown	45	2	4.4
Dark brown	10	0	0.

embryos enveloped by white capsules are viable than are those enveloped by brown capsules.

The fact that two of the embryos enveloped by brown capsules hatched out showed that melanization does not necessarily begin after the parasite embryos have died from some other cause. Melanization of the capsules may be the factor or one of the factors responsible for the ultimate death of the parasite embryos.

H. Histology of the Capsule.

A brief attempt was made to section the capsules formed around M. tenthredinis eggs within larch sawfly

Figure 13.

Section of a Capsule, Chorion and Embryo Removed, Showing the Fibrous Nature of the Inner Layer.
(stained with Mallory's triple stain)

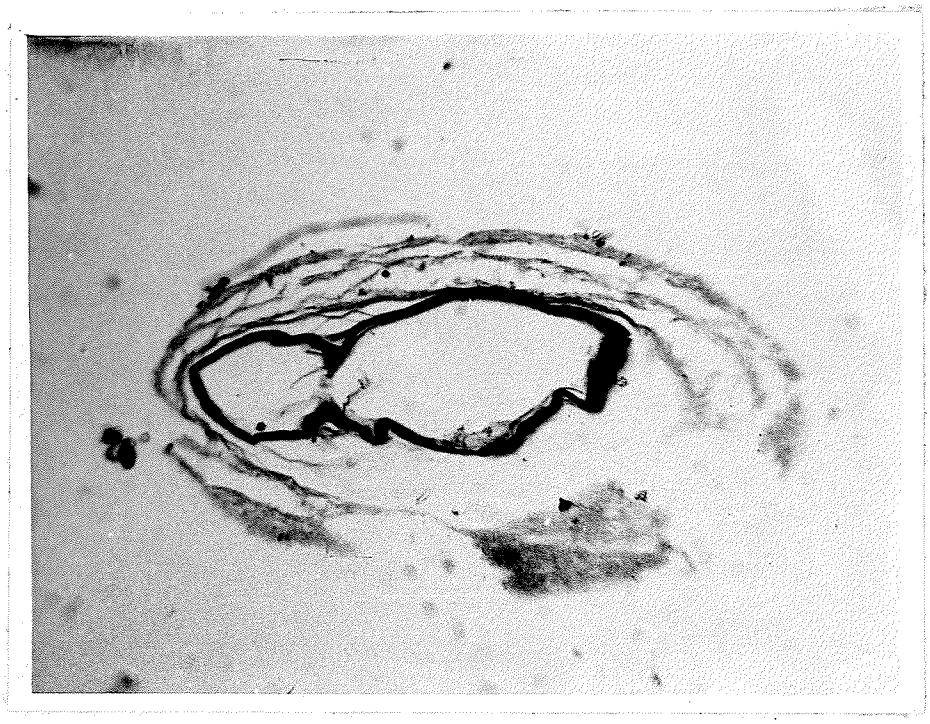


Figure 14.

Upper Row - A M. tenthredinis Egg Teased from its Capsule (21X).

Left - The outer cellular layer.

Center - The inner fibrous-like, apparently non-cellular layer.

Right - The embryo within its chorion. This embryo was probably viable before it was fixed in formalin.

Lower Row - A M. tenthredinis Egg Teased from its Capsule (21X). This capsule was melanized when removed from the host and the embryo was dead.

Figure 15.

Upper Left - A White Capsule Around M. tenthredinis Embryo (21X).

