NATURAL ENEMIES OF PHYLLOPHAGA SPP. (COLEOPTERA: SCARABAEIDAE) IN SOUTHERN QUEBEC, WITH SPECIAL REFERENCE TO ENTOMOPATHOGENS

by

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This thesis is dedicated to my families, the Milejs and the Poprawskis, and especially to Natalia and Kalinka.

Suggested short title:

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TADEUSZ JERŻY POPRAWSKI

ABSTRACT

Ph.D. Tadeusz Jerzy Poprawski Entomology

NATURAL ENEMIES OF PHYLLOPHAGA SPP. (COLEOPTERA: SCARABAEIDAE) IN SOUTHERN QUEBEC, WITH SPECIAL REFERENCE TO ENTOMOPATHOGENS

A systematic survey of the microbial and invertebrate natural enemies of Phyllophaga spp. was conducted from 1979 to 1981 in 45 localities in southern Quebec. Chronic but low (non-epizootic) rates of biotic regulation were found in all host life stages except eggs. The natural enemies included 36 species of predatory and parasitic insects, 15 mite species, six fungal species, five bacterial species, three different nematodes, one virus, one gordian worm, and one sporozoan. Microorganismal pathogenicities to white grubs were demonstrated by infectivity tests and laboratory bioassays using four methods of inoculation. Field-type microplot studies on the fungus Metarhizium anisopliae and the nematode Mikoletzkya aerivora were undertaken in 1982. The fungus and the nematode showed potential as biological suppressants of Phyllophaga grubs by causing 91% and 68% mortality, respectively. Twenty-seven chemicals were tested as attractants against P. anxia adults in 1981 in four localities in southern Quebec. Hexanoic acid was consistently the most attractive chemical to beetles of both sexes.

RESUME

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Les microorganismes et invertébrés, ennemis naturels des Phyllophaga spp., furent systématiquement dépistés de 1979 à 1981 en 45 localités du Québec méridional. On constata dans tous les stades du cycle de l'hôte, l'oeuf excepté, des taux de régularisation biotique chroniques mais faibles (non-épizootiques). Les ennemis naturels comprirent 36 espèces d'insectes prédateurs et parasites, 15 espèces d'acariens, six espèces de cryptogames, cinq espèces de bactéries, trois nématodes différents, un virus, un ver nématomorphe, et un sporozoaire. Quatre méthodes d'inoculation des vers blancs furent utilisées au laboratoire pour démontrer la pathogénicité microbienne en essais d'infectivité et en tests biologiques. Des études en micro-parcelles sur le cryptogame Metarhizium anisopliae et le nématode Mikoletzkya aerivora furent entreprises en 1982. Le cryptogame et le nématode, en tuant respectivement 91% et 68% des vers blancs, révélèrent leur potentialité comme agents biologiques de lutte contre les larves de Phyllophaga. L'attraction de 27 composés chimiques pour les adultes de P. anxia fut éprouvée en 1981 en quatre localités du Québec méridional. L'acide hexanoique fut constamment le plus attrayant pour les scarabées des deux sexes.

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Finally, to my wife Natalia I wish to express my most sincere appreciation and profound respect for her patience and

support during all these years and for her invaluable help in typing this thesis.

CLAIM TO ORIGINALITY

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

1. The June beetles, Phyllophaga fraterna (Harris) and P. rugosa (Melsheimer) were collected in southern Quebec for the first time.

2. A new small iridescent virus, Phyllophaga anxia iridescent virus (PaIV), was found to infect white grubs. Phyllophaga anxia (LeConte). The first record of a naturally occurring virus disease of Phyllophaga spp. for North America. The first record of a natural virus of Scarabaeidae for North America.

3. PaIV virus was isolated, purified, characterized and its antiserum was prepared for the first time.

4. PaIV was shown to cause mortality of P. anxia grubs in infectivity tests.

5. A eugregarine sporozoan, Actinocephalus sp., was found associated with all stages of Phyllophaga spp. except eggs for the first time in Canada.

6. The eugregarine, Actinocephalus sp., was demonstrated to be chronically present in populations of grubs of Phyllophaga spp. throughout southern Quebec. A distribution map of Actinocephalus sp. in populations of grubs of Phyllophaga spp has been produced for southern Quebec.

7. The bacteria Bacillus popilliae Dutky, Pseudomonas aeruginosa (Schroeter) Migula and Serratia marcescens Bizio were found for the first time in several stages of Phyllophaga spp. in Canada and in North America for the latter two bacteria.

8. Five bacterial species: B. popilliae, P. aeruginosa, S. marcescens, Bacillus cereus Frankland and Frankland, and Micrococcus nigrofasciens Northrup were shown to be pathogenic to grubs of Phyllophaga spp. in force-feeding and intrahemocoelic injection tests for the first time in Canada.

9. A local isolate of B. popilliae and the commercial preparation DOOM (milky disease spores) were assayed against grubs of Phyllophaga spp. for the first time in Canada, in comparative laboratory bioassays, using four methods of inoculation for the test grubs.

10. The susceptibility of grubs of Phyllophaga spp. to five fungal species: Metarhizium anisopliae (Metschnikoff) Sorokin, Beauveria bassiana (Balsamo) Vuillemin, Fusarium sp. near F. solani (Martius) Appel and Wollenweber, Aspergillus Micheli: Fries and Penicillium Link: Fries was demonstrated for the first time in infectivity tests.

11. The fungi B. bassiana and M. anisopliae were tested in the laboratory against grubs of Phyllophaga spp. for the first time, using four methods of inoculation of the test grubs.

12. Field microplot studies on a local isolate of M. anisopliae as a potential control agent of Phyllophaga grubs were conducted for the first time.

13. A gordian worm, a rhabditid and an aphelenchoidid nematode were found from Phyllophaga individuals for the first time. New host records.

14. The parasitic nature of the entomogenous, diplogasterid nematode, Mikoletzkya aerivora (Cobb) was demonstrated for the first time in behavior studies.

15 The survival of M aerivora was found to depend on regular passages through an insect host, eg , Phyllophaga grubs ,

16 An improved apparatus for extraction of live M aerivora from its insect host was built and its efficiency was demonstrated

17 Field microplot studies on M aerivora as a poténtial control agent of Phyllophaga grubs were conducted for the first time

18 A systematic survey of mites associated with Phyllophaga spp was conducted for the first time in southern Quebec

19 Several species of mites were found for the first time from Phyllophaga spp in Canada

20 An undescribed species of mite, Scarabaspis sp n , was found from P anxia adults

21 Several species of predatory and parasitic insects were found for the first time to attack Phyllophaga spp

22 The natural enemies' complex of Phyllophaga spp was qualified and quantified for the first time in Quebec

23 Twenty-seven chemicals were tested as attractants against P anxia adults for the first time

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I. GENERAL INTRODUCTION

Phyllophaga spp. are well known to the layman as May or June beetles which defoliate ornamental trees and shrubs and are a nuisance around outdoor lights and lighted windows during their flight periods. The larvae, commonly known as white grubs, are polyphagous soil-dwellers that are major pests of lawns and other turf grasses and such agricultural commodities as pasture and hay grasses, strawberries, corn, potato tubers and young nursery trees (Metcalf et al , 1962; Beirne, 1971).

Phyllophaga anxia (LeConte) is the most common, and consequently destructive, species of June beetle in the province of Quebec (Hammond, 1940, Toohey, 1977; Lim, 1979), but is also found in several other Canadian provinces and American states (Luginbill and Painter, 1953) Most of the damage occurs in the second year or "white grub year" of the three year life cycle of P. anxia in Quebec and Ontario, and is essentially caused by heavy feeding of third instar grubs on plant roots (Hammond, 1948a, 1954). A note on these important pests appeared in 36 of the Annual Reports (Proceedings) of the Entomological Society of Ontario between 1900 and 1937 and most of them commented on the economic damage caused by white grubs and on the extreme difficulty of controlling them. The reports for 1948, 1951, 1954 and 1957 were similar, covering the years of widespread occurrence of the second year larvae of June beetles. Crop damage by white grubs in Eastern Canada peaked in the 1930's and 1940's. Losses of up to 75% in fodder corn were common in Eastern Canada (Hammond, 1934a); many thousands of ha of

sod were destroyed in Ontario in 1945 (Hammond, 1946a); annual losses of $500,000 were estimated to be caused by white grubs from Manitoba eastwards (Hammond, 1948a).

There was no control for white grubs until 1949 on agricultural land except crop rotations, repeated discing and other cultural methods, whereas applications of up to 645 pounds of lead arsenate per acre per season were recommended for lawns and golf greens in Ontario and several American states (Anonymous, 1974). Thereafter and until 1957, chlordane, lindane, aldrin, dieldrin and heptachlor were used to control white grubs on lawns and golf greens but damage to pasture still occurred. (Anonymous, 1974). From 1958 to 1967 several cyclodiene insecticides and lindane were recommended for white grub control except on pasture, and chlordane continued to prevent loss on lawns and turf. However, for unknown reasons, the populations of white grubs decreased dramatically after 1960 on pasture land although insecticide treatments were not widely applied in Ontario and Quebec (Anonymous, 1974; Morrison in Toohey, 1977). Such a situation also occurred in New York state where Phyllophaga spp.were almost "endangered species" in the late 1970's (Gyrisco, G. G., 1980 personal communication); Gyrisco (1980) found a very high correlation between dry weather in the flight year and subsequent reduction of grub populations, but this did not account for the almost complete disappearance of June beetles in New York and Ontario. However, populations of white grubs were still destroying chlordane-treated potato crops near Nicolet, in Quebec Province (Morrison, 1971), strawberry fields at Pierreville, Quebec (per-

sonal observation, 1979 - see Figure 2; Anonymous, 1981a) and Letendre (1982) wrote that widespread white grub infestations have been observed recently in Quebec pastures. In Quebec, there is at present no chemical control recommendation for white grubs (Anonymous, 1981b); instead, farmers are advised to resort to cultural and mechanical practices. In North America, many of the persistent chlorinated hydrocarbon insecticides, once used to control white grubs, have been de-registered for agricultural use and studies in the Unites Stated have shown that several Phyllophaga spp. have developed resistance to some of those insecticides (Plapp and Frankie, 1976). Although alternative insecticides, especially the organophosphates and carbamates, have been receiving attention recently for control of white grubs, (Reinert, 1979; Letendre, 1982), none has been registered for use against Phyllophaga spp., in Quebec.

Biological control agents are among the alternatives to use of chemical pesticides. These agents can be used against a pest through manipulation of native entomogenous species or through introduction of exotic species. Prior to introduction or manipulation of entomopathogenic microbes and entomophagous arthropods into any ecosystem, it is essential that an inventory of the natural enemies of the target species be known in order to provide a basis for future management strategies (Pschorn-Walcher, 1977).

The present study of Phyllophaga spp., principally P. anxia, was conducted in 1979, 1980, 1981 and 1982 in southern Quebec. The main objectives of the research were:

1. to inventory the non-vertebrate natural enemy complex of Phyllophaga spp.
2. to determine the impact of biotic components (parasitic and predatory arthropods, etiological agents of diseases) of the complex upon the pests species.
3. to evaluate the potential of biocontrol agents of selected natural enemies of Phyllophaga spp.
4. to determine the feasibility of autocidal control by means of behavior-modifying chemicals.

The results of the present study should provide a better understanding of the biological regulation of Phyllophaga in southern Quebec. They should also provide a basis for future management strategies against the pest by supplementing local crop protection recommendations with microbial control agents and behavior-modifying chemicals.

II. LITERATURE REVIEW

"June beetle" is one of the vernacular names given to adults of the large, near cosmopolitan scarabaeid genus Phyllophaga Harris 1827. Members of this genus have been reported from the Americas, the West Indies, eastern and southern Asia, and the islands of the Pacific and Indian Oceans. The root-feeding larvae, or "white grubs", are among the most destructive pests of numerous cultivated crops, grasses and trees in many countries, including Canada.

The literature on Phyllophaga is voluminous. Miner (1952) reviewed it up to 1950. Pike et al. (1976) listed 680 titles in their world bibliography of the genus. Toohey (1977), Lim (1979), Lim et al. (1980a) and Letendre (1982) have reviewed the literature on Phyllophaga with special reference to P. anxia (Le Conte) in Quebec.

This review deals with P. anxia since it is the major pest species of the genus in Quebec (Hammond, 1940; Maheux and Gauthier, 1944; Toohey, 1977; Lim, 1979); relevant publications on other Phyllophaga spp. are also included.

A. SYSTEMATIC HISTORY AND SYNONYMY

According to Benoit (1975), the common name for Phyllophaga anxia (LeConte) is the common June beetle. This insect has at various times since its description been known under several combinations of names, due mainly to changes in generic

concepts throughout the years. The American entomologist Harris assigned the generic name Phyllophaga in 1827, but the following year the British entomologist Hope gave the name Lachnosterna (Maheux and Gauthier, 1944). To end the growing confusion, LeConte (1850) reviewed the generic names given to species of June beetle and concluded that Phyllophaga was the valid name. However, in 1856, Lacordaire rejected the name Phyllophaga Harris 1827 as nomen nudum and Blatchley's (1910) and Dalla Torre's (1912) catalogs still opted for Lachnosterna. Finally, Glasgow (1916) revised the synonymy for North American species of June beetle and Phyllophaga Harris 1827 was adopted, although the name Lachnosterna was used for a long time in Canada (Hammond, 1948a). As recently as 1976, Pike et al. stated that "taxonomically, the genus Phyllophaga does not have a solid classification base; satisfactory generic and subgeneric divisioning have not been achieved". Each of the following names are or have been considered a valid element of the genus Phyllophaga: Phyllophaga sensu stricto, Ancylonycha, Chirodines, Chlaenobia, Clemora, Cnemarchis (=Abcrana), Endrosa, Eugastra, Gynnis, Holotrichia (=Brahmina), Lachnosterna, Listrochelus, Melolontha, Phytalus, Rhizotrogus, Tostegoptera, Trichestes and Triodonyx (Maheux and Gauthier, 1944; Pike et al., 1976). Species within the genus Phyllophaga are numerous; Dalla Torre's (1912) catalog listed 232 species worldwide, and according to Luginbill and Painter (1953) 152 species of Phyllophaga occur in North America north of Mexico with about 25 of them known from Canada.

Phyllophaga anxia (LeConte), a native species of North

America (Hammond, 1948a; Ritcher, 1949a; Neiswander, 1963), was first described by LeConte (1850) under the name Lachnosterna anxia. Luginbill and Painter (1953) listed the following synonyms for P. anxia:

Lachnosterna anxia Leconte 1850
Ancylonycha brevicollis Blanchard 1850
Ancylonycha puncticollis Blanchard 1850
Lachnosterna cephalica LeConte 1856
Ancylonycha uninotata Walker 1866
Lachnosterna dubia Smith 1888
Lachnosterna insperata Smith 1889
Lachnosterna alpina Linell 1896
Phyllophaga anxia Glasgow 1916

The taxonomic position of P. anxia is as follows (Ritcher, 1966):

Order: Coleoptera
Suborder: Polyphaga
Superfamily: Scarabaeoidea
Family: Scarabaeidae
Subfamily: Melolonthinae
Tribe: Melolonthini
Genus: Phyllophaga
Subgenus: Phyllophaga
Species: anxia

Published vernacular names used to designate various June beetles are abundant; adults are known as June beetles, June bugs, dor bugs, hardbacks, May beetles, chafers, cockchafers; larvae are referred to as white grubs, grub worms, potato grubs, garden grubs. In Quebec, the June beetle is known as hanneton, hanneton de St.-Jean, barbeau, man; and

the white grub as ver blanc, ver des jardins, turc, ver matis, engraisse-poules.

P. anxia has a wide distribution in North America, having been recorded in every Canadian province and in 43 of the continental American states (Luginbill and Painter, 1953).

Keys for identification of adult P. anxia and other Phyllophaga spp. are based on external morphological characters such as form of antennae, form of clypeus, surface and form of pronotum, spur and hind tibia of males, surface and structure of abdominal sterna (Horn, 1887; Dillon and Dillon, 1961; Nairn and Wong, 1965), and especially structure of the male and female genitalia (Smith, 1889; Langston, 1927; Luginbill, 1928; Sim, 1928, Ritcher, 1940; Böving, 1942; Luginbill and Painter, 1953; Chagnon and Robert, 1962). Luginbill and Painter (1953) distinguished a northern and southern form for P. anxia on the basis of morphological variability in the male phallic structures.

Excellent keys for identification of scarabaeid larvae, including P. anxia, were published by Böving (1942) and Ritcher (1940, 1949a, 1966), but Ritcher (1949a) emphasized that positive identification of Phyllophaga grubs was difficult because of the considerable within-species variation and overlap between taxonomic characters. The only field key to Phyllophaga adults in Canada was prepared by Nairn and Wong (1965) for Manitoba. Lim (1979) proposed a key, based on genital characters of both sexes, for Phyllophaga spp. of southern Quebec.

The following species were recorded in Quebec agricultural lands by Toohey (1977) and Lim (1979): P. anxia (LeConte),

P. drakii (Kirby), P fusca (Froelich), P futilis (LeConte), P. marginalis (LeConte) and P. nitida (LeConte). All these species were found in the present study except P marginalis, in addition, P. fraterna (Harris) and P. rugosa (Melsheimer) were recorded in several localities of southern Quebec by this author

B. MORPHOLOGY, ANATOMY, HISTOLOGY AND EMBRYOLOGY

LeConte (1850) provided the original description of adult P. anxia Further descriptions of adults of this and other species are found in numerous publications, especially in Luginbill and Painter (1953) and Dillon and Dillon (1961) Lim et al (1980a) summarized the morphological characters of adult P anxia and of third instar grubs, the latter having been originally described in detail by Böving (1941, 1942). Descriptions of all the life stages of several Phyllophaga spp. are detailed in numerous technical and research bulletins or reports of North American agricultural research stations and extension services. A partial list of pertinent publications is indexed in Pike et al (1976). For Quebec, Lim (1979) described all stages of P anxia, including the prepupa and the teneral adult Lim (1979) also listed measurements for eggs and adults and gave measurements of head width that are used to distinguish the three larval instars

The anatomy and histology of reproductive, nervous, respiratory and digestive systems of adult and third instar grub of P. anxia were studied by Hammond (1944a), and Berberet and Helms (1972).

The only contribution to the knowledge of the embryology of the June beetle was prepared by Luginbill (1953).

C. LIFE HISTORY AND BIOLOGY

1 Life History

The complete life cycle of P. anxia takes three years in eastern Canada and in most American states (Forbes, 1916; Hammond, 1931, 1940, 1948a, 1954; Hammond and Maheux, 1934; Maheux and Gauthier, 1944; Jarvis, 1966; Toohey, 1977, Lim, 1979, Lim et al , 1981b). The life history is summarized as follows.

Overwintering adults begin to emerge from the soil and to fly to neighbouring trees and shrubs during the last three weeks of May, when approximately 176 degree-days have accumulated above a base of 5°C in southern Quebec (Lim et al., 1979). Peak flight activity occurs in late May or in early June and flight is usually terminated at the end of June (Criddle, 1918; Maheux and Gauthier, 1944, Hammond, 1948a, 1954; Sutton and Stone, 1974, Lim et al., 1979) June beetles are nocturnal in habits, emerging at dusk to fly to the preferred food plants where mating also takes place, returning at dawn to hide under rocks and debris or just below the surface of the soil (Maheux and Gauthier, 1944). This pattern of activity apparently continues daily until the insects die of natural causes, succumb to their natural enemies, or until the females often die from exhaustion in their oviposition burrows (Grüner, 1973). About

ten days after mating, females lay eggs in grassy areas, at a depth of about 10 cm, eggs are enclosed in an earthen ball constructed by the females (Hudson, 1920, Hammond, 1940, 1948a, Maheux and Gauthier, 1944) First instar grubs hatch about 30 days later and feed on decaying organic matter, soil fungi and fine roots of plants (Maheux and Gauthier, 1944, Hammond, 1948a, 1954, Miner, 1952) Molting to the second instar takes place 6 to 8 weeks later and after feeding on plant roots for a short time, the second instar grubs migrate downwards and overwinter at depths of 15 to 90 cm, depending on the locality and the climate (Johnson, 1942, Hammond, 1948a)

In early spring of the second year of the life cycle, the overwintering second instar grubs migrate upwards to the root zone, feed on roots for a short period, and molt to the third instar, usually in late July This final larval stage feeds voraciously until the fall, severely damaging the roots and underground stems of a wide variety of plants, the second year of the cycle is therefore referred to as the white grub year (Hammond, 1940, 1948a, 1954) The second winter of the life cycle is spent by the third instar grubs in burrows at various depths in the soil (Sutton and Stone, 1975)

The third instar, third year grubs remain relatively inactive in the subsoil and feed very little or not at all (Hammond, 1941, 1944a, 1948a) Pupation takes place in July, following a short prepupal (yellow grub) stage Pupae transform to the teneral adult stage in August Teneral adults remain in the pupa's earthern cell until emergence in the following spring (Hammond, 1948a, Lim et al , 1979)

2 Oviposition

Several workers have reported that females of some Phyllophaga spp , including P anxia, preferred to oviposit in ground covered with grasses rather than legumes (Davis, 1916, 1918, Forbes, 1916; Hayes, 1920, Luginbill, 1938). Although eggs were deposited in legume fields, mortality was high presumably because the taproot system of legumes and the hard dry soil in which they were growing were unfavorable for larval feeding and tunneling (Chamberlin and Callenbach, 1943) Although Sweetman (1927) and Miner (1944) stated that females of 8 species of June beetle, including P anxia, did not exhibit preferences for oviposition sites on the basis of cover vegetation, Hammond (1954) found that females of P anxia preferred grassy land. Sweetman (1927) also concluded that females preferred easily-penetrated moist soils to lay their eggs, and Sweetman (1927), Miner (1944) and Toohey (1977) wrote that P anxia and other Phyllophaga spp. showed a preference for thick stands of vegetation and soils containing roots Slightly acid soils, a soil moisture of between 28 and 58% and an optimal temperature of about 25°C were found to be ideal conditions for oviposition of P. anxia females (Sweetman, 1931, Hammond, 1948a, 1949b). Females of Phyllophaga spp usually lay an average of 55 eggs (Maheux and Gauthier, 1944)

3 Distribution and Movement

Horizontal movement of Phyllophaga spp. was studied by Forbes (1907) in Illinois He concluded that there was no

evidence of significant migration of June beetles in any stage or under any circumstances The most marked movements were the evening flights of adults to their food plants, and the morning dispersal from trees to fields in which females laid their eggs. Hammond (1944a, 1948a), however, wrote that second year grubs migrated 30 m or more when searching for new roots on which to feed Spatial distribution of white grubs was studied by Lim (1979) in two pastures of southern Quebec; he concluded that P anxia individuals in the two populations were not distributed randomly but were aggregated

Vertical distribution and movement of white grubs as related to overwintering depths vary with the locality, species preference, soil texture and moisture, drainage and temperature Criddle (1918) reported that grubs of P anxia overwintered at depths of from 36 to 64 cm in wet land but as deep as 112 cm in dry woodland soils Overwintering of the grub stages of P anxia took place at 15 to 38 cm below the surface of the soil in eastern Canada (Hammond, 1941), somewhat deeper (20 to 61 cm) in western Quebec (Guppy and Harcourt, 1970), and third instars of P anxia spent the winter at a depth of 60 cm (Sutton and Stone, 1975). Granovsky (1956) reported that Phyllophaga grubs, including P anxia, overwintered at depths of 76 cm in silt loam soil and much deeper in sandy soil Non-overwintering third instar, third year grubs of P anxia were usually found at depths of 10 to 20 cm in Quebec (Hammond, 1941) and 20 to 40 cm in Manitoba (Ives and Warren, 1965)

Pupal cells of P anxia, and hence overwintering adults,

were found at less than 20 cm below the surface of the ground in Quebec (Gauthier, 1944), at depths of 10 to 23 cm (Guppy and Harcourt, 1970) in western Quebec, and as deep as 30 cm in southern Quebec (Lim, 1979).

4 Broods

Torre-Bueno (1937) defined "brood" as "all the individuals that hatch at about one time, from eggs laid by one series of parents, and which normally mature at about the same time".

The concept of brood, and hence brood zones, was used for many years, concurrently with the knowledge of the duration of the life cycle of Phyllophaga spp., to predict "white grub years" several years in advance and thus prepare preventive control measures for a given brood zone (eg. cropping rotation).

Davis (1918) recognized three distinct broods (A, B and C) for Phyllophaga spp in northeastern United States. A similar pattern was recognized for various Phyllophaga spp. in several other states (Chamberlin et al., 1938; Luginbill, 1938; Ritcher, 1940, 1949b). There was little or no overlap of broods in each locality mapped.

Hammond (1931) provided the first distribution maps for Quebec and Ontario populations of P. anxia. Three distinct zones (A,B,C) were found to exist with little or no overlap (Hammond, 1931, 1948a, 1954). Hammond and Maheux (1934) identified three brood zones in Quebec and their map was long used to predict local "outbreaks" of white grubs in Quebec's agricultural lands. In 1944, Maheux and Gauthier redefined these brood zones and

found small pockets of brood C within zone A, while Hammond (1946b) reported that there was no brood B in eastern Ontario. Toohey (1977) re-assessed the geographical location of the 3 broods of P anxia in Quebec and stated that zone C appeared to have largely merged with zone B; she strongly suggested the abandonment of the term brood zone in favor of "flight year". The value of recommendation maps for white grubs issued by Quebec Agriculture was questioned by Toohey (1977) and studies by Lim (1979) on the concept of non-overlapping brood zones for P. anxia in Quebec showed it to be invalid, thus confirming the opinion of Shenefelt and Simkover (1951).

5. Diapause

Teetes and Wade (1974) for P. crinita (Burmeister), and Toohey (1977) for P. anxia, suggested that the delay they observed from third instar grub to pupa, reared under laboratory conditions, was evidence of a facultative diapause in the third instar grub stage.

6. Rearing Phyllophaga spp.

There have been many attempts at rearing grubs of root-feeding scarabs, including Phyllophaga spp., individually in the field and mass rearing in the laboratory Field rearing was usually difficult and mass rearing was impractical, many grubs being lost to cannibalism (Girault, 1914, Davis, 1915, Hayes, 1920, Miner, 1948, 1952; Howe and Campbell, 1953).

Partial success at rearing Phyllophaga spp. in the

laboratory, often on artificial diets, was reported by Sanders and Frackers (1916), Sweetman (1931), Reinhard (1940, 1946), Ritcher (1940), Miner (1948), Teetes and Wade (1974), Toohey (1977) and Lim (1979).

7. Food, Feeding Behavior and Type of Injury

The flight of June beetles in spring coincides essentially with refoliation of many plants. Adults have been reported to feed on the leaves of numerous species of deciduous trees and shrubs, including fruit trees and berry shrubs, and of coniferous trees (Hammond, 1948a). Beetles were also observed to feed on the flower buds and young fruits of fruit trees (Hammond, 1948a), and on the leaves of various weeds, flowers, and vegetable crops (Scheibner, 1978).

Although the feeding habit of the beetles is polyphagous, a few primary plant species seem to be more attractive to one Phyllophaga sp. than to another and to the same species at different localities (Davis, 1913, 1918; Chamberlin et al., 1938). In Alabama, P. prunonculina Burmeister and P. micans Knoch apparently feed only on longleaf pine (Forbes, 1916). Forbes (1916) also noted that P. anxia, a dominant species in southern Illinois, was collected from elm, willow, poplar and apple trees; in Kentucky, the same species was mostly found on ash, oaks, sumac and blackberry (Ritcher, 1940). Feeding preference of P. anxia appeared to be for oaks, pines and grape-vine at sea level, but for maple and walnut at higher altitudes (Luginbill, 1938). Hammond (1936) reported that the worst damage caused by

the feeding of P. anxia adults in Ontario was to elm, hawthorn, rose and oaks. Elm, ash, cherry tree, hawthorn, willow and raspberry were favored by P. anxia in Quebec (Maheux and Gauthier, 1944). These authors stated that the common June beetle adapts itself to environmental conditions and will feed on trees and shrubs which are dominant in the locality; grasses, legumes and weeds growing at the edges of fields are also important host plants, especially for female P. anxia. 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 and prefer the more tender young foliage of various plants. They usually feed on the crowns of trees, and defoliate the top twigs first by gnawing inwards from the edge of leaves to the petiole (Davis, 1918; Hammond, 1948a).

Host plants may be completely defoliated as a result of the feeding of adult June beetles. The denudation caused by beetles may result in the death of trees, impoverished growth of timber and, when flower buds are eaten, in poor yield of fruits (Riley, 1869; Smith, 1889; Davis, 1918; Hammond, 1948a). According to Luginbill and Painter (1953), serious damage to trees is more common in northern latitudes than in southern areas, because of differences in weather patterns. In northerly areas, the weather remains cold and wet until late spring, then suddenly becomes dry and hot, thus triggering the sudden emergence of large numbers of beetles, whereas in southern areas, the steady rise in spring temperature causes the beetles to emerge and feed more gradually.

As early as 1857, Dupont (alias Abbé L. Provancher) wrote: "in Quebec, white grubs do not respect any root; they are the terror of the gardener and the despair of the farmer" (in Maheux and Gauthier, 1944). Although primarily grass feeders, white grubs are polyphagous and few cultivated plants are immune to their attacks (Dustan, 1932; Gibson, 1934). They feed on the underground parts of a wide variety of plants, including flowering plants, without a given species appearing to have preferred hosts (Hammond, 1948a). However, fibrous-rooted plants, such as timothy, Kentucky bluegrass, redtop, corn, strawberries and small grains are favored as foods, whereas tap-rooted plants such as clovers and alfalfa are attacked and injured to a lesser extent (Hammond, 1940, 1948a). White grubs will eat potato tubers and roots of a variety of tree nursery stock (Hammond, 1948a); they are occasional but serious pests in greenhouses when they are brought in with potting soil (Haseman and Jones, 1934).

White grubs feed about 5 cm under the soil surface (Hammond, 1941) and move forward in a horizontal plane to new food sources (Hammond, 1948a). According to Hammond (1944a), the grubs of P. anxia and other Phyllophaga spp. are capable of shaking off soil particles adhering to roots, to avoid intake of too much soil.

The presence of white grub infestations in crops is often not detected until their damage appears as areas of wilted or dead seedlings and areas of stunted plants; these areas are commonly scattered across the field, circular in shape, and

vary in size from a few meters to almost an entire field (Hammond, 1960). White grub injury to potato and other root crops is typical; they eat out large shallow circular holes and leave no overhanging epidermis; the injury is usually unoticed until harvest time (MacLeod and Rawlins, 1933; Goble, 1957). Grubs typically cut the main stem or root of vegetable seedlings and transplants so that the plants wilt and die (Goble, 1957). By feeding on grass roots, white grubs seriously reduce the ability of sod to withstand the stress of hot or dry weather conditions, the damaged sod develops large dead patches and can be rolled back like a carpet (Scheibner, 1978). Fibrous roots are sheared through and only partially devoured. This results in death of the plants, evidenced at first by sudden wilting; where the roots are not completely destroyed, the plants may survive but are browned or dwarfed (Metcalf et al., 1962). White grubs consume the smaller roots of tree seedlings and eventually girdle the larger ones, reducing growth, weakening and finally killing the trees (Fowler and Wilson, 1971a, 1974). Shrubs and saplings may be killed by massive root girdling when grub infestations are heavy (Hammond, 1960).

8. Physical Factors

The activity, survival of individuals, and population size of P. anxia and other Phyllophaga spp. are affected by physical factors of the environment. Besides the availability of food plants, soil moisture, structure and texture, appear to be important factors in influencing the attraction of adult

Phyllophaga spp. to an oviposition site and in survival of the offspring. Sweetman (1931) reported that female Phyllophaga spp. laid very few eggs in dry fields and that females died within a few days when confined to dry soil. Adult P. lanceolata (Say) deposited more eggs in moist than dry soil in Iowa (Travis, 1939). Chamberlin and Callenbach (1943) believed that the usual preference of June beetles for grass rather than legumes was a result of different soil conditions produced by the two crops. Miner (1944) found that the oviposition rate of P. crassissima (Blanchard) was higher in soil covered with straw than in bare soil. He also observed that different soil types with uniform moisture conditions had little effect on oviposition rates of P. fervida (Fabricius) and P. crassissima. However, heavy soils, such as clay, were relatively free of white grubs in Ontario and Quebec (Hammond, 1931; Gauthier, 1936; Maheux and Gauthier, 1944), but sandy loam and silt loam favored the multiplication of populations of P. anxia in eastern Canada (Hammond, 1948a). High densities of grubs of P. anxia and P. fusca were found in soil with a pH between 5.3 and 6.2 (Hammond, 1949b) and addition of lime to acid soils resulted in a decrease of grub populations. Sweetman (1931) found that the optimal conditions for incubation of eggs of P. anxia was 25°C and 20-73% of water saturation of the soil, whereas optimal conditions for development of grubs was about 28°C and 25-75% of soil saturation. The majority of grubs were found in the sandhill areas of Nebraska where the soil moisture ranged from 20 to 40% (Jarvis, 1966). Hot and dry weather caused abrupt drops in white grub populations in Wisconsin in 1936 and 1937, but cold seemed to have

no marked effect on grub survival (Clark and Hoveland, 1938). Hammond (1948a, 1954) found that reduction in populations of P. anxia and other Phyllophaga spp. in eastern Canada within each generation was largely caused by weather conditions. Extreme dry weather for a prolonged period destroyed large numbers of grubs. Excessive rainfall (and flooding of pastures for a period of several weeks) reduced oviposition and hatching rates, and also the survival of the three larval instars of P anxia Gaylor and Frankie (1979) demonstrated that, in Texas, female P. crinita (Burmeister) did not oviposit in very wet or very dry soil, and egg and first instar grub survival was poor in very wet or dry soil.

Air temperature and rainfall affected the activity of adults of several Phyllophaga spp. (Hudson, 1920), as did relative humidity for P. anxia (Maheux and Gauthier, 1944). Light intensity controlled the activity pattern of adult P. anxia, and direction of flight and feeding site were influenced by strong winds in Minnesota (Sweetman, 1931) Gruner (1973, 1975a) suggested that the period and pattern of activity of P pleei Blanchard adults were dictated by temperature, rainfall and the moon. The site of oviposition of P anxia and other Phyllophaga spp. was possibly being influenced by the moon in Quebec (Toohey, 1977). Gaylor and Frankie (1979) showed that the flight activity of P. crinita was correlated with rainfall in Texas; in Quebec, temperature had an effect on the emergence of P. anxia (Lim, 1979), and temperature and light intensity regulated the nocturnal activity patterns of P. anxia and P. fusca in western

Quebec (Guppy, 1982). June beetles, P. anxia, were killed when soil temperature dropped to -5°C (Sweetman, 1931), and Ritcher (1958) and Howden (1966) stated that soil temperature was apparently the major factor affecting the developmental rate of all stages of P. anxia and other Phyllophaga spp.

D. ECONOMIC IMPORTANCE

For the past 130 years, white grubs, Phyllophaga spp., have been often referred to as "the most destructive insect pests of the year" in numerous Canadian provinces and American states, as documented in the Annual Reports of the Entomological Society of Ontario and the publications of U.S. Agricultural Experiment Stations. In Ontario and Quebec, most of the references on crop damage by white grubs are for P. anxia (Hammond, 1948a).

White grubs live in the soil and feed on roots, underground stems, rhizomes and tubers of a great variety of agricultural, horticultural and greenhouse crops, and of nursery stock. Much of the actual injury is not recognized and is frequently ascribed to winter killing, sun scorching or some unfavorable soil condition (Hammond, 1934a).

Several studies of economic losses due to grub damage have been made. Damage to corn, timothy and potato by white grubs in Iowa, Wisconsin and Illinois in 1912 was estimated at over $12,000,000 (Davis, 1913, 1918). Losses of several millions were recorded in strawberries, nursery stock, lawns and other

crops in the same states in 1912 (Davis, 1913), a year referred to as "as the most serious outbreak of white grubs in the history of American agricuture" According to Twinn (1934), P anxia grubs were the most serious crop pests in eastern Ontario in 1933, and crop losses, especially timothy, corn and potatoes, were "many" thousands of dollars Gauthier (1936) estimated losses caused by white grubs at about $216 for each of 45 surveyed farms in 1935 in Quebec He also estimated that over $100,000 damage was caused in prairie land in Quebec, in 1935 Over 2400 km^2 of bluegrass pasture was destroyed in southern Wisconsin in 1933 (Fluke and Ritcher, 1938) The average loss was $188 per farm during an outbreak of white grubs in eastern Ontario and western Quebec in 1933 (Hammond, 1940). In eastern Ontario, pastures were either completely destroyed in 1942, or they had their stock carrying capacity reduced by 75% due to white grub attacks; up to 80% of corn and potato crops were lost in eastern Ontario, and oats and barley sustained losses of up to 90% in several counties of southern Ontario (Hammond, 1943) Hammond (1943) also pointed to a secondary problem resulting from grub damage in pastures in that sod was replaced by noxious weeds, adding to the farmers' costs White grub infestations on 78 farms in the Eastern Townships of Quebec resulted in an average loss of $108 per farm in 1938 (Maheux and Gauthier, 1944). The 1944 outbreak of white grubs in the Niagara Peninsula caused losses of $250,000 in crops and nursery stock (Hammond, 1948a) Schwartz and Klassen (1981) averaged the reported mean losses due to white grubs over 10 years in the U S A and found

that grub damage yielded losses of 43 and 39% in corn and grain sorghum, respectively

As adults, Phyllophaga spp have been reported to cause, during their flight years, considerable damage to trees and shrubs by their nocturnal feeding activity. Newly established plum and cherry orchards were completely destroyed by adult beetles feeding on leaves and flower buds, and gnawing at petioles and bark in Virginia in 1886 (Anonymous, 1889c). Hundreds of km^2 of tracts of timber were completely defoliated in Wisconsin, Iowa and Illinois in 1911, and numerous trees were dead in those tracts in 1912 as a result of the loss of foliage the year before (Davis, 1913). Twinn (1935, 1937) reported severe defoliation of deciduous trees and shrubs over 7,000 km^2 across Quebec in 1934 and 1936. Hammond (1936) estimated that general defoliation of large trees had taken place on over 2,000 km^2 in Ontario

In years of abundance, Phyllophaga adults are also sometimes serious nuisances Linton (1889) complained about a "huge swarm" 2 5 km wide and up to 3 m high that occurred in May 1887 In eastern Montana in 1917 Phyllophaga beetles were so abundant that "campers were greatly annoyed by their hitting the tent and alighting on the bed" (Cooley, 1918). Large sums of money had to be spent in cleaning streets in Montreal (Cooley, 1918) and in Wisconsin (Davis, 1918), where beetles were attracted to lights in such numbers that they had to be hauled away by the wagon load each morning for two weeks

E TRAPPING AND SAMPLING

Various forms of electromagnetic radiation have been used extensively to attract Phyllophaga spp adults for detection, survey and control purposes (Forbes, 1916, Henry and Heit, 1940, Ritcher, 1940, Maheux and Gauthier, 1944, Sanderson, 1944, Chandler et al , 1955, Neiswander, 1963, Rao, 1964, Teetes et al 1976, Veeresh, 1977) Blacklight trapping has been useful for monitoring adult P anxia populations and other Phyllophaga spp (Chandler et al , 1955, Neiswander, 1963, Cantello et al , 1973, 1974, Gruner, 1975a, Teetes et al , 1976, Lim et al , 1979, Toohey and Morrison, 1981)

Several sampling methods for immature stages of Phyllophaga spp have been developed The sample unit usually consisted of a block of soil with an area of 0 09 m^2 dug to a depth of 30 5 cm Optimum sample sizes for population density estimation of Phyllophaga spp grubs, teneral adults and eggs have been calculated by Ives and Warren (1965) for cut-over and burnt-over forest land in Manitoba, Jarvis (1966) for sandhills in Nebraska, Guppy and Harcourt (1970, 1973) for permanent meadows in western Quebec, Fowler and Wilson (1971a) for red pine plantations in Michigan and Wisconsin, Teetes (1973), Teetes and Sterling (1976), and Teetes et al (1976) for grain sorghum and wheat in the Texas high plains, Gruner (1975b) for sugar cane in Guadeloupe, Lim (1979) for pastures in southern Quebec The number of samples taken varied according to the purpose of the survey and the level of sampling precision required

F NATURAL ENEMIES

1 Entomopathogens and Entomophagous Invertebrates

Practically all of the known invertebrate natural enemies of P anxia and other Phyllophaga spp have been discussed by Davis (1919) for the American states, Petch and Hammond (1925, 1926) for Ontario and Quebec, and Lim et al (1980a, 1981a) for southern Quebec A detailed literature review on invertebrate natural enemies of Phyllophaga spp is presented in the appropriate chapters of this thesis

2 Vertebrates

Numerous authors have reported that vertebrates are the most important natural enemies of Phyllophaga spp Wild birds and mammals, and farm animals such as pigs and fowls have long been credited as the most efficient biotic regulators of Phyllophaga populations in North America and elsewhere. Both grubs and adult beetles are a part of the usual diet of a variety of species of vertebrates, and grubs and pupae are also eaten in large quantity by some other species when exposed by soil cultivation Hogs and especially wild hogs such as long-nosed prairie-rooters have been praised for the work they did in destroying grubs in infested farm land (Townley, 1867) Riley (1869) mentioned badgers, weasels, skunks, crows and different hawks as important natural checks and destroyers of grubs. To this list, Lintner (1888), Anonymous (1889a), Fogg (1889),

Hargitt (1890) and Forbes (1891) added domesticated pigs, toads, frogs, racoons, foxes, gophers, martins, bats, rats and birds such as robins, brown thrushes, jays, blackbirds and others as invaluable help in the control of grubs and adults of Phyllophaga spp All of these reports were not mere visual observations but were based upon stomach and crop examinations of the predators Osborn (1894) insisted on the importance of frogs and skunks as destroyers of adult beetles whereas Devereaux (1890) advocated that the farmer should protect skunks, hedgehogs, coyotes, wolves and bears because they rescue his crops from "many thousands of dollars damage" by eating grubs Crows, blackbirds, skunks, moles, ground squirrels, amphibians and pigs were considered as the principal enemies of white grubs and adult beetles by Forbes (1894, 1896, 1907, 1908) Crows were said to rank highest on the list of natural enemies of Phyllophaga spp. in Manitoba (Criddle, 1914) Cosens (1915) wrote that white grubs were becoming more numerous in Ontario's market gardens because of the destruction of star-nosed moles by the gardener and also because wild birds were forced to nest in other localities following the removal of trees in garden areas Davis (1919) listed 52 species of birds as largely benefiting the farmer by destroying June beetles and their progeny, crows, robins and blackbirds were again top of the list Frogs, salamanders, and skunks, were considered to be most efficient enemies of Phyllophaga, as well as domesticated pigs and other farm animals such as turkeys, chickens and dogs (Davis, 1919). Skunks and moles were among the most active farmer's helpers

and ought to be protected according to Davis (1919). Gulls, shrews, deer mice, grasshopper mice were extremely efficient white grub and pupal destroyers (Sweetman, 1936). Maheux and Gauthier (1944) calculated that nine pigs devoured over 500,000 grubs when pastured for 3 weeks on an infested meadow in Quebec, and McAtee (1946) estimated that blackbirds destroyed over 90% of grubs exposed at ploughing time in California. Hammond (1948a) called for the protection of skunks in eastern Canada for their important role in the destruction of June beetles and white grubs. Hammond (1948a) also wrote that migratory birds, many of which feed upon grubs and beetles, should be encouraged by working the land to expose the insects in the period when these predators are present Snelling (1968) found that Phyllophaga spp were an important part of the natural diet of grackles and redwinged blackbirds. Vance and App (1971) stated that moles, skunks and birds should not be hunted from lawns infested with grubs Pigs, skunks and crows thus appear to be considered the most important vertebrate natural enemies of Phyllophaga spp. in North America

G CONTROL MEASURES

1. Chemical Control

Effective chemical control for Phyllophaga spp. has been sought since the 1880's Until the late 1940's, food trees and shrubs of the adults were sprayed with a variety of inorganic

insecticides such as sulfur, sodium fluosilicate, lead, copper and calcium arsenates (Fluke and Ritcher, 1934, 1935, Travis, 1936, André, 1937, Luginbill, 1938, Hammond, 1940, 1948a, Neiswander, 1951) Later, benzene hexachloride (BHC) and DDT were used as foliar sprays to control the adults (Hammond, 1948b). Sulfur was also dusted on potential oviposition sites to deter egg laying (Forbes, 1907) All those chemicals were unsatisfactory in controlling Phyllophaga spp , although partial success was obtained with lead arsenate (Hammond, 1940) and BHC and DDT (Hammond, 1948b, 1952, Marshall, 1951)

Most efforts to achieve preventive and remedial chemical control of Phyllophaga spp were directed at the grub stage Early attempts to destroy white grubs relied on soil application of kerosene, petroleum oils and botanical insecticides (Alwood, 1889, Maheux and Gauthier, 1944) Inorganic insecticides, especially sulfur and lead arsenate, were used for grub control in a variety of crops (Luginbill, 1938; Hammond, 1940, Kerr, 1941, Schwardt, 1942; Marshall, 1951, Neiswander, 1951). Phyllophaga grubs were also tentatively controlled with soil fumigants such as chloropicrin, paradichlorobenzene, methyl bromide, dichlorethylether and ethylene dibromide; prohibitive costs and slow mode of action accounted for the abandonment of soil fumigation as a means of control (Johnston and Eaton, 1942, Schwardt, 1942, Hammond, 1945)

Chlorinated hydrocarbon insecticides were recommended for control of white grubs from the late 1940's. DDT, BHC, aldrin, endrin, dieldrin, chlordane, heptachlor, toxaphene and

others gave partial control in various commodities (Hammond, 1948a, 1949a, 1960; Shenefelt and Simkover, 1951; Burhardt, 1955; Pass, 1964; Polivka, 1965; Daniels, 1966a, 1971; Fowler and Wilson, 1971b, 1974; Sutton and Stone, 1974). The development of resistance to the chlorinated hydrocarbon insecticides by several Phyllophaga spp (Teetes, 1973, 1975; Fuchs et al., 1974; Plapp and Frankie, 1976) and legislative restrictions on the use of these persistent chemicals on crops and in the soil have reduced the number of insecticides available for white grub control Testing of alternatives has been mainly with carbamate and organophosphorous insecticides. Diazinon, fensulfothion, and carbofuran, were effective for grub control in grain sorghum and wheat (Teetes, 1973). Fuchs et al. (1974) reported effective control of white grubs, Phyllophaga crinita, using fonofos, diazinon, carbofuran and fensulfothion. Greenhouse tests showed that an organophosphate, CGA 12223, was effective for control of white grubs. Pike et al. (1978) demonstrated the efficacity of carbofuran for control of second and third instar grubs of P. anxia. Reinert (1979) showed consistent control of white grubs on Bermuda grass in Florida with isofos, fonofos and carbofuran. Fensulfothion, fonofos, isofenphos and WL 24073 were toxic to third instar white grubs, Phyllophaga spp., (Lim et al., 1980b). Fenvalerate and cypermethrin were highly toxic to adult Phyllophaga spp (Letendre, 1982). Chlorpyrifos and fonofos were effective in controlling white grubs, both as a contact and soil insecticide (Letendre, 1982). Lim et al. (1980b) and Letendre's (1982) bioassays were conducted

in the laboratory on field-collected Phyllophaga individuals, in Quebec.

2. Non-Chemical Control

a. Cultural control

Damage by white grubs to susceptible plants such as potatoes, corn, strawberries and nursery stock has been prevented by not planting them in ploughed-up pasture or abandoned fields (Crosby and Leonard, 1918; Pettit, 1930; Hammond, 1940; Neary, 1950; Fowler and Wilson, 1971a; Sutton and Stone, 1974; Anonymous, 1981a,b). Injury of white grubs in strawberry beds has been prevented by burying tobacco stems in the beds and by placing ashes on the ground before setting the plants (Lintner, 1888).

