BIONOMICS OF THE COMMON JUNE BEETLE, *PHYLLOPHAGA ANXIA* (LECONTE) (COLEOPTERA: SCARABAEIDAE), WITH PARTICULAR REFERENCE TO DISTRIBUTION, LIFE HISTORY AND NATURAL ENEMIES IN SOUTHERN QUEBEC

by

©

Kiok-Puan Lim

A thesis submitted to the Faculty of Graduate Studies and Research of McGill University in partial fulfilment of the requirements for the degree of Doctor of Philosophy

Department of Entomology,
McGill University,
Montreal, Quebec,
Canada.

August 1979

TO MY FAMILY

Suggested short title:

THE COMMON JUNE BEETLE IN SOUTHERN QUEBEC

KIOK-PUAN LIM

ABSTRACT

Ph.D. Kiok-Puan Lim Entomology

BIONOMICS OF THE COMMON JUNE BEETLE, PHYLLOPHAGA ANXIA (LECONTE) (COLEOPTERA: SCARABAEIDAE), WITH PARTICULAR REFERENCE TO DISTRIBUTION, LIFE HISTORY AND NATURAL ENEMIES IN SOUTHERN QUEBEC

The bionomics of *Phyllophaga anxia* (LeConte) were studied in southern Quebec during 1975, 1976, and 1977. Spring emergence of adults required approximately 176 degree-days accumulated above a base of 5° C. *Phyllophaga anxia* was the most numerous and widely distributed of the six species of *Phyllophaga* found. The life cycle of *P. anxia* in southern Quebec was determined. Overlapping of broods was found to occur in most of the study areas. The dispersion of *P. anxia* generally fitted the theoretical negative binomial distribution. Optimum sample sizes were calculated for population density estimation. Parasites, predators and pathogens of *P. anxia* were found in the field. A diplogasterid nematode was shown to cause mortality of *P. anxia* grubs in laboratory tests. A possible infection of white grubs by entomopoxvirus was studied. Of ten insecticides tested, two organophosphates, fonofos and WL 24073, showed highest contact toxicity for third instars by two different bioassay methods.

RESUME

Ph.D. Kiok-Puan Lim Entomologie

La bionomie de *Phyllophaga anxia* (LeConte) fut étudiée au Québec méridional en 1975, 1976 et 1977. L'émergence printanière des adultes requit approximativement 176 degré-jours accumulés au-dessus d'un seuil de 5° C. Six especes de *Phyllophaga* furent piégées; *P. anxia* fut capturé plus fréquemment que les autres especes. Le cycle vital de *P. anxia* a été élucidé pour le Québec méridional. Un Chevauchement des zones d'émergence fut observé dans la plupart des régions d'échantillonnage. La distribution spatiale de *P. anxia* cadrait généralement avec la loi binomiale négative. On calcula les tailles d'échantillons optimaux pour estimer la densité de population. Parasites, prédateurs et pathogènes de *P. anxia* furent récoltés sur le terrain. En essais de laboratoire, on démontra qu'un nématode diplogastéride cause la mort des larves de *P. anxia*. La possibilité d'une infection entomopoxvirale fut également examinée. Lors de tests biologiques sur dix insecticides, deux organophosphorés de contact, fonofos et WL 24073, s'avérerent les plus toxiques envers le troisième stade larvaire.

ACKNOWLEDGEMENTS

I wish to thank my research supervisors, Dr. W.N. Yule and Dr. R.K. Stewart, for their guidance, continued interest and encouragement during this research, and for the many invaluable suggestions made during the critical reviewing of the draft of this thesis; also to the late Dr. F.O. Morrison, who initiated and supervised the first year of this research project.

I would like to thank Dr. H.F. Howden, Department of Biology, Carleton University, Ottawa, Ontario, for the determination of _Phyllophaga_ species, and to Dr. J. McNamara, Biosystematics Research Institute (B.R.I.), Ottawa, Ontario, for the determination of _P. anxia_; and to Dr. C.C. Loan, Dr. L. Masner, Dr. A. Smetana, Dr. D.M. Wood, all of B.R.I., for the determination of the parasitic and predatory insects, and to Dr. C.T. Oseto, Department of Entomology, North Dakota State University, Fargo, North Dakota, U.S.A., for the identification of the mites; and to Dr. B.M. Arif and Dr. F.T. Bird, both of Insect Pathology Research Institute (now Forest Pest Management Institute), Sault Ste. Marie, Ontario, for their positive comments about the entomopoxvirus; Dr. R.H. Estey, Department of Plant Science, Macdonald Campus of McGill University, Ste. Anne de Bellevue, Quebec, for the determination of Diplogasteridae; Dr. D.M. MacLeod, Insect Pathology Research Institute, for the identification of the green muscardine fungus; Dr. G.O. Poinar, Jr. and Mr. G.M. Thomas, Division of Entomology and Parasitology, University of California, Berkeley, California, U.S.A., for the identification of the diplogasterid nematode and pathogens, respectively.

Thanks are also due to Dr. T.A. Angus, Insect Pathology Research Institute, Canadian Forestry Service, Sault Ste. Marie, Ontario, for his encouragement and for providing the facilities for studying the techniques for the identification of pathogens of P. anxia during my visit from March 21 to April 2, 1976; and to Dr. C.R. Harris, Soil Insecticides Research Section, Research Institute, Agriculture Canada, London, Ontario, for his suggestions and for providing the facilities to carry out toxicity tests of soil insecticides during my visit from September 7 to 14, 1977.

I wish also to thank the following families or members of the Experimental Stations for the use of their facilities and for operating light traps: Ste-Clothilde (Mr. J.J. Jasmin and G. Perron), Cowansville (The Goettels), Valleyfield (The Isiks), L'Acadie (Mr. J.J. Jasmin and Mr. G. Routhier), Lennoxville (Dr. C.S. Bernard, Dr. D.P. Hilton and assistant), Ste. Anne-de-Bellevue (The Watsons), Pointe Claire (The Tooheys), St. Hyacinthe (Mr. M. Guibord), L'Assomption (Dr. P.P. Lukosevicius and Mr. M. Dupré), Nicolet (The Cotés), Ste-Augustin (Mr. M.A. Richard), La Pocatiere (Mr. R. Rioux), Normandin

(Mr. J.P.F. Darisse and assistant), and Les Buissons (Dr. G.J. Banville and Mr. R. Avoine).

Sincere thanks are also expressed to Dr. M.A. Fanous, Department of Agricultural Physics and Chemistry, and Mr. Habib Khoury, formerly of Statistics Laboratory, both of Macdonald Campus, for advice and assistance in statistical analyses, also to Miss Karen Toohey, who collaborated in field collecting and identifying adult beetles in 1975 and 1976, and to Mr. Terry Caunter, who assisted with the field collecting and prepared the final drafting of many line drawings, also to Mrs. S.A. Turnbull, Research Institute, Agriculture Canada, London, Ontario, for her excellent technical assistance in toxicity testing of soil insecticides, to Mr. T.J. Poprawski for the French translation of the abstract, and to Mrs. Jean Vauthier for the typing of this thesis.

I acknowledge with appreciation the financial assistance provided for this research by Conseil des Recherches et Services Agricoles du Quebec.

CLAIM TO ORIGINALITY

The following findings from the present study, in the author's opinion, provide original knowledge on *Phyllophaga anxia* in Quebec.

1. The concept of non-overlapping brood zones for *P. anxia*, upon which current cultural control recommendations have been based, has been shown to be invalid.

2. Temperature accumulation requirements for first spring flight of *P. anxia* have been determined for the first time.

3. Temperature accumulation has been shown to be inadequate for forecasting 50% adult emergence.

4. An illustrated key for identification of males of six species, and females of three of the same species of *Phyllophaga* existing in southern Quebec, has been produced.

5. Effects of egg mortality, and overwintering mortality of third instar grubs of *P. anxia*, on population density have been determined.

6. A forecast map of broods of *P. anxia* in southern Quebec has been developed for 1980.

7. Variance/mean ratio and Morisita's index for determination of spatial distribution of *P. anxia* grubs have been used for the first time and shown to be valid.

8. An optimum sample size for population density estimates of *P. anxia* in pasture has been developed.

9. A diplogasterid nematode, *Mikoletzkya aerivora* (Cobb), was found for the first time in *P. anxia* grubs in Canada.

10. The de Man's formula of *M. aerivora* is presented for the first time.

11. *M. aerivora* was shown to cause mortality of *P. anxia* grubs in laboratory tests.

12. An entomopathogenic bacterium, *Bacillus cereus* Fr. & Fr., was found in *P. anxia* grubs for the first time. A new host record.

13. The green muscardine fungus, *Metarhizium anisopliae* (Metsch.) Sorok., was found for the first time from *P. anxia* adults and grubs in Canada.

14. The white muscardine fungus, *Beauveria bassiana* (Bals.) Vuill., was found from *P. anxia* adults and grubs for the first time. A new host record.

15. Two species of fungi, *Fusarium* sp. and *Penicillium* sp., were found on *P. anxia* for the first time.

16. A possible infection of *P. anxia* grubs by entomopoxvirus was found.

17. Organophosphate insecticides, WL 24073, chlorfenvinphos, and Bay 92114, were tested for the first time against *P. anxia* grubs.

TABLE OF CONTENTS

Table of Contents (cont'd)

Table of Contents (cont'd)

Table of Contents (cont'd)

LIST OF TABLES

List of Tables (cont'd)

LIST OF FIGURES

List of Figures (cont'd)

I. GENERAL INTRODUCTION

June beetles, *Phyllophaga* spp., are well known as pests of agricultural crops, pasture and trees. The adults feed at night on the leaves of trees, such as elm, oak, aspen, and poplar. The larvae, known as white grubs, live in the soil and feed on the roots of corn, pasture grasses, strawberries, young nursery trees, and the tubers of the potato (Metcalf *et al.*, 1962; Beirne, 1971).

Phyllophaga anxia (LeConte) is the most common and injurious species of June beetle in the Province of Quebec (Hammond, 1940). It is also found in every other province of Canada and in almost every state in the United States (Luginbill and Painter, 1953). Hammond (1948a) estimated annual losses of $500,000 were caused by white grubs from Manitoba to the Maritimes.

In 1971, a population of white grubs completely destroyed a potato crop near Nicolet, Quebec, despite the fact that this field had been treated with chlordane for control of white grubs (Morrison, 1971). In Quebec, there is at present no chemical control recommendation for white grubs in the potato field (Anonymous, 1979a).

Chlorinated hydrocarbon insecticides such as DDT, aldrin, endrin, dieldrin, heptachlor, and chlordane used to be recommended for control of white grubs (Shenefelt and Simkover, 1951; Hammond, 1960; Pass, 1964; Polivka, 1965; Fowler and Wilson, 1971). In the United States, however, several Phyllophaga spp. have already developed resistance to chlorinated hydrocarbon insecticides (Frankie et al., 1973; Teetes, 1973,1975; Fuchs et al., 1974; Plapp and Frankie, 1976), and in recent years, this whole group of insecticides has been largely restricted in use for insect control in North America. Not only because of the problem of resistance, but also due to their undesirable persistance in the ecosystem, this group of insecticides will have little chance of being reintroduced for controlling soil insect pests in agriculture. Alternative insecticides are being tested for control of white grubs (Rivers et al., 1977; Pike et al., 1978).

In general, the newer organophosphate insecticides and carbamates have shorter residual toxicity than the chlorinated hydrocarbon insecticides, thus the timing of application is critical. Thus, there is an urgent need for a good knowledge of the bionomics of P. anxia, in order to provide a basis for future management strategies under Quebec conditions.

The present study of P. anxia was conducted in 1975, 1976, and 1977 in the agriculturally-important areas of southern Quebec. The objectives of the research were the determination of geographic distribution, field life history, and a survey of natural enemies. The results of the present study should give a better understanding of the bionomics of *P. anxia* in southern Quebec.

II. LITERATURE REVIEW OF *PHYLLOPHAGA ANXIA* (LECONTE)

The genus *Phyllophaga* Harris 1827 includes those numerous insects commonly known as "May beetles", "June beetles", "June bugs" and their larvae, the "white grubs". In Quebec, the adults are commonly known as "hannetons" and their larvae as "vers blanc".

The literature on *Phyllophaga* is enormous, and it is impractical to summarize all of it here. According to Pike *et al*. (1976), there were 680 publications on *Phyllophaga*.

In the present review, emphasis will be placed on *P. anxia*, which is the only species of importance in Quebec (Hammond, 1940), and any relevant publications on other *Phyllophaga* spp. will be included.

A. TAXONOMY

The common name for *Phyllophaga anxia* is the common June beetle (Benoit, 1975). It is a native species of North America (Hammond, 1948a; Ritcher, 1949; Neiswander, 1963), and it has not been reported to occur outside North America.

Luginbill and Painter (1953) listed the following as synonyms for *P. anxia*:

Lachnosterna anxia LeConte 1850

Ancylonycha brevicollis Blanchard 1850

Ancylonycha puncticollie Blanchard 1950

Lachnosterna cephalica LeConte 1856

Ancyclonycha uninotata Walker 1866
Lachnosterna dubia Smith 1888
Lachnosterna insperata Smith 1889

Lachnosterna alpina Linell 1896

Phyllophaga anxia Glasgow 1916

An important taxonomic paper is that of Glasgow (1916), who reviewed the taxonomic status of June beetles in North America, and he concluded that the proper generic name for this group was *Phyllophaga*. In spite of this, the name *Lachnosterna dubia* was used for a long time in Canada (Hammond, 1948a).

According to Dillon and Dillon (1961), and Ritcher (1966), the taxonomic position of *P. anxia* is as follows:

Order: Coleoptera

Suborder: Polyphaga

Superfamily: Scarabaeoidea

Family: Scarabaeidae

Subfamily: Melolonthinae

Tribe: Melolonthini

Genus: *Phyllophaga*

Subgenus: *Phyllophaga*

Species: *anxia*

The adult of *P. anxia* can be separated from other *Phyllophaga* spp. by external morphological characters which include the surface and form of pronotum, form of clypeus, form of antenna, form of spur on hind tibia of male, and surface and structure of abdominal sterna (Horn, 1887; Dillon and Dillon, 1961; Nairn and Wong, 1965), structure of the male genitalia, particularly whether the phallus is symmetrical or asymmetrical, and the presence and shape of the pubic process of the female genitalia (Langston, 1927; Luginbill, 1928; Sim, 1928; Ritcher, 1940; Böving, 1942; Luginbill and Painter, 1953; Chagnon and Robert, 1962).

Keys for identification of white grubs of P. anxia have been published by Böving (1942) and Ritcher (1940,1949,1966). However, taxonomic characters found on the mandibles, epipharynx, and raster have considerable overlaps and variation. Keys to Phyllophaga spp. of a limited geographical area will tend to be more accurate (Ritcher, 1949).

B. MORPHOLOGICAL CHARACTERS, ANATOMY AND HISTOLOGY

The original description of adult P. anxia was published in Latin by LeConte (1850). The morphological characters of P. anxia were also described by Luginbill and Painter (1953), Dillon and Dillon (1961). These are summarized as follows:

> Body oblong ovate; dark brown, shining. Clypeus emarginate. Pronotum slightly wider at middle than at base, strongly narrowed apically. Elytra finely, densely,

punctate, punctures shallow. Metasternum with long, dense hairs. Antennae 10-segmented. Claws with a strong median tooth. Lower spur of hind tibia of male about one-half as long as the upper, unarticulated. Length 17-21 mm.

The morphological characters of third stage larva of P. anxia were described by Böving (1942) and summarized as follows:

Posterior part of labrum with transverse series of 5 to 7 long setae on each side. Dorso-molar region of right mandible with a patch of about 30 setae. Epipharynx with about 12 heli. Raster with anterior third of septula oval, tapering posteriorly into a subrectangular part; palidium with one very irregular series of double row of pali numbering from 20 to 30 or more; palus compressed, with concave sides and hooked at the tip; majority of pali separated by a distance half as long as a palus or shorter; preseptular setae 6 or more.

Details of the anatomy and histology of digestive, nervous, respiratory and reproductive systems of third stage larvae and adults of P. anxia were given by Berberet and Helms (1972). They found that the digestive systems of larvae and adults are quite similar. The nervous system of larvae and adults have concentrated all ganglia of the ventral nerve cord in the proand meso-thoracic segments. Many air sacs are associated with the tracheae of adults. Primordial gonadal tissues are present in third stage larvae.

C. ECONOMIC IMPORTANCE

Most of the references on crop damage by white grubs in southern Quebec and Ontario are for P. anxia (Hammond, 1948a).

White grubs live in the soil and feed on plant roots such as timothy, Kentucky bluegrass, corn, beans and strawberries. Other fibrous-rooted crops are severely attacked and injured (Davis, 1918; Luginbill, 1938; Hammond, 1940,1948a). Potato is considered as a preferred food for white grubs. The entire crop may be destroyed if potatoes are planted after heavily infested pasture. White grubs feed gregariously on fibrous-rooted plants, moving forward in a horizontal plane, about 5 cm under the soil surface, chewing off the roots and killing the plants (Hammond, 1948a). Injury to grass sod, meadow or turf by white grubs is due to the grubs chopping the roots, thereby weakening, stunting or killing the grasses (Hammond, 1940; Jarvis, 1966).

White grubs have also destroyed seedlings of red pine, Japanese larch and red oak (Stone and Schwardt, 1943), and during outbreaks of white grubs, shrubs and saplings may be killed by massive root girdling (Hammond, 1960). They also cause high mortality and loss of vigor in red pine seedlings (Fowler and

Wilson, 1974), and more than 50 per cent of young white pine have been killed by white grubs in a plantation (Sutton and Stone, 1974).

The adult beetles are nocturnal and feed on foliage of elm, oak, poplar, rose, ash, aspen, raspberry, basswood, willow, pear, snowball, chokecherry, alder, walnut, birch, dogwood, cherry, and other plants. They also feed on the petals of flowers, such as apple and lilac (Hammond, 1948a).

Several studies of monetary losses due to grub damage have been made. According to Davis (1918), damage to corn, timothy and potato by white grubs in Iowa, Wisconsin and Illinois in 1912 was conservatively estimated at $7,000,000. Gauthier (1963) surveyed 45 farms in Quebec and estimated losses caused by white grubs at about $216 per farm in 1935. During an outbreak of white grubs in eastern Ontario and the Upper Ottawa Valley in Quebec in 1933, the average loss was $188 per farm (Hammond, 1940). During 1938, an average loss of $108 per farm was caused by white grubs in 78 infested farms in the Eastern Townships of Quebec (Maheux and Gauthier, 1944). In 1944, the damage by white grubs in Niagara Peninsula cost the area over $250,000 in crop and nursery stock (Hammond, 1948a).

D. BIOLOGY AND ECOLOGY

1. Life History

P. anxia has a three-year life cycle (Forbes, 1916;

Hammond, 1931,1940,1948a,1954; Jarvis, 1966). The life history is as follows.

In the first or flight year, the adult beetles emerge during the middle of May and fly at dusk to the preferred food plants where mating takes place. In the daytime, the beetles remain hidden under rocks, leaves, trash, or in the soil. The peak of flight usually occurs in June (Criddle, 1918; Maheux and Gauthier, 1944; Hammond, 1948a,1954; Sutton and Stone, 1974). Eggs are laid in grass sod about 10 days after mating (Maheux and Gauthier, 1944); these usually occur towards the end of May (Hammond, 1948a). The eggs are laid singly in a ball of soil at a depth of about 10 cm (Hudson, 1919; Hammond, 1940), and about 30 days later hatch into first instar grubs (Maheux and Gauthier, 1944; Hammond, 1948a). The first instar grubs feed upon decaying vegetation (Criddle, 1918), fungal hypae (Miner, 1952) and rootlets of plants (Hammond, 1948a,1954; Miner, 1952). In six to eight weeks they moult into second instar and feed on plant roots. After feeding for a short period, the second instar grubs burrow into the soil to varying depths for overwintering (Hammond, 1948a).

In the second year, known as the white grub year, the second instar grubs move closer to the soil surface in early spring, feed on the roots of plants for a short period, and moult into third instar in July. The third instar grubs feed voraciously and descend into the soil for the winter (Hammond, 1948a,1954).

In the third year, the third instar grubs are mostly inactive and feed very little in the spring. They pupate in July, and the teneral adults emerge from the pupae but remain in the soil until the following spring (Hammond, 1948a,1954).

2. Food Preference and Feeding Behavior

Forbes (1916) noted that the majority of *P. anxia* adults were collected from elm, willow, poplar and apple trees. Chamberlin *et al.* (1938) stated that most *P. anxia* adults were collected from willow. Ritcher (1940) reported that *P. anxia* was found on ash, willow, persimmon, oak, sumac and blackberry. According to Hammond (1948a), the preferred food plants of *P. anxia* were elm, oak, poplar, lilac, rose, ash, aspen, butternut, apple and raspberry.

June beetles feed at night on the foliage of trees, and the noise of dropping excrement sound like hail (Davis, 1918). June beetles prefer tender, young foliage. They feed inwards from the edge of the leaf to the petiole. They usually feed on the crown of the tree and defoliate the top twigs first. They also feed on flower petals of lilac and apple (Hammond, 1948a).

White grubs feed underground on the roots of a wide variety of plants. Fibrous-rooted plants, such as timothy, redtop, Kentucky bluegrass, corn, strawberries are favored as foods by white grubs. Tap-rooted plants, such as clover and alfalfa are attacked and injured to a lesser extent (Hammond, 1940). Potato is a preferred food, and roots of young evergreens and young fruit trees are also taken as foods by white grubs (Hammond, 1948a). In the sandhill areas in Nebraska, grasses are the most important and possibly the only hosts of grubs of *P. anxia* (Jarvis, 1966).

White grubs in sod usually feed at a depth of 5 cm or less (Hammond, 1941). White grubs are capable of shaking or combing off the soil adhering to the roots to avoid intake of too much soil into the digestive tract (Hammond, 1944a).

3. *Oviposition*

Sweetman (1927) concluded that females of some *Phyllophaga* spp., including *P. anxia*, did not select places for oviposition according to the cover vegetation. However, Hammond (1954) stated that females of *P. anxia* preferred a grassy surface on a well-drained, warm, light soil as well as proximity to food trees for oviposition. Recently, Toohey (1977) concluded that *P. anxia* preferred thicker stands of plants for oviposition.

Females of *P. anxia* prefer to oviposit in acid soil (Hammond, 1948a). The optimal temperature for oviposition is about 25°C, and soil moisture is between 28 and 58 per cent (Sweetman, 1931).

In Quebec, eggs of *P. anxia* are deposited at a depth of 2.5 to 10 cm (Criddle, 1918). Females of *Phyllophaga* spp. deposit an average of 55 eggs each at depths varying from 5 to 17 cm (Maheux and Gauthier, 1944).

4. Vertical Movement and Distribution

The depth for overwintering of white grubs depends on the locality. In Manitoba, grubs of *P. anxia* go down to an average depth of 112 cm in dry woods soils, and from 36 to 64 cm in wet land for overwintering (Criddle, 1918). In eastern Canada, the second and third instars of *Phyllophaga* spp., including *P. anxia*, pass the winter at depths varying from 15 to 38 cm (Hammond, 1940). In western Quebec, the second and third instars of *P. anxia* pass the winter at depths of 20 to 61 cm; and the adults at a depth of 10 to 23 cm (Guppy and Harcourt, 1970). In Ontario, third instars of *P. anxia* are capable of surviving the winter at a depth of 60 cm (Sutton and Stone, 1975). Based on results of ten year's observations, Granovsky (1956) reported that the depth for hibernation of *Phyllophaga* grubs, including *P. anxia* was dependent on the type of soil. Granovsky found that in silt loam soil, the grubs moved downward for winter hibernation to about 76 cm; while in sandy soil, they stayed at depths between 76 and 91 cm.

During the growing season in Quebec, the majority of third year grubs of *P. anxia* stay between depths of 10 and

25 cm (Hammond, 1941). During June and July in Manitoba, most of the population of grubs of P. anxia can be found at a depth of 30 cm (Ives and Warren, 1965).

In Quebec, third instar grubs of P. anxia construct their pupal cells less than 20 cm from the surface of the ground (Gauthier, 1944). In Kentucky, the mean pupation depths of several Phyllophaga spp. range from 8.3 cm to 39.6 cm; species preference, soil texture, drainage and moisture are the factors that may affect the pupation depths (Ritcher, 1939,1940).

5. Physical Factors

Physical factors can affect the survival of individuals and the size of population of P. anxia. Optimal condition for incubation of eggs of P. anxia is about 25°C and 20 to 73 per cent of water saturation of the soil. For the larval stages, optimal condition for development is about 28°C and 25 to 75 per cent of soil saturation (Sweetman, 1931). In the sandhill areas of Nebraska, the majority of the grubs are found in areas where the soil moisture ranged from 20 to 40 per cent (Jarvis, 1966).

Light soils, such as sandy loam and silt loam, favour the multiplication of populations of P. anxia (Gauthier, 1936; Hammond, 1948a). High density of white grubs of P. anxia and P. fusca are found in soil with a pH between 5.35 and 6.25 (Hammond, 1948b).

Excessive rainfall during the period of oviposition, eclosion, and early development of *P. anxia* critically affects the size of populations of the next generation. It appears that excessive rainfall reduces the oviposition, reduces the hatching rate, and causes high mortality to the first instars (Hammond, 1954).

6. Brood

The term "brood" was used by Davis (1918) to indicate the developmental cycle of *Phyllophaga* spp. in the northeastern United States. Insects of the same brood were in the same developmental stage. Davis (1918) found that *Phyllophaga* spp. had a three-year life cycle, and there were three distinct broods, designated as broods A, B and C. Broods A, B and C emerged as adult beetles in 1914, 1915 and 1916, respectively. A given stage would reappear at three-year intervals in a given locality. There was little or no overlapping of broods in each locality.

In Eastern Canada, there are claimed to be three broods, A, B and C of *P. anxia*, located in three well-defined life-cycle zones, with very little overlapping of broods within each zone (Hammond, 1931,1948a), and Hammond (1954) stated that there was no overlapping of generations of *P. anxia* within a brood area. Hammond and Maheux (1934) reported that there were three broods of *P. anxia* in Quebec, and designated the areas occupied by each brood as Zone 1, Zone 2 and Zone 3. In a study of *Phyllophaga* spp. in Quebec, Maheux and Gauthier (1944) found that there were small pockets of brood C within Zone A.

In Eastern Ontario, there were only two broods, A and C, of *Phyllophaga* spp. as reported by Hammond (1946a).

Shenefelt and Simkover (1951a) questioned the validity of the use of the terms "Brood A", "Brood B" and "Brood C", in the sense in which these had been used by some workers. They proposed the use of the word "flight" instead of the word "brood" for indicating the population of adult June beetles for a given year.

7. Rearing Techniques

Rearing techniques of *Phyllophaga* spp. have been described by several investigators. Sanders and Frackers (1916) supplied young corn plants as food for white grubs, and the mortality was about 16 per cent during the six-week experiment. Reinhard (1940) used a commercial breakfast cereal "Grapenuts" to feed the grubs of *P. lanceolata* (Say) and *P. crinita* (Burmeister); 43.8 per cent of *P. lanceolata* and 97.6 per cent of *P. crinita* had successfully developed from first instar to pupal stage. Ritcher (1940) placed wheat kernels in the rearing containers as food for first-stage white grubs. Miner (1948) gave a pinch of ground "Grapenuts" and some rye grass as food for the first-stage white grubs. Teetes and Wade (1974) prepared a diet mixture to rear _P. crinita_; the diet composed of 60 per cent soil, 35 per cent grass clippings and 5 per cent "Grapenuts". Of 176 eggs kept on the diet, 28 hatched and developed to the third instars. Toohey (1977) used "Grapenuts" to rear _P. anxia_ and her results showed that 42 per cent of the first instars molted into second instars.

E. TRAPPING AND SAMPLING METHODS

Light traps using various types of light sources have been used for collecting _Phyllophaga_ spp. adults (Forbes, 1916; Sanders and Frackers, 1916; Henry and Heit, 1940; Ritcher, 1940; Maheux and Gauthier, 1944; Sanderson, 1944; Chandler _et al._, 1955; Neiswander, 1963; Teetes _et al._, 1976). Light traps using blacklight lamps as attractants have been found useful for collecting _P. anxia_ (Chandler _et al._, 1955) and other _Phyllophaga_ spp. (Teetes _et al._, 1976). Light traps for collecting _Phyllophaga_ spp. are set at heights of approximately 1.5 m (Neiswander, 1963) and 2.1 m above the ground (Henry and Heit, 1940).

Several sampling methods for immature stages of _Phyllophaga_ spp. have been developed. The sample unit usually consists of an area of one square foot (0.09 sq m), with a depth of 12 inches (30.5 cm). Number of samples required varies according to the purpose and the level of sampling precision

(Ives and Warren, 1965; Jarvis, 1966; Guppy and Harcourt, 1970, 1973; Fowler and Wilson, 1971a; Teetes, 1973; Teetes et al., 1976).

For estimating density of white grub populations in the red pine plantations, two sampling plans were used by Fowler and Wilson (1971a). In the first plan, they took four samples (one cubic foot each) per line at regular intervals along four parallel lines across the plantation. In the second plan, they took 30 samples along five parallel lines within a 4-acre (1.6 ha) block.

For estimating density of immature stages of *P. fusca* (Froelich) and *P. anxia* in a permanent meadow in western Quebec, more than 100 samples had to be examined in order to have a precision level of about 10 per cent; for eggs, a similar level of precision required a minimum of 200 samples (Guppy and Harcourt, 1973).

F. NATURAL ENEMIES

The most comprehensive study of the natural enemies of *Phyllophaga* spp. was published by Davis (1919). Unfortunately, Davis did not specify which species of *Phyllophaga* he was working with in most of his descriptions of natural enemies. Information about natural enemies of *P. anxia* is summarized in Table 1.

Table 1. Known Natural Enemies of *Phyllophaga anxia*

Natural Enemy	Taxonomic Status	Stages Attacked	Locality	Frequency & Importance	Reference
Tiphia sp.	Hymenoptera: Tiphiidae	3rd instar	Nebraska	33%	Jarvis, 1966
Tiphia inornata Say	"	"	Quebec	13.2%	Petch & Hammond 1925
Tiphia inornata Say	"	"	Quebec	--	Petch & Hammond 1926
Tiphia berbereti Allen	"	"	Nebraska	Significant Role	Berberet & Helms 1970
Pelecinus polyturator Drury	Hymenoptera: Pelecinidae	Unknown	Quebec	--	Petch & Hammond 1925
Pelecinus polyturator Drury	"	Grub	Quebec	--	Petch & Hammond 1926
Microphthalma michiganensis Townsend	Diptera: Tachinidae	2nd and 3rd instar	Quebec	45.2%	Petch & Hammond 1926
Microphthalma michiganessis Townsend	"	3rd instar	Nebraska	Not important	Jarvis, 1966
Microphthalma phyllophagae Curran	Diptera: Tachinidae	3rd instar	Quebec	9.8 to 15.4%	Petch & Hammond 1926
Eutrixa exile Coq.	"	Adult	Ontario, Mo., NY, Va., Ind., Mich., Ohio, Wisc.	--	Davis, 1919
Asilid larvae	Diptera:Asilidae	Grub	Quebec	--	Petch & Hammond 1926
Asilus snowi Hine.	"	Grub	Quebec	--	Petch & Hammond 1926

Table 1 - Continued.

Pyrgota undata Wied.	Diptera: Ortalidae	Adult	Quebec	0.1%	Petch & Hammond 1926
Pyrgota valida Harris	"	Adult	Ontario, Ill., Kans., Ind., Mich., Tex.	Beneficial	Davis, 1919
Coxiella popilliae Dutky & Gooden	Rickettsiales: Rickettsiaceae	Grub	N.J., Maryland	--	Dutky & Gooden 1952
Gregarine sp.	Gregarinida: Gregarinidae	Grub, Adult	Nebraska	--	Berberet & Helms 1969
Actinocephala sp.	Gregarinida: Actinicephalidae	Grub, Adult	Nebraska	--	"
Bacillus popilliae Dutky	Eubacteriales: Bacillaceae	Grub	Laboratory	--	Dutky, 1941
Bacillus popilliae Dutky	"	Grub	New Jersey	--	Dutky, 1963
Bacillus popilliae Dutky	"	Grub	New York	--	Tashiro & Steinkraus, 1966
Bacillus popilliae Dutky	"	2nd and 3rd instar	Laboratory	--	Jarvis, 1966
Cordyceps ravenellii Berk. & Curt.	Ascomycetes: Hypocreales	3rd instar	Quebec, Ontario	Occasional to 59%	Hammond, 1961
Metarhizium anisopliae (Metsch.) Sorokin	Deuteromycetes: Moniliales	Grub	Nebraska	--	Jarvis, 1966

In addition to these natural enemies, hypopal nymphs of mites, such as *Tyroglyphus armipes* Bls., *Rhizoglyphus phylloxerae* Riley, *Caloglyphus* sp., *Caloglyphus phyllophaginus* Oseto and Mayo, have often been found attached to the grubs of *P. anxia* and have generally been considered as scavengers (Petch and Hammond, 1925,1926; Jarvis, 1964,1966; Oseto and Mayo, 1975).

G. CONTROL MEASURES

1. Chemical Control

Different groups of insecticides havebeen used for control of white grubs. In the 1930's, inorganic insecticides, such as lead arsenate and sulfur, were tested or used for control of grubs of *Phyllophaga* spp. in forest tree nurseries (Shenefelt and Simkover, 1951b), in golf links and lawns (Luginbill, 1938; Neiswander, 1951), in vegetable gardens (Hammond, 1940,1948a), and in strawberry fields (Hammond, 1940; Kerr, 1941; Marshall, 1951). Lead arsenate had also been suggested for control of June beetles by applying to the food trees (Fluke and Ritcher, 1935; Hammond,1940,1946b,1948c). Botanical insecticides and derivatives, such as derris and nicotine sulfate, were also recommended for control of white grubs in turf (Maheux and Gauthier, 1944).

Later, in the 1940's and 50's, chlorinated hydrocarbon insecticides, such as DDT, BHC, aldrin, endrin, dieldrin, chlordane, heptachlor and toxaphene were tested and recommended for control of white grubs (Hammond, 1948a,1949,1960; Shenefelt and Simkover, 1951b; Burkhardt, 1955; Pass, 1964; Polivka, 1965; Daniels, 1971; Fowler and Wilson, 1971b,1974; Sutton and Stone, 1974).

In more recent years, organophosphate insecticides and carbamates have been tested and used as alternatives to the chlorinated hydrocarbon insecticides. According to Pass (1964), some of the organophosphates were as effective as some of the chlorinated hydrocarbons for control of white grubs. Frankie *et al*. (1973) recommended the application of granular diazinon, an organophosphate, for control of white grubs in lawns. Teetes (1973) reported that two organophosphates, diazinon and fensulfothion, and one carbamate, carbofuran, were effective for control of white grubs in grain sorghum and wheat. Fuchs *et al*. (1974) developed a new technique for screening insecticides against white grubs and found that diazinon, fonofos, fensulfothion and carbofuran were among the more effective insecticides tested. Rivers *et al*. (1977) reported that an organophosphate, CGA 12223, was most effective for control of white grubs in a greenhouse test. Pike *et al*. (1978) found that second and third stage white grubs of *P. anxia* were more susceptible to carbamate and organophosphate insecticides than to chlorinated hydrocarbons.

2. <u>Non-Chemical Control</u>

a. Cultural Control

Prevention by avoiding crops of susceptible plants such as corn, strawberries, potatoes and white pine in ploughed-up pasture or abandoned fields, has been suggested for escaping the potential damage by white grubs (Pettit, 1930; Hammond, 1940; Sutton and Stone, 1974; Anonymous, 1979a,1979b).

Ploughing is one of the common methods for control of white grubs. Infested fields are ploughed and followed by several cultivations with disk harrows in order to kill the grubs by physical injuries or exposing to natural enemies and adverse weather (Davis, 1916,1918; Criddle, 1918; Drake *et al*., 1932; Hammond and Maheux, 1934; Hammond, 1933,1940,1946b,1948a, 1960; Maheux and Gauthier, 1938,1944; Gauthier, 1944; Anonymous, 1979a,1979b). Timing of ploughing is also important. It is recommended that the best time to till the soil to kill second-year grubs is from early May to late June, and the best time to till it to kill first-year grubs is from late July to late September. At either time the grubs are near the surface and within easy reach of the plough or disk (Hammond, 1960). Recently, however, Rivers *et al*. (1977) noticed that there were more white grubs of *P. anxia* found in the soil around corn plants with conventional tillage than with minimum tillage. They suggested that the grubs preferred to feed on roots of the grass left between rows in the minimum tillage areas, rather than on corn when both were available.