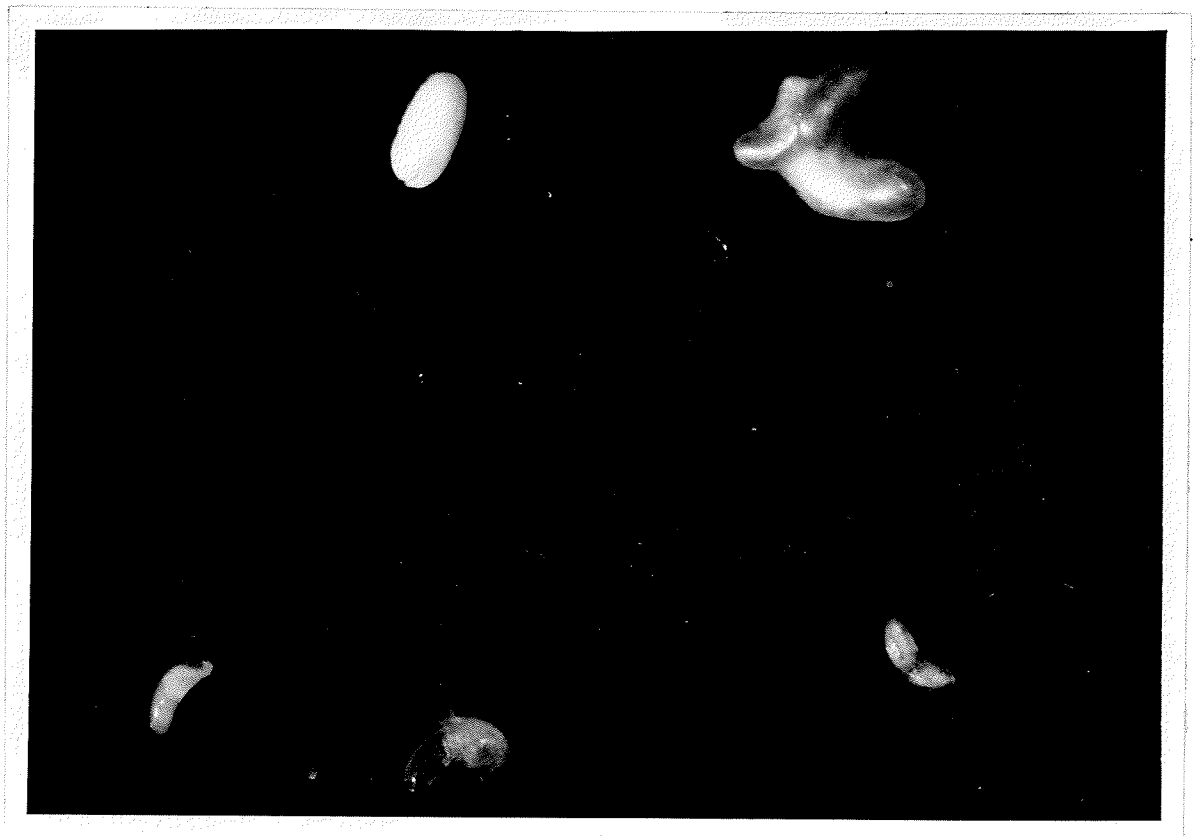
Upper Right - A Cluster of Brown Melanized Capsules around Three or Four M. tenthredinis Eggs (21X).

Lower Row (21X) -

Left - A dead M. tenthredinis embryo within its chorion.

Center - An empty melanized capsule showing the transparent, thin layer that is laid down adjacent to the chorion.

Right - A dead partly melanized M. tenthredinis embryo within its chorion.



larvae but the sections were not of a sufficiently high quality to permit an accurate description of the histological structure of the capsule. It is felt, however, that good sections could be obtained without too much difficulty. Sectioning should be done at different stages during the life of the capsule.

The first stage in the process of the encapsulation of M. tenthredinis eggs within larch sawfly larvae is, seemingly, the gathering of living cells, probably phagocytes, at the surface of the egg. Early in the development of the capsule an apparently non-cellular substance seems to be laid down adjacent to the chorion of the parasite egg and within the outer covering of living cells. The cells adjacent to this non-cellular layer appear to be spindle-shaped whereas the cells of the outer layer of the capsule seem to be ovoid or round. The inner, apparently non-cellular, layer seems to be the one in which melanization first occurs. The earliest melanized capsules recorded were found 12 days after parasitization in "unhealthy" host larvae. In most cases melanization of the capsules did not take place until at least one month after parasitization. The majority of the capsules removed from sawfly larvae in February or later were melanized, but some capsules remained non-

melanized for a considerable period. In one case, seven out of 35 capsules removed seven months after parasitization were non-melanized. Some of the host larvae dissected at this time contained both melanized and non-melanized capsules.