Prevention of damage by reducing populations of white grubs has been achieved by capturing and destroying the adults during flight years; tree shaking at night (Lintner, 1888; Forbes, 1907; Comstock, 1940), attraction to lighted lanterns suspended over a pan filled with water and kerosene (Fogg, 1889; Dustan, 1932), hand-collecting (Veeresh, 1977), and attraction to blacklight traps (Rao, 1964; Cantelo et al., 1973, 1974) are some of the means that have been used to reduce populations of adult June beetles.

Hand-picking and destroying grubs and pupae ("grubbing") when preparing land have been recommended as preventive measures in small fields and gardens (Fogg, 1889; Dustan, 1932;

Gibson, 1934) and as remedial measures (Neary, 1950).

Compacting the surface of the ground by the use of heavy rollers or by treading it with sheep and cattle has been said to give protection from deposition of eggs and prevention of easy passage of grubs from root to root (Lintner, 1888; Gauthier, 1936). Heavy fertilizing has been credited for reduction of injury by white grubs in susceptible crops (Anonymous, 1889b; Goble, 1957; Hammond, 1960). Destruction of food shrubs for adults in the vicinity of cultivated land has been advocated as a deterrent means of control (Maheux and Gauthier, 1944). Hammond (1948) reported that flooding of pastures and meadows for a period of several weeks when white grubs were active eliminated the pest.

Ploughing is one of the commonest methods of white grub control, and ploughing of infested fields followed by repeated cultivations with disk harrows has been shown to reduce population levels of white grubs and pupae by physical injury or exposure to adverse weather and natural enemies, especially vertebrates (Davis, 1916, 1918; Criddle, 1918; Drake et al., 1932; Hammond and Maheux, 1934; Hammond, 1934b, 1940, 1948a, 1960; Maheux and Gauthier, 1938, 1944; Gauthier, 1936, 1944; Hodgson et al., 1974). Timing of the operation is important for maximum effect and proper timing is achieved by thorough knowledge of the life cycle of the pest, principally of the seasonal vertical distribution of the soil-dwelling stages (Maheux and Gauthier, 1938, 1944; Gauthier, 1944; Hammond, 1960; Hodgson et al., 1974).

Short rotation of crops in which land will not be left in sod for more than two or three years has also been recommended for control of white grubs. A rotation of corn or clover and small grains (oats and barley) was recommended by Davis (1918) with the suggestion that clover or corn should be planted at the flight year of Phyllophaga spp. Hammond (1940), and Chamberlin and Fluke (1947) recommended planting crops resistant to grub damage, such as clovers and alfalfa, during flight years. A deterrent effect resulting in reduced oviposition was demonstrated when pastures of grass were planted in combination with clovers or alfalfa (Fluke et al , 1932; Fuelleman and Graber, 1937). Hammond (1940) suggested a five year rotation sytem: hoed crops in the first year, grains the second, clover hay the third, grains the fourth , and clover hay or pasture the fifth. Maheux and Gauthier (1944) however concluded that, though partially successful in lightly infested land, no system of crop rotation would control Phyllophaga spp. in heavily infested land.

b. Biological control

There have been only a few attempts at using entomopathogens and entomophagous insects for the control of Phyllophaga spp. outside North America (Clausen, 1978) and most, if not all, were not successful. Although native invertebrate natural enemies of Phyllophaga spp. have been found, there is little published information on their manipulation and use for the biological control of June beetles in North America; the relevant

information is reviewed in the appropriate chapters of this thesis.

Farm and other animals, however, have long been advocated and put to work as biotic control agents of grubs and adults, in North America and elsewhere. Domestic fowls, especially turkeys, have been successful in controlling white grubs and pupae of Phyllophaga spp., whenever allowed the run of freshly ploughed infested fields; poultry released in orchards and timber lots destroyed adult June beetles in flight years (Lintner, 1888; Fletcher, 1905; Davis, 1913, 1918, 1919; Huard, 1916; Walton, 1917). Sanderson (1911) even advocated the "training" of a flock of chickens or turkeys to follow the plow and pick up exposed white grubs. Pigs have always been considered to be the most useful assistant in man's fight against Phyllophaga spp. and more than one pasture or meadow has been cleaned of white grubs by pigs allowed to run free and root out the pests Similarly, freshly ploughed fields have been successfully stocked with pigs to feed on the exposed grubs and pupae; populations of Phyllophaga spp. were reduced by pigs rooting out adults hiding below the soil surface in orchards, timber lots and shrubs fencing cultivated land (Townley, 1867; Riley, 1869; Lintner, 1888, Forbes, 1891, 1907; Fletcher, 1905; Walton, 1917; Davis, 1918, 1919; Maheux and Gauthier, 1944; Chamberlin and Fluke, 1947; Hammond, 1948a). Davis (1919), and Chamberlin and Fluke (1947) warned, however, against extensive rooting of white grubs by pigs in pasture land to avoid erosion and to protect against the danger of pigs becoming infested with spiny-headed

worms which have white grubs as intermediate hosts Devereaux (1890) reported to have trained "several kinds" of domestic dogs to follow the plough and eat white grubs He concluded that dogs rendered a better service than crows and ravens at plow time Davis (1919) recommended the use of dogs to root out white grubs in infested fields

III. APPROACH TO THE STUDY OF BIOTIC REGULATORS OF PHYLLOPHAGA SPP

A. INTRODUCTION

The limitations imposed on population growth of Phyllophaga spp by the abiotic components of the environment are obvious and will not be discussed here The principal biotic factors influencing the presence of Phyllophaga in a given habitat and affecting the population density of the beetles are availability of preferred host plants for adults, availability of breeding sites for grubs, intraspecific and interspecific competition, predators, parasites, diseases, man's manipulation of the agro-ecosystem It would have been idealistic to encompass all these factors in the study of biotic regulation of Phyllophaga reported in this thesis However, time, money and manpower limitations dictated a more realistic approach and a selection of the biotic factors to be studied had to be made

Until 1979, most of the information on the biotic regulation of Phyllophaga spp. was available in Davis' (1919) "Contributions to a Knowledge of the Natural Enemies of Phyllophaga " and in a few subsequent publications of a lesser scope It is only recently that an ecological-type survey of the natural enemies of Phyllophaga was reported, Lim's (1979) doctoral thesis included two chapters presenting his studies on the complex of natural enemies of P. anxia in southern Quebec, later summarized

by Lim et al (1981a).

In view of these facts, it was considered worthwhile to continue Lim's (1979) preliminary studies and to survey, both qualitatively and quantitatively, Phyllophaga populations of southern Quebec in a search for their invertebrate parasites and predators, etiological agents of disease, and of any non-vertebrate organism associated in various relationships with this beetle genus

Due to the exploratory nature of the research, the main emphasis was placed on the field survey of Phyllophaga populations No particular sampling method of these populations was retained, the ultimate objective of the research being to collect the maximum number of Phyllophaga individuals, as often as feasible and in as many localities as possible. This emphasis on large host samples (Phyllophaga, occasions, localities) should result in correspondingly high numbers of natural enemies, both in relative abundance and species diversity The ecological observations and the field collections of specimens were then followed up with studies of hosts and potential natural enemies in the laboratory and, in a few cases, in field microplots Figure 1 presents the progressive stages of the research, emphasizing the study of pathogens.

Due to the broad scope of the project and the diversity of organisms found associated with Phyllophaga, it was considered desirable to present the data in the form of successive chapters, each of them dealing with a particular organism or group of organisms Nevertheless, I emphasize here that the field recoveries and laboratory follow-up reported in the following sections of

Field- collection of all stages of Phyllophaga and of organisms associated with them *

Isolation and identification of associated organisms *

Assessment of natural enemy status of the organisms isolated from Phyllophaga *

Infectivity tests with isolated microorganisms *

Laboratory bioassays with selected pathogenic microorganisms *

Microplot tests with selected pathogenic microorganisms

Recommendation for future needs in research

Recommendations for integration with current cultural and chemical controls

Figure 1 Progressive stages of research (*concurrent) on the natural enemies of Phyllophaga spp. in southern Quebec 1979 to 1982

the thesis were concurrent in space and time during the survey

Surface (1908) wrote that "A knowledge of the natural enemies of insects may be exceedingly valuable in helping to preserve those which are beneficial and destroy those which are obnoxious" DeBach (1964) wrote that "the prime requisite of integrated pest control is basic ecological knowledge of the entire complex involved, including the extent of biological control of each host insect that occurs in the abscence of treatment" These two quotations were always kept in mind during my research and were encouraging when frustration was stronger than passion

B MATERIALS AND METHODS

Collecting trips in southern Quebec were planned according to the observations made by Lim (1979) in his studies of the life cycle and occurrence of "brood zones" of P anxia in that part of the province Lim's (1979) forecast of broods for 1980 was used for that year and extrapolated for 1981 This method made it possible to know with relative accuracy where and when to collect a particular stage of Phyllophaga, given that the site to be visited was an habitat suitable for Phyllophaga (Figure 2)

Eggs of Phyllophaga were obtained by digging in the soil with a hand shovel and by maintaining a colony of field-collected adults in oviposition cages in the laboratory (Figures 3 and 4) Grubs of the three instars were collected by hand-pulling infested plants, digging, plowing, sod cutting (Figure 5) and "carpet

Figure 2 Strawberry field destroyed by third instar, second year white grubs, Phyllophaga sp Photograph taken at Pierreville, Quebec, September 1979

Figure 3 Eggs of Phyllophaga anxia laid in the laboratory by field-collected females Some eggs are shown in their earthen cells opened intentionally The arrow points at a newly emerged first instar white grub. 2 5X

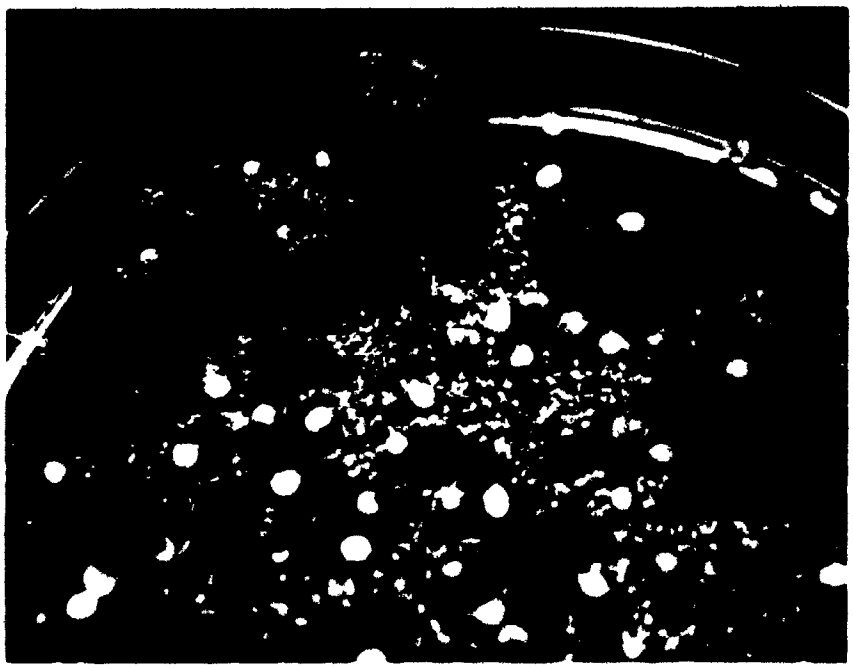


Figure 4. Rearing cages used for obtaining eggs and first instar grubs of Phyllophaga anxia. The oviposition boxes were wooden containers filled with sandy loam soil seeded with red fescue and Kentucky blue grass.

Figure 5. Sod-cutting, a rapid method for collecting large numbers of Phyllophaga grubs. Robins, crows and gulls were competing with man to pick up the exposed grubs.

rolling" (Figure 6) the ground vegetation of badly infested grazing land, first instar grubs were also obtained from eggs hatching (Figure 3) in the oviposition cages. Prepupae, pupae and teneral adults were usually found by digging, although plowing was also used A few adults were hand-collected while flying to lighted windows and found by digging, but the bulk was trapped in Ward's 4-baffled insect traps fitted with an 8-watt blacklight fluorescent tube (GE F8T5. BL)* and equipped with a collecting funnel and a nylon bag at the tube end of the funnel Site, particulars of habitat, date and method of collection were recorded for each specimen of Phyllophaga.

With the exception of some adults, live specimens of Phyllophaga were brought to the laboratory on the day of collection for purposes of rearing under close observation. Adults trapped in blacklight traps located at considerable distances from the laboratory were collected by volunteers each morning and kept in paper bags at 5°C for 3 days but sometimes up to one week. All stages of live Phyllophaga, except adults trapped in blacklight traps, were transported to the laboratory in horticultural flats filled with local soil and covered with grass uprooted at the site of collection, the trays were placed in styrofoam coolers containing bags of ice. Adults collected in blacklight traps were transported in the nylon collecting bags kept in a refrigerated cooler.

Potential predatory insects and parasitic gordian worms found in close association with Phyllophaga specimens collected in the ground, were placed individually in plastic vials with a

* Ward's Natural Science Establishment, Inc., Rochester, N.Y.

Figure 6 "Carpet rolling" of damaged grass, a method for collecting large numbers of Phyllophaga grubs, especially during the white grub year Also shown is the serious damage (severed roots and dead grass leaves) inflicted by the feeding grubs

label recording all pertinent data, they were transported to the laboratory for purposes of identification when collected in the adult stage and for purposes of rearing prior to identification when collected in the immature stages Specimens of live Phyllophaga found to be infested with Acarines at the time of collection were transported to the laboratory individually in plastic vials Transportation in individual vials was also the mode for specimens of Phyllophaga found dead or dying - with or without external symptoms of disease - and for those showing abnormal behaviour All Phyllophaga specimens wounded by collecting tools were discarded in the field and were not accounted for as having been collected Wounds, usually decapitation, were rather common in grubs exposed by the cutting machines used on sod farms in the vicinity of Mirabel With the exception of light-trap catches of adults which were kept in domestic refrigerators, Phyllophaga specimens reached the laboratory within 4 to 5 hours after collection

In the laboratory, live insects were confined singly in numbered plastic vials Pasteurized sandy loam was added to vials receiving eggs and pupae Food for grubs consisted of an artificial diet for insects used by Toohey (1977) for rearing P anxia grubs, and of a piece of lettuce Adults were fed leaves of oak or chestnut growing near the laboratory Prepupae and teneral adults were not fed One regularly-moistened cotton dental wick was added to each of the containers which were kept in an environmental chamber set at usually 22±1°C, 40-50% RH and 16h photophase Thus, after their removal from the original habitat, the

insects were kept in isolation in order to prevent any subsequent contamination as well as transmission of disease As a matter of routine the specimens were examined every other day for emergence of parasitic insects and nematodes (Figure 7), symptoms of disease and eventual death Five to six weeks of isolation were considered sufficient for manifestation of parasites and infectious agents, at the end of the quarantine, apparently healthy insects were killed by decapitation and dissected under a binocular stereoscopic microscope for purposes of a final detailed examination, haemolymph smears were routinely taken during the post-mortem examination

Field-collected and laboratory-quarantined dying and abnormally-behaving insects were allowed to die in isolation Haemolymph smears were taken upon their death and, following surface sterilization in 70% ethanol and 3 passages in sterile distilled water, the cadavers were incubated on nutrient agar plates at 25°-27°C for 7 days Plates were checked daily for colonization of the agar by bacteria, fungi, nematodes and by emerging mites and parasitic insects The remnants of the insects incubated on the colonized plates and the cadavers of the insects from the non-colonized plates were then subjected to a final examination under a dissecting microscope Field-collected and laboratory-quarantined dead but asymptomatic insects were processed in a manner similar to that described for dying insects Subcultures of plates colonized by bacteria and fungi were performed until pure cultures were obtained

Figure 7 Nematodes, Mikoletzyka aerivora (Cobb) emerging from a field-collected, moribund, third instar Phyllophaga grub which died in quarantine 5X

Field and laboratory dead insects for which a detectable manifestation of potential microbial disease was present (such as mycelial growth or milky-colored body) were processed using standard microbiological techniques Pure cultures were obtained after several subcultures of the original inoculum (Figure 8) As a matter of precaution for survival of spore-forming bacteria, which grow poorly in vitro, inocula were also preserved as air-dried smears of aseptically-obtained diseased haemolymph Isolation media were prepared as recommended in the Difco Manual (1977) Isolation techniques for potentially pathogenic bacteria, fungi, protozoans and nematodes, and staining techniques for microorganisms were as described in Martignoni and Steinhaus (1961), Steinhaus (1963), Burges and Hussey (1971), Cantwell (1974), Poinar (1975, 1979), Poinar and Thomas (1978), Burges (1981), Cole and Kendrick (1981) More specialized manuals were also used and are cited in the appropriate chapters of the thesis

Ecto and endoparasitic insects found in the larval and pupal stages were, whenever feasible, reared to the adult stage in order to facilitate their identifications Potentially predatory insects and parasitic insects were mounted as adults and preserved in alcohol as larvae and pupae Adults were identified, often to the species level, using various keys Larvae were identified to the family level but pupae were not identified Finally, all specimens of predatory and parasitic insects were submitted to specialists for confirmation and, in the case of larvae and pupae, for further identification Mites were mounted in some cases, preserved in other cases, and submitted for

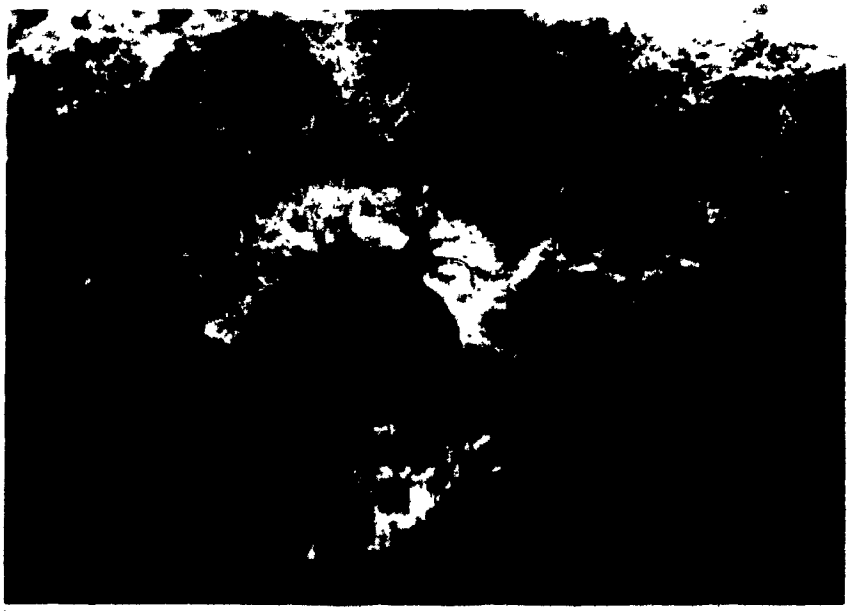
Figure 8 The entomopathogenic fungus Metarhizium anisopliae (Metsch.) Sorok, in pure culture Fungus isolated from a field-collected, dead, Phyllophaga grub

identification. Nematodes were identified using characteristics provided by Lim (1979) and Poinar (1975, 1979) Bacteria and fungi were identified using the keys of Barnett and Hunter (1972), Poinar and Thomas (1978), Burges (1981) Both agar slants and microscope slide mounts were submitted to specialists for confirmation of the identifications Protozoans were mounted on slides and identified to the genus level A new virus was identified by specialists.

Voucher specimens of all organisms found associated with Phyllophaga were deposited in the collection of the appropriate depository; duplicates were deposited in my collection. Keys used for the identifications, names of the specialists and locations of the voucher specimens are provided in the appropriate chapters.

The identification of specimens of Phyllophaga collected in the course of this study was made soon after collection. The grubs, prepupae and eggs (through the hatching grubs) were identified to the genus and in some instances to the species levels using keys published by Böving (1942) and Ritcher (1940, 1949a, 1966). Measurements of the cephalic capsules allowed me to sort the grubs into one of three instars and the body appearance permitted distinction of prepupae from third instar grubs (Lim,1979). Second and third instar grubs were further designated as second instar/first year, second instar/second year, third instar/second year and third instar/third year grubs accordingly to Lim (1979). Species of Phyllophaga adults, teneral adults and pupae were separated from each other using descriptions and keys published by Horn (1887); Langston (1927); Luginbill (1928), Sim (1928);

Figure 9 A common soil fungus, Rhizopus sp , colonizing the cadaver of a white grub Causes of death unknown Findings similar to this one were not accounted for since definite identification of the insect was not possible



Ritcher (1940), Böving (1942), Luginbill and Painter (1953), Dillon and Dillon (1961), Chagnon and Robert (1962), Nairn and Wong (1965), and the unpublished key of Lim (1979) for southern Quebec The identifications were confirmed by Dr J McNamara, Biosytematics Research Institute (B R I), Ottawa, Ontario and Dr. E R Hoebeke, Department of Entomology, Cornell University, Ithaca, New York Voucher specimens were deposited with the B R I and the Lyman Museum and Research Laboratory, MacDonald College, Ste.-Anne-de-Bellevue, Quebec

Specimens of Phyllophaga collected in the field in an advanced stage of decomposition, rendering generic identification impossible, were not taken into account in the course of the present study (Figure 9)

Finally, field microplot tests of two pathogenic micro-organisms were conducted, details are given in the appropriate chapters

C. RESULTS

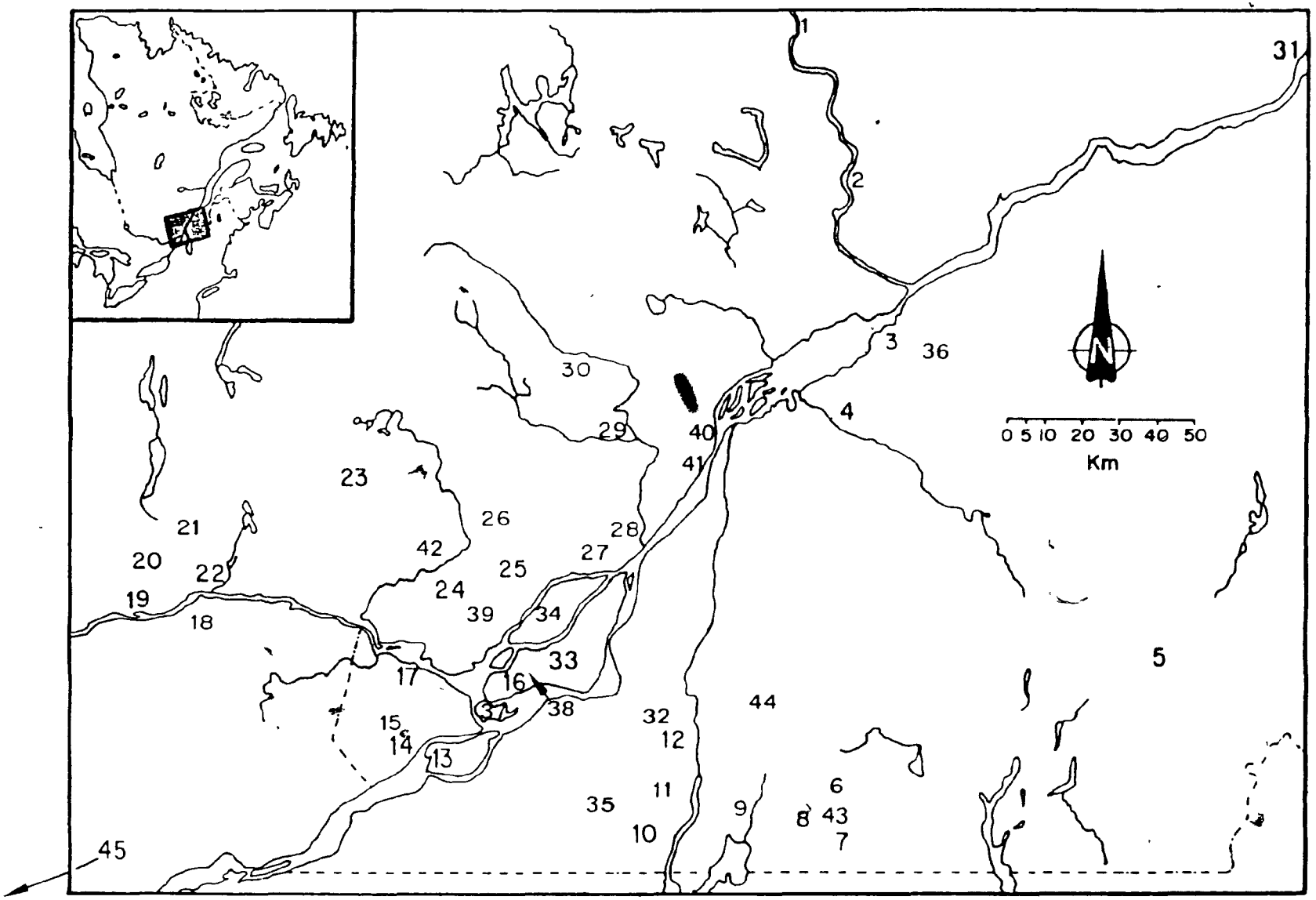
The results of the three-year survey of the natural non-vertebrate enemies of Phyllophaga in southern Quebec are presented and discussed in detail in the next chapters Data pertaining to host's collection are however summarized here for sake of continuity in the format of the thesis

Forty-five collection sites were visited on several occasions in southern Quebec from 1979 to 1981 (Figure 10) and all stages of the life cycle of Phyllophaga, especially P. anxia

Figure 10. Locations of collection sites for Phyllophaga spp. in southern Quebec, 1979 to 1981.

Site		Abbreviation used in other tables	Description of site
1	Mattawin	-	Old pasture
2	Grand'Mère	-	Old pasture
3.	Nicolet	-	Old pasture
4	Pierreville	-	Strawberry field
5.	East Angus	-	Meadow
6.	Cowansville	-	Meadow
7.	Frelighsburg	-	Farm land
8	Stanbridge East	Stanbridge	Abandoned corn field
9.	St.-Sébastien	St. Sébastien	Corn field
10.	Lacolle	-	Highway banks
11.	Napierville	-	Sod farm
12.	St.-Jean-Richelieu	St. Jean	Meadow
13.	Valleyfield	-	Strawberry field
14.	Coteau Station	Coteau	Sod farm
15.	St -Clet	St. Clet	Sod farm
16	Ste Anne-de-Bellevue	Ste Anne	Pasture
17	Rigaud	-	Old pasture
18	Alfred [a]	-	Meadow
19	Papineauville	-	Hay field
20.	St -André-Avalin	St. André	Backyard garden
21	Notre-Dame-de-la-Paix	Notre-Dame	Potato field
22.	Montebello	-	Meadow
23	Lac-des-Seize-Isles and Montford	Montford	Meadow
24	Mirabel-Ste.-Monique	Mirabel	Sod farm
25.	St.-Janvier	-	Sod farm
26.	Ste.-Sophie and New Glasgow	Ste. Sophie	Sod farm
27	Mascouche	-	Old pasture
28.	L'Assomption	-	Mixed vegetable fields
29.	Joliette	-	Meadow
30	Lac Cloutier	-	Old pasture
31	Quebec	-	Hay field
32.	L'Acadie	-	Mixed vegetable fields
33.	Pointe-Claire	Pte. Claire	Lawn
34.	Duvernay	-	Lawn
35.	Ste.-Clotilde	Ste. Clotilde	Mixed vegetable fields
36.	Grand-St.-Esprit	Gd. St. Esprit	Potato field
37.	Pincourt	-	Lawn
38	Baie d'Urfé	-	Lawn
39.	Le Luc	-	Meadow
40.	Berthierville	-	Tree nursery
41.	Lanoraie	-	Old pasture
42.	Bellefeuille	-	Sod farm
43.	Dunham	-	Apple orchard
44.	Ste.-Angèle	Ste. Angèle	Corn field
45.	Silver Lake [a]	-	Old pasture

[a] Eastern Ontario Province.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(Figure 11), were found at one or more of the collection sites, all the life stages of the beetle were not necessarily found at any one given location

A grand total of 33,468 Phyllophaga individuals were collected during the survey and examined for associated organisms (Table 1) The 8,895 adult males, 2,647 adult females, 73 teneral adult males, 37 teneral adult females, 1,289 pupa males and 897 pupa females reported in Table 1 were determined to belong to the species, Phyllophaga anxia Sixty-six adults other than P anxia were also collected, six species were thus found to occur in southern Quebec, besides P anxia P drakii (Kirby), P fraterna (Harris), P fusca (Froelich), P futilis (LeConte), P nitida (LeConte), P rugosa (Melsheimer) (Table 2) Since those 66 adults were killed for mounting purposes upon collection, they were not examined for the presence of associated organisms and therefore are not tallied in Table 1 By comparing numbers for adults and teneral adults in Table 1 to numbers for adults in Table 2 it was deduced that P anxia adults represented 99 44% of all Phyllophaga adults collected in southern Quebec during this three-year survey This observation agreed with earlier reports, Toohey (1977) stated that 98% of the June beetles trapped by her in 1975 and 1976 in southern Quebec were P. anxia, Lim (1979) also found that P anxia was the predominant Phyllophaga sp. in southern Quebec in 1976 and 1977, in some instances representing 100% of all Phyllophaga adults trapped in the course of his studies It thus seems safe to consider that at least 98% of the white grubs collected by me and the same percentage of

Figure 11 Life stages of Phyllophaga anxia (LeConte) A egg B first instar grub C second instar D third instar E prepupa F male pupa G female teneral adult, 1h old H male (left) and female (right) teneral adults, 3h old I female (left) and male (right) adults A to G, 2 5X H and I, 2X

Table 1. Numbers of Phyllophaga[a] spp individuals collected in 45 localities of southern Quebec in 1979, 1980 and 1981.

Insect stage and sex	Year of collection 1979	1980	1981	Total for three years
Adult male	2636	552	5707	8895
Adult female	1511	258	878	2647
Egg	1870	0	655	2525
First instar grub	1556	0	276	1832
Second instar year 1	1010	0	642	1652
Second instar year 2	1130	370	1	1501
Third instar year 2	7064	1106	4	8174
Third instar year 3	84	3598	89	3771
Prepupa	1	166	8	175
Pupa male	3	1259	27	1289
Pupa female	0	888	9	897
Teneral Adult male	5	23	45	73
Teneral adult female	3	11	23	37
All stages combined	16873	8231	8364	33468

[a] All adults, teneral adults and pupae determined to be P. anxia (LeConte); by combining numbers of all adults tallied in this Table and Table 2, it was deduced that 99.44% of all adults collected were P anxia; adults other than P. anxia are not tallied in Table 1 but are in Table 2.

Table 2. Numbers of Phyllophaga spp. adults other than P. anxia collected by blacklight traps in southern Quebec, in 1979, 1980 and 1981

Species	Collection date	Site	Numbers collected and sex
P. drakii	Jun. 5,1980	Frelighsburg	1 male
	Jun 14,1980	St.André	1 male
	May 27,1981	Ste Anne	2 males
	Jun 6,1981	Ste Anne	1 male
P. fraterna	Jun.12,1980	Frelighsburg	1 male
	Jun 14,1980	Frelighsburg	5 males
	Jun 15,1980	Pincourt	1 male
	May 29,1981	St.Jean	1 male
	Jun. 6,1981	L'Acadie	1 male
P. fusca	May 16,1979	Nicolet	6 males
	May 18,1979	Silver Lake	1 male
	May 20,1979	Silver Lake	6 males
	May 20,1979	Nicolet	1 male
	May 24,1979	Ste.Anne	1 male
	Jun.12,1980	L'Acadie	1 male
	May 27,1981	Ste Anne	1 male
	Jun 4,1981	Ste Anne	1 female
P. futilis	May 27,1979	Ste Anne	1 male
	May 10,1981	Baie d'Urfé	1 male
	May 29,1981	Pincourt	1 male
	May 30,1981	Ste.Anne	1 female
	May 30,1981	Pte.Claire	1 male
	Jun.10,1981	Pincourt	1 male
P. nitida	Jun.21,1979	Joliette	1 male
	May 28,1981	Pte Claire	1 male
	May 31,1981	Pincourt	1 male
P. rugosa	May 16,1979	Nicolet	1 male
	May 18,1979	Silver Lake	23 males
	May 31, 1980	Silver Lake	1 male

eggs (through the hatching grubs) were P. anxia. From now on it should be understood that when "Phyllophaga sp./spp." is mentioned when reporting my studies, it is implied that I am referring to P. anxia; this rider applies to grubs and eggs only, since all other life stages were actually determined to be P. anxia. However small (0.56%) the number of Phyllophaga adults other than P anxia found by me, it indicated that at least a comparable percentage of collected grubs and eggs might have been non-anxia; hence the use of "Phyllophaga spp." and not P. anxia in the title of this thesis.

Detailed data for numbers of males and females of P.anxia collected in 1979, 1980 and 1981 are presented in Tables 3, 4 and 5 respectively. Very few adults were collected other than by lighttrapping. Females represented 36.43 and 31.85% of adults collected in 1979 and 1980, respectively, but were less (13.33%) in 1981. On the other hand, 33.63% of teneral adults found over the three-year period were females (Table 6), a percentage in agreement with those reported for adult females collected in 1979 and 1980. Moreover, 41.03% of all pupae collected during the survey were females (Table 6). It thus appeared that approximatively one third of P. anxia populations in southern Quebec in 1979 to 1981 was composed of females. These numbers are about the average of numbers reported in the literature for P. anxia and other Phyllophaga adults collected in blacklight traps: 53% P. anxia were females in New York (Henry and Heit, 1940); 0% P. anxia were females in Indiana (Chandler et al., 1955); 5% Phyllophaga spp. were females in eastern Quebec (Gauthier, 1944); only male

Table 3. Numbers of males and females of Phyllophaga anxia collected in southern Quebec, 1979.

Site	Collection technique	Collection dates	Males	Females	Total
Baie d'Urfé	Digging	Apr. 4	3	3	6
	Light trap	May 7-May 14	12	0	12
Nicolet	Light trap*	May 10-Jun.25	1978	1195	3173
	Digging	Jul.20	0	1	1
Ste.Anne	Digging	Jul.26	0	1	1
Gd.St.Esprit	Light trap	May 16-May22+	230	148	378
Joliette	light trap	Jun.26	26	10	36
Lac Cloutier	Digging	Apr.27	22	10	32
Silver Lake	Light trap	May 18-May20	109	11	120
Quebec	Light trap	Jun.19-Jun.25	138	98	236
St.André	Light trap	May 12-Jun.28	1	0	1
Alfred	Light trap	May 18-May 21	89	11	100
Pierreville	Digging	Aug.28	0	2	2
Lanoraie	Light trap	May 25-May 27	13	8	21
East Angus	Digging	May 3	13	12	25
Berthierville	Digging	May 1	2	1	3
All sites			2636	1511	4147

* Indicates two non-interfering blacklight traps operating at this site.

\+ Discontinued on this date because of lack of adequate storage facilities for trapped beetles.

Table 4. Numbers of males and females of Phyllophaga anxia collected in southern Quebec, 1980.

Site	Collection technique	Collection dates	Males	Females	Total
Pte. Claire	Digging	May 27	3	0	3
	Light trap	May 10-Jun. 27	11	6	17
Pincourt	Digging	May 5	9	2	11
	Light trap*	May 16-Jul. 8	0	0	0
L'Acadie	Light trap*	May 14-Jul. 7	91	42	133
St. André	Light trap	May 12-Jul. 26	56	44	100
Baie d'Urfé	Digging	May 10	4	4	8
	Light trap*	May 17-Jul. 13	7	2	9
St. Jean	Light trap	May 15-Jul. 7	146	84	230
Ste. Anne	Light trap	May 21-Jul. 2	27	14	41
L'Assomption	Light trap*	May 14-Jun. 29	6	3	9
Frelighsburg	Light trap	May 14-Jun. 26	103	38	141
Alfred	Light trap	May 20-Jun. 13	13	1	14
Mattawin	Digging	Jun. 4	48	13	61
Silver Lake	Light trap	May 31	3	0	3
Grand'Mère	Digging	Jun. 4	25	5	30
All sites			552	258	810

* Indicates two non-interfering blacklight traps operating at these sites.

Table 5. Numbers of males and females of Phyllophaga anxia collected in southern Quebec, 1981.

Site	Collection technique	Collection dates	Males	Females	Total
Duvernay	Digging	Apr.11	1	1	2
	Light trap	May 15-Jun. 8	38	5	43
St.Sébastien	Digging	Apr.23	19	6	25
Baie d'Urfé	Digging	Apr.19	2	2	4
	Light trap*	May 5-Jun.24	65	12	77
Pte.Claire	Light trap	May 19-Jun.14	41	3	44
Pincourt	Light trap	May 20-Jun.30	84	9	93
Stanbridge	Light trap	May 12-Jun.30	886	204	1090
Ste.Anne	Digging	May 15	2	4	6
	Light trap	May 18-Jun.27	436	189	625
Dunham	Water pans	May 5-Jun.27	26	4	30
L'Acadie	Light trap*	May 5-Jun.24	1846	199	2045
St.Jean	Light trap	May 5-Jun. 8+	2109	199	2308
Ste.Clotilde	Light trap	May 28-Jun.21	121	33	154
Lac Cloutier	Light trap	May 22-May 26	12	0	12
St.André	Light trap	May 30-Jun.12	19	8	27
All sites			5707	878	6585

* Indicates two non-interfering blacklight traps operating at these sites.

\+ Discontinued on this date to avoid interference with the June beetle attractants experiment (Chapter XI).

Table 6. Numbers of eggs, prepupae, pupae and teneral adults of Phyllophaga spp.[a] collected in southern Quebec, in 1979, 1980 and 1981.

Site	Collection dates	Insect stage					
		Egg	Prepupa	Pupa		Teneral adult	
				Male	Female	Male	Female
Ste.Anne	Jun. 8-Jun.24,1979[c]	1070[b]	0	0	0	0	0
Nicolet	Jun.21-Jul. 3,1979	618	0	0	0	0	0
Gd.St.Esprit	Jun.17-Jul. 6,1979[d]	182	0	0	0	0	0
Pierreville	Aug.23,1979	0	1	1	0	1	1
Ste.Anne	Oct.25,1979	0	0	0	0	3	2
Mirabel	Jul.30,1979	0	0	2	0	0	0
St.André	Sep.28,1979	0	0	0	0	1	0
Stanbridge	Aug. 9,1980	0	166	1259	888	17	9
Ste.Anne	Oct. 2,1980	0	0	0	0	3	2
Pte.Claire	Sep.24,1980	0	0	0	0	3	0
Duvernay	Sep.30,1981	0	0	0	0	0	1
Ste.Anne	Jun.14-Jul.20,1981[e]	272	0	0	0	0	0
	Oct.12,1981 [f]	0	0	0	0	3	3
Stanbridge	Jun.23-Jul.24,1981	383	0	0	0	0	0
	Aug.26,1981	0	0	0	0	0	3
Nicolet	Aug. 8,1981	0	8	27	9	21	9
Mirabel	Sep. 4,1981	0	0	0	0	0	1
Pincourt	Sep. 9,1981	0	0	0	0	21	6
All sites	All years	2525	175	1289	897	73	37

[a] Teneral adult is defined here as an adult collected in the third year of development of the life cycle.
[b] Pupae and teneral adults identified as P. anxia.
[c] Eggs laid in the laboratory by females trapped at Nicolet, thus identified as P. anxia.
Fifteen occasions. [d] Eleven occasions. [e] Seven occasions. [f] Seventeen occasions.

June beetles were attracted to light in eastern Ontario and Quebec (Hammond, 1960); 96.6% P. anxia were females in Ohio (Neiswander, 1963); from 0 to 45% P. anxia were females in one or the other locality of southern Quebec (Toohey, 1977; Lim, 1979).

A total of 2525 eggs were secured and examined during rearing; 1070 of them were laid in the laboratory by female P. anxia trapped at Nicolet in June 1979 and confined in cages with male P. anxia from the same location (Table 6). Of these 1070 eggs, 490 hatched successfully (45.6%), 41.3% formed embryos but failed to hatch and the remainder were apparently sterile. Failure to hatch was not elucidated but no parasitic insects or microorganisms were found upon examination; embryonic grubs thus probably died from constitutional weaknesses, genetic defects, idiopathic diseases or because of improper rearing conditions. Teetes and Wade (1974) obtained 15.9% successful hatching in rearing experiments with P. crinita. Only 132 (9.07%) of the 1455 field-collected eggs hatched in the laboratory; the first instar grubs were determined to be Phyllophaga spp. Here again, causes behind the failure to hatch for the 926 eggs with formed embryos were not found; the hypotheses advanced for the laboratory-laid eggs probably applied here too with additional causes such as breakage of the protective earthen cells at digging time, cold storage during journey to the laboratory and manipulation. The remainder of the field-collected eggs were found to be sterile. Drawing conclusions for egg mortality is difficult although neither infectious diseases nor parasitic insects were responsible

for the high rate of mortality observed. Egg predation was not observed when digging for eggs.

Very few pupae were found during the survey and most of them were collected on a single occasion at Stanbridge East, in August 1980 (Table 6).

Data for white grubs are summarized in Tables 7, 8 and 9 for 1979, 1980 and 1981, respectively. A total of 16930 grubs, including 490 first instars which hatched from eggs in the laboratory, were examined. Slightly over 48% of all grubs were third instars/second year and 8.86% were second instars/second year. The second year of the life cycle of P. anxia is known as the "white grub year"; most damage to cultivated and other plants occurs in that year (Hammond, 1948a, 1954) and is principally caused by the feeding of the voracious third instars/second year. Grubs of the second year and especially third instars were thus of particular interest in this survey. The 132 first instar grubs obtained from field-collected eggs hatching in the laboratory were not tallied in Tables 7, 8 and 9 since they were killed soon after hatching to become voucher specimens.

All data pertaining to invertebrate predators and parasites, microorganisms (including nematodes) and Acarina found in the course of this survey are reported separately in succeeding chapters.

I emphasize here that bacteria and fungi found associated with Phyllophaga individuals for which Koch's postulates (Steinhaus, 1949) failed in infectivity tests, were not considered to be entomopathogenic, thus not natural enemies, and were not

Table 7. Number of white grubs, Phyllophaga spp., collected in southern Quebec, 1979.

Site	Collection dates	Instar [a] L1	L2,1	L2,2	L3,2	L3,3	Total
Ste.Anne	Jun.21-Jul.19	490[b]	0	0	0	0	490
Nicolet	Jul.28	813	96	0	0	0	909
	Aug. 7	28	914	0	0	0	942
Quebec	Aug. 6	0	0	0	0	84	84
Pierreville	Aug.28-Nov. 6[c]	0	0	7	4194	0	4201
Bellefeuille	Jul.12	0	0	8	155	0	163
Valleyfield	Aug.20	0	0	0	93	0	93
Mirabel	Jun.20-Aug. 6[d]	42	0	607	1662	0	2311
Napierville	Jul.29	0	0	112	170	0	282
Gd.St.Esprit	Jul.21	183	0	0	0	0	183
Ste.Sophie	Jul.16	0	0	112	183	0	295
Rigaud	Jul.23	0	0	37	0	0	37
Papineauville	Aug. 9	0	0	28	44	0	72
Montebello	Aug. 9	0	0	93	153	0	246
Notre-Dame	Aug. 9	0	0	126	77	0	203
Montford	Aug. 3	0	0	0	79	0	79
St.Janvier	Aug.13	0	0	0	226	0	226
Mascouche	Aug.13	0	0	0	28	0	28
All sites		1556	1010	1130	7064	84	10844

[a] L1: first instar grub; L2,1: second instar, first year; L2,2: second instar, second year; L3,2: third instar, second year; L3,3: third instar, third year.

[b] Hatched from eggs laid in the laboratory by females collected at Nicolet.

[c] Nine occasions.

[d] Eight occasions.

Table 8. Numbers of white grubs, Phyllophaga spp., collected in southern Quebec, 1980.

Site	Collection dates	Instar [a] L1	L2,1	L2,2	L3,2	L3,3	Total
Pincourt	May 16	0	0	0	0	4	4
Ste. Anne	May 28	0	0	0	3	2	5
Le Luc	May 29	0	0	0	0	2	2
Ste. Angèle	May 29	0	0	0	0	2	2
Nicolet	Aug. 5	0	0	328	245	0	573
	Aug. 6	0	0	42	38	0	80
	Aug. 20	0	0	0	820	0	820
Cowansville	Sep. 4	0	0	0	0	324	324
St. Sébastien	Aug. 24	0	0	0	0	38	38
Lacolle	Aug. 24	0	0	0	0	57	57
Coteau	Aug. 28	0	0	0	0	123	123
St. Clet	Aug. 28	0	0	0	0	213	213
Montebello	Aug. 9	0	0	0	0	188	188
Mirabel	Sep. 2	0	0	0	0	934	934
St. Janvier	Sep. 2	0	0	0	0	173	173
Stanbridge	Jun. 12	0	0	0	0	1354	1354
	Jul. 9	0	0	0	0	184	184
All sites		0	0	370	1106	3598	5074

[a] L1: first instar grub; L2,1: second instar, first year; L2,2: second instar, second year; L3,2: third instar, second year; L3,3: third instar, third year.

Table 9. Numbers of white grubs, Phyllophaga spp., collected in southern Quebec, 1981.

Site	Collection dates	Instar[a] L1	L2,1	L2,2	L3,2	L3,3	Total
Stanbridge	Aug. 9-23 *	40	195	1	0	0	236
	Oct. 10	0	43	0	1	0	44
Ste. Anne	Apr. 16	0	0	0	3	0	3
Mirabel	Jul. 28	6	4	0	0	0	10
Nicolet	Jul. 30	0	0	0	0	89	89
St. André	Aug. 16	217	184	0	0	0	401
Notre-Dame	Aug. 16	13	216	0	0	0	229
All sites		276	642	1	4	89	1012

[a] L1: first instar grub; L2,1: second instar, first year; L2,2: second instar, second year; L3,2: third instar, second year; L3,3: third instar, third year.

* Three occasions.

studied further as biotic regulators of Phyllophaga spp. in southern Quebec.

IV A NEW SMALL IRIDESCENT VIRUS (PaIV) FOUND INFECTING WHITE GRUBS, PHYLLOPHAGA ANXIA IN SOUTHERN QUEBEC

A INTRODUCTION

In recent years, the study of virus diseases of insects has advanced very rapidly, descriptions and characterization of the viruses, ecological aspects of the diseases, industrial production of the etiological agents, control of insect pests with viruses, have all been intensively studied

The literature on insect virology is voluminous and several excellent reviews have been prepared in the past 40 years. Specific and detailed treatments of the icosahedral cytoplasmic deoxyriboviruses, including the iridescent viruses, were prepared by Smith (1963), Bellett (1968), Vago (1968), Kelly and Robertson (1973), Stoltz (1973), McAuslan and Armentrout (1974); Lee (1977), Goorha and Granoff (1979); Harrap and Payne (1979), Kelly (1981), Payne and Kelly (1981) Martignoni and Iwai (1981) have compiled a useful computer-based catalog of virus diseases of insects, mites and ticks, updated on a regular basis

Classification and nomenclature of viruses have slowly evolved from an early chaotic state to a relative stability

and a general consensus, thanks to the efforts of the International Committee on Taxonomy of Viruses. This committee has unified to some extent the numerous systems and names advocated by virologists and viruses of invertebrates have been included in the reorganization (Gibbs et al., 1966; Lwolff and Tournier, 1971; Wildy, 1971, Fenner et al., 1974, Fenner, 1975, 1976; Matthews, 1979, 1981, 1982; Fenner and Gibbs, 1983).

An interim nomenclature system for the iridescent group of insect viruses was proposed by Tinsley and Kelly (1970); the system is still interim at the time of writing although over 25 new types have been isolated since 1970.