Crop rotation has also been recommended for control of white grubs. Davis (1918) suggested a rotation of oats or barley, clover and corn. Davis further suggested that clover or corn should be grown at the flight year of the June beetle, since the beetles would not lay eggs in such land, but preferred land with small grains. Hammond (1940) suggested two rotation systems for control of white grubs. The first system was a three year rotation: corn or root crops are planted the first year, grain the second, and clover hay or pasture the third year. The second system was a five year rotation: hoed crops are planted in the first year, grain the second, clover hay the third, grain the fourth, and clover hay or pasture the fifth.

b. Biological Control

Although many natural enemies of Phyllophaga spp. have been found, it is surprising to find that only domestic animals have been suggested for their biological control. Davis (1918) pointed out that poultry could be released in a ploughed field to feed on white grubs. It was also suggested by Davis (1919) that hogs should be pastured in fields during the flight year to destroy the beetles, and in other years to control the white grubs. Both Pettit (1930) and Hammond (1948a) supported the idea of using hogs for control of white grubs.

III. A MONITORING PROGRAM FOR THE COMMON JUNE BEETLE *PHYLLOPHAGA ANXIA* (LECONTE) (COLEOPTERA: SCARABAEIDAE) IN SOUTHERN QUEBEC

A. INTRODUCTION

Chemical control of white grubs with a persistent soil insecticide used to be aimed at newly-hatched grubs, or at active grub stages feeding near the surface of sod (Miner, 1952; Hammond, 1960), or else adults were controlled while on their food trees (Fluke and Ritcher, 1935; Luginbill, 1938; Hammond, 1940,1946,1960). The persistent chlorinated hydrocarbon insecticides are now restricted in use for control of soil insects in agriculture, and non-persistent organophosphate insecticides and carbamates are likely the alternatives for use in the future. Due to their lack of persistence, however, the timing of application is more critical than before, and a monitoring technique which allows the forecasting of adult emergence will be valuable.

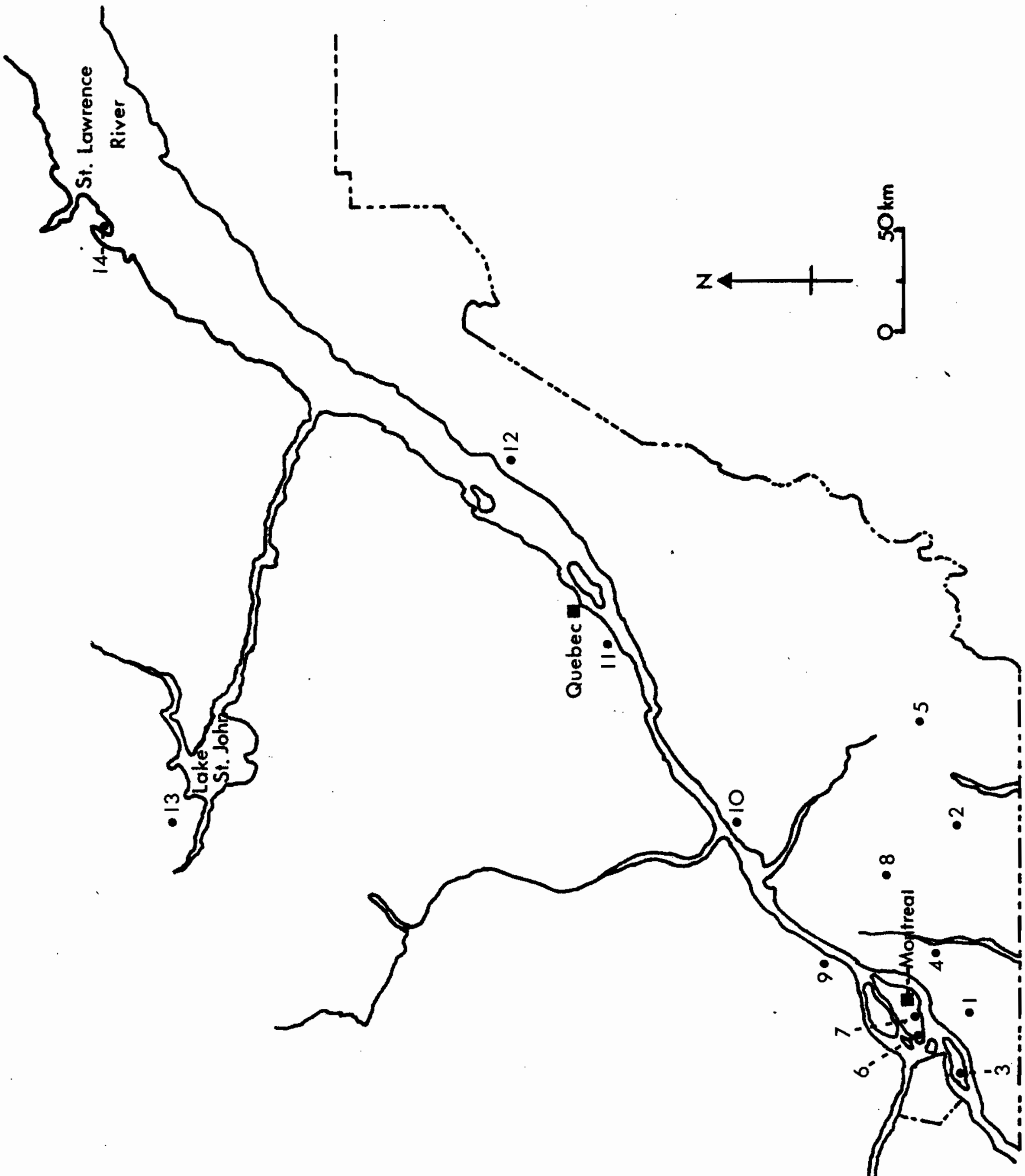
From 1975 to 1977 inclusive, I used blacklight traps distributed throughout Quebec to monitor the flight period of *P. anxia*, and to estimate the corresponding accumulated heat units required for flight to begin.

B. MATERIALS AND METHODS

Blacklight traps are effective for collecting June beetles (Chandler et al., 1955; Teetes and Wade, 1974). I used Ward's 4-baffled insect trap (Ward's Natural Science Establishment, Inc., Rochester, N.Y.), fitted with an 8-watt blacklight fluorescent tube (GE F8T5.BL). A galvanized metal collecting funnel (upper diameter: 30 cm, funnel opening 3.5 cm) was added to the base of the trap and a galvanized metal cone was fitted to the top to keep out rain. A nylon net bag was tied to the base of the cone funnel to collect insects. Each bag was supplied with a recording slip for date and site information, and traps were set at a height of 1.2 m, measured from ground level to the upper rim of the funnel. In 1975, only one trap was operated in Ste-Anne-de-Bellevue in June, and later in Pointe-Claire (Figure 1). In 1976, with the cooperation of nine Federal and Provincial Agricultural Stations, and five other private cooperators, I had 14 traps in operation throughout the southern part of Quebec. Traps were operated from early May to mid-July. The monitoring stations were Ste. Clothilde (45°10'N, 73°41'W), Cowansville (45°12'N, 72°45'W), Valleyfield (45°15'N, 74°08'W), L'Acadie (45°19'N, 73°21'W), Lennoxville (45°22'N, 71°51'W), Ste-Anne-de-Bellevue (45°24'N, 73°57'W), L'Assomption (45°50'N, 73°25'W), Nicolet (46°13'N, 72°37'W), St-Augustin (46°43'N, 71°28'W), La Pocatiere (47°22'N, 70°02'W), Normandin (48°50'N, 72°32'W) and Les Buissons (49°06'N, 68°23'W). In 1977 seven monitoring stations were in operation. These were L'Acadie,

Figure 1. Locations of stations with blacklight traps for monitoring *Phyllophaga* spp. in southern Quebec.

1. Ste-Clothilde
2. Cowansville
3. Valleyfield
4. L'Acadie
5. Lennoxville
6. Ste-Anne-de-Bellevue
7. Pointe Claire
8. St-Hyacinthe
9. L'Assomption
10. Nicolet
11. St -Augustin
12. La Pocatière
13. Normandin
14. Les Buissons


St. Lawrence
River
14
12
Quebec
11
Lake
St. John
13
10
5
2
8
Montreal
9
4
1
7
6
3
N
0
50km

Ste-Anne-de-Bellevue, Lennoxville, Nicolet, St-Augustin, Normandin and Les Buissons. Counting, sexing and identification of beetles were carried out in the laboratory at Ste-Anne-de-Bellevue.

I used air temperature data, supplied by the respective Experimental Stations or from the Monthly Record (Meteorological Observations in Canada, Atmospheric Environment Service, Environment Canada), to calculate degree-day accumulations for each collection area, using the method of Lindsey and Newman (1956). This method is simple and practical (Sevacherian *et al.*, 1977) and the formulae used are:

1. If Min. $> 5^{\circ}C$

$$\text{Degree-days} = \frac{\text{Max.} + \text{Min.}}{2} - 5^{\circ}C$$

2. If Max. $> 5^{\circ}C \geqslant$ Min.

$$\text{Degree-days} = \frac{(\text{Max.} - 5^{\circ}C)^2}{2(\text{Max.} - \text{Min.})}$$

3. If Max. $\leqslant 5^{\circ}C$

$$\text{Degree-days} = 0$$

where Max. = daily maximum temperature

Min. = daily minimum temperature

A base temperature of 5°C was used, as this is within the range usually applied to insects (Hardwick, 1971). April 1 was used as the starting date for calculating temperature accumulation, since in this region the temperature is rarely above the threshold prior to that date.

C. RESULTS AND DISCUSSION

In 1975, only one trap was available for operation. The trap was set up on June 9 in Ste-Anne-de-Bellevue. After four trapping nights, six males and one female of *P. anxia* had been collected. The trap was then moved to Pointe-Claire on June 18, but there no June beetles were collected from a total of nine trapping nights.

Dates of first and last capture of *P. anxia* by blacklight traps in 1976 and 1977 are summarized in Table 2. In 1976, ten of the fourteen stations had their first captures within the first three weeks of May. In 1977, four of the six stations had their first captures within the same period. *P. anxia* emerged later in the northern stations such as Normandin and Les Buissons. In both years, most of the stations had their last capture before the end of June (Table 2). Similar flight periods of *P. anxia* had been reported in Manitoba (Criddle, 1918), Quebec and Ontario (Hammond and Maheux, 1934; Hammond, 1940,1948; Maheux and Gauthier, 1944), New York (Henry and Heit, 1940), Arkansas (Sanderson, 1944), Indiana (Chandler *et al*., 1955), and Nebraska (Jarvis, 1966).

Later dates for first spring flight of *P. anxia* in the monitoring stations in the higher latitudes were observed than those in lower latitudes, this generally agreed with the

Table 2. First and last dates of capture of the common June beetle, *Phyllophaga anxia*, by blacklight traps in southern Quebec, 1976 and 1977.

Station	First capture [1]		Last capture	
	1976	1977	1976	1977
Ste-Clothilde	May 15	-	May 30	-
Cowansville	May 15	-	June 15	-
Valleyfield	May 7	-	June 21	-
L'Acadie	May 16-17	May 16	June 9	May 31
Lennoxville	May 28	May 13-15	June 15	June 6
Ste-Anne-de-Bellevue	May 10	May 11	June 9	June 30-July 3
Pointe-Claire	May 15	-	June 9	-
St-Hyacinthe	May 16	-	June 18-20	-
L'Assomption	May 13	-	June 7	-
Nicolet	May 10	0	June 22	0
St-Augustin	May 25	May 20-30	June 22	June 16
La Pocatière	May 18	-	July 7	-
Normandin	May 29	May 23	July 4	June 12
Les Buissons	May 30	May 27-29	July 8	July 13

[1] — Represents no operation in 1977.

principle of the "Bioclimatic Law of Latitude, Longitude and Altitude" of Hopkins (1918).

For example, the time of occurrence of a biological phenomenon between the southernmost station, Ste-Clothilde, and the northernmost station, Les Buissons, was estimated through the use of the "Bioclimatic Law" (Table 3). There should be a difference of 18.4 days between these two stations. The observed difference in the date of first flight of P. anxia between these two stations was 15 days. Hopkins (1918) pointed out that all conditions were never exactly equal in two or more biological climatic regions; there were always departures from the theoretical time constant. Chapman (1931) suggested that all departures from the calculated dates for a periodic event were due to local factors. With regard to the first flight of P. anxia, it is likely that a rainy night or a short cold spell in spring could delay the scheduled flight of beetles.

In 1976, the mean of the cumulative degree-days for first adult emergence was 185.68 $\pm$ 48.41 (Table 4). In 1977, it was 166.52 $\pm$ 30.79. A Student's t-test showed that there was no significant difference between the means of 1976 and 1977 at the 0.05 level. For practical purposes, I calculated the mean of these two values and postulated a thermal accumulation of 176.1 $\pm$ 13.55 was required for adult emergence. Six stations with 1976 and 1977 results were used to determine if there was

Table 3. Expected difference in the time of occurrence of a biological phenomenon between Ste-Clothilde and Les Buissons, as estimated by the use of the "Bioclimatic Law".

	Ste-Clothilde	Les Buissons	Expected difference (days)
Latitude	$45^{o}10'N$	$49^{o}06'N$	+15.6
Longitude	$73^{o}41'W$	$68^{o}23'W$	+ 4.2
Altitude	185 ft.	50 ft.	- 1.4
		Total	18.4

Table 4. Dates of first *Phyllophaga anxia* adults captured by blacklight trap and the corresponding cumulative degree-days, 1976 and 1977.

Station	1976		1977	
	Date	Cumulative Degree-days (base 5^{o}C)	Date	Cumulative Degree-days (base 5^{o}C)
Ste-Clothilde	May 15	203.0	-	-
Cowansville	May 15	247.4	-	-
Valleyfield	May 7	152.2	-	-
L'Acadie	May 16-17	214.0	May 16	206.7
Lennoxville	May 28	282.0	May 13-15	143.8
Ste-Anne-de-Bellevue	May 10	166.9	May 11	189.9
Pointe-Claire	May 15	212.9	-	-
St-Hyacinthe	May 16	229.4	-	-
L'Assomption	May 13	172.1	-	-
Nicolet	May 10	124.2	zero catch	
St-Augustin	May 25	185.8	May 20-23	184.5
La Pocatière	May 18	128.4	-	-
Normandin	May 29	161.1	May 23	135.0
Les Buissons	May 30	120.1	May 27-29	139.2
Mean ± S.D.		185.68 ± 48.41		166.52 ± 30.79

any difference in the thermal accumulation required for the first adult emergence at the different stations. Each station was considered as a treatment. The years, 1976 and 1977, were considered as replicates. An Analysis of Variance of the results showed that there was no significant difference among the stations at the 0.05 level.

In 1976, the mean of the cumulative degree-days for 50% adult emergence was 323.45 $\pm$ 48.13 (Table 5). In 1977, it was 229.89 $\pm$ 43.13. A Student's t-test showed that they were significantly different at the 0.01 level. This indicated that thermal accumulation cannot be used for forecasting 50% emergence of P. anxia adults.

There is no other report on thermal accumulation data for spring emergence of P. anxia that can be used for comparison. Sweetman (1931) mentioned that first emergence of P. implicita and P. futilis depended upon the cumulative effects of heat, but he did not mention how many degree-days were required.

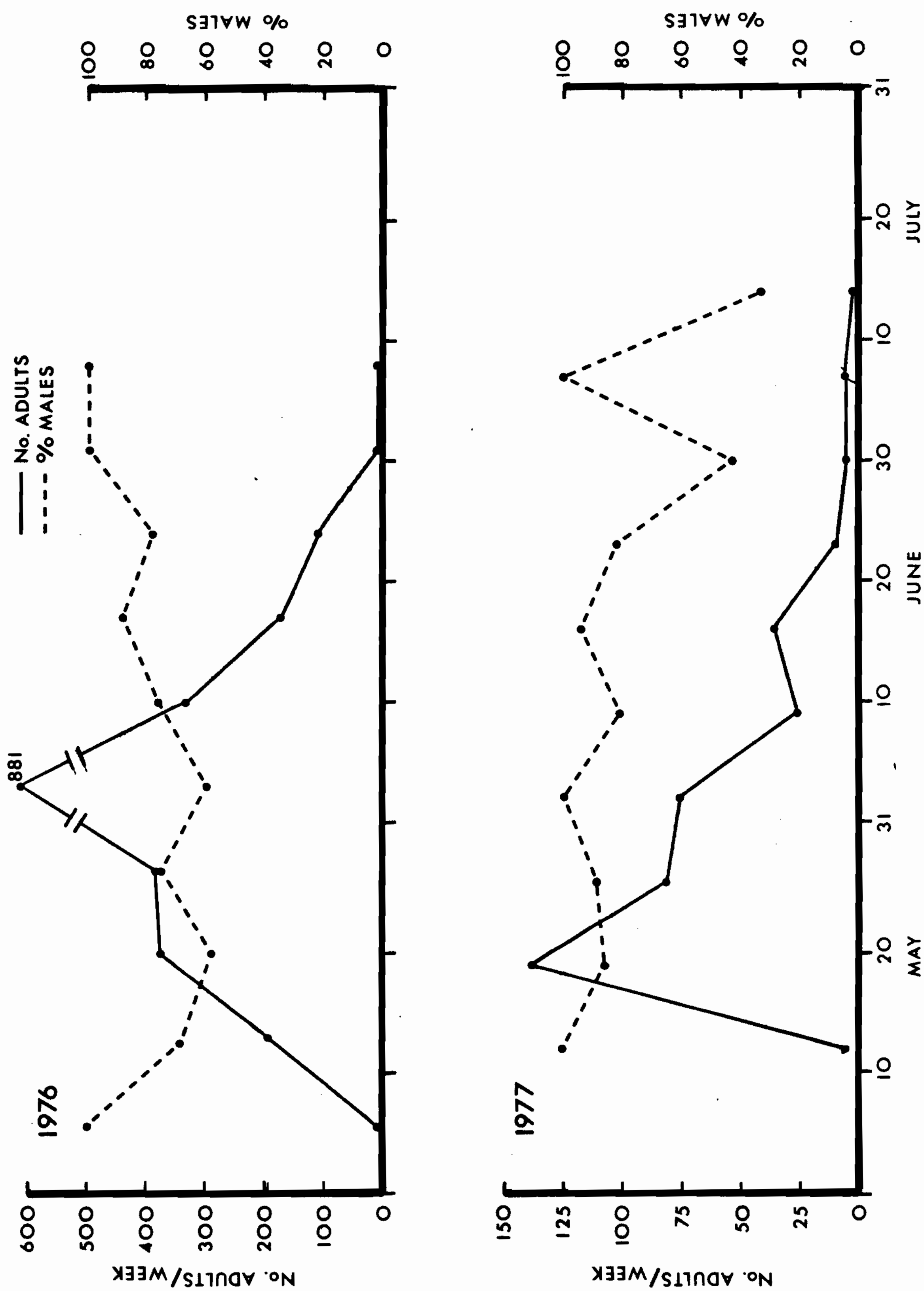
In 1976, the flight peak occurred during the first week of June (Figure 2). In 1977, the peak occurred during the week of May 16. In both years, adult captures ceased in mid-July although traps were operating.

In 1976, the greatest percentage of captured females (42%) were taken during the week of May 31 through June 6 (Figure 2), which was also the week of peak of flight for that year.

Table 5. Dates of 50% *Phyllophaga anxia* adults captured by blacklight trap and the corresponding cumulative degree-days, 1976 and 1977.

Station	1976		1977	
	Date	Cumulative Degree-days (base 5^oC)	Date	Cumulative Degree-days (base 5^oC)
Ste-Clothilde	May 26	260.8	-	-
Cowansville	May 26	325.6	-	-
Valleyfield	May 30	340.8	-	-
L'Acadie	May 27	284.8	May 20-22	262.7
Lennoxville	June 6-9	370.5	May 20-22	211.5
Ste-Anne-de-Bellevue	June 4	387.1	May 20-22	297.0
Pointe-Claire	May 30	339.5	-	-
St-Hyacinthe	May 28-30	320.0	-	-
L'Assomption	June 6	394.2	-	-
Nicolet	May 31	328.3	zero catch	
St-Augustin	June 7	324.4	May 20-23	184.5
La Pocatière	June 5	254.6	-	-
Normandin	June 15	357.5	June 3-5	229.9
Les Buissons	June 15	240.2	June 2	193.5
	Mean ± S.D.	323.45 ± 48.13		229.85 ± 43.13

Figure 2. Seasonal flight activity of *Phyllophaga anxia* adults as monitored by blacklight traps in southern Quebec, 1976 and 1977.


1976
1977
No. ADULTS
% MALES
881
No. ADULTS/WEEK
% MALES
0
100
200
300
400
500
600
25
50
75
100
125
150
20
40
60
80
10
20
31
30
MAY
JUNE
JULY

Males were more common than females at the beginning and end of the flight period. In 1977, males were also more common at the beginning of the flight period, but at the end of this flight period, only 33% of the collection was male.

In Table 6 the numbers of males and females of _P_. _anxia_ collected by blacklight traps from each station are shown. In 1976, all of the stations, except Nicolet, had captured mostly males. This result confirms most of the reports of light trapping of _Phyllophaga_ spp. which indicate that usually many more males than females are caught. Chandler et al. (1955), in Indiana, reported that among the 28 _P_. _anxia_ collected, no females were found. According to Gauthier (1944), in the Eastern Townships of Quebec, 95% of his light trap collection of _Phyllophaga_ spp. were males. Hammond (1960) stated that only male June beetles were attracted to light. Neiswander (1963), in Ohio, collected 59 individuals of _P_. _anxia_, 57 of them were males (96.6%). But, Henry and Heit (1940), in New York, reported that 53% of the _P_. _anxia_ adults collected were females. In the present study, 44.7% of _P_. _anxia_ collected in Nicolet were females and this shows that blacklight traps can sometimes be effective for collecting both sexes of _P_. _anxia_, thus supporting the results of Henry and Heit (1940).

Table 6. Numbers of males and females of *Phyllophaga anxia* captured by blacklight traps in southern Quebec, 1976 and 1977.

Station	1976		1977 [1]		Percentage of Males in Total	
	Males	Females	Males	Females	1976	1977
Ste-Clothilde	11	1	-	-	91.67	-
Cowansville	68	7	-	-	90.67	-
Valleyfield	19	1	-	-	95.00	-
L'Acadie	58	2	33	0	96.66	100
Lennoxville	54	10	45	16	84.38	73.77
Ste-Anne-de-Bellevue	5	0	52	11	100	82.54
Pointe-Claire	21	2	-	-	91.30	-
St-Hyacinthe	62	4	-	-	93.94	-
L'Assomption	5	1	-	-	83.33	-
Nicolet	1045	843	0	0	55.35	-
St-Augustin	80	2	69	1	97.56	98.57
La Pocatière	90	3	-	-	96.77	-
Normandin	133	7	100	8	95.0	92.59
Les Buissons	36	0	50	7	100	87.82

[1] - Represents no operation of light trap in these Stations in 1977.

IV. A KEY FOR IDENTIFICATION OF ADULT JUNE BEETLES, *PHYLLOPHAGA* SPP., FOUND IN SOUTHERN QUEBEC

Keys for the identification of certain adult June beetles, *Phyllophaga* spp., have been published for Quebec (Chagnon and Robert, 1962) and Manitoba (Nairn and Wong, 1965). In a study of seasonal flight pattern of *Phyllophaga anxia* (LeConte) in Quebec (Lim *et al.*, 1979a), five species of *Phyllophaga* were collected in blacklight traps. These were *P. anxia*, *P. fusca* (Froelich), *P. drakii* (Kirby), *P. futilis* (LeConte), and *P. nitida* (Leconte). In addition to the above, a single male specimen of *P. marginalis* (LeConte) was caught by hand by Mr. L. Crozier at Lac Carre in the Laurentians. A total of 3075 adult June beetles were identified according to the descriptions of earlier reports (Sim, 1928; Ritcher, 1940; Böving, 1942; Luginbill and Painter, 1953; Chagnon and Robert, 1961). However, the Chagnon and Robert (1961) key included only three of these species.

The following keys, based on the genitalia, were prepared to allow identification of the above six species using males, and three of the same species using females, of *Phyllophaga* spp. that were found in southern Quebec. The key for females is only partially complete, as females of only three of the species were found in the present study. Photographs are used to supplement the given descriptions of the genitalia. Males can be distinguished from the females externally by the presence of small ridges on the penultimate abdominal sternum.

Key to male adults of <u>Phyllophaga</u> spp. found in southern Quebec (viewed from the caudal aspect).

1.	Parameres symmetrical	2
1'.	Parameres asymmetrical	5
2(1).	Parameres with distinct keeled .... protrusion	<u>P</u>. <u>anxia</u> (Fig. 3a)
2'.	Parameres without distinct keeled .... protrusion	3
3(2').	Parameres with rounded tips	<u>P</u>. <u>futilis</u> (Fig. 6)
3'.	Parameres with pointed tips	4
4(3').	Distal opening of parameres, ... almost circular	<u>P</u>. <u>fusca</u> (Fig. 4a)
4'.	Distal opening of parameres, ovate	<u>P</u>. <u>drakii</u> (Fig. 5a)
5(1').	Parameres with pointed process on .... inner edge	<u>P</u>. <u>nitida</u> (Fig. 7)
5'.	Parameres with blunt protruberance .... on inner edge	<u>P</u>. <u>marginalis</u> (Fig. 8)

Key to female adults of <u>Phyllophaga</u> spp. found in southern Quebec (viewed from the ventral aspect).

1.	Pubic process single stemmed	<u>P</u>. <u>fusca</u> (Fig. 4b)
1'.	Pubic process bifurcate	2
2(1').	Pubic process V-shaped; stems of .... pubic process broad	<u>P</u>. <u>anxia</u> (Fig. 3b)
2'.	Pubic process Y-shaped; stems of .... pubic process thin	<u>P</u>. <u>drakii</u> (Fig. 5b)

Figure 3. Genitalia of _P_. _anxia_.

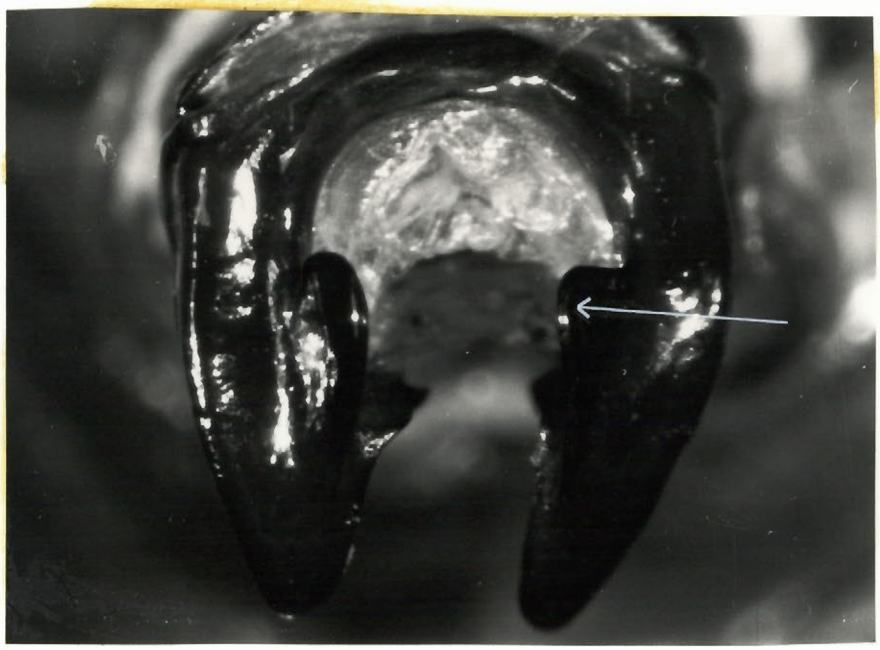
a. Caudal view of male genitalia

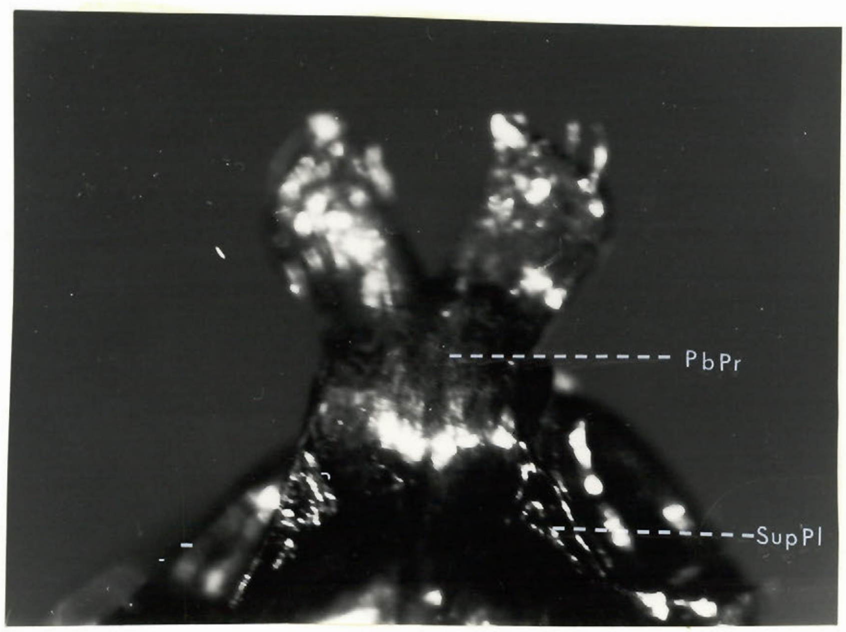
Arrow: Keeled protrusion

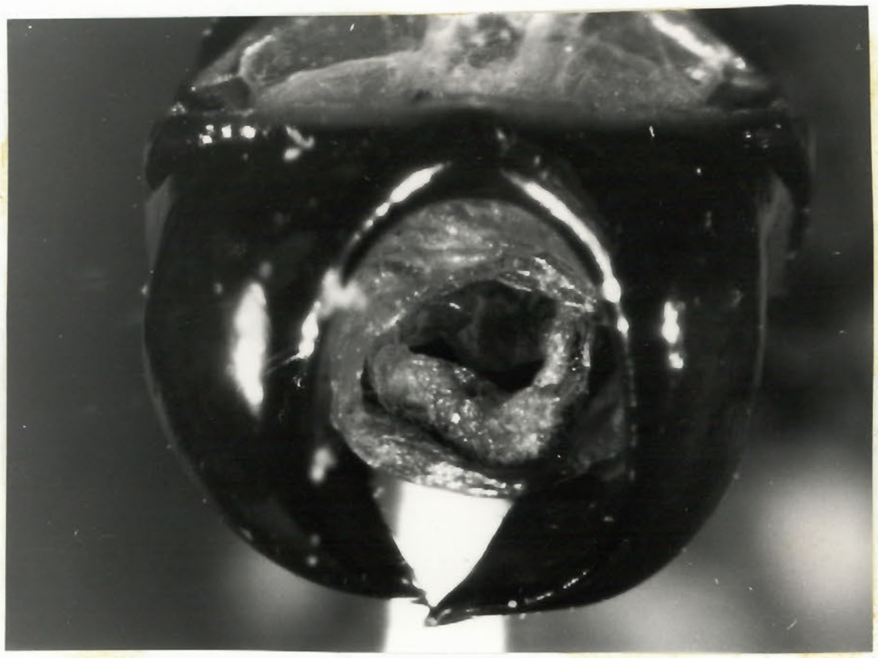
b. Ventral view of female genitalia

PbPr: pubic process

SupPl: superior plate




PbPr
SupPl



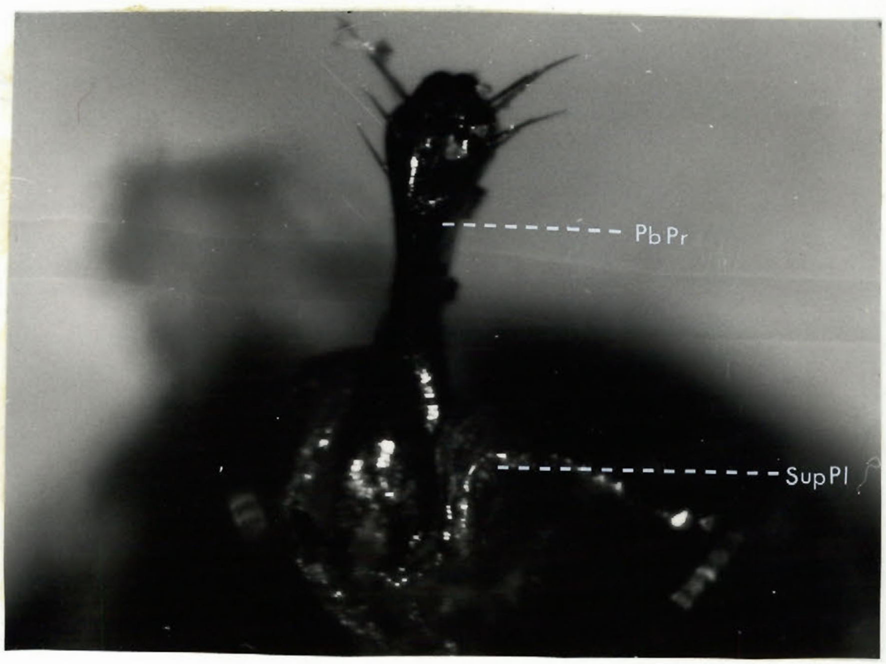

PbPr
SupPl

Figure 5. Genitalia of *P. drakii*.

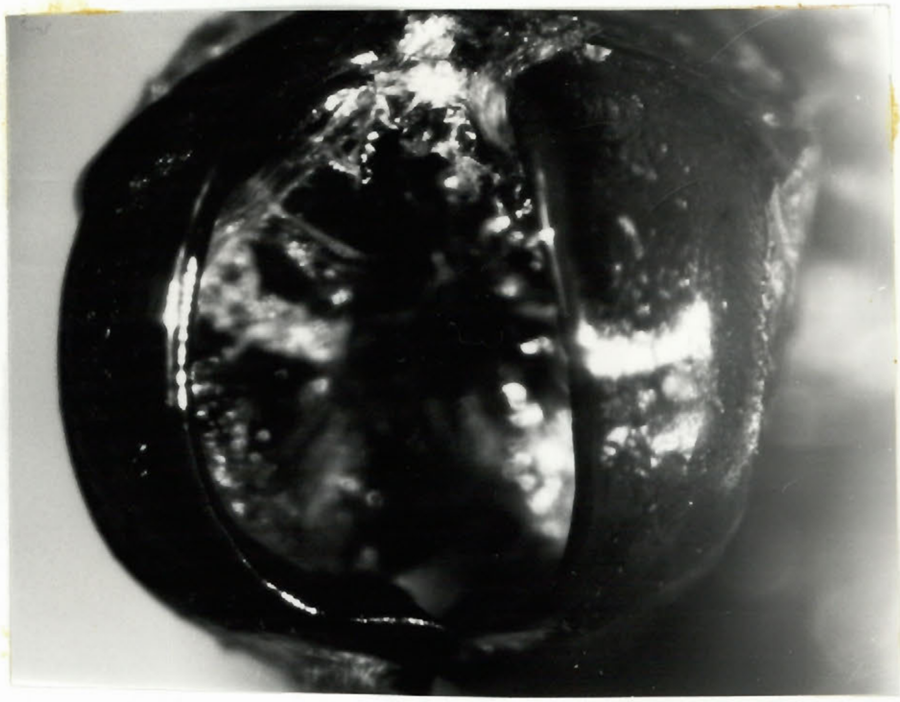
a. Caudal view of male genitalia

b. Showing distal opening of parameres, ovate

b. Ventral view of female genitalia

PbPr: pubic process

SupPl: superior plate



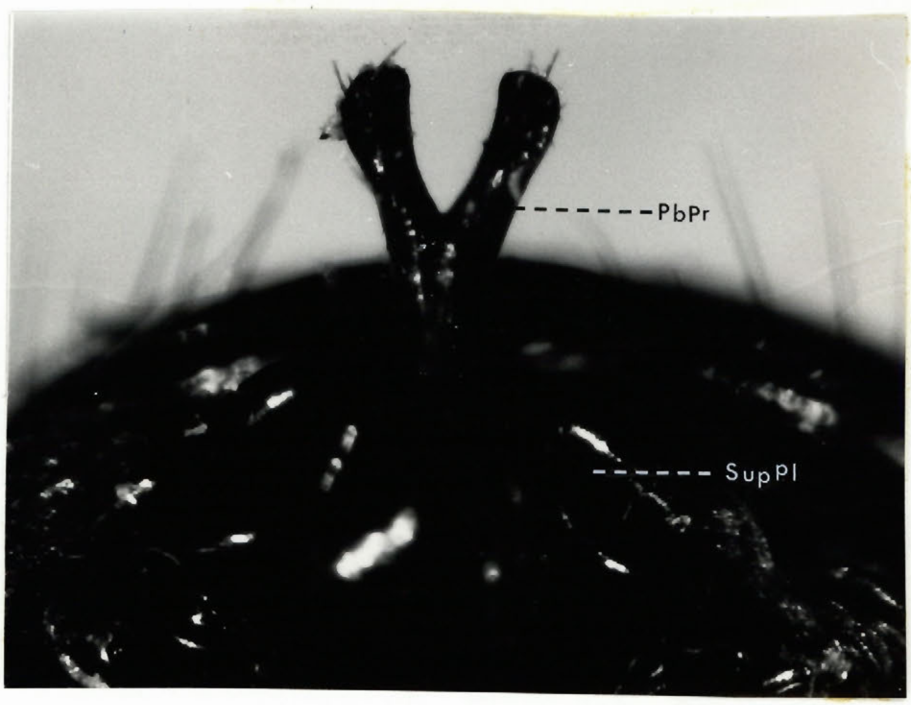

PbPr
SupPl

Figure 6. Caudal view of male genitalia of *P. futilis*.