The histological structure of the capsules formed within the larch sawfly is approximately the same as that described by Bess (5) for the capsules formed in Pseudococcus gahani, and by Meyer (59, 60, 61) for the capsules formed in Pieris rapae. It is not like that described by Paillot (65) for the capsules formed in corn borer larvae. Paillot described two distinct layers; the outer composed of spindle-shaped flattened cells, and the inner of round cells with indistinct nuclei.

It is of interest to note that Lazarenko (46) considered that the connective tissue membranes enclosing the fat bodies, ovaries, basement membrane of epidermis, etc., in insects and also the capsules formed around parasites are all composed of phagocytes which gather at the surface and fuse into a syncytium. Lartschenko (44) was of a similar opinion except that he believed the cells responsible were "mesenchyme" cells rather than phagocytes because of the presence of vacuoles in these cells. Thorpe (85) was of the opinion that Lartschenko's

distinction between phagocytic and mesenchyme cells is not completely satisfactory and that the interpretation of Lazarenko is preferable.

I Blood Count Studies on Larch Sawfly Larvae.

It was hoped that during the summer of 1951 a study of the changes in blood count of larch sawfly larvae following parasitization could be carried out. By the time the study was begun, however, the emergence of M. tenthredinis adults was almost over and therefore a study of the changes in blood count following the introduction of thorns rather than of parasite eggs was carried out.

Tips of rose thorns were introduced into larch sawfly larvae from both Manitoba and British Columbia. Each tip was introduced through one of the host's prolegs. Using tweezers, the tip of the thorn was forced into a proleg to a distance of one to two mm. and this tip was then cut off at the integument using scissors. The thorn tip was then pushed into the body cavity of the sawfly larva using a slender insect pin.

Results and Discussion.

It was found that the sawfly larvae from both

Manitoba and British Columbia possessed the ability to deposit capsules about these thorn tips. Some larvae from Manitoba were dissected two to six days after the introduction of the thorns. Typical capsules were not found around the thorns removed from these larvae. Small bits of tissue were often found adhering to these thorns. The thorns removed from larch sawfly larvae from both Manitoba and British Columbia six to 19 days following introduction were usually surrounded by typical capsules.

From the period August 13 to September 22, 93 blood counts were made on larch sawfly larvae from Manitoba and British Columbia. The counts were made using a Levy counting chamber with the improved double Nebauer ruling. All counts were made on fifth-instar sawfly larvae. The following tentative results were obtained:

Mean count of 16 untreated feeding larvae from Manitoba = 7,051 cells per cubic mm. (Range = 3,940 to 14,307).

Mean count of 9 untreated feeding larvae from British Columbia = 8,080 cells per cubic mm. (Range = 4,440 to 15,300).

Blood counts were made on larvae into which thorn tips had been introduced. These counts were made at various intervals following the introduction of these thorns. The results of these counts are given in Table XV.

TABLE XV

Relation Between Time Spent by Thorn Tips in Sawfly Larvae and the Blood Count of These Larvae.

No. of days after introduction of thorn	No. of counts made	Cells per cubic mm. (mean value)	Range of individual counts
2	2	13,476	12,675 - 14,278
3	4	17,715	11,125 - 21,620
5	1	14,440	-
6	1	35,231	-
7	2	30,837	21,375 - 40,300
9	1	42,825	-
10	4	18,269	10,200 - 34,040
17	1	11,700	-
18	4	11,181	6,420 - 19,225
19	1	8,025	-

The thickness of the capsules found around the thorns in relation to the blood count is shown in Table XVI.

TABLE XVI

Relation Between Thickness of Capsule and Blood Count in Four Sawfly Larvae Examined Ten Days after the Introduction of Thorn Tips.

Number	Cells per cubic mm.	Thickness of capsule
1	34,040	very thin
2	16,075	thin
3	12,760	medium-sized
4	10,200	relatively thick

Conclusions.

Larch sawfly larvae from both Manitoba and British Columbia possess the ability to deposit capsules about thorn tips introduced into their bodies.