Insect virology is a very dynamic field due to the continual discovery of new viruses and new host-pathogen relationships. One of the first published catalogs of insects attacked by virus diseases listed 42 species of hosts (Sweetman, 1936) and one of the first attempts to classify entomopathogenic viruses recognized one family and four genera (Steinhaus, 1949). As of September 1984 (Martignoni, 1984, personal communication), the updated Martignoni's and Iwai's (1981) computer catalog listed over 1020 host species, each reported to have one or more of 22 virus diseases or disease groups, for a total of about 1500 natural host-virus records. Matthews (1982) recognized eleven families and groups of entomopathogenic viruses, numerous subgroups and genera. Interestingly, although the order Coleoptera contains the bulkiest number of species in the insect world, beetles accounted for only 4.7% of host-virus records in Sweetman's (1936) catalog and, today, still represent only 5.7% of all records

(Martignoni, 1984, personal communication) About half of beetle-virus records concern species of the family Scarabaeidae (Martignoni and Iwai, 1981). From 1981 to September 1984, only three new host-virus records for Scarabaeidae were added to Martignoni's and Iwai's (1981) catalog, one of which was a new host, Phyllophaga anxia (LeConte) (Lim et al., 1981a). However, although Lim et al. (1981a) reported a suspected spheroidosis (entomopox virus) for P. anxia, Martignoni (1984, personal communication) listed their record in the " presumed virosis " category of the catalog. Martignoni and Iwai (1981) warned the users of their catalog that such entries should be considered with scepticism.

Scarabaeid grubs are known to be infected with the following virus diseases: Malaya disease, densonucleosis, watery disintegration, spheroidosis, iridescent virosis and " other non-occluded-virus disease " To my knowledge, none of these confirmed virus diseases occurring in scarabaeids is a North American record. The scarabaeid hosts, all grubs, are listed in Martignoni and Iwai (1981) together with the viruses infecting them

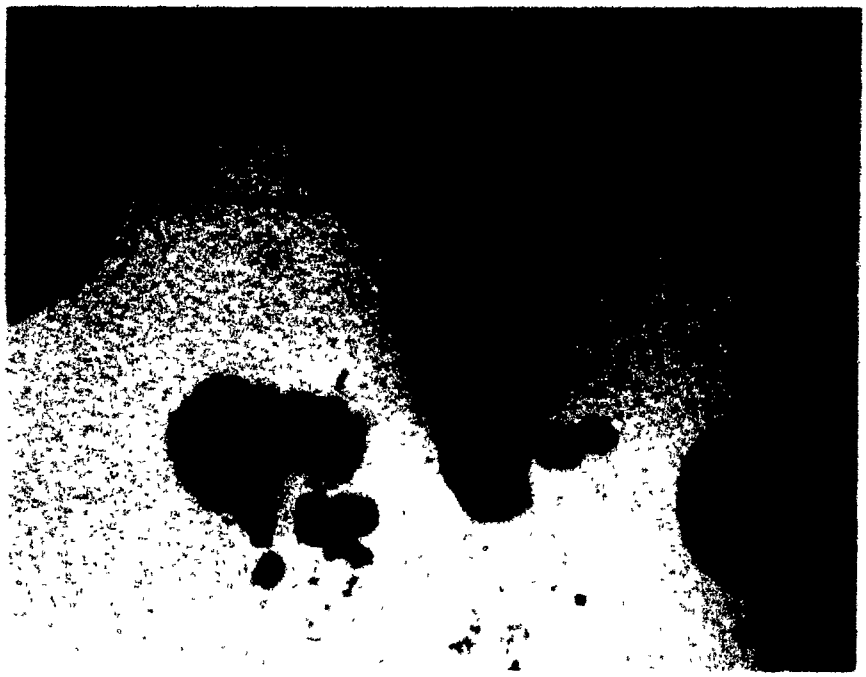
As far as could be ascertained by consulting the available literature, I report here the first naturally-occurring virus disease of P. anxia grubs whose etiological agent I propose to name Phyllophaga anxia iridescent virus (PaIV) This is also the first record of a natural virus disease of Scarabaeidae for North America

B. MATERIALS AND METHODS

Third instar, second year white grubs were collected by the sod cutting technique described earlier, at the farm of Mr. C. Lorrain (Bellefeuille, Quebec) on July 12, 1979. The grub population was quite dispersed since only 155 grubs were uncovered in the removal of 45000 m^2 of sod. Most of the grubs were aggregated at the lower part of the red fescue and Kentucky bluegrass field at a depth of 6 cm, 2 to 3 m from the bank of a small creek. The grubs were healthy-looking (sensu Berberet and Helms, 1972), although several subsequently died of fungal and bacterial infections. Two grubs however were de situ noticed for their aberrant aspect. One dead grub displayed an emerald green opalescence on the four apical segments of the abdomen while the remainder of the body was in an advanced stage of decomposition. The second grub was alive but sluggish; its response to probing with the pointed end of a pencil was not immediate and, at rest, it did not assume the typical C-shaped scarabaeid posture but lay outstretched on its dorsum. Instead of being creamy white in color, the body had large areas of an azure to turquoise blue iridescence on the ventro-thoracic segments and on the dorsum and ventrum of the abdomen (Figure 12).

The dead grub was frozen at -18°C and the 154 live grubs were placed in individual milk cups and held in an incubator set at 15°C, 20-25% RH and complete darkness. No food was provided in order to stress the grubs, which were checked daily for manifestations of latent virosis.

Figure 12 A field-collected third instar white grub, Phyllophaga anxia, infected with Phyllophaga anxia iridescent virus (PaIV)
Note the typical blue hue (arrow).



All the live grubs were identified as Phyllophaga anxia and, therefore, the dead grub was also considered to be P. anxia.

Eight days of daily examination of the quarantined grubs did not reveal aberrant aspect or behavior besides an accentuation and an extension of the blue iridescence of the affected grub, especially on the ventro-thoracic area. On July 20, the grub was still alive but the blue color had spread to most parts of the body, being more intense on the ventral side, from head to pygidium; the intersegmental folds were more intensely colored than the body segments. The grub was then killed by freezing and both iridescent grubs were shipped, for diagnostic analysis, in dry ice to Dr. G. O. Poinar, Jr., Division of Entomology and Parasitology, University of California at Berkeley. The two specimens were forwarded by Dr. Poinar to Dr. A. Cole, Department of Plant Pathology, University of California at Berkeley, who processed them for E M studies.

Isolation and purification of the etiological agent responsible for the blue iridescence were performed following the method of Kalmakoff and Tremaine (1968). Methods for E M of fat body and hemocytes and, later, of the purified agent were similar to those reported by Cole and Morris (1980) for diseased isopods (Cole, 1979, personal communication). Antiserum preparation and serology tests were similar to those of Cole and Morris (1980). The Koch's postulates for viruses (Rivers, 1937) were fulfilled using 50 healthy third instar, second year grubs which I shipped to Dr. Cole on November 22, which had been collected from a strawberry field at Pierreville, Quebec, on November 6. Voucher iso-

lates of the etiological agent were deposited with Dr. J. Morris, Department of Plant Pathology, University of California at Berkeley. The antiserum, prepared in rabbits by injection of the purified etiological agent from P. anxia, was deposited with Dr. J. Peterson, Department of Plant Science, MacDonald College of McGill University, Ste.-Anne-de-Bellevue, Quebec, Canada.

All E M studies were performed on a Philips E M 300 electron microscope operated at 60 kV.

C. RESULTS AND DISCUSSION

The gross symptomatology of the aberrant grubs recovered at Bellefeuille was at first indicative of a possible rickettsial infection described from grubs of several scarab beetles under the vernacular name of "blue disease" (maladie bleue in Europe). Such rickettsiosis is known to be caused by Rickettsiella popilliae (Dutky and Gooden) Philip in grubs of Popillia japonica Newman, Rhizotrogus majalis (Razoumowsky), Phyllophaga anxia (LeConte) and P. ephilida (Say), in New Jersey and Maryland, (Dutky and Gooden, 1952) and by R. melolonthae (Krieg) Philip in grubs of several Melolontha species in Europe (Hurpin and Vago, 1958). Krieg (1963) reported that the bluish-green iridescence (greyish-blue in Melolontha), similar to that of Tipula iridescent virus but not so intense, is due to Tyndall scattering and Bragg reflection, due to the smallness and orientation of rickettsiae within the fat body cells of diseased grubs.

However, preliminary E M study of a crude homogenate of

embedded tissues revealed, in both Bellefeuille grubs, the presence of non-occluded cytoplasmic, symmetrical (hexagonal) virus particles, morphologically similar to those of members of the family Iridoviridae or icosahedral cytoplasmic deoxyriboviruses (Matthews, 1982). Isolation, purification and subsequent E M study of the particles confirmed that both grubs suffered from an iridovirus infection. Payne and Kelly (1981) emphasized that the presence of iridescent viruses can be confirmed visually only by E M.

The large virus particles found in thin sections of embedded tissues occurred only in the cytoplasm of infected cells, never in the nucleus, and averaged 110 nm (n=32) in diameter. Particles of purified virus, stained with 2% potassium phosphotungstate, pH 6.8, averaged 138.6 nm in diameter (n=30; range 135-140 nm) (Figure 13).

The peculiar optical properties of the virus particles in infected grubs and in purified virus pellets, the cytoplasmic confinement of the virions in infected cells, the non-inclusion of the virions, their shape, isometry and size were all indicative that the virus isolated from P. anxia grubs was indeed an icosahedral cytoplasmic deoxyribovirus (family Iridoviridae) of the group of the small iridescent insect viruses, genus Iridovirus, for which the proposed type species (Matthews, 1982) is the Tipula iridescent virus (TIV) or insect iridescent virus Type 1, the first such virus ever reported (Xeros, 1954). The other members of the insect iridescent viruses belong to the genus Chloriridovirus or large iridescent insect virus group (particle diameter = 180 nm)

Figure 13. Electron micrograph of Phyllophaga anxia iridescent virus; purified particles negatively stained in 2% potassium phosphotungstate, pH 6.8. Magnification on print = 102200 times. Note the hexagonal shape of the particles, the capsid enclosing the densely staining core, some empty shells and the apparent second membrane (inner row of protein subunits).

whose type species is the mosquito iridescent virus, regular strain (RMIV) or insect iridescent virus Type 3 (Matthews, 1982).

Since P. anxia is a new natural host for a virus of the genus Iridovirus, I propose to designate the Bellefeuille isolate PaIV or Phyllophaga anxia iridescent virus. It is also legitimate to designate PaIV as Type 33 in the interim nomenclature system proposed by Tinsley and Kelly (1970) for the iridescent group of insect viruses. Thus, PaIV becomes iridescent virus Type 33 because the last published type designation was Type 32 from the sow bug, Porcellio dilatatus, from southern California (Federici, 1980). Several authors have published new natural host records for insect iridescent viruses since Federici (1980), but they have failed to assigne new types (Popelkova, 1982; Avery and Bauer, 1984). The cryptogram for PaIV is similar to the cryptogram of TIV, the type species of the genus Iridovirus (Fenner and Gibbs, 1983) and reads:

(D/2:126/L$^-$:S/S:I/*)

in which the first two sets describe the genome: (D) DNA/(2) double-stranded: (126) molecular weight in Mdaltons/(L$^-$) linear non-infectious isolated nucleic acid; the third set describes the virion shape: (S) spherical outline shape of virion/(S) spherical outline shape of nucleocapsid in the virion; the fourth set gives some biological characters of the virus: (I) invertebrate host/(*) mode of transmission unknown.

Viruses with properties in common with the insect (including terrestrial isopods) iridescent group, other than iridescence in vivo, have been isolated from the protozoan,

Entamoeba histolytica; the mollusc, Octopus vulgaris; the annelid, Nereis diversicolor; the midge, Chironomus plumosus, the daphnid, Simocephalus expinosus; frogs, newts, tadpoles, swine and fish (Tinsley and Kelly, 1970; Federici, 1980; Matthews, 1982) Viruses of the family Iridoviridae (the name is misleading, since the inclusion within the family does not necessarily denote iridescence) are thus recognized as ubiquitous pathogens in populations of several Phyla in many parts of the world and more particularly (genera Iridovirus and Chloriridovirus) in invertebrate populations of Isopoda, Ephemeroptera, Orthoptera, Diptera, Lepidoptera, Hymenoptera, Heteroptera and Coleoptera. Within the Coleoptera, iridescent viruses have been identified in species of the families Buprestidae (1), Cerambycidae (1), Chrysomelidae (1), Coccinellidae (1), Curculionidae (2), Lucanidae (2), Tenebrionidae (1) and Scarabaeidae (6) (Martignoni and Iwai, 1981).

All isolates of insect iridescent viruses are lethal to their hosts (Kelly et al., 1979), the viruses having a cytocidal effect by rapid inhibition of host cell macromolecular synthesis (Matthews, 1982) and death of the host (for TIV) occurs 2 to 4 weeks or more after the disease becomes detectable (Xeros, 1954).

The physics of the peculiar properties of TIV were described in detail by Williams and Smith (1957) and Smith and Williams (1958). In vivo and in vitro iridescent hue in reflected light depends on the state of hydration of infected tissues or of virus pellets and may thus be purple, blue, green or orange but the color is always orange in transmitted light (Xeros, 1954;

Smith and Williams, 1958, Lipa, 1967; Smith, 1976), Bellett (1968), Lee (1977), Kelly and Robertson (1973), McAuslan and Armentrout (1974), Payne and Kelly (1981) reviewed the structure, biochemistry and serological relationships of insect iridescent viruses and Matthews (1982) summarized their main characteristics.

The route of infection of insect iridescent viruses is uncertain. Evans and Harrap (1982) wrote that in nature it has been difficult to substantiate that iridescent virus infection is acquired by mouth, as for other insect viruses. Kelly (1981) and Evans and Harrap (1982) remarked that iridescent viruses are far more infectious when injected into the hemocoel of an insect than when ingested but Kelly (1981) also suggested that iridescent viruses are likely transmitted, in the field, by cannibalism on infected hosts and, according to Evans and Harrap (1982), accidental injury or predatory behavior might be important in iridescent viruses gaining access to susceptible tissues but it would be perhaps less likely than cannibalism or feeding on infected cadavers. It should be said here that, in my three-year survey of natural enemies of Phyllophaga in Quebec, I found only the two cases of iridescent virosis reported in this chapter. Both infected grubs were collected on the same day and in close proximity (2 to 3 cm) of each other. Moreover, one of the grubs was dead and the live grub was in an early stage of infection. In my opinion, these facts strongly suggest that contact between the two grubs took place sometime after the death of the first grub. It is known that Phyllophaga grubs have a tendency to cannibalism (Miner, 1948). My observations on PaIV occurrence in the field appear to agree

with the suggestions advanced by Kelly (1981) and Evans and Harrap (1982)

The replication of iridescent viruses is summarized in Kelly (1981) and Matthews (1982) and will not be discussed here. All students of insect iridescent viruses agree that the first site of infection and virus replication of the iridescent viruses of insects are the fat body cells, that other tissues become gradually infected and that infection finally results in systemic infection and death of the host (Kelly, 1981). This systemic infection was observed in the live grub that I quarantined after field collection, the blue iridescence, an indication of iridescent virus infection, spread gradually throughout the body of the grub, as mentioned earlier

Indirect enzyme-linked immunosorbent assay used by Cole and Morris (1980) to define serological relationships between PaIV, TIV and IIV (isopod iridescent virus) indicated clearly a distant relationship between TIV and IIV and an intermediate relationship for PaIV, with however a greater affinity to TIV. This relationship was confirmed by titration of the sera with the three antigens, using the indirect test.

Infectivity tests on healthy third instar grubs to fulfil Koch's postulates were conclusive and transmission of PaIV by intra hemocelic injection was readily demonstrated (Cole, 1980, personal communication) Of 40 healthy P anxia grubs injected with 2 to 3 µl of pure PaIV suspension, 37 became infected and died The grubs began to show symptoms of infection (a bluish iridescence) 8 to 10 days after inoculation with PaIV and died 19 to 21 days after injection. IIV purified from an isopod also readily infected

another series of third instar grubs; 15 days after injection with IIV, the grubs developed purplish coloration and died 7 to 9 days later (Cole, 1980, personal communication; Cole and Morris, 1980) The results of the IIV experiment are in agreement with previous observations on insect iridescent viruses summarized by Matthews (1982): viruses of the genus Iridovirus are apparently not specific and infect a wide range of insect species.

The yield of purified virus in the PaIV transmission tests was very high, averaging 5 mg per grub, the average weight of a healthy third instar, second year grub being 0.65 g (n=38; personal measurements) Insect viruses are well known for the very high yield of virus often produced in infections (Evans and Harrap, 1982) and insect iridescent viruses outperform all insect viruses as well as other animal viruses (Williams and Smith, 1957; Day and Mercer, 1964, Longworth et al., 1979, Kelly, 1981).

I returned to the Bellefeuille sod field on several occasions in the summer and fall of 1979 but never collected any other PaIV infected grubs. Moreover, the three-year survey in 45 localities of southern Quebec (over 16900 grubs collected) did not result in other PaIV isolations. Iridescent viruses are thus far from being important natural regulators of Phyllophaga anxia and allied species in southern Quebec. It is true that the marker of blue iridescence as a measure of frequency of infection probably would under-estimate the prevalence of the virus since early stages of infection are not detected by the naked eye, but, I must recall here, that every grub collected during the survey was routinely quarantined in the laboratory for periods of time

much longer than the 8 to 10 days it took, in the PaIV transmission tests, to notice the first color symptoms of iridescent virus infection

The incidence rate of PaIV in the Bellefeuille field was 1 29% (2 cases of virosis in 155 grubs examined) on July 12, 1979, whereas the prevalence rate of viral disease in the grub populations (16930 grubs examined) over 3 years in 45 localities of southern Quebec was less than 0.02%. Although several authors reported relatively high infection rates in several insect species, most reports of new insect iridescent virus types concerned one or two, rarely more, specimens, especially for scarab beetles, and Longworth et al (1979) concluded that " none of the 30 or so iridoviruses that have been described so far has been identified as causing major mortality in natural populations of its specific host, so iridoviruses are not regarded as promising candidates for biological control "

V. A EUGREGARINE PROTOZOAN, ACTINOCEPHALUS SP., ASSOCIATED WITH PHYLLOPHAGA SPP. IN SOUTHERN QUEBEC

A. INTRODUCTION

Protozoa-insect relationships range from symbiosis to obligate parasitism in nature and the species involved are numerous. Early reviews of Protozoa-insect associations, including lethal infection, were made by Paillot (1933), Steinhaus (1946, 1949) and Aoki (1957). Lipa (1963) reviewed the entomopathogenic ciliates, flagellates and amoebae, Weiser (1963) the Sporozoa, and Lipa (1967) general protozoonoses of insects. Considerations of pathogenic Protozoa as potential control agents for insect pests were given by Tanada (1959, 1963); Hall (1963); McLaughin (1971, 1973); Pramer and Al-Ribiai (1973); Brooks (1974); Canning (1981); Henry and Oma (1981); Maddox et al. (1981); Wilson (1981, 1982). The Microsporidia, Coccidia and Neogregarinida appear to be the most potentially useful pathogenic Protozoa for insect control.

In spite of this bulky literature, there is only scattered reference to the Protozoa associated with scarabaeid beetles (Table 10). Forbes (1916), Davis (1919), Travis and Becker (1931), and Berberet and Helms (1969) are the few authors who reported associations between Protozoa and Phyllophaga spp. As shown in Table 10, most of these reports are of a taxonomic or descriptive nature. To supplement this information, I report here the first record of

Table 10. Known Protozoa associated with some important scarabaeid pests.

Protozoan species	Host insect[a]	Country	Nature of information[b]	Reference
Unspecified	Phyllophaga spp.	USA	E	Forbes 1916
Unspecified	Phyllophaga spp.	USA	E	Davis 1919
Polymastix melolontae (Grassi) Bütschli	Phyllophaga spp.	USA	T,M,	Travis & Becker 1931
P. phyllophagae Travis & Becker	Phyllophaga spp.	USA	T,M	Travis & Becker 1931
Monocercomas melolontae Grassi	Phyllophaga spp.	USA	T,M	Travis & Becker 1931
Eutrichomastix passali Tanabe	Phyllophaga spp.	USA	T,M	Travis & Becker 1931
E. phyllophagae Travis & Becker	Phyllophaga spp.	USA	T,M	Travis & Becker 1931
Embadomonas phyllophagae Travis & Becker	Phyllophaga spp.	USA	T,M	Travis & Becker 1931
Vahlkampfia, Arcella and Allantion sp.	Phyllophaga spp.	USA	T,M	Travis & Becker 1931
Retortamonas sp.	Phyllophaga spp.	USA	T	Lipa 1963

Table 10. (cont'd)

Protozoan species	Host insect	Country	Nature of information[b]	Reference
Gregarina, Actinocephalus sp.	Phyllophaga anxia	USA	M,B	Berbert & Helms 1969
Actinocephalus sp.	P. anxia adult	USA	Record	Berberet & Helms 1969
Gregarina cetoniae Foerster	Cetonia sp.	Germany	T,B	Foerster 1938
Monocercomas mackinnoni Kowalczyk	Popillia japonica	USA	T,M	Kowalczyk 1938
Nosema melolonthae (Krieg)	Melolontha sp.	Germany	T,M	Krieg 1955
N. melolonthae (Krieg)	Melolontha melolontha	France	B,E	Hurpin & Vago 1958 Hurpin 1965
Adelina sericesthis Weiser & Beard	Sericesthis pruinosa	Australia	T,B,P	Weiser & Willie 1959
Monocystis sp.	Hoplia sp.	Switzerland	T,B	Weiser & Willie 1960
Retortamonas sp.	Popillia japonica	USA	B	Lipa 1963

Table 10. (cont'd)

Protozoan species	Host insect	Country	Nature of information[b]	Reference
Polymastix melolonthae (Grassi) Bütschli and related species	Melolontha melolontha, Popillia japonica, Anomala orientalis, Oryctes nasicornis	Unspecified	B	Lipa 1963
Monocystis sp.	Melolontha melolontha	France	B	Weiser 1963
Adelina sericesthis Weiser & Beard	Melolontha hippocastani	Czechoslovakia	P	Weiser 1963
Gregarina melolonthae Lankester	Melolontha melolontha	Unspecified	T,B	Lipa 1967
Eugregarinida	Costelytra zealandica	New Zealand	T,B	Allison 1969
Nosema costelytrae n. sp.	Costelytra zealandica	New Zealand	T,B	Hall et al. 1977

[a] Grub stage when not specified.

[b] E: epizootiology; T: taxonomy; M: morphology; B: biology; P: pathology.

a eugregarine protozoan found parasitizing Phyllophaga spp. in Canada.

B. MATERIALS AND METHODS

In August 1979, when dissecting third instar Phyllophaga grubs collected from Pierreville, Quebec, I noticed that several specimens harbored relatively large, extracellular, white organisms, which, after searching the literature, were tentatively identified as representatives of the Protozoa.

All specimens of Phyllophaga spp. that were collected in 1979-1981 from the 45 collection sites in southern Quebec (Figure 10) were thus examined for the presence of the conspicuous mature trophozoites observed at the onset of the study and for other signs of protozoan invasion. Methods of examination are described in Chapter III. The abdominal cavity and alimentary tract of the insects were specifically searched for the presence of protozoans. Eggs of Phyllophaga spp. were not dissected but examined with a dissecting microscope set at the highest magnification.

The following data were recorded for each specimen examined presence and number of trophozoites, sex and stage of the specimen, collection date and locality. Some trophozoites were permanently mounted on microscope slides after coloration with Giemsa or hematoxylin stains as recommended by Martignoni and Steinhaus (1961); Weiser and Briggs (1971); Thomas (1974); Poinar and Thomas (1978). Blood smears, routinely taken during the examination, were treated in the same way.

It can be assumed that over 98% of specimens of Phyllophaga collected in Quebec were P. anxia (Toohey, 1977; Lim, 1979; Chapter III).

C. RESULTS AND DISCUSSION

Study of the mounted specimens under a bright field microscope confirmed that the organisms previously seen were the mature, extracellular stage of a eugregarine protozoan. The gregarine was further identified to the generic level Actinocephalus (order Eugregarinida, suborder Cephalina, family Actinocephalidae). The identifications were based on descriptions and keys published by Watson-Kamm (1916); Jahn and Jahn (1949); Grassé (1953); Hall (1953); Honigberg et al. (1964); Kudo (1966); Berberet and Helms (1969); Sleigh (1973); Poinar and Thomas (1978); Levine et al. (1980). The fact that sporadins were never observed in syzygy and hence were solitary gregarines, confirmed that they belonged to the genus Actinocephalus and not to the closely related genus Gregarina, whose members are biassociative (Laird, 1959; Berberet and Helms, 1969). Although voucher specimens were submitted to protozoologists (Drs. M. Akbarioh, Département des Sciences Biologiques, Université de Montréal and G. G. Wilson, Forest Pest Management Institute, Sault Ste. Marie), they were unable to identify the gregarines to the species level.

I measured a total of 42 extracellular mature trophozoites (sporadins) isolated from the gut lumen and hemocoel of grubs collected at various localities during the three-year survey. The

mean length was 1400 μm (S.D. = 160, range 1100-1800), with the deutomerite being 6 to 8 times longer than the protomerite. The single nucleus was always located in the deutomerite, this latter compartment of the cephaline sporadin being separated from the protomerite by an ectoplasmic septum (Figures 14 and 15). The mean width, measured at the widest part of the deutomerite, was 280 μm (S.D. = 40, range 220-360). The above measurements are within the ranges reported for gregarines by Steinhaus (1949) and Berberet and Helms (1969), the latter authors having described a Gregarina sp. and an Actinocephalus sp. from P. anxia grubs in Nebraska.

The number of gregarines recovered from any single insect specimen was consistently low, averaging 3 to 5 per specimen, with a maximum of 12 in 6 cases, whatever the host stage, year, time of the year and locality sampled. Steinhaus (1949) and Weiser (1963) wrote that insect hosts are not intensely infected by eugregarines since these lack merogony (reproduction is by gamogony) and no actual multiplication occurs within a single host. Steinhaus (1949) also observed that the numbers of gregarines (unspecified) in one individual of a species of insect may vary according to the season of the year, being much larger (up to 100 or more) in the fall than in the earlier seasons (from 1 to 10 gregarines). Furthermore, a greater number of insects in a given ecological niche are infected in the fall than earlier in the year (Steinhaus, 1949). Such seasonal variations at the host and host population levels were not observed in the course of this study. The relative abundance of Actinocephalus sp. that I found appeared

Figure 14. Three mature trophozoites (sporadins) from the midgut lumen of a white grub, Phyllophaga sp Giemsa staining. N = nucleus, P = protomerite, D = deutomerite. 50X.

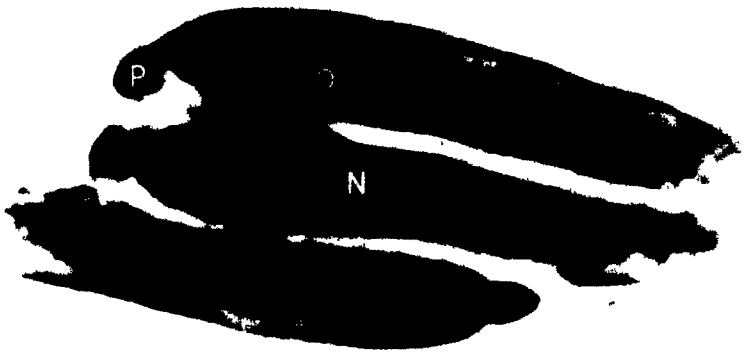

P
N

Figure 15 A sporadin prior to encystment. Giemsa staining
Note the shortening and thickening of the deutomerite
180X

to have been fairly constant over the years in geographical distribution and in host stage specificity (Figure 16 and Table 11)

The short duration of the first instar grub stage explains the low numbers of individuals found harboring the gregarine, since these grubs had less opportunity to ingest the infective stage of the parasite Pasteurization of the soil used in the laboratory rearing trays probably killed the infective stage of the gregarine, hence, first instar grubs derived from laboratory rearing were free of the parasite (Table 11)

The relatively long duration of the second and third grub stages and the active feeding activity of these larvae may have accounted for their relatively higher infection rates with gregarines (Table 11)

The profound metamorphic changes taking place at the onset of pupation and the quiescence of the non-feeding pupal stage and teneral adult would result in parasite-free individuals; only one prepupa, one pupa and one teneral adult were found parasitized during this study (Table 11)

The phyllophagous habits of the flying adults might explain why, with one exception (Table 11), they were parasite-free indeed, even if gregarine spores did occur on foliage, it is unlikely that they could survive the lethal action of solar radiation and other adverse physical factors None of the blood and tissue smears taken routinely during this study showed the presence of immature gregarine stages in P anxia adults, pupae and prepupae, although Berberet and Helms (1969) found cysts of an Actinocephalus sp in the mesenteron of P anxia adults collected in

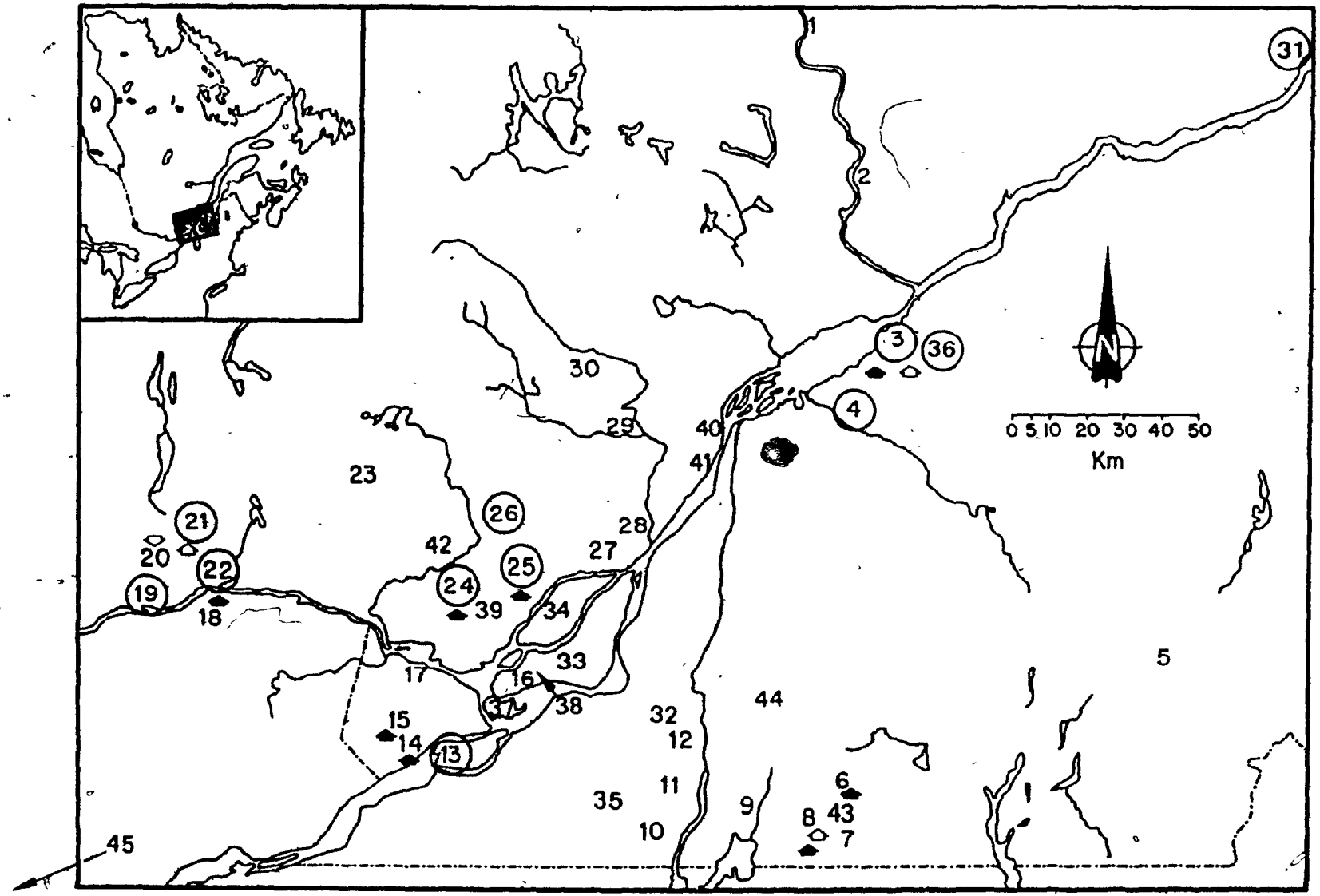
Figure 16 Distribution of the gregarine, Actinocephalus sp., associated with grubs of Phyllophaga spp. in southern Quebec, 1979-1981.

○ 1979

● 1980

⌓ 1981

Numbers assigned to collection sites are explained in Figure 10.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45
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0 5 10 20 30 40 50
Km

Table 11. Relative abundance of the gregarine, Actinocephalus sp., in populations of Phyllophaga spp. of southern Quebec, 1979 to 1981.

Collection year	Insect stage[a] collected	Number collected	Number harboring Actinocephalus	Infection rate (%)
1979	Egg	1870	0	0
	L1	1556[b]	9[c]	0.60
	L2	2140	91	4.25
	L3	7148	232	3.25
	PP	1	0	0
	P male	3	0	0
	P female	0	-	-
	T A male	5	0	0
	T A female	3	0	0
	A male	2636	0	0
	A female	1511	0	0
1980	Egg	0	-	-
	L1	0	-	-
	L2	370	14	3.78
	L3	4704	387	8.23
	PP	166	1	0.60
	P male	1259	1	0.08
	P female	888	0	0
	T A male	23	0	0
	T A female	11	0	0
	A male	552	0	0
	A female	258	0	0
1981	Egg	655	0	0
	L1	276	2	0.72
	L2	643	31	4.82
	L3	93	0	0
	PP	8	0	0
	P male	27	0	0
	P female	9	0	0
	T A male	45	1	2.22
	T A female	23	0	0
	A male	5707	1	0.02
	A female	878	0	0

[a] P: pupa;, PP: prepupa; A; adult; TA: teneral adult; L1, L2, L3: first, second, third instar grub, respectively.

[b] Including 490 L1 from laboratory rearing.

[c] None of the 490 L1 from laboratory rearing.

Nebraska.

No gregarines were found associated with the eggs of Phyllophaga spp.

Over 95% of gregarines recovered by me were located in the gut lumen of the host, at the mesenteromic level, and on most occasions, were found free in the gut and not attached to its epithelium. In a few instances, however, trophozoites were found attached to the mesenteromic epithelium and free sporadins were recovered from the host hemocoel in the midgut region (Figure 17). Poinar and Thomas (1978) reported that the entry of most entomophilic protozoans into the hemocoel occurs through the midgut wall.

Excellent reviews on the morphology and biology of gregarines were prepared by Léger (1892); Watson-Kamm (1916, 1917, 1918); Kamm (1922); Steinhaus (1946, 1949); Théodoridès (1959); Théodoridès and Jolivet (1959); Weiser (1963); and Brooks (1974), but these topics will not be discussed further here.

The effects of Actinocephalus on individual Phyllophaga specimens and on field populations are not clear. During this study, I could not identify any specific symptom in grubs harboring the protozoan; all grubs examined were healthy and behaved normally, but this is not unusual according to Poinar and Thomas (1978). None of the dissections revealed any particular sign that could have been the manifestation of disease caused by a pathogenic condition. However, it is true that the destruction of grubs for dissection purposes did not allow prognosis and pathogenesis studies in the event of a latent infection although the location of Actinocephalus found in the insect body would exclude patho-

Figure 17. Gregarine (circled) in the mesenteromic region of a third instar grub, Phyllophaga sp. 2.5X.

genesis. Equally true is that some damage must have taken place in the gut wall of grubs which had sporadins present in the hemocoel. This type of mechanical damage, if not fatal by itself, could result in death of the grub by septicemia following the invasion of the hemolyph by the general bacterial flora. Honigberg et al. (1964) considered cephalines to be parasites of the gut and body cavity of insects, but this does not necessarily imply that they are pathogenic. On the other hand, the majority of workers involved with the study of cephaline-insect associations, have concluded that gut-inhabitating cephalines are non-pathogenic commensals of their hosts (Laird, 1959 ; Weiser, 1963; Weiser and Briggs, 1971; Brooks, 1974). At least one worker (Weiser, 1963) stated that the adhesion of cephalines to the host's gut must result in a decrease of the absorptive capability of the gut and must contribute to a lowered vitality and shorter life of the host, although symptoms of disease are seldom pathognomonic. The lack of apparent pathogenicity of cephalines for their hosts should not be taken for granted. As for many other Sporozoa, only inadequate methods of detection are responsible for inadequate knowledge of their importance in natural balance of insect populations. In the field most infected grubs disappear, consumed by saprobes and other predators and most protozoan infections escape detection (Weiser, 1963).

I have demonstrated that the Actinocephalus eugregarine found in this study is chronically present in Phyllophaga populations of southern Quebec. The analysis of data in Table 11 show that 4.52% of 16930 white grubs collected in the three-year survey were infected with the gregarine. Infection rates for the other

stages of Phyllophaga spp. were insignificant. The "infection" thus appears to be endemic to the larval stages of the June beetle but enzootic in grub populations throughout southern Quebec, never reaching epizootic dimensions. The true nature of the Actinocephalus-Phyllophaga association remains to be demonstrated at the host level as does the impact on host populations.

VI. BACILLUS POPILLIAE DUTKY AND OTHER BACTERIAL PATHOGENS OF PHYLLOPHAGA SPP. IN SOUTHERN QUEBEC

A. INTRODUCTION

Hundreds of bacterial species have been associated with insects (Steinhaus, 1946, 1949) and a vast number of bacteria have been recorded as causing disease in insects (Milner, 1980). However, primary consideration for use in pest suppression has fallen upon the order Eubacteriales, especially members of the families Enterobacteriaceae, Micrococcaceae, and Bacillaceae, along with several genera in the order Pseudomonales (Bucher, 1960). The Enterobacteriaceae, Micrococcaceae and Pseudomonales are all nonsporeformers and are particularly sensitive to drying and sunlight, vary greatly in their virulence to insects and are very often pathogenic for vertebrates. There is, therefore, little hope for these bacteria as potentially useful pathogens for insect pests (Milner, 1980). According to Dulmage (1981), the only bacteria used or proposed for use in the foreseeable future as microbial insecticides belong to the genus Bacillus, of which four species are considered entomophagous: B. thuringiensis (Berliner), B. popilliae Dutky, B. moritai (Aizawa and Fujiyoshi) and B. sphaericus (Neide). For some reason, Dulmage (1981) did not include the sporeforming obligate pathogen B. lentimorbus Dutky in his listing, although the eighth edition of Bergey's

Manual of Determinate Bacteriology (Buchanan and Gibbons, 1974) recognized B. lentimorbus as a species distinct from its close relative B. popilliae. Only B. thuringiensis is produced commercialy in vitro. Bacillus popilliae and B. lentimorbus, the agents of the milky disease(s) of scarab beetles, were the first microbial insecticides to be registered in the United States (in 1948) although commercial production is possible only in vivo.

Comprehensive reviews of bacterial associations with insects and of the practical use of pathogenic species of bacteria were prepared by Steinhaus (1946, 1949), Bucher (1960, 1963), Briggs (1963); Hall (1963); Heimpel and Angus (1963); Lysenko (1963); Tanada (1963); Jacques (1964); Angus (1965, 1974); Falcon (1971); Dulmage and Rhodes (1971); Faust (1974), Bulla et al., (1975); Cherwonogrodzky (1980); Dulmage (1981); Singer (1981).

In 1940, Dutky published the original description of two bacteria which he named Bacillus popilliae (Type A milky disease) and B. lentimorbus (Type B milky disease), both sporeformers and obligate pathogens which sporulated profusely in the haemolymph of living grubs, Popillia japonica Newman, collected in New Jersey. All infected larvae eventually succumbed to the disease. The term "milky disease" arose because the spore-filled blood of grubs in the final stage of the septicemia developed an opaque white appearance, although overwintering grubs infected with Type B organisms were brown or black in color. Milky diseases, caused by similar but distinct pathogens, have subsequently been reported from scarab beetles in several parts of the world. The genera Popillia, Anomala, Rhizotrogus (Amphimallon)

Melolontha, Costelytra, Cyclocephala, Ataenius, Sericesthis, Odontria, Heteronychus and Phyllophaga are among those with species that have been reported to be naturally infected with milky disease organisms (Dutky, 1963; Tashiro et al., 1969; Milner, 1980). Surprisingly, similar pathogens have not been found in other groups of insects whether they are soil inhabiting or not. Numerous laboratory and field studies of the milky diseases have followed Dutky's (1940) descriptions of these highly pathogenic and specific insect pathogens. Klein et al. (1976) compiled a bibliography of the bacteria associated with P. japonica and closely related Scarabaeidae, including Beard's (1945) publication reporting notable field studies of milky diseases. Excellent reviews of B. popilliae and related forms were written by Briggs (1963), Dutky (1963); Steinkraus and Tashiro (1967); Dulmage and Rhodes (1971); St. Julian and Bulla (1973); Bulla et al. (1975, 1978), Klein (1981a).

The published information relevant to associations between bacteria and Phyllophaga spp. is meagre. Among the obligate pathogenic bacteria, only B. popilliae has been reported to naturally occur in populations of grubs of Phyllophaga spp. Dutky (1963) listed the following species of June beetles as natural hosts for B. popilliae: P. anxia, P. fusca, P. futilis, P. fraterna, P. hirticula (Knoch) and P. inversa (Horn). However, Dutky (1963) did not give further information besides the names of the hosts and it is not known if any of these records referred to the work of Wheeler (1946) who isolated B. popilliae from P. anxia, P. fusca and P. hirticula; the strains anxia, fusca and

hirticula have been isolated in New York, New Jersey and Kentucky, respectively Tashiro and Steinkraus (1966) isolated B. popilliae from a milky grub of P. anxia in New York and designated it strain anxia-2 In Nebraska, Jarvis (1966) found B. popilliae in laboratory-reared second and third instar grubs of P. anxia.

Two species of true facultative pathogenic or potentially pathogenic bacteria have been found associated with Phyllophaga spp. Micrococcus nigrofasciens Northrup has been reported to infect white grubs in Michigan, Illinois, Maryland and North Carolina (Northrup, 1914). Davis (1919) confirmed Northrup's (1914) findings and stated that the bacterium was generally distributed throughout the United States in populations of Phyllophaga grubs Duporte (1915) found the bacterium from Phyllophaga grubs in Quebec Finally, Lim (1979) wrote that he often collected P. anxia grubs in southern Quebec which died from bacterial infection after various holding times, the bacterium involved, although Koch's postulates were not fulfilled, was identified as Bacillus cereus Frankland and Frankland.

Several species of bacteria have been isolated from diseased and dead Phyllophaga individuals in southern Quebec from 1979 to 1981 I report these findings in this chapter Only bacterial species for which Koch's postulates were fulfilled were considered to be pathogenic or potentially pathogenic and are discussed here Several other species of common soil inhabitants, usually found on decomposed Phyllophaga individuals, were not further studied and are not reported here The results of pathogenicity tests with pathogenic or potentially pathogenic bacterial

species are included in the discussion

B MATERIALS AND METHODS

1 Recovery and Identity of Bacteria

Individuals of all stages of Phyllophaga spp., including P anxia, were collected in southern Quebec in 1979 to 1981 and field-collected live specimens were maintained in the laboratory as reported in Chapter III Dead (but not decomposed) and moribund specimens for which bacteriosis was suspected were retained for diagnosis Insect specimens were surface-sterilized by dipping them in a solution of Hyamine 2389 and water (1:75) for 60 seconds, rinsed twice for 30 seconds in sterile distilled water (Morris, 1979), aseptically opened, and samples of tissues and body contents were inoculated on nutrient agar plates and in nutrient broth test tubes Care was taken not to puncture the alimentary tract of the insects when taking samples. In a few cases (eggs and first instar grubs) whole specimens were inoculated on the bacteriological media. Bacterial colonies representative of discernible morphological types were selected from plates and tubes after a 48 hour aerobic incubation period at 30°C and sub-cultured until pure cultures were obtained, Blood smears were also aseptically taken from all processed insects, air-dried on microscope slides and stored at 0°C for further studies. Bacteriological media were used and bacteriological tests were performed according to directions given in the Manual of Microbiological Methods (Society of American Bacteriologists 1957); Martignoni and Steinhaus (1961); Wittig (1963);

Weiser and Briggs (1971), Buchanan and Gibbons (1974), Faust (1974), Thomas (1974), Difco Laboratories (1977), Poinar and Thomas (1978).

As identification and classification tools I mainly used Bucher (1963, 1981), Dutky (1963), Lysenko (1963), Gibbs and Skinner (1966), Edwards and Ewing (1972), St Julian and Bulla (1973), Buchanan and Gibbons (1974), Cowan (1978); Milner (1981) The identifications of bacterial species isolated were confirmed by Mr G M Thomas, Division of Entomology and Parasitology, University of California, Berkeley, California, and Dr.C. Blackwood (retired), Department of Microbiology, Macdonald College of McGill University, Ste -Anne-de-Bellevue, Quebec. Voucher specimens were deposited with Mr G M Thomas and within my collection

2 Infectivity Tests to Fulfill Koch's Postulates

The pathogenicity of the bacteria isolated from Phyllophaga specimens was determined using two methods of inoculation; force-feeding and intrahemocoelic injection of bacterial suspensions. All infectivity tests were performed against healthy third instar, second year white grubs collected at Pierreville, Quebec, in September 1979

Inocula of sporeforming bacteria (Bacillus spp) were prepared by sterilizing the surface of a field-collected diseased grub, puncturing the hemocoel through the dorsal body region and suspending the haemolymph loaded with spores in sterile 0 1% tryptone, a medium recommended by St Julian et al. (1973)

because of its minimal harmful effect on both insects and bacteria Prior to injection, the spore suspensions were heated at 70°C for 15 minutes to destroy the vegetative cells. For non-sporeformers (Pseudomonas, Serratia and Micrococcus spp.), inocula were prepared by rinsing bacterial growth from the surface of agar cultures with sterile distilled water All bacterial suspensions were serially diluted in the appropriate diluent to obtain a final concentration of 2 to 3 x 10^4 cells or spores ml^{-1} of suspension Bacterial counts were made using a Petroff-Hausser counting chamber

For each bacterial species, sterilized 1 ml serological syringes fitted with a 27-gauge needle were used to inject, following agitation, 0 1 ml of the bacterial suspension into the left side of 30 surface-sterilized test grubs, just behind the penultimate abdominal spiracle Ten control grubs were injected with 0 1 ml of sterile distilled water, ten with 0 1 ml of 0 1% tryptone, ten were punctured with a 27-gauge needle, and ten did not receive any treatment

A pipetting gun fitted with a blunt sterilized glass tip was used to force-feed 0 1 ml of a bacterial suspension to white grubs Each bacterial suspension was fed to 30 test grubs. Ten control grubs were fed 0 1 ml of 0.1% tryptone, ten were fed 0 1 ml of sterile distilled water and ten did not receive any treatment

After each treatment, grubs were placed in individual cardboard cups containing a piece of sterile artificial diet (Toohey, 1977) and the experimental units were incubated at 25°C, 60-65% RH and 16h photophase. Grubs were checked every second

day for 29 days for symptoms of bacterial disease and mortality Blood samples were examined from diseased and dead grubs, and from all apparently healthy grubs at the end of the infectivity tests Reisolation of the test bacterium from the haemolymph of test grubs was considered a positive case of infection.

3 Bioassays of Two Isolates of Bacillus popilliae

The bacterial cultures used in these bioassays were (1) a local isolate of B popilliae isolated from diseased third instar, second year Phyllophaga grubs collected in July 1979 at Mirabel, Quebec, and preserved as air-dried blood smears on microscope slides, (2) the commercial product DOOM manufactured by Fairfax Biological Laboratory Inc , Clinton Corners, New York. DOOM is advertised as a milky disease spore powder, produced in vivo in grubs of Popillia japonica against which it is recommended as a microbial insecticide According to the label, 1g of the inert carrier (talcum) contains no less than 100 x 10^6 spores of either or both B popilliae or B lentimorbus

Three methods of inoculation were used with both isolates in this study. force-feeding, intrahemocoelic injection and topical application. The commercial product DOOM was also tested as soil inoculum.