Showing parameres with rounded tips

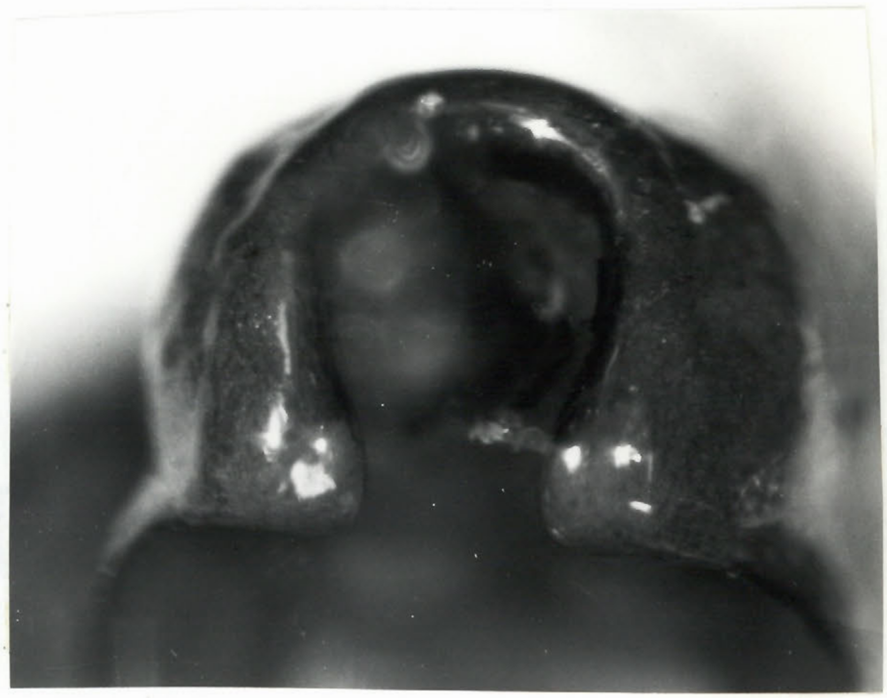


Figure 7. Caudal view of male genitalia of *P. nitida*.

PP: pointed process

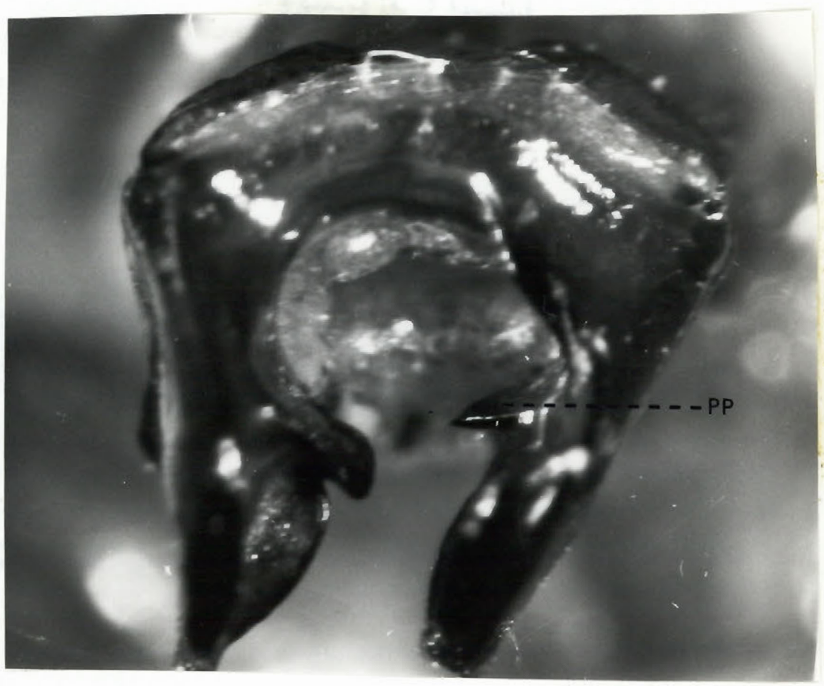
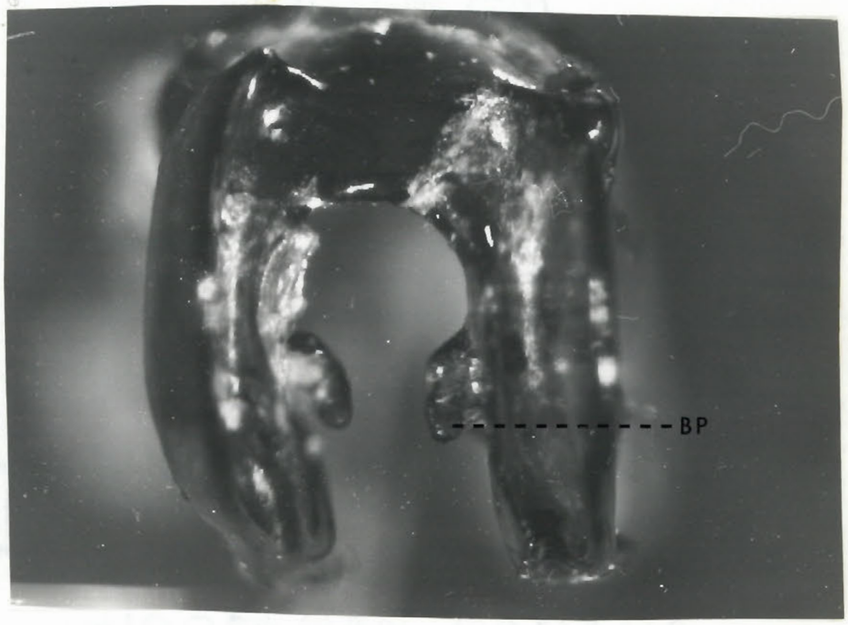

PP

Figure 8. Caudal view of male genitalia of P. marginalis.

BP: blunt process


BP

V. DISTRIBUTION AND LIFE HISTORY OF PHYLLOPHAGA ANXIA (LECONTE) IN SOUTHERN QUEBEC

A. INTRODUCTION

Phyllophaga anxia (LeConte) has been reported as the most common and injurious species of June beetles in Quebec; it's life history had been investigated several decades ago in Ontario and Quebec (Hammond, 1940, 1948a; Maheux and Gauthier, 1944), and in Nebraska (Jarvis, 1966). In recent years, reports of damage by it's larval stage (white grubs) in potato crops, pasture, and home gardens have increased in the Province of Quebec. For example, a population of white grubs completely destroyed a potato crop near Nicolet, despite the field having been treated with chlordane for soil insect control (Morrison, 1971). Recently, Toohey (1977) studied the bionomics of *P. anxia*, in which parts of the data were collected jointly with the present author. Beside these reports, there appeared to be no recent and comprehensive studies on the distribution and life history of *P. anxia* in this region. The present study was conducted from 1975 to 1977 in southern Quebec.

B. MATERIALS AND METHODS

June beetles were trapped in blacklight traps (Lim *et* al., 1979a), and identified according to the key of Lim et al. (1979b). Soil samples (quadrats) were taken in pastures for life stages of P. anxia. Quadrats were taken near each light trap station and other collection points (Figure 9). Each quadrat consisted of 0.09 sq m (1 sq ft) of soil and sod, with about 30 cm deep. Samples were taken in one or more habitats on each survey trip and the samples were hand sorted in the field and specimens were counted in the laboratory.

Two pastures, in Valleyfield and Nicolet, with measurable infestations of different stages of P. anxia were used for intensive study of the seasonal history of the species. Normally two samples were taken each month from each site during the growing season.

C. RESULTS AND DISCUSSION

1. Distribution

Five species of Phyllophaga were collected by the black-light traps in the present study. These were P. anxia, P. fusca (Froelich), P. drakii (Kirby), P. futilis (LeConte), and P. nitida (LeConte). In addition to the above, a single specimen of P. marginalis (LeConte) was caught by hand by Mr. L. Crozier at Lac Carré in the Laurentians.

P. anxia was found in every light trap station in the study (Figure 10). In 1976, in seven of the fourteen stations,

Figure 9. Locations of collection points at which soil samples were taken to examine for Phyllophaga anxia in southern Quebec. (Locations with less than two survey trips are not plotted).

1. Ste. Clothilde	9. St. Hyacinthe
2. Cowansville	10. L'Assomption
3. Valleyfield	11. Lavaltrie
4. L'Acadie	12. Nicolet
5. Lennoxville	13. St-Augustin
6. Dorion	14. La Pocatiere
7. Ste. Anne de Bellevue	15. Rimouski
8. Pointe Claire	16. Normandin
	17. Les Buissons

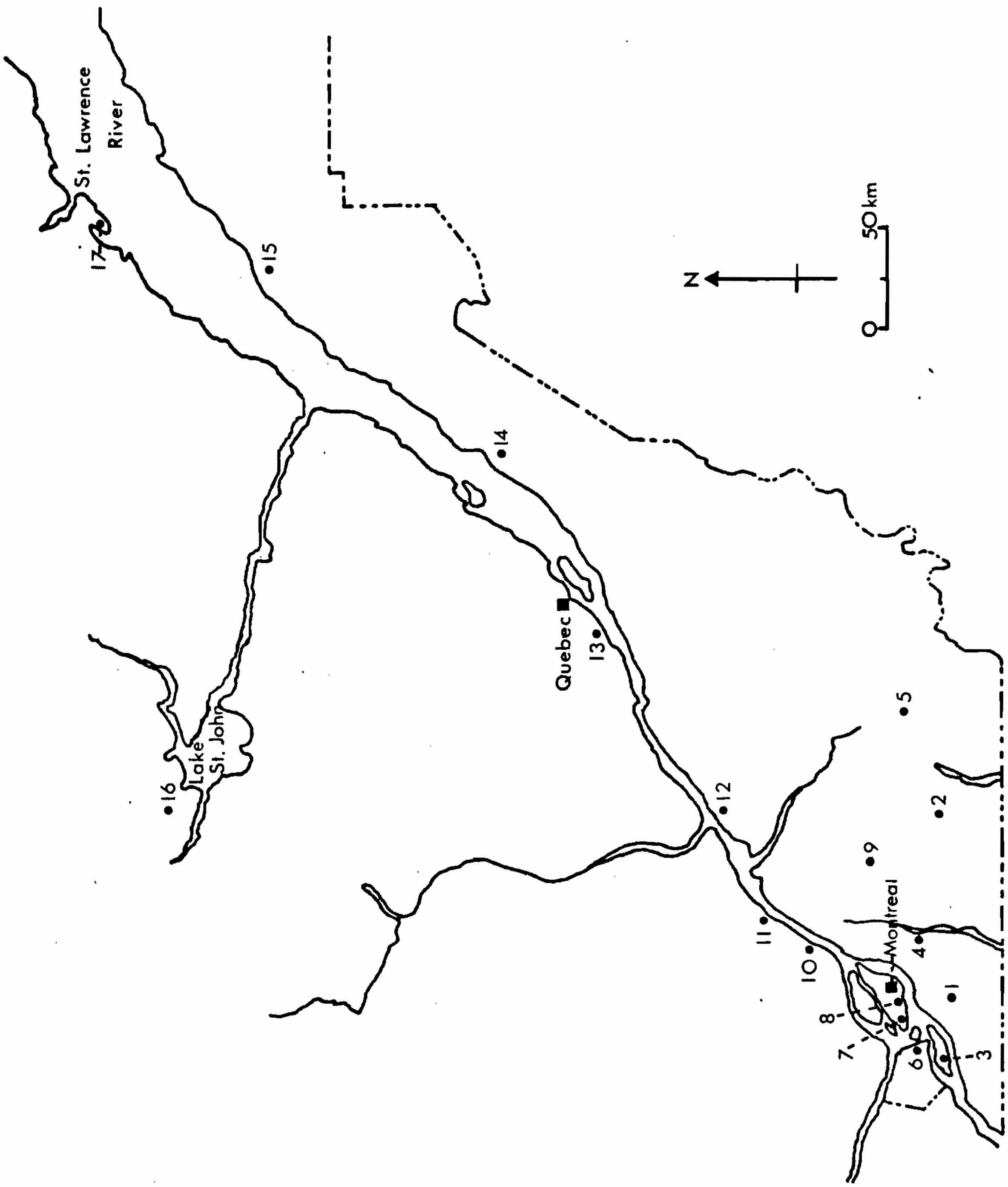
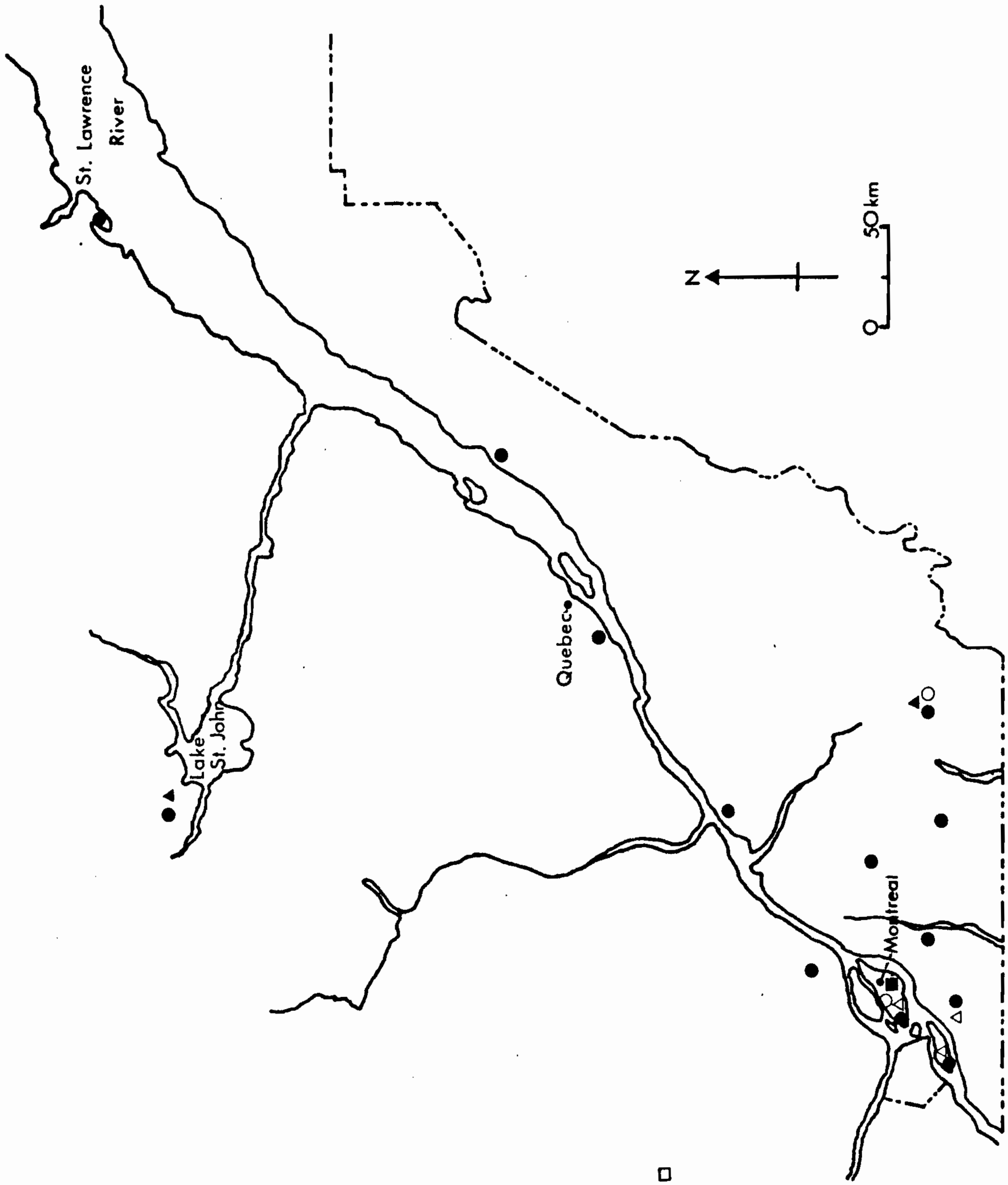

St. Lawrence River
Lake St. John
Quebec
Montreal
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
N
0
50 km

Figure 10. Distribution of *Phyllophaga* spp. in southern Quebec.

● *P. anxia* △ *P. futilis*

○ *P. fusca* ■ *P. nitida*

▲ *P. drakii* □ *P. marginalis*


St. Lawrence
River
Lake
St. John
Quebec
Montreal
N
0
50 km

P. anxia was the only species captured by the traps and in the other stations it was the most numerous species collected (Table 7). In 1977, P. anxia was again collected in every station. The ability of P. anxia to distribute over a wide area had been noticed by Hammond (1948) and Luginbill and Painter (1953).

The relative abundance of Phyllophaga spp. collected by blacklight traps at each station is presented in Table 8. The results show that species other than P. anxia might be important in some areas in different years, as shown at Lennoxville and Normandin in 1976 and 1977 (Table 8). Similar results have been observed in Illinois by Forbes (1916). Based on the results of 1975 and 1976, Toohey (1977) concluded that 98% of the June beetles trapped were P. anxia.

2. Description of Life Stages

a. Adult

The adult (Figure 11)varies from light to dark brown in colour. The mean size for 20 males was 20.70 ± 0.61 mm (length) and 11.05 ± 0.39 mm (width) and for 20 females was 20.50 ± 0.76 mm (length) and 10.95 ± 0.69 mm (width).

b. Egg

The newly-laid egg of P. anxia is white, ellipsoidal,

Table 7. Numbers of *Phyllophaga* spp. adults collected by blacklight traps in southern Quebec, 1976 and 1977.

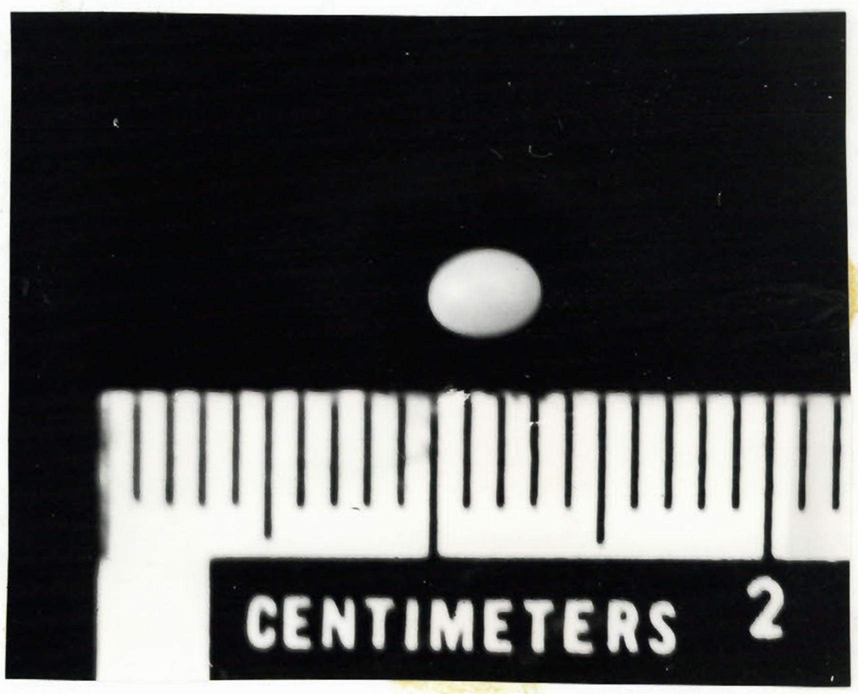
Station	Species	1976		1977	
		Males	Females	Males	Females
Ste. Clothilde	*P. anxia*	11	1	-	-
	P. futilis	1	0	-	-
Cowansville	*P. anxia*	68	7	-	-
Valleyfield	*P. anxia*	19	1	-	-
	P. futilis	5	0	-	-
L'Acadie	*P. anxia*	58	2	33	0
Lennoxville	*P. anxia*	54	10	45	16
	P. fusca	2	1	12	8
	P. drakii	3	0	0	0
Ste-Anne-de-Bellevue	*P. anxia*	5	0	52	11
	P. fusca	2	0	0	0
	P. futilis	1	0	6	0
	P. nitida	1	0	0	0
Pointe Claire	*P. anxia*	21	2	-	-
	P. nitida	4	0	-	-
St-Hyacinthe	*P. anxia*	62	4	-	-
L'Assomption	*P. anxia*	5	1	-	-
	P. drakii	1	0	-	-
Nicolet	*P. anxia*	1045	843	0	0
St-Augustin	*P. anxia*	80	2	69	1
La Pocatiere	*P. anxia*	90	3	-	-
Normandin	*P. anxia*	133	7	100	8
	P. drakii	24	1	33	1
Les Buissons	*P. anxia*	36	0	50	7

Table 8. Relative abundance of *Phyllophaga* spp. adults collected by blacklight traps in southern Quebec, 1976 and 1977.

Station	Year	% of Each Species					Total No. Collected
		P. anxia	*P. drakii*	*P. fusca*	*P. futilis*	*P. nitida*	
Ste. Clothilde	76	92.3			7.7		13
Cowansville	76	100					75
Valleyfield	76	80			20		25
L'Acadie	76	100					60
	77	100					33
Lennoxville	76	91.4	4.3	4.3			70
	77	75.3		24.7			81
Ste-Anne-de-Bellevue	76	55.6		22.2	11.1	11.1	9
	77	91.3			8.7		69
Pointe Claire	76	85.2				14.8	27
Ste-Hyacinthe	76	100					66
L'Assomption	76	85.7	14.3				7
Nicolet	76	100					1888
St-Augustin	76	100					82
	77	100					70
La Pocatiere	76	100					93
Normandin	76	84.9	15.1				165
	77	76.1	23.9				142
Les Buissons	76	100					36
	77	100					57

Figure 11. Adult of *P. anxia*.

Figure 12. Egg of *P. anxia*.


CENTIMETERS 2

and with a pearly lustre (Figure 12). Measurements of 20 eggs showed that the eggs range in length from 2.31 to 2.82 mm (mean = 2.53 $\pm$ 0.15 mm) and in width from 1.77 to 2.07 mm (mean = 2.02 $\pm$ 0.08 mm). As development progresses the eggs increase in size and become spherical and the reddish mandibles of the fully developed embryo can be seen through the chorion.

c. Larva

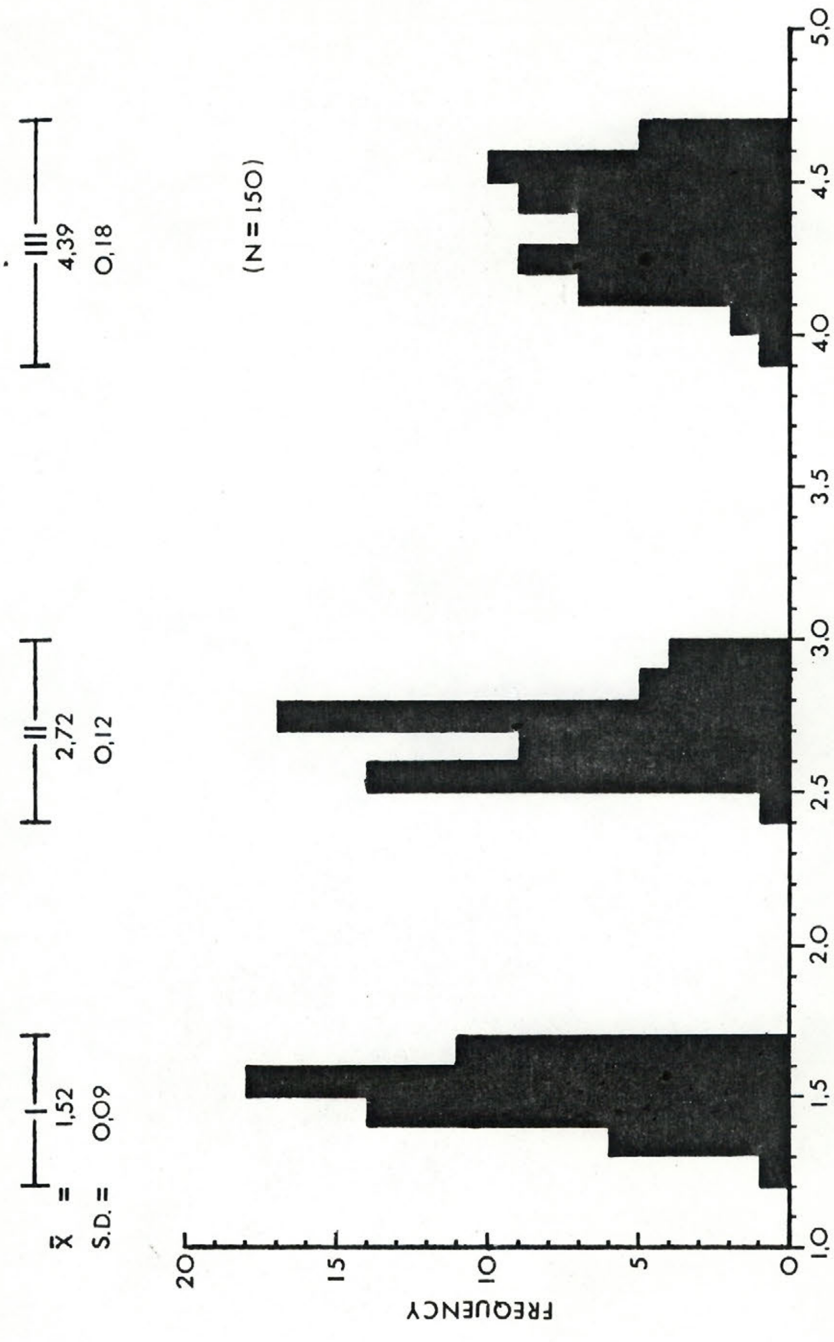
Earlier reports (Hammond, 1940,1948a,1954;Maheux and Gauthier, 1944) used body size to distinguish the three larval instars of P. anxia. In the present study, widths of head capsules of 150 larvae were measured (Figure 13). Three distinct distributions of measurements were found. This demonstrates the presence of three larval instars.

The newly hatched first instar is partially translucent and is a typical scarabaeiform larva (Figure 14). After feeding, a dark region is visible through the integument of the anterior colon as described by Berberet and Helms (1972). The second instar (Figure 15)is white in colour and the dark anterior colon is more distinct than is the first instar. The third instar is very similar to the second instar but with a measurable increase in size (Figure 16).

d. Prepupa

The prepupa (Figure 17) can be differentiated from the

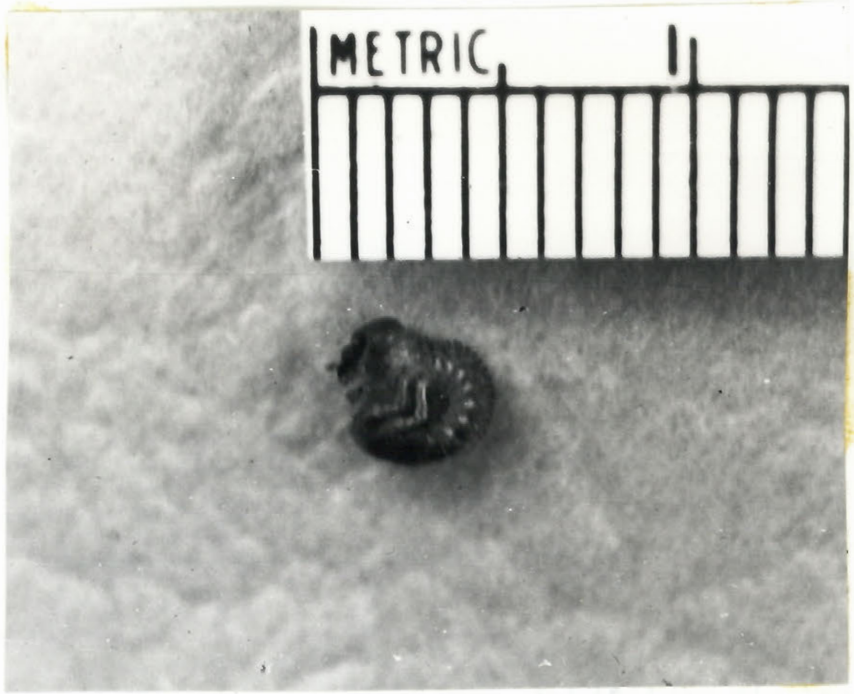
Figure 13. Frequency histogram of head width of grubs of *Phyllophaga anxia* collected in southern Quebec.



x̄ =
S.D. =
I
1,52
O,O9
II
2,72
O,12
III
4,39
O,18
(N = 15O)
FREQUENCY
HEAD WIDTH (MM)

Figure 14. First instar of P. anxia.

Figure 15. Second instar of P. anxia.


METRIC
1

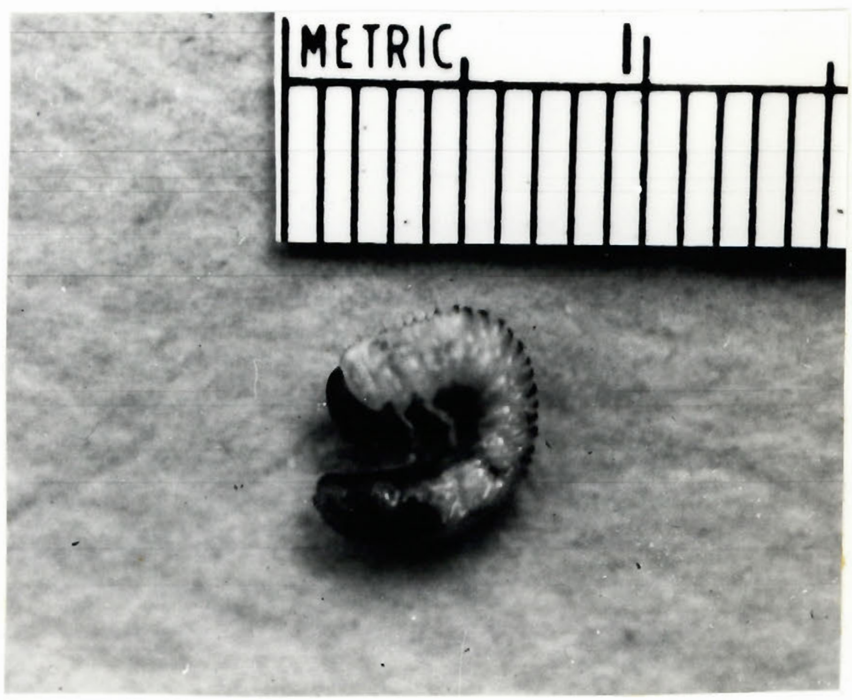

METRIC
1

Figure 16. Third instar of *P. anxia*.

Figure 17. Prepupa of *P. anxia*.


METRIC
1
2


2
3

third instar by the wrinkled, shrunken integument on the posterior end of the body. No yellowing of the body of the prepupal stage as described by Criddle (1918) was noticed on the prepupae obtained in the present study.

e. Pupa

The pupa is of the exarate type (Figure 18). All pupae were found in earthern pupal cells (Figure 19) and typically responded to touch by twisting of the body. Exuviae of the prepupae were found at the posterior end of the pupal cells. The newly formed pupae were yellowish white in colour, gradually becoming creamy yellow. When a pupa was crushed mechanically, copious amounts of thick, white material, believed to be fat body were seen. Just a few days before the adult emerged, the appendages of the pupa changed to a reddish brown colour (Figure 20a,b).

Sexual differences of the pupae are distinguishable on the ventral side of the caudal segments. In the male (Figure 21a), a prominent conical structure (future phallic organ) is present just anterior of the anal opening. In the female (Figure 21b), no such structure is found. Similar structures on other Phyllophaga spp. have been observed by Hayes and McColloch (1920). Of 297 pupae examined in the present study, 150 were males and 147 were females.

Figure 18. Pupae of *P. anxia*.

Male on left. Female on right.

Figure 19. Pupae in earthern pupal cells.

Figure 21. Ventral view of caudal segments of pupae of *P. anxia*.

a. Male
Note the conical structure (future phallic organ)

b. Female

f. Teneral Adult

The adult is soft with translucent elytra (Figure 22) when it first emerges from the pupa. The elytra hardened and developed a brown colour after a few hours. These adults stayed in the soil until the following spring and are termed teneral adults (Guppy and Harcourt, 1970).

3. Life Cycle and Habits of *P. anxia* in Southern Quebec

A total of 4351 specimens of *P. anxia* were collected by soil sampling in various parts of southern Quebec (Table 9). By using the first and last dates of finding different stages of *P. anxia* (Table 10), a life cycle of *P. anxia* in southern Quebec was developed (Figure 23).

In the present study, adults were found as early as April 29 in 1976 at Nicolet, at depths of up to 30 cm from the soil surface. Adult beetles are nocturnal, and the flight period is from early May to mid-July depending on the locality and the season (Hammond, 1940,1948a; Jarvis, 1966; Lim *et al.*, 1979a). Eggs were found most commonly in June (Table 11). Eggs found in July and August were usually from habitats in more northerly latitudes. Under field conditions, eggs are known to require about 30 days to hatch (Hammond, 1948a ; Maheux and Gauthier, 1944). First instars were abundant in July and August


METRIC 1 2

Table 9. Total numbers of *Phyllophaga anxia* collected by soil sampling (0.09 sq m each) in southern Quebec.

Vicinity[a]	Year	No. of Visits	No. of Quadrats Taken	Total No. of *P. anxia*
Ste. Clothilde	75	1	15	9
	76	3	129	119
Cowansville	75	3	44	4
	76	4	105	36
Valleyfield	75	1	20	0
	76	11	523	1361
	77	8	424	312
L'Acadie	75	3	109	330
	76	3	50	58
	77	4	150	261
Lennoxville	76	2	102	12
	77	3	120	10
Dorion	76	8	118	184
Ste-Anne-de-	75	16	316	80
Bellevue	76	5	106	60
	77	4	110	11
Pointe Claire	75	3	60	32
	76	8	226	147
St-Hyacinthe	75	1	25	1
	76	2	80	19
	77	3	144	41
L'Assomption	75	2	37	5
	76	2	73	19
	77	3	130	87
Lavaltrie	75	3	52	4
	76	2	68	31
Nicolet	75	1	50	14
	76	8	223	266
	77	7	390	324
St-Augustin	76	2	98	18
	77	2	132	16
La Pocatiere	76	1	148	0
	77	2	174	60
Rimouski	77	2	76	78
Normandin	76	2	112	22
	77	2	124	54
Les Buissons	76	1	180	15
	77	2	174	168
Others	75	3	119	41
	76	6	104	42

[a]Collections from locations with only one survey trip each are pooled together under "others".

Table 10. First and last dates of finding different stages of *Phyllophaga anxia* by soil sampling in southern Quebec, 1975 to 1977.