The number of cells per cubic mm. of larch sawfly blood appears to increase to a maximum at about nine days after introduction and then to decline to about the normal concentration at about 20 days after introduction.

At ten days after the introduction of thorns the thinnest capsules were found on the thorns removed from the larvae having the highest concentration of cells.

J. Theoretical Considerations Arising out of and Pertaining to this Problem.

A possible method of proving that the capsules are the primary factor responsible for the death of the M. tenthredinis embryos within larch sawfly larvae might be found if a technique could be developed whereby the living cells of the outer layers of the capsule could be kept alive after the transfer of the encapsulated eggs from host larvae to a physiological solution.

In connection with the melanization of the capsules, a theory has appeared in the literature of late (79) which attempts to explain why it is that the normal insect does not blacken despite the simultaneous presence of tyrosine, oxygen, and enzyme in it. It is claimed that tyrosinase activity is prevented by the low oxidation-reduction potential of the blood. Dehydrogenase activity has been suggested to be responsible for the maintainance of the low potential. There is some evidence that the puparium-invoking hormone in Calliphora destroys the reducing power of dehydrogenases by binding their sulfhydryl (-SH) groups in the -S-S- form to the point where the oxidation products of tyrosinase can accumulate.

Experiments attempting to change the redox

potential of the capsules formed around M. tenthredinis eggs by the larch sawfly might be attempted, in vivo and in vitro, using such substances as methyl alcohol and glutathione. The effect of such substances on the rapidity of melanization of the capsule might be observed.

It was pointed out on pages 57 and 58 that the mechanism by which the growth and development of M. tenthredinis embryos is inhibited may be similar to that which inhibits the development of insects in diapause. C. M. Williams and co-workers (94) have recently found that in the diapausing Cecropia silkworm pupa and secretion of the "growth and differentiation" hormone terminates diapause. Williams considers that this hormone brings about repair of a biochemical defect in the diapausing tissues of the Cecropia silkworm pupa. This biochemical defect he believes to be the absence of the cytochrome mechanism. The possibility exists that the inhibition of embryonic development of M. tenthredinis eggs in larch sawfly larvae is due to a blocking of the cytochrome mechanism in the cells of the embryo. If this could be shown to be the case, the search for the explanation of the immunity mechanism in the larch sawfly would be narrowed down to a search for the specific factor that blocks the cytochrome system.

VI SUMMARY

M. tenthredinis larvae were found in third-, fourth-, and fifth-instar larch sawfly larvae. No obvious relation was found between the host instar attacked and the effective parasitism. M. tenthredinis larvae were found in host sawfly larvae throughout July and August. No obvious relation was found between the seasonal period of attack and the effective parasitism.

Embryonic development of the M. tenthredinis eggs began shortly after they were deposited into the host larvae and continued for a period of three to four days at which time growth appeared to be abruptly inhibited. The factor responsible for preventing the parasite eggs from developing and hatching in the host larvae did not kill the parasite eggs outright but only inhibited growth and development.

A proportion of the encapsulated M. tenthredinis eggs dissected out of larch sawfly larvae from Manitoba and Saskatchewan hatched out after they were transferred to Ringer's invertebrate solution. This was true whether or not the capsules were removed from these eggs before they were placed in the solution. The longer the encapsulated M. tenthredinis eggs remained in the host

larvae, the lower was the proportion of them that hatched after they were placed in Ringer's solution. Viable parasite embryos were found in host larvae seven months after the eggs were deposited. Observations indicate that none of the encapsulated parasite eggs removed from host larvae eight or more months after parasitization were viable.

The decreasing viability of the M. tenthredinis eggs may be due to a detrimental effect caused by the capsules but this was not definitely proved by this study. Unencapsulated parasite eggs were found in host larvae up to 15 days after parasitization. Since the normal incubation period of these eggs is approximately seven to ten days this evidence appears to discredit the theory that the phagocytic capsules are the sole factor responsible for the immunity of the larch sawfly to M. tenthredinis.