To prepare the inoculum of the local isolate of B popilliae, spores preserved as dried blood smears were flushed off the slides with 10 ml of warm sterile 0 1% tryptone, a spore count was made and the suspensions were adjusted to 3 x 10^9 spores ml^{-1}. The final dosages retained for the tests were

obtained after serial dilutions in 0 1% tryptone. The milky disease spores of the commercial product were separated from the carrier by 3 successive centrifugations (2000 rpm for 5 minutes) of 6g of the spore powder suspended in 10 ml of 0.1% tryptone. The final dosages were obtained as for the local isolate. The inoculum for soil application tests was the spore powder DOOM.

For each test, except the soil application trials, a standard volume (0.1 ml per grub) of various spore dosages was inoculated to a standard number of test grubs (2 replicates of 20) Two replicates of 15 control grubs in each test were inoculated with 0 1 ml of 0.1% tryptone.

a Force-feeding tests

The experimental procedure, including post-treatment conditions, was as outlined in section 2. Spore dosages of 2×10^7 and 3×10^7 spores ml^{-1} of the local isolate were used against second instars (collected at Nicolet in August 1979) and third instars (collected at Pierreville in September 1979), respectively 3×10^7 spores ml^{-1} of DOOM against second (Nicolet, August 1979) and third instars (pierreville, September 1979); 2×10^7 spores ml^{-1} of DOOM against first instars (Grand St.-Esprit, July 1979). The tests were terminated after 30 days and mortality was recorded

b Intrahemocoelic injection

The experimental procedure was similar to that described in section 2. The local isolate was assayed at a spore dosage of 3×10^7 spores ml^{-1} against second and third instar grubs. The

product DOOM was tested at dosages of 4×10^7 spores ml^{-1} against first and second instars, and 3×10^7 spores ml^{-1} against third instars. The sources of the grubs used in these tests were the same as those of the force-feeding trials Post-treatment conditions were as reported in section 2 The tests were terminated after 30 days and mortality recorded.

c. Topical application

The experimental procedure was as outlined in section 2 for the force-feeding infectivity tests, but the inoculum was pipetted on the dorsum of surface-sterilized test grubs instead of being introduced into the alimentary tract. Second and third instar grubs were inoculated with 6×10^7 and 1×10^8 spores ml^{-1}, respectively, of the local isolate. DOOM was assayed at dosages of 5×10^7, 6×10^7 and 8×10^7 spores ml^{-1} agianst first, second and third instars, respectively. Test grubs originated from the collection sites and on the dates reported for the force-feeding trials. The tests were terminated after 30 days and mortality recorded.

d. Soil inoculation

Sandy loam soil (1:1) was steam-sterilized, oven-dried, and distributed in amounts of 1.75 kg to 30 x 30 x 10 cm ($0.009 m^3$) boxes. The soil in each box was then seeded with about 10g of a red fescue and Kentucky bluegrass mixture, and inoculated with either 1g (1×10^8 spores g^{-1}) or 3g (1×10^8 spores g^{-1}) of the commercial product DOOM. The contents of the boxes were hand-mixed and watered; boxes were covered

with a plastic sheet and one week was allowed for the germination of the grass seeds. The soil in the boxes intended to receive control grubs was treated in the same way but was inoculated with 1 or 3g of commercial talcum. Tests started on August 28, 1981 when the grass in the boxes was 3 to 4 cm tall. Five healthy first instar grubs, collected at St.-André-Avellin on August 16, were placed on the soil surface of each of 10 boxes inoculated with 1g of DOOM. Five healthy second instar, first year grubs, collected at Stanbridge East on August 9, were placed on the soil surface of each of 10 boxes inoculated with 3g of DOOM. Two control boxes containing 1g of talcum received 5 first instars each and two control boxes containing 3g of talcum received 5 second instar, first year grubs each. Grubs which did not bury themselves within 2 hours were replaced. All the boxes were then incubated in an environmental chamber set at 27±1°C, 60-65% RH and 16h photophase. The soil in the boxes was kept moist, but not saturated, by regular watering with sterile distilled water. Tests were terminated after 42 days when the boxes were emptied and all data pertaining to mortality of test grubs were recorded.

C. RESULTS AND DISCUSSION

1. Recovery and Identity of Bacteria

About 25 species of bacteria were found associated with Phyllophaga individuals in the course of this three-year survey in southern Quebec. However, only those obligate, facultative and potential entomopathogenic bacterial species (on the basis

of literature review and results of infectivity tests reported in section 2) were considered to be natural enemies of Phyllophaga spp. and are discussed here. Field-collected and laboratory-obtained diseased or dead Phyllophaga specimens were thus found to be infected by five entomogenous bacteria: Bacillus cereus Frankland and Frankland, B. popilliae Dutky, Pseudomonas aeruginosa (Schroeter) Migula, Serratia marcescens Bizio and Micrococcus nigrofasciens Northrup (Table 12a). Bacillus cereus (Figures 18 and 19) was found to infect all host stages except the egg. It was also isolated on more occasions (sites and dates) and from higher numbers of hosts than the other bacteria (Table 12a). These observations agreed with Lim (1979) who reported that B. cereus was abundant among populations of Phyllophaga grubs in southern Quebec in 1975-1977. Bacillus popilliae (Figure 20) appeared to be the second most important bacterial pathogen of Phyllophaga although it was isolated at only 4 collection sites and only from second and third instar grubs (Table 12a). Serratia marcescens was widespread throughout southern Quebec although the numbers of grubs infected with the bacterium were low. Micrococcus nigrofasciens was isolated from only 9 grubs out of the 16,930 grubs examined in 1979-1981 (Table 12a). The latter observation contradicted Northrup's (1914) report on the wide distribution of M. nigrofasciens in populations of Phyllophaga spp. grubs across most of the American states. One June beetle egg was found naturally infected with P. aeruginosa (Table 12a); this was the only pathological condition observed among populations of eggs of Phyllophaga in the course of this

Figure 18. Adult male (top left) and adult female (top right), Phyllophaga anxia (LeConte), naturally infected with the bacterium, Bacillus cereus Frankland and Frankland. Bottom: healthy adult male, P. anxia. 2X.

Figure 19. Third instar white grub, Phyllophaga sp., killed by B. cereus.

1 cm

Table 12 a. Entomopathogenic bacteria isolated from Phyllophaga spp.[a] in southern Quebec, 1979 to 1981.

Bacterial species	Host stage[b] (number infected)	Collection site[c]	Date of collection[d]
P. aeruginosa	E(1)	Nicolet	June 79
	L2,1(4)	Nicolet	Aug. 79
	L3,2(2)	Pierreville	Sept. 79
	L2,2(2)	Mirabel	July 79
	L2,2(7)	Ste. Sophie	July 79
S. marcescens	L3,2(2)	St.-Janvier	Aug. 79
	L3,3(1)	Cowansville	Sept. 80
	L3,3(4)	Stanbridge	June 80
	L2,2(1)	Nicolet	Aug. 81
	L2,1(3)	Stanbridge	Aug. 81
	L2,2(4)	Mirabel	July 79
M. nigrofasciens	L3,2(1)	Montebello	Aug. 79
	L2,1(3)	Stanbridge	Aug. 81
	L2,1(1)	St. André	Aug. 81
	L1,(28)	Nicolet	July 79
	TA male(1)	Ste. Anne	Oct. 79
	L2,1(36)	Nicolet	Aug. 79
	L2,2(3)	Rigaud	July 79
B. cereus	L2,2(17)	Mirabel	July 79
	L3,2(54)	Pierreville	Sept. 79
	L3,2(39)	Mirabel	Sept. 79
	PP(1)	Stanbridge	Aug. 80
	P male (1)	Stanbridge	Aug. 80

Table 12 a - Continued.

Bacterial species	Host stage (number infected)	Collection site	Date of collection
	P female(1)	St. Jean	Aug. 80
	A female(3)	Nicolet	June 80
	L2,2(11)	Nicolet	Aug. 80
	L3,2(22)	Mirabel	Aug. 80
	L3,3(33)	Stanbridge	Sept.80
B. cereus	L3,3(28)	St.-Janvier	July 80
	L3,3(17)	Stanbridge	Sept.80
	A male(1)	Stanbridge	June 81
	L2,1(1)	Stanbridge	Aug. 81
	L2,1(9)	St. André	Aug. 81
	P female(1)	Nicolet	Aug. 81
	L3,2(1)	Pierreville	Aug. 79
	L2,2(10)	Mirabel	July 79
	L3,2(18)	Mirabel	July 79
	L3,2(16)	St.-Janvier	Aug. 79
B. popilliae	L3,3(34)	Mirabel	Sept.80
	L3,3(22)	St.-Janvier	Sept 80
	L3,3(48)	Stanbridge	July 80
	L2,1(1)	Mirabel	July 81
	L2,1(26)	Stanbridge	Aug. 81

[a] Adults, teneral adults and pupae were P. anxia.

[b] E: egg; L1: first instar grub; L2,1: second instar, first year; L2,2. second instar, second year; L3,2: third instar, second year; L3,3: third instar, third year; PP: prepupa; P: pupa; A: adult.

[c] See figure 10.

[d] Date of collection of host, with or without symptoms of bacterial disease.

Figure 20. Left: third instar grub, Phyllophaga sp., in advanced stage of milky disease showing uniform opacity all over body. Right: healthy third instar grub. 1.4X.

study. Bacterial infections in populations of Phyllophaga were prevalent in the summer months of each year of the survey (Table 12a)

Table 12b lists bacterial infection rates among populations of Phyllophaga spp in southern Quebec from 1979 to 1981. Except for second and third instar grubs, the incidence of bacterial diseases appeared to be very low, and the natural regulation of Phyllophaga populations by entomopathogenic bacteria was not important during the years of the survey. Only 3% of 16930 grubs examined were diagnosed to have been killed by bacteria The low incidences of bacterial diseases reported here are in agreement with observations made by all earlier workers, except for Northrup, 1914, for M nigrofasciens, that Phyllophaga spp , which spend the greatest part of their life in the soil, are relatively free of bacterial diseases. No bacterial disease ever gained epizootic dimensions in Phyllophaga spp. populations.

Duporte (1915) isolated M nigrofasciens from P anxia grubs near Montreal, Quebec Bacillus popilliae, P. aeruginosa and S. marcescens were here found for the first time from Phyllophaga hosts in Canada. To my knowledge, Phyllophaga spp are also new North American host records for the bacteria P aeruginosa and S marcescens

2 Infectivity Tests

Although rates of septicemia caused by test bacteria were relatively low, except for B. cereus in injection tests, test

Table 12 b Incidence of five entomopathogenic bacterial species in natural populations of Phyllophaga spp[a], southern Quebec, 1979 to 1981

Host stage	Number examined	Percentage infected with[b]				
		P a	S m	M n	B c	B p
Adult male	8895	-[c]	-	-	0 01	-
Adult female	2647	-	-	-	0 11	-
Egg	2525	0 04	-	-	-	-
First instar grub	1832	-	-	-	1 53	-
Second instar year 1	1652	-	0 42	0 24	2 78	1 63
Second instar year 2	1501	-	0 66	0 26	2 06	0 66
Third instar year 2	8174	-	0 05	0 01	1 40	0 43
Third instar year 3	3771	-	0 13	-	2.07	2 76
Prepupa	175	-	-	-	0 57	-
Pupa male	1289	-	-	-	0 08	-
Pupa female	897	-	-	-	0 22	-
Teneral adult male	73	-	-	-	1 37	-
Teneral adult female	37	-	-	-	-	-

[a] Adults, teneral adults and pupae identified as P anxia

[b] P. aeruginosa (P a), S marcescens (S m), M nigrofasciens (M n), B cereus (B c), B popilliae (B p)

[c] Not isolated (-) from this host stage

grubs were susceptible to the five bacterial species (Table 13) and Koch's postulates for proof of etiological agents of disease (Steinhaus, 1949) were fulfilled

The two sporeformers, B cereus and B. popilliae, were infectious when injected or force-fed to white grubs (Table 13). However, it was surprising that the only obligate pathogen tested, B. popilliae, found in Phyllophaga grubs, induced much lower rates of septicemia than the facultatively pathogenic (Bucher, 1960, Jacques, 1964) B cereus Bacillus popilliae is discussed in section 3 of this chapter Bacillus cereus (Bacillaceae) is a common soil saprophyte, closely related to B. thuringiensis, but does not produce a crystalline parasporal body (Milner, 1980) Many strains of B cereus are known, and some of these have been shown to be pathogenic for insects Steinhaus (1949), Tanada (1959), Bucher (1960, 1963), Jacques (1964), Milner (1980), and others considered B. cereus to be a facultative entomopathogen since it possesses some mechanism (exoenzymes) for damaging host tissue or for invading a tissue of a susceptible host, multiplies extracellularly in the hemocoel of insects and produces a lethal septicemia However, in contrast to obligate pathogens, B. cereus grows readily in vitro and attacks a wide range of insect species both in nature and experimentally. According to Heimpel and Angus (1963), the pathogenicity of B. cereus strains was found to be correlated with production of lecithinase C which has been implicated as a toxin Although Milner (1980) wrote that the pathogenicity of B cereus is low, Angus (1974) stated that there is quite a range of pathogenicity depending on the isolate.

Table 13 Susceptibility of third instar, second year grubs, Phyllophaga sp , to five entomogenous bacteria

Method of testing [a]	Bacterial species	Percentage of diseased or dead grubs [b] Total	Septicemia
Injection [c]	B. popilliae	13.3	13 3
	B cereus	83 3	80 0
	P aeruginosa	10 0	6 6
	S marcescens	53 3	46 6
	M nigrofasciens	56 6	50 0
Control [d]		7 5	0
Force feeding [c]	B popilliae	16 6	16 6
	B cereus	60 0	43 3
	P aeruginosa	3.3	0
	S marcescens	6 6	0
	M nigrofasciens	10 0	0
Control [e]		0	0

[a] Inoculum 0 1 ml of 2 to 3 x 10^4 spores (Bacillus spp) or cells (other bacteria) ml^{-1} of bacterial suspension

[b] After 29 days.

[c] Thirty grubs for each test bacterium

[d] Four times 10 grubs, see Materials and Methods for details

[e] Three times 10 grubs, see Materials and Methods for details

The results of my infectivity tests agreed with Angus (1974) since B. cereus isolated from Phyllophaga grubs caused from 43.3 to 80% infection in experimentally exposed grubs of that genus (Table 13) The results of the force-feeding tests with B. cereus (Table 13) also agreed with Heimpel and Angus (1963) who considered the bacterium as being capable of causing frank infection through a normal portal of entry (per os) as distinct from an experimental infection (intrahemocoelic inoculation) which artificially circumvents the insect's defenses.

Pseudomonas aeruginosa (Pseudomonadaceae), S. marcescens (Enterobacteriaceae) and M nigrofasciens (Micrococcaceae), which are all non-sporeformers, were not infectious when force-fed to white grubs, but induced septicemia when injected to grubs at relatively low doses (2000 to 3000 cells) (Table 13). Bucher (1960) defined potential pathogens as organisms that can multiply in the hemocoel of insects from small inocula and produce a fatal septicemia, but are not actively invasive and do not flourish or multiply significantly in the gut of insects. Therefore, and although Bucher (1960) considered P. aeruginosa as facultatively entomopathogenic, the strain isolated from a Phyllophaga egg (probably as a wound parasite) and assayed against grubs in the infectivity tests was considered as a weak potential pathogen The pathogenicity of P. aeruginosa injected to test grubs was very low (6.6% infection rate) (Table 13); moreover, the bacterium was never found occurring naturally in populations of Phyllophaga grubs in this survey (Tables 12a and 12b). These observations agreed with Jacques (1974) who wrote that P. aeruginosa infects insects usually only under extraordinary

circumstances Pseudomonas aeruginosa as entomopathogen has been reviewed by Steinhaus (1949), Bucher (1963); Angus (1965); Faust (1974); Milner (1980)

Micrococcus nigrofasciens caused septicemia in 50% of the test grubs when injected to the hemocoel (Table 13), the bacterium had also been isolated from naturally infected white grubs (Tables 12a and 12b) and was thus considered as a potential pathogen of Phyllophaga grubs There is very little published information on M nigrofasciens besides a study by Northrup (1914) and a brief review by Steinhaus (1949) Although M. nigrofasciens has been experimentally transmitted to cockroaches and the eastern tent caterpillar, its natural hosts appear to be the grubs of several scarabaeid beetles, including Phyllophaga spp (Northrup, 1914, Duporte, 1915, Petch and Hammond, 1925, Steinhaus, 1949) According to Northrup (1914), the route of transmission of M nigrofasciens is unusual in that it apparently occurs through the integument of June beetle grubs, and the likelihood of infection is increased when the integument is injured by parasitic insects, fungi or mechanical means. Petch and Hammond (1925) believed that the young maggots of the parasitic tachinid fly, Microphthalma phyllophagae Curran, introduced M. nigrofasciens into their grub hosts when becoming established. Davis (1919) considered M. nigrofasciens as a wound parasite of minor importance in the control of Phyllophaga grubs.

Serratia marcescens, a potential entomopathogen (Bucher, 1960, 1963), produced infection in 46 6% of the test grubs when injected directly to the hemocoel (Table 13). Death usually

occurred 4 to 6 days after injection. Many strains of this bacterium are known; most of them produce red-pigmented colonies *in vitro* and give a pinkish-red hue to the body of infected insects. This ubiquitous saprophytic microorganism has been isolated from over 50 species of natural insect hosts in the field, including the scarabs *Oryctes rhinoceros* (L.) (Steinhaus, 1959) and *Melolontha melolontha* (L.) (Hurpin and Vago, 1958). *Serratia marcescens* was said to be highly pathogenic for a wide range of insects when injected (Bucher, 1960), including for grubs of the Japanese beetle (Fleming, 1970). In nature, it has however limited invasive powers and it probably is not an important factor in the control of scarabaeid grubs (Fleming, 1970). *Serratia marcescens* as an entompathogen has been reviewed by Steinhaus (1959) and Bucher (1963). Safety problems preclude the use of *S. marcescens* and *P aeruginosa* in pest control because they cause septicemia, and respiratory, urinary and intestinal infections in mammals (Angus, 1965; Milner, 1980).

3. Bioassay of Two Isolates of *B. popilliae* (Bacillaceae)

Contrary to expectations, and although *B. popilliae* was found in natural populations of *Phyllophaga* spp. (Table 12a and 12b) and killed some individuals in the experimental infections reported ere, *Phyllophaga* grubs were relatively resistant to infection by both isolates of *B. popilliae* tested. This was true notwithstanding the age of the test grubs, the origin of the bacterial isolates, the spore concentrations of the inocula, or

the method of inoculation (Table 14). The results reported in Table 14 agreed with the results of infectivity tests with B. popilliae (Table 13), although infection rates obtained in the latter tests were slightly higher. For the present, I cannot explain these low rates of lethal infection (Table 14), especially for the local isolate of B. popilliae.

In New Zealand, Dumbleton (1946) found that grubs of Odontria zealandica White were naturally and experimentally attacked by isolates of B. popilliae from a local grub and the Japanese beetle but, in a series of bioassays similar to mine, lethal infection rates obtained were very low. Moreover, infectivity tests with the Odontria bacillus, using very heavy spore dosages by injection into the Japanese beetle and Phyllophaga hirticula, were negative. A great variability in susceptibility of grubs of five scarabaeid species to infection by several isolates of B. popilliae was also reported by Hurpin (1959). Some isolates induced high rates of mortality in a particular test species of beetle whereas other isolates were non-infectious, either per os or by intrahemocoelic injection. Dutky (1941) experimentally infected grubs of several species of Phyllophaga with the Japanese beetle isolate of B. popilliae by injection and by feeding in inoculated soil, but grubs of other species of Phyllophaga were not infected by feeding in inoculated soil (Dutky, 1963); the latter constitutes the natural route of infection for the milky disease bacteria (Beard, 1945; Milner, 1980). Injection of spores into third instar grubs of the European chafer, Rhizotrogus majalis (Razoumowsky), of several strains

Table 14. Pathogenicity of two isolates of Bacillus popilliae for Phyllophaga grubs: mortality response to various spore dosages and methods of exposure to spores.

Isolate and method of exposure [a]	Grub parameters: First instar Dosage	First instar %Mortality [b]	Second instar Dosage	Second instar %Mortality [b]	Third instar Dosage	Third instar % Mortality [b]
Local isolate						
A	- [c]	-	2	5.0	3	7.5
B	-	-	3	2.5	3	5.0
C	-	-	6	0	10	2.5
DOOM						
A	2	5.0	3	7.5	3	5.0
B	4	2.5	4	5.0	3	2.5
C	5	0	6	0	8	2.5
D	100	2.0	300	4.0	-	-

[a] A: Force-feeding; x 10^6 spores per grub. Test duration: 30 days.
B: Intrahemocoelic injection; x 10^6 spores per grub. Test duration : 30 days.
C: Topical application; x 10^6 spores per grub. Test duration: 30 days.
D: Soil inoculation; x 10^6 spores per 0.009 m^3 of soil. Test duration : 42 days.

[b] Methods A, B, and C: average for 2 replicates of 20 grubs per dosage; Method D: average for 10 replicates of 5 grubs per dosage; all mortalities corrected for control mortality by Abbott's formula.

[c] Not tested.

of B. popilliae (including a strain from P. anxia) produced septicemia in over 70% of the grubs; injections of spores of another anxia strain did not produce infection (Tashiro and Steinkraus, 1966) Tashiro et al. (1969) showed that Japanese beetle grubs were highly resistant to strains of B. popilliae and B. lentimorbus that were most virulent to the European chafer. From the above examples retrieved from the literature and from the results of my experiments (Tables 13 and 14), it is safe to list the following reasons as possible causes explaining the low rates of lethal infection observed in my tests: resistance or immunity of grubs to B. popilliae, low order of magnitude in the pathogenicity of the milky disease organisms tested, avirulence of the strains, or poor rate of germination of spores used in my bioassays resulting in poor growth of vegetative cells and thus in non-invasion of the hemocoel by the bacilli. Dutky (1963) wrote that selected strains of B. popilliae from the Japanese beetle were frequently much more virulent for another host insect than the indigenous strain recovered from that host. According to Dulmage and Rhodes (1971), milky disease spores from the haemolymph of insects germinate poorly and aged spores must be used to infect grubs as the germination rate of fresh spores is low. On the other hand, St. Julian et al. (1978) found that spores of B. popilliae stored at refrigerator temperature lost 84% of their viability after only one week and concluded that the low infectivity of the bacterium for the Japanese beetle in their feeding tests was primarily due to the inability of the bacterium to reproduce itself within the alimentary tract of

the grubs in sufficient numbers for the chance invasion into the haemolymph. In my tests, however, I used spores (for both isolates) which had been in storage for several months. Milner (1981) stated that prolonged storage induces dormancy in milky disease spores and that dormant spores usually may not germinate without their aging, as a water suspension, at room temperature for several months. I was not aware of this phenomenon at the time of the bioassays, and this fact might partially explain the low lethal infection rates obtained.

VII ENTOMOGENOUS FUNGI ASSOCIATED WITH PHYLLOPHAGA SPP IN SOUTHERN QUEBEC

A INTRODUCTION

The associations of fungi and insects cover the entire range of relationships, from mutualism to parasitism Among the entomogenous fungi (sensu Steinhaus, 1949), those which are distinctly entomopathogenic are found in virtually all major taxonomic groups of true fungi Mastigomycotina, Zygomycotina, Ascomycotina, Basidiomycotina and Deuteromycotina or Fungi Imperfecti Exclusive of the very large group of ectoparasitic Laboulbeniales, over 700 species in about 90 fungal genera are considered as entomopathogenic (Roberts and Humber, 1984), and the majority of the species involved are contained in the order Entomophthorales, the pyrenomycete genus Cordyceps, and the class Hyphomycetes. The muscardine diseases are caused by several fungi in the latter group Fungal pathogens of insects differ from most other entomopathogens in that they invade the host by direct penetration of the cuticle, rather than per os, and in that a saprophytic phase follows the parasitic phase in the life cycle of most species

Detailed reviews of the biology of fungal pathogens and parasites of insects were given by Steinhaus (1949), MacLeod (1963), Madelin (1963, 1966), McEwen (1963), Roberts and Yendol (1971), Amouriq (1973), Bell (1974), McCoy (1974),

Ferron (1975, 1978, 1981), Roberts and Humber (1981, 1984), Humber (1985)

Entomogenous fungi are known from practically all insect orders (Charles, 1941), although plant sucking insects and the Coleoptera seem to be more susceptible to fungal diseases than other groups of insects Despite the numerous reports of fungal diseases among species of the beetle family Scarabaeidae, few of them concerned members of the vast and widespread genus Phyllophaga

Most fungal infections of Phyllophaga spp were of the soil-inhabiting grub stages these larvae seemed to be especially subject to lethal infection by Cordyceps, Beauveria and Metarhizium species Beauveria bassiana (Balsamo) Vuillemin, the etiological agent of the white muscardine disease, killed Phyllophaga grubs in many parts of southern Illinois (Davis, 1919), Lim et al (1981a) found only one adult and four grubs of P anxia infected with B bassiana over three years of sampling populations of Phyllophaga in southern Quebec The green muscardine fungus, Metarhizium anisopliae var anisopliae (Metschnikoff) Sorokin, appears to have been isolated from individuals of natural populations of Phyllophaga spp more often than B bassiana. Davis (1919) stated that isolated cases of green muscardine infection were found on Phyllophaga grubs in various counties of Wisconsin, Illinois, Indiana and Michigan Hammond (1940) reported this fungus from grubs of P anxia, P drakii, P fusca, P futilis and P rugosa in eastern Canada, and Charles (1941) stated that it attacked the same Phyllophaga

spp in other parts of Canada Jarvis (1966) found that M. anisopliae killed three grubs of P anxia in the sandhills of Nebraska, and Veen (1968) listed several Phyllophaga spp as natural hosts for M anisopliae outside North America During a 3-year survey of P anxia in Quebec, Lim et al (1981a) collected only 10 grubs and 7 adults of P anxia infected with M anisopliae It appears, from this review of the literature, that muscardine diseases are rather uncommon in populations of Phyllophaga spp and that no such disease ever gained epizootic dimensions The uncommoness of muscardine diseases also applies for eastern Canadian situations, Hammond (1961) stated that references to fungi parasitic on white grubs in Ontario and Quebec were few

References to the endoparasitic pyrenomycetous fungi of the genus Cordyceps Fries Fries attacking North American white grubs, Phyllophaga spp , are more numerous than references to hyphomycetous fungi Within the genus Cordyceps, the "white grub fungus", Cordyceps ravenelii Berkeley and Curtis, appears to be a highly specific entomopathogen since its only known hosts are grubs of a few species of Phyllophaga beetles (Overholts, 1938) The first mention of C ravenelii as a white grub pathogen seems to have been made by Cist (1824) Cordyceps epizootics were reported among populations of P quercina Knoch grubs in Iowa (Walsh, 1867) and in Missouri, Ohio and Virginia (Riley, 1869) Although C ravenelii was rare in Kansas (Anonymous, 1880), the fungus occurred over large areas in Alabama (Riley, 1880) and in Iowa and Wisconsin (Lintner, 1888)

Riley (1880) concluded that the fungus distribution over a large extent of the United States was probably co-extensive with the distribution of the grubs that it attacked, P fusca and other Phyllophaga spp Piers (1889) reported Cordyceps infections among grubs of P quercina in Nova Scotia, and Forbes (1894) stated that Cordyceps infections were the only natural contagious diseases of local white grubs in Virginia, Iowa and several other North American states Cordyceps infections occurred among populations of Phyllophaga grubs in the state of New York (Pettit, 1895) and in the Canadian province of Ontario (Fletcher, 1902) According to Fletcher (1902), outbreaks of the fungus C ravenelii, although occurring rarely in Ontario, reduced the numbers of grubs of Phyllophaga spp rapidly and perceptibly where they occurred Although C ravenelii attacked grubs of Phyllophaga spp in Illinois (Davis, 1919), and of P anxia in eastern Ontario and southern Quebec (Hammond, 1931), the fungus was found only in rare instances However, Hammond (1961) noted a few fields of central Quebec where C. ravenelii was responsible for a spectacular reduction in numbers (up to 60%) of P anxia and P. fusca grubs Cordyceps ravenelii was a rather abundant entomopathogen in restricted localities of Pennsylvania (Overholts, 1938) Scheibner (1978) wrote that Cordyceps infections aided in controlling grubs of Phyllophaga spp in Kentucky

Lim et al (1981a) stated that non-identified species of Fusarium and Penicillium were among the natural enemies of P anxia in southern Quebec, but this observation must be taken

with moderate scepticism because of the fungal genera involved and because infectivity tests to demonstrate the pathogenicity of the fungi to P anxia were not performed

I report in this chapter the re-discovery of several species of entomopathogenic fungi found to regulate natural populations of Phyllophaga spp , principally P anxia, in southern Quebec I also discuss the results of infectivity and pathogenicity tests with some of the fungi

B MATERIALS AND METHODS

1 Recovery, Identity and Infectivity of Fungi

Individuals of all stages of Phyllophaga spp , including P anxia, were collected in southern Quebec from 1979 to 1981 and maintained in the laboratory as reported in Chapter III Dead and moribund specimens for which mycosis was suspected were retained for diagnosis Several techniques were used to isolate suspected entomopathogenic fungi Fungal inoculum was taken from specimens showing abundant mycelial growth and transferred to Sabouraud dextrose agar plus 1% yeast extract (SDAY) plates, bacterial growth was inhibited by addition of penicillin and streptomycin to the medium as recommended in the Difco Manual (1977) Insect specimens were also surface-sterilized by dipping them in 5% sodium hypoclorite or 70% ethanol for 5 minutes, then rinsed three times in sterile distilled water Some sterilized specimens were placed directly on SDAY medium, and others

were dissected and a portion of the insect tissues was transferred to SDAY medium If more than one fungal species was found growing on the agar plates, a portion of the mixed culture was suspended in sterile water containing Tween 80 (1 10,000), shaken for 5 minutes, and aliquots of the suspension were spread on SDAY medium Pure cultures were obtained by subsequent reinoculations of each of the isolated fungal colonies

Identification of the fungi isolated was accomplished with the assistance of Gilman (1957), Raper and Fennell (1965), Raper and Thom (1968), Weiser and Briggs (1971), Barnett and Hunter (1972), Poinar and Thomas (1978), Jong (1981), Samson (1981) Cultures on SDAY slants were sent to Dr D. M MacLeod, Forest Pest Management Institute, Sault Ste Marie, Ontario, for confirmation of my identifications and voucher specimens were deposited with that institution

A standardized procedure was adopted to test Koch's postulates for representatives of the genera Beauveria, Metarhizium, Fusarium, Penicillium and Aspergillus Infectivity tests against healthy third instar, second year white grubs were conducted in September 1979 (grubs collected at Pierreville, August 1979) and in August 1980 (grubs collected at Nicolet, August 1980) Inoculating materials were prepared from local isolates as described by Yendol and Paschke (1965). Ten white grubs were placed on an actively growing isolate of the genera mentioned above, for 3 hours Each treatment was replicated 3 times. Following inoculation, grubs were transferred individually to cardboard cups containing Toohey's (1977) artificial diet. The

cups were incubated for 21 days at 28°C, 95% RH and 16h photophase Reisolation of the test fungi was attempted with each dead or moribund grub An uninoculated grub group (10 insects) treated in a similar manner served as a control for each fungus tested

2 Bioassays of Beauveria bassiana and Metarhizium anisopliae

a Laboratory studies

Local isolates of B bassiana and M anisopliae for which Koch's postulates were fulfilled were further assayed against white grubs in pathogenicity tests

Four methods of inoculation were used with both fungal species in this study force-feeding, intrahemocoelic injection, topical application, and soil inoculation

To prepare the fungal inocula, conidia were aseptically harvested from the surface of 10 day old SDAY cultures of M anisopliae and 21 day old SDAY cultures of B bassiana The conidia were suspended in sterile water containing Tween 80 (1 10,000) and the final spore concentrations used in the tests were obtained after serial dilutions in the same diluent Spore counts were made using a Howard-Mold cell-counting chamber.

A pipetting gun fitted with a blunt sterilized glass tip was used to force-feed 0 1 ml of a given conidial suspension to the test grubs Each conidial suspension was fed to 20 grubs and each treatment was replicated 3 times. Following inoculation, grubs were transferred in individual cardboard cups supplemented with Toohey's (1977) artificial diet The cups were incubated

for 22 days at 28 °C, 90-95% RH and 16h photophase Reisolation of the test fungi was attempted with each dead or moribund grub. For each treatment, ten control grubs were force-fed 0 1 ml of sterile aqueous Tween 80 (1 10,000) Spore dosages tested were 3×10^7 spores ml^{-1} (B bassiana) and 4×10^7 spores ml^{-1} (M. anisopliae) against second instars (collected at Nicolet in August 1979) and third instar grubs (Pierreville, September 1979).

The experimental procedure for the topical application tests was as outlined for the force-feeding tests, but 0.1 ml of the inoculum was pipetted on the dorsum of 20 surface-sterilized grubs (3 replicates) instead of being introduced into the alimentary tract Spore dosages tested were 5×10^7 spores ml^{-1} of B bassiana and M anisopliae against second and third instar grubs of the same provenance as grubs used in the force-feeding tests Each spore dosage of both fungi was assayed 3 times against 20 grubs of each instar Control grubs (10 for each treatment) were inoculated 0 1 ml of sterile aqueous Tween 80 (1: 10,000).

Sterilized serological syringes fitted with a 27-gauge needle were used to inject 0.1 ml of a given conidial suspension behind the penultimate spiracle on the left abdominal side of surface-sterilized grubs Spore dosages tested were 5×10^7 spores ml^{-1} and 4×10^7 spores ml^{-1} of B. bassiana against second and third instar grubs, respectively, and 3×10^7 spores ml^{-1} and 4×10^7 spores ml^{-1} of M. anisopliae against second and third instar grubs, respectively. The provenance of the grubs was the same as that of grubs used in the force-feeding

tests. Each treatment was replicated 3 times (20 grubs per replicate) Control grubs (10 per treatment) were injected 0 1 ml of aqueous Tween 80 (1 10,000) Post-treatment conditions were as described for force-feeding tests

Wooden boxes (30 x 30 x 10 cm) were filled to the rim with 1 75 kg of a steam-sterilized mixture of sand and loam (1.1), seeded with 10 to 15 g of a mixture of red fescue and Kentucky bluegrass, and set aside for 7 to 10 days to allow for the germination of the grass seeds The soil in the boxes was then inoculated with conidial suspensions (in Tween 80, 1:10,000) of B. bassiana or M. anisopliae and 5 healthy grubs were placed on the soil surface of each box Grubs which did not bury themselves rapidly were replaced All boxes were incubated at 28 °C, 80-85% RH and 16h photophase The soil in the boxes was kept moist by regular watering with sterile tap water. The boxes were emptied after 34 days and data pertaining to grub mortality were recorded Spore dosages tested were. 6 x 10^7 spores of B bassiana per 1 75 kg of soil against second instar grubs collected at Nicolet in August 1980; 6 x 10^6 spores of M anisopliae per 1 75 kg of soil against second instar grubs (Nicolet, August 1980) and 5 x 10^6 spores of M. anisopliae per 1 75 kg of soil against third instars (Nicolet, August 1980) Each treatment was replicated 8 times. An uninoculated grub group (2 boxes containing 5 grubs of the proper instar) served as a control for each spore dosage of each fungus tested.

b. Field microplot studies on M. anisopliae

On the basis of the pathogenicity to Phyllophaga grubs

of M. anisopliae observed in the laboratory soil application tests, microplot trials took place on the Macdonald College seed farm in June and July 1982.

On June 14, 1982, twelve 2 by 1 m microplots containing a sandy loam soil (40% sand, 49% loam) were cleared of tall vegetation and thoroughly watered. Forty evenly spaced soil plugs (15 cm deep, 8 cm in diameter) were removed with a core sampler from each microplot. One healthy second instar, second year grub collected at Mirabel (June 4, 1982) was placed in each hole and covered with 1 to 2 cm of soil. Four treatments, in a complete random design, were then applied to the twelve microplots. Each hole in 3 microplots was inoculated with 4.275 g of talcum containing either 0 (control), 2.5×10^6, 6.3×10^6 or 19.0×10^6 spores of M. anisopliae g^{-1} of talcum (the inert carrier). Conidia used in these tests were produced on SDAY from a mycosed grub collected at Stanbridge East in July 1980. Holes were replugged and microplots were watered every second day for the duration of the experiment. The plots were dug out by hand on July 24, 1982 and all recovered white grubs were brought to the laboratory for diagnosis.

The amounts of spore powder used in the microplots were equivalent to applications of 214×10^6 (dosage of 2.5×10^6 spores g^{-1} of talcum), 538×10^6 (dosage of 6.3×10^6 spores g^{-1} of talcum) and 1625×10^6 (dosage of 19.0×10^6 spores g^{-1} of talcum) spores of M. anisopliae per m^2 of soil.

C. RESULTS AND DISCUSSION

1 Recovery, Identity and Infectivity of Fungi

The pyrenomycetous genus Cordyceps (Figure 21) was found from one second and one third instar grub in July 1979 (Table 15) Although infectivity tests were not performed with Cordyceps sp., the latter was considered as a natural enemy of grubs of Phyllophaga spp. on the basis of the published information pertaining to Cordyceps spp. (see introduction). Obviously, Cordyceps infections were rare in southern Quebec during the three years of the present survey. Although Cordyceps ravenelii was noted to be occasionally abundant and capable of decimating white grub populations in North America up to the 1910's, the occurrence of this fungus seems to have gradually become less frequent. Hammond (1961) wrote that Cordyceps-infected white grubs have been found only rarely in Ontario since 1945. Cordyceps infections in insects were reviewed by Mains (1958), McEwen (1963) and Humber (1985).

Five genera of hyphomycetous fungi, for which Koch's postulates for infectivity were fulfilled, were found, on many occasions, as naturally occurring pathogens of Phyllophaga spp. in southern Quebec, from 1979 to 1981 (Table 15). In order of decreasing rate of field-incidence, these entomogenous fungi were Metarhizium anisopliae var. anisopliae (Metschnikoff) Sorokin, Beauveria bassiana (Balsamo) Vuillemin, Fusarium sp. near F. solani (Martius) Appel and Wollenweber, and one species each within the genera Penicillium Link:Fries and Aspergillus

Figure 21. Cordyceps infection in third instar white grub, Phyllophaga sp. Napierville, Quebec, July 1979. 4X.

Table 15. Entomogenous fungi recovered from Phyllophaga spp.[a] in southern Quebec, 1979 to 1981.

Fungal species	Host stage[b] (number infected)	Collection site[c]	Date of collection[d]
Cordyceps sp.	L2,2 (1)	Napierville	July 79
	L3,2 (1)	Napierville	July 79
	L1 (9)	Nicolet	July 79
	L2,1 (2)	Nicolet	Aug. 79
	L2,2 (7)	Mirabel	June 79
	L3,2 (11)	Mirabel	Aug. 79
Aspergillus sp.	L3,2 (1)	Montebello	Aug. 79
	L3,2 (2)	Notre-Dame	Aug. 79
	L2,2 (2)	Nicolet	Aug. 80
	L3,2 (7)	Nicolet	Aug. 80
	L3,3 (4)	Stanbridge	June 80
	L2,1 (1)	Stanbridge	Aug. 81
	L3,3 (4)	Nicolet	July 81
	L2,1 (17)	Nicolet	Aug. 79
	L3,2 (20)	Pierreville	Sept. 79
	L3,2 (1)	Bellefeuille	July 79
	L2,2 (11)	Ste. Sophie	July 79
	L3,3 (1)	Quebec	Aug. 79
	L3,2 (2)	St.-Janvier	Aug. 79
Penicillium sp.	L2,2 (4)	Nicolet	Aug. 80
	L3,2 (1)	Nicolet	Aug. 80
	L3,3 (2)	Cowansville	Sept. 80
	L3,3 (1)	Mirabel	Sept. 80
	L3,3 (2)	Stanbridge	June 80
	L2,1 (4)	St. André	Aug. 81
	L2,1 (12)	Stanbridge	Aug. 81

Table 15 - Continued

Fungal species	Host stage[b] (number infected)	Collection site[c]	Date of collection[d]
	L2,1 (4)	Nicolet	July 79
	L2,2 (1)	Pierreville	Aug 79
	L3,2 (43)	Pierreville	Aug 79
	L2,2 (18)	Mirabel	June 79
	L3,2 (19)	Mirabel	July 79
	L2,2 (1)	Rigaud	July 79
Fusarium sp near	L3,2 (1)	Montebello	Aug 79
F. solani	L3,2 (5)	St -Janvier	Aug. 79
	L2,2 (6)	Nicolet	Aug. 80
	L3,2 (4)	Nicolet	Aug. 80
	L3,3 (2)	St Sébastien	Aug. 80
	L3,3 (7)	St -Janvier	June 80
	L2,1 (18)	Stanbridge	Aug 81
	L2,1 (3)	St André	Aug 81
	L2,1 (7)	Notre-Dame	Aug 81
	L2,1 (16)	Nicolet	July 79
	L2,1 (43)	Nicolet	Aug 79
	L3,2 (127)	Pierreville	Aug -Sept 79
	L3,2 (6)	Bellefeuille	July 79
	L2,2 (9)	Mirabel	June 79
	L3,2 (73)	Mirabel	Aug 79
Beauveria bassiana	TA male (1)	Ste Anne	Oct 79
	L2,2 (2)	Ste.Sophie	July 79
	L2,2 (4)	Montebello	Aug. 79.
	L3,2 (4)	Montebello	Aug. 79
	L3,2 (11)	St -Janvier	Aug. 79
	TA male (1)	Stanbridge	Aug. 80
	P male (7)	Stanbridge	Aug. 80
	P female (8)	Stanbridge	Aug. 81

Table 15 - Continued

Fungal species	Host stage[b] (number infected)	Collection site[c]	Date of collection[d]
	P male (1)	Nicolet	Aug 81
	L2,2(9),L3,2(11)	Nicolet	Aug 80
	L3,3 (2)	Coteau	Aug. 80
Beauveria	L3,3 (10)	St Clet	Aug 80
bassiana	L3,3 (31)	Stanbridge	June 80
	L2,1 (2)	Notre-Dame	Aug 81
	L3,3 (4)	Nicolet	July 81
	L1 (18)	Gd St. Esprit	July 79
	L2,1 (39)	Nicolet	Aug 79
	L3,2 (164)	Pierreville	Aug.-Nov. 79
	L2,2 (45)	Mirabel	June 79
	L3,2 (37)	Mirabel	July 79
	A male (38)	Nicolet	June 79
	A female (46)	Nicolet	June 79
	L3,2 (21)	Ste Sophie	July 79
	L2,2 (2)	Papineauville	Aug. 79
	A male (2)	East Angus	May 79
Metarhizium	L3,2 (17)	St-Janvier	Aug. 79
anisopliae	PP (1)	Stanbridge	Aug. 80
	P male (9)	Stanbridge	Aug 80
	P female (17)	Stanbridge	Aug. 80
	TA male (6)	Stanbridge	Aug. 80
	L2,2 (32)	Nicolet	Aug 80
	L3,2 (28)	Nicolet	Aug 80
	L3,3 (1)	Lacolle	Aug. 80
	L3,3 (17)	St. Clet	Aug. 80
	L3,3 (44)	Mirabel	Sept. 80
	L3,3 (78)	Stanbridge	June 80
	TA male (13)	Nicolet	Aug. 81

Table 15 - Continued

Fungal species	Host stage[b] (number infected)	Collection site[c]	Date of collection[d]
Metarhizium anisopliae	TA male (8)	Pincourt	Sept 81
	L1 (2)	St André	Aug 81
	L3,3 (2)	Nicolet	July 81

[a] Adults, teneral adults and pupae were P anxia

[b] L1 first instar grub, L2,1 second instar, first year, L2,2 second instar, second vear, L3,2 third instar, second year, L3,3 third instar, third year, PP prepupa, P pupa, A adult

[c] See Figure 10

[d] Date of collection of host, with or without symptoms of fungal infection, which may have developed in quarantine

Micheli Fries (Tables 15 and 16) Metarhizium anisopliae was isolated on more occasions and from more host stages than the other fungi (Tables 15 and 16) Beauveria bassiana, although frequently isolated, seemed to have been more specifically pathogenic to second and third instar grubs (Tables 15 and 16). Penicillium sp , Aspergillus sp and Fusarium sp near F. solani were isolated on only a few occasions and only from the grub stages of Phyllophaga spp (Tables 15 and 16) Fungal diseases had a rate of field-incidence of 7% among the 16,930 grubs and 4% among the 33,468 individuals of Phyllophaga spp examined during the survey (Table 16) Interestingly, over 26% of the teneral adults were killed by the two muscardine diseases and M anisopliae, which infected 24 5% of the teneral adults, was the single most important biotic regulator of a single stage of Phyllophaga spp (P anxia in this study) recorded in southern Quebec from 1979 to 1981 (Table 16) Although no epizootics due to fungal diseases was observed, the two truly entomopathogenic fungi, M anisopliae and B bassiana (Figures 22, 23, 24A, 24B), appeared to be chronically present and endemic in populations of Phyllophaga spp. throughout southern Quebec (Table 15)

The results of the laboratory infectivity tests are summarized in Table 17 Third instar, second year white grubs were moderately to highly susceptible to local isolates of the five entomogenous species tested Not surprisingly, rates of lethal infection were higher with the two truly pathogenic fungi, M anisopliae and B bassiana, than with the other fungi The muscardine fungi are discussed in the next section of

Table 16 Incidence of six entomogenous fungal species in natural populations of Phyllophaga spp [a], southern Quebec, 1979 to 1981

Host stage	Number examined	Percentage mycosis[b]					
		C	A	P	F.	B.b	M.a.
Adult male	8895	-[c]	-	-	-	-	0.43
Adult female	2647	-	-	-	-	-	1.74
Egg	2525	-	-	-	-	-	-
First instar grub	1832	-	0 49	-	-	-	1.09
Second instar year 1	1652	-	0 18	1 99	1.93	3.69	2 36
Second instar year 2	1501	0 06	0 59	0 99	1 73	1.59	5.26
Third instar year 2	8174	0 01	0 25	0 29	0 88	2.83	3.22
Third instar year 3	3771	-	0 21	0 16	0 23	1.24	3.76
Prepupa	175	-	-	-	-	-	0.57
Pupa male	1289	-	-	-	-	0.62	0.69
Pupa female	897	-	-	-	-	0.89	1.89
Teneral adult male	73	-	-	-	-	2.73	36.98
Teneral adult female	37	-	-	-	-	-	-

[a] Adults and teneral adults identified as P [illegible]ia

[b] Cordyceps sp. (C), Aspergillus sp (A); Penicillium sp. (P), Fusarium sp near F solani (F), Beauveria bassiana (B.b), Metarhizium anisopliae (M a)

[c] Not isolated (-) from this host stage

Table 17 Susceptibility of third instar, second year grubs, Phyllophaga spp., to five entomopenous fungi: mortality response following 3 hours of exposure to actively growing cultures of the fungi [a]

Fungal species[b]	Number of grubs exposed[c]	Number of dead grubs	
		Total	Mycosis (%)
M. anisopliae	30	27	26 (86 6)
B bassiana	30	23	23 (76 6)
Fusarium sp near F solani	30	17	14 (46 6)
Aspergillus sp	30	19	15 (50 0)
Penicillium sp	30	10	10 (33 3)
Control	10[c]	2	0 (0)

[a] Tests terminated after 21 days of incubation at 28° C and 95% RH.