	First		Last	
	Date	Vicinity	Date	Vicinity
Adult	-	-	Jul.13[a]	Les Buissons
Egg	Jun.10	Nicolet	Aug.11	Rimouski
1st L.	Jul.13	Nicolet	Sept.22	Ste-Anne-de-Bellevue
2nd L.	Aug.6	L'Acadie	Overwintering	
2nd L. (2nd year)	-	-	Aug.11	Rimouski
3rd L. (2nd year)	Jun.20	Nicolet	Overwintering	
3rd L. (3rd year)	-	-	Aug.10	Normandin
Prepupa	Jun.27	Valleyfield	Aug.11	Rimouski
Pupa	Jul.7	St-Hyacinthe	Aug.24	Valleyfield
Teneral adult	Aug.3	St-Hyacinthe	Overwintering	

[a]The last date for finding adults was determined by blacklight trap.

Figure 23. Generalized life cycle of *Phyllophaga* *anxia* (LeConte) in southern Quebec.

Å – Adult

○ – Egg

△ – First instar

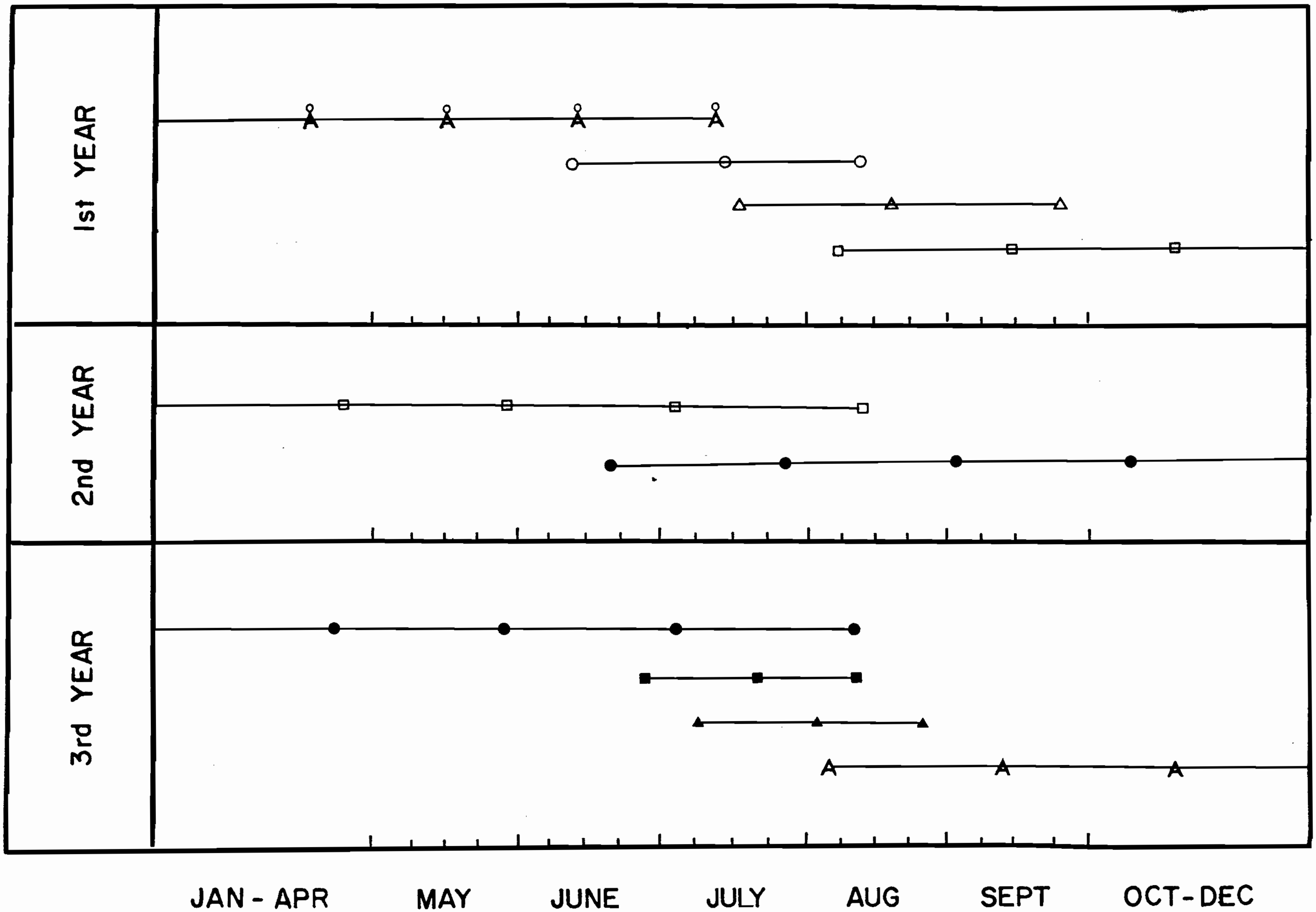
□ – Second instar

● – Third instar

■ – Prepupa

▲ – Pupa

A – Teneral adult


1st YEAR
2nd YEAR
3rd YEAR
JAN - APR
MAY
JUNE
JULY
AUG
SEPT
OCT - DEC

(Table 11). After feeding for five to eight weeks, they molted to second instar. Similar observations have been reported by Hammond (1948a). First instars were found to have begun molting to second instar on August 6, 1975 at L'Acadie and also on August 17, 1976 at Nicolet. Consequently, any second instars found later than August were usually in the second stadium of their first year (Figure 23, Table 10). In the second year, the second instars were found near the root zones of grasses in the spring. Second instars were found in the process of molting to third instar on June 21, 1976 at Valleyfield and the same was observed on June 20, 1977 at Nicolet. However, Hammond (1948a) stated that the second molt into third instar occurred in late July in Quebec and Ontario. In Kentucky, Ritcher (1940) reported that <u>Phyllophaga</u> spp. had the second molt in early June. In the study areas, the second molt occurred in late June, except at Normandin and Les Buissons, both in the north, where the second molt occurred as late as August. The third instars of the second year (Figure 23) are the most voracious, and caused heavy damage to plants (Hammond, 1948a). This is also the stage that overwinters in the second year. Third instars found before June were likely to be the third instars of the third year (Figure 23, Table 10). If a third instar is found in July or early August, it can be differentiated into

Table 11. Occasions of finding various stages of *Phyllophaga anxia* at different periods by soil sampling in southern Quebec, 1975 to 1977.

Period	No. of Quadrats Taken	Occasions of finding						
		Egg	1st L.	2nd L.	3rd L.	Prepupa	Pupa	Adult
April	107	0	0	3	2	0	0	2
May	1232	0	0	20	26	0	0	8
June	1390	6	0	29	28	4	0	5
July	963	3	6	7	15	6	7	1
August	1412	3	11	19	34	2	12	9
Sept.	249	0	2	5	9	0	0	3
Oct.	88	0	0	3	2	0	0	1

the second year/third instar or the third year/third instar by its physical appearance. The recently-molted second year/third instar has a bristly appearance, due to the proximity of the setae on the body. This appearance is absent in well-grown third year/third instar. In the third year, the third instars feed very little (Hammond, 1948a) and develop into prepupae. Prepupae were found on June 27, 1977 at Valleyfield. By July 11, in the same habitat, all had developed into pupae. Prepupae were found as late as August 11 in 1977 at Rimouski. In Manitoba, prepupae began to appear about June 26 (Criddle, 1918). In these areas, pupae were found only in July and August (Table 11). Teneral adults were found from August onwards (Table 11). However, Criddle (1918) stated that teneral adults of _P. anxia_ were found by the middle of July in Manitoba. Jarvis (1966) carried out his study in Nebraska, where teneral adults of _P. anxia_ were found from August onwards. The data of Hammond (1948a) showed that teneral adults of _P. anxia_ were first found on August 5 in Quebec and Ontario.

From the information gathered in the present study, the seasonal history of _P. anxia_ can be best summarised as follows: In the first year, adults stay near the soil surface in April and begin to fly in early or mid-May. Eggs are laid in June, and first instars are abundant in July and molt into second instars in early August. The second instars spend the first winter.

In the second year, second instars molt into third instars around the third week of June. The third instars spend the winter of the second year. In the third year, the third instars develop into prepupae in late June. Pupation occurs around mid-July. Teneral adults begin to emerge from the pupae in early August and stay in the soil until the following spring.

Our results indicate that *P. anxia* has a three-year life cycle in Quebec, which confirms the earlier conclusions of Hammond (1940,1948a), Maheux and Gauthier (1944) and Jarvis (1966).

4. Seasonal History of *P. anxia* in Pastures at Valleyfield and Nicolet

Results of periodic soil samplings from Valleyfield and Nicolet are presented in Tables 12 and 13 , respectively.

In Valleyfield, almost all specimens found in 1976 were in the second and third larval stages (Figure 24). In the following year, prepupae and pupae were the stages found during June and July. By August, 1977, teneral adults were the most common stage encountered. These results indicate that in 1977 the population of *P. anxia* in Valleyfield was in the third year of the life cycle. The population density of *P. anxia* in Valleyfield decreased from 3.3 individuals/0.09 sq m to 0.6 individuals/0.09 sq m, between September of 1976 and May of 1977; this suggests that there was a high mortality (about 82%)

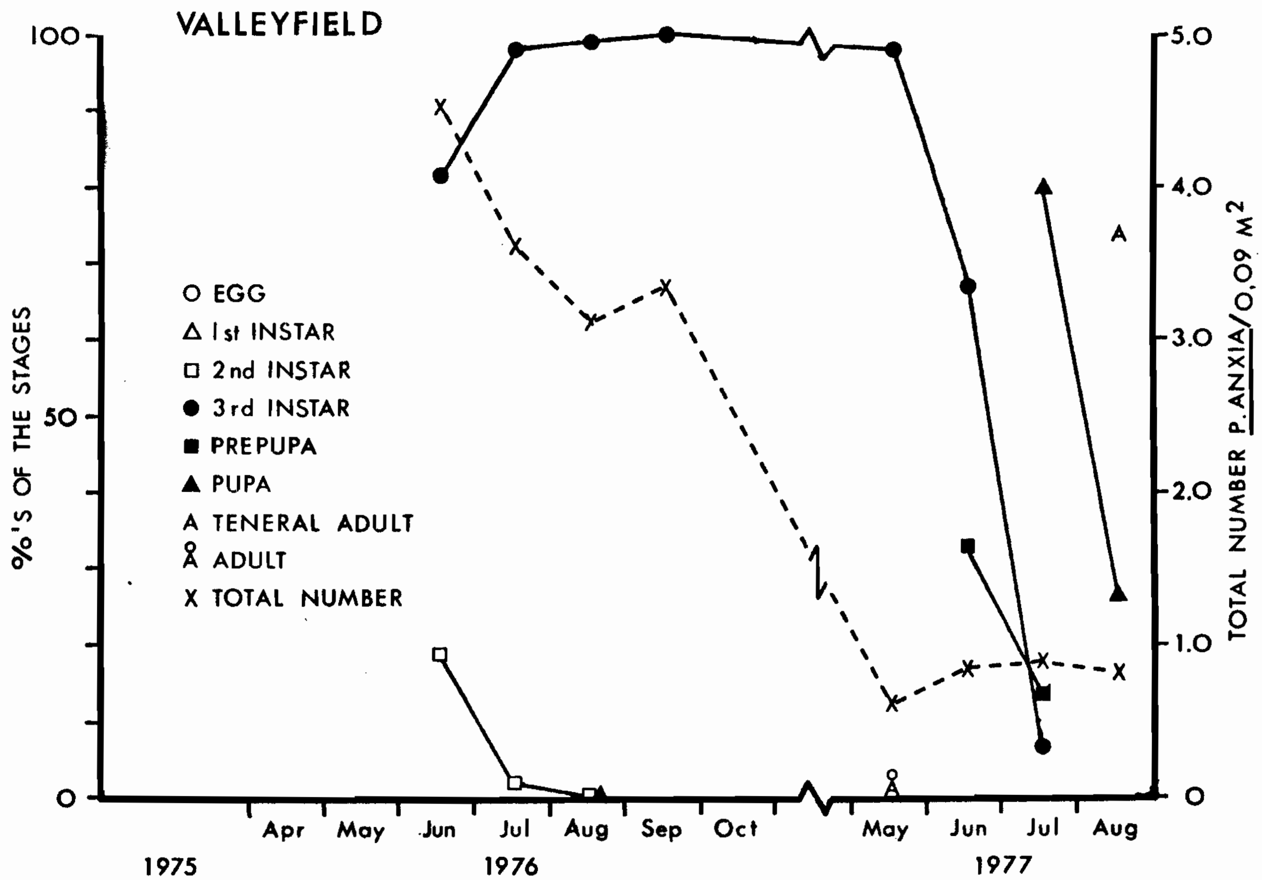
Table 12. Number of various life stages of <u>Phyllophaga anxia</u> found by soil sampling in a pasture in Valleyfield, 1976 to 1977.

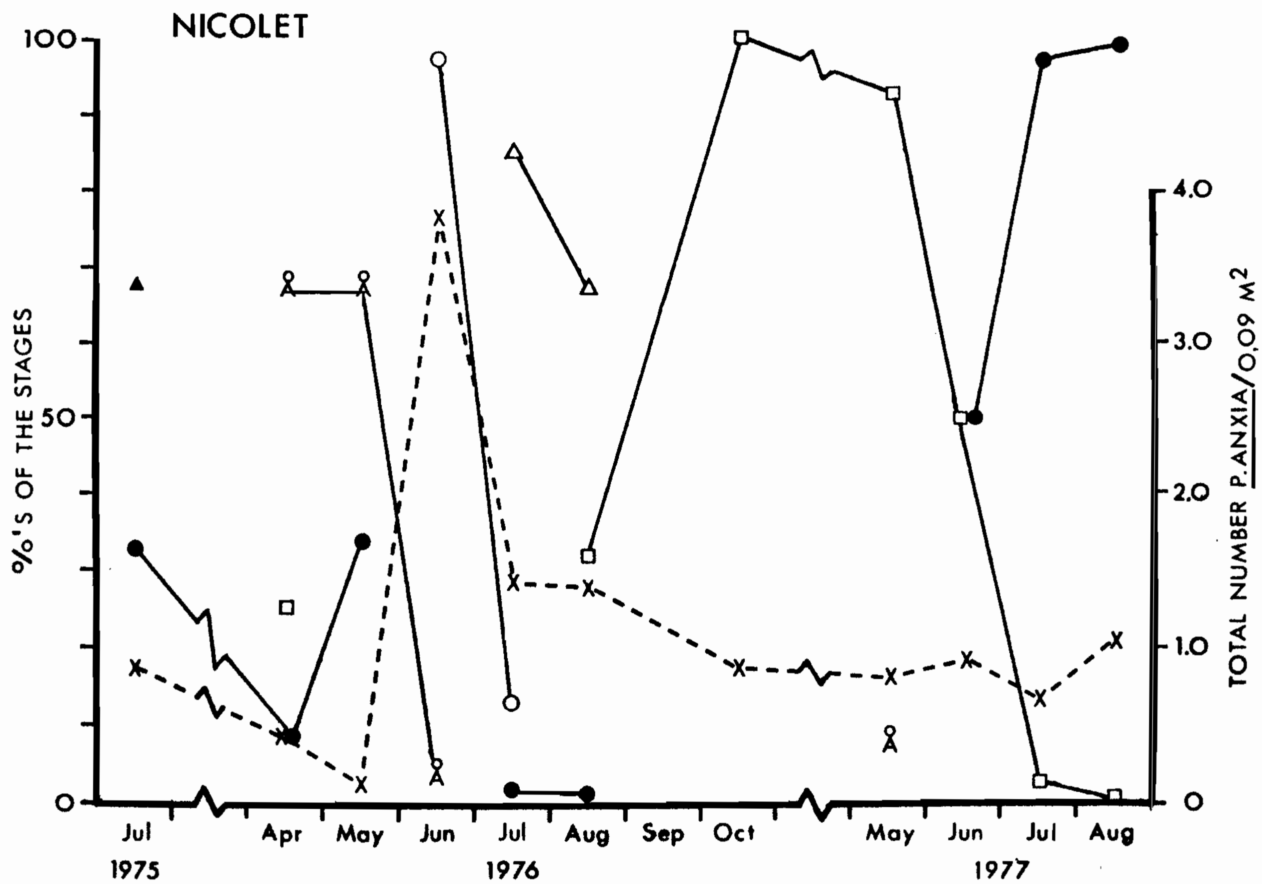
Date	No. Found							No. Quadrats Taken
	Egg	1st L.	2nd L.	3rd L.	Prepupa	Pupa	Adult	
1976								
June 21			61	278				75
July 5			3	192				65
July 20			4	144				30
Aug. 3			2	90		1		39
Aug. 11				122				38
Aug. 24				118		1		30
Sept. 7				231				55
Sept. 22				43				27
1977								
May 9				31			1	50
May 31				17				30
June 13				49				50
June 27				6	30			50
July 11				6	12	25		50
July 25						46		50
Aug. 8						22	34	50
Aug. 23							27	50

Table 13. Number of various life stages of *Phyllophaga anxia* found by soil sampling in a pasture in Nicolet, 1975 to 1977.

Date	No. Found							No. Quadrats Taken
	Egg	1st L.	2nd L.	3rd L.	Prepupa	Pupa	Adult	
1975								
July 11				14		29		50
1976								
Apr. 29			3	1			8	30
May 25				1			2	30
June 10	67						2	18
July 13	7	26						17
July 27	1	27		1				27
Aug. 17		27	18	1				36
Aug. 31		30	9					25
Oct. 1			35					40
1977								
May 11			38				3	50
June 6			31	1				50
June 20			15	45				50
July 4			2	23				50
July 18				42				50
Aug. 2			1	56				50
Aug. 15				48				50

Figure 24. Population development and seasonal occurrence of the life stages of *Phyllophaga anxia* (LeConte) in pastures at Valleyfield and Nicolet.


VALLEYFIELD
100
50
0
%'S OF THE STAGES
5.0
4.0
3.0
2.0
1.0
0
TOTAL NUMBER P. ANXIA/0,09 M²
O EGG
△ 1st INSTAR
□ 2nd INSTAR
● 3rd INSTAR
■ PREPUPA
▲ PUPA
A TENERAL ADULT
ADULT
X TOTAL NUMBER
Apr May Jun Jul Aug Sep Oct May Jun Jul Aug
1975
1976
1977


NICOLET
100
50
0
%'S OF THE STAGES
4.0
3.0
2.0
1.0
0
TOTAL NUMBER P. ANXIA/0,09 M²
Jul Apr May Jun Jul Aug Sep Oct May Jun Jul Aug
1975
1976
1977

for the third instar grubs during the winter months of 1976-1977. Thereafter, there was not much change in the population density during the prepupal, pupal and teneral adult stages (Figure 24).

In Nicolet, specimens found in 1976 comprised mainly adults, eggs, and first and second instar grubs (Figure 24). In 1977, second instar grubs were the most common stage in May. By August, 99 per cent of the specimens collected were in the third larval stage. These results indicate that in 1977 the population was in the second year of the life cycle. The initial increase of population density observed was due to the large numbers of eggs deposited by the adults (Figure 24). Thereafter, the population density dropped from 3.7 individuals/ 0.09 sq m to 1.2 individuals/0.09 sq m between June and July of 1976. This apparent egg mortality may have been due to unfavourable environmental conditions, such as insufficient moisture, for successful egg development and eclosion. For example, there was only 63.0 mm of precipitation in June of 1976 in the Nicolet area (Monthly Record, Meteorological Observations in Canada, Atmospheric Environment Service, Ottawa), and this may not have been sufficient to provide the moisture conditions of 20 to 73 per cent of soil saturation required for egg eclosion of *Phyllophaga* spp. (Sweetman, 1931). The population density thereafter remained steady for the remainder of 1976 and 1977 (Figure 24).

The results obtained with the Valleyfield and Nicolet populations indicate that egg mortality and overwintering mortality of third instars were the two major factors that reduced population density in these particular habitats during 1976 and 1977.

5. Broods

Davis (1918) used the term "brood" to indicate the developmental cycle of *Phyllophaga* spp. in the northeastern United States. Insects of the same brood were in the same developmental stage and a given stage would reappear at three-year intervals in a given locality.

In Quebec and Ontario, it is claimed that three broods of *P. anxia* exist, located in three well-defined life-cycle zones, with little overlapping of broods (Hammond, 1931,1948a; Hammond and Maheux, 1934), or no overlapping of broods within each zone (Hammond, 1954).

In the present study, based on the collection data from each study area, it shows that most of the study areas had overlapping of broods (Table 14), but populations in Valleyfield, L'Acadie, L'Assomption and Nicolet had a dominant brood with very little overlapping of other broods. By using the present results (Table 14), a forecast of broods of *P. anxia* in southern Quebec is presented in Figure 25. The maps of brood zones as

Table 14. Broods of *Phyllophaga anxia* in southern Quebec, 1975 to 1977.

Vicinity	Year	PERCENTAGE OF STAGES FOUND IN EACH BROOD[1]		
		1st Year	2nd Year	3rd Year
Ste-Clothilde	1975	55.6	44.4	
	1976		97.5	2.5
Cowansville	1975	50		50
	1976	11.1	88.9	
Valleyfield	1976		97.4	2.6
	1977	0.3		99.7
L'Acadie	1975	99.7		0.3
	1976	8.1	91.9	
	1977	1.2	0.4	98.5
Lennoxville	1976		83.3	16.7
	1977		10	90
Dorion	1976		89.1	10.9
Ste-Anne-de-Bellevue	1975	55.0	33.8	11.2
	1976		100	
	1977		54.5	45.5
Pointe Claire	1975	93.8	6.3	
	1976		46.9	53.5
St-Hyacinthe	1975			100
	1976	31.6	63.2	5.2
	1977	24.4		75.6
L'Assomption	1975	80.0	20.0	
	1976		100	
	1977	4.7		95.3
Lavaltrie	1975	75		25
	1976		100	
Nicolet	1975			100
	1976	97.4	1.9	0.8
	1977	1.2	98.8	

Cont'd

Table 14 (cont'd)

Vicinity	Year	PERCENTAGE OF STAGES FOUND IN EACH BROOD[1]		
		1st Year	2nd Year	3rd Year
St-Augustin	1976	27.8	72.2	
	1977	38.1	28.6	33.3
La Pocatiere	1977	65	18.3	16.7
Rimouski	1977	43.8	16.3	40.0
Normandin	1976	4.5	72.7	22.7
	1977	37.0	5.6	57.4
Les Buissons	1976	6.7	6.7	86.7
	1977	28	3	69

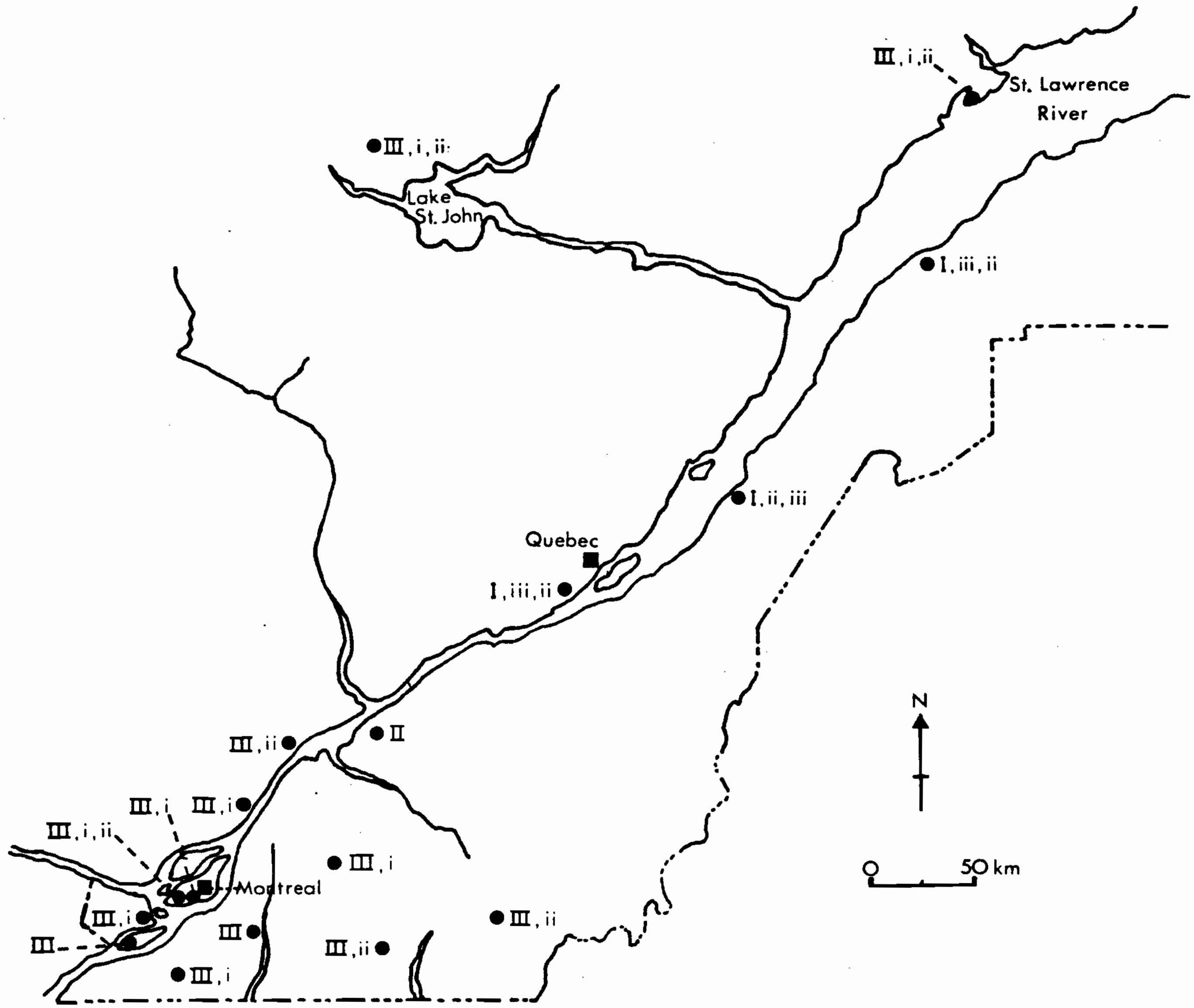
[1]Refer to Table 9 for numbers of *P. anxia* collected in each locality.

Figure 25. A forecast of broods of *Phyllophaga anxia* (LeConte) in southern Quebec for 1980.[a,b,c]

a. I,II,III represent the brood of *P. anxia* in each vicinity in the first, second and third year of development of the life cycle, respectively.

b. Roman numerals in lower case represent *P. anxia* in that brood which is only present in a small proportion.

c. For example, III i means the majority of individuals of *P. anxia* in that vicinity are in the third year of development of the life cycle. The population is also mixed with a small proportion of individuals of *P. anxia* which are in the first year of the development of the life cycle.



St. Lawrence River
Lake St. John
Quebec
Montreal
III, i, ii
III, i, ii
I, iii, ii
I, ii, iii
I, iii, ii
II
III, ii
III, i
III, i
III, i, ii
III, i
III, i
III
III
III, i
III, ii
III, ii
III, i
N
0
50 km

suggested by earlier investigators (Hammond, 1931,1948a,1954; Hammond and Maheux, 1934) cannot be used generally for white grub control or damage avoidance by crop rotation. In the future, any locality which is being questioned with respect to the brood of *P. anxia* should preferably be surveyed by taking soil samples before the end of May.

VI. SPATIAL DISTRIBUTION OF *PHYLLOPHAGA ANXIA* (LECONTE) IN PASTURES, WITH AN ESTIMATE OF OPTIMUM SAMPLE SIZE

A. INTRODUCTION

The description of the pattern of distribution of insects in space has been termed as spatial pattern or spatial distribution (Harcourt, 1965) or dispersion (Southwood, 1978).

The spatial distribution of the insect in the field must be determined in order to decide on an optimum sample size for estimate of population density (Sevacherian and Stern, 1972; Karandinos, 1976).

As part of the studies on the bionomics of *P. anxia* in southern Quebec, a sampling program was carried out in order to investigate the spatial distribution of *P. anxia*, and determine the number of samples required to estimate the density of *P. anxia* with a given level of accuracy.

B. MATERIALS AND METHODS

The work was conducted during 1977 with two populations of *P. anxia* in pastures at Valleyfield (45°15'N, 74°0'W) and Nicolet (46°13'N, 72°37'W) in southern Quebec. In the Valleyfield plot, the soil was well-drained sandy loam. The plot size was 0.08 hectare (40 x 20 m). In 1977, the population of *P. anxia* was in the third year of the life cycle so that there were third instar grubs in June, prepupae in July, pupae in July and early August, and teneral adults in August. In the Nicolet plot, the soil was also sandy loam with a higher content of humus. The plot size was 0.13 hectare (85 x 15 m). In 1977, the population of *P. anxia* was in the second year of the life cycle giving second instar grubs in May, June and early July, and third instar grubs in June, July, August and September.

A shovel was used for sampling soil in the present study as recommended by Burrage and Gyrisco (1954) for sampling pasture fields. A sample unit of 0.09 sq m (one square foot) dug to a depth of 30 cm, was utilized, as recommended for most efficient sampling of soil-inhabiting stages of *P. fusca* (Froelich) and *P. anxia* (Guppy and Harcourt, 1973), and other scarabs (Fleming and Baker, 1936; Burrage and Gyrisco, 1954).

On each sampling occasion, 50 quadrats were taken in a systematic pattern in the plot. In Valleyfield, quadrats were taken in five lines across the field, with 10 quadrats per line. The distance between the first and the successive sample was approximately 3 m. In Nicolet, due to the long, narrow shape of the plot, quadrats were taken in three lines. Seventeen quadrats were taken from the two outside lines, and sixteen quadrats were taken from the center line. The distance between the first and the successive samples was approximately 4 m. All the insects were found by sorting through the sod and soil by hand. Numbers of insects from each quadrat were recorded on a chart in their actual positions.

Theoretical distributions commonly used to describe insect spatial distribution are the normal, Poisson, positive, binomial, logarithmic, lognormal, Neyman type A, and negative binomial; the first three are random, the others are nonrandom (Waters, 1959). Poisson and negative binomial distributions have been widely accepted for fitting with the field counts (Anscombe, 1949; Wadley, 1950; Bliss and Fisher, 1953; Bliss, 1958; Waters and Henson, 1959; Harcourt, 1960,1961,1963,1965; Ives and Warren, 1965; Guppy and Harcourt, 1970; Sevacherian and Stern, 1972; Hammond and Pedigo, 1976; Doane, 1977; Simonet and Pienkowski, 1979; Jackson, 1979). In the present study, the counts of P. anxia from each sampling date for both Valleyfield and Nicolet were fitted to the Poisson and negative binomial distributions.

The expected frequencies of Poisson distribution were obtained by multiplying the number of samples (50) by the respective Poisson probability mass function, which was calculated by use of a preprogrammed scientific notation calculator (Commodore (R) SR 9190R, Commodore Business Machines Ltd., Agincourt, Ontario). A chi-square test for goodness-of-fit was then used for comparing the observed and expected frequencies (Zar, 1974; Southwood, 1978).

Estimates of parameter k of the negative binomial distribution were calculated from one of the three methods listed by Southwood (1978). The appropriate method was chosen with an efficiency of 90% or better, based on the criteria suggested by Bliss and Fisher (1953). The expected frequencies of the negative binomial distribution were calculated by the method described in detail by Bliss and Fisher (1953). Then, a chi-square test for goodness-of-fit was also used for comparing the observed and expected frequencies.

Morisita (1950) proposed an index of dispersion (I) for expressing the distribution of individuals. Recently, Myer (1978) pointed out that the Morisita's coefficient (index) of dispersion was not influenced by population density and is a good measure of dispersion. In the present study, Morisita's index of dispersion was calculated and tested for significance with the procedure of Morisita (1950).

There are several methods to determine optimal sample size for a specific distribution (Karandinos, 1976). In the present study, estimates by the use of coefficient of variability (C.V.) was employed with the use of the formulae as listed by Karandinos (1976).

C. RESULTS AND DISCUSSION

In 1977, the total counts of P. anxia found in the pastures in Valleyfield (Figure 26) and Nicolet (Figure 27) were apparently not distributed in random.

Besides the chi-square test for goodness-of-fit, three other methods were used to determine the type of spatial distribution. These were variance/mean ratio, k of the negative binomial, and Morisita's index of dispersion.

For the variance/mean ratio, if the variance is equal to the mean, this implies the distribution is random; if the variance is less than the mean, this implies a more regular (or uniform) distribution than Poisson series; and if the variance is larger than the mean, then the distribution is contagious (Southwood, 1978).

The parameter k has been suggested by Waters (1959) as a valid mean of aggregation. The larger the value of k (over about 8) indicates that the distribution is approaching a random distribution; whereas the smaller the value k the greater the extent of aggregation (Waters, 1959; Harcourt, 1961; Southwood, 1978).

For Morisita's index of dispersion, when the dispersion of individuals in an area are at random (Poisson), the index will give a value of unity; when the individuals are distributed

Figure 26. The distribution of total seasonal counts of _Phyllophaga_ _anxia_ in a pasture in Valley-field, 1977.

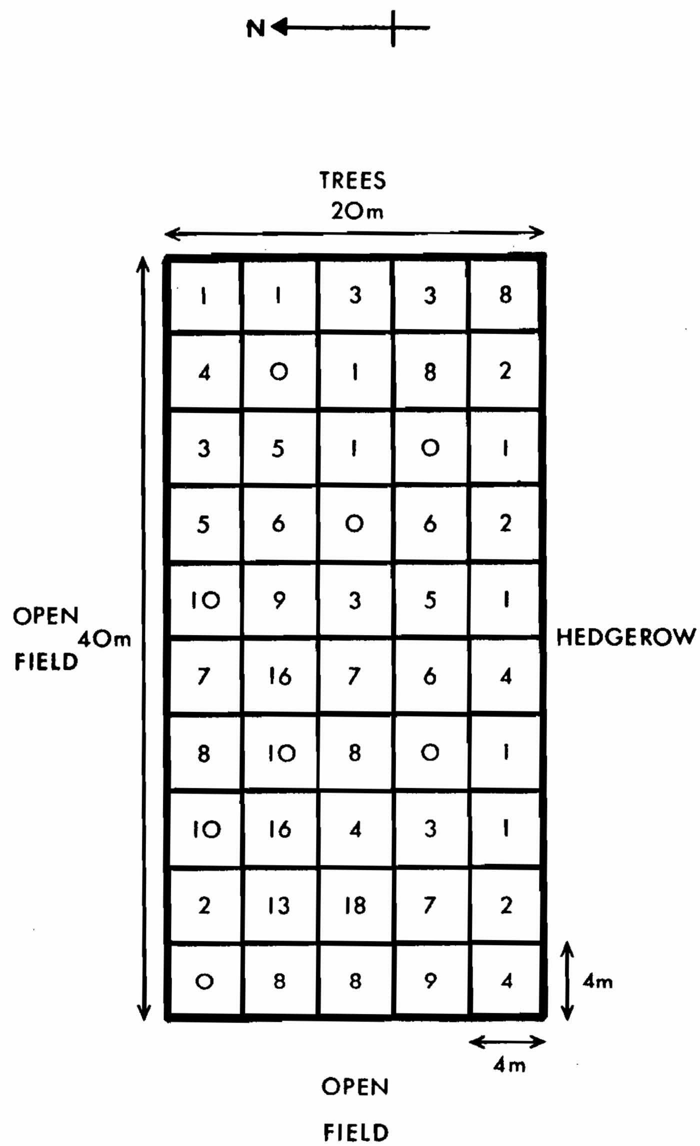
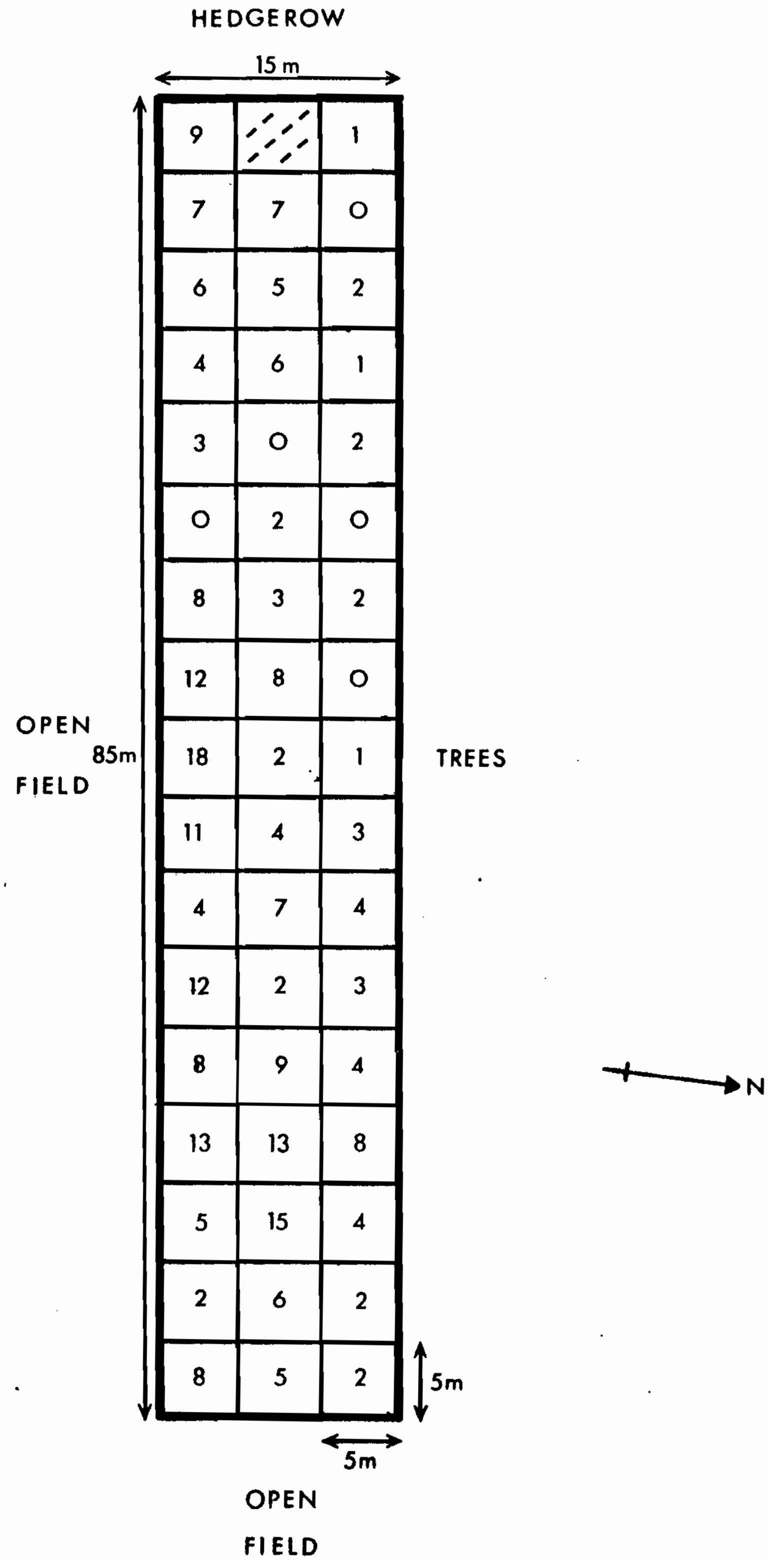

N
TREES
20m
OPEN FIELD
40m
HEDGEROW
1 1 3 3 8
4 0 1 8 2
3 5 1 0 1
5 6 0 6 2
10 9 3 5 1
7 16 7 6 4
8 10 8 0 1
10 16 4 3 1
2 13 18 7 2
0 8 8 9 4
4m
4m
OPEN
FIELD

Figure 27. The distribution of total seasonal counts of *Phyllophaga anxia* in a pasture in Nicolet, 1977.