The average time required for the parasite eggs to hatch in Ringer's at first decreased as the time spent by the eggs in sawfly larvae increased because the first part of the parasite's embryonic development occurs in the host larva. As the time spent by the parasite embryos in the host increased over 14 days, the time required for the embryos to develop in Ringer's increased proportionately.

Encapsulation of the M. tenthredinis eggs began about the second day after oviposition. The proportion of encapsulated eggs found increased as the time spent by these eggs in the host larvae increased to about ten days at which time approximately 100 percent encapsulation occurred. Some evidence was obtained to indicate that, proportionately speaking, eggs were encapsulated more rapidly in fourth-instar larvae than in third-instar larvae, and more rapidly in third-instar larvae than in fifth-instar larvae. This evidence does not support the theory that the rate of capsule-formation increases during a moulting period. Parasite eggs, from which the capsules were removed, developed and hatched more rapidly after transfer to Ringer's than did eggs from which the capsules were not removed. Unencapsulated parasite eggs found within host larvae developed and hatched more rapidly after transfer to Ringer's than did the eggs from which the capsules were removed.

There was some evidence that more M. tenthredinis eggs hatched in unhealthy host larvae than in healthy host larvae. Evidence also indicated that fewer parasite eggs were encapsulated in unhealthy host larvae than in healthy host larvae. No definite indication was obtained

that host larvae which were excessively parasitized were less effective in inhibiting development of M. tenthredinis than were the lightly parasitized ones.

A fairly consistent yearly correlation in trend was obtained between high summer temperatures and the percent hatch of M. tenthredinis eggs for several locations in Manitoba and Saskatchewan; but inconclusive results were obtained in small-scale laboratory studies on the effect of rearing temperature following parasitization on the hatch of the parasite eggs.

There was some evidence that the melanization of the capsules formed around M. tenthredinis eggs did not necessarily begin after the parasite embryos died. The indications were that a greater percentage of the embryos found to be enveloped by non-melanized capsules were viable than were the embryos found to be enveloped by melanized capsules. Melanization of the capsules may have been an important factor in causing the actual death of the embryos.

Some larch sawfly of British Columbia origin were studied and 96 percent of these were found to be susceptible to M. tenthredinis stock from the Prairies and British Columbia. Furthermore, it was found that host material from Manitoba and Saskatchewan formed capsules

readily around parasite eggs deposited by M. tenthredinis from British Columbia. Of 53 parasitized larvae from British Columbia dissected, only two did not contain at least one living parasite larva and each of these two contained an encapsulated parasite egg. No other encapsulated eggs were found in host larvae from British Columbia. Sawfly larvae from both Manitoba and British Columbia were found to possess the ability to deposit capsules about thorn tips introduced into their bodies.

The number of cells per cubic mm. of larch sawfly blood was found to increase to a maximum at about nine days after the introduction of thorns. The cell concentration then declined to about normal at approximately 20 days after the introduction of thorns. At ten days after the introduction of thorns, the thinnest capsules were found on the thorns removed from larvae having the highest concentration of cells in the blood.

The results of this study do not support the theory that the immunity of the larch sawfly to M. tenthredinis is the result of an unsuitable food medium in the host since the parasites are killed before they begin to feed. Neither do they support the theory that the prime cause of immunity is the inability of the parasite to secrete substances that repel or paralyze the

phagocytes. The hypothesis that a successful parasite is so similar to its host in "physical being" that its presence is not resented (86, 77, 78) is not definitely refuted. The M. tenthredinis eggs probably offer identical stimuli, possibly chemotactic, to the phagocytes in both varieties of host and it may be that the phagocytes of the hosts from British Columbia are not able to respond to these stimuli whereas the hosts from Manitoba and Saskatchewan are able to do so.

The results obtained do not solve the problem as to whether the phagocytes actually attack and encapsulate living parasite eggs and larvae and are a primary cause of their death or whether they merely play a secondary role, accumulating about the parasite after the primary cause of immunity, possibly chemical, has acted upon the parasite. The writer feels that the evidence favours the former interpretation and that the capsules play a more important part than that of simply protecting the host against injury from weakened and dead parasite eggs and larvae.

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