[b] Local isolates

[c] Test grubs collected at Pierreville, August 1979, except for the Aspergillus sp test (grubs collected at Nicolet, August 1980) Ten control grubs were included in the Aspergillus sp test, no mortality was observed in the control group

Figure 22 Field-collected white grub, Phyllophaga sp., killed by Beauveria bassiana, the white muscardine entomopathogenic fungus 1.5X.

Figure 23. Mummified white grub, Phyllophaga sp., killed by the green muscardine fungus, Metarhizium anisopliae. 2X.

Figure 24 A. *Metarhizium anisopliae* infection in a field-collected pupa, *Phyllophaga* sp.

Figure 24 B. *Beauveria bassiana* infection in a field-collected pupa, *Phyllophaga* sp.


1 cm

this chapter. Fusarium, Penicillium and Aspergillus spp. are all ubiquitous saprophytic or phytopathogenic organisms but, under exceptional circumstances, some species have been said to be able to infect and kill susceptible host insects, usually acting as wound parasites or opportunists, eg. stressed hosts in environments such as bee hives, wood tunnels or insect rearing chambers which are suitable for conidial germination and fungal growth. Generally, many if not most normally non-pathogenic species of fungi may induce infection in an insect host once the obstacle of the insect exocuticle has been overcome. In the infectivity tests reported here, however, insects were healthy and their cuticle was, as far as could be ascertained, not wounded; no particular stress was placed upon the test grubs. It thus appeared that under the experimental conditions, Fusarium, Penicillium and Aspergillus spp. were actively capable of infecting some of the test grubs and that these three fungi (which were isolated from field-collected Phyllophaga grubs) were likely potential weak entomopathogens. Lim (1979) discussed Fusarium and Penicillium spp. in their relationships to P. anxia and other insects. Steinhaus (1949) and Samson (1981) considered certain species in the genera Fusarium, Aspergillus and Penicillium as containing insect pathogens, although Roberts and Humber (1981, 1984) did not include the genus Penicillium in their lists of entomogenous fungi. Teetor-Barsch and Roberts (1983), and Claydon and Grove (1984) reviewed the entomogenous Fusarium species; the former authors concluded that, among the many Fusarium spp. associated

with insects, a large number are potentially entomopathogenic, although most species are only weakly pathogenic and most frequently mutualists.

2. Bioassays of B. bassiana and M. anisopliae

a. Laboratory studies

Regardless of the instar tested, white grubs were relative resistant to infection by B. bassiana and M. anisopliae in per os bioassays (Table 18). This was not surprising and is well documented; the per os route is a non-natural route of invasion for entomopathogenic fungi. If the infective unit (conidium) enters the alimentary tract of an insect, its germination is usually inhibited by the gut conditions or simply, the conidium, is ejected with the feces (Steinhaus, 1949; Madelin, 1963; Veen, 1968).

Intrahemocoelic injection of conidial suspensions of B. bassiana did not kill white grubs; M. anisopliae was only moderately pathogenic, killing 20 and 42% of second and third instar grubs, respectively (Table 18). The reasons behind these apparent low pathogenicities were not clear, but it is likely that some defense mechanism was involved, such as encapsulation or lysis of the conidia before their germination and subsequent mycelial colonization of grub's tissues.

Topical application of conidial suspensions induced green muscardine mycosis in 64 and 52% of second and third instar grubs, respectively, but rates of infection were lower with B. bassiana (Table 18). Both fungi, however, mycotized a lower percentage of the test grubs than in the infectivity tests (Table 17) even

Table 18. Pathogenicity of two entomogenous fungi for Phyllophaga grubs: mortality response to various spore dosages and methods of exposure.

Fungus species and method of exposure[a]	Grub parameters			
	Second instar		Third instar	
	Dosage	%Mortality by mycosis[b]	Dosage	%Mortality by mycosis
M. anisopliae				
A	4	2	4	18
B	3	20	4	42
C	5	64	5	52
D	6	82	5	97
B. bassiana				
A	3	0	3	4
B	5	3	4	2
C	5	39	5	28
D	60	24	-[c]	-

[a] A: Force feeding; x 10^6 spores per grub. Test duration: 22 days.
B: Intrahemocoelic injection; x 10^6 spores per grub. Test duration: 22 days.
C: Topical application: x 10^6 spores per grub. Test duration: 22 days.
D: Soil inoculation; x 10^6 spores per 1.75 kg of soil. Test duration: 34 days.

[b] Methods A, B, and C: average for 3 replicates of 20 grubs per dosage; method D: average for 8 replicates of 5 grubs per dosage; all mortalities corrected for control mortality by Abbott's formula; percentages rounded up to the next unit.

[c] (-) not tested.

though the same fungal isolates were used in both cases. The quantity of inoculum certainly affected the incidence of mycoses produced in the two integumental contamination tests. Although spore dosages in the infectivity tests were not determined, they were obviously much higher than the spore dosages assayed in the topical application tests (Table 18) Also, the ages of the test grubs were different in both experiments; this fact, which is well-documented for hyphomycetous fungi, affected the rates of infection observed

Soil application of relatively low spore dosages of M. anisopliae resulted in 82 and 97% control of second and third instar grubs, but a 10-fold higher spore dosage of B. bassiana achieved only 24% control of second instars (Table 18). These significant differences in pathogenicity between the two muscardine fungi, in tests duplicating the natural habitat of white grubs, mirrored the field observations (Tables 15 and 16) that M anisopliae was more commonly encountered than B. bassiana among Phyllophaga grubs in southern Quebec. Lim (1979) also observed from 1975 to 1977 that M anisopliae was much more common than B. bassiana among populations of P anxia in southern Quebec It can be concluded that grubs of Phyllophaga spp are more susceptible to infection by M anisopliae than by B. bassiana or, at least, that local isolates of the latter fungus are either less virulent (or have more fastidious growth requirements) to Phyllophaga grubs than isolates of M. anisopliae. Studies on the pathogenicity, host specificity and practical use for controlling some scarabaeid pests have been carried with M. anisopliae and B. bassiana by Latch, 1965, 1976;

Hurpin and Robert, 1972; Gruner, 1973b; Ferron et al., 1975; Fargues, 1976; Latch and Fallon, 1976; Coles and Pinnock, 1982.

b. Field microplot studies on M. anisopliae

White grubs, Phyllophaga spp., were susceptible to the infectious activity of M. anisopliae (Figure 25) applied to the soil of field microplots under natural environmental conditions, and a clear dose-response was evident (Table 19). The LD_{50} value for M. anisopliae was graphically estimated to be 10.2×10^6 spores per m^2 of soil or 10.2×10^{11} spores per ha of soil and the LD_{90} value was estimated at 2.5×10^9 spores per m^2 of soil or 2.5×10^{13} spores per ha. The results of this experiment agreed with results for the laboratory pathogenicity tests using soil application of conidia of M. anisopliae against second and third instar grubs (Table 18). It was concluded that M. anisopliae (local isolate Ma 104, deposited with the fungal collection of the Forest Pest Management Institute, Sault Ste Marie) is a highly potent potential suppressant of white grubs, Phyllophaga spp , in southern Quebec.

Table 19 Field evaluation of M anisopliae[a] against second instar white grubs, Phyllophaga sp

Spore dosage x 10^6 per m^2 of soil	Grub parameters: Total grubs added to 3 microplots	Total grubs recovered after 40 days	Dead grubs with green muscardine symptoms	Percentage mortality due to mycosis[b]
214	120	108	81	64 42
538	120	117	100	80 12
1625	120	101	92	91 63
Control	120	113	0	4 42 (Total)

[a] Isolated from a field-mycotized white grub

[b] After Abbott's correction

Figure 25 Second instar grub, Phyllophaga sp., recovered 40 days after inoculation of a microplot with Metarhizium anisopliae (214 x 10^6 spores per m^2). Notice the heavy fungal sporulation on the anterior half of the cadaver and bacterial decomposition of the posterior half.

VIII MIKOLETZKYA AERIVORA (COBB), A DIPLOGASTERID NEMATODE PARASITIC IN GRUB AND PUPAL STAGES OF PHYLLOPHAGA BEETLES IN SOUTHERN QUEBEC

A INTRODUCTION

Nematodes (Nematoda) are best known for their noxious nature in animals and cultivated plants and for the major agricultural and economic problems that they cause However, many species are beneficial in that they infect, debilitate and kill pest insects. nematoses are diseases caused by nematodes and Nematomorpha or gordian worms Many other species of nematodes associated with insects are not detrimental to their hosts, the coexistence being incidental, phoretic or commensalistic in nature Although the insect-associated nematodes belong to the Metazoa and, as such, should be regarded as other macroparasites, it is common policy to place them with the microbial pathogens in the microbiology and pathology of insects and to consider the definite parasitic forms as microbial agents of pest population suppression This is done more by default than by logic (Welch, 1963), one of the main reasons being the great similarity of the morbid processes and techniques of observation In this chapter, I have decided to follow the general trend and this justifies the use of the term "infection' rather than "infestation", although the latter would be more appropriate when referring to insect-parasitic nematodes (Steinhaus and Martignoni,1970)

Published information on insect-associated nematodes has proliferated since van Zwaluwenberg's (1928) synopsis of some 420 published insect-nematode relationships to 1926 Comprehensive treatises, monographs and reviews on insect-nematode taxonomy, life history, biology, natural occurrence, culture and pathology were prepared by Sweetman (1936), Bovien (1937), Christie (1941), Filipjev and Schuurmans Stekhoven (1941), LaRivers (1949), Steinhaus (1949), Rühm (1956), Welch (1963, 1965), Lipa (1967), Massey (1974), Nickle (1974, 1984), Shephard (1974), Poinar (1972, 1975, 1977) The use of insect-nematodes in pest suppression programs was adequately reviewed by Welch (1958, 1962), Briand and Welch (1963), Poinar (1971, 1979), Webster (1972), Gordon and Webster (1974), Finney (1981), Nickle (1981), Hominick and Tingley (1984)

There is a greater frequency of nematode occurrence in those insect orders that are associated with aquatic or at least moist habitats Among the Coleoptera, the greater frequency of such occurrence is in the soil-inhabiting Scarabaeidae and wood-inhabiting Scolytidae (Welch, 1962) The host list of insect-nematode associations compiled by Poinar (1975) included some 500 entries (out of 3100) pertaining to nematode citations for scarab beetles Surprisingly, the large and noxious genus Phyllophaga, whose species mostly have a very long subterranean life cycle (eg three years), is cited only a few times in natural association with nematodes Criddle (1918) reported non- identified Mermithidae from grubs of Phyllophaga anxia, P drakii, P nitida and P rugosa in Manitoba In Wisconsin, Davis (1919)

found the diplogasterid, Mikoletzkya aerivora (Cobb), a non-identified mermithid and possibly a Cephalobus sp. (Cephalobidae) in grubs of Phyllophaga spp. Mermis nigrescens Duj. was cited as a parasite of a Phyllophaga sp. in Vermont by Sweetman (1936), and Chamberlin (1944) reported a Diplogaster sp. and a Neoaplectana sp as associates of Phyllophaga spp in Wisconsin. Toohey (1977) wrote that up to 50 percent of grubs of P anxia collected in southern Quebec and kept in the laboratory were infected with nematodes Finally, Lim (1979) was the first to isolate M. aerivora in Canada from grubs of P anxia collected in four localities of southern Quebec.

Experimental infection with Neoaplectana glaseri Steiner was achieved against grubs of Phyllophaga spp. by Glaser et al. (1940) Chamberlin (1944) conducted field trials of Neoaplectana spp. including N glaseri against white grubs of Phyllophaga spp. in Wisconsin, but infection was not obtained. Poinar (1978) was more successful in field trials with N. glaseri against grubs of Phyllophaga spp. in the midwest U.S

There have been numerous reports in the literature of association between representatives of the large family Diplogasteridae and insects. The majority of the associations in which definite or semi-parasitism and pathogenesis resulted from diplogasterid nematodes' activity have received little attention besides the discussions by Bovien (1937), Weingärtmer (1955); Poinar (1969, 1972, 1979), Massey (1974).

Mikoletzkya aerivora, one of the few entomopathogenic nematodes in the family Diplogasteridae, was first described by

Cobb in Merrill and Ford (1916) from the head of the termite, Leucotermes lucifugus Rossi, in Kansas; Merrill and Ford (1916) noticed that when infections were heavy, the termites became sluggish and often died Cobb, in Merrill and Ford (1916), observed M. aerivora feeding on grasshopper eggs in Kansas Banks and Snyder (1920) found juveniles of M. aerivora in the head of live termites, Reticulitermes flavipes (Kollar), and adult nematodes in sick and dead insects in several southern states. Pupae and larvae of the noctuid, Helicoverpa zea (Boddie), were found infected with M. aerivora in Kansas (Winburn and Painter, 1932). Swain (1945) reported that M. aerivora was found in widespread association within live larvae of the white-fringed beetles, Pantomorus spp., in southeastern U S.A., the association was tentatively assumed to be primarily of a saprophagous nature although definite fatal parasitism was observed on occasion. M. aerivora was reported to have killed grubs of Phyllophaga spp. in Wisconsin (Davis, 1919) and grubs of P. anxia in Quebec (Lim, 1979)

In my study of biotic regulators of Phyllophaga spp. in southern Quebec, M. aerivora was found on many occasions in live, moribund and dead grubs and in live pupae I report these findings in this chapter and present the results of nematode maintenance and extraction in the laboratory, some characteristics of its behavior, and laboratory and field plot pathogenicity tests against Phyllophaga grubs.

B. MATERIALS AND METHODS

1. Recovery of Nematodes

The various stages of Phyllophaga were collected as described earlier. Field-collected moribund and non-putrefied dead grubs having a greyish appearance, a symptom of nematosis (Figure 26), were processed for nematode recovery according to the scheme reported by Lim (1979). The same procedure was followed for grubs and pupae which developed symptoms of nematosis in the laboratory. Recovery of nematodes from field-collected putrid dead grubs was immediate since the cadavers were usually covered with crawling nematodes. Detection of nematode infections within Phyllophaga individuals collected live and which died under quarantine without symptoms of disease or which were sacrificed when still alive at the end of the quarantine, was possible only by dissection. Diagnosis of nematosis based on evidence of color alteration of the body was only possible with the grub stage of Phyllophaga.

Several adult nematodes of both sexes were retained for identification purposes at each occasion of recovery. After fixation for 24h in FAA (120 ml distilled water, 60 ml 95% ethanol, 18 ml 40% formaldehyde solution, 3 ml glacial acetic acid, 2 ml glycerin), nematodes were processed to pure glycerin and mounted in glycerin or lactophenol-cotton blue (Massey, 1974).

Figure 26. Pathogenesis of M aerivora in a white grub, Phyllophaga sp. A: field-collected, moribund grub, 2 days before death; notice the symptomatic greyish body color B: the same grub 10h after death, notice the flaccid and shrunken cadaver, and the absence of external nematodes. C: the same grub, 13 days after death; nematodes have multiplied greatly. All photographs approx. 4X.

2. Behavior Studies

A series of laboratory experiments was begun to examine the behavioral response of M. aerivora when exposed to the stimulus of Phyllophaga grubs. Several two-compartmented plastic petri dishes (100x15 mm; divider height: 7.5 mm) were prepared for this purpose. A strip (100x15 mm) of transparent plastic sheet was glued to one side of the divider to increase its height to 15 mm. Five holes were drilled in the divider with a number 3 insect pin heated over a flame. Holes were drilled 10 mm apart at a height of 5 mm from the bottom of the dish. The modified petri dishes were sterilized by dipping for 5 minutes in 70% ethanol, then rinsed twice in sterile distilled water, assembled, and left overnight under an ultra-violet lamp. Following sterilization, half-strength nutrient agar was poured aseptically into the bottom half of the dishes up to the level of the holes of the divider, taking care not to plug them.

A 0.2 ml aliquot (450 to 500 nematodes of mixed stages) of nematodes harvested from dead grubs was pipetted into one of the compartment of each of 60 dishes which were then treated as follows. (1) Ten dishes did not receive further treatment since they were assigned to be controls. (2) One healthy, surface- sterilized third instar grub was placed in the nematode-free compartment of ten dishes. (3) A 0.1 ml aliquot of haemolymph aseptically secured from healthy third instar grubs was pipetted in the nematode-free compartment of ten dishes, diluted with 1 ml of sterile distilled water and spread over the agar by gentle rotation. (4) One healthy third instar grub, killed by freezing

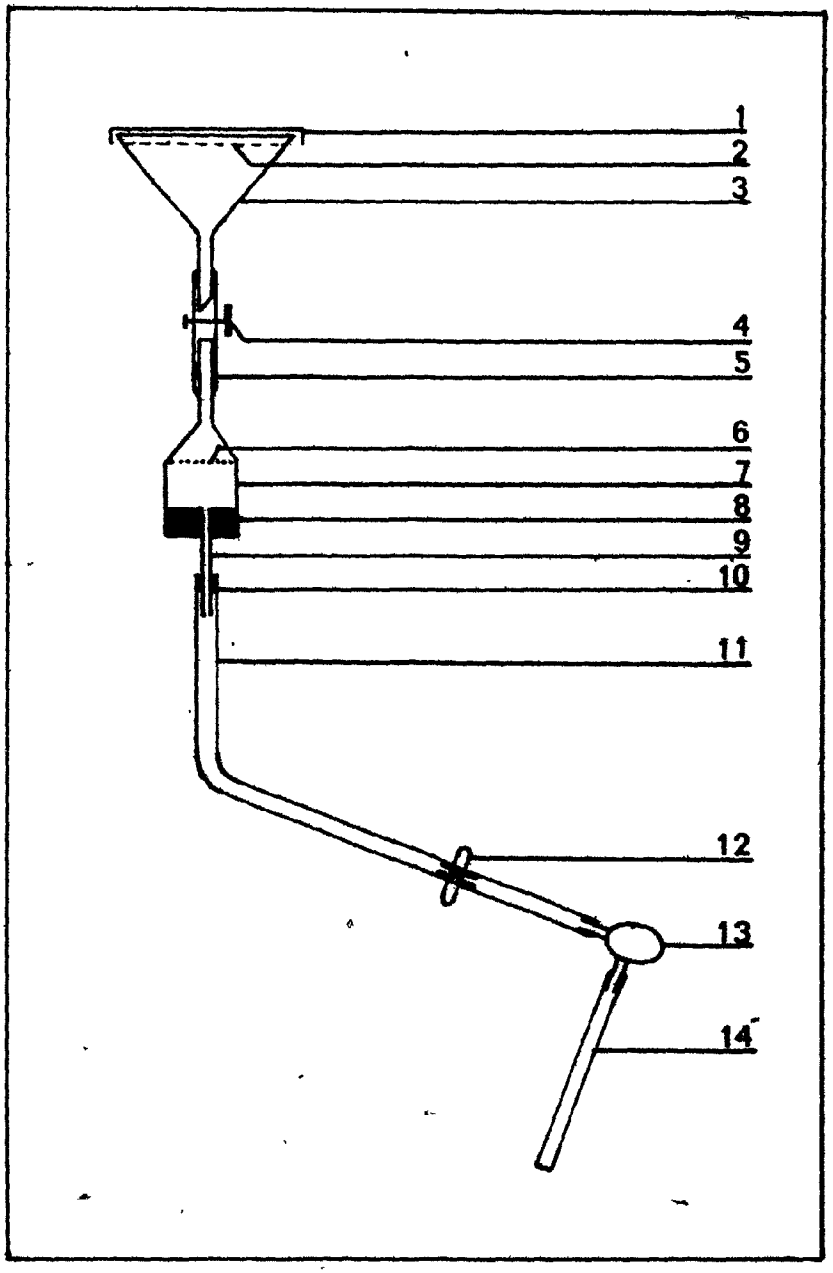
and left to decay for one week was placed in the nematode-free compartment of each of ten dishes. (5) The nematode-free compartment of each of ten dishes received one surface-sterilized healthy third instar grub whose anus and mouthparts were plugged by dipping in lukewarm melted histological grade paraffin. (6) Five dishes were "inoculated" with 1 ml of melted paraffin and five others with 1.1 ml of sterile distilled water, always in the nematode-free compartment. The time of introduction of non-nematode material in the dishes was recorded; all dishes were kept at room temperature (21±1°C) and in full artificial light; each dish was observed under the binocular microscope (40X) seven times, at hourly intervals, after the start of the experiments and once more 24h later (31h elapsed time). Numbers of nematodes found in each half of the experimental dishes were recorded at each infection. The experiment was repeated twice, at weekly intervals, in July 1980.

3. Techniques of Extraction

In the fall of 1979, three extraction methods were tested for their efficiency in yielding the maximum number of live M. aerivora individuals per infected grub. The extraction methods used were the classical funnel proposed by Baermann (1917), the apparatus designed by Carne and Reed (1964) for harvesting infective stage nematodes emerging from their insect hosts, and a modification of the Baermann apparatus that is shown in Figure 27. The modification is simple and can be made rapidly with materials found in most research laboratories. Its principle is based on the oxygen requirements of nematodes for survival (Lee

Figure 27. Modified Baermann funnel for harvesting M. aerivora from its insect host (not to scale).

1. Top of petri dish
2. Nylon screening supporting white grub on filter paper
3. 15 cm glass funnel
4. Hoffman open side pinchcock
5. Rubber tubing
6. Grid of 0.45 µm millipore filter with accumulated nematode harvest
7. 0.45 µm millipore filter
8. rubber stopper
9. 100 µl micropipette
10. Rubber stopper
11. Rubber tubing
12. Two-way valve
13. Bench compressed air valve
14. Free rubber tubing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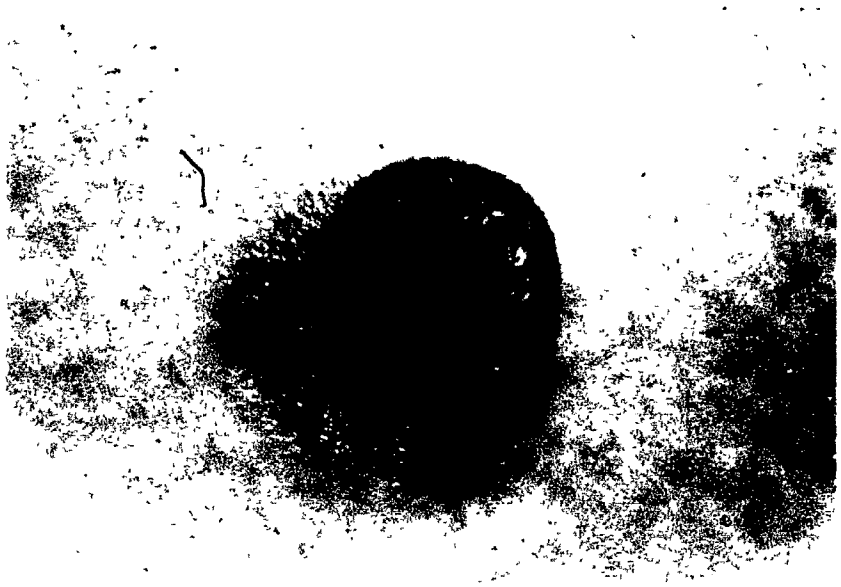
and Atkinson, 1976) Oxygenation of the water in my apparatus is achieved as follows the bench compressed air valve is opened to a minimum, the two-way valve is opened to a maximum, compressed air is pushed through the micropipette into the water held in the "upper chamber" millipore filter, the glass funnel and the tubing connection between them, creating a strong turbulence By trial and error, using the two-way valve, the air flow can be regulated in such a manner that tiny air bubbles are continuously forced into the water with minimal disturbance, allowing both the oxygenation of water and the accumulation of nematodes in the "upper chamber" of the millipore filter Nematodes are collected simply by shutting off the air flow with the two-way valve and allowing a standing time of 1-1 5 h for the nematodes to settle in the millipore filter The pinchcock is closed and the millipore filter is pulled from the rubber tubing, the rubber stopper is pulled from the millipore filter which is then inverted over a collecting container A gentle flow of air from the free rubber tubing is blown over the lower surface of the grid A known volume of nematode suspension is obtained if desired through prior calibration of the "upper chamber" and end tubing of the millipore filter Subsequent washing of the millipore filter is not needed

The experimental procedures to test the efficiency of the extraction methods were standardized Third instar grubs collected at Pierreville on August 30, 1979 were quarantined individually for seven days Healthy grubs were then selected, surface sterilized, placed on half-strength nutrient agar in a

petri dish and inoculated on the mouthparts with a 2 ml suspension (4500 to 5000 individuals) of freshly-harvested juvenile nematodes, less than 5% of adults were present in the inocula, no food was provided and the dishes were left at room temperature Ten days later, the grubs were removed from the dishes, decapitated and the body was placed on a moist filter paper in a petri dish for 2 to 3h (Figure 28) This allowed confirmation of the presence of internal nematodes, which were flushed out with the oozing haemolymph The filter paper and contents were then placed on the nylon screening of extraction apparatus The water level in the glass funnel of the apparatus was adjusted by pipette rinsing the surface of the agar A desk lamp (60 W incandescent bulb) was positioned 20 cm above the collecting units

Estimates of nematode densities in the water suspensions were made 8, 24 and 48h after the start of the experiments At each inspection, all the water from the extraction apparatus was transferred to a 250 ml beaker, nematodes were flushed as described earlier in the case of the modified Baermann funnel. One ml of each nematode suspension was pipetted into 9 ml of dechlorinated tap water in a test tube and 0 1 ml aliquots were taken from the diluted suspension and placed on 10 microscope slides The number of nematodes was counted under a binocular dissecting microscope and total number and number of dead nematodes were recorded Counts were averaged, allowing to determine the nematode density in the water of the extraction apparatus Fresh dechlorinated water was added to the funnels after the 8 and 24h extractions After the 48h extraction, grub cadavers were opened

Figure 28 Juveniles of M aerivora flushed out with the haemolymph of a laboratory-infected white grub 10 days after inoculation Photograph taken 1h after decapitation of the moribund but live grub 2 5X



lengthwise and, while held with dissecting forceps, were shaken for 2 minutes in 20 ml of tap water to dislodge any nematodes that were trapped in the grubs' remnants Counts were also made for this final harvest Each extraction method was tested 7 times

4 Maintenance of Nematodes

Maintenance of M aerívora in the laboratory was achieved by storage in water and in agar culture

Storage in water, at 5°C and complete darkness was conducted as described by Lim (1979) The only modification was the addition of 20 units of penicillin and 40 µg of streptomycin per ml of sterile water The contents of the storage erlenmyer flasks were oxygenated aseptically by agitation with a sterile glass rod at monthly intervals

Agar culture of M aerivora was made on half-strength nutrient agar in petri dishes The agar cultures were subcultured, approximately every 10 to 15 days, by transferring a small piece of the original culture medium with its nematodes to a fresh nutrient agar plate Two subcultures were made each time One was left as such and the other was supplemented with a healthy grub killed by decapitation Maintenance took place under fluctuating laboratory ambient conditions

5 Infectivity Tests

Experimental infections and pathogenicity studies with M aerivora were performed and Koch's postulates for nematodes

(Poinar, 1975) were fulfilled in the laboratory and in the field using three techniques, topical application, infection chamber and soil inoculation in microplots.

Topical application tests were conducted in 1979 and 1980 and consisted of 2 replicates of 20 grubs per replicate per treatment and test Healthy first instar and second instar grubs collected at Nicolet and Mirabel respectively were used in 1979, and third instars collected at Nicolet were used in 1980. A 0.5 ml nematode suspension of one inoculum level was applied topically to the mouthparts of the test grubs, 0.5 ml sterile distilled water was applied to control grubs. After treatment, the grubs were held individually at $22 \pm 1°C$, 40-50% RH, 16h photophase in cardboard cups supplied with a lettuce leaf and Toohey's (1977) artificial diet The grubs were monitored for symptoms of disease and mortality for five weeks Apparently non-infected grubs were then killed and incubated on half-strength nutrient agar (laboratory ambient conditions) for possible emergence of nematodes.

Infection chamber tests were performed with third instar, third year grubs collected at Stanbridge East on July 9, 1980. Cardboard coffee cups were sterilized with 70% alcohol and filled to a height of 6 cm with steam-sterilized sandy loam (1:1). One surface-sterilized healthy grub was introduced in each of 20 cups, gently pushed into the soil and covered with a layer of sterile soil. Ten ml of sterile tap water were spread over the surface of the soil and 5 ml of a nematode suspension (estimated density: 3.1×10^3 nematodes ml^{-1}) were added to the experimental unit. Only water was added to cups containing the control grubs. Cups

were stoppered with a double layer of parafilm for the duration of the experiment and held at 22 ± 1°C and 16h photophase. Grubs were not fed for 5 days and then a chunk of sterile artificial diet (Toohey, 1977) was aseptically pushed below the surface of the soil. Five ml of sterile tap water were added to the infection chambers at weekly intervals. The experiment was repeated after one week. The tests were terminated after 32 days when test insects were examined for the internal presence of nematodes

Microplot trials took place on the Macdonald College seed farm from September 9 to October 6, 1982. Three 2 by 1 m microplots containing the local clay-loam soil were cleared of tall vegetation and thoroughly watered. Forty evenly spaced soil plugs (15 cm deep, 8 cm in diameter) were removed with a core sampler from each microplot. One healthy third instar, second year white grub, collected at Mirabel, was introduced into each hole and covered with 1 to 2 cm of soil. One of three treatments was then applied to each hole in one microplot: addition of (1) 20 ml of tap water, (2) 20 ml of nematode suspension (estimated density 2.8×10^3 nematodes ml^{-1}), (3) 20 ml of nematode suspension (estimated density 0.7×10^3 nematodes ml^{-1}). Holes were replugged and microplots were watered every second day until October 6 when they were dug out by hand; recovered grubs were brought to the laboratory and examined for presence of M. aerivora.

An important remark must be made here: for all infectivity tests reported in this chapter, a grub, treated or not, was

considered to have been attacked by M. aerivora on the absolute condition that the test nematode, whatever its stage, had to be present internally in dead, moribund or surviving hosts. Situations in which M. aerivora was observed externally only were not considered positive cases of infection, although the infection process might indeed have been at the initiation stage.

C. RESULTS AND DISCUSSION

1. Recovery and Identity of Nematodes and Allied Organisms

At least three species of nematodes were recovered from Phyllophaga individuals in the survey of southern Quebec.

Rhabditid nematodes were isolated from two second instar, second year and one third instar, second year moribund grubs collected on a sod farm at Mirabel, July 2, 1979. Aphelenchoidids were found infecting one third instar, second year moribund grub on July 16, 1979, and one third instar, third year grub on September 2, 1980, both at Mirabel. The characteristics listed by Goodey (1963) were used to identify the nematodes to the family level. Voucher specimens were sent to Dr. G.O. Poinar, Jr., Division of Entomology and Parasitology, University of California at Berkeley, who confirmed my identifications. Although several species of Rhabditidae and Aphelenchoididae are known insect parasites (Poinar, 1979), no infectivity tests were made as all the specimens were killed for diagnostic purposes. These recoveries

are reported here for the record, as is the occurrence of gordian worms (Nematomorpha) associated with dead Phyllophaga individuals in an old field at Stanbridge East, in July and August 1980. Gordian worms were found on seven occasions in the earthen cell of young pupae and once each in close proximity to a prepupa, a third instar, third year grub, and a teneral female of P. anxia. The size and free-living status of the worms indicated that they were adults (Figure29). Since the juveniles of all species of Nematomorpha are obligate insect parasites (Steinhaus, 1949; Lipa, 1967) and since the worms were found in Phyllophaga pupal cells, it is likely that they were responsible for the death of their hosts. The female, prepupa and grub were also considered to have been killed by these worms. Voucher specimens were submitted for diagnosis to Dr. M. Laird, Memorial University, St. John's, Newfoundland. No reply had been received at the time of writing this thesis.

A third species of nematode was recovered from live, moribund and dead grubs and from live pupae of Phyllophaga spp. on many occasions and at several localities during this three-year survey in southern Quebec (Figures 26 and 30). As a rule, juveniles only were present in the live hosts but all stages were found in moribund and especially in dead grubs. Field-collected dead grubs were invaded by soil microorganisms, as well as the nematodes above, the most common being Rhizopus, Fusarium and Pseudomonas spp., and by saprobic mites. On one occasion I found the entomopathogenic bacterium, Serratia marcescens Bizio associated with the nematodes in a recently dead third instar

Figure 29. A gordian worm recovered from the earthen cell of a dead pupa, Phyllophaga sp.

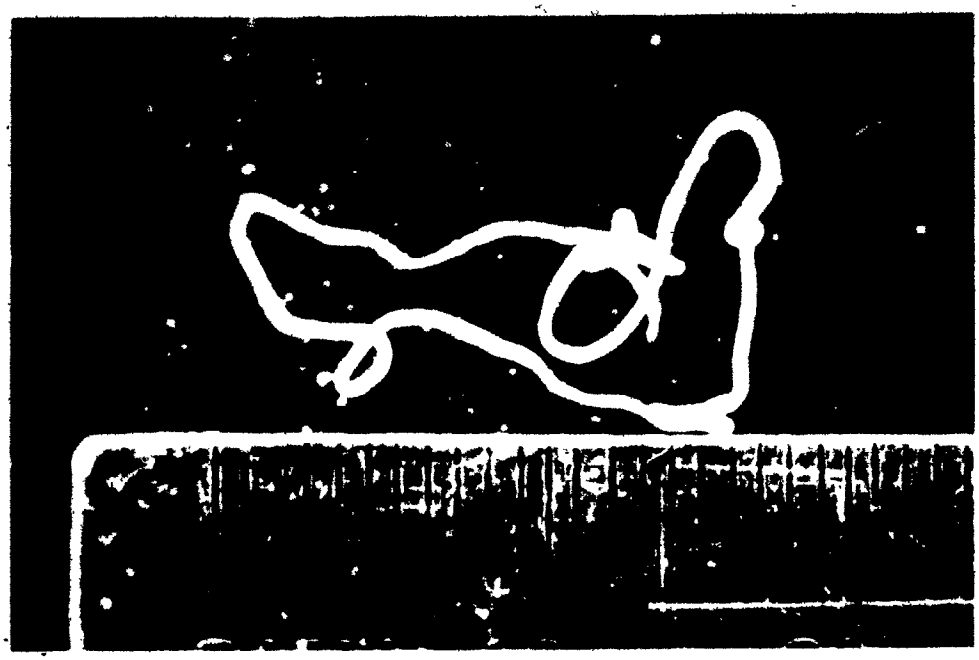


Figure 30. Water suspension of adults and juveniles of M. aerivora recovered from a field-collected moribund white grub, Phyllophaga sp. Nematodes extracted 3 days after the death of the grub. 80X.

grub (Pierreville, September 6, 1979). Several species of bacteria have been demonstrated to be associated with insect- parasitic nematodes (Poinar, 1978), including S. marcescens (Swain, 1945) The third species of nematode was identified to belong to the family Diplogasteridae by the characteristics listed by Goodey (1963) and Poinar (1977) Dr R H Estey, MacDonald College of McGill University, Ste -Anne-de-Bellevue, Quebec, confirmed the identification At the time of the first nematode findings (June 1979), Dr K -P Lim , presently of the Canadian Forestry Service, St John's, Newfoundland, was working at MacDonald College We examined the nematodes found at several localities in southern Quebec (Tables 20 and 21) and we agreed that they were representatives of the species Mikoletzkya aerivora (Cobb). Our identification was made by comparing the characteristics and measurements of the nematodes I have isolated, with descriptions and measurements reported for M. aerivora by Merrill and Ford (1916), Poinar (1979), and Lim (1979) who gave a detailed description, including measurements and the de Man formula, for males and females of M. aerivora Although rarely present in pupae, M. aerivora appeared to be widespread in grubs and since it was found in each year of the survey, it was considered to be endemic in natural populations of Phyllophaga spp. It also appeared to be more prevalent in third instar grubs and more commonly found in the month of August. Five percent of the 16,930 grubs examined over the three-year survey were infected with M. aerivora. Davis (1919) reported that 90% of Phyllophaga grubs were killed by M aerivora in one field in Wisconsin My findings

Table 20. Recovery of the nematode Mikoletzkya aerivora from Phyllophaga grubs and pupae in southern Quebec, 1979 to 1981

Locality	Date of recovery	Host stage[a]
Nicolet	July 28, 79	L1
	Aug 7, 79	L2,1
Pierreville	Sep 9, 79	L3,2
Mirabel	Jun 26, 79	L2,2
	Aug. 6, 79	L3,2
Montebello	Aug 9, 79	L3,2
St.-Janvier	Aug 13, 79	L3,2
Stanbridge	Jun 12, 80	L3,3
Nicolet	Aug. 5, 80	L3,2
	Aug 20, 80	L3,2
Stanbridge	Aug. 9, 80	P
St Clet	Aug 28, 80	L3,3
Mirabel	Sep 2, 80	L3,3
St -Janvier	Sep 2, 80	L3,3
Stanbridge	Aug. 23, 81	L2,1
Nicolet	July 30, 81	L3,3

[a]L1 first instar grub, L2,1 second instar, first year, L3,2 third instar, second year, L3,3 third instar, third year, P: pupa

Table 21 Incidence rate of the nematode Mikoletzkya aerivora in natural populations of Phyllophaga spp , in southern Quebec, 1979 to 1981

Host developmental stage	Number examined	Percent nematosis
Adult male	8895	0
Adult female	2647	0
Egg	2525	0
First instar grub	1832	1 74
Second instar, year 1	1652	2 42
Second instar, year 2	1501	0
Third instar, year 2	8174	7 04
Third instar, year 3	3771	5.40
Prepupa	175	0
Pupa male	1289	0 31
Pupa female	897	0 22
Teneral adult male	73	0
Teneral adult female	37	0

support Lim's (1979) suggestion that M aerivora was widespread in P anxia populations of southern Quebec in 1975 and 1976 Lim (1979) also found that M aerivora was more commonly associated with third instar grubs than with other instars, he did not isolate the nematode from other stages of P anxia

2 Behavior of M aerivora

The results of the laboratory experiments on the behavioral response of M aerivora exposed to various stimuli are presented in Table 22 Untreated units, and paraffin and water treated units did not attract nematodes On the contrary, there was clear evidence that grubs and grubs' tissues stimulated nematode positive taxis, although the response was variable and probably triggered by different mechanisms The pattern of attraction (time and numbers) of juveniles was about the same when they were exposed to live grubs whether plugged or not In both these cases, the live grubs were surface-sterilized, so that attraction to the external microflora of the insects is excluded One plausible source of attraction for the juveniles might have been the metabolic carbon dioxide produced by live grubs, CO_2 is known to stimulate the activity of several nematode species (Lee and Atkinson, 1976) No adult nematodes were attracted by live grubs but this was not surprising, considering that none of the Diplogasteridae are obligate parasites However, those species which are parasitic on insects are facultative parasites, the adult being the free-living stage (Poinar, 1969, 1975) Decaying grubs attracted, after 3lh, the whole population of juveniles and

Table 22 Response, at various time intervals, of M aerivora[a] exposed to various stimuli

Treatment[b] (stimulus)	Estimated density[c] of nematodes in treated compartment[d]							
	1	2	3	4	5	6	7	31
None	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Healthy grub	0,0	0,0	1,0	1,0	2,0	4,0	4,0	5,1
Haemolymph	0,0	0,0	0,0	2,0	2,0	3,0	3,1	3,3
Decaying grub	0,0	0,1	0,2	0,2	1,3	2,3	4,5	5,5
Plugged healthy grub	0,0	0,0	1,0	1,0	3,0	4,0	4,0	5,1
Paraffin	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Sterile water	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0

[a] 450 to 500 nematodes (mixed stages) pipetted on the surface of one compartment of a 2-section petri dish

[b] Three replicates of 10 (5 for paraffin and sterile water) experimental units per treatment

[c] Average, for each treatment, expressed as the estimated percentage of the nematode population which migrated to the treated compartment Symbols: 0, 1, 2, 3, 4, 5 for 0, 10, 25, 50, 75, 100%, respectively, left digit represents juveniles, right digit represents adults

[d] Data recorded 1, 2, 3, 4, 5, 6, 7 and 31h after treatment

adults, although attraction was fastest for adults In this case, it was evident that the nematodes were attracted to the grub's decomposing tissues and the microflora that they supported Such saprozoic and microbiovorous habits in part of the life cycle of facultative entomoparasitic nematodes have been documented frequently, including the Diplogasteridae (Poinar, 1969, Nickle, 1974) The host's decaying body becomes a source of food and the site of reproduction for large numbers of diplogasterics Sterile haemolymph stimulated the movement of both adult and juvenile nematodes Attraction to haemolymph was stronger than attraction to live grubs for adult nematodes but weaker for juveniles The only cause for this apparent attraction might be that haemolymph was the only food source available to the nematodes under the conditions of the experiment Mikoletzyka aerivora was thus found to be capable of actively searching for a potential host, although the triggering mechanism is not clear The process of host's invasion by the infective juvenile stage was not investigated but only two alternatives were possible once host-parasite contact was established active invasion through a natural opening in the host, and ingestion by the feeding host (passive invasion) In either case, the mouthparts of the grubs appeared to be the natural route of penetration since, in over 80% of my observations, juveniles were found in heavier density near and on the head of grubs than near and on other body parts

3 Techniques of Extraction

The results of these experiments are shown in Table 23

Knowing that each grub had been inoculated with the same number of nematodes (4500 to 5000), of which at least 95% were juveniles, and assuming that no multiplication took place between the time of inoculation and the start of extraction, each sacrificed grub should have yielded an average of 4500 to 5000 nematodes. However, after 48h of extraction, efficiencies of the extraction methods were 40-44%, 48-53% and 60-66% for the Baermann funnel, the Carne and Reed apparatus, and the modified Baermann funnel, respectively (Table 23) Losses occurred between the time of inoculation and the final rinse, eventually by bursting of the nematodes when decapitating the grubs (Poinar, 1965). Losses appeared also to have been due to the extraction method itself, at least for the least efficient Baermann funnel method (Table 23) The three methods yielded the same number of nematodes (total and live) after 8h of extraction, but large differences in yields were observed at the second occasion of sampling. The modified Baermann funnel was much more efficient for total and live nematodes extracted than the other two methods. The same trend was observed at the third occasion of sampling, the Baermann funnel, and the Carne and Reed apparatus yielding respectively 58 and 65% of live nematodes, compared to 90% for the modified apparatus The final rinse of the grubs' remnants confirmed the lower efficiency of the Baermann funnel and of the Carne and Reed apparatus, both having extracted fewer nematodes compared to the modified Baermann funnel. The latter procedure proved to be over 93% efficient for total numbers of nematodes extracted and over 98% efficient for live recovery. Oxygenation

Table 23. Average number[a] of juveniles of M. aerivora recovered from artificially infected white grubs[b], using three methods of extraction[c]

Occasions of sampling (hours after start)	Number of nematodes recovered[d]		
	Baermann funnel	Carne & Reed apparatus	Modified Baermann funnel
8	800 (96)	900 (100)	900 (100)
24	400 (78)	800 (82)	1600 (100)
48	800 (58)	700 (65)	500 (90)
Final rinse[e]	600	700	200

[a] Average for 7 grubs with each method, numbers off to nearest one hundred.

[b] All grubs in the same developmental condition, inoculated with 4500-5000 nematodes.

[c] Extractions started 10 days after inoculation

[d] Numbers in parenthesis show the percentage of nematodes recovered alive.

[e] Remnants of grubs were searched for non-extracted nematodes after the 48h occasion of sampling.

of the water with the modified Baermann funnel procedure was thus beneficial in 3 ways; higher yield of total nematodes, higher yield of live nematodes and shorter time needed for extraction, compared with the other two methods.

4. Maintenance of Nematodes

Thirteen suspensions of nematodes of all stages were prepared in sterile water on July 13, 1980. The nematodes were extracted from infected third instar grubs collected at Stanbridge East, June 12, 1980. The aqueous suspensions were stored at 5°C until May 7, 1982 when they were checked for viability of nematodes. All suspensions contained live nematodes after 22 months in cold storage. The viability averaged 44% and most live nematodes were third stage juveniles ensheathed in the second juvenile's cuticle. My findings confirmed Lim's (1979) observations on the feasibility of storing M. aerivora juveniles for extended periods at 5°C.

M. aerivora was also successfully maintained on half-strength nutrient agar under ambiant laboratory conditions for 27 months if the agar was supplemented, at 10 to 15 days intervals, with a decapitated grub. Petri dishes of nutrient agar only supplemented with the natural microflora of the water of the first extraction were less efficient for maintaining M. aerivora; the nematode population usually died after 7 to 10 subcultures. It was also observed that over 95% of the female nematodes reared on agar supplemented with grubs were oviparous whereas eggs began hatching within the body of females reared on agar only. The switch

in the developmental pattern of M. aerivora occurred as soon as the second subculture and no later than the sixth. Survival of M. aerivora was thus dependent on regular passages through its host; this observation confirmed the parasitic nature of the nematode.

5a. Infectivity by Topical Application

White grubs were susceptible to nematosis induced by topical application of M. aerivora, but there was a significant difference in susceptibility between the three instars (Table 24). Second instar grubs (test II in 1979) appeared to be much less susceptible than the two other instars, especially the third (Table 24). The third instar, for which 85% mortality due to nematosis was recorded in this laboratory test (Table 24), was also the instar most commonly found infected with M. aerivora in the field (Tables 20 and 21). Mortalities due to causes other than the nematode were high in the first and second instars and very low in the third instar, but no pathological agent was isolated in either case. No nematosis was observed in the control lots although mortalities were high for all three instars. Here again, no disease was observed, with the exception of two cases of mycosis (green muscardine) in the 1980 test. Koch's postulates were thus fulfilled for M. aerivora against the three larval stages of Phyllophaga grubs (Figure 31). According to Poinar (1979) it is the "dauer" stage juvenile of M. aerivora (non-feeding third stage juvenile) which is associated with living insects and is capable of initiating lethal infections of the host.

Table 24. Lethal infection rates of Phyllophaga grubs by topical applications of M. aerivora.

Year tested [a] (instar)	Dosage [b] (nematodes ml^{-1})	Percent mortality [c] Total	Nematosis
1979 (first)	2.6×10^3	75.0	45.0
	0 (control)	27.5	0
1979 (second)	2.4×10^3	55.0	22.5
	0 (control)	30.0	0
1980 (third)	2.4×10^3	87.5	85.0
	0 (control)	15.0	0

[a] Tests terminated 5 weeks after treatment.

[b] 0.5 ml water suspension of nematodes applied topically on mouthparts of treated grubs (0.5 ml sterile water on control grubs).

[c] Average for 2 replicates of 20 grubs per treatment per test.

Figure 31 Infection studies with M aerivora Remnants of a white grub, Phyllophaga sp (arrow), killed by induced infection Photograph taken 28 days after inoculation Notice the numerous adult nematodes on the bottom of the container Approx 4X D. artificial diet for grub


D

5b. Infection Chamber Test

Results of the infection chamber test are presented in Table 25. Fifty-five percent of the grubs died of nematosis in the infection chambers treated with M. aerivora, 10% died of unknown causes and the remaining grubs had pupated by the end of the experiment. No nematosis was found in the treated grubs which had pupated No nematosis was found in the control lots and all survivors (95%) had pupated Third instar grub mortality due to nematosis was lower (55%) in the infection chamber test than in the topical application test (Table 24), although the inoculum level of the former was higher However, it is clear from the results of both experiments that Phyllophaga grubs are susceptible to infection by M. aerivora The variability in results obtained with the two experiments was likely due to the differences in experimental procedures but also to the different developmental stage of test grubs used in the experiments, third instar, third year grubs in the infection chamber test, and third instar, second year in the topical application tests

5c. Field Microplot Trials

The pathogenicity of M aerivora for Phyllophaga grubs was also demonstrated under field conditions (Table 26) Several grubs were not accounted for from the 3 microplots at the conclusion of the trials, predation or human error in finding or counting grubs were possible causes of non-recovery. Nematosis was relatively high in both treated microplots and one grub (2.7% of recovered grubs) was infected with M. aerivora in the control

Table 25. Susceptibility of Phyllophaga third instar, third year grubs exposed to M. aerivora in infection chambers.[a]

Dosage[b] (nematodes ml^{-1})	Percent mortality[c]	
	Total	Nematosis
3 1 x 10^3	65 0	55.0
0 (control)	5 0	0

[a] Experiment terminated 32 days after inoculation.