HEDGEROW
15 m
9 1
7 7 O
6 5 2
4 6 1
3 O 2
O 2 O
8 3 2
12 8 O
18 2 1
11 4 3
4 7 4
12 2 3
8 9 4
13 13 8
5 15 4
2 6 2
8 5 2
OPEN
85m
FIELD
TREES
N
5m
5m
OPEN
FIELD

uniformly (binomial), the index will be less than one; when the distribution of individuals is contagious (negative binomial), the index will be greater than one (Morisita, 1959).

In Valleyfield, all the variance/mean ratios were greater than one (Table 15). This indicates that the individuals in the population were not distributed randomly. Morisita's indices were greater than one and significantly different from the random expectations. All the k values were less than 8, this further indicated that the individuals were not distributed randomly. For the sampling of June 13, the result of chi-square test showed that the data had no significant difference from both Poisson and negative binomial distributions. Waters (1959) noted that for many insects there would be some field counts which apparently fit both the Poisson and negative binomial distributions. Waters (1959) further suggested that in these cases, the value of k could be used for indicating the relative degree of aggregation. As in the present case of June 13, k was 0.86, this indicates that there was aggregation. In addition, both the variance/mean ratio and Morisita's index were also suggested of aggregation. For the sample of August 8, the result of chi-square test showed that the data differed significantly from both the Poisson and negative binomial distributions. Nevertheless all the other tests showed that the distribution was not at random. Field counts of Phyllophaga spp. that did not fit

Table 15. Determination of the spatial distribution of *Phyllophaga anxia* in pasture, by variance/mean ratio, Morisita's index, k of the negative binomial series, and the chi-square test for goodness-of-fit, and with an estimate of optimum sample size, Valleyfield, 1977.

					Goodness of Fit[a]					Optimal Sample Size		
					Poisson		Negative Binomial			10%	20%	30%
Date	$\bar{x} \pm SE$	s^2	$s^2/\bar{x}$	I	df	2	df	k	2	C.V.	C.V.	C.V.
June 13	0.98±0.21	2.26	2.31	2.34**	2	5.81NS	3	0.86	0.79NS	218	55	24
										(102)	(25)	(11)
June 27	0.72±0.15	1.14	1.58	1.83**	2	10.58**	2	0.79	2.13NS	265	66	29
July 11	0.86±0.14	0.98	1.14	1.50*	2	3.41NS	1	6.16	1.47NS	133	33	15
										(116)	(29)	(13)
July 25	0.92±0.22	2.48	2.70	2.85**	2	14.70**	3	0.56	1.71NS	287	72	32
Aug. 8	1.12±0.20	1.90	1.70	1.62**	3	30.06**	3	0.66	16.51**	241	60	27
										(89)	(22)	(10)
Aug. 23	0.54±0.15	1.15	2.13	2.13**	1	13.81**	2	0.28	5.16NS	542	136	60

[a]NS - Not significantly different at the 0.05 level.
* - Significantly different at 0.05 level.
** - Significantly different at 0.01 level.

[b]Numbers in parentheses are sample estimates based on Poisson distribution.

both Poisson and negative binomial distributions had also been reported in Manitoba (Ives and Warren, 1965) and western Quebec (Guppy and Harcourt, 1970).

In Nicolet, most of the counts did not differ significantly from the theoretical negative binomial distribution (Table 16). On July 4, due to the low number of frequency classes found in the field on that date, the results could not be evaluated meaningfully by chi-square test for goodness-of-fit. Similar results had been reported on the redbacked cutworm, Euxoa ochrogaster (Guenee) by Danielson and Berry (1978), also on white grubs, Phyllophaga spp. by Ives and Warren (1965). On August 15, the data did not differ significantly from both Poisson and negative binomial distributions. All the other tests showed that the spatial distribution was not random.

Optimum sample sizes were calculated for Valleyfield (Table 15) and Nicolet (Table 16) with the coefficient of variability (CV) of 10, 20, 30%. In general, in Valleyfield, six man-hours were needed to collect and examine the fifty sample units in the field. This was equivalent to 7.2 man-minutes per sample. For example, in Valleyfield, on June 13, for estimating the mean number of P. anxia per 0.09 sq m with a coefficient of variability of 10%, I would have had to take 218 sample units. This would have required 26.16 man-hours. For the same date, for a coefficient of variability of 30%, only 24 sample units

Table 16. Determination of the spatial distribution of Phyllophaga anxia in pasture, by variance/mean ratio, Morisita's index, k of the negative binomial series, and the chi-square test for goodness-of-fit, and with an estimate of optimum sample size, Nicolet, 1977.

Date	$\bar{x} \pm SE$	s^2	$s^2/\bar{x}$	I	Goodness of Fit[a]					Optimal Sample Size		
					Poisson		Negative Binomial			10%	20%	30%
					df	2	df	k	2	C.V.	C.V.	C.V.
June 6	0.76±0.19	1.82	2.39	2.84**	2	12.70**	3	0.45	2.88NS	354	88	39
June 20	1.20±0.22	2.41	2.01	1.84**	3	15.67**	4	1.05	3.30NS	179	45	20
July 4	0.36±0.10	0.48	1.33	1.96NS	1	1.11NS	0	19.0	- [c]	(278)	(69)	(31)
July 18	0.84±0.25	3.08	3.66	4.18**	2	11.19**	4	0.39	2.96NS	375	94	42
Aug. 2	1.52±0.27	3.76	2.48	1.96**	3	29.09**	5	0.73	9.95NS	203	51	24
Aug. 15	0.88±0.18	1.54	1.75	1.85**	2	3.82NS	2	1.16	0.93NS	200	50	22
										(114)	(28)	(13)

[a]NS - Not significantly different at 0.05 level.
 ** - Significantly different at 0.01 level.

[b]Numbers in parentheses are sample estimates based on Poisson distribution.

[c]Significance cannot be evaluated.

would need to have been taken. This would have required 2.88 man-hours.

In general, a larger number of sample units was required for the similar level of precision in the present study than that reported by Guppy and Harcourt (1973). This might be due to the lower density of *P. anxia* in my study areas than in theirs.

VII. AN INVESTIGATION OF OVERWINTERING MORTALITY OF THE THIRD INSTAR GRUBS OF *PHYLLOPHAGA ANXIA* (LECONTE)(COLEOPTERA: SCARABAEIDAE) IN SOUTHERN QUEBEC

A. INTRODUCTION

Phyllophaga anxia (LeConte) has a three-year life cycle. The first, second and third winters are passed by the second, third instars and the teneral adults, respectively (Hammond, 1940, 1948a; Maheux and Gauthier, 1944; Guppy and Harcourt, 1970). The depth of overwintering of *P. anxia* varies according to the locality. In Manitoba, grubs of *P. anxia* went down to an average depth of 112 cm in dry woods and from 36 to 64 cm in wet land for overwintering (Criddle, 1918). In eastern Canada, grubs of *P. anxia* overwintered at depths varying from 15 to 38 cm (Hammond, 1940). In western Quebec, the second and third instars of *P. fusca* and *P. anxia* passed the winter at depths of 20 to 61 cm (Guppy and Harcourt, 1970). In Ontario, third instars of *P. anxia* are capable of surviving the winter at a depth of 60 cm (Sutton and Stone, 1975).

Fall plowing has been suggested for control of white grubs (Criddle, 1918; Davis, 1918; Hammond, 1933,1960; Anonymous, 1979a,1979b). The usual depth of plowing is about 15 cm (McColloch *et al.*, 1928). Thus it is important to know if the grubs could overwinter above the plow-line in this region. An experiment was set up in Ste-Anne-de-Bellevue for the winter of 1976-1977 to determine the overwintering mortality of the third instar larvae buried to a depth above the plow-line.

B. MATERIALS AND METHODS

In 1976, second year third instar grubs of *P. anxia* collected between July and September inclusively from Valleyfield were divided into a treated group and a check group. Clay flower pots (top diameter 20 cm, height 20 cm) were filled to about 3 cm from the rim with pasteurized sandy loam soil and sown with seeds of a mixture of Kentucky bluegrass and creeping red fescue. Holes at the bottom of the pots were blocked by using pieces of broken pots. Ten grubs were introduced into each pot at about 1 cm below the soil surface. A total of 18 pots with grubs were prepared. These were kept in a rearing room at 24°C, 25-30% R.H. and 16-hour photophase until October 12, 1976. Pots were watered as necessary to keep the soil moist. On the above date, half of the pots (treated group) was transferred outdoors to a vacant land and sunk to ground level. Pots were covered with wire screen (with 6 mm a mesh) to prevent entry of predators. All pots from the treated group were recovered in the following spring. Numbers of live individuals and stages of development were recorded for both treated and check group on the same day. The official records of Daily Soil Temperature Data, Environment Canada, of the Ste-Anne-de Bellevue station were used in the present study.

C. RESULTS AND DISCUSSION

Pots from the treated group were brought into the laboratory on April 27, 1977. Examinations of the insects in the pots of treated and check groups were carried out promptly. Results of the experiment are summarized in Table 17. Mean mortalities of treated and check groups were 51% and 54%, respectively. A Student's t-test showed that there was no significant difference between the mean mortalities of these two groups at the 0.05 level. There were many obvious differences in the physical conditions between the field (treated) and the laboratory (check), such as fluctuating winter temperatures (Table 18) versus constant 24^{o}C in the laboratory, natural winter precipitation versus artificial watering, and winter short-day photophase versus 16-hour photophase in the laboratory, but the mortalities were similar. However, the mortality factors during the winter may have been very different. Part of the mortality in the laboratory may be due to the artificial conditions which might have disrupted the normal physiological process of diapause, whereas field mortality could have been due to a number of unknown factors. The problem of diapause of *P. anxia* has

Table 17. Results of an overwintering experiment on third instars of *Phyllophaga anxia*.

Replicate[1]	Group	No. Started	No. found alive in spring 3rd L.	Prepupa	Pupa	Total	% Mortality[2]
I (Jul.20,76)	Treated	20	2	-	-	2	90
	Check	20	3	-	-	3	85
II (Aug.3,76)	Treated	10	7	-	-	7	30
	Check	10	1	2	3	6	40
III (Aug.24,76)	Treated	20	13	-	-	13	35
	Check	20	3	2	4	9	55
IV (Sept.7,76)	Treated	30	18	-	-	18	40
	Check	30	2	8	8	18	40
V (Sept.22,76)	Treated	10	4	-	-	4	60
	Check	10	-	2	3	5	50

[1]Date within parentheses represents date of field collection of the experimental insects.

[2]No significant difference between the mean mortalities of the treated and check groups at the 0.05 level, Student's t-test.

not been investigated.

For the winter of 1976-1977 in Ste-Anne-de-Bellevue, the lowest soil temperature was -6.7°C recorded at a depth of 20 cm under a snow cover of 15.2 cm on both December 31, 1976 and January 1, 1977. The coldest temperatures during the winter of 1976-1977 were always recorded at a depth of 20 cm (Table 18). In the spring, 92 per cent of the live *P. anxia* were found within 3 cm of the bottom of the pots of both groups and it is assumed that during the winter the insects also stayed near there. The grubs therefore would be about 14 cm from the soil surface. Sweetman (1931) reported that young grubs (unspecified stage) of *Phyllophaga* sp. were able to withstand temperature of -4.0°C in Minnesota. Granovsky (1956) stated that grubs of *Phyllophaga* spp. might become lethally frozen with winter soil temperature of about -4° or -5°C. Sutton and Stone (1975) demonstrated that 75% of the third instars of *P. anxia* overwintered successfully near Ottawa, Ontario, in cages at a depth of 60 cm.

In the present study, all 44 individuals of *P. anxia* that survived the winter in the field (treated group) remained as third instars (Table 17). Among the 41 individuals that survived in the laboratory during the same period, 78% (32 individuals) had developed into either prepupal or pupal stage.

Table 18. Monthly mean soil temperatures in Ste-Anne-de-Bellevue, Oct. 1976 to Apr. 1977.[1,2]

Depth (cm)	Mean Temperature (C°)						
	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.
5	6.5	1.6	-0.9	-1.7	-1.3	0.2	3.9
10	6.9	1.8	-0.8	-2.1	-1.7	-0.6	3.3
20	5.6	0.4	-3.1	-4.3	-3.9	-2.3	1.6
50	7.6	2.6	-0.3	-2.2	-2.3	-2.2	0.4
100	9.7	5.4	2.6	0.6	0	-0.7	0.4

[1]Based on the official records of Daily Soil Temperature Data, Environmental Canada, of the Ste-Anne-de-Bellevue Station.

[2]Temperatures of 8 a.m. are used.

VIII. A SURVEY OF NATURAL ENEMIES OF PHYLLOPHAGA ANXIA (LECONTE) (COLEOPTERA: SCARABAEIDAE) IN SOUTHERN QUEBEC

A. INTRODUCTION

The common June beetle, *Phyllophaga anxia* (LeConte), is an indigenous pest of agricultural crops in North America (Hammond, 1948a; Ritcher, 1949; Neiswander, 1963). Chlorinated hydrocarbon insecticides such as aldrin provided effective and economical control for *P. anxia* larvae in soils in the past (Hammond, 1948a,1949,1960; Polivka, 1965; Fowler and Wilson, 1971,1974). However, in more recent years, several *Phyllophaga* spp. have developed resistance to organochlorines in the United States (Frankie *et al*., 1973; Teetes, 1973; Fuchs *et al*., 1974; Plapp and Frankie, 1976), and this occurrence, together with persistence problems, has encouraged a search for alternative chemicals and supplementary control methods for this pest. Among the several possible alternatives, biological control by parasites, predators and pathogens has long been recognized as an important component of a pest management program (Anonymous, 1969). There is scattered information on the natural enemies of *P. anxia* (Davis, 1919; Petch and Hammond, 1925,1926; Dutky, 1941,1963; Dutky and Gooden, 1952; Hammond,1944b,1961; Jarvis, 1966; Tashiro and Steinkraus, 1966; Berberet and Helms, 1969, 1970), but knowledge about the natural enemies of *P. anxia* in the Province of Quebec is limited to the reports by Petch and Hammond (1925,1926) and Hammond (1944b, 1961).

To supplement this information, a survey of the natural enemies of *P. anxia* was conducted in southern Quebec from 1975 to 1977.

B. MATERIALS AND METHODS

Adults of *P. anxia* were collected using blacklight traps, and grubs were collected by digging using a shovel. Adults and grubs were kept in a rearing room at 24^{o}C, 25-30% RH, with 16 hours of light for emergence of parasites or appearance of diseases. Usually 20 adults from the same collection were kept in a covered clay flower pot (diameter 20 cm, height 20 cm) containing sterilized soil to a depth of 16 cm. Leaves of white birch were supplied as food. Grubs were kept individually in covered styrofoam cups (top diameter 6 cm, height 6 cm) with a piece of moistened dental roll to provide moisture. Grubs were fed lettuce and this also provided additional moisture. Predatory insects such as carabids and asilids found feeding on, or in close proximity of *P. anxia* in the field were collected. When dead or diseased insects were found, microorganisms recovered were tentatively identified using the diagnostic techniques of Weiser and Briggs (1971) and Thomas (1974). Dissected fat bodies, mid-gut and entire grubs were fixed in alcoholic Bouin fluid for histological studies. After fixation, specimens were dehydrated through an ethanol series, cleared in benzene, infiltrated and embedded in Paraplast$^{(R)}$ (m.p. 56-57^{o}C, Brunswick Co., St. Louis, Mo., U.S.A.). Sections were cut at 5-8 μ with a rotary microtome and then affixed on slides coated with Mayer's albumin, allowed to dry and stained by the method of Hamm (1966).

Parasitic and predatory insects were identified by taxonomists of the Biosystematics Research Institute, Agriculture Canada, Ottawa, Ontario. Mites were identified by Dr. C.Y. Oseto, North Dakota State University, Fargo, North Dakota. Microorganisms and nematodes were identified by Mr. G.M. Thomas and Dr. G.O. Poinar, Jr., respectively, Division of Entomology and Parasitology, University of California, Berkeley, California.

C. RESULTS AND DISCUSSION

The blacklight traps collected 2968 individuals of *P. anxia* adults from 1975 to 1977. Of these, 1670 individuals were kept for recovery of parasites. I examined a total of 5440 sample units and found 4351 individuals of soil-inhabiting stages of *P. anxia*, and of these, 2893 individuals were kept individually in containers for recovery of parasites and diseases.

A total of sixteen species of parasitic and predatory insects, two species of mites, one species of nematode, and six different microorganisms were found in the study (Table 19). Results for parasites and diseases are mainly based on the parasite emergence data and the disease symptoms which appeared during the laboratory rearing. This gives only qualitative information, as many host insects may have died before the parasites emerged or disease symptoms appeared. The quantitative aspect of the natural enemies of P. anxia is being investigated in 1979 by Mr. Tadeusz Poprawski, a postgraduate student in this department.

1. Insect Parasites

a. Diptera: Tachinidae

Table 20 shows the tachinid parasites reared from adults and grubs of P. anxia.

Eutrixa exilis Coq. was reared from adult of P. anxia (Figure 28). I found eight adults emerged from a single host cadaver, and dissection showed a further two intact pupae inside the dead host. This tachinid species has been reported as a parasite of P. anxia in Quebec (Petch and Hammond, 1925,1926) and Manitoba (Criddle, 1918). In the United States, it has been reared from P. anxia adults and fourteen other Phyllophaga spp. and it has been suggested that the tachinids oviposit on the beetles while they are on trees at night (Davis, 1919).

Table 19. Natural enemies of *Phyllophaga anxia* collected in southern Quebec, 1975 to 1977.

Organisms	Host Stage
Insect Parasites	
Diptera	
Tachinidae	
Eutrixa exilis Coq.	adult
Cryptomeigenia sp.	adult
Microphthalma michiganensis Tnsd.	larva
Ptilodexia sp.	larva
Hymenoptera	
Pelecinidae	
Pelecinus polyturator (Drury)	larva
Tiphiidae	
Tiphia sp.	larva
Insect Predators	
Diptera	
Asilidae	
Diogmites sp.	larva
Asilus sp.	larva
Coleoptera	
Carabidae	
Agonum placidum Say	larva
Carabus serratus Say	larva
Anisodactylus discoideus Dej.	larva
Pterostichus leconteianus Lutz.	larva
Chlaenius sericeus Forst.	larva
Harpalus caliginosus F.	larva
Harpalus longicollis Lec.	larva
Harpalus bicolor F.	larva
Acarines	
Acari	
Acaridae	
Tyrophagus sp.	larva, adult
Anoetidae	
unidentified anoetid mite	larva, adult
Pathogens	
Nematoda	
Diplogasteridae	
Mikoletzkya aerivora (Cobb)	larva

Table 19 (cont'd)

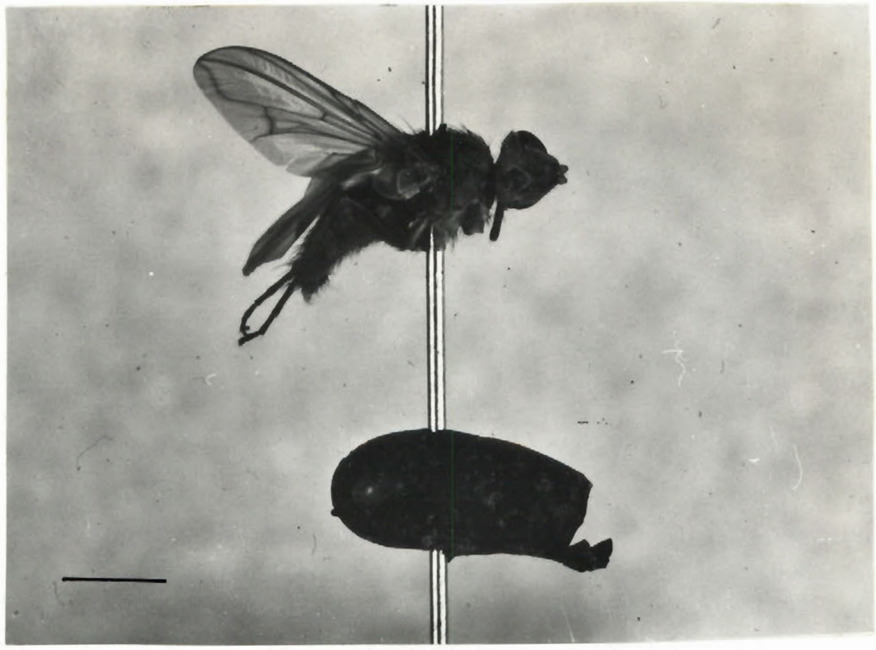
Organisms	Host Stage
Eubacteriales	
Bacillaceae	
Bacillus cereus Fr. & Fr.	larva
Deuteromycetes	
Moniliales	
Metarhizium anisopliae (Metsch.) Sorok.	larva, adult
Beauveria bassiana (Bals.) Vuill.	larva
Fusarium sp.	larva
Penicillium sp.	larva
Virus	
Entomopoxvirus (?)	larva

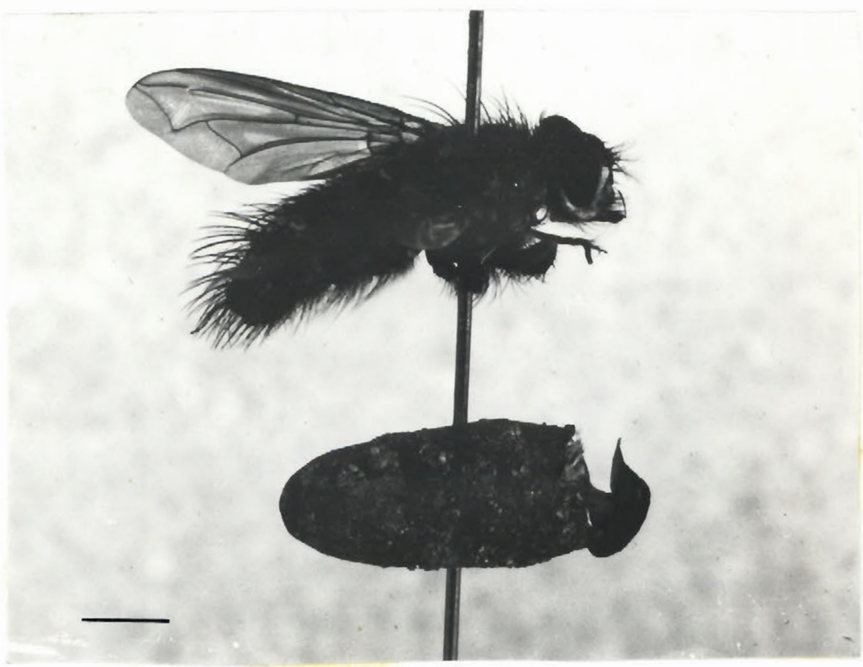
Table 20. Tachinid parasites recovered from *Phyllophaga anxia* adults and grubs collected in southern Quebec, 1975 to 1977.

Species	Location	Collection Date	Stage Found: Host	Stage Found: Parasite
Eutrixa exilis	Nicolet	May 11, 77	adult	pupa
Eutrixa sp.	Cowansville	May 18, 75	adult	pupa
Cryptomeigenia sp.	Cowansville	May 18, 75	adult	pupa
Microphthalma michiganensis	Les Buissons	June 12, 76	3rd L.	larva
Ptilodexia sp.	Valleyfield	Sept.23, 76	3rd L.	larva
Tachinid pupa	Nicolet	July 11, 75	adult	pupa
	Valleyfield	May 21, 76	adult	pupa
	Ste. Clothilde	May 27, 76	adult	pupa
	Dorion	June 4, 76	adult	pupa
	Les Buissons	June 12, 76	3rd L.	larva
	Valleyfield	June 21, 76	adult	pupa
	Valleyfield	July 5, 76	adult	pupa
	Valleyfield	Aug. 3, 76	adult	pupa

Figure 28. Adult and empty pupal case of *Eutrixa exilis* Coq. (scale: 2 mm).

Figure 29. Adult and empty pupal case of *Microphthalma michiganensis* Tnsd. (Scale: 2 mm).





One pupa of _Cryptomeigenia_ sp. was found in an adult _P. anxia_ and did not develop into the adult stage. Fifty per cent of the adult beetles of _Phyllophaga_ spp. were killed by _Cryptomeigenia theutis_ Walker in 1914 in Manitoba (Criddle, 1918). Females of _C. theutis_ oviposit in the side of abdomen of the host beetle, and the larvae enter and develop within the abdomen, and from one to seven larvae may develop in a single host. Host records include thirteen _Phyllophaga_ spp., but _P. anxia_ was not among them (Davis, 1919). Petch and Hammond (1925,1926) did not find this species in Quebec. If it does not exist or is rare in Quebec, it should be considered for introduction to supplement existing natural enemies of _P. anxia_ adults.

Microphthalma michiganensis Tnsd. was reared from a third instar of _P. anxia_ (Table 20, Figure 29). Davis (1919) reared _M. michiganensis_ from grubs of _P. anxia_ collected from Framingham, Mass., but considered it of little economic importance in controlling white grubs due to it's scarcity. In a loose muck soil of an unspecified area in Quebec, in 1924, 15.4 per cent of _P. anxia_ grubs were parasitized by _M. michiganensis_ (synonym, _M. phyllophagae_ Curran) (Petch and Hammond, 1925), but in 1925, the level of parasitism was much lower (Petch and Hammond, 1926). In the sandhills area of Nebraska, Jarvis (1966) reared four _M. michiganensis_ adults from the third instar larvae in three years and considered this parasite was not important during his studies.

Ptilodexia sp. was reared from a third instar of *P. anxia* (Table 20, Figure 30). This parasite had not been found in Quebec (Petch and Hammond, 1925,1926). *Ptilodexia abdominalis* Desv., and *P. harpasa* Walk. (Synonym, *P. tibialis* Desv.) were reared from *Phyllophaga* grubs in Manitoba (Criddle, 1918). Parasitism of *Phyllophaga* grubs by *P. harpasa* reached 35 per cent in a sample collected in Vermont (Davis, 1919).

Tachinid pupae were found in several occasions at different collection sites (Table 20) but failed to emerge as adults in the laboratory.

b. Hymenoptera: Pelecinidae

Previously, *Pelecinus polyturator* (Drury) has been reported as a parasite of grubs of *Phyllophaga* spp. (Davis, 1919; Petch and Hammond, 1925,1926; Brues, 1928; Hammond, 1944b), but the phylogenetic position is still a mystery (Masner, 1979). In the present study, only one prepupa of *P. polyturator* (Figure 31) was found on August 12, 1977 in St. Augustin (Table 21). The elongate abdomen of the prepupa was attached posteriorly to the ventro-posterior end of a shrivelled brown cadaver of the third instar grub of *P. anxia*. Eyes and appendages of the prepupa were barely distinguishable. Hammond (1944b) noted that parasite larvae emerged through a slit from the mid-ventral

Figure 30. Adult and empty pupal case of Ptilodexia sp. (Scale: 3 mm).

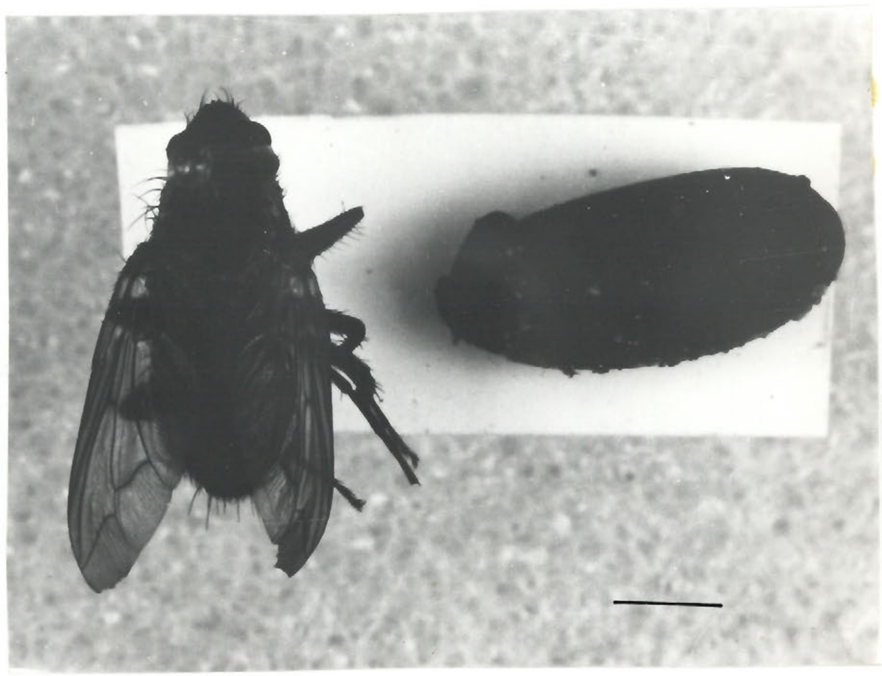


Figure 31. Prepupa of *Pelecinus polyturator* (Drury) attached to a *Phyllophaga anxia* (Lec.) third instar cadaver. (Scale: 5 mm).

Figure 32. Pupa of *P. polyturator* emerged from a third instar of *P. anxia*. (Scale: 4 mm).

Table 21. *Pelecinus polyturator* (Drury) recovered from *Phyllophaga anxia* grubs collected in southern Quebec, 1975 to 1977.

Location	Collection Date	Stage Found	
		Host	Parasite
Nicolet	July 11, 75	3rd L.	pupa
Nicolet	July 16, 76	3rd L.	pupa
Nicolet	July 27, 76	3rd L.	pupa
Ste-Anne-de-Bellevue	June 15, 77	3rd L.	pupa
L'Acadie	July 20, 77	3rd L.	pupa
St. Augustin	Aug. 12, 77	3rd L.	prepupa pupa

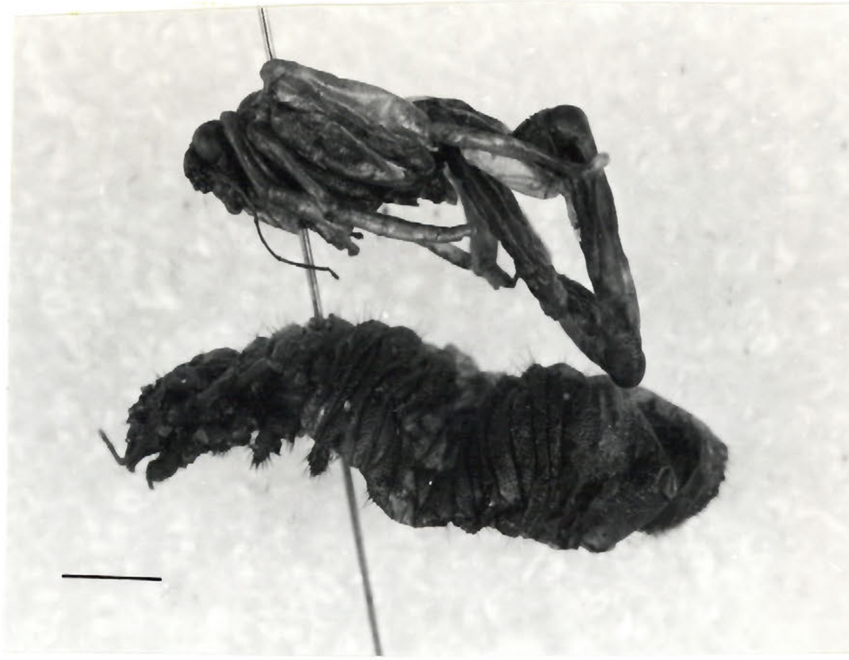
line of the host grub. However, we observed that the terminal segment of the parasite pupa was connected with the everted rectum of the host grub. Thus, it appeared that the parasite emerged through the anal opening of the host and remained there until pupation (Figure 32). When a recently formed pupa was detached from the host cadaver, an unidentified white sticky substance exuded from the body of the host. The pupa is of the exarate type (Figure 33) and resembles the shape of the adult (Figure 34). A female adult emerged from a pupa in the laboratory on July 29, 1976, two days after collection at Nicolet. Under field conditions in Quebec and Ontario, adult emergence occurs between late July and late August (Hammond, 1944b). The female adult lived for 14 days in the laboratory with moistened raisin and water as food. Although second and third stage white grubs were presented to the female, no attempt at oviposition was observed, and dissection of the grubs after two weeks revealed no signs of immature stages of the parasite. All the parasite pupae we found emerged from the third instar of the host. No male specimen was found in the present study, and it is known to be extremely rare (Brues, 1928).

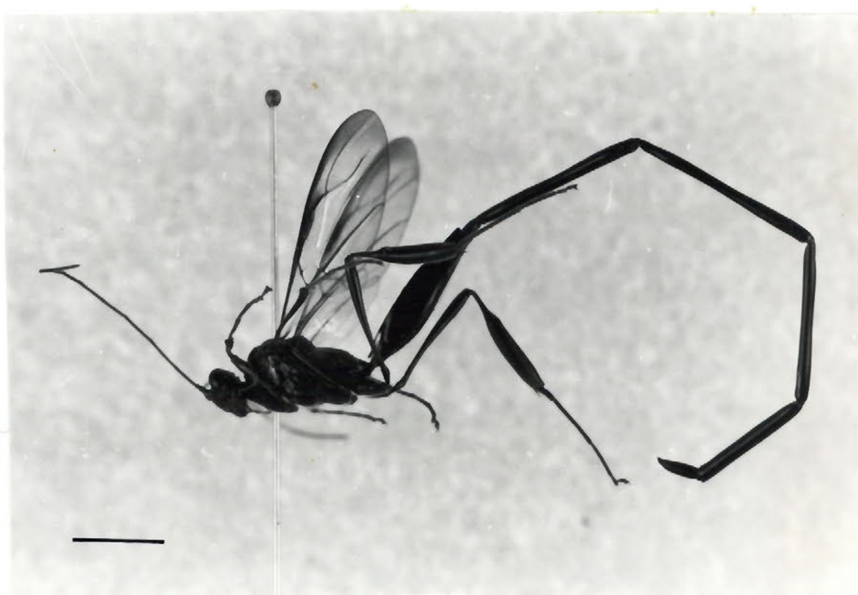
c. Hymenoptera: Tiphiidae

A total of four cocoons of Tiphia sp. were collected in the soil, but no adults emerged in the laboratory. Two were

Figure 33. Pupa of _P. polyturator_, with a shrivelled cadaver of _P. anxia_ (third instar) which it had parasitized. (Scale: 4 mm).

Figure 34. Female adult of _P. polyturator_. (Scale: 5 mm).





from Valleyfield and collected on September 7 and September 27, 1976, and the other two were from Ste. Augustin and La Pocatiere, both collected on May 18, 1977. In Manitoba, tiphiids were not common (Criddle, 1918), whereas in the United States the ecto-parasitic tiphiids, *Tiphia punctata* Rob., *T. inortata* Say, *T. transversa* Say, and *T. vulgaris* Rob., were the most efficient and abundant of the many parasites known to attack *Phyllophaga* grubs (Davis, 1919). In Quebec, parasitism of *P. anxia* by *T. inornata* ranged from 3 to 49 per cent depending on the areas (Petch and Hammond, 1925), and the males and females of *P. inornata* emerged in early- and mid-June, respectively (Petch and Hammond, 1926). In Nebraska, eggs of *Tiphia* sp. were found on second and third instar grubs of *P. anxia*, and parasitism reached 33 per cent in one field (Jarvis, 1966). In northcentral Nebraska, *T. berbereti* Allen parasitizing *P. anxia* larva had a one-year life cycle and the density of the parasite cocoons reached 4-6/yd^2 (Berberet and Helms, 1970). Adults of *Tiphia* spp. are known to feed on nectar and pollen of some wild plants (Davis, 1919; King and Holloway, 1930; Clausen *et al.*, 1933) and presence of these wild plants in a grub-infested field may increase the degree of parasitism.

2. Insect Predators

a. Diptera: Asilidae

Two species of asilids were found (Table 22), *Diogmites*

Table 22. Asilids collected while feeding or in close proximity to *Phyllophaga anxia* in southern Quebec, 1975 to 1977.

Species	Location	Collection Date	Stage Found	
			Host	Predator
Diogmites sp.	Nicolet	Apr. 29, 76	2nd L.	larva
	Nicolet	July 27, 76	-	adult
	Nicolet	Aug. 17, 76	-	adult
Asilus sp.	Valleyfield	May 4, 76	2nd L.	larva
Asilid larva	Ste. Clothilde	Aug. 7, 75	3rd L.	larva
	Valleyfield	Aug. 3, 76	3rd L.	larva
	Nicolet	May 11, 77	2nd L.	larva
	Rimouski	Aug. 11, 77	3rd L.	larva
	La Pocatiere	Aug. 11, 77	2nd L.	larva

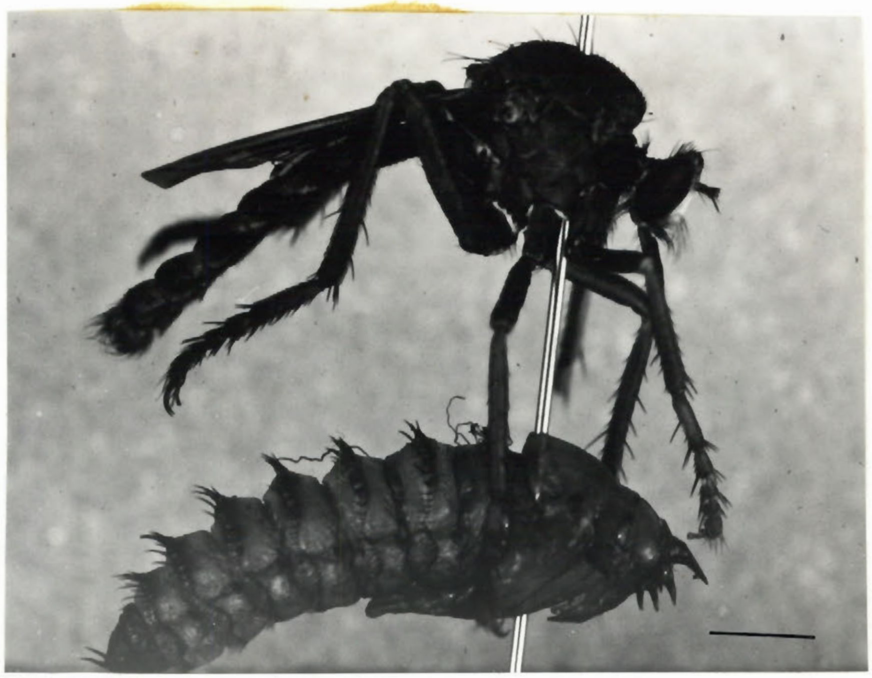
sp. (Figure 35), and *Asilus* sp. (Figure 36). On one occasion, an asilid larva was observed feeding on a third instar white grub (Romouski, August 11, 1977). The asilid larva detached from the moribund host grub leaving a feeding mark caused by the puncture of the mouth hook (Figure 37). Davis (1919) saw the larva of the asilid, *Diogmites winthemi* (Wied.), feeding on a *Phyllophaga* pupa, and he also found the larvae of *Asilus paropus* Walker and *A. lecythus* Walker to be predaceous on *Phyllophaga* grubs. Petch and Hammond (1925) observed asilid larvae feeding on white grubs of *P. anxia* in rearing containers. Petch and Hammond (1926) stated that 98 per cent of robber flies in southern Quebec were *Asilus snowi* Hine. Ritcher (1940) estimated that about 12 per cent of the pupae of five *Phyllophaga* spp. (*P. anxia* was not among them) were destroyed by larvae of an asilid, *Diogmites discolor* Loew at Lexington of Kentucky. The adults of *Diogmites* spp. stay in rather dense, low ground vegetation in damp areas (Hull, 1962). More recently, Daniels (1966) found asilid larvae to be predatory on grubs of *Phyllophaga koehleriana* (Saylor) in Texas.

b. Coleoptera: Carabidae

According to Larochelle (1976), there are 440 species of carabids in Quebec and many of these are predators. Eight species of carabids were found in the vicinity of the white

Figure 35. Adult and empty pupal case of _Diogmites_ sp. (Scale: 4 mm).

Figure 36. Adult and empty pupal case of _Asilus_ sp. (Scale: 3 mm).



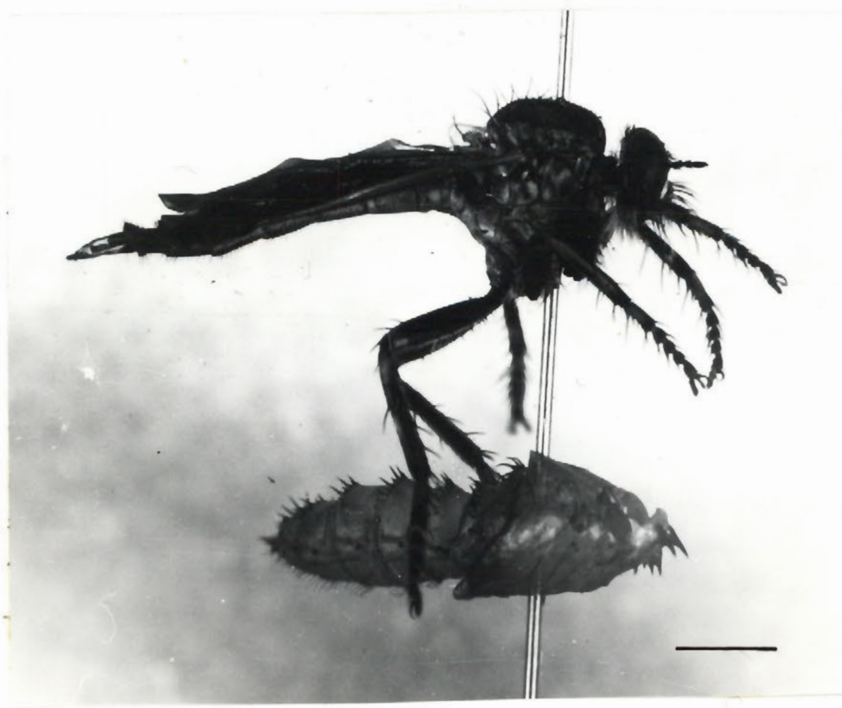


Figure 37. An asilid larva and a moribund third instar of *Phyllophaga anxia*. Note a feeding mark on the grub.

grubs. These were Agonum placidum Say, Carabus serratus Say, Anisodactylus discoideus Dej., Pterostichus leconteianus Lutz., Chlaenius sericeaus Forst., Harpalus caliginosus F., Harpalus longicollis LeC., and Harpalus bicolor F. Davis (1919) observed adults of H. caliginosus feeding on a dead Phyllophaga adult, and larvae and adults of Harpalus pennsylvanicus Dej. were seen attacking Phyllophaga grubs. Seaton (1939) reported that larvae and adults of Pterostichus chalcites (Say) fed on eggs and grubs of Phyllophaga sp. Larochelle (1974) recorded that C. sericeaus fed on the grubs of the Japanese beetle, Popillia japonica Newman, dead slugs, earthworms and unidentified insects.

3. Acarines Associated with P. anxia

Mites found on the femora of grubs and the thoracic sterna and femora of adults of P. anxia, were identified by Dr. C.Y. Oseto, as Tyrophagus sp. and anoetid mites. Criddle (1918) believed that mites of Tyrophagus heteromorphus Felt and other mites caused the death of many white grubs and Davis (1919) considered that mites of Rhizoglyohus phylloxerae Riley frequently killed white grubs. Petch and Hammond (1925) found hypopi of Tyroglyphus on 100 per cent of the grubs in the Hemmingford district of Quebec. They also found R. phylloxerae but did not consider either species as predaceous. In the following year, Petch and Hammond (1926) found that both species of mites were scarce. Jarvis (1964,1966) noted that second- and third-instar larvae, pupae and adults of *P. anxia* in the sandhills area in Nebraska were infested by hypopi of *Caloglyphus* and the mites did not feed on live hosts. Daniels (1966) stated that *Caloglyphus* mites fed on injured or dead grubs of *P. koehleriana*. Oseto and Mayo (1975) described a new species of mite, *Caloglyphus phyllophagianus*, which was collected from larvae of *P. anxia*. Olynyk and Freitag (1979) found hypopi of *Anoetus* sp. on carabids and they suggested the mites were phoretic. In the present study, the *Tyrophagus* sp. mites and the anoetid mites are also considered to be phoretic.

4. *Pathogens*

a. Nematoda: Diplogasteridae

Nematodes were recovered from both live and dead grubs and were identified as belonging to the family Diplogasteridae by the characteristics listed by Goodey (1963). They were then sent to Dr. G.O. Poinar, Jr., University of California, Berkeley, for further identification. He said they closely resembled *Mikoletzkya aerivora* (Cobb), and pointed out that the original description of this species published by Merril and Ford (1916) was inadequate. I examined my specimens again in 1978 using a key published by Poinar (1977), and I identified it as *M. aerivora*. This nematode was recovered by us from white grubs collected from Valleyfield, Ste-Clothilde, Ste-Anne-de-Bellevue and Nicolet, and is considered widespread in southern Quebec. The host cadaver usually had a grey colour, which could be due to bacterial septicemia, as bacteria have been associated with insect-parasitic nematodes (Poinar, 1966,1975a,1978). M. aerivora was first described and reported to be parasitic in the head of the termite, Leucotermes lucifugus Rossi, and in grasshopper eggs in Kansas (Merrill and Ford, 1916). It was also found in Phyllophaga grubs in Wisconsin (Davis, 1919). It has not been reported in white grubs in Canada.

b. Eubacteriales: Bacillaceae

Many grubs of P. anxia collected from the field died after various holding times, ranging from several hours to more than a month. The dead grubs were a solid black colour (Figure 38) and had a putrid smell. Blood smears from the grubs stained with Gram's stain (Thomas, 1974) and examined by Mr. G.O. Thomas, University of California, Berkeley, showed them to be of Bacillus cereus Fr. & Fr. This organism has been isolated from numerous diseased insects of the order Coleoptera, Hymenoptera and Lepidoptera (Heimpel and Angus, 1963). B. cereus is a soil organism, and a number of varieties are pathogenic when ingested by susceptible insect hosts (Angus, 1965). There is no available published information about B. cereus infecting grubs

Figure 38. Diseased third instar of *Phyllophaga anxia* infected by *Bacillus cereus* Fr. & Fr. (Scale: 5 mm).

of *P. anxia*. However, there are two known bacterial diseases of *Phyllophaga* grubs caused by *Micrococcus nigrofaciens* Northrup and *Bacillus popilliae* Dutky (Northrup, 1914; DuPorte, 1915; Davis, 1919; Dutky, 1963; Tashiro and Steinkraus, 1966).

c. Deuteromycetes: Moniliales

Four species of fungi (Table 23) were found on cadavers of grubs and adults of *P. anxia*. Fungi were identified using the descriptions of Barnett and Hunter (1972) and Bell (1974) and then confirmed by Mr. G.O. Thomas.

Among the fungi recovered, the green muscardine fungus, *Metarhizium anisopliae* (Metsch.) Sorok. was the most commonly encountered (Table 23). Mummified grubs (Figure 39) and adults (Figure 40) were found covered with masses of green conidia. Conidia were stained and mounted in 0.1% lactophenol-cotton blue. The conidia are cylindrical in shape, truncate at both ends (Figure 41). I measured 20 conidia. The mean length was 8.2 μm (S.D.=0.7, range 6.5-9.5) and the mean width was 2.9 μm(S.D.=0.4, range 2.3-3.6). According to Latch (1976), the species *M. anisopliae* has two forms, a short spore form, with conidia of 5 to 8μm long; and a long spore form, with conidia of 9 to 14 μm long. The short spore form corresponds to the dimension of *M. anisopliae* var. *anisopliae* as described by Tulloch (1976). In the present study, the size of conidia is similar to *M. anisopliae*

Table 23. Entomopathogenic fungi recovered from Phyllophaga anxia adults and grubs collected in southern Quebec, 1975 to 1977.

Species	Location	Collection Date	Host Stage
Metarhizium anisopliae	Nicolet	May 15, 75	adult
	Nicolet	July 11, 75	3rd L.
	Valleyfield	Aug. 7, 75	adult
	Nicolet	Apr. 29, 76	adult
	Nicolet	May 10, 76	adult
	Nicolet	May 25, 76	3rd L.
	St. Hyacinthe	June 10, 76	adult
	Nicolet	Aug. 17, 76	2nd L.
	Nicolet	Aug. 31, 76	1st L.
	Nicolet	June 10, 77	3rd L.
	L'Assomption	June 23, 77	3rd L.+adult
	Les Buissons	Aug. 10, 77	3rd L.
	Rimouski	Aug. 11, 77	3rd L.
	Nicolet	Aug. 17, 77	2nd L.
	Valleyfield	Aug. 23, 77	adult
	Nicolet	Sept. 6, 77	3rd L.
Beauveria bassiana	Ste. Clothilde	May 27, 76	3rd L.
	Nicolet	June 10, 76	2nd L.
	Valleyfield	June 21, 76	3rd L.
	Valleyfield	Sept. 23, 76	adult
	Les Buissons	Aug. 10, 77	3rd L.
Fusarium sp.	Valleyfield	May 21, 76	3rd L.
	L'Assomption	June 8, 76	2nd L.
	L'Acadie	June 16, 76	2nd L.
	Valleyfield	June 21, 76	3rd L.
	Valleyfield	July 5, 76	3rd L.
Penicillium sp.	Nicolet	May 10, 76	adult
	Valleyfield	July 5, 76	3rd L.
	Rimouski	Aug. 11, 77	adult

Figure 39. Mummified cadaver of third instar of *Phyllophaga anxia* infected with green muscardine fungus, *Metarhizium anisopliae* (Metsch.) Sorok. (Scale: 5 mm).

Figure 40. Mummified cadaver of adult of *Phyllophaga anxia* infected with green muscardine fungus, *M. anisopliae*. (Scale: 3 mm).

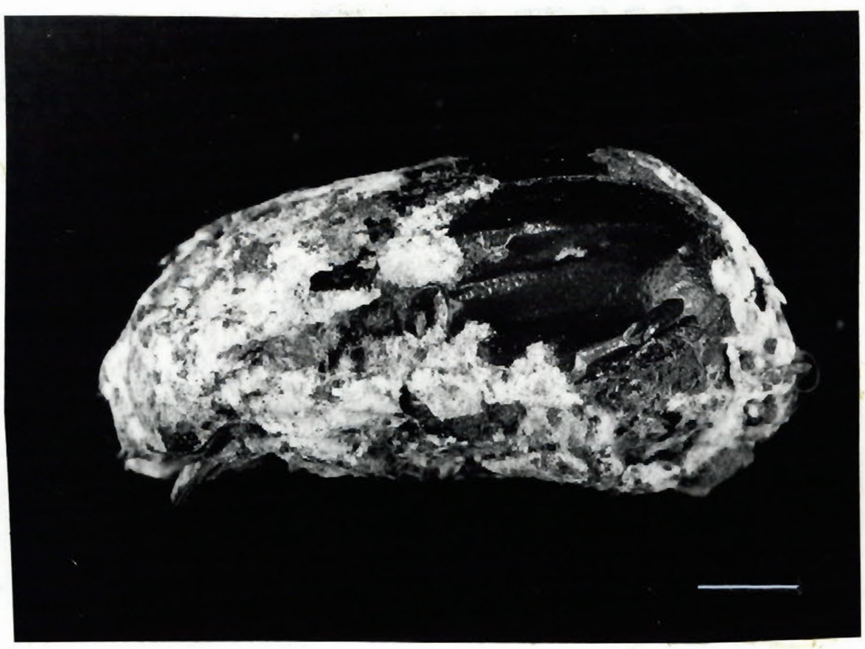
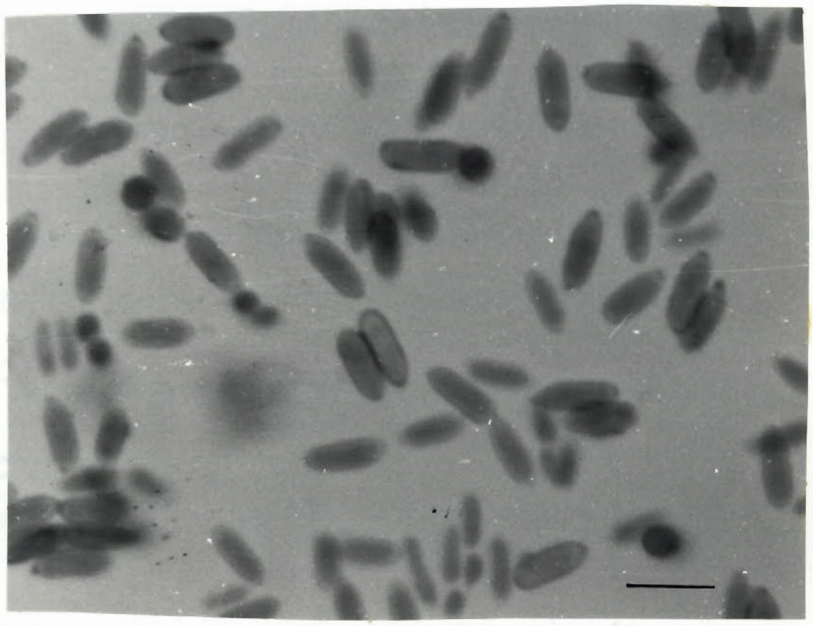


Figure 41. Conidia of *M. anisopliae* recovered from third instar of *P. anxia*. (Scale: 10 μm).



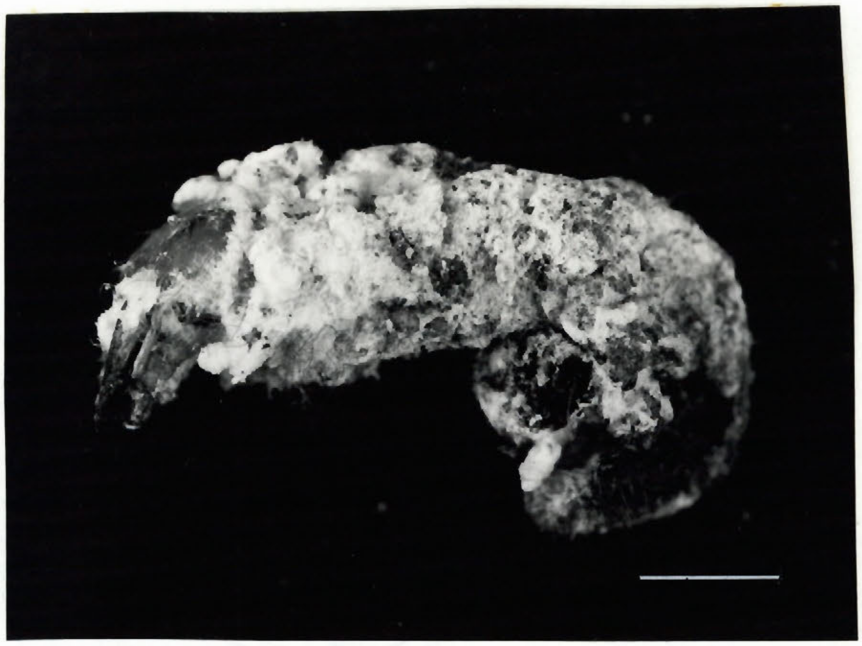
var. *anisopliae*. The conidia of *M. anisopliae* germinated within two days of being inoculated on Sabouraud dextrose agar with 0.2% yeast extract (SDA+Y) at 27°C. This SDA+Y medium has been used successfully for culturing various fungi recovered from insects by Thomas (1974). Conidia formed 5 days after inoculation. Conidia in the culture dishes were still viable after 10 months at room temperature. Suspensions of conidia of *M. anisopliae* were inoculated on to autoclaved grains of barley (ratio 1.5 ml water/1.0 g grain) in Erlenmeyer flasks gave profuse sporulation after one week. A similar method had been used by Latch (1976) for producing large quantities of *M. anisopliae* on oats to use as an inoculum for field use. Records of *M. anisopliae* on *Phyllophaga* spp. are few. Davis (1919) stated that isolated cases of infection of *M. anisopliae* were found on *Phyllophaga* grubs in various parts of the United States. Jarvis (1966) found a total of three cases of third instar grubs of *P. anxia* infected by *M. anisopliae* in three years in a field in Nebraska. *M. anisopliae* has a wide host range, according to Veen (1968), at least 204 species of insects were recorded or susceptible to *M. anisopliae* under natural conditions. Recently, Ferron (1978) reviewed the practical field application of *M. anisopliae* and other fungi for control of insect pests. Studies on the susceptibility, host specificity of *M. anisopliae*, and its use for controlling on some scarabaeids have been carried out elsewhere (Nirula, 1957; Latch, 1965,1976; Gruner, 1973; Ferron et al., 1975; Fargues, 1976; Latch and Fallon, 1976). I suggest similar research could be carried out in the future in a pest management programme objective.

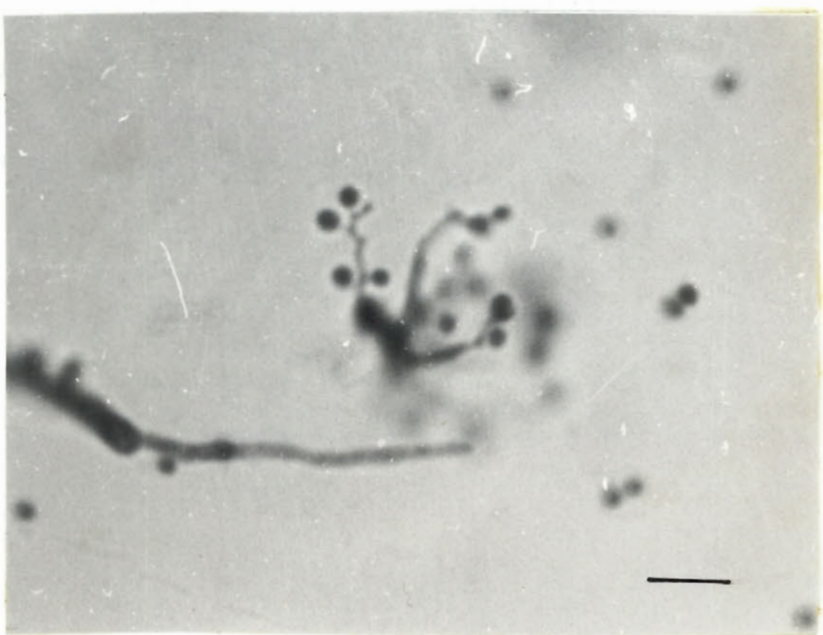
The white muscardine fungus, Beauyeria bassiana (Bals.) Vuill. was found on grubs and adults of P. anxia (Table 23). Cadavers were covered with white conidia, which had a fine powdery appearance (Figure 42). The conidia are borne singly on the zigzag phialides (Figure 43). I measured twenty conidia. The mean size was 3.4 μm(S.D.=1.1, range 1.8-5.0). MacLeod (1954) reported that the diameter of globose spores of B. bassiana was 1.0 to 4.0 μm. Beauveria spp. has been tested for control of soil-inhabiting scarabaeids. In New Zealand, Latch (1976) found that B. bassiana killed larvae of the coconut rhinoceros beetle, Oryctes rhinoceros L., at the time of pupation. In France, Hurpin and Robert (1972) showed that among five different pathogenic organisms tested, a fungus, Beauveria tenella (Delacr.) Siem. had caused the highest mortality of the common cockchafer, Melolontha melolontha L. This species is a possible biological control agent for P. anxia.

Grubs of P. anxia were found infected with a fungus, Fusarium sp. on five occasions (Table 23). The fungus was localized at the abdomen of the host grub. The cadavers were not mummified as with muscardine diseases. The fungus had a

Figure 42. Mummified cadaver of third instar of _Phyllophaga anxia_ infected with white muscardine fungus, _Beauveria bassiana_ (Bals.) Vuill. (Scale: 5 mm).

Figure 43. Conidia and phialides of _B. bassiana_ recovered from third instar grub of _P. anxia_. (Scale: 15 µm).



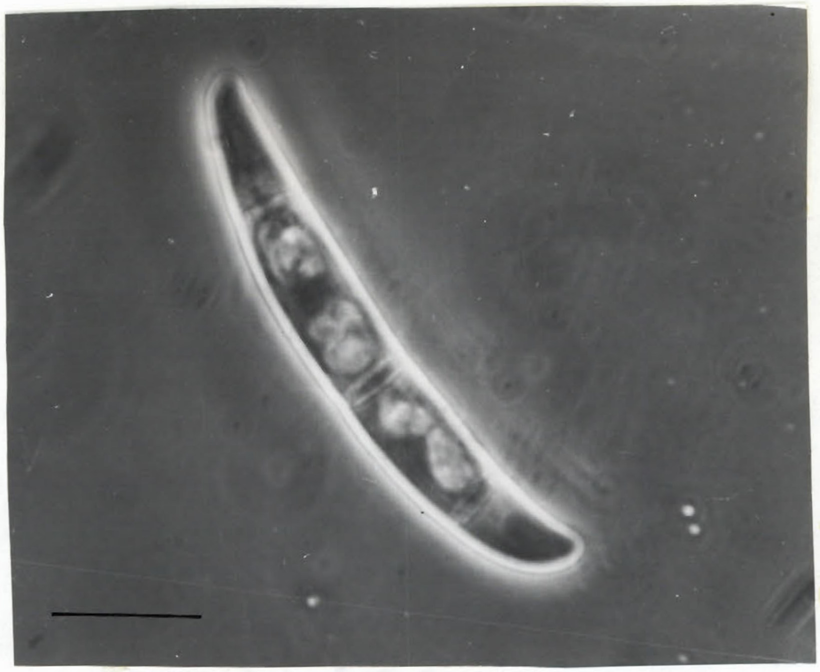


yellow appearance on the host. The macroconidia of the *Fusarium* sp. are canoe-shaped, and with three septa (Figure 44). Some *Fusarium* spp. had been reported to infect insects such as scale insects, white flies (Steinhaus, 1949); the desert locust (madelin, 1963); mosquito larva (Hassan and Vego, 1972); and the bagworm (Berisford and Tsao, 1975). Larvae and adults of the common cockchafer, *M. melolontha* have also been reported and infected by *Fusarium* sp. in a natural environment in France (Hurpin and Vago, 1958).

Grubs and adult of *P. anxia* were found infected by *Penicillium* sp. on three separate occasions (Table 23). The cadavers were covered with light green conidia and were not mummified. Phialides are borne in groups on the conidiophores and conidia are in short chains and globose in shape (Figure 45). Smirnoff (1968) recovered *Penicillium thomii* from larvae of the larch sawfly in Quebec. Sen *et al.* (1970) reported that *Penicillium citrinum* Thom. caused mycosis in the Indian silkworm. Berisford and Tsao (1975) found that two *Penicillium* spp. were among the most effective biological control agents for a bagworm. Previously, both *Fusarium* sp. and *Penicillium* sp. have been recovered from many species of insects and are generally considered to be saprophytic or surface contaminant (Steinhaus, 1951; Steinhaus and Marsh, 1962; Thomas and Poinar, 1973).

Figure 44. A macroconidium of _Fusarium_ sp. recovered from third instar of _Phyllophaga anxia_. (Scale: 10μm).

Figure 45. _Penicillium_ sp. recovered from third instar of _Phyllophaga anxia_. P: Phialide, C: Conidiophore. (Scale: 20 μm).




P
C

d. Entomopoxvirus (?)

A possible infection of grubs of _P. anxia_ by entomopox-virus was found. The external signs of the disease are quite distinct. The infected grub shows lethargy, an elongated body, and beige body colour (Figure 46). Later, the body is fully distended, with a translucent appearance, spiracles are black in colour (Figure 47). Diarrhea is common at this stage of the disease. In some late severe cases, the rectum is completely prolapsed (Figure 48). After this, the body may become brown in colour, shrunk and mummified. It is known that two types of inclusions are commonly produced by entomopoxvirus; a large ovoid to irregular-shaped virus containing "spheroid", and a smaller "spindle" devoid of virus particles (virions) (Granados, 1973; Goodwin and Filshie, 1975; Smith, 1976; Poinar and Thomas, 1978). By examination of histological sections stained as described by Hamm (1966), no infected cells were found in the mid-gut of the healthy-looking third instar grub (Figure 49). Mid-gut from a third instar grub with symptom of prolapsed rectum was in a state of hyperplasia (Figure 50). The infected cells appeared to be smaller in size, compared to the healthy cells, due probably to their rapid division. Spheroids were ovoid in shape, developed in the nuclei, and stained red in colour (Figure 51). Healthy nuclei stained green. Hamm's method (1966) is recommended by Smith (1967) for diagnosis of virus diseases

Figure 46. Third instar of _Phyllophaga anxia_.

Left: Grub infected with entomopoxvirus

Right: Healthy specimen

Figure 47. Third instar of _P. anxia_ showing signs of infection of entomopoxvirus. Note the slight prolapse of rectum. (Scale: 5 mm)

Figure 48. Moribund third instar of P. anxia infected with entomopoxvirus. Note the complete prolapse of rectum. (Scale: 5 mm).

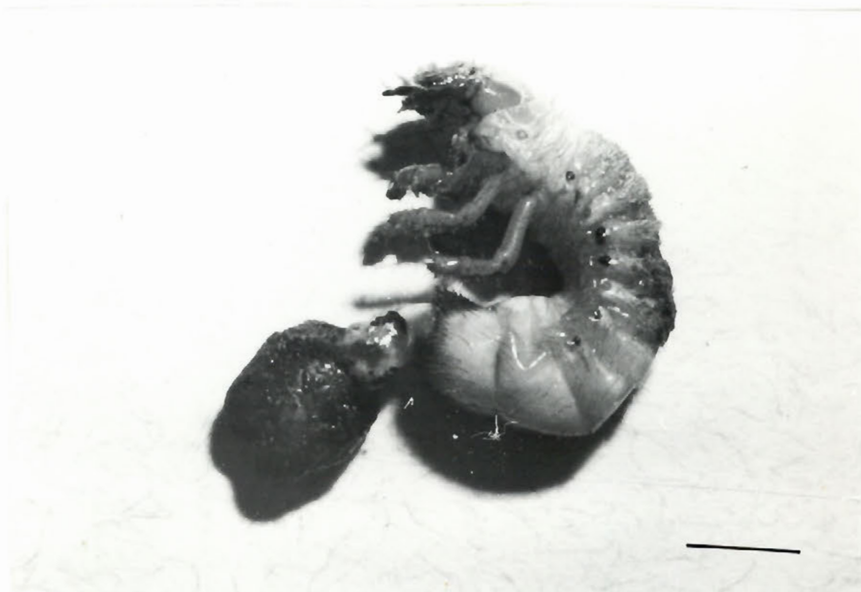


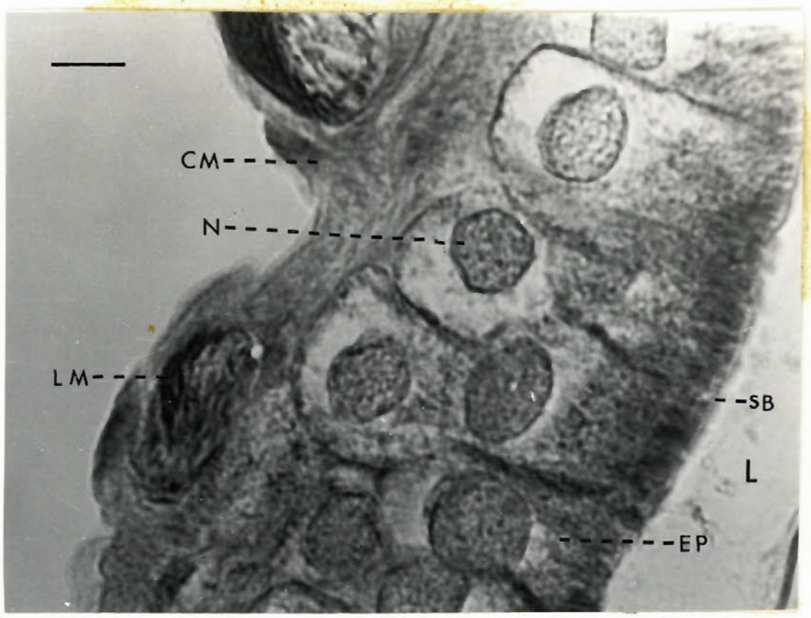
Figure 49. Cross section of mid-gut of healthy third instar of *Phyllophaga anxia*.

CM: Circular muscle
EP: Epithelial cell
L: Lumen
LM: Longitudinal muscle
SB: Striated border

Scale: 10 μm

Figure 50. Cross section of mid-gut of third instar of *P. anxia* infected by entomopoxvirus. Note the gut wall is thickened due to hyperplasia of the epithelial cells.