[b] 5 ml water suspension of nematodes applied to the soil of infection chambers (5 ml of sterile water applied to the soil of control chambers).

[c] Average for 2 replicates of 20 grubs per treatment.

Table 26 Pathogenicity of M aerivora for Phyllophaga third instar, second year grubs in field microplot tests [a]

Dosage [b] (nematodes ml^{-1})	No of grubs recovered/40	Percent mortality due to nematosis
2.8×10^3	34	64.7
0.7×10^3	38	68.4
0 (control)	37	2.7

[a] Tests concluded 28 days after treatment

[b] Forty times 20 ml of one of the nematode water suspensions added to one microplot (2 by 1 m), controls: 40 times 20 ml of tap water added to one microplot of similar size

plot, the latter case suggested that M aerivora might have been present in the plot or introduced by me as a contaminant The experiments were not replicated and definite field conclusions are not possible However, the results reported in Table 26 suggested that mortality was independent of the inoculum level, since no clear dose-response effect was obtained in the trials The results of these small scale field trials confirmed the results of laboratory experiments and may also demonstrate sufficient tolerance to environmental stress by M aerivora for it to be considered for field control of white grubs, or at least, for retesting on a larger scale

In summary, M aerivora was found to occur widely in field populations of Phyllophaga spp throughout southern Quebec, as suggested by Lim (1979) My studies have helped establish the feasibility of using M aerivora as a biological control agent against soil stages of Phyllophaga, its natural occurrence in the damaging grub stages of Phyllophaga is frequent and widespread in southern Quebec, the nematode is easy to rear and to maintain in the laboratory, it readily infects susceptible host stages in the laboratory and in the field M aerivora was found to be an important biotic regulator of Phyllophaga field populations in southern Quebec, although no major epizootics were observed during this three-year study

IX ACARINES ASSOCIATED WITH PHYLLOPHAGA SPP IN SOUTHERN QUEBEC

A INTRODUCTION

Acari (the Parasitiformes or gamasid mites and ticks, and the Acariformes or "true" mites) are more ubiquitous than any other single group of arthropods and have colonized many marine and terrestrial habitats (Kevan, 1965, Lindquist, 1979a) Mites are common inhabitants of soils, where they show predatory, parasitic, saprotrophic, scavenging or phoretic habits in their associations with other soil dwellers However, it appears from a review of the literature, that mites associated with June Beetles and white grubs have not been studied to any great extent, except for occasional observations, when they were reported to be conspicuously present

Criddle (1918) reported that Tyroglyphus heteromorphus Felt and other mites killed large populations of white grubs in the field in Manitoba Perkins (1892) and Davis (1919), while recognizing its saprotrophic habits, found that Phyllophaga grubs in the field and in rearing cages were frequently infested with Rhizoglyphus phylloxerae Riley, and noted that grubs heavily infested with this mite were weakened and even killed, especially under field conditions Tyroglyphus armipes Banks and a species of Parasiticus have been found infesting white grubs in Texas, Utah and South Dakota (Davis, 1919) Phoretic nymphal stages of

an unspecified Uropoda species, probably neither parasitic nor predaceous, have been collected on adult June beetles (Davis, 1919) Petch and Hammond (1925, 1926), Jarvis (1964, 1966) and Oseto and Mayo (1975) observed that grubs, pupae, and adults of P anxia were frequently infested with hypopi of Caloglyphus sp including C phyllophaginus Oseto and Mayo, and also R phylloxerae and T armipes, all these species appeared to be scavengers or saprotrophs Daniels (1966b) reported that Caloglyphus acarines fed on injured or dead grubs of P koehleriana Lim et al (1981a) found phoretic Tyrophagus sp and unspecified anoetids attached to the femora of grubs and the thoracic sterna and femora of adults of P anxia in southern Quebec

To supplement this meagre information, a survey of mites associated with P anxia and other June beetles was conducted in southern Quebec from 1979 to 1981

B MATERIALS AND METHODS

The various stages of Phyllophaga were collected from 1979 to 1981 at the localities and using the techniques reported earlier Each individual collected was observed under a dissecting microscope and the following data were recorded, collection site and date, host stage, presence of mites and their developmental stage, numbers and position on the host Samples of mites were set aside from each host and preserved in Oudeman's fluid (McDaniel, 1979) until they were permanently mounted on microscope slides, as described by Krantz (1978) Mite specimens were

identified by Drs V Behan and E Lindquist from the Biosystematics Research Institute (B R I), Agriculture Canada, Ottawa, Ontario Voucher specimens were deposited at the B R I and with Dr B O'Connor, Museum of Zoology, University of Michigan, Ann Arbor, Michigan

Mite terminology used by McDaniel (1979) has been followed here, and Krantz (1978) and Lindquist et al (1979) have been followed for classification above the generic level

The status of mites recovered from Phyllophaga in the course of this survey could not be established definitely, but some interpretations are made using information retrieved from the literature

C RESULTS AND DISCUSSION

Mites were found associated with all stages of Phyllophaga spp , except the egg and prepupa, at most of the collecting sites and times covered by this survey (Table 27) As already stated, only adult beetles and pupae were identified to species level, immature stages were keyed-out to the generic level

At least twelve genera of mites, representing six families and fifteen species, were found to infest Phyllophaga spp in southern Quebec and these were present in most of their stages and forms (Tables 27 and 28) Although mite eggs are not recorded in Table 27, they were present on most hosts or within gravid female mites Some of the mite identifications are incomplete (Table 27) because the species was undescribed at the time

Table 27 Acari associated with Phyllophaga spp. in southern Quebec, 1979 to 1981

Acari species	Stage found[a]	Collection site[b]	Year of collection	Host stage[c]
ACARIDAE				
	F,M	3,4,36	79	M,F,L1,L3
	F.M,PM	16	79,80,81	M,F
	H	4,11,17,23,25	79	L3
	H	3	79	L1,L2
	H	16	80 81	L3
	H	6,8,9,10,15,24,25,37	80	L3
Sancassania sp	F,M	2,7,16,17	80	M,F
near michaeli	H	8,34,43	81	L2,L3,M
	H	20,21	81	L2
	F,M,H	8	81	M,F,L1
	H	3	81	P,TM,TF
	H	16	79	TM
	PhM,F	31	79	M,F
	H	31	79	L3
Sancassania sp.	M,F,	16	79	M
near chelone	M,F,H,PhF,PhM	4,19,21,24,26	79	L3
	M,F,H	3,8,24,25,44	80	L3

Table 27 - Continued

Acari species	Stage found[a]	Collection site[b]	Year of collection	Host stage[c]
Sancassania sp.	H,M,F,PM	3,16,31	79	M,F
	H,M,F	12,16,32,33	80,81	M,F
	H,M,F	35	81	M,F
Tyrophagus putrescentiae	All	All except 1,2,9,10, 17,19,20,27,28,34	79,80,81	All except E and PP
Tyrophagus sp.	M,F,H	3,30,31	79	M,F
	M,F,H	3,8,14	80	L3
	M,F,H	8,20,24	81	L1,L2
Acarus sp [d]	All	laboratory	79,80,81	All except E and PP
Caloglyphus sp	N	3	80	L3
Rhizoglyphus sp.	N	24,25	79	L3
ANOETIDAE and HISTIOSTOMIDAE	N	22,26	79	L3
Histiostoma sp	N,F	4,31	79	L3

Table 27 - Continued

Acari species	Stage found[a]	Collection site[b]	Year of collection	Host stage[c]
EVIPHIDIDAE				
Scarabaspis sp.n.	M,N,	3	79	M
	F	12	81	F
MACROCHELIDAE				
Macrocheles glaber	F,N	12	81	F
PARASITIDAE				
Parasitus coleoptratorum	D	3	79	M
Poecilochirus necrophori	D	12	81	M,F
Poecilochirus sp.	D	12	81	M,F
Trachygamasus sp.	D	3	79	M,F

Table 27 - Continued

[a] F: female; M: male; PM: pleomorphic male; H: hypopus; PhM: pharate male; PhF: pharate female; N: nymph; D: deutonymph.

[b] See figure 10 (Chapter III).

[c] M: male; F: female; P: pupa; PP: prepupa; TM: teneral male; TF: teneral female; E: egg; L1, L2, L3: first, second, third instar grub, respectively. Adults, teneral adults and pupae determined as P. anxia.

[d] Never field-collected; probably opportunists in rearing facilities.

Table 28. Higher classification of mite species found associated with Phyllophaga spp. in southern Quebec, 1979 to 1981.

Order	Suborder	Cohort	Superfamily	Family	Number of genera found
Parasitiformes	Mesostigmata (Gamasida)	Gamasina	Parasitoidea	Parasitidae	3
			Eviphidoidea	Eviphididae	1
				Macrochelidae	1
Acariformes	Acaridiae (Astigmata)	-	Acaroidea	Acaridae	5
			Anoetoidea	Anoetidae	1
				Histiostomidae	1

or because specific identification depended upon characters found only in stage or sex that was not collected.

Several species of mites were of widespread occurrence throughout southern Quebec, while other species appeared to be rarer and more localized in distribution (Table 27) Species of the Acaridae were recovered from most of the stages of the hosts, with the exception of Caloglyphus and Rhizoglyphus spp., but the Anoetidae, Histiostomidae, Eviphididae, Macrochelidae, and Parasitidae seemed to be more specifically associated with only one host stage, often the adult beetle. Acaridae, especially Sancassania spp., were collected on more occasions and at more stations than other acarines Acarids were also found in more life stages and on more host stages than the other mites, which occurred in decreasing frequency as follows: Parasitidae, Macrochelidae, Anoetidae, Histiostomidae and Eviphididae. Lindquist et al. (1979) gave the following numbers of mite species known from Canada. Acaridae (45), Parasitidae (35), Macrochelidae (15), Anoetidae including Histiostomidae (20), and Eviphididae (5). Second and third instar grubs were infested by mites more frequently than first instar larvae and pupae. The frequencies of infestation among teneral and flying adult beetles were lower than those of second and third instar grubs, but higher than those of other stages Mites were more often collected on flying adult females than on the males (Table 27)

The legs and anal region were the most common sites of infestation of white grubs, although mouth parts and spiracles were occasionally invaded by mites The sub-elytral area was

the most common infestation site in adult June beetles of both sexes. The antennae, mouth parts, spiracles, genital and anal regions, thoracic setae and occipital region were found to be infested only occasionally.

Species of Tyrophagus mites were found internally in dead grubs and adults. Mixed infestations of Tyrophagus, Poecilochirus, and Sancassania species were commonly found in Phyllophaga cadavers. Macrocheles glaber (Müller) was isolated from the genital chamber of a live female P. anxia. The percentages of field-collected Phyllophaga on which mites were found in various associations are given in Table 29 The relatively high percentages of infested host stages are somewhat in contradiction with earlier literature reports, except for those of Petch and Hammond (1925, 1926). These authors reported up to 100% infestation of some field samples of white grubs in southern Quebec.

The high percentage of infestation in second and third instar grubs might be due to the relatively long life span of these stages of Phyllophaga, whereas in flying adults it suggests phoretic association The low infestation rates of the egg, prepupal and pupal host stages may be a reflection of their short duration or comparatively good protection (Davis, 1919).

A brief review of Family characteristics is given now as a basis for interpretation of mite-Phyllophaga associations.

Table 29 Numbers of individuals of Phyllophaga spp found infested with mites in southern Quebec, 1979 to 1981.

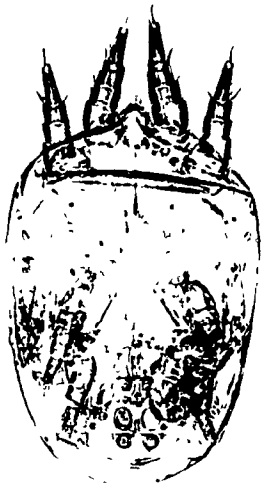
Host stage	Number collected	Number infested	Percentage infested
Egg	2525	0	0
First instar grub	1832	173	9 44
Second instar grub	3153	1326	42 05
Third instar grub	11945	6521	54 59
Prepupa	175	0	0
Pupa male	1289	113	8 76
Pupa female	897	22	2 45
Teneral adult male	73	23	31 50
Teneral adult female	37	10	27 02
Adult male	8895	3037	34 14
Adult female	2647	1038	39 21
All stages	33468	12263	36 64

Acaridae (Figures 32 to 39)

As members of the Acaridiae (or Astigmata), acarid mites are generally non-predatory and have four active immature stages The second stage (deutonymph or hypopus) is highly modified for phoresy (Krantz, 1978) on invertebrate or vertebrate hosts, and if present, may have a facultative or obligatory host association Acaridae belong to the supercohort Acaridia which is formed of species in which adults are free living or parasitic upon invertebrates and the hypopus is usually present (Lindquist et al., 1979b) Most acarids are saprophagous, but some are fungivorous or phytophagous, acarids of many species are associated with insects, especially Coleoptera and Hymenoptera Samšiňák (1970) stated that Sancassania spp are often associated with scarab beetles as phoretic hypopodes in the heteromorphic deutonymphal stage, and on the death of the carrier, as succeeding saprophagous stages Krantz (1978), citing various authors, reported that Sancassania spp are either mycophagous (fungivorous) mites or necrophagous and saprophagous on dead soil insects Members of the genera Acarus and Tyrophagus occupy a wide range of niches and are common fungivorous microphytophages T putrescentiae (Schrank), a cosmopolitan stored product mite, is also a pest on laboratory animal cultures, and some hypopodial Acarus spp are phoronts on insects (Baker 1962, Krantz, 1978, Lindquist et al., 1979b) Most Rhizoglyphus spp exhibit phytophagy, some as fungivorous microphytophages and some as macrophytophages, but a few species are however associated with insects, often as phoronts

Figure 32 Acarines associated with Phyllophaga spp in southern Quebec Sancassania sp , hypopus (Acaridae) 500X

Figure 33 Acarines associated with Phyllophaga spp in southern Quebec Sancassania sp near michaeli (Oudemans) sensu Turk and Turk, hypopus (Acaridae) 500X



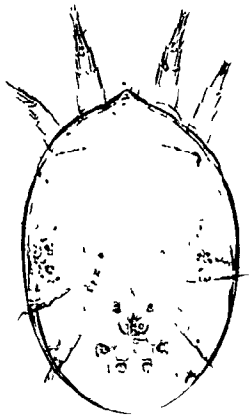


Figure 34 Acarines associated with Phyllophaga spp in southern Quebec Sancassania sp near michaeli, male (Acaridae) 300X

Figure 35 Acarines associated with Phyllophaga spp in southern Quebec Sancassania sp near michaeli, female (Acaridae) 200X

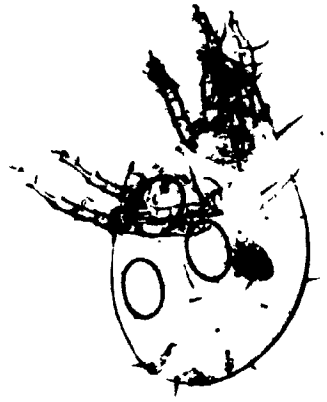
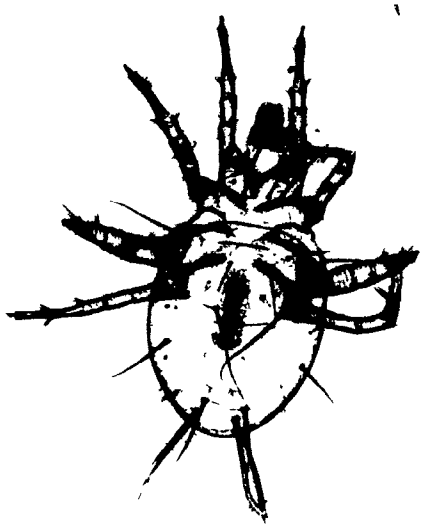


Figure 36 Acarines associated with Phyllophaga spp in southern Quebec Sancassania sp near chelone Oudemans, male (Acaridae) 200X

Figure 37 Acarines associated with Phyllophaga spp in southern Quebec Sancassania sp near chelone, female (Acaridae) 200X



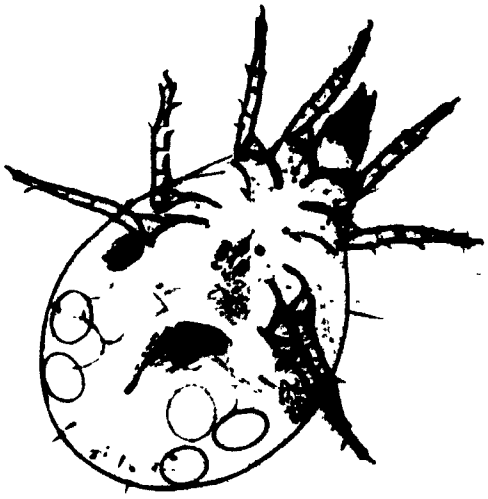


Figure 38 Acarines associated with Phyllophaga spp. in southern Quebec Tyrophagus putrescentiae (Schrank), male (Acaridae) 500X

Figure 39 Acarines associated with Phyllophaga spp. in southern Quebec Tyrophagus putrescentiae, female (Acaridae) 500X

and scavengers (Lim et al., 1980a).

Histiostomidae (Figure 40) and Anoetidae

Krantz (1978) classified the genus Histiostoma as anoetid or slime mites Behan and Lindquist (1982, personal communication) considered them as members of the Histiostomidae. In either case, Anoetidae and Histiostomidae are worldwide in distribution and primarily microphytophagous as adults, larvae and non-hypopodial nymphs (Krantz, 1978) One anoetid species from Michigan is a parasitoid of earthworm eggs and at least two Histiostoma spp. feed on leeches (Oliver, 1962) Scheucher (1959) and Mahunka (1972) reported that hypopodial anoetids are often attached to various arthropods Phoretic hypopodial Anoetus sp. were collected on carab beetles by Olynyk and Freitag (1979). Anoetids of one or two species are occasionally pests in substrates of neglected insect cultures (Lindquist et al , 1979b)

Macrochelidae and Eviphididae (Figures 41 and 42)

Eviphidids and macrochelids belong to the suborder Mesostigmata (Gamasida) and as such have only three active, immature stages (one larval, two nymphal, no hypopodial) The large cohort Gamasina includes numerous species of mostly free-living mites predaceous on nematodes, small insects and other mites (Lindquist, 1979b) Eviphididae are generally free-living, nonphoretic acarines Some members of this family, however, have

Figure 40. Acarines associated with Phyllophaga spp. in southern Quebec. Histiostoma sp., female (Histiostomidae). 300X.

Figure 41. Acarines associated with Phyllophaga spp. in southern Quebec. Macrocheles glaber (Müller), female (Macrochelidae). 120X.

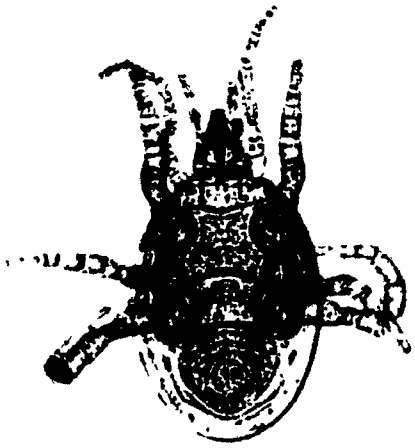
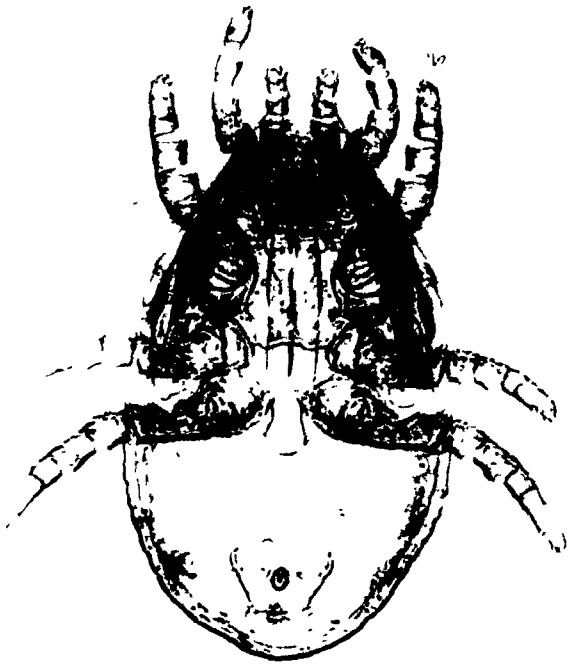


Figure 42. Acarines associated with Phyllophaga spp. in southern Quebec. Scarabaspis sp.n., female (Eviphididae). 500X.



established phoretic relationships with insects and amphipods (Krantz, 1978) Lindquist (1979b) stated that eviphidids are associated with insects in edaphic habitats of arable land and pastures Deutonymphs and adults of Scarabaspis and related genera are commonly found on dung beetles and coprophagy is probably exhibited by some Scarabaspis spp (Krantz, 1978) Evans (1969), and Potter and Johnston (1976), found that some eviphidid species are predatory in temporary habitats and are also phoretic on arthropods exploiting these habitats

Three females and one nymph Scarabaspis isolated from P anxia adults in the course of this study are probably representatives of a new species (Lindquist, 1982 personal communication) One female and one nymph were found on an apical abdominal spiracle of a P anxia male at Nicolet on June 7, 1979 Two females were collected on the apical sternite of one P anxia female at St Jean-sur-Richelieu on May 25, 1981 The biology of the family Macrochelidae has been reviewed by Evans and Browning (1956), Costa (1967), Krantz and Mellott (1972), and Treat (1975) Macrochelids live in various soil habitats where they are free-living and predaceous upon litter microfauna and nematodes Some species are associated with synanthropic fly maggots in compost and manure and some species are found within the nest of social insects such as bees and ants (Rodriguez et al , 1970, Krantz, 1978) Female and deutonymphal forms of several members of the genera Holocelaeno Macrocheles, and Neopodocinum are specific phoronts of scarabaeid beetles, silkworm caterpillars and muscid flies, particularly dung-visiting flies Predation, phoresy, and coprophagy are thus usual habits exhibited by members of this family

Some phoretic macrochelids and eviphidids are known to be predaceous on various life stages of their insect carriers Among various phoretic acarine groups, macrochelids are site specific (Krantz, 1978)

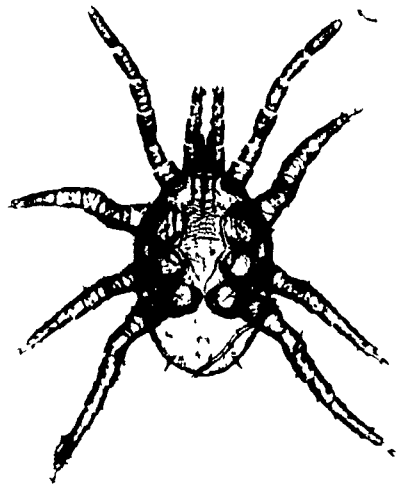
Most of the species of mites discussed so far, including those collected on Phyllophaga spp in the course of this study, are either free-living or phoretic but a few are predaceous upon insects and thus likely participate in the regulation of natural populations of Phyllophaga in southern Quebec

Parasitidae (Figures 43 to 46)

The free-living species discussed now are all members of the predatory family Parasitidae The Parasitoidea live in edaphic, nidicolous, subcortical, and carrion habitats, some parasitids are in close association with insects, and deutonymphs of numerous species are phoretic on insects (Lindquist 1979b) Rapp (1959) and Krantz (1978) listed bumblebees, ground beetles and carrion beetles as usual carriers of phoretic parasitids Parasitid species are recorded as preying upon nematodes, mites, bark beetles, nidicolous and stored product arthropods It is also known that most parasitids have an adverse effect upon the prey population Berry (1973) and Krantz (1978) concluded that the Parasitidae and other predaceous species (e g Macrochelidae) are beneficial to man since they usually prey upon harmful insects and other noxious invertebrates Among typical predaceous ground mites, Parasitus coleoptratorum (L) is a common predator

Figure 43 Acarines associated with Phyllophaga spp in southern Quebec Trachygamasus sp , deutonymph (Parasitidae) 120X

Figure 44 Acarines associated with Phyllophaga spp in southern Quebec Poecilochirus sp , deutonymph (Parasitidae) 120X



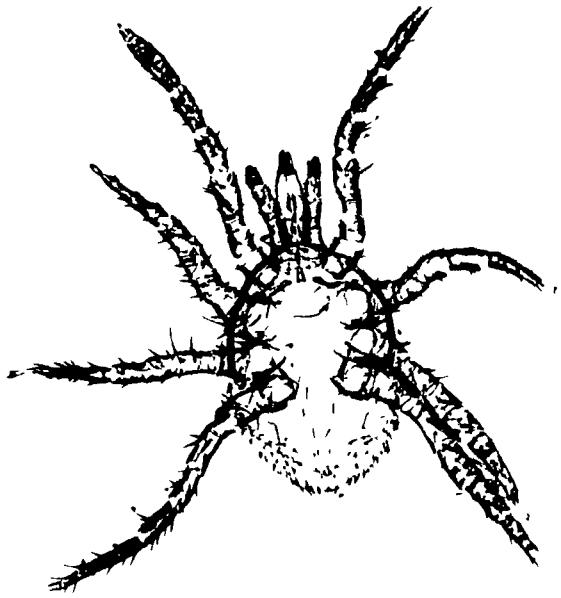
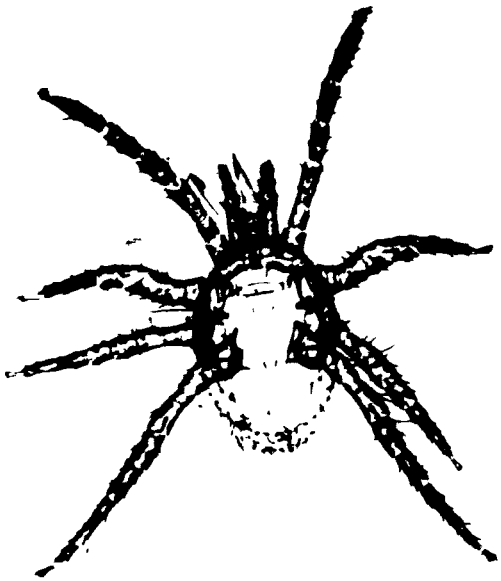
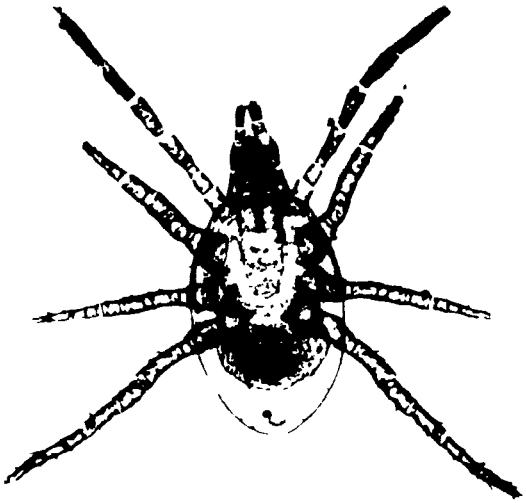


Figure 45 Acarines associated with Phyllophaga spp in southern Quebec Poecilochirus necrophori Vitzthum, deutonymph (Parasitidae) 80X

Figure 46 Acarines associated with Phyllophaga spp in southern Quebec Parasitus coleoptratorum (L), deutonymph (Parasitidae) 80X





of fly maggots and beetle larvae (Wernz and Krantz, 1976) Poecilochirus and Trachygamasus spp are known to prey on various soil-inhabiting arthropods (Krantz, 1978)

In summary, the present survey indicated that mite occurrences in association with Phyllophaga spp were much more common in southern Quebec than I expected to find from literature reports All Phyllophaga stages except the egg and prepupa were prone to mite infestation Although it was not possible to determine the nature of most associations with certainty, and thus to assess the real impact of the mites involved in these associations, it is likely that the macrochelid, parasitid and possibly eviphidid mites that were found were acting as natural regulators of local Phyllophaga populations Acarid, anoetid, and histiostomid mites appeared likely to have a phoretic or saprobic relation with Phyllophaga, and thus were probably not harmful to their host The fact that more than 60% of the hosts that I found killed by entomopathogenic microorganisms, were also infested with Sancassania, Tyrophagus, and Rhizophagus spp of mites reinforces the hypothesis of saprobic habits for these mites even if microphytophagy (fungivory) is considered as their usual diet

Most of the mite species found during this survey have not previously been reported in Canada occurring on Phyllophaga beetles

X FIELD SURVEY OF INSECTS ASSOCIATED WITH PHYLLOPHAGA SPP IN SOUTHERN QUEBEC, 1979 TO 1981

A INTRODUCTION

Detailed accounts of entomophagous insects and of their biology are included in numerous publications and especially in Böving and Craighead (1930-1931), Balduf (1935), Sweetman (1936), Clausen (1940), Doutt (1959), Wallwork (1970), Askew (1971) Information is also found in entomological books and reviews treating the applied aspects of the biological control of arthropod pests, and in the lists and indices compiled by the Commonwealth Agricultural Bureaux Among others, Davis (1919), Petch and Hammond (1925, 1926), Ritcher (1940, 1958) and Lim (1979) reviewed the known predatory and parasitic insects of Phyllophaga spp in North America

I report in this chapter my observations on entomophagous insects found associated with Phyllophaga spp in southern Quebec, from 1979 to 1981

B MATERIALS AND METHODS

All stages of Phyllophaga spp , P anxia in particular, were collected at 45 localities of southern Quebec (Figure 10) as described in Chapter III Insects found in the field either feeding on or in very close proximity to Phyllophaga specimens

were collected and classed as predators or potential predators Insects that emerged from field-collected Phyllophaga specimens in the laboratory and insects that were found upon dissection of Phyllophaga specimens were also collected and classed as parasites All recovered entomophagous insects were identified by me to the genus and, in some cases, to the species level (Chapter III) Specimens were then submitted to taxonomists (Agriculture Canada, Biosystematics Research Institute, Ottawa) for confirmation of my identifications or for further identification The following assisted with identifications Drs. Y Bousquet, H Goulet, L Lesage and A Smetana, coleopterists; Drs H J Teskey, H C Walker and D M Wood, dipterists, Dr L. Masner and Mr M Ivanochko, hymenopterists Voucher specimens were deposited with the Biosystematics Research Institute and with the Lyman Museum and Research Laboratory, McGill University Ste -Anne-de-Bellevue, Quebec

C RESULTS AND DISCUSSION

Insect predators and parasites recovered from Phyllophaga spp are listed in Table 30. Table 30 also gives the stages of both the natural enemy and the host, the relation of enemy to host, and the number of occasions of finding the enemy. Since the study reported here was aimed at disclosing qualitative and quantitative aspects of the biotic regulation of Phyllophaga spp. in southern Quebec, the numbers of recovered entomophagous insects are also given in Table 30. It is interesting

Table 30. Insect predators and parasites of Phyllophaga spp collected in southern Quebec, 1979 to 1981

Entomophagous insect	Stage found [a]	No of occasions hosts attacked	Relation to host [b]	No. of enemies recovered	Host stage [c]
COLEOPTERA					
CICINDELIDAE					
Cicindela sp.	L	28	PR	28	L2, L3
Cicindela punctulata Oliv	A	1	PR	1	TAF
Cicindela repanda Dej	A	1	PR	1	TAF
CARABIDAE					
Agonum cupripenne Say	A	9	PR	9	L2, L3
Agonum placidum Say	A	2	PR	2	L3
Amara cupreolata Putzeys	A	12	PR	14	L2, L3
Amara patruelis Dej	A	3	PR	5	L3
Anisodactylus discoideus Dej	A	19	PR	19	L2, L3
Anisodactylus harrisi LeC.	A	8	PR	9	L2, L3
Anisodactylus nigrita Dej	A	4	PR	4	L3
Bembidion versicolor (LeC.)	A	1	PR	1	L1
Calosoma frigidum Kby.	A	1	PR	1	L3
Carabus serratus Say	A	26	PR	26	L2, L3
Chlaenius sericeus Forst	A	15	PR	15	L2, L3
Chlaenius tricolor Say	A	35	PR	36	L2, L3
Harpalus bicolor F	A	48	PR	48	L2,L3

Table 30 - Continued

Entomophagous insect	Stage found [a]	No of occasions hosts attacked	Relation to host [b]	No. of enemies recovered	Host stage [c]
Harpalus caliginosus F.	A	3	PR	5	L2
Harpalus herbivagus Say	A	4	PR	4	L3
Harpalus longicollis LeC.	A	10	PR	10	L2, L3
Harpalus pleuriticus Kirby	A	2	PR	3	L2, L3
Pterostichus leconteianus Lutz.	A	18	PR	19	L2, L3
Pterostichus lucublandus Say	A	19	PR	19	L2, L3
ELATERIDAE	L	17	PR	17	L2, L3
CURCULIONIDAE	L	2	PR	2	L3
STAPHYLINIDAE					
Philonthus fuscipennis Mnnh.	A	1	PR	1	L2
SCARABAEIDAE					
Aphodius sp.	L	3	PR	3	L3
Phyllophaga spp.	L	>100	CA	>100	L2, L3
TENEBRIONIDAE					
Tenebrio molitor L.	A	2	PR	3	L3
HYMENOPTERA					
PELECINIDAE					
Pelecinus polyturator (Drury)	A	18	ENPA	18	grub [d]

Table 30 - Continued

Entomophagous insect	Stage found[a]	No. of occasions hosts attacked	Relation to host[b]	No. of enemies recovered	Host stage[c]
TIPHIIDAE					
Tiphia sp.	L	18	ECPA	18	L3
Tiphia inornata Say	L	2	ECPA	2	L3
Tiphia micropunctata Allen	L	1	ECPA	1	L3
DIPTERA					
MUSCIDAE					
Pararicia pascuorum Mg.	P	1	ENPA ?	1	AF
PYRGOTIDAE	P	2	ENPA	2	AF
SARCOPHAGIDAE	L	3	ENPA	3	L3
TACHINIDAE					
Tachinid sp	L	19	ENPA	48	AF
Eutrixa exilis (Coq)	P	48	ENPA	123	AF
Microphthalma sp.	L	7	ENPA	18	L3
Cryptomeigenia sp near C. theutis (Wlk.)	P	9	ENPA	15	AF
Cryptomeigenia sp	L	22	ENPA	53	AF
Cryptomeigenia sp	P	5	ENPA	12	AF
Cryptomeigenia theutis (Wlk)	P	49	ENPA	113	AF

Table 30 - Continued

Entomophagous insect	Stage found [a]	No of occasions hosts attacked	Relation to host [b]	No of enemies recovered	Host stage [c]
Microphthalma michiganensis Tnsd.	L	2	ENPA	4	L3
Microphthalma michiganensis	P	1	ENPA	1	PF
Cryptomeigenia theutis	L	14	ENPA	31	AF
Cryptomeigenia sp.	L	1	ENPA	1	AF
Cryptomeigenia theutis	P	1	ENPA	1	AM
Eutrixa exilis	P	1	ENPA	1	AM
ASILIDAE					
Asilus sp.	L	3	PR	4	L2, L3
Diogmites basalis (Walker)	A	2	PR	2	-

[a] L: larva, A: adult, P: pupa.

[b] PR: predator, ENPA: endoparasite, ECPA ectoparasite, CA cannibalism.

[c] L1: first instar grub; L2: second instar, L3 third instar, AM adult male, AF adult female, TAF teneral adult female.

[d] The 18 adult females of P. polyturator were caught flying above grub-infested areas, in its immature stages, P polyturator is a known endoparasite of scarabaeid grubs, including Phyllophaga spp grubs (Lim et al., 1980c)

to note that in many cases especially among parasitic tachinid flies, more than one individual was recovered on a given occasion from a single host

Insect predators and parasites of Phyllophaga spp found in southern Quebec belonged to three orders and 14 families Twenty-one genera and 30 species were identified 23 of which were predatory or potentially predatory (Table 30) Most of the predators were found attacking Phyllophaga grubs, all of the ectoparasitic Hymenoptera were recovered from white grubs and most endoparasites were found from adult female P anxia Although fewer parasite species were found, they occurred on about the same number of occasions and in slightly higher number of hosts than were predators Most predators found in this study were adults whereas all parasites were immatures Some of these immatures were successfully reared to maturity in the laboratory All pelicinid wasps and one Microphthalma michiganensis Tnsd were found only in the adult stage but since their status as enemies of Phyllophaga is documented, they are included in Table 30

Percentage parasitization of Phyllophaga and numbers preyed on were each calculated from the data in Tables 1 and 30 (Table 31) Out of a total of 33,468 Phyllophaga individuals examined, only 0 74% were parasitized and 297 were attacked by predatory insects Percentage parasitization was higher for adult females (7 84%) than for other stages of Phyllophaga and third instar grubs were preyed on more often than the other stages (Table 31)

Table 31 Percentage parasitization and number of predated Phyllophaga[a] individuals found in southern Quebec 1979 to 1981

Host stage	Numbers examined	Percentage parasitized[b,c]	Number attacked by predators[d]
Egg	2525	0	0
First instar grub	1832	0	1
Second instar grub	3153	0 10	32
Third instar grub	11945	0 22	262
Prepupa	175	0	0
Pupa male	1289	0	0
Pupa female	897	0 04	0
Teneral adult male	73	0	0
Teneral adult female	37	0	2
Adult male	8895	0 02	0
Adult female	2647	7 84	0
All stages	33468	0 74	297

[a] P anxia in over 98% of individuals collected in the survey

[b] Based on the number of occasions of findings, not the number of parasites

[c] Including 18 grubs assumed to have been hosts for 18 Pelecinus polyturator (Drury)

[d] Excluding cannibalism and the potential prey of two adult asilid flies

The survey clearly shows that insect enemies have little impact on the size of Phyllophaga field populations in southern Quebec. This situation could be summarized in Askew's (1971) words: "not all natural enemies of a pest necessarily contribute towards bringing about a reduction in its number." Although Lim (1979) did not express his observations in percentages, analysis of the data presented in his tables leads to a very similar conclusion: entomophagous insects do not appear to be an important mortality factor or biotic regulator of June beetles in southern Quebec.

To my knowledge, this is the first time that such a detailed survey has been made on Canadian Phyllophaga species. It should be emphasized however that this study dealt principally with P. anxia in agricultural lands (Chapter III) and that the bionomics of the dozen or so other Phyllophaga spp. of Quebec are not well understood (Goulet, 1980, personal communication).

D. CASE DETAILS

CARABIDAE AND CICINDELIDAE (COLEOPTERA)

At least two cicindelid species and 19 carabid species were observed attacking white grubs and teneral adults of P. anxia or found in close proximity to white grubs (Table 30). It would be tedious to list here data pertaining to each occasion of predation observed during this study. These data appear on the labels of voucher specimens deposited in the collections named in "Materials and Methods" for this chapter.

Nearly all known species of Carabidae and Cicindelidae are predaceous upon other arthropods and upon many other forms of small animal life Both larvae and adults of ground beetles and tiger beetles are non-selective entomophagous insects and will attack and prey upon any host occurring in their habitat (Clausen 1940) Clausen (1940) considered the Carabidae to be among the most important insect predators of insect pests in the agro-ecosystem

Davis (1919), Seaton (1939) and Lim (1979) listed the species of carabid species that have been observed to prey upon various stages of Phyllophaga spp Davis (1919) insisted on the importance of carabid larvae as predators of white grubs, Phyllophaga spp

To my knowledge there are no published records of cicindelid predation upon Phyllophaga spp other then those reported by me for southern Quebec (Table 30) New records of carabid predation upon grubs of Phyllophaga in southern Quebec are Agonum cupripenne, Anisodactylus harrisi, A nigrita, Bembidion versicolor Harpalus pleuriticus and Pterostichus lucublandus (Table 30)

ELATERIDAE (COLEOPTERA)

During this three-year survey, 17 elaterid larvae were collected while feeding upon live second and third instars white grubs (Table 30) Tentative rearing of the wireworms to the adult stage was a failure The occasions of finding were

6 predators at Pierreville in July and August 1979, 1 predator at Notre Dame-de-la-Paix in early August 1979, 4 predators at Mirabel in mid-summer 1979, 4 predators at Nicolet in June-July 1980, and 2 predators at Coteau-Station in late August 1980

Records of wireworm predation upon white grubs are few Davis (1919) reported that larvae of the elaterid beetle, Pyrophorus luminosus Ill , were reared on several occasions upon Phyllophaga grubs Davis (1919) stated that Puerto Rican cane fields heavily infested with white grubs were also supporting dense populations of elaterid adults and the author concluded that the presence of click beetles in such numbers indicated the predaceous activity of their immature stages Clausen (1940) included the elaterid, Monocrepidius pallipes Esch among the most important natural enemies of white grubs in Fiji Clausen (1940) also reported the predaceous habit of larvae of Monocrepidius exsul Sharp upon cane grubs in various Pacific islands and that of Pyrophorus luminosus Ill larvae upon various scarabaeid cane grubs in the West Indies The latter elaterid have been used in attempts to control scarabaeids in several tropical countries Predation by wireworms upon white grubs, Phyllophaga spp , has not been previously reported in North America

CURCULIONIDAE (COLEOPTERA)

An unusual case of predation was observed at Pierreville, August 24, 1979 One curculionid grub was found feeding on one live third instar Phyllophaga grub (Table 30)

The taxonomy of the larval forms of weevils is an area that requires much study (Bright, 1979) and unfortunately Dr Lesage was unable to key the submitted specimen All known members of the Curculionidae being phytophagous (Jacques, 1951, Dillon and Dillon, 1961, Metcalf et al, 1962, Bright 1979) this predation record is probably a first though it may be purely accidental like the case of tenebrionid beetles (Lesage, 1980 personal communication)

STAPHYLINIDAE (COLEOPTERA)

On one occasion in this study one adult Philonthus fuscipennis Mnnh was found with its mouthparts embedded into the abdomen of a second instar white grub (Stanbridge East, August 8 1981), therefore demonstrating true predation (Table 30)

The Staphylinidae is a large family of scavenging beetles and many species are very abundant in decaying organic matter (Dillon and Dillon, 1961) However according to Campbell and Peck (1979), a considerable number of staphylined species are entomopredaceous in the larval and adult stages Reviews of the host preferences and habits of the rove beetle family were given by Mank (1923) Voris (1934), Balduf (1935) and Clausen (1940)

To my knowledge, the literature does not mention records of staphylinid predation upon Phyllophaga spp

SCARABAEIDAE (COLEOPTERA)

Grubs of Aphodius spp and Phyllophaga spp (probably

P anxia) are listed in Table 30 as predators of white grubs. Phyllophaga spp Three Aphodius larvae were found attacking Phyllophaga grubs in a hay field at St -Sébastien, in late summer 1980 Most of my observations on the cannibalism of Phyllophaga were made in a strawberry field at Pierreville, from midsummer to late September 1979

Most species of the large superfamily Scarabaeoidea are phytophagous, coprophagous or saprophagous in habit (Luginbill and Painter, 1953, Dillon and Dillon, 1961, Howden, 1979) The predaceous habits of several scarabaeoid species have been however reported by Hayward (1936), Denier (1936)and Clausen (1940) Cannibalism is not uncommon among many insect species and occurs under field conditions and in laboratory confinement facilities when food is scarce or when overcrowding results in competition for space The strawberry field at Pierreville was badly infested with third instar grubs in 1979 Food was plentiful and, thus, competition for space likely explained the unusual incidence of cannibalism observed among white grubs, on the average, each strawberry plant supported 10 grubs, a high population density for a small niche (Letendre, 1982) Since Table 30 lists only those cases of actually observed zoophagy, the role of Phyllophaga spp and other scarab beetles as biotic regulators of Phyllophaga spp should be recognized, however small it may be.

TENEBRIONIDAE (COLEOPTERA)

Three adult beetles, Tenebrio molitor L , were found on two occasions in close proximity to third instar white grubs in

an old field at Pierreville, August 24 1979, about 15 cm deep in the soil (Table 30)

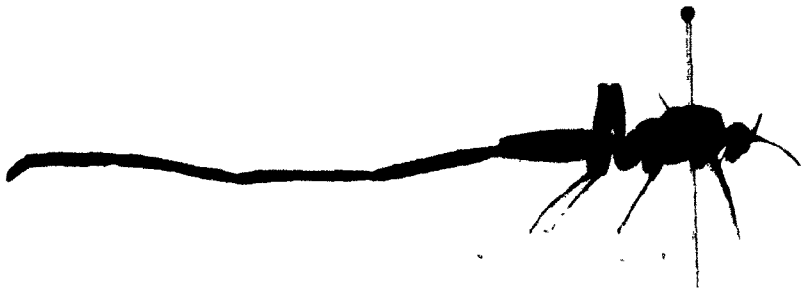
Although the family Tenebrionidae is essentially composed of species of scavengers (Dillon and Dillon, 1961), some tenebrionids, without actually being predators, could probe an alternative food source such as insect larvae (Goulet, 1980, personal communication) Since one of the grubs found in close proximity to two T molitor was covered with fresh oozing scratches, it is likely that the wounds were the consequence of repeated assaults by the tenebrionids

PELICINIDAE (HYMENOPTERA)

Eighteen Pelecinus polyturator (Drury) females (Figure 47) were caught in the course of this survey in fields known to support white grub populations (Table 30) Thirteen females were collected on a single day (July 29, 1979) at Nicolet above a pasture, three specimens were caught when sweeping a small meadow at Ste Anne-de-Bellevue (July 17, 1980) and three individuals were found at ground level in a flowering field at Stanbridge on August 6, 1980

Forbes (1894), Davis (1919), Petch and Hammond (1925, 1926), Brues (1928), Hammond (1944b) and Lim et al (1980c) reported that P polyturator is a parasite of Phyllophaga spp. grubs including P anxia Quantitative data unfortunately are few and it is difficult to assess with accuracy the impact of the pelecinids on natural white grub populations Davis' (1919) and Lim

Figure 47. Female adult of Pelecinus polyturator (Drury). 1.5X.



et al. (1980c) findings as well as this study suggest that this impact is very low. According to Clausen (1940), the true hosts of P. polyturator would be beetle larvae in decaying wood and parasitism upon scarabaeid grubs would only be incidental.

TIPHIIDAE (HYMENOPTERA) (Figures 48 and 49)

Twenty-one white grubs were found parasitized by Tiphia wasps in the course of this survey of the natural enemies of Phyllophaga spp. (Tables 30 and 32). This represented 0.13% parasitization among the 16,930 grubs of all instars examined from 1979 to 1981 or 0.18% of 11,945 third instar grubs, the only instar found parasitized by tiphiids. No parasitism by Tiphia wasps was recorded for 1981 (Table 32) and only three of the parasitic larvae were successfully reared to the adult stage (Table 12).

The first mention of parasitism by Tiphia wasps upon grubs of the genus Phyllophaga was made by Riley (1874) who studied the habits and life history of Tiphia inornata Say. Forbes (1907), Davis (1913, 1919) and Criddle (1918) wrote that T. inornata and several other species of tiphiid wasps were important ectoparasites of white grubs in North America, although Criddle (1918) stated that tiphiids were uncommon insects. Petch and Hammond (1925, 1926) reported that third instar white grubs, P. anxia, were parasitized by T. inornata in Quebec; rates of parasitism ranged from three to 49% in some areas of the province. Jarvis (1966) found eggs of Tiphia sp. on second and third instar grubs of P. anxia in Nebraska, where 33% of

Table 32. Tiphiids collected while parasitizing third instar Phyllophaga grubs in southern Quebec, 1979-1980.[a,b]

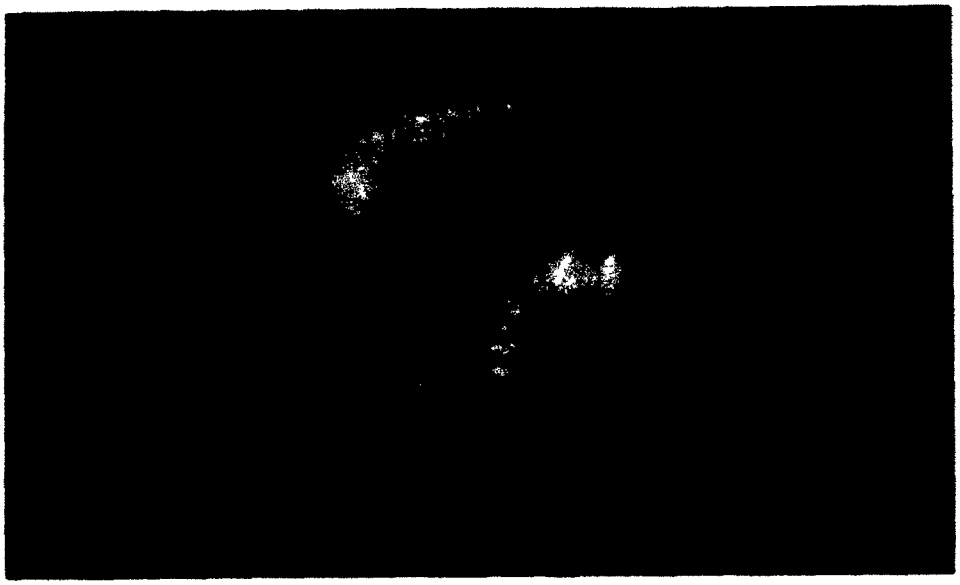
Tiphiid species	Collection site	Collection date	Occasions of finding[c]			Stage reached in laboratory
Tiphia inornata Say	Stanbridge	July 10, 80	1	3	1	adult (Sept. 3,80)
Tiphia inornata Say	Stanbridge	July 16, 80	1	5	1	adult (Sept.22,80)
Tiphia micropunctata Allen	Cowansville	July 13, 80	1	3	1	adult (Aug. 11,80)
Tiphia sp.	Stanbridge	July 11, 80	6	17	1(x6)	pupa (late July, 80)
	Nicolet	Aug. 2, 80	3	13	1(x3)	pupa (mid-Aug., 80)
	Mirabel	July 7, 80	1	3	1	pupa (July 17, 80)
	Napierville	July 4, 79	2	8	1(x2)	pupa (mid-July, 79)
	Mirabel	July 24, 79	4	18	1(x4)	pupa (early-Aug., 79)
	New Glasgow	June 28, 79	1	5	1	pupa (mid-July, 79)
	Pierreville	Aug. 6, 79	1	3	1	pupa (Aug.18, 79)

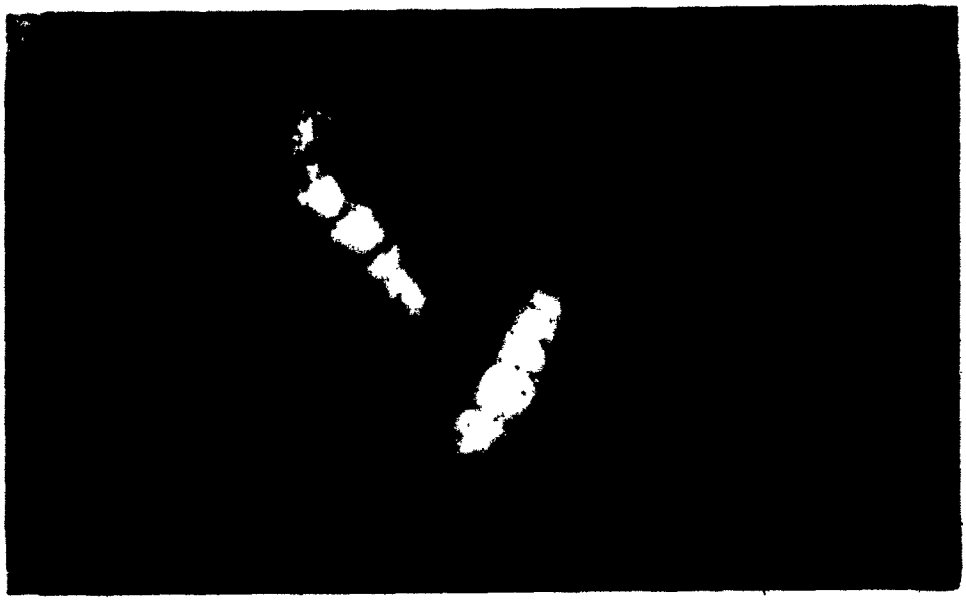
[a] All tiphiids collected as 1st instar ectoparasitic larvae.

[b] None found in 1981.

[c] Left column: number of hosts found parasitized on a given date, at a given site.
Middle column: number of parasites found on a given host; or, in case of more than one host found per given date and site, total parasites for the total of hosts.
Right column : number of parasites present per host after 36 hours of rearing at 20°C, 80% RH and no light.

Figure 48. Tiphia sp., an ectoparasite of third instar white grubs, Phyllophaga spp., in southern Quebec.
Top: parasitic larva (pointer), three days old. 2.5X.
Middle: mature larvae, 14 days old, still attached to the shrivelled cadavers of their hosts. 2X.
Bottom: pupal cocoon of Tiphia sp., with remnants of host.





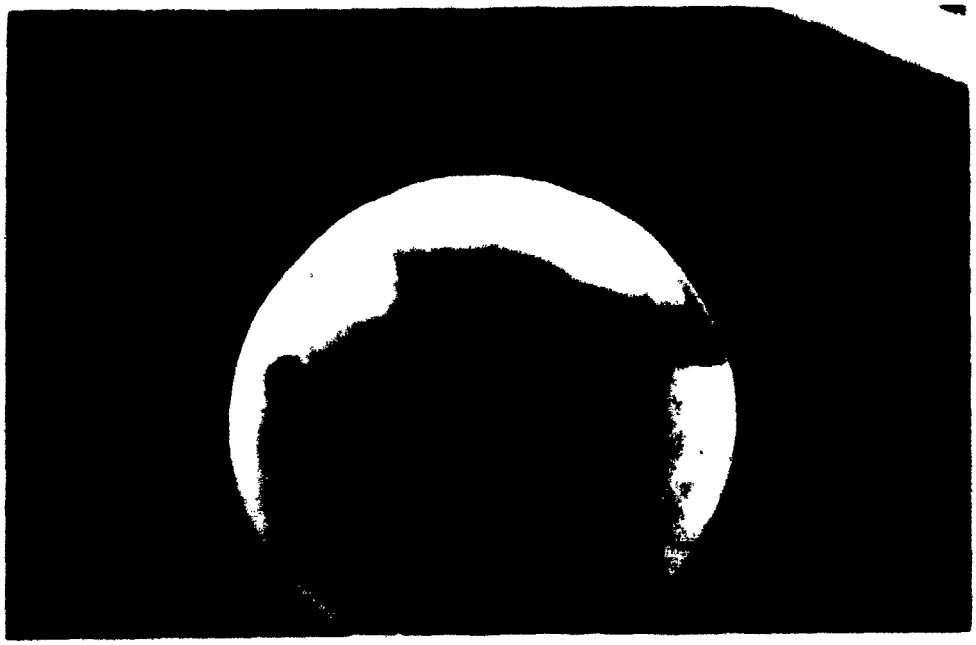
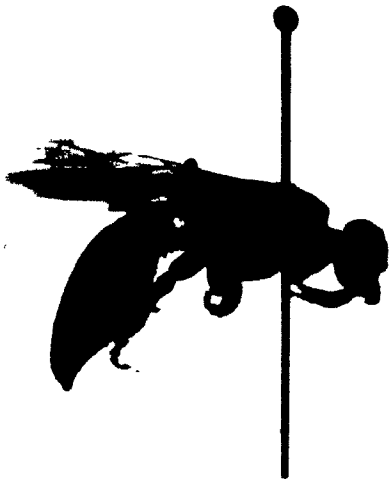


Figure 49. Adult of Tiphia inornata Say. 3.2X.



white grubs in one field were parasitized Berberet and Helms (1970) calculated that the density of cocoons of T berbereti Allen, a parasite of P anxia, was 5 to 7 per m^2 in northcentral Nebraska Askew (1971) and DeLong et al (1976) commented on the important regulating role of tiphiid wasps on natural populations of several species of scarabaeid beetles In Quebec, Lim (1979) found several Tiphia cocoons in the soil of pasture land infested with grubs of P anxia

The biology, habits and host relationships of species of Tiphiidae have been studied by Davis (1919), Clausen et al , (1927, 1932, 1933), Clausen (1940), Askew (1971)

Tiphiid wasps were not important biotic regulators of grubs of Phyllophaga spp in southern Quebec from 1979 to 1981 (Tables 30 and 32)

MUSCIDAE (DIPTERA)

A puparium of the muscid fly Pararicta pascuorum Mg was recovered from the abdominal cavity of one female P anxia cadaver collected in a pasture at Nicolet, August 20 1980, about 10 cm deep in the soil (Figure 50) The adult fly emerged from the puparium six days later This relationship is probably incidental To my knowledge, there is no authenticated record of muscid parasitization or predation on Phyllophaga spp but there is an exceedingly wide range of food habits in muscid maggots Many species are filth-inhabiting saprovores or scavengers, some are phytophagous, a few are vertebrate parasites (Riley and

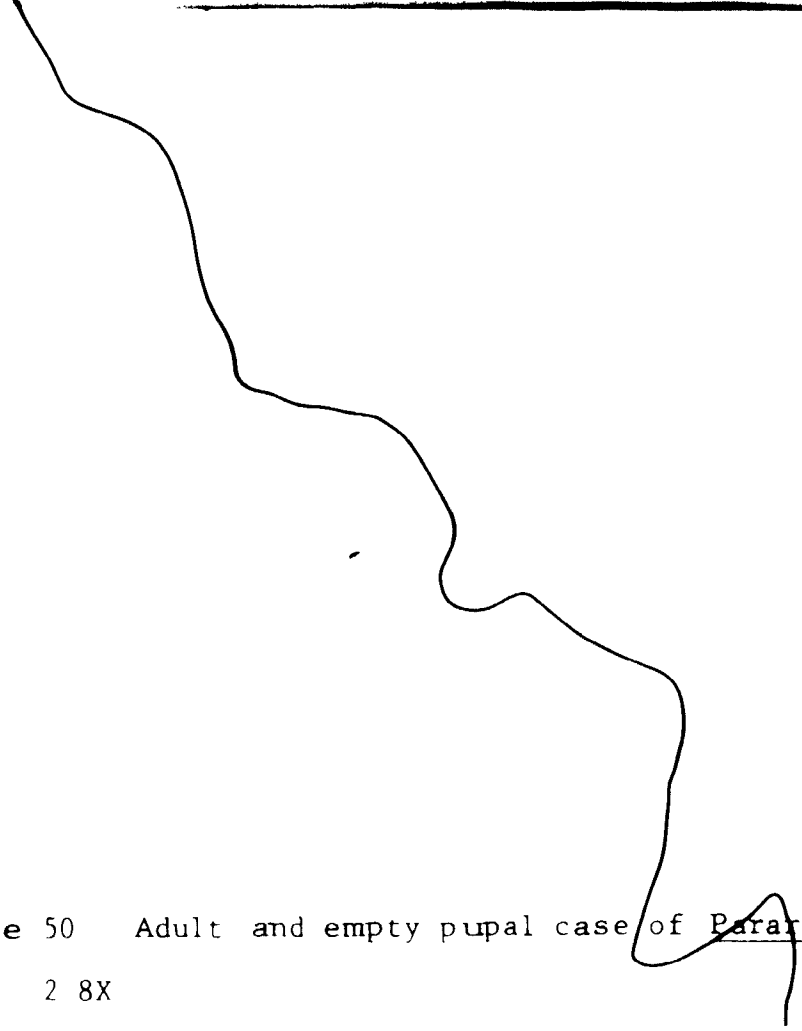


Figure 50 Adult and empty pupal case of Paraticia pascuorum

Mg 2 8X

Wallace, 1959) and several are insect predators (Vockeroth, 1979)

PYRGOTIDAE (DIPTERA)

Two cases of endoparasitism by an unidentified pyrgotid fly were found during this study The parasites were isolated, as pear-shaped puparia, from the abdominal cavity of the cadaver of two mature females P anxia collected at Pierreville, August 26, 1979

The pyrgotids (ortalids) are poorly known entomoparasites of scarab, especially melolonthine beetles Among the first published records of Pyrgota undata Weid as a parasite of Phyllophaga adults were those of Forbes (1907 1908) Davis (1913) reared Pyrgota valida Harris from adult June beetles Davis (1913, 1919) considered pyrgotid flies to be highly beneficial parasites and regulators of Phyllophaga populations, at least locally , in several American states, Ontario and Quebec Pyrgota undata appeared to be the most common species and was reared from nine species of Phyllophaga but not P anxia, collected in numerous localities including Quebec (Davis, 1919) Pyrgota valida was bred "many times" from 13 species of June beetles including P anxia, collected in several American states but not Quebec Petch and Hammond (1926) reported Pyrgota undata as a parasite of P anxia adults in Quebec, the authors stated that only about 0 1% of the beetles were attacked by this pyrgotid

SARCOPHAGIDAE (DIPTERA)

Unidentified sarcophagid fly maggots were recovered on three occasions from third instar Phyllophaga grubs (Table 30). The endoparasitic larvae were collected at Stanbridge East, (July 7, 1980), from the abdominal cavity of three live white grubs Davis (1919) reported the sarcophagids have been isolated from Phyllophaga adults but he questioned the actual parasitic status of several of the sarcophagids He did not report sarcophagid parasitism on other stages of Phyllophaga. True parasitism can only be assessed when the suspected parasites are recovered from a live host (Davis, 1919) The three solitary sarcophagid fly larvae that I recovered from live grubs can thus be given parasitic status

TACHINIDAE (DIPTERA) (Figures 51 and 52)

Parasitic tachinid flies were among the most common arthropod regulators of Phyllophaga spp. recorded during this survey A total of 421 tachinid larvae and pupae within their puparia were recovered from 179 of 33,468 Phyllophaga individuals collected over three years in southern Quebec (Tables 30 and 33). This represented 0 53% parasitization of examined Phyllophaga individuals Besides one pupa, only the grub and adult stages of Phyllophaga were found parasitized and the 179 cases of parasitization were 0 05% of 16,930 grubs or 0.07% of 11,945 third instar grubs, the only larval instar parasitized; 1.58% of 11,652 adults or 1 59% of 11,542 adults on the wing (no teneral

Table 33. Average number of tachinid flies, parasitic on Phyllophaga, found in a single host, in southern Quebec, 1979 to 1981.

Tachinid	Parasite stage	Host stage	Number of hosts found parasitized	Number of parasites found	Average number per host
Tachinid sp.	pupa	adult female	19	48	2 5
Eutrixa exilis	pupa	adult female	48	123	2 5
Eutrixa exilis	pupa	adult male	1	1	1
Cryptomeigenia sp.	maggot	adult female	22	53	2.5
Cryptomeigenia sp.	pupa	adult female	5	12	2.5
Cryptomeigenia sp.	maggot	adult female	1	1	1
Cryptomeigenia sp. (theutis?)	pupa	adult female	9	15	1 5
Cryptomeigenia theutis	pupa	adult female	49	113	2.5
Cryptomeigenia theutis	maggot	adult female	14	31	2
Cryptomeigenia theutis	pupa	adult male	1	1	1
Microphthalma sp.	maggot	3rd instar grub	7	18	2.5
Microphthalma michiganensis	maggot	3rd instar grub	2	4	2
Microphthalma michiganensis	pupa	pupa female	1	1	1

Figure 51. Top: two maggots, Cryptomeigenia theutis (Wlk.), in the abdominal cavity of a P. anxia female. 5X. Bottom: pupa of Eutrixa exilis (Coq.) in the abdominal cavity of a P. anxia female. 2X.

Figure 52. Adult of Microphthalma michiganensis Tnsd. 2.8X.

adults were parasitized); the latter number can be subdivided as 7.75% parasitization of 2,647 adult females and 0.02% of 8,895 adult males; one case (0.04%) of parasitization was observed among 2,186 pupae. Tables 30 and 33 summarize the above data and list the species of Tachinidae recovered from their hosts. Occasions of findings are too numerous to be reported here. Data pertaining to localities and dates appear on the labels of voucher specimens deposited in the collections named in "Materials and Methods" for this chapter.

The Tachinidae are, from an economic point of view, the most important of the dipterous families of entomophagous habits The biology, habits and hosts of numerous species have been reviewed by Criddle (1918); Davis (1919); Petch and Hammond (1925, 1926); Sweetman (1936); Clausen (1940); Curran (1947); Van Emden (1954); Stone et al. (1965); Jarvis (1966); Askew (1971). The family will not be further discussed here.

From the point of view of natural control of P. anxia, it is important to point out that, Microphthalma spp. excepted, all species of tachinid flies discussed here were found from adult beetles and that female P. anxia accounted for 98.8% of the 169 parasitized adults (Table 33).

To my knowledge, P. anxia is a new host record for Cryptomeigenia theutis.

ASILIDAE (DIPTERA)

Four maggots of this almost exclusively entomopredatory family of flies were found feeding on grubs in the course of

this study (Table 30) Attempts to rear the larvae to maturity were unsuccessful, the maggots were kept alive in moist sand for several months but they refused to feed on grubs presented to them and they all died eventually Two of the asilids were collected on third instar grubs in an old field at Stanbridge East (July 18, 1980), one was recovered at Nicolet from a second instar grub, (July 6, 1980) and one at Mirabel (July 26, 1980) from a third instar Phyllophaga grub None of the maggots has been identified below the family rank, but two adult Diogmites basalis (Walker) were caught in flight over the same Stanbridge East field, July 9, 1980

Davis (1919) reported that several species of larval asilids are predaceous insect enemies of Phyllophaga grubs He treated six asilid genera (Promachus, Erax, Deromyia, Asilus, Ceraturgus and Proctacanthus) as actual, probable, or possible predaceous enemies of white grubs Davis (1919) was more affirmative when he stated that Promachus vertebratus Say larvae were important predators of white grubs in several American states. Davis (1919) working with P vertebratus experienced the same difficulties as Felt (1915), working with P fitchii O S . to rear the recovered larvae to the adult stage. It appeared that both asilids had a probable three-years' life cycle The observations of these two workers are in accord with the difficulties encountered during this study in attempts to rear asilid maggots Petch and Hammond (1925, 1926) stated that Asilus snowi Hine and some unspecified asilid larvae were predaceous upon white grubs in Quebec Ritcher (1940) wrote that

the pupae of five Phyllophaga spp. were preyed upon by the maggot of Diogmites (=Deromyia) discolor Loew in Kentucky and Hull (1962) studied the ecology of Diogmites (=Deromyia) spp. In Texas, Daniels (1966b) observed that asilid larvae were predators of Phyllophaga koehleriana (Saylor) grubs Lim (1979) collected unspecified asilid larvae, and maggots and adults of Diogmites spp and one Asilus larva in southern Quebec All the larvae were found feeding on or in close proximity to P anxia second and third instar grubs from late spring to mid-summer

Most reports are vague about actual rates of predation The only specific quantitation was made by Ritcher (1940) who estimated that about 12% of the pupae of five Phyllophaga spp were killed by the maggots of an asilid fly in Kentucky. In my three-year study in southern Quebec, less than 0 02% of all the white grubs collected and no pupae, were found to have been attacked by Asilidae (Tables 30 and 33)

XI FIELD EVALUATION OF CHEMICAL ATTRACTANTS FOR THE COMMON JUNE BEETLE, PHYLLOPHAGA ANXIA (LECONTE), DURING THE 1981 FLIGHT SEASON IN FOUR LOCALITIES OF SOUTHERN QUEBEC

A INTRODUCTION

Semiochemicals, including attractants other than true sex attractants, have been searched for, assayed and used in pest control programs for several purposes The main uses of semiochemicals in agricultural insect control are the study of population distribution and movements, crop monitoring and warning systems, and the direct and indirect suppression of noxious insects. One aspect of indirect suppression of a pest by means of semiochemicals is the dissemination of entomopathogenic microorganisms by the target insect itself lured to traps baited with an attractant combined with infective or resistant stages of pathogens This means of control has sometimes been referred to as "autocidal control", both parents and progeny may contact a disease, the former by self and cross-contamination, the latter by vertical transmission and by inoculation of its breeding and feeding grounds by the parental activity This appealing method of insect control has been advocated by Daum et al (1967), Beroza (1972), Glass (1975), Burkholder (1977, 1980), Roelofs (1979), Shorey (1981), and others and its use has been explored against several pest species

McLaughlin (1966, 1967) demonstrated the effective use of a bait containing a phagostimulant and the sporozoan, Mattesia grandis McLaughlin, in spreading the pathogen throughout cotton fields for the control of the boll weevil, Anthonomus grandis Boheman McLaughlin et al (1969) further field tested a bait containing a feeding stimulant and the sporozoans M grandis and Glugea gasti McLaughlin against the same cotton pest Field control of Heliothis spp has been attempted with a nuclear polyhedrosis virus combined with a bait developed for the boll weevil (McLaughlin et al , 1971) Experiments with traps baited with a potent attractant, ethyl chrysanthemumate, and Rhabdionvirus oryctes Huger virus were conducted by Maddison et al (1973) in an attempt to demonstrate the feasibility of the autodissemination of the pathogen by its host, the coconut rhinoceros beetle, Oryctes rhinoceros (L) Schwalbe et al (1974) evaluated in laboratory and field assays the concept of utilizing inoculation devices treated with a pheromone and spores of Mattesia trogodermae Canning to control a stored-product pest, Trogoderma glabrum (Herbst) Campion (1975) reported the possibility of controlling the Egyptian cotton leafworm, Spodoptera littoralis Boisduval, by attraction to traps baited with pheromones and various virus preparations Burkholder (1977) and Shapas et al (1977) reported the efficacy of sex pheromones-pathogenic protozoans combinations in the control of store-product pests, especially T glabrum Knipling (1979) suggested the suppression of the imported fire ants, Solenopsis invicta and S richteri (Forel) by attracting them to a phagostimulant bait

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contaminated with a suitable pathogen Bedford (1981) advanced the idea of transmitting baculoviruses into populations of O rhinoceros by attractant-trapping of a few adults using ethyl chrysanthemumate

The literature on attractant-trapping of scarabaeid pests is voluminous, especially for the Japanese beetle, Popillia japonica Newman Table 34 summarizes the information most relevant to this investigation with Phyllophaga anxia

The primary objective of the study reported here was to ascertain the relative attractancy to P anxia adults of single natural and synthetic lures selected for their attractiveness to other scarab beetles "Natural lure" is defined here as a chemical known to be present in an insect species, phenol falls in this category (Beroza, 1972) "Synthetic lure" is a chemical that is not yet known to be part of the natural stimulus world of an insect species, although empirical screening has demonstrated that individuals of a given species are attracted to these chemicals (Shorev, 1981)

B MATERIALS AND METHODS

1 Test Fields

Four localities were selected to conduct the experiments (Table 35) All test fields were chosen in southern Quebec since heavy flights of the common June beetle were forecast for this region for 1981 (Lim, 1979) The fields were unique because they had received neither cultural nor chemical treatments for at

Table 34 Approach to the study of potential attractants for Phyllophaga anxia, justification of the choice of chemicals selected for the 1981 field trials, based on their known attractancy to other scarab beetles, including reports on attraction to other compounds, sex attractants and their mimics, and females of various Scarabaeidae [a]

Insect species	Attractants [b,c,d]	References
	Females	Smith & Hadley, 1926, Goonewardene et al , 1970, Ladd, 1970; Klein et al , 1972, 1973, Tumlinson et al , 1977, Doolittle et al , 1980
	Sex attractants	Adler & Jacobson, 1971
	Female sex pheromone	Blum, 1977
	Japonilure	Doolittle et al , 1980; Klein, 1981b.
Popillia japonica Newman	Japonilure + PEP eugenol (3 7)	Klein et al , 1981
	Japonilure + PEP eugenol geraniol (3.7 3)	Ladd et al , 1981
	Eugenol, geraniol	Richmond, 1927, Langford et al , 1943
	Geraniol eugenol (10 1)	Metzer, 1934

Table 34 - Continued

Insect species	Attractants	References
	Geraniol eugenol (10 8)	Fleming & Burgess, 1940.
	Geraniol eugenol (9 1)	Fleming et al , 1940
	Anethole eugenol (9 1)	Fleming & Chisholm, 1944
	Eugenol, caproic acid, PEB, geraniol (various blends)	Langford & Cory, 1946, Schwartz et al , 1966
	Eugenol, anethole, geraniol, eugenol anethole (9 1)	Fleming, 1969
Popillia japonica Newman	MCP eugenol (9 1)	McGovern et al , 1970b
	PEP, PEP eugenol (9 1)	McGovern et al., 1970c
	Geraniol PEB eugenol (9 9 2), geraniol PEB phenyl isovalerate eugenol (9 9 9 3)	Schwartz et al , 1970
	MCP eugenol geraniol (1 1 1)	Ladd, 1971
	PEP eugenol (7 3) + virgin females	Klein et al , 1973, Klein, 1981b.

Table 34 - Continued.

Insect species	Attractants	References
	MCP, PEP, eugenol, geraniol (various blends)	Ladd et al., 1973, 1974, 1975, 1976.
	Eugenol; caproic acid	Ladd, 1980.
Popillia japonica Newman	PEP: eugenol . geraniol (3:7:3)	Ladd & McGovern, 1980.
	PEP + eugenol, methyl cyclohexanecarboxylate + eugenol	McGovern, 1981 (personal communication).
	Java citronella oil	Whitcomb, 1947; Tashiro & Fleming, 1954.
	Java citronella oil . eugenol (3:1)	Tashiro & Fleming, 1954; Tashiro et al., 1969.
Rhizotrogus (Amphimallon) majalis (Razoumowsky)	Butyl sorbate	Beroza & Green, 1963a, 1963b; Beroza & Sarmiento, 1964; McGovern et al., 1966; Tashiro et al., 1964, 1969, 1970.
	Benzoic acid; chrysanthemumic acid; citronellal;	Tashiro et al., 1964.

Table 34 - Continued:

Insect species	Attractants	References
	cyclohexanol, eugenol; geraniol, phenol, propionic acid, menthol	Tashiro et al., 1964.
	Propyl 1,4-benzodioxan-2-carboxylate	Roelofs et al., 1967, McGovern et al., 1970a.
Rhizotrogus (Amphimallon) majalis (Razoumowsky)	Isobutyl 1,4-benzodioxan-2-carboxylate	McGovern et al., 1970a; Tashiro et al., 1970.
	Cyclohexanol, anthranilic acid; benzoic acid esters; decanoic acid; sodium benzoate; propionic acid; oil of thyme, rosemary oil; menthol; oil of laurel	Tashiro et al., 1970.
Oryctes rhinoceros L.	Unspecified	Cumber, 1956.
	Chrysanthemumic acid derivatives	Catley, 1966

Table 34 - Continued.

Insect species	Attractants	References
	Ethyl dihydrochrysanthemumate	Barber et al., 1971; Maddison, 1971.
Oryctes rhinoceros L.	Ethyl chrysanthemumate	Maddison, 1973; Maddison et al., 1973.
	(±)-des-N-morphinan	Vander Meer et al., 1979.
	Females	Kelsey, 1967.
	Oil from elder flowers	Osborne & Hoyt, 1968.
Costelytra zealandica (White)	Phenols; phenolic resins; fluorophenols	Henzell et al., 1969; Henzell, 1970; Henzell & Lowe, 1970; Osborne & Hoyt, 1969, 1970; Hoyt et al., 1971; Lauren, 1979; Osborne et al., 1979.
	Vanillin	Henzell, 1970.
Plectris aliena Chapin	Females	Roberts, 1968.

Table 34 - Continued.

Insect species	Attractants	References
Macrodactylus subspinosus (Fabricius)	Unspecified bait	Johnson, 1940.
Phyllophaga spp.	Isoamyl acetate	Jacobson, 1965.
Phyllophaga pleei Blanchard	Eugenol; anethol; eugenol: anethol (9:1); phenol; phenolic resins; anethol: eugenol (9:1)	Gruner, 1973, 1975a.
Phyllophaga patrueloides Paulian	Phenol; anethol; eugenol; isoamyl acetate; anethol: eugenol (9:1)	Gruner & Marival, 1974; Gruner, 1975a, 1975b.
Phyllophaga lanceolata (Say)	Isoamyl acetate; females	Travis, 1939; Jacobson, 1965.
Rhopaea spp.	Females	Soo Hoo & Roberts, 1965.
Melolontha vulgaris (Fabricius)	Females	Hauser, 1880.
Polyphylla decemlineata (Say)	Females	Lilly & Shorthouse, 1971.

Table 34 - Continued.

Insect species	Attractants	References
Cyclocephala insulicola Arrow	Anethol; eugenol; anethol: eugenol (9:1); isoamyl acetate; phenol	Gruner, 1971, 1975a; Chalumeau & Gruner, 1974; Gruner & Marival, 1974.
Cyclocephala rubiginosa Burmeister	Phenol; anethol:eugenol (9:1)	Gruner, 1975a.
Cyclocephala tridentata Fabricius	Phenol	Gruner & Marival, 1974.
Unspecified South African Scarabaeidae	β-ionone; cinnamyl alcohol	McGovern, 1981 (personal communication).

[a] List restricted to publications reporting results of field trials; only the best performing lures are tabulated here.

[b] Chemical names as they appear in the literature; synonymy is given in Table 36.

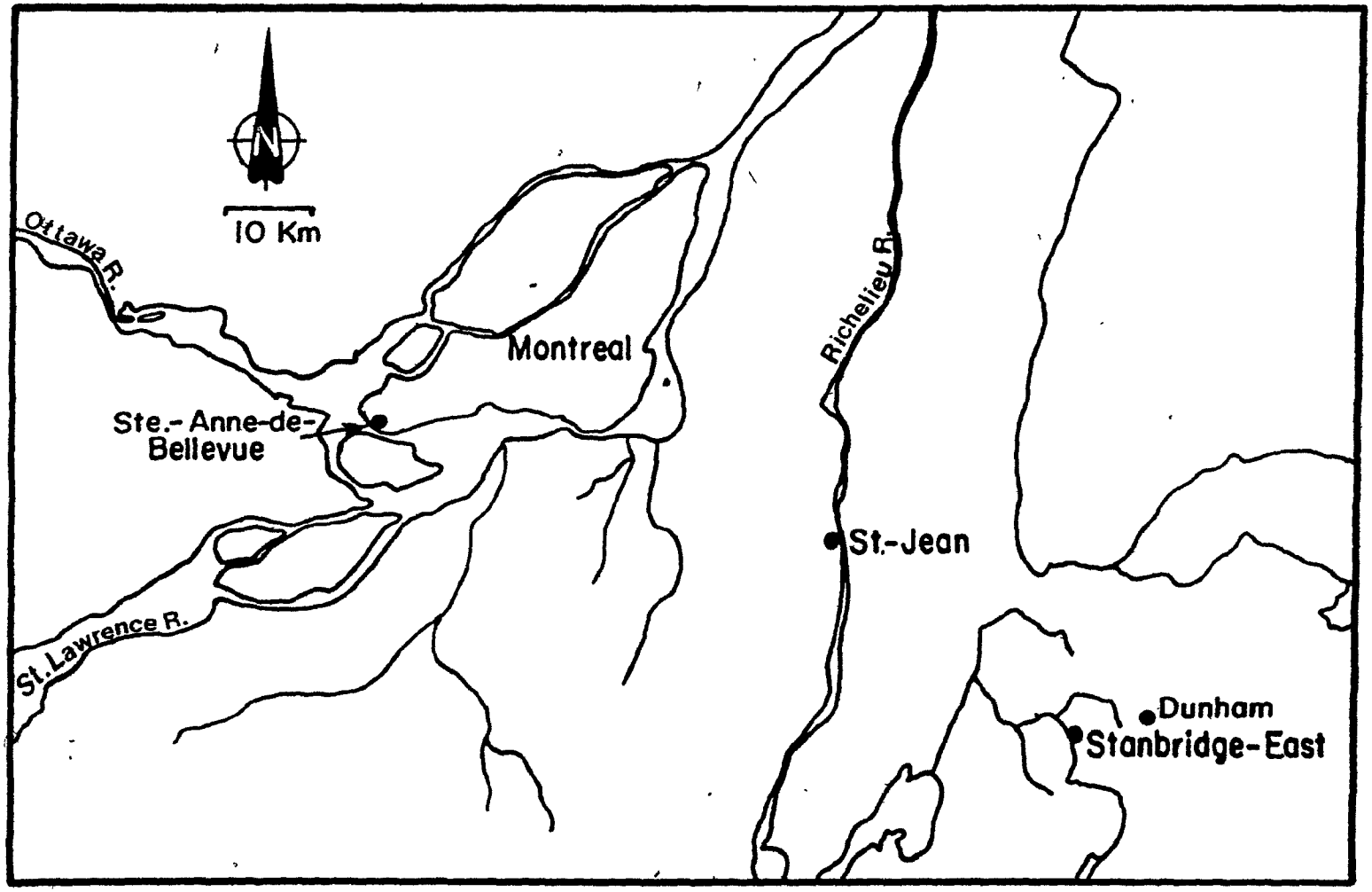
[c] Numbers in parentheses refer to the composition (V./V.) of mixtures.

[d] PEB = phenylethyl butyrate; PEP = phenylethyl propionate; MCP = methyl cyclohexane porpionate.

Table 35. Locations of June beetle attractants trials, southern Quebec, 1981.

Field location and number on map	Prevailing winds[a]	Field description	Soil	Number of experiments
1. Ste-Anne-de-Bellevue	SW	Hay field	Clay	3
2. St-Jean-sur-Richelieu	SW	Hay field	Sandy loam	1
3. Stanbridge East	SW	Abandoned corn field	Sandy loam	2
4. Dunham	ESE	Meadow in apple orchard	Sandy loam	2

[a] Late spring and early summer 1981.


N
10 Km
Ottawa R.
Montreal
Ste.-Anne-de-Bellevue
Richelieu R.
St.-Jean
St. Lawrence R.
Dunham
Stanbridge-East

least five years and were guaranteed to stay undisturbed, e.g. by hay cutting, for the duration of the experiments Surrounding woodland, acting as a natural windbreak, protected the habitats against strong winds, excepting at St.-Jean-sur-Richelieu. None of the fields had a slope greater than 5 percent. The fields (Figure 53) were free of natural obstacles and were covered with medium to dense ground vegetation composed mainly of various grasses, clovers, Artemisia, Chrysanthemum, Solidago, and Taraxacum spp.

2. Test Traps

Traps (Figure 54) modified from the European chafer trap designed by Tashiro and Fleming (1954) were built for the experiments. The main body of the traps consisted of four masonite baffles (2) (length : 36 cm; width : 15 cm) secured at right angles, a collecting polyethylene funnel (4) (height : 15 cm; radius : 15 cm) to which was attached an end cylinder (length : 3 cm; radius : 2 cm), and a plastic collecting bag (5) secured to the cylinder by a rubber band. Early in the experiments, the bag was replaced by a fine mesh muslin bag to prevent the accumulation of rain water and to allow better ventilation and a resting surface for trapped beetles. The rubber bands, cracked after a few days in the sun, were replaced by more weather-resistant cotton strings. A window (3.7 x 11 5 cm for each baffle) cut in the center of the main body of the trap held a galvanized square-weave chicken wire (0.5 cm mesh) cylinder (3) supported by a small masonite platform affixed to the bottom part of the

Figure 53. Experimental field at St -Jean-sur-Richelieu, Quebec (location 2).

Figure 54 Modified European chafer trap (after Tashiro and Fleming, 1954)

1 Horizontal branch of gallow

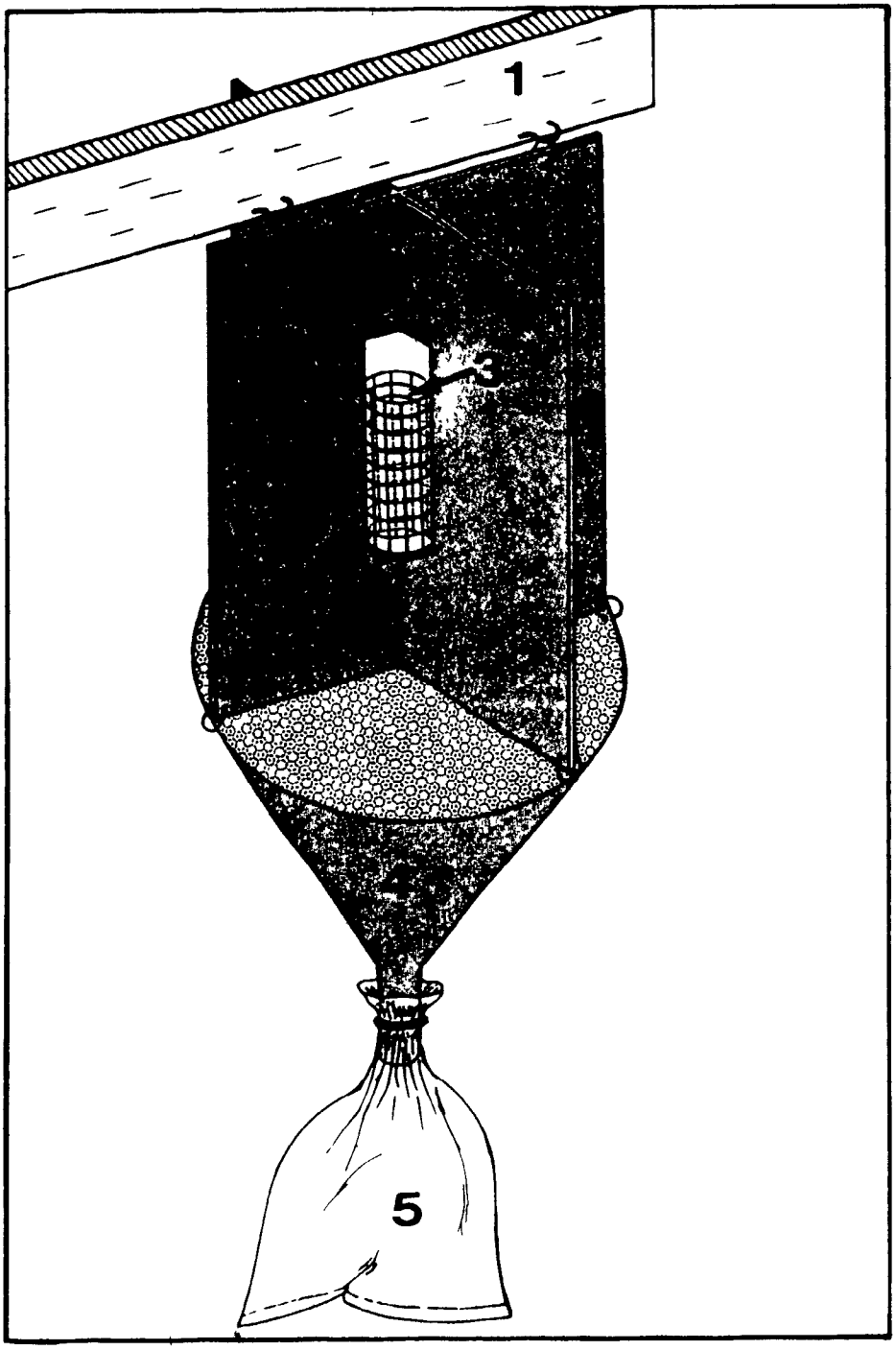
2 Baffle

3 Dispensing chamber

4 Collecting funnel

5 Collecting bag

Dimensions are given in the text


1
5

window With the exception of the collecting bags, all parts of the traps were painted with a commercial glossy black enamel, a color used by Tashiro and Fleming (1954), Tashiro et al (1964, 1969) when trapping the European chafer Each trap was screwed to the extremity of the 60 cm long horizontal branch (1) of a spruce timber gallow (vertical branch 180 cm long, including 30 cm driven in the soil) Traps were thus suspended at 150 cm above ground level, a standard height recommended for blacklight traps in June beetle surveys (Lim, 1979) The overall cost per trap was $10 00

3 Test Compounds

Most of the chemicals tested as attractants for P anxia were selected for their known attractiveness to other scarabaeid species (Table 34) Table 36 lists the names of the 27 chemicals tested, their synonyms and formulae, and the physical state and grade in which they were assayed in the field Natural aromatic oils were purchased from the Wide World of Herbs store, Montreal Anlure, Chrislure, butyl sorbate, cinnamyl alcohol and β-ionone were obtained from the U S Department of Agriculture, Organic Chemical Synthesis Laboratory, Beltsville, Maryland, through the courtesy of Dr McGovern The other products were purchased from chemical supply houses

As shown in Table 36, each chemical was assayed in the field in its original form and concentration, a procedure followed by most workers in initial empirical screening of candidate lures Some of the chemicals, however, needed treatment

Table 36. Chemicals tested in the field as attractants for Phyllophaga anxia (LeConte) adults; southern Quebec, 1981.

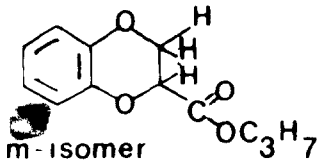
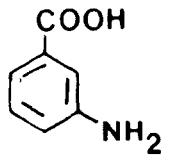
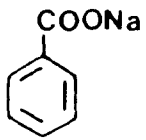
Chemical	Synonyms[a]	Empirical and/or[a] structural formulae	Physical state	Grade
Amlure®	propyl 1,4-benzodioxan-2-carboxylate	$C_{12}H_{14}O_4$  m-isomer	slightly viscous liquid	99% pure
Anthranilic acid	2-aminobenzoic acid O-aminobenzoic acid	$C_6H_4(NH_2)(CO_2H)$ 	solid, soluble in alcohol	99% pure
Benzoic acid (sodium salt)	benzenecarboxylic acid oracylic acid phenylformic acid carboxybenzene	C_6H_5COONa  Na benzoate	solid, soluble in water	99% pure

Table 36. cont'd

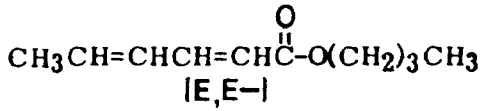
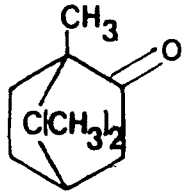
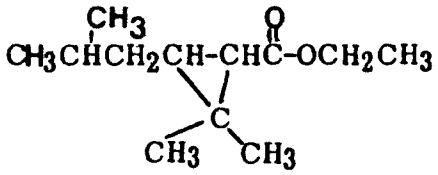
Chemical	Synonyms[a]	Empirical and/or[a] structural formulae	Physical state	Grade
Butyl sorbate	butyl(E-E)-2-4-hexadienate	$C_{10}H_{16}O_2$  (E,E-)	viscous liquid	99% pure
Camphor	1,7,7-trimethylbicyclo=(2.2.1) heptan-2-one 2-bornanone 2-camphanone 2-keto-1,7,7-trimethyl-norcamphane	$C_{10}H_{16}O$ 	solid, soluble in alcohol	technical
Chrislure®	ethyl dihydrochrysanthemumate ethyl 3-isobutyl-2, 2-dimethyl=-cyclopropanecarboxylate	$C_{12}H_{22}O_2$  cis, trans mixture	liquid	95% pure

Table 36. cont.

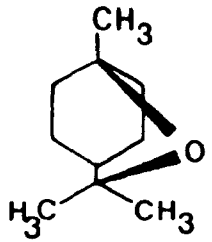
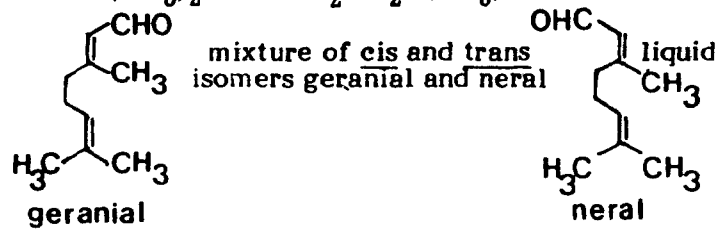
Chemical	Synonyms[a]	Empirical and/or[a] structural formulae	Physical state	Grade
Cineol(e)	1,3,3-trimethyl-2-oxabicyclol (2.2.2)octane 1,8-epoxy-p-menthane eucalyptol cajeputol	$C_{10}H_{18}O$ 	liquid	99% pure
Cinnamyl alcohol	3-phenyl-2-propen-1-ol cinnamic alcohol styryl carbinol γ-phenylallyl alcohol phenylallylic alcohol	$C_9H_{10}O$ $C_6H_5CH{=}CHCH_2OH$	solid, melting by warming in hot water	99% pure
Citral (commercial=mixture of α and β isomers)	3,7-dimethyl-2,6-octadienal geranial geranialdehyde	$(CH_3)_2C{=}CHCH_2CH_2C(CH_3){=}CHCHO$  mixture of cis and trans isomers geranial and neral geranial neral	viscous liquid	95% pure

Table 36. cont.

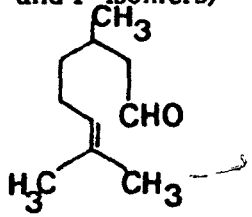
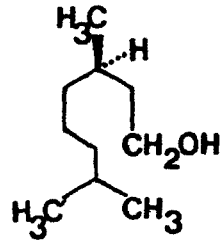
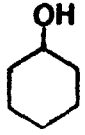
Chemical	Synonyms[a]	Empirical and/or[a] structural formulae	Physical state	Grade
(Java) citronella oil		mixture of citronellal (50%) and geraniol (45%)	volatile oil	95% pure
Citronellal	3,7-dimethyl-6(or 7)-octenal	$C_9H_{17}CHO$ (d- and l- isomers) 	liquid	99% pure
Citronellol	3,7-dimethyl-6(or 7)-octen-1-ol 2,6-dimethyl-2-octen-8-ol β-citronellol cephrol rhodinol	$CH_2=C(CH_3)(CH_2)_3CH(CH_3)-CH_2CH_2OH$ (7-octone form) 	liquid	99% pure
Cyclohexanol	hexalin hexahydrophenol	$C_6H_{11}OH$ 	solid, soluble in alcohol	99% pure

Table 36. cont.

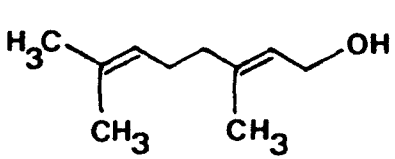
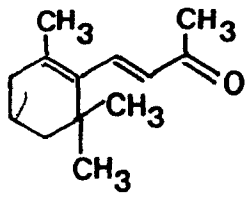
Chemical	Synonyms[a]	Empirical and/or[a] structural formulae	Physical state	Grade
Decanoic acid	n-capric acid decylic acid decoic acid	$CH_3(CH_2)_8\ COOH$	solid, soluble in alcohol	99% pure
Geraniol	(E)-3,7-dimethyl-2,6-octadien-1-ol 2,6-dimethyl-2,6-octadien-8-ol lemonol	$C_{10}H_{18}O$ 	viscous liquid	99% pure
Hexanoic acid	n-caproic acid hexylic acid hexoic acid	$CH_3(CH_2)_4COOH$	viscous liquid	99.5% pure
β-Ionone	4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one irisone β-cyclocitrylidenacetone	$C_{13}H_{20}O$ 	liquid	99% pure

Table 36. cont.