Scale: 20 μm


CM
N
LM
SB
L
EP

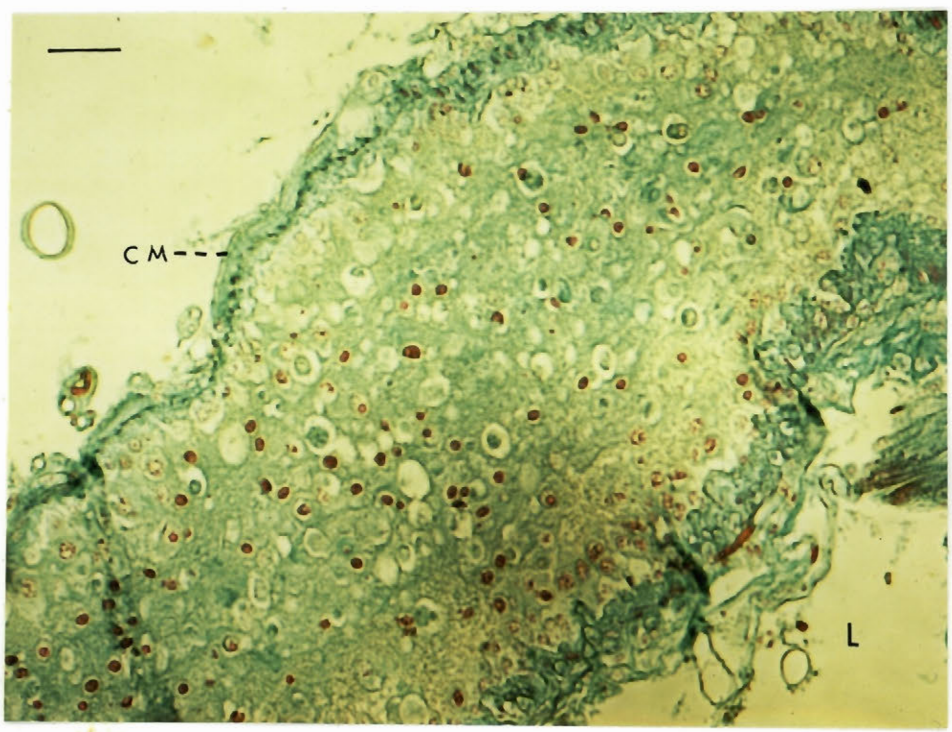
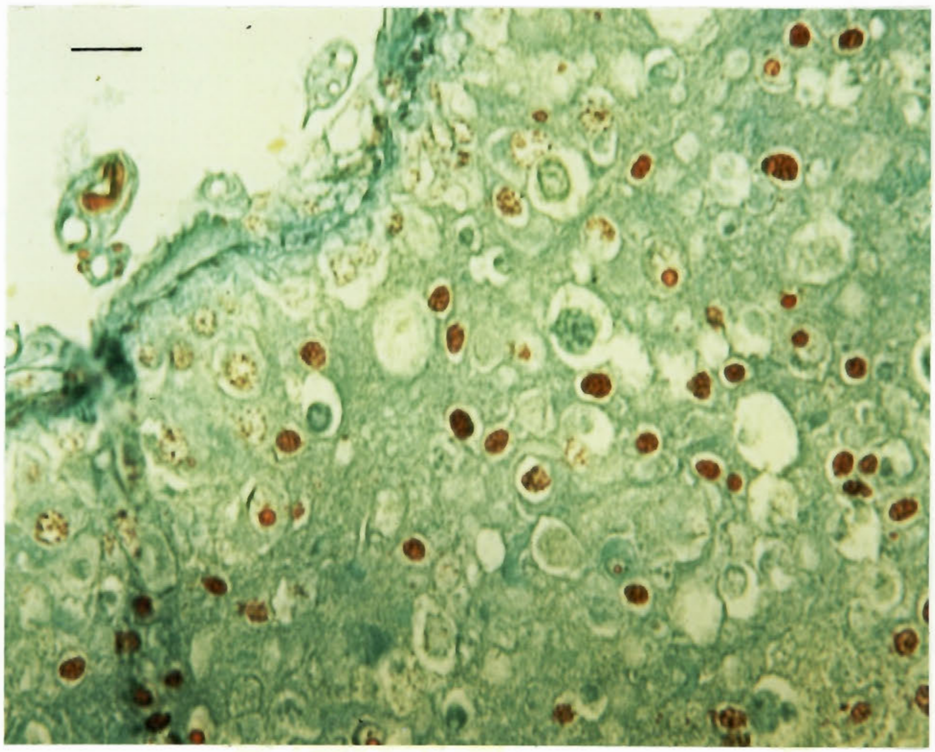

CM
L

Figure 51. Spheroids of entomopoxvirus in the nuclei of mid-gut cells of third instar of *Phyllophaga anxia*.

spheroids, red
healthy nuclei, green

Scale: 10 μm



of insect. Since then, it has been used successfully by other investigators to study baculovirus of cabbage looper (Vail and Hall, 1969), and entomopoxvirus and two baculoviruses of a hepialid moth (Crawford and Kalmakoff, 1977). I measured 30 spheroids. The mean length was 7.5 μm(S.D.=1.2, range 5.9-10.1). The mean width was 6.2 μm(S.D.-1.1, range 4.6-9.3). The size of the spheroids of *P. anxia* is smaller than that reported for *M. melolontha*, listed as 10-20 μ by Granados (1973). The size of spheroids of entomopoxvirus of an Australian scarab, *Othnonius batesi* Oll. was 5-10 μ (Goodwin and Roberts, 1975). The above histological sections were sent to Insect Pathology Research Institute (now Forest Pest Management Institute), Sault Ste. Marie, Ontario and examined by Drs. F.T. Bird and B.M. Arif, and they considered that this may have an entomopoxvirus infection but could not be certain. Sections were also examined by Mr. G.M. Thomas, University of California at Berkeley, and, he also considered it was probably an entomopoxvirus infection. Various virus diseases have been suggested or tested for control of other scarabaeids (Marschall, 1970; Hurpin and Robert, 1972; Ferron and Hurpin, 1974; Milner and Lutton, 1975; Bedford, 1976; Zelazny, 1977). Recently, the use of viruses as pesticides and possible ecological hazards are discussed in detail by Tinsley (1979). Future work on this entomopoxvirus should include electron microscopy study, infectivity on various host stages and ultimately a field trial.

IX. A DIPLOGASTERID NEMATODE, *MIKOLETZKYA AERIVORA* (COBB), RECOVERED FROM *PHYLLOPHAGA ANXIA* (LECONTE) (COLEOPTERA: SCARABAEIDAE) IN SOUTHERN QUEBEC

A. INTRODUCTION

The diplogasterid nematode, *Mikoletzkya aerivora* (Cobb), was originally found as a parasite of the termite, *Leucotermes lucifuges* Rossi, and also grasshopper eggs (Merrill and Ford, 1916). It has also killed larvae of *Phyllophaga* spp. in Wisconsin (Davis, 1919; Chamberlin, 1944). It was formerly known as *Diplogaster aerivora* Cobb (Baker, 1962; Poinar, 1969,1975b). Some other diplogasterid nematodes have also been reported as facultative parasites of insects (Swain, 1945; Poinar, 1969,1972; Fedorko, 1971; Fedorko and Stanuszek, 1971; Massey, 1974; Poinar *et al*., 1976; Weiser, 1977).

In a study of the natural enemies of *Phyllophaga anxia* (Le Conte) by the present author, *M. aerivora* was found in larvae of *P. anxia* in the field. This chapter presents the results of the field recovery, additional descriptions of adult males and females, infection tests and the storage viability of the nematodes.

B. MATERIALS AND METHODS

Moribund grubs of *P. anxia* having a greyish appearance that were collected from various areas of southern Quebec were examined in the laboratory under a dissecting microscope to detect the possible presence of nematodes on the external surface. If no nematodes were found on the external surface, grubs were then incubated on nutrient agar in petri dishes for possible recovery of nematodes from the body cavity. Nutrient agar was prepared at half strength as recommended for bacteriological purposes (Southey, 1970). Plates were incubated at 25°C without light. Incubated samples were checked for nematodes for at least a week before being discarded.

Nematodes recovered from insect hosts were used for diagnostic measurements. They were heat-killed, fixed in TAF (Courtney *et al*., 1955) and processed using Baker's method for mounting (Southey, 1970).

Infection tests were carried out in order to fulfill Koch's postulates for confirmation of a disease causal organism (Steinhaus, 1949). *M. aerivora* was cultured on nutrient agar. Subsequent subcultures were obtained by transferring the nematodes accompanied by a small amount of the previous culture medium into a fresh nutrient agar plate. Nematode suspensions were prepared by adding sterile water to the culture plate and transferring this to a 50-ml beaker. Estimates of density of nematodes suspension were done by pipetting one milliliter of this suspension to 9 ml of sterile water in a test tube. Five separate 0.1 ml volumes were then pipetted from the diluted suspension in the test tube and placed on glass slides. The number of nematodes was counted under a stereoscopic dissecting microscope. The average of these five counts was taken as the number of nematodes per 0.1 ml of the diluted suspension in the test tube. This estimate was then used for determination of the density of the suspension in the beaker. Grubs collected from the same locality on the same date were used in each test. Grubs were kept individually in styrofoam cups for at least a week before testing, with a lettuce leaf supplied as food as well as a moisture source. These grubs were kept in a rearing room at 24^{o}C, 25-30% RH, with 16 hours of light. After a week, only healthy looking grubs were chosen for experiments. An inoculum of 0.5 ml of the nematode suspension was then applied topically by an automatic pipette, Biopette$^{(R)}$ (Schawarz Bioresearch, Orangeburg, N.Y.) between the prothoracic legs. Sterile water (0.5 ml) was administered to check grubs. Grubs were then returned to the rearing room and these were examined every two days for the presence of nematodes on the diseased individual for up to two months.

Nematode suspensions were kept in Erlenmyer flasks. The level of suspension in the flask was adjusted to a depth of 3 cm by addition of distilled water, the flask was then covered with aluminium foil and stored at 5^{o}C.

C. RESULTS AND DISCUSSION

Nematodes recovered from grubs of _P. anxia_ were identified as belonging to the family Diplogasteridae, using the descriptions of Goodey (1963). This identification was confirmed by Dr. R.H. Estey, Macdonald Campus of McGill University, Ste-Anne-de-Bellevue, Quebec. Later, these specimens were sent to Dr. G.O. Poinar, Jr. for further identification. He considered these to closely resemble _M. aerivora_. In 1978 I examined more specimens using a key developed by Poinar (1977) and agreed with his earlier tentative identification of specimens, as _M. aerivora_.

1. Recovery Sites

M. aerivora was recovered from second and third instar grubs of _P. anxia_ (Table 24). No nematodes were found on or in other stages of _P. anxia_.

In all specimens examined, female nematodes were larger than males (Table 25, Figure 52). Cobb's formula was used for indicating characteristics of the nematode in the original description of _M. aerivora_ (Merrill and Ford, 1916). However, the de Man formula has been preferred rather than the Cobb formula by most workers since 1940 (Southey, 1970). The de Man formula for _M. aerivora_ is listed in Table 26.

Table 24. Recovery of the diplogasterid nematode, *Mikoletzkya aerivora* (Cobb), from *Phyllophaga anxia* grubs in southern Quebec, 1975 to 1977.[a]

Vicinity	Date of Collection	Stage of Host
Ste. Clothilde	Aug. 7, 75	3rd instar
Ste-Anne-de-Bellevue	Sept. 29, 75	3rd instar
Ste. Clothilde	May 27, 76	3rd instar
Valleyfield	June 21, 76	2nd and 3rd instars
Valleyfield	July 5, 76	3rd instar
Nicolet	July 27, 76	2nd instar
Valleyfield	Aug. 11, 76	3rd instar
Valleyfield	Sept. 7, 76	3rd instar
Valleyfield	Sept. 23, 76	3rd instar

[a]No nematodes were found in 1977.

Table 25. Measurements of males and females of *Mikoletzkya aerivora* (Cobb) from *Phyllophaga anxia* grubs.

Character (μm)	Male (n=4)		Female (n=8)	
	$\bar{X}$	Range	$\bar{X}$	Range
Total Length	1020	890-1160	1370	1100-1880
Greatest width	72.8	61.4-83.2	82.4	59.4-101.0
Length stoma	6.1	4.6-7.6	6.9	4.6-9.5
Width stoma	7.6	5.0-9.0	7.9	6.5-9.9
Length head to base of esophagus	184.9	176.2-191.2	194.1	178.5-223.6
Body width at base of esophagus	50.0	41.1-56.1	60.8	48.2-77.4
Width at vulva	-	-	81.6	56.1-93.2
Length head to vulva	-	-	692.8	483.1-851.4
Length cloaca to anteriormost part of testis	408.9	277.2-631.6	-	-
Length head to anus	947.4	833.6-1077.1	1199.2	958.3-1637.5
Width at anus	37.6	19.8-51.5	38.6	19.8-47.5
Length tail	67.8	55.4-79.2	169.5	128.7-176.2

Figure 52. Adults of *Mikoletzkya aerivora* recovered from third instar of *Phyllophaga anxia*.

Left: Female

Right: Male

Scale: 120 μm

Table 26. de Man formula for <u>Mikoletzkya aerivora</u> (Cobb).

Male	Female
n = 4	n = 8
L = 1020 μm	L = 1370 μm
a = 14.0	a = 16.6
b = 5.5	b = 7.1
c = 15.0	c = 8.1
T = 40.1	V = 50.6

n = number of specimens

L = total body length in mm or μm

a = body length ÷ greatest body width

b = body length ÷ distance from anterior end to junction of esophagus and intestine

c = body length ÷ tail length (anus or cloaca to tail terminus)

T = distance from cloaca to anteriormost part of testis x 100 ÷ body length

V = distance of vulva from anterior end x 100 ÷ body length

2. Description of Adult Males and Females

The following descriptions of the adult males and females are based on the specimens recovered from *P. anxia* in the present study. Nematological terms are those of Bird (1971).

The structure of the stoma and the pharyngeal region is the same in males and females (Figure 53). The stoma is reduced, short and wide. Cheilorhabdions are represented as weakly sclerotized areas lining the inside of the lip region and a small tooth-like structure is originated from the telostoma. Pro-, meso-, meta- and telorhabdions are indistinct and the esophagus is typically diplogasteroid. The esophagus is composed of an elongate corpus, valvated metacorpus, indistinct istmus, and a pyriform basal bulb. A nerve ring surrounds the isthmus. The base of the pharynx is connected with the anterior part of the intestine.

Adult males (Figures 54a,54b). Body slender. Cuticle smooth. Body assumed J-shape after being heat-relaxed. Anterior intestine broad but posteriorly it is pushed aside by the testis towards the dorsal wall of the body. The intestine opens to a cloaca. Testis monorhic, reflexed. Spicules paired, symmetrical and curved. Manubrium of spicule distinguishable. Gubernaculum short. Bursa absent. The tail consists of two parts, a conical and a thin sharp spine.

Adult females (Figures 55a,55b,55c). Body robust.

Figure 53. Pharyngeal region of _Mikoletzkya aerivora_.

S:	stoma
C:	corpus
MC:	metacorpus
I:	isthmus
NR:	nerve ring
BB:	basal bulb

Scale: 18 μm

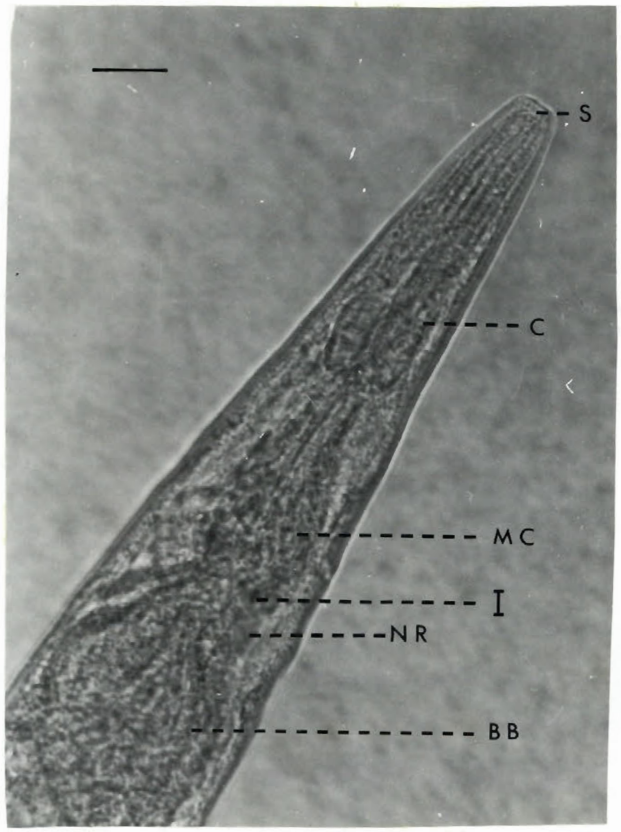

S
C
MC
I
NR
BB

Figure 54a. Adult male of <u>Mikoletzkya</u> <u>aerivora</u>.

Scale: 90 μm

Figure 54b. Tail of adult male of <u>M</u>. <u>aerivora</u>.

M: manubrium of spicule
Sp: spicule
G: gubernaculum

Scale: 14 μm


M
Sp
G

Figure 55a. Adult female of *Mikoletzkya aerivora*.

Scale: 125 μm

Figure 55b. Vulva region of adult female of _M. aerivora_.

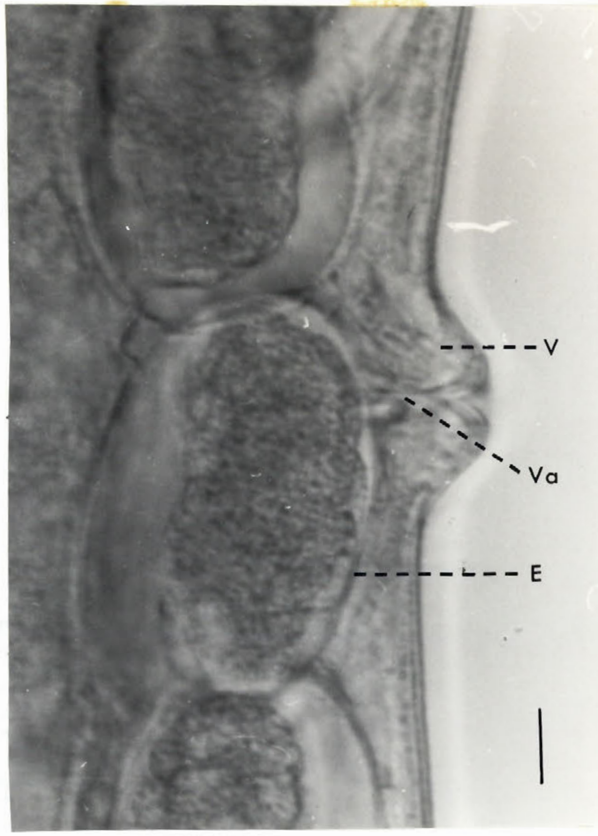
V: vulva
Va: vagina
E: egg

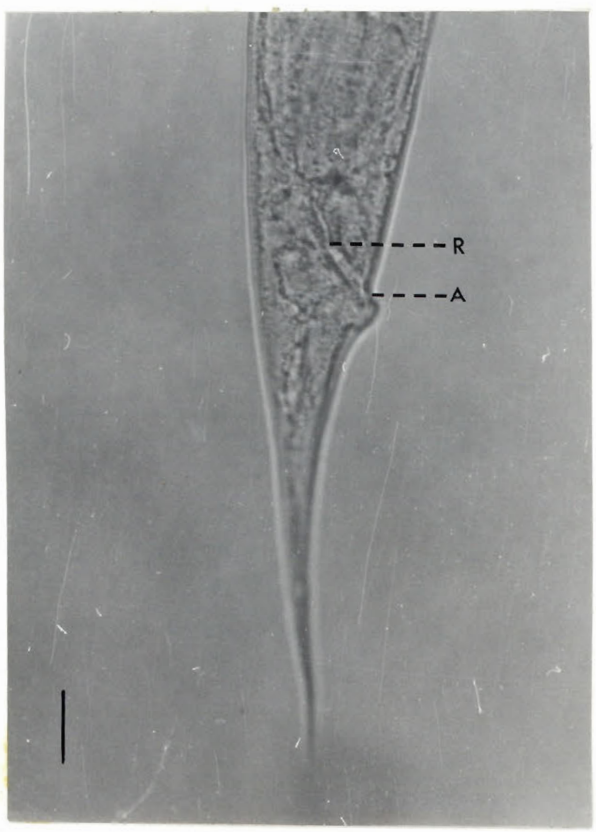
Scale: 8 μm

Figure 55c. Tail of adult female of _M. aerivora_.

R: rectum
A: anus

Scale: 22 μm


V
Va
E


R
A

Slightly curved after being heat-relaxed. The intestine is also a simple tube form. Rectum short and distinct. Anus slightly elevated from the cuticle. Ovaries amphidelphic, usually reflexed. Vulva prominent, median in location, generally protruding from the body surface. Vagina short and straight. Tail tapering to fine tip.

3. Infection Test

Results of the infection tests are listed in Table 27. The cadavers infested with nematodes in the tests were either a normal (creamy white) colour or grey. As the tests were carried out in the laboratory, the grubs were subjected to stress conditions and high mortalities were found in treated and check groups. Many grubs might have died due to shortage of food and diseases other than nematode infection. A single case of a grub in the check group was found to be infected with nematodes. This might be explained by the fact that all the grubs used in the experiments were collected from the field. The grubs could have been infected in the field by the nematode which then built up to large numbers during the experimental period. Positive infections, however, were shown in both tests.

4. Storage Viability at $5^{o}C$

A total of 25 nematode suspensions were harvested between February 2, 1976 and August 20, 1976 and stored in Erlenmyer

Table 27. Results of the infection tests on grubs of *Phyllophaga anxia* by the diplogasterid nematode, *Mikoletzkya aerivora*.[a,b]

Test	Group	No. Grubs Tested	Dosage of Inoculum (nemas/ml)	No. of Cadavers with nemas	No. of Cadavers without nemas
I	Treated	10	2.0×10^3	3	2
	Check	10	-	0	3
II	Treated	10	2.4×10^3	4	6
	Check	10	-	1	8

[a]Both tests concluded 78 days after inoculation.

[b]Second- and third-instar grubs were used for test I, and II, respectively.

flasks at 5^{o}C. These were then brought from the cold on March 14, 1978 and examined for their viability. It was found that 18 out of the 25 suspensions contained live specimens. Most of the adults were dead but juveniles were found moving actively only about five minutes after removal from the cold. This showed that M. aerivora can be stored successfully at 5^{o}C for a period of at least one and half years. Juveniles of many species of diplogasterid nematodes are able to survive periods of adverse conditions (Poinar, 1969).

The potential of M. aerivora as a biological control agent for P. anxia should be investigated. It would also be of interest to investigate the application of a combination of M. aerivora and the milky disease organism, Bacillus popillae Dutky, for control of larval stages of P. anxia. It is hoped that the nematode may act as a carrier of the bacteria and actively search for the host.

X. TOXICITY OF SOME INSECTICIDES TO THE THIRD INSTAR GRUBS OF *PHYLLOPHAGA ANXIA* (LECONTE) (COLEOPTERA: SCARABAEIDAE)

A. INTRODUCTION

White grubs, the larvae of many species of June beetles, *Phyllophaga* spp., cause economic loss to a wide range of crops as well as turf and trees in Canada and the United States (Metcalf *et al.*, 1962; Beirne, 1971). In 1971, a population of white grubs completely destroyed a potato crop near Nicolet in the Province of Quebec (Morrison, 1971). It is known that in Quebec the most common and injurious species of June beetles is the common June beetle, *Phyllophaga anxia* (LeConte) (Hammond, 1940). In the past, organochlorine insecticides were used for control of white grubs (Shenefelt and Simkover, 1951; Hammond, 1960; Pass, 1964; Polivka, 1965; Fowler and Wilson, 1971), but in the last few years several *Phyllophaga* spp. had developed resistance to the organochlorine insecticides (Frankie *et al.*, 1973; Teetes, 1973,1975; Fuchs *et al.*, 1974; Plapp and Frankie, 1976), which persisted in some soils and transferred residues to some crops (Edwards, 1973; Moriarty, 1975; Brown, 1978). In recent years, alternative insecticides for control of white grubs have been tested in the United States (Teetes, 1973; Fuchs *et al.*, 1974; River *et al.*, 1977; Pike *et al.*, 1978).

Insecticides are useful in a pest management program when other carefully planned control measures have not been completely successful, and also when emergency corrective treatment is necessary (Metcalf, 1975). A preliminary study was conducted in 1977 to investigate the potential of some insecticides for future use in a white grub management program. At the present time, there are no available insecticides recommended to supplement preventive control of white grubs in Quebec potato fields (Anonymous, 1979a).

B. MATERIALS AND METHODS

Third instar larvae of *P. anxia* were collected on September 6, 1977 from a pasture in Nicolet. These were transported by aircraft the following day, packed in field soil in a cooler, to the Soil Pesticide Research Section, Research Institute, Agriculture Canada, London, Ontario, where by arrangement with Dr. C.R. Harris, a series of tests were carried out within 48 hours of arrival in the Section. Only active, healthy-looking third instar larvae were selected for the tests. Two test methods were used for determining the toxicity of the insecticides to the grubs. These were the direct contact exposure and soil treatment.

1. Direct Contact Exposure

The direct contact exposure was done using a Potter spray tower. Details of the procedures have been described by Harris and Mazurek (1961). Solutions of technical grade insecticides (95-99% purity) were made up in 19:1 acetone:olive oil solvent mixture (V/V). Solutions of 1% were used for the test. Two replicates of five larvae each were used for each insecticide treatment. Larvae were placed on filter paper in 9-cm glass petri dishes and sprayed with 5 ml of each insecticide. Checks were sprayed with solvent only. Following treatment, grubs were transferred onto the surface of 200 g moist Plainfield sand (5% water, 0.5% organic matter) in plastic pots (11.5 cm diam., 7.6 cm high). The observation containers were then covered with glass plates.

2. Soil Treatment

Procedures for soil treatment have been outlined by Harris and Mazurek (1966). Plainfield sand was used as representative of mineral soil. Insecticides were dissolved in chromatographed, distilled n-pentane and pipetted onto the soil contained in 24-fluid oz jars. Soils with 50 ppm of insecticides were prepared. Checks were treated with solvent only. After the addition of the solutions, the jars were rolled for about 10 minutes in order to mix the insecticides and the soil homogenously and also allow the pentane to evaporate. The treated soils were then weighed and placed 200 g each in plastic pots. Two replicates of five grubs each were used for each treatment.

For both the direct contact exposure and the soil treatment, the observation pots were held in a holding room at 27 $\pm$ 1^{o}C, 65 $\pm$ 5% RH and 24 hours of light. Mortality counts were made at 24-hour intervals after treatment. Grubs were lightly touched with thin forceps on the abdomen, those which failed to respond by moving at least one of the antennae or legs were considered dead. In both tests, the mortality data from the duplicates were averaged. Corrections for natural mortality were made using Abbot's formula (Abbot, 1925).

C. RESULTS AND DISCUSSION

Mortality counts of grubs were recorded for each of 5 days after the treatments. As mortality in most of the checks exceeded 30% after 48 hours, it was decided that only data collected after 24 h and 48 h would be used for evaluation of the toxicity.

1. Direct Contact Exposure

In the direct contact exposure test, fensulfothion was the most toxic, followed by fonofos, and Bay 92114 (Table 28). Most of the insecticides tested were more toxic by contact than dieldrin. It has to be pointed out that although grubs treated

Table 28. Direct contact toxicity of 10 insecticides (1.0% solution) to third instars of *Phyllophaga anxia*.

Insecticide[a]	Average Corrected % Mortality[b] 24 Hours	48 Hours
Fensulfothion	89	100
Fonofos	46	86
Bay 92114	46	86
WL 24073	25	73
Chlorfenvinphos	14	59
Diazinon	25	48
Isozophos	14	32
Dieldrin	14	32
Terbufos	14	32
Chlorpyrifos	0	32

[a]Chemical designations of experimental insecticides without accepted common names are:

Bay 92114: Isopropyl salicylate 0-ester with 0-ethyl isopropyl-phosphoramidothioate

Shell WL 24073: 0- 2-chloro-1-(2,5-dichloropheny)vinyl 0-methyl-ethylphosphonothioate

[b]Corrected by Abbott's formula.

with terbufos were able to move the legs or antennae during the mortality counts, they definitely had been weakened. The body colour changed to light brown and the movements of appendages were tremulous.

The high contact toxicity of fensulfothion agreed with the result reported by Pike *et al*. (1978), that the second instar grubs of *P. anxia* were most susceptible to carbofuran and fensulfothion.

2. Soil Treatment

In the soil treatment test, WL 24073 was the most toxic, followed by diazinon and fonofos (Table 29). Fensulfothion was the least toxic in the soil treatment. Dieldrin was the fourth most toxic among the insecticides tested in the soil treatment.

By comparing results of the direct contact exposure and soil treatment, it appears that both WL 24073 and fonosfos had consistent high toxicity. Fonofos has also been reported quite effective in control of third instar grubs of *P. anxia* in greenhouse experiment (River *et al*., 1977). Fensulfothion has been reported as an effective soil insecticide for control of *Phyllophaga crinita* (Burmeister) in Texas (Teetes, 1973; Fuchs *et al*., 1974) and recently it was found to be markedly less effective in soil (Kinoshita *et al*., 1978). The complexity of the factors that affect the effectiveness of soil insecticides has been discussed by Harris (1972).

Table 29. Toxicity of 10 insecticides when applied as soil treatment (50 ppm) to third instars of *Phyllophaga anxia*.

Insecticide	Average Corrected % Mortality[a]	
	24 Hours	48 Hours
WL 24073	40	100
Diazinon	47	75
Fonofos	58	63
Dieldrin	26	63
Chlorfenvinphos	37	5
Bay 92114	5	50
Isozophos	26	38
Terbufos	16	38
Chlorpyrifos	16	38
Fensulfothion	16	13

[a]Corrected by Abbott's formula.

XI. SUMMARY AND CONCLUSIONS

A monitoring program for adults of Phyllophaga anxia (LeConte) using blacklight traps for determining spring emergence and the seasonal flight activity was established. The flight period started in the last three weeks of May and terminated before the end of June in most parts of southern Quebec in the years 1975-1977. Adults emerged at the southernmost collecting station (Ste. Clothilde), 15 days in advance of those at the northernmost station (Les Buissons). The adults emerged after a thermal accumulation of 176.1 ± 13.55 degree-days above a base of $5^{o}C$. Thermal accumulation could not be used for forecasting of 50% emergence of P. anxia, as the emergence rate varied from year to year. The flight peak occurred in late May or early June, depending on the season. Males emerged prior to females. In most stations, more males than females were collected by light traps.

There are six species of Phyllophaga present in southern Quebec. A pictorial key has been prepared for the identification of males (6 spp.) and females (3 spp.) of Phyllophaga spp. present in southern Quebec. P. anxia was the most numerous and widely distributed.

Freshly laid eggs of _P. anxia_ are white and ellipsoidal, but change to spherical as development progresses. First, second and third instar grubs have mean head capsule widths of 1.52 mm, 2.72 mm, and 4.39 mm, respectively. Prepupae stay in the earthern pupal cells and are recognized by the wrinkled appearance of the posterior end of the body. Male pupae only have a conical structure (future phallic organ) on the ventral side of the caudal segments of abdomen. _P. anxia_ has a three-year life cycle in Quebec. In the first year, adults begin to fly in early May. Eggs are laid between June and July. First instars are abundant between mid-July and mid-August. The first molt to second instar occurs in mid-August. The first winter is spent as the second instar. In the second year, second instars molt into third instars about the third week of June. The second winter is spent as third instar. In the third year, the third instars develop into prepupae in late June. Pupation occurs in mid-July. Teneral adults emerge from the pupae in August and stay in the soil until the following spring. During 1976 and 1977, summer egg mortality and overwintering mortality of the third instar grubs were the two critical factors that affected population density in pastures at Valleyfield and Nicolet. Most of the study areas had overlapping of broods, but in Valleyfield, L'Acadie, L'Assomption and Nicolet, there were distinct broods.

Spatial distribution and optimum sample size were determined in pastures in Valleyfield and Nicolet. Most of the counts of frequency distribution were significantly different from the theoretical Poisson distribution, but not significantly different from the negative binomial distribution. The variance/mean ratio, Morisita's index and parameter k of the negative binomial distribution also indicated that the spatial distribution was not random. Optimum sample size was determined at the coefficient of variability of 10, 20 and 30%.

An overwintering experiment of third instar grubs of _P_. _anxia_ was conducted in Ste-Anne-de-Bellevue for the winter of 1976-1977. There was no significant difference between the mortalities of treated (field) and check (indoor) groups. Some grubs in the treated group were able to survive the winter at a depth less than 15 cm. When recovered in the spring, grubs in the treated group remained in the third stage, while most of those in the check group had developed into prepupal or pupal stage.

Sixteen species of parasitic and predatory insects were found attacking or in close proximity to _P_. _anxia_. These were: Tachinidae (4 spp.), Pelecinidae (1 sp.), Tiphiidae (1 sp.), Asilidae (2 spp.) and Carabidae (8 spp.). Two species of mites, _Tyrophagus_ sp. and an anoetid mite, were found on grubs and adults of _P_. _anxia_ and are considered to be phoretic. One species of diplogasterid nematode, _Mikoletzkya_ _aerivora_ (Cobb), was also recovered from P. anxia grubs. Pathogens found included one bacterium, Bacillus cereus Fr. & Fr., and four species of fungi, Metarhizium anisopliae (Metsch.) Sorok., Beauveria bassiana (Bals.) Vuill., Fusarium sp., and Penicillium sp. A possible infection by entomopoxvirus was also found.

Among the many natural enemies found, the diplogasterid nematode was chosen for further study, as little is known about this organism. Measurements of important characteristics for identification were made and the de Man formula was calculated. Males and females were described. M. aerivora killed second and third instar grubs of P. anxia in laboratory tests. M. aerivora can be stored at 5°C for a period of at least one and a half years.

Laboratory bioassay tests on toxicity of ten insecticides to the third instar grubs of P. anxia were carried out. In a direct contact exposure test, fensulfothion, fonofos and Bay 92114 were the most toxic. In a soil treatment test, WL 24073, diazinon and fonofos were more toxic. Fensulfothion was the least toxic in soil.

XII. REFERENCES CITED

(Title abbreviations according to Biosis list of serials)

Abbot, W.S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol., 18: 265-267.

Angus, T.A. 1965. Symposium on microbial insecticides. I. Bacterial pathogens of insects as microbial insecticides. Bacteriol. Rev., 29: 364-372.

Anonymous. 1969. Insect pest management and control. Principles of plant and animal pest control. Vol. 3. U.S. Natl. Acad. Sci. Publ. 1695. 508 p.

_______. 1979a. Pomme de terre. Protection. Min. Agric. Que. CPVQ Agdex 161. 18 p.

_______. 1979b. Petits fruits. Guide de Protection. Min. Agric. Que. CPVQ Agdex 230. 14 p.

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

Baker, A.D. 1962. Check list of nematode superfamilies Dorylaimoidea, Rhabditoidea, Tylenchoidea, and Aphelenchoidea. E.J. Brill, Leiden. 261 p.

Barnett, H.L. and B.B. Hunter. 1972. Illustrated Genera of Imperfect Fungi. Third edition. Burgess, Minneapolis, Minn. 241 p.

Bedford, G.O. 1976. Use of a virus against the coconut palm rhinoceros beetles in Fiji. PANS, 22: 11-25.

Beirne, B.P. 1971. Pest insects of annual crop plants in Canada. I. Lepidoptera, II. Diptera, III. Coleoptera. Mem. Entomol. Soc. Can.,78. 128 p.

Bell, J.V. 1974. Mycoses, p. 185-236 in G.E. Cantwell, ed. Insect Diseases. Vol. 1. Marcel Dekker, New York.

Benoit, P. 1975. French names of insects in Canada with corresponding Latin and English names. Min. Agric. Que. QA38-R4-30. 214 p.

Berberet, R.C. and T.J. Helms. 1969. Two Eugregarina, _Gregarina_ sp. and _Actinocephalus_ sp., associated with the scarab _Phyllophaga anxia_, as observed in histological sections. J. Invertebr. Pathol., 14: 395-401.

_______. 1970. Notes on the biology of _Tiphia berbereti_ (Hymenoptera: Tiphiidae), a parasite of larval _Phyllophaga anxia_. Ann. Entomol. Soc. Am., 63: 471-473.

_______. 1972. Comparative anatomy and histology of selected systems in larval and adult _Phyllophaga anxia_ (Coleoptera: Scarabaeidae). Ann. Entomol. Soc. Am., 65: 1026-1053.

Berisford, Y.C. and C.H. Tsao. 1975. Distribution and pathogenicity of fungi associated with the bagworm, _Thyridopteryx ephereraeformis_ (Harworth). Environ. Entomol., 4: 257-261.

Bird, A.F. 1971. The Structure of Nematodes. Academic Press, New York. 318 p.

Bliss, C.I. 1958. The analysis of insect counts as negative binomial distributions. Proc. 10th Int. Congr. Entomol., Montreal, 2: 1015-1032.

Bliss, C.I. and R.A. Fisher. 1953. Fitting the negative binomial distribution to biological data and note on the efficient fitting of the negative binomial. Biometrics, 9: 176-200.

Böving, A.G. 1942. A classification of larvae and adults of the genus Phyllophaga (Coleoptera: Scarabaeidae). Mem. Entomol. Soc. Wash. 2. 96 p.

Brown, A.W.A. 1978. Ecology of Pesticides. John Wiley, New York. 525 p.

Brues, C.T. 1928. A note on the genus _Pelecinus_. Psyche, 35: 205-209.

Burkhardt, C.C. 1955. Preliminary studies on the control of white grubs in wheat. J. Kans. Entomol. Soc., 28: 60-63.

Burrage, R.H. and G.G. Gyrisco. 1954. Distribution of third instar larvae of the European chafer and the efficiency of various sampling units for estimating their populations. J. Econ. Entomol., 47: 1009-1014.

Chagnon, G. and A. Robert. 1962. Principaux Coleopteres de la Province de Quebec. Second edition. Les Presses de l'Universite de Montreal. 440 p.

Chamberlin, T.R. 1944. Observations on nematodes associated with white grubs. J. Econ. Entomol., 37: 313-314.

Chamberlin, T.R., C.L. Fluke, L. Seaton, J.A. Callenbach and P.O. Ritcher. 1938. Population and host preferences of June beetles in southern Wisconsin in 1935, 1936 and 1937. Insect Pest Surv. Bull. (Supplement to No. 4), 18: 225-240.

Chandler, L., J.G. Taylor and H.O. Deay. 1955. Phyllophaga collected at light traps in Indiana (Scarabaeidae, Coleoptera). Proc. Indiana Acad. Sci., 65: 149-158.

Chapman, R.N. 1931. Animal Ecology with Special Reference to Insects. McGraw-Hill, New York. 464 p.

Clausen, C.P., H.A. Jaynes and T.R. Gardiner. 1933. Further investigations of the parasites of Popillia japonica in the Far East. U.S. Dep. Agric. Tech. Bull. 366. 58 p.

Courtney, W.D., D. Polley and V.L. Miller. 1955. TAF, an improved fixative in nematode technique. Plant Dis. Rep., 39: 570-571.

Crawford, A.M. and J. Kalmakoff. 1977. A host-virus interaction in a pasture habitat. Wiseana sp. (Lepidoptera: Hepialidae) and its baculoviruses. J. Invertebr. Pathol., 29: 81-87.

Criddle, N. 1918. The habits and control of white grubs in Manitoba. Agric. Gaz. Can., 5: 449-454.

Daniels, N.E. 1966. The association of a rangeland grub, Phyllophaga koehleriana (Coleoptera: Scarabaeidae), with asilid larvae and with mites. Ann. Entomol. Soc. Am., 59: 1021.

_______. 1971. Detection of insecticidal residue and control of soil insects. J. Econ. Entomol., 64: 175-177.

Danielson, S.D. and R.E. Berry. 1978. Redbacked cutworm: sequential sampling plans in peppermint. J. Econ. Entomol., 71: 323-328.

Davis, J.J. 1916. A progress report on white grub investigations. J. Econ. Entomol., 9: 261-281.

Davis, J.J. 1918. Common white grubs. U.S. Dep. Agric. Farmers' Bull. 940. 28 p.

_______. 1919. Contributions to a knowledge of the natural enemies of Phyllophaga. Ill. Nat. Hist. Surv. Bull., 13: 53-138.

Dillon, E.S. and L.S. Dillon. 1961. A Manual of Common Beetles of Eastern North America. Row Peterson and Co., Evanston, Illinois. 884 p.

Doane, J.F. 1977. Spatial pattern and density of Ctenicera destructor and Hypolithus bicolor (Coleoptera: Elateridae) in soil in spring wheat. Can. Entomol., 109: 807-822.

Drake, C.J., B.J. Travis and E.V. Collins. 1932. A preliminary report on the control of white grubs with a rotary plow. J. Econ. Entomol., 25: 199-206.

DuPorte, E.M. 1915. A disease of the white grub. Que. Soc. Prot. Plants Rep., 7: 83-85.

Dutky, S.R. 1941. Susceptibility of certain scarabaeid larvae to infection by type A milky disease. J. Econ. Entomol., 34: 215-216.

_______. 1963. The milky diseases, p. 75-115 in E.A. Steinhaus, ed. Insect Pathology. An Advanced Treatise. Vol. 2. Academic Press, New York.

Dutky, S.R. and E.L. Gooden. 1952. Coxiella popilliae n. sp., a rickettsia causing blue disease of Japanese beetle larvae. J. Bacteriol., 63: 743-750.

Edwards, C.A. 1973. Environmental Pollution by Pesticides. Plenum Press, New York. 542 p.

Fargues, J. 1976. Spécifité des champignons pathogènes imparfaits [Hyphomycetes] pour les larves de Coléoptès [Scarabaeidae et Chrysomelidae]. Entomophaga 21: 313-323.

Fedorko, A. 1971. Nematodes as factors reducing the populations of Colorado beetle, Leptinotarsa decemlineata Say. Acta Phytopathol. Acad. Sci. Hung., 6: 175-179.

Fedorko, A. and S. Stanuszek. 1971. Pristionchus uniformis sp. n. (Nematoda, Rhabditida, Diplogasteridae), a facultative parasite of Leptinotarsa decemlineata Say and Melolontha melolontha L. in Poland. Morphology and biology. Acta Parasitol. Pol., 19: 95-112.

Ferron, P. and B. Hurpin. 1974. Effets de la contamination simultanee ou successive par Beauveria tenella et par Entomopoxvirus melolontha des larves de Melolontha melolontha (Col. Scarabaeidae). Ann. Soc. Entomol. Fr., 10: 771-779.

Ferron, P., P.H. Robert and A. Deotte. 1975. Susceptibility of Oryctes rhinoceros adults to Metarhizium anisopliae. J. Invertebr. Pathol., 25: 313-319.

Fleming, W.E. and F.E. Baker. 1936. A method for estimating populations of larvae of the Japanese beetle in the field. J. Agric. Res., 53: 319-331.

Fluke, C.L. and P.O. Ritcher. 1935. Encouraging results in spraying oak trees to reduce white grubs. Wis. Agric. Exp. Stn. Bull., 430: 7-10.

Forbes, S.A. 1916. A general survey of the May beetles (Phyllophaga) of Illinois. III. Agric. Exp. Stn. Bull., 186: 215-257.

Fowler, R.F. and L.F. Wilson. 1971a. White grubs populations related to damaged red pine seedlings in Michigan and Wisconsin plantations. Mich. Entomol., 4: 23-28.

_______. 1971b. Evaluation of three aldrin application methods for white grub, Phyllophaga spp., control (Coleoptera: Scarabaeidae). Mich. Entomol., 4: 89-91.

_______. 1974. Injury to aldrin-treated and untreated red pine by white grubs (Coleoptera: Scarabaeidae) and other agents during first five years after planting. Great Lakes Entomol., 7: 81-88.

Frankie, G.W., H.A. Turney and P.J. Hamman. 1973. White grubs in Texas turfgrass. Tex. A & M Univ., Ext. Serv. L-1131. 3 p.

Fuchs, T.W., J.A. Harding and T. Dupnik. 1974. New technique for evaluation of insecticides against Phyllophaga crinita. J. Econ. Entomol., 67: 455-456.

Gauthier, G. 1936. Observations concerning the white grubs in Quebec, in 1935. Entomol. Soc. Ont. Annu. Rep., 66: 45-48.

_______. 1944. White grub control. Entomol. Soc. Ont. Annu. Rep., 74: 26.

Glasgow, R.D. 1916. Phyllophaga Harris (Lachnosterna Hope): a revision of the synonomy, and one new name. Ill. Nat. Hist. Surv. Bull., 11: 365-379.

Goodey, T. 1963. Soil and Freshwater Nematodes. Second edition. Metheun, London. 544 p.

Goodwin, R.H. and B.K. Filshie. 1975. Morphology and development of entomopoxviruses from two Australian scarab beetle larvae (Coleoptera: Scarabaeidae). J. Invertebr. Pathol., 25: 35-46.

Goodwin, R.H. and R.J. Roberts. 1975. Diagnosis and infectivity of entomopoxviruses from three Australian scarab beetle larvae (Coleoptera: Scarabaeidae). J. Invertebr. Pathol., 25: 47-57.

Granados, R.R. 1973. Insect poxviruses: pathology, morphology, and development. Misc. Publ. Entomol. Soc. Am., 9: 73-94.

Granovsky, A.A. 1956. Ecological studies on vertical movements in the life cycle of Phyllophaga. Proc. 10th Int. Congr. Entomol., Montreal 3: 375-383.

Gruner, L. 1973. Sensibilisation des larves de Phyllophaga pleei Bl. et de P. patrueloides Pa. (Coleoptera: Scarabaeidae) a la mycose a Metarrhizium anisopliae Sorokin au moyen d'une faible dose d'insecticide ou d'un autre agent infectieux. Ann. Zool.-Ecol. Anim., 5: 335-349.

Guppy, J.C. and D.G. Harcourt. 1970. Spatial pattern of the immature stages and teneral adults of Phyllophaga spp. (Coleoptera: Scarabaeidae) in a permanent meadow. Can. Entomol., 102: 1354-1359.

Guppy, J.C. and D.G. Harcourt. 1973. A sampling plan for studies on population dynamics of white grubs, *Phyllophaga* spp. (Coleoptera: Scarabaeidae). Can. Entomol., 105: 479-483.

Hamm, J.J. 1966. A modified azan staining technique for inclusion body viruses. J. Invertebr. Pathol., 8: 125-126.

Hammond, G.H. 1931. The white grub situation in eastern Canada. Entomol. Soc. Ont. Annu. Rep., 61: 69-73.

________. 1933. Ploughing and discing for the control of white grubs in eastern Canada. Entomol. Soc. Ont. Annu. Rep., 64: 47-51.

________. 1934. A study of white grub losses to individual crops under mixed farming conditions. Que. Soc. Prot. Plants Annu. Rep., 25: 72-74.

________. 1940. White grubs and their control in eastern Canada. Can. Dep. Agric. Publ. 668. 18 p.

________. 1941. Feeding habits and vertical movements of third year grubs of *Phyllophaga anxia* LeConte. Entomol. Soc. Ont. Annu. Rep., 71: 20-22.

________. 1944a. The feeding habits of *Phyllophaga* larvae. Entomol. Soc. Ont. Annu. Rep., 74: 23-26.

________. 1944b. Economic importance and host relationship of *Pelecinus polyturator* Drury. Can. Entomol., 76: 150.

________. 1946a. White grub infestations in Ontario during 1945. Entomol. Soc. Ont. Annu. Rep., 76: 15-18.

________. 1946b. How to foretell and control outbreaks of white grubs. Can. Dep. Agric. Proc. Publ., 42. 5 p.

________. 1948a. The distribution, life-history and control of *Phyllophaga anxia* Lec. in Quebec and Ontario. Sci. Agric. (Ottawa) 28: 403-416.

________. 1948b. Soil pH and intensity of *Phyllophaga* infestation. Entomol. Soc. Ont. Annu. Rep., 78: 13-18.

Hammond, G.H. 1948c. Control of Phyllophaga spp. beetles with DDT and benzene hexachloride. Entomol. Soc. Ont. Annu. Rep., 78: 24-28.

_______. 1949. Benzene hexachloride (BHC) as a rapid control agent for white grubs (Phyllophaga spp.). Can. Entomol., 81: 200-201.

_______. 1954. Long-term fluctuations in populations of white grubs (Phyllophaga spp.) in sod in eastern Ontario. Emp. J. Exp. Agric., 22: 59-64.

_______. 1960. Control of white grubs in eastern Canada. Can. Dep. Agric. Publ. 1069. 8 p.

_______. 1961. Observations on infection of white grubs, Phyllophaga spp., by Cordyceps ravenellii Berk. & Curt. in eastern Canada. Can. Field-Nat., 175: 41-42.

Hammond, G.H. and G. Maheux. 1934. Comment lutter contre les vers blanc dans Quebec. Min. Agric. Que. Bull. 130. 15 p.

Hammond, R.B. and L.P. Pedigo. 1976. Sequential sampling plans for the green cloverworm in Iowa soybeans. J. Econ. Entomol., 69: 181-185.

Harcourt, D.G. 1960. Distribution of the immature stages of the diamondback moth, Plutella maculipennis (Curt.) (Lepidoptera: Plutellidae), on cabbage. Can. Entomol., 92: 517-521.

_______. 1961. Spatial pattern of the imported cabbageworm, Pieris rapae (L.) (Lepidoptera: Pieridae), on cultivated Cruciferae. Can. Entomol., 93: 945-952.

_______. 1963. Population dynamics of Leptinotarsa decemlineata (Say) in eastern Ontario. I. Spatial pattern and transformation of field counts. Can. Entomol., 95: 813-820.

_______. 1965. Spatial pattern of the cabbage looper, Trichoplusia ni, on crucifers. Ann. Entomol. Soc. Am., 58: 89-94.

Hardwick, D.F. 1971. The "phenological date" as an indicator of the flight period of noctuid moths. Can. Entomol., 103: 1207-1216.

Harris, C.R. 1972. Factors influencing the effectiveness of soil insecticides. Annu. Rev. Entomol., 17: 177-198.

Harris, C.R. and J.H. Mazurek. 1961. Bioassay of organic insecticides, in terms of contact toxicity to the black cutworm, Agrotis ypsilon (Rott.). Can. Entomol., 93: 812-819.

_______. 1966. Laboratory evaluation of candidate materials as potential soil insecticides. J. Econ. Entomol., 59: 1215-1221.

Hassan, S. and C. Vago. 1972. The pathogenicity of Fusarium oxysporum to mosquito larvae. J. Invertebr. Pathol., 20: 268-271.

Hayes, W.P. and J.W. McColloch. 1920. Some observations on the genitalia of Lachnosterna. Ann. Entomol. Soc. Am., 13: 75-82.

Heimpel, A.M. and T.A. Angus. 1963. Diseases caused by spore-forming bacteria, p. 21-73 in E.A. Steinhaus, ed. Insect Pathology. An Advanced Treatise. Vol. 2., Academic Press, New York.

Henry, H.K. and C.E. Heit. 1940. Flight records of Phyllophaga (Coleoptera: Scarabaeidae). Entomol. News, 51: 279-282.

Hopkins, A.D. 1918. Periodic events and natural law as guides to agricultural research and practice. U.S. Monthly Weather Rev. Suppl. 9. 42 p.

Horn, G.H. 1887. Revision of the species of Lachnosterna of America north of Mexico. Trans. Am. Entomol. Soc., 14: 209-296.

Hudson, H.F. 1919. Some notes on the life-history of our common June beetles. Entomol. Soc. Ont. Annu. Rep., 50: 81-83.

Hull, F.M. 1962. Robberflies of the world. The genera of the family Asilidae. U.S. Natl. Mus. Bull. 224. 2 Vols. 907 p.

Hurpin, B. and P. Robert. 1972. Comparison of the activity of certain pathogens of the cockchafer Melolontha melolontha in plots of natural meadowland. J. Invertebr. Pathol., 19: 291-298.

Hurpin, B. and C. Vago. 1958. Les maladies du hanneton commun (Melolontha melolontha L.) (Col. Scarabaeidae). Entomophaga, 3: 285-330.

Ives, W.G.H. and C.L. Warren. 1965. Sequential sampling for white grubs. Can. Entomol., 97: 596-604.

Jackson, D.M. 1979. Codling moth egg distribution on unmanaged apple trees. Ann. Entomol. Soc. Am., 72: 361-368.

Jarvis, J.L. 1964. An association between a species of Caloglyphus (Acarine: Acaridae) and Phyllophaga anxia (Coleoptera: Scarabaeidae). J. Kans. Entomol. Soc., 37: 207-210.

_______. 1966. Studies of Phyllophaga anxia (Coleoptera: Scarabaeidae) in the sandhills area of Nebraska. J. Kans. Entomol. Soc., 39: 401-409.

Karandinos, M.G. 1976. Optimum sample size and comments on some published formulae. Bull. Entomol. Soc. Am., 22: 417-421.

Kerr, T.W., Jr. 1941. Control of white grubs in strawberries. Cornell Univ. Agric. Exp. Stn. Bull. 770. 40 p.

King, J.L. and J.K. Holloway. 1930. Tiphia popilliavora Rohwer, a parasite of the Japanese beetle. U.S. Dep. Agric. Circ. 145. 11p.

Kinoshita, G.B., C.H. Harris, H.J. Svec and F.L. McEwen. 1978. Laboratory and field studies on the chemical control of the crucifer flea beetle, Phyllotreta cruciferae (Coleoptera: Chrysomelidae), on cruciferous crops in Ontario. Can. Entomol., 110: 795-803.

Langston, J.M. 1927. Phyllophaga of Mississippi. Miss. Agric. Exp. Stn. Tech. Bull. 15. 103 p.

Larochelle, A. 1974. A world list of prey of Chlaenius (Coleoptera: Carabaeidae). Great Lakes Entomol., 7: 137-142.

_______. 1976. Manuel d'identification des Carabidae du Quebec. Cordulia. Suppl. 1. 127 p.

Latch, G.C.M. 1965. Metarrhizium anisopliae (Metschnikoff) Sorokin strains in New Zealand and their possible use for controlling pasture-inhabiting insects. N.Z. J. Agric. Res., 8: 384-396.

Latch, G.C.M. 1976. Studies on the susceptibility of Oryctes rhinoceros to some entomogenous fungi. Entomophaga, 21: 39-48.

Latch, G.C.M. and R.E. Fallon. 1976. Studies on the use of Metarhizium anisopliae to control Oryctes rhinoceros. Entomophaga, 21: 39-48.

LeConte, J.L. 1850. General remarks upon the Coleoptera of Lake Superior, p. 201-42 in L. Agassiz, ed. Lake Superior: Its Physical Character, Vegetation and Animals. Gould, Kendall and Lincoln Co. Boston.

Lim, K.P., W.N. Yule and R.K. Stewart. 1979a. A monitoring program for the common June beetle, Phyllophaga anxia (LeConte) (Coleoptera: Scarabaeidae) in southern Quebec. Accepted for publication by Can. Entomol.

Lim, K.P., R.K. Stewart and W.N. Yule. 1979b. A key for identification of adult June beetle, Phyllophaga spp., found in southern Quebec. Can. Entomol. Under reviewing.

Lindsey, A.A. and J.E. Newman. 1956. Use of official weather data in spring time temperature analysis of an Indiana phenological record. Ecology, 37: 812-823.

Luginbill, P. 1928. The beetles of the genus Phyllophaga inhabiting South Carolina. Ann. Entomol. Soc. Am., 21: 47-91.

_______. 1938. Control of common white grubs. U.S. Dep. Agric. Farmers' Bull. 1798. 19 p.

Luginbill, P., Sr., and H.R. Painter. 1953. May beetles of the United States and Canada. U.S. Dep. Agric. Tech. Bull. 1060. 102 p.

MacLeod, D.M. 1954. Natural and cultural variation in entomogenous fungi imperfecti. Ann. N.Y. Acad. Sci., 60: 58-70.

Madelin, M.F. 1963. Diseases caused by hyphomycetes fungi, p. 233-271 in E.A. Steinhaus, ed. Insect Pathology. An Advanced Treatise. Vol. 2. Academic Press, New York.

Maheux, G. and G. Gauthier. 1938. The most vulnerable stage of the life cycle of June beetles. Entomol. Soc. Ont. Annu. Rep., 69: 90-93.

_______. 1944. Recherches sur le hanneton commun *Phyllophaga* spp. Mom. Min. Agric. Que. 1. 69 p.

Marschall, K.J. 1970. Introduction of a new virus disease of the coconut rhinoceros beetle in Western Samoa. Nature (Lond.), 225: 288-289.

Marshall, G.E. 1951. White grub control in strawberries. J. Econ. Entomol., 44: 668-674.

Masner, L. 1979. Pelecinoidea, p. 498 *in* H.V. Danks, ed. Canada and Its Insect Fauna. Mem. Entomol. Soc. Can. 108.

Massey, C.L. 1974. Biology and taxonomy of nematode parasites and associates of bark beetles in the United States. U.S. Dep. Agric., Agric. Handb. 446. 233 p.

Merrill, J.H. and A.L. Ford. 1916. Life history and habits of two new nematodes parasitic on insects. J. Agric. Res., 6: 115-127.

Metcalf, R.L. 1975. Insecticides in pest management, p. 235-273 *in* Metcalf, R.L. and W.H. Luckmann, eds. Introduction to Pest Management. Wiley-Interscience, New York.

Metcalf, C.L., W.P. Flint and R.L. Metcalf. 1962. Destructive and Useful Insects. Their Habits and Control. Fourth Edition. McGraw-Hill, New York. 1087 p.

Milner, R.J. and G.G. Lutton. 1975. The pathogenicity of an entomopoxvirus from *Othnonius batesi* (Col.: Scarabaeidae) and its possible use as a control agent. Entomophaga, 20: 213-220.

Miner, F.D. 1948. Rearing techniques for white grubs. J. Kans. Entomol. Soc., 21: 58-59.

_______. 1952. Biology of the prairie white grub *Phyllophaga crassissima*. Arkansas Agric. Exp. Stn. Bull. 521. 75 p.

Moriarty, F. 1975. Organochlorine Insecticides: Persistent Organic Pollutants. Academic Press, New York. 302 p.

Morisita, M. 1959. Measuring of the dispersion of individuals and analysis of the distributional patterns. Mem. Fac. Sci., Kyushu Univ., Ser. E (Biol.), 2: 215-235.

Morrison, F.O. 1971. Additional information on insects in Quebec in 1971. Ann. Entomol. Soc. Que., 17: 64.

Myer, J.H. 1978. Selecting a measure of dispersion. Environ. Entomol., 7: 619-621.

Nairn, L.D. and H.R. Wong. 1965. Field key to adult June beetles (Phyllophaga spp.) attacking coniferous plantations in Manitoba. Proc. Entomol. Soc. Man., 21: 33-35.

Neiswander, C.R. 1951. Duration of the effectiveness of lead arsenate applied to turf for white grub control. J. Econ. Entomol., 44: 221-224.

_______. 1963. The distribution and abundance of May beetles in Ohio. Ohio Agric. Exp. Stn. Res. Bull. 951. 35 p.

Nirula, K.K. 1957. Observations on the green muscardine fungus in populations of Oryctes rhinoceros L. J. Econ. Entomol., 50: 767-770.

Northrup, Z. 1914. A bacterial disease of June beetle larvae, Lachnosterna spp. Mich. Agric. Exp. Sta. Tech. Bull. 18. 37 p.

Olynyk, J.E. and R. Freitag. 1979. Some phoretic associations of ground beetles (Coleoptera: Carabidae) and mites (Acarina). Can. Entomol., 111: 333-335.

Oseto, C.Y. and Z.B. Mayo. 1975. A new species of Caloglyphus (Acarina: Acaridae) associated with Phyllophaga anxia (coleoptera: Scarabaeidae). J. Kans.Entomol.Soc., 48: 212-213.

Pass, B.C. 1964. Effectiveness of insecticides against white grubs in bluegrass lawn. J. Econ. Entomol., 57: 1002-1003.

Petch, C.E. and G.H. Hammond. 1925. Notes on insect parasites of Phyllophaga anxia in the Province of Quebec. Entomol. Soc. Ont. Annu. Rep., 55: 24-28.

_______. 1926. Parasites of white grubs in southern Quebec. A progress report. Entomol. Soc. Ont. Annu. Rep., 56: 85-91.

Pettit, R.H. 1930. June beetles or white grubs in Michigan. Mich. Agric. Exp. Stn. Circ. Bull. 132. 10 p.

Pike, K.S., R.L. Rivers and Z.B. Mayo. 1978. Topical toxicity to field-collected _Phyllophaga anxia_. J. Econ. Entomol., 71: 7-8.

Pike, K.S., R.L. Rivers, C.Y. Oseto and Z.B. Mayo. 1976. A world bibliography of the genus _Phyllophaga_. Univ. Nebr. Misc. Publ. 31. 21 p.

Plapp, F.W., Jr. and G.W. Frankie. 1976. Residues of chlorinated insecticides in white grubs and soils treated for grub control in urban areas of Texas. Tex. Agric. Exp. Stn. Prog. Rep. 3376C. 10 p.

Poinar, G.O., Jr. 1966. The presence of _Achromobacter nematophilus_ in the infective stage of a _Neoaplectana_ sp. (Steinernematidae: Nematoda). Nematologica, 12: 105-108.

_______. 1969. Diplogasterid nematodes (Diplogasteridae: Rhabditida) and their relationship to insect disease. J. Invertebr. Pathol., 13: 447-454.

_______. 1972. Nematodes as facultative parasites of insects. Annu. Rev. Entomol., 17: 103-122.

_______. 1975a. Description and biology of a new insect parasitic rhabditoid, _Heterorhabditis bacteriophora_ n. gen., n. sp. (Rhabditida: Heterorhabditidae n. fam.). Nematologica, 21: 463-470.

_______. 1975b. Entomogenous nematodes. A manual and host list of insect-nematode associations. E.J. Brill, Leiden. 317 p.

_______. 1977. CIH key to the groups and genera of nematode parasites of invertebrates. Commonw. Agric. Bur., Farnham Royal, England. 43 p.

_______. 1978. Generation polymorphism in _Neoaplectana glaseri_ Steiner (Steinernematidae: Nematoda), redescribed from _Strigoderma arboricola_ (Fab.) (Scarabaeidae: Coleoptera) in North Carolina. Nematologica, 24: 105-114.

Poinar, G.O. Jr., and G.M. Thomas. 1978. Diagnostic Manual for the Identification of Insect Pathogens. Plenum Press, New York. 218 p.

Poinar, G.O. Jr., O. Triggiani and R.W. Merritt. 1976. Life history of Eudiplogaster aphodii (Rhabditida: Diplogasteridae), a facultative parasite of Aphodius fimetarius (Coleoptera: Scarabaeidae). Nematologica, 22: 79-86.

Polivka, J.B. 1965. Effectiveness of insecticides for the control of white grubs in turf. Ohio Agric. Dev. Cent. Res. Circ. 140. 7 p.

Reinhard, H.J. 1940. The life history of Phyllophaga lanceolata (Say) and Phyllophaga crinita Burmeister. J. Econ. Entomol., 33: 572-578.

Ritcher, P.O. 1939. Observation on white grub pupation. J. Kans. Entomol. Soc., 12: 64-69.

_______. 1940. Kentucky white grubs. Ky. Agric. Exp. Stn. Bull. 401. 157 p.

_______. 1949. Larvae of Melolonthinae with keys to tribes, genera and species (Coleoptera: Scarabaeidae). Ky. Agric. Exp. Stn. Bull. 537. 36 p.

_______. 1966. White grubs and their allies. Oreg. State Monogr. Stud. Entomol. 4. 219 p.

Rivers, R.L., K.S. Pike and Z.B. Mayo. 1977. Influence of insecticides and corn tillage systems on larval control of Phyllophaga anxia. J. Econ. Entomol., 70: 794-796.

Sanders, J.G. and S.B. Fracker. 1916. Lachnosterna records in Wisconsin. J. Econ. Entomol., 9: 253-261.

Sanderson, M.W. 1944. Distribution and hosts of Arkansas Phyllophaga. J. Kans. Entomol. Soc., 17: 14-21.

Seaton, L. 1939. A feeding record of Pterostichus (Poecilus) chalcites (Say) upon June beetle eggs and grubs. J. Econ. Entomol., 32: 151-152.

Sen, S.K., M.S. Jolly and T.R. Jammy. 1970. A mycosis in the Indian tasar silworm, Anthreaea mylitta Drury, caused by Penicillium citrinum Thom. J. Invertebr. Pathol., 15: 1-5.

Sevacherian, V. and V.M. Stern. 1972. Spatial distribution patterns of lygus bugs in California cotton fields. Environ. Entomol., 1: 695-704.

Sevacherian, V., V.M. Stern and A.J. Mueller. 1977. Heat accumulation for timing *Lygus* control measures in a safflower-cotton complex. J. ECON. ENTOMOL. 70: 399-402.

Shenefelt, R.D. and H.G. Simkover. 1951a. Notes on habits and "broods" of June beetles. Entomol. News, 62: 219-223.

_______. 1951b. Insecticides for control of white grubs. J. Econ. Entomol., 44: 359-362.

Sim, R.J. 1928. *Phyllophaga* (Scarabaeidae) of the United States and Canada. N.J. Dep. Agric. Circ. 145. 60 p.

Simonet, D.E. and R.L. Pienkowski. 1979. A sampling program developed for potato leafhopper nymphs, *Empoasca fabae* (Homoptera: Cicadellidae), on alfalfa. Can. Entomol., 111: 481-486.

Smirnoff, W.A. 1968. Diseases of the larch sawfly, *Pristiphora erichsonii*, in Quebec. J. Invertebr. Pathol., 10: 417-424.

Smith, K.M. 1967. Insect Virology. Academic Press, New York. 256 p.

_______. 1976. Virus-insect Relationships. Longman, London. 291 p.

Southey, J.F. 1970. Laboratory methods for work with plant and soil nematodes. Min. Agric. Fish. Food, London Tech. Bull. 2. 128 p.

Southwood, T.R.E. 1978. Ecological Methods with Particular Reference to the Study of Insect Populations. Second edition. Chapman and Hall, London. 524 p.

Steinhaus, E.A. 1949. Principles of Insect Pathology. McGraw-Hill, New York. 757 p.

_______. 1951. Report on diagnoses of diseased insects. 1944-1950. Hilgardia, 20: 629-678.

Steinhaus, E.A. and G.A. Marsh. 1962. Report of diagnoses of diseased insects 1951-1961. Hilgardia, 33: 349-390.

Stone, E.L., Jr., and H.H. Schwardt. 1943. White grub injury to young plantations in New York. J. For., 41: 842-843.

Sutton, R.F. and E.L. Stone, Jr. 1974. White grubs: a description fo foresters, and an evaluation of their silvicultural significance. Can. For. Serv., Inf. Rep. O-X-212. 21 p.

_______. 1975. White grubs and white spruce establishment. Tree Planters' Notes, 26: 9-11.

Swain, R.B. 1945. The association of nematodes of the genus Diplogaster with white-fringed beetles. J. Econ. Entomol., 38: 488-490.

Sweetman, H.L. 1927. A preliminary report on the factors controlling the oviposition of May beetles in Minnesota (Phyllophaga, Scarabaeidae, Coleoptera). J. Econ. Entomol., 20: 783-794.

_______. 1931. Preliminary report on the physical ecology of certain Phyllophaga. Ecology, 12: 401-422.

Tashiro, H. and K.H. Steinkraus. 1966. Virulence of species and strains of milky disease bacteria in the European chafer, Amphimallon majalis (Razoumowsky). J. Invertebr. Pathol., 8: 382-389.

Teetes, G.L. 1973. Phyllophaga crinita: damage assessment and control in grain sorghum and wheat. J. Econ. Entomol., 66: 773-776.

Teetes, G.L. and L. Wade. 1974. Seasonal biology and abundance of the white grub, Phyllophaga crinita Burmeister, in the Texas high plains. Tex. Agric. Exp. Stn. Prog. Rep. 3261. 4 p.

Teetes, G.L., L.J. Wade, R.C. McIntyre and C.A. Schaefer. 1976. Distribution and seasonal biology of Phyllophaga crinita in the Texas high plains. J. Econ. Entomol., 69: 59-63.

Thomas, G.M. 1974. Diagnostic techniques, p. 1-48 in G.E. Cantwell, ed. Insect Diseases. Vol. I. Marcel Dekker, New York.

Thomas, G.M. and G.O. Poinar, Jr. 1973. Report of diagnoses of diseased insects 1962-1972. Hilgardia, 42: 261-360.

Tinsley, T.W. 1979. The potential of insect pathogenic viruses as pesticide agents. Annu. Rev. Entomol., 24: 63-87.

Toohey, M.K. 1977. Laboratory and field studies on the bionomics of Phyllophaga anxia (LeConte) (Coleoptera: Scarabaeidae). M.Sc. thesis (unpublished), McGill University. 151 p.

Tulloch, M. 1976. The genus Metarhizium. Trans. Br. Mycol. Soc., 66: 407-411.

Vail, P.V. and I.M. Hall. 1969. The histopathology of a nuclear polyhedrosis in larvae of the cabbage looper, Trichoplusia ni, related to symptoms and mortality. J. Invertebr. Pathol., 13: 188-198.

Veen, K.H. 1968. Recherches sur la maladie, due a Metarrhizium anisopliae chez le criquet pelerin. Meded. Landbouwhogesch. Wageningen, 68: 1-77.

Wadley, F.M. 1950. Notes on the form of distribution of insect and plant populations. Ann. Entomol. Soc. Am., 43: 581-586.

Waters, W.E. 1959. A quantitative measure of aggregation in insects. J. Econ. Entomol., 52: 1180-1184.

Waters, W.E. and W.R. Henson. 1959. Some sampling attributes of the negative binomial distribution with special reference to forest insects. For. Sci., 5: 397-412.

Weiser, J. 1977. An Atlas of Insect Diseases. Second edition. Dr. W. Junk, The Hague. 81 + 240 p.

Weiser, J. and J.D. Briggs. 1971. Identifications of pathogens in Burges, H.D. and N.W. Hussey, eds. Microbial Control of Insects and Mites. Academic Press, New York. 861 p.

Zar, J.H. 1974. Biostatistical Analysis. Prentice-Hall, Englewood Cliffs, N.J. 620 p.

Zelazny, B. 1977. Oryctes rhinoceros populations and behavior influenced by a baculovirus. J. Invertebr. Pathol., 29: 210-215.

APPENDIX

List of manuscripts and oral presentations derived from the present thesis (as of August 1979).

Manuscripts accepted for publication:

Lim, K.P., W.N. Yule and R.K. Stewart. 1979. A monitoring program for the common June beetle, Phyllophaga anxia (LeConte) (Coleoptera: Scarabaeidae) in southern Quebec. Can. Entomol.

Lim, K.P., W.N. Yule and R.K. Stewart. 1979. A note on Pelecinus polyturator (Drury) (Hymenoptera: Pelecinidae), a parasite of Phyllophaga anxia (LeConte) (Coleoptera: Scarabaeidae). Can. Entomol.

Manuscripts submitted for publication:

Lim, K.P., R.K. Stewart and W.N. Yule. 1979. A key for identification of adult June beetles, Phyllophaga spp., found in southern Quebec. Can. Entomol.

Lim, K.P., R.K. Stewart and W.N. Yule. 1979. A historical review of Phyllophaga anxia (LeConte) (Coleoptera: Scarabaeidae), with special reference to Quebec. Ann. Entomol. Soc. Que.

Manuscripts in preparation:

Lim, K.P., W.N. Yule and R.K. Stewart. Distribution and life history of Phyllophaga anxia (LeConte) (Coleoptera: Scarabaeidae) in southern Quebec.

Lim, K.P., R.K. Stewart and W.N. Yule. Spatial distribution of Phyllophaga anxia (LeConte) (Coleoptera: Scarabaeidae) in pastures, with an estimate of optimum sample size.

Lim, K.P., R.K. Stewart and W.N. Yule. A survey of natural enemies of Phyllophaga anxia (LeConte) (Coleoptera: Scarabaeidae) in southern Quebec.

Lim, K.P., W.N. Yule and R.K. Stewart. A diplogasterid nematode, Mikoletzkya aerivora (Cobb), recovered from Phyllophaga anxia (LeConte) (Coleoptera: Scarabaeidae) in southern Quebec.

Lim, K.P. and C.R. Harris. Contact and soil bioassays of 10 insecticides for controlling white grubs, Phyllophaga anxia (LeConte) (Coleoptera: Scarabaeidae).

Oral presentations:

Lim, K.P. and W.N. Yule. 1978. The use of blacklight traps for monitoring of June beetles in southeastern Quebec. Paper presented at the 28th Annual Meeting of the Entomological Society of Canada, August 23rd, Ottawa, Ont.

Lim, K.P. 1979. Natural enemies of Phyllophaga anxia in southern Quebec. Paper presented at the 71st Annual Meeting of Quebec Society for Plant Protection, April 12th, Ste-Anne-de-Bellevue, Que.

Lim, K.P., W.N. Yule and R.K. Stewart. 1979. Basic studies towards a pest management scheme for white grubs in Quebec. A paper to be presented at the 29th Annual Meeting of the Entomological Society of Canada, Oct. 1-4, Vancouver, B.C.