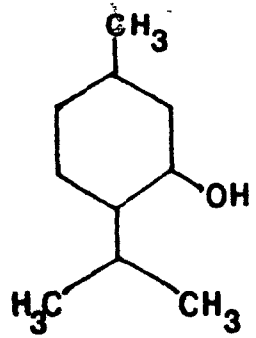
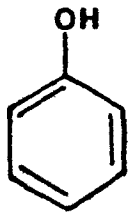
Chemical	Synonyms[a]	Empirical and/or[a] structural formulae	Physical state	Grade
Lauric acid	dodecanoic acid	$CH_3(CH_2)_{10}COOH$	solid, soluble	99.5% pure
	dodecoic acid		in alcohol	
	laurostearic acid			
Menthol	5-methyl-2-(1-methylethyl)= cyclohexanol	$C_{10}H_{20}O$ 	solid, soluble	99% pure
	3-p-menthanol		in alcohol	
	hexahydrothymol			
	para-menthan-3-ol			
	peppermint camphor			
	methylhydroxyisopropyl= cyclohexane			
Phenol	carbolic acid	C_6H_5OH 	solid, soluble	99% pure
	phenic acid		in alcohol	
	phenylic acid			
	hydroxybenzene			
	phenyl hydroxide			
	oxybenzene			
	benzophenol			

Table 36. cont.

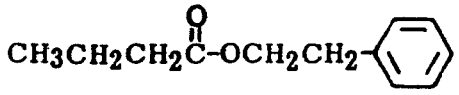
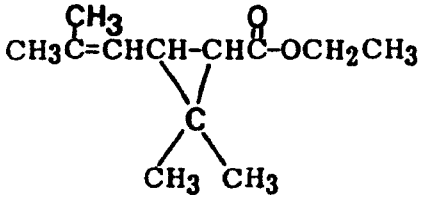
Chemical	Synonyms[a]	Empirical and/or[a] structural formulae	Physical state	Grade
β-Phenylethyl butyrate	phenethyl butyrate	$C_{12}H_{16}O_2$ 	liquid	technical
Rhinolure®	ethyl chrysanthemumate ethyl 2,2-dimethyl-3-(2-methylpropenyl)cyclopropane-carboxylate	$C_{12}H_{20}O_2$  cis, trans mixture	liquid	95% pure
Rosemary oil		mixture of 10% total borneol and 2.5% bornyl acetate, camphor, eucalyptol, pinene, camphene	volatile oil	99% pure

Table 36. cont.

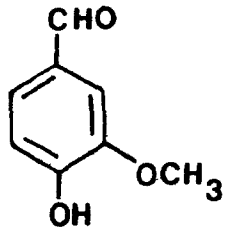
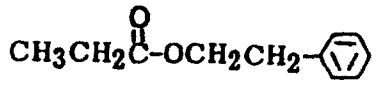
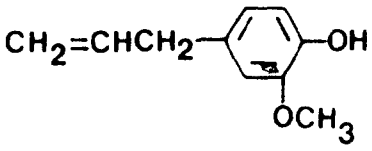
Chemical	Synonyms[a]	Empirical and/or[a] structural formulae	Physical state	Grade
White thyme oil		mixture of 20–40% by volume of thymol and carvacrol, cymene, pinene, linalool and bornyl acetate	volatile oil	99% pure
Vanillin	4-hydroxy-3 methoxybenzaldehyde vanillic aldehyde 3-methoxy-4-hydroxybenzaldehyde methylprotocatechnic aldehyde		solid, soluble in alcohol	99% pure
β-Phenylethyl propionate[b]	phenethyl propionate(PEP) 2-phenylethyl propionate	$C_{11}H_{14}O_2$ 	liquid	technical

Table 36. cont.

Chemical	Synonyms[a]	Empirical and/or[a] structural formulae	Physical state	Grade
Eugenol[b]	2-methoxy-4-(2-propenyl)phenol 4-allyl-2-methoxyphenol 4-allylguaiacol eugenic acid caryophyllic acid	$C_{10}C_{12}C_2$ 	liquid	99%

[a] Sources: Kenaga and End (1974); the Merck Index (1976); Hawley (1981).

[b] Tested as the arbitrary standard bait in a 9:1 (v./v.) mixture of PEP and eugenol.

before being applicable to dispensing strips Cinnamyl alcohol, a low melting solid, was melted by warming in hot tap water Butyl sorbate and sodium benzoate were dissolved in acetone and distilled water, respectively All other solids were dissolved in 95% ethanol

Five drops of each potential attractant were evenly distributed with a pipette on a Whatman filter paper strip (11 x 3.5 cm) and time was allowed for the lures and the solvents to spread over the entire strip (Tashiro et al , 1964, 1969, 1970) Dispensing strips loaded with dissolved products were placed under the gentle draft of a chemical hood and the solvents were allowed to evaporate. All the treated strips were kept in sealed glass jars until they were taken to the field for testing purposes (Tashiro et al., 1964, 1970) The dispensing strip method was preferred to the most widely used wick-fitted bottle dispenser because some of the compounds were obtained in only very small amounts.

4 Field Layouts

Eight experiments (cut design in space and time) were conducted in four locations The basic field layout for each location is represented in Figures 55 to 58 One experiment was performed at location 2, two experiments at locations 3 and 4, and three experiments at location 1 (Table 35). The layout of the second experiment at location 3 was similar to the layout of the first experiment but three traps in each block were removed from the western side of the field and were used in the

Figure 55 Field layout of attractants trials at Ste -Anne-de-Bellevue (location 1), southern Quebec, 1981

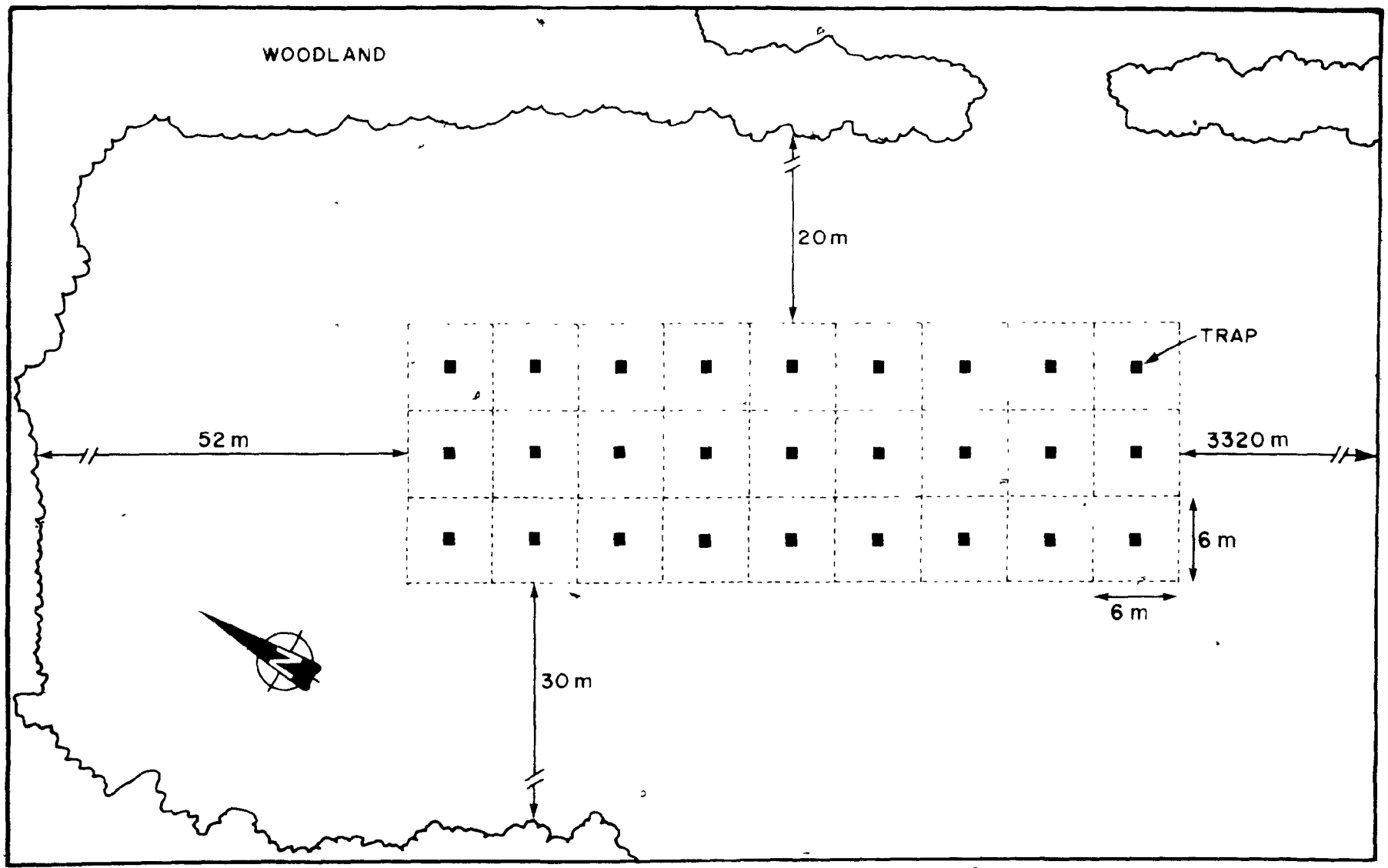

WOODLAND
20m
TRAP
52m
3320m
6m
6m
30m

Figure 56 Field layout of attractants trials at St -Jean-sur-Richelieu (location 2), southern Quebec, 1981

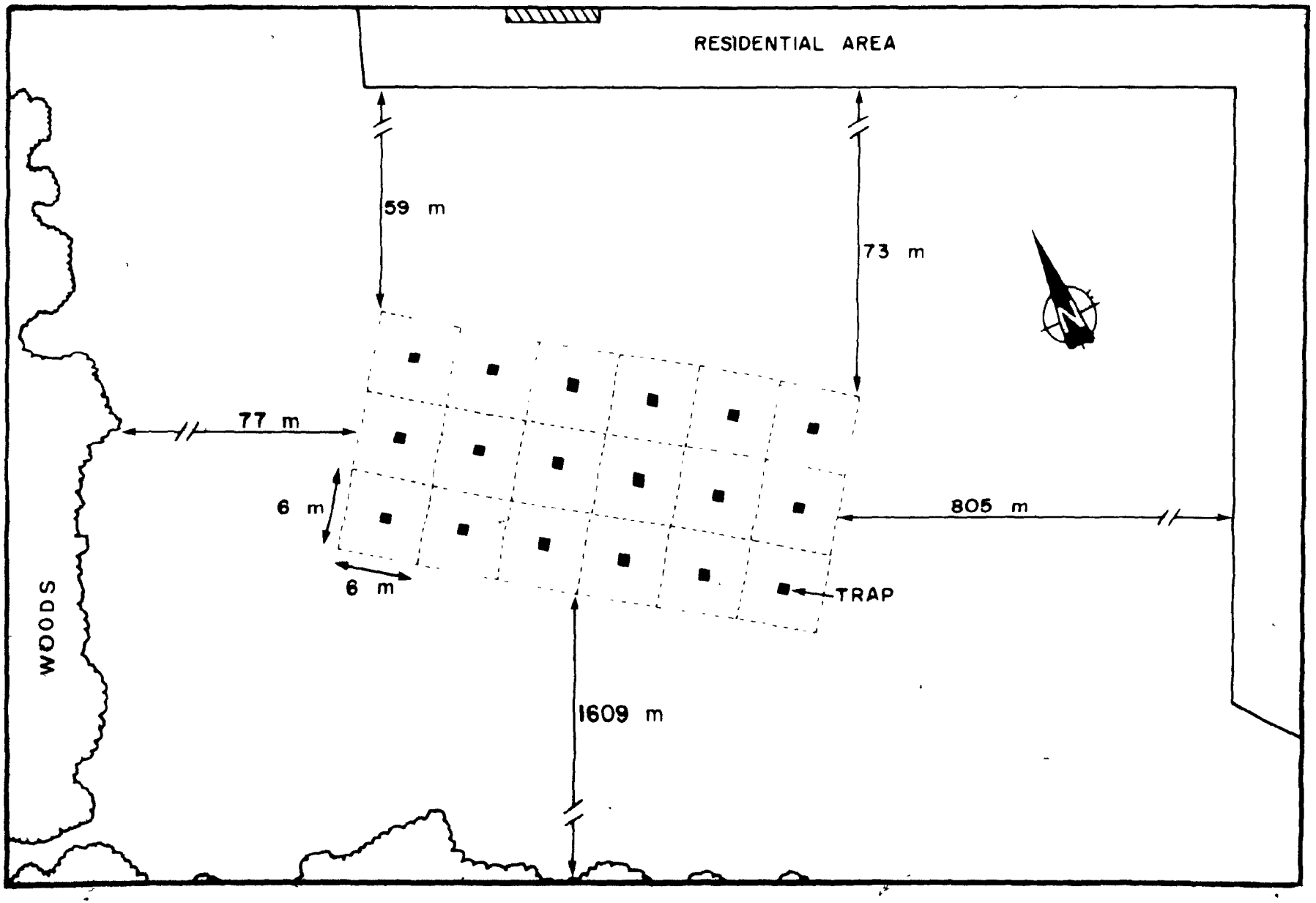

RESIDENTIAL AREA
59 m
73 m
77 m
6 m
6 m
805 m
TRAP
WOODS
1609 m
N

Figure 57 Field layout of attractants trials at Stanbridge East (location 3), southern Quebec, 1981

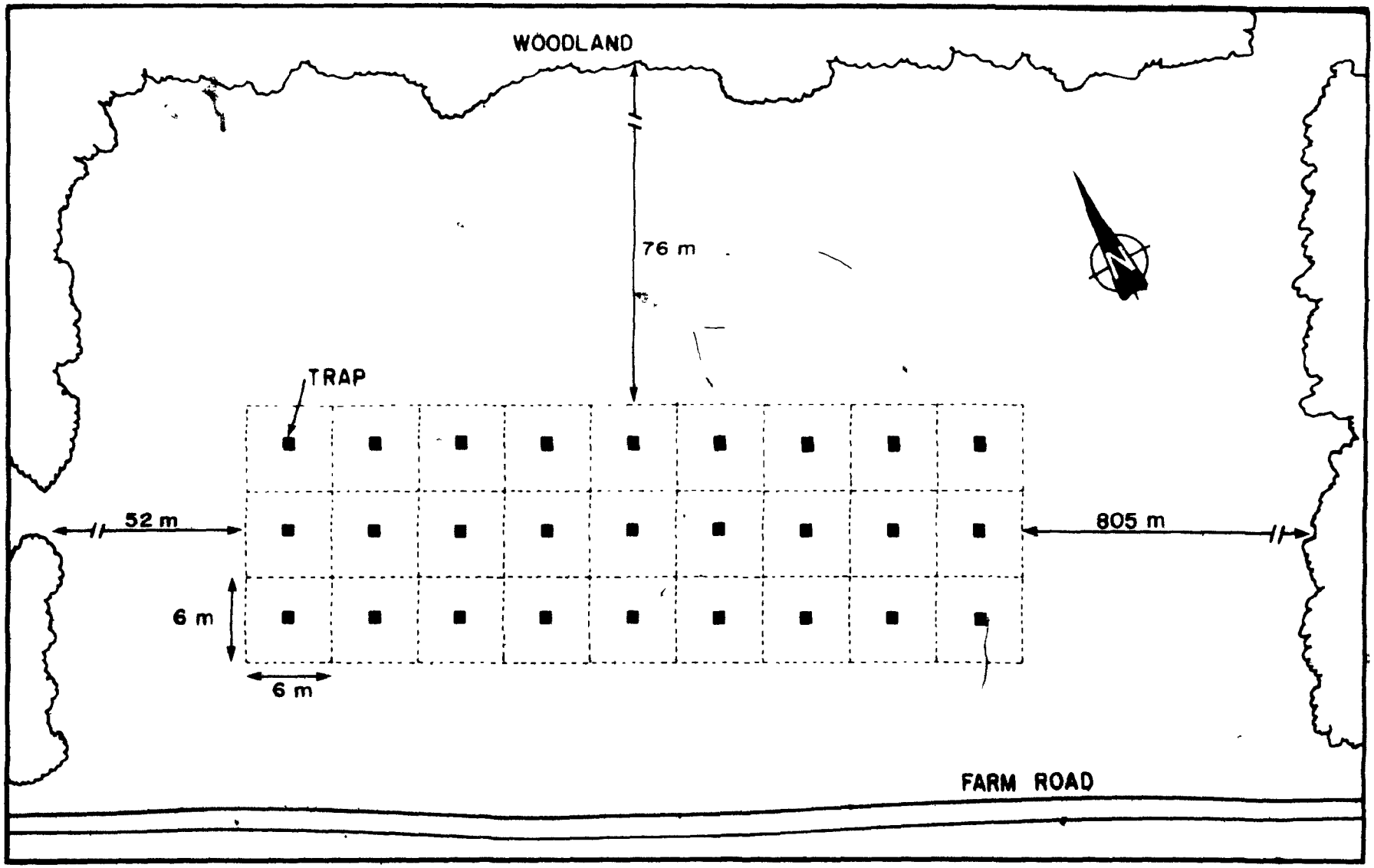
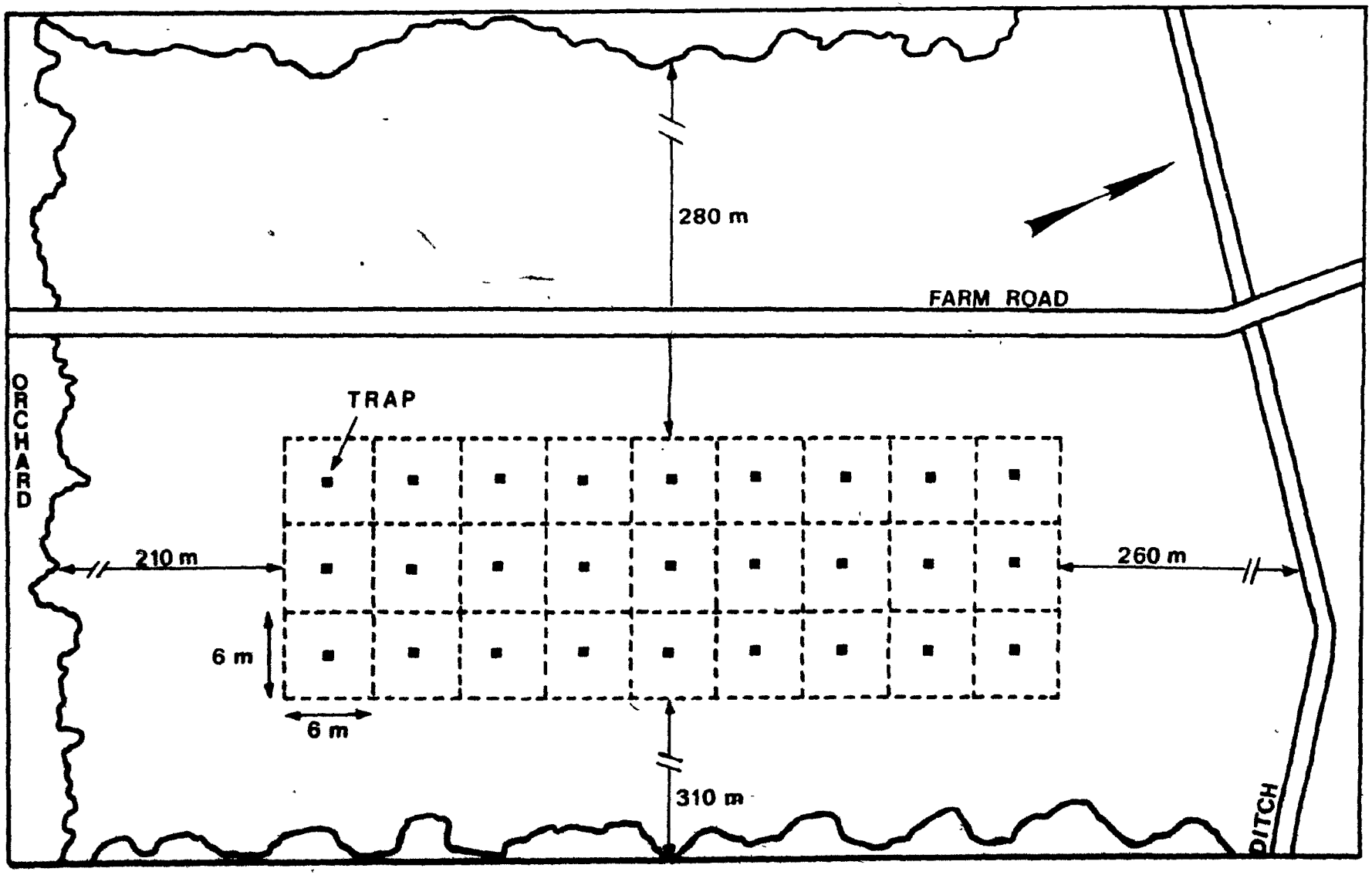

WOODLAND
76 m
TRAP
52 m
805 m
6 m
6 m
FARM ROAD

Figure 58 Field layout of attractants trials at Dunham (location 4), southern Quebec, 1981


280 m
FARM ROAD
ORCHARD
TRAP
210 m
260 m
6 m
6 m
310 m
DITCH

experiment at location 2, later in the season. In each layout the test traps were distributed in a 3-replicate randomized block design (RBD) The traps were spaced at 6 m intervals in the rows and columns (Tashiro et al., 1964, 1970) A new RBD was generated every third day within each experiment in order to minimize the effects of extrinsic factors such as direction of prevailing winds and proximity of food sources for emerging beetles, also the effects of intrinsic factors such as trap interactions and clumping of beetles within the field layouts. A different RBD was also generated at the start of each experiment at each location. The 3-replicate RBD permitted the simultaneous exposure of up to seven candidate attractants plus one standard and one control, the latter consisting of unbaited traps No buffer traps were used in the experiments.

The presence of adequate June beetle populations was confirmed by monitoring blacklight traps in the vicinity of the test fields at all locations. The light traps were not visible from any point of the test fields and were operated for the duration of the experiments on attractants.

5 Timing of the Experiments

In southern Quebec, adult June beetles emerge from the ground about mid-May and are actively on the wing until mid-July (Lim, 1979). My observations in 1979 and 1980 (Chapter III) confirmed Lim's (1979) reports and agreed with the existence of a peak activity during the first three weeks of June Field experiments on June beetle attractants were thus conducted to

coincide with the adult flight period The first field test started at location 1 on the night of May 19 and the last test was terminated at location 3 on the morning of June 23. Detailed timing data on the eight field experiments for the four locations are provided in Table 37.

6. Experimental Procedure

Each evening, one to two hours before beetle flight began, one test strip was placed into the screened cage of the appropriate trap. Care was taken to handle the baited strips with forceps to minimize chances of contamination of strips or traps. Strips were replaced every night to accomodate variability in rates of volatilization of chemicals and to reduce the effects of extrinsic factors such as dilution by rain water and photooxidation of some or all of the materials. The screened cages and their stands were thoroughly washed in acetone for reuse before starting and at each change within an experiment. Beetles caught in traps were collected the following morning and either brought to the laboratory (locations 1 and 2) or kept refrigerated and collected weekly (locations 3 and 4). Beetles were identified, sexed, and counted. Records of numbers caught were kept and all of the data were pooled at the end of each experiment.

The attractiveness of each candidate bait was determined by comparing numbers of beetles lured by that bait with numbers of beetles caught with the arbitrary standard. The standard was a mixture of β-phenylethyl propionate (PEP) and eugenol (9:1,

Table 37. Occasions of testing chemical attractants* for the common June beetle at four locations of southern Quebec in 1981.

Location	Ste. Anne			St.Jean	Stanbridge		Dunham	
Dates tested	May 19-May 27	May 29-June 27	June 8-June 21	June 9-June 21	May 27-June 8	June 10-June 22	May 22-June 6	June 8-June 19
Chemical tested[a]								
Anthranilic acid			*					
Amlure		*						
Benzoic acid	*				*		*	
Butyl sorbate		*		*		*		
Camphor			*					
Chrislure								*
Cineole		*						
Cinnamyl alcohol		*				*		
Citral								*
Java citronella oil				*				
Citronellal			*					
Citronellol			*					
Cyclohexanol	*				*		*	
Decanoic acid			*					
Geraniol	*				*		*	
Hexanoic acid	*	*	*	*	*	*	*	*
β-Ionone		*						
Lauric acid								*

Table 37 - Continued

Location	Ste. Anne			St.Jean	Stanbridge		Dunham	
Dates tested	May 19-May 27	May 29-June 27	June 8-June 21	June 9-June 21	May 27-June 8	June 10-June 22	May 22-June 6	June 8-June 19
Chemical tested[a]								
Menthol								*
Phenol	*	*		*	*	*	*	
β-Phenylethyl butyrate								*
Rhinolure	*				*		*	
Rosemary oil	*				*		*	
White thyme oil								*
Vanillin			*					
Standard[b]	*	*	*	*	*	*	*	*
Control	*	*	*	*	*	*	*	*

[a] Three replicates for each chemical.

[b] Mixture of β-phenylethyl propionate and eugenol (9:1, V./V.)

V /V) selected for its attractiveness to several scarabaeid beetles (Table 34) The index of relative attractiveness for each test bait was calculated at the end of each experiment The best performing lures were further tested against the standard and a series of untested compounds, in a second or third experiment No further tests were conducted with products which had performed poorly in the first series of experiments The indices of relative attractiveness (I) were defined as

$$I = \frac{B}{S} \times 100$$

where B was the relative value of each test bait (including the control) and S was the relative value of the arbitrary standard bait The values B and S were calculated as follows B was the number of beetles captured in traps by each test bait as compared with the catch in unbaited (control) traps in the test block, S was the number of beetles captured in traps by the standard test bait as compared with the catch in unbaited traps in the test block

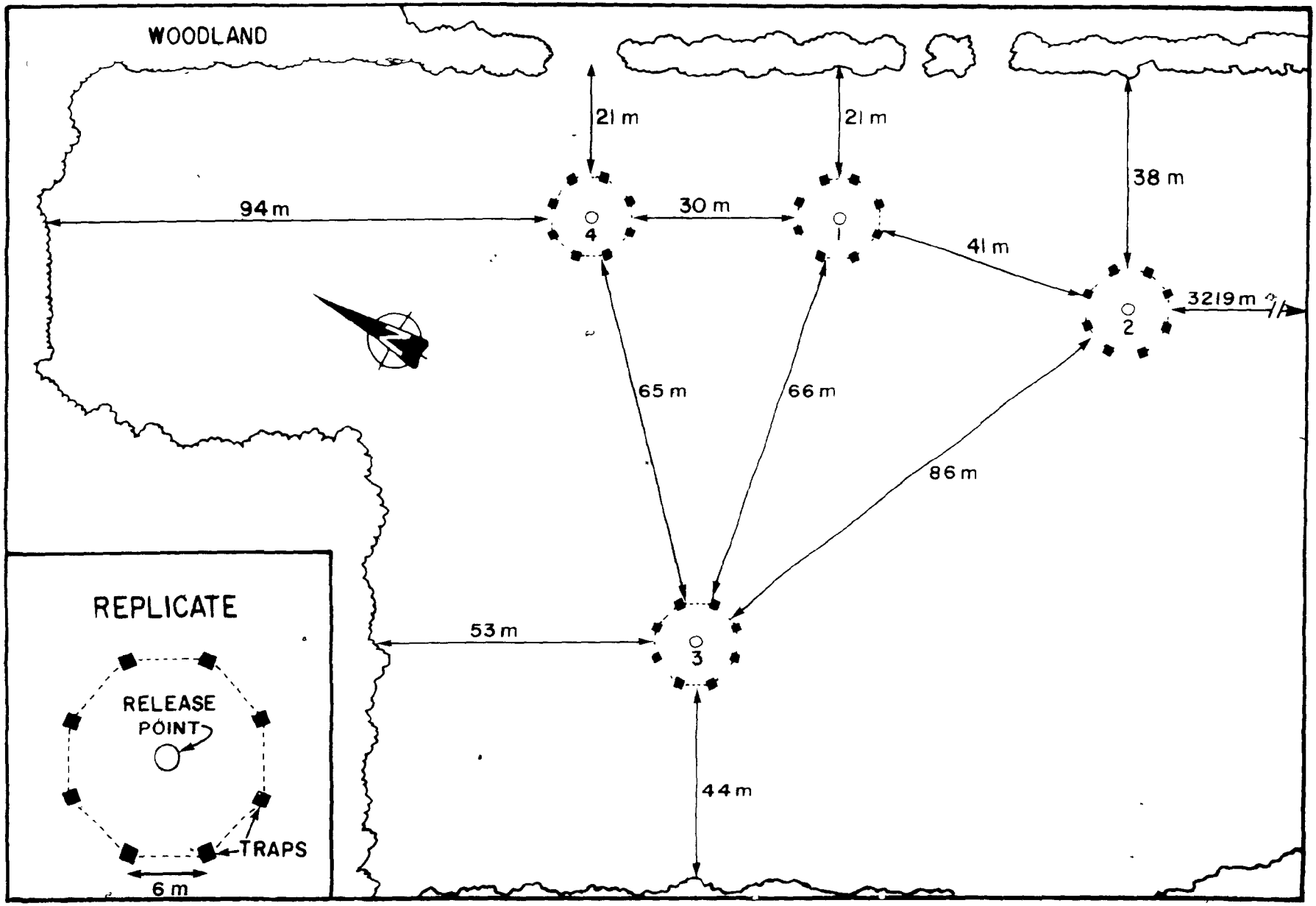
7 Follow-up Experiment

The experiments described above were designed to test, empirically, a number of potential attractants for undefined natural populations of *P anxia*, and of unknown distribution I therefore decided, before the start of these experiments, that a follow-up experiment would be conducted to assess the attractiveness of the best-performing attractants to a defined beetle population of known distribution

Location 1 (Table 35) was chosen as the site of the follow-up experiment for its proximity to the laboratory During the last week of June 1981, June beetle traps described earlier were placed in a 4-replicate randomized block design (Figure 59) in a freshly cut hay field The experimental units within each replicate consisted of one trap either baited with hexanoic acid, decanoic acid, phenol, cinnamyl alcohol, phenylethyl propionate, anthranilic acid, Chrislure or unbaited (see results and discussion section for the choice of the chemicals) No standard bait was selected for the experiment whose objective was to test the effects of the treatments on beetle response rather than to evaluate indices of attractiveness Beetles collected in light traps operated concurrently with the attractant-trapping experiments were accumulated before use in this experiment and kept in a controlled environment chamber (5°C, 40% RH, no light).

The close monitoring of weather forecasts allowed me to schedule the date of the experiment The refrigerated beetles were gradually brought to conditions of 20°C and full daylight on the morning of July 7, 1981 One thousand active beetles were randomly picked and placed in batches of 250 (♂ ♀,1 1) in 4 large plastic trays Traps were baited around 7 pm and at 9 pm one tray was placed at the center of each replicate Field conditions were considered to be ideal for beetle release 21°C, 80% RH, covered sky and gentle breeze Collecting bags were brought to the laboratory the next morning and all pertinent data were recorded

Figure 59 Field layout of follow-up attractants trials at Ste -Anne-de-Bellevue, southern Quebec, 1981


WOODLAND
21 m
21 m
38 m
94 m
30 m
41 m
3219 m
65 m
66 m
86 m
53 m
44 m
4
1
2
3
REPLICATE
RELEASE POINT
TRAPS
6 m

C RESULTS AND DISCUSSION

1 Empirical screening of Chemicals

The results of the eight field trials are summarized in Tables 38 to 45 The tables report the average numbers of males and females lured to the traps of each treatment Indices of relative attractiveness of the standard bait, the unbaited trap (control) and the candidate baits were calculated from the total average number of beetles caught in the course of each trial

On a per trial basis 23 of the candidate baits were consistently less attractive than the standard bait (index 100) and than the control notwithstanding the location and the timing of the trials (Tables 38 to 45), although most of these products are attractive to other scarab beetles (Table 34) Phenol, a highly potent attractant to other Phyllophaga species and to Costelytra zealandica (Table 34), acted erratically, tested in six experiments, it was more attractive than the standard bait in three cases but less attractive in the other cases The three commercialized lures for scarab beetles, Amlure (for Rhizotrogus majalis), Chrislure and Rhinolure (for Oryctes rhinoceros) were not potent attractants for P anxia Hexanoic acid was consistently more attractive than the standard bait and was the overall best performer, except in the second experiment at location 3 (Table 43) Hexanoic (caproic) acid had a strong attraction to Popillia japonica (Langford and Cory, 1946, Schwartz et al ,1966 Ladd, 1980) and Macrodactylus subspinosus (Williams and Miller, 1982,

Table 38 Attractiveness of chemicals[a] in June beetle traps to Phyllophaga anxia, Ste -Anne-de-Bellevue, Quebec, May 19 to May 27, 1981

Treatment	Number of beetles trapped[b]			Index of relative attractiveness
	Males	Females	Total	
Standard[c]	17	8	25	100
Control (unbaited)	11	12	23	92
Benzoic acid	12	7	19.	76
Cyclohexanol	5	4	9	36
Geraniol	7	7	14	56
Hexanoic acid	17	13	30	120
Phenol	12	14	26	104
Rhinolure	8	10	18	72
Rosemary oil	10	11	21	84

[a] At least 95% pure and undiluted

[b] Average for three replicates per treatment

[c] Phenylethyl propionate eugenol (9 1, V /V)

Table 39 Attractiveness of chemicals[a] in June beetle traps to Phyllophaga anxia, Ste -Anne-de-Bellevue, Quebec, May 29 to June 7, 1981

Treatment	Number of beetles trapped[b]			Index of relative attractiveness
	Males	Females	Total	
Standard[c]	14	12	26	100
Control (unbaited)	9	10	19	73
Amlure	8	10	18	69
Butyl sorbate	6	7	13	50
Cineole	7	2	9	34
Cinnamyl alcohol	7	10	17	65
Hexanoic acid	15	13	28	108
β-Ionone	12	6	18	69
Phenol	8	12	20	77

[a] At least 95% pure and undiluted

[b] Average for three replicates per treatment

[c] Phenylethyl propionate eugenol (9 1, V /V)

Table. 40 Attractiveness of chemicals[a] in June beetle traps to Phyllophaga anxia, Ste -Anne-de-Bellevue, Quebec, June 8 to June 21, 1981

Treatment	Number of beetles trapped[b]			Index of relative attractiveness
	Males	Females	Total	
Standard[c]	10	7	17	100
Control (unbaited)	7	5	12	71
Anthranilic acid	7	5	12	71
Camphor	0	2	2	12
Citronellal	2	0	2	12
Citronellol	3	1	4	24
Decanoic acid	7	8	15	88
Hexanoic acid	11	12	23	135
Vanillin	0	0	0	0

[a] At least 95% pure and undiluted

[b] Average for three replicates per treatment

[c] Phenylethyl propionate eugenol (9 1, V /V)

Table 41 Attractiveness of chemicals[a] in June beetle traps to Phyllophaga anxia, St -Jean-sur-Richelieu, Quebec, June 9 to June 21, 1981

Treatment	Number of beetles trapped[b]			Index of relative attractiveness
	Males	Females	Total	
Standard[c]	9	7	16	100
Control (unbaited)	6	5	11	69
Butyl sorbate	4	5	9	56
Citronella oil	0	1	1	6
Hexanoic acid	11	12	23	144
Phenol	6	11	17	106

[a] At least 95% pure and undiluted

[b] Average for three replicates per treatment

[c] Phenylethyl propionate eugenol (9 1, V /V)

Table 42 Attractiveness of chemicals[a] in June beetle traps to Phyllophaga anxia, Stanbridge East, Quebec, May 27 to June 8, 1981

Treatment	Number of beetles trapped[b]			Index of relative attractiveness
	Males	Females	Total	
Standard[c]	17	22	39	100
Control (unbaited)	18	12	30	77
Benzoic acid	9	15	24	62
Cyclohexanol	3	7	10	26
Geraniol	6	7	13	33
Hexanoic acid	22	20	42	108
Phenol	14	11	25	64
Rhinolure	11	9	20	51
Rosemary oil	8	10	18	46

[a] At least 95% pure and undiluted.

[b] Average for three replicates per treatment.

[c] Phenylethyl propionate· eugenol (9:1, V /V.).

Table 43. Attractiveness of chemicals[a] in June beetle traps to Phyllophaga anxia, Stanbridge East, Quebec, June 10 to June 22, 1981

Treatment	Number of beetles trapped[b]			Index of relative attractiveness
	Males	Females	Total	
Standard[c]	10	8	18	100
Control (unbaited)	6	9	15	83
Butyl sorbate	4	7	11	61
Cinnamyl alcohol	10	7	17	94
Hexanoic acid	6	8	14	78
Phenol	8	12	20	110

[a] At least 95% pure and undiluted

[b] Average of three replicates per treatment

[c] Phenylethyl propionate: eugenol (9 1, V /V)

Table 44. Attractiveness of chemicals[a] in June beetle traps to Phyllophaga anxia, Dunham, Quebec, May 22 to June 6, 1981.

Treatment	Number of beetles trapped[b]			Index of relative attractiveness
	Males	Females	Total	
Standard[c]	21	17	38	100
Control (unbaited)	15	12	27	71
Benzoic acid	13	10	23	61
Cyclohexanol	4	3	7	18
Geraniol	6	3	9	24
Hexanoic acid	25	22	47	124
Phenol	13	16	29	76
Rhinolure	12	10	22	58
Rosemary oil	3	6	9	24

[a] At least 95% pure and undiluted.

[b] Average of three replicates per treatment.

[c] Phenylethyl propionate: eugenol (9:1, V./V.)

Table 45 Attractiveness of chemicals [a] in June beetle traps to Phyllophaga anxia, Dunham, Quebec, June 8 to June 19, 1981

Treatment	Number of beetles trapped [b]			Index of relative attractiveness
	Males	Females	Total	
Standard [c]	10	12	22	100
Control (unbaited)	8	5	13	59
Chrislure	10	6	16	73
Citral	2	5	7	32
Hexanoic acid	14	12	26	118
Lauric acid	2	1	3	14
Menthol	0	2	2	9
β-Phenylethyl butyrate	5	3	8	36
Thyme oil	0	4	4	18

[a] At least 95% pure and undiluted

[b] Average of three replicates per treatment

[c] Phenylethyl propionate eugenol (9 1, V /V)

Williams et al , 1982), a neutral effect on most of the other scarab beetles against which it has been tested, and a repellent effect on Rhizotrogus majalis (Tashiro et al , 1969, 1970) The blend phenylethyl propionate eugenol (9 1, V /V) appeared to have been a good choice as the standard bait since the numbers of beetles lured by it were consistently higher than the numbers attracted by the other baits, except for hexanoic acid, and, in three experiments, for phenol This also denoted that the blend or at least one of its ingredients was, besides hexanoic acid, a relatively potent attractant to P anxia The combination phenylethyl propionate-eugenol has been, in various proportions, the standard bait in numerous attractant-trapping field tests against several scarab beetles (Table 34)

A clear picture of the overall experiment is obtained when the data of the eight trials are pooled in space and time and mean indices of relative attractiveness are calculated (McGovern et al , 1970a) (Table 46) Hexanoic acid excepted (mean index 117), none of the candidate lures outperformed the standard bait (index 100), although phenol, decanoic acid, cinnamyl alcohol, Chrislure and anthranilic acid surpassed or approached the performance of the unbaited control (index 74) Thirteen chemicals tested had a mean index of attractiveness of 37 or less, vanillin (index 0) being completely unattractive, if not repellent All the aromatic oils or their main components were poor performers, this was surprising and contradicted results obtained by other workers in their tests with the same oils against various scarab beetles (Table 34) These food-type

Table 46 Mean index of attractiveness to adult Phyllophaga anxia of 25 chemicals[a] tested against a standard and a control in eight field trials, southern Quebec, 1981

Chemical	Number of nights tested	Mean index of attractiveness
Hexanoic acid	104	117
Standard[b]	104	100
Phenol	78	89
Decanoic acid	14	88
Cinnamyl alcohol	23	79
Chrislure	12	73
Anthranilic acid	14	71
Amlure	10	69
β-Ionone	10	69
Benzoic acid	37	66
Rhinolure	37	60
Butyl sorbate	41	55
Rosemary oil	37	51
Geraniol	37	37
β-Phenylethyl butyrate	12	36
Cineole	10	34
Citral	12	32
Cyclohexanol	37	26
Citronellol	14	24
Thyme oil	12	18
Lauric acid	12	14
Camphor	14	12
Citronellal	14	12
Menthol	12	9
Citronella oil	18	6
Vanillin	14	0
Unbaited (control)	104	74

[a] Undiluted and at least 95% pure

[b] Phenylethyl propionate eugenol (9 1 V /V)

lures were particularly attractive to Rhizotrogus majalis (Tashiro et al , 1970), a chafer with adult feeding preferences similar to those of P anxia (Tashiro et al , 1969)

The probability that the outstanding chemical, hexanoic acid, might be a component of a sex attractant to the common June beetle was tested by the Student t-test on the means of males and females lured in the eight field trials The means were not significantly different at the 0 05 level (t=0 41, 14 df) and thus both males and females were attracted equally to hexanoic acid, rejecting the probability of a sex attractant mimic Hexanoic acid is comparatively attractive to both sexes of several scarab beetles (Table 34) but it is also the sex attractant for Trogoderma glabrum (Herbst)(Yarger et al , 1975), and a trail pheromone for Lasius fuliginosus (Latreille) (Huwyler et al , 1975) and Zootermopsis nevadensis (Hagen) (Karlson et al , 1968) In my experiments, hexanoic acid likely acted as a food-type lure or as an aggregant semiochemical The Student t-test was also performed on the means for phenol, the second best attractant to P anxia The results of the analysis indicated that phenol did not act as a sex attractant since the means were not significantly different at the 0 05 level (t=-1 6, 10 df) Phenol, however, is known to be the sex attractant for Cyclocephala insulicola (Gruner and Marival, 1974) and Costelytra zealandica (Lauren, 1979), and a strong attractant for male Phyllophaga patrueloides (Gruner, 1975b) Decanoic acid, the third best performer, is attractive to several Scarabaeidae (Table 34) and is also a trail pheromone for L fuliginosus

(Huwyler et al 1975) Hexanoic and decanoic acids are closely related chemicals (Table 36)

2 Follow-up Experiment

The results of the follow-up experiment were disappointing From the total of 1000 beetles released, 584 were found dead or moribund in the traps or in close proximity to them Another 79 beetles were recovered alive and buried 5 to 7 cm deep near the trays or were hiding under them Seventeen males and 11 females were found at the base of the gallows supporting traps baited with hexanoic acid, phenol, anthranilic acid and Chrislure Two females were caught in the phenol baited traps of replicate 4 The missing beetles could not be found they were either eaten by birds and insectivorous mammals were hiding in the ground outside the field layout, or flew to the trees bordering the hay field, although the latter possibility is contradicted by the high number (584) of beetles which were unable to take flight The high number of dead beetles and the low number of beetles which were able to fly up to otherwise attractive baits indicated an impaired metabolism perhaps due to prolonged cold storage In follow-up experiments, Gruner (1975a) released 3000 marked Cyclocephala insulicola, a scarab beetle responding very strongly to attraction to phenol One thousand of the released beetles did not take flight and only 4 4% of the remainder were caught in the phenol traps In a similar experiment, Barber et al (1971) obtained better results since 25% of marked and released Oryctes rhinoceros adults were caught in traps

baited with Chrislure, but Klein et al (1972) captured only 8 of 3164 marked Popillia japonica in 740 traps baited with phenylethyl propionate eugenol (7 3)

3 Conclusion

Twenty-five chemicals, selected for their attractancy to several scarabaeid pests, were field-evaluated in June beetle traps as potential attractants for adult P anxia in 4 localities of southern Quebec the field trials included an arbitrary binary standard bait and an unbaited lure Attractant-trapping of P anxia had never been assayed before the experiments reported here It was also a first attempt against any scarab beetle in Canada

Hexanoic acid consistently lured the larger number of beetles of both sexes and was more attractive than the standard bait which it should replace in future attractant-trapping trials with P anxia Hexanoic acid and phenol did not act as mimics of a female sex pheromone

Despite its demonstrated attractiveness to P anxia adults, hexanoic acid was about 40 times less efficient than blacklight trapping, the nightly average number of beetles caught per trap baited with hexanoic acid was 0 10 beetle as compared to 3 72 beetles per blacklight trap (1341 beetles trapped in 4 blacklight traps operating for 90 days)

Minks (1979) distinguished, next to population density, other factors which could have an effect on the numbers of insects caught in semiochemical traps site of the population,

insect behavior - including biotic and abiotic factors-, trap density in the area, trap design and concentration of chemical per trap In my experiments, the relative high numbers of beetles caught by the unbaited traps suggested a high physical (visual) attraction to the traps themselves In future tests trap catches could be maximized by evaluating different trap designs size, shape color, finish, position above ground, type of bait dispenser are all factors to be taken into consideration On the other hand, the large number of beetles caught by the unbaited traps emphasizes the difficulty of attempting to separate the effect of the bait from that of the trap, as a trap becomes a baited trap once beetles are attracted to it I have also noticed that, soon after the onset of the field trials, most traps were soiled with bird droppings, this suggested that a number of beetles lured to the traps were preyed by birds so I concluded that traps should be protected by some wiring

The poor performance of about half of the candidate lures especially the aromatic oils, suggested a possible repellence, anti-attraction, inhibition or arrestment Non-attractancy (neutral effect) is dismissed since traps baited with these lures were much less efficient than the unbaited traps In my empirical screening, I had no other choice than to use pure, undiluted chemicals Chemical concentration, chemical load and release rate should be given attention in future tests It is also possible that the relative potency of hexanoic acid had a masking effect on the other test baits this could be

minimized by evaluating bait interactions, factors to consider would be density of traps in the study area, spacing of traps within the field layout, statistical design Barber et al (1971) and Maddison et al (1973) used a cycling program, and Perry et al (1980) recommended Latin square designs for such experiments

XII SUMMARY AND CONCLUSIONS

Forty-five localities in southern Quebec were visited on several occasions from 1979 to 1981 and all life stages of Phyllophaga spp., especially P. anxia, were found at the collection sites. A grand total of 33,468 Phyllophaga individuals were collected during the survey and examined for associated organisms that were potential regulators of Phyllophaga spp. The 8,895 adult males, 2,647 adult females, 73 teneral adult males, 37 teneral adult females, 1,289 pupa males and 897 pupa females belonged to the species Phyllophaga anxia (LeConte). Sixty-six adults other than P. anxia were also collected in southern Quebec, they belonged to the species P. drakii (Kirby), P. fraterna (Harris), P. fusca (Froelich), P. futilis (LeConte), P. nitida (LeConte) and P. rugosa (Melsheimer). Phyllophaga fraterna and P. rugosa were new records for southern Quebec. Also collected during the three-year study were 2,525 eggs, 1,832 first instar grubs, 1,652 second instars/first year, 1,501 second instars/second year, 8,174 third instars/second year, 3,771 third instars/third year, and 175 prepupae. Since P. anxia adults represented 99.44% of all Phyllophaga adults collected in southern Quebec, it was assumed that a similar proportion of white grubs were P. anxia.

Rates of incidence of individual organisms associated with each life stage of Phyllophaga spp. are presented in Table 47.

Two third instar/second year grubs were found infected with a new small iridescent virus, which was designated as Phyllophaga

Table 47 Incidence rates (%)[a] of invertebrate organisms recovered from field-collected Phyllophaga[b] spp from 1979 to 1981 in 45 localities in southern Quebec

Organism	Host stage[c] (number examined)												
	AM (8895)	AF (2647)	E (2525)	L1 (1832)	L2,1 (1652)	L2,2 (1501)	L3,2 (8174)	L3,3 (3771)	PP (175)	PM (1289)	PF (897)	TAM (73)	TAF (37)
Phyllophaga anxia iridescent virus	-[d]	-	-	-	-	-	0 02	-	-	-	-	-	-
Actinocephalus sp	0 01	-	-	0 60	←4 31→		←5 18→		0 57	0 07	-	1 37	-
Bacillus cereus	0.01	0.11	-	1 53	2 78	2 06	1 40	2 07	0 57	0 08	0 22	1 37	-
B. popilliae	-	-	-	-	1 63	0 66	0 43	2 76	-	-	-	-	-
Pseudomonas aeruginosa	-	-	0.04	-	-	-	-	-	-	-	-	-	-
Serratia marcescens	-	-	-	-	0 42	0 66	0 05	0 13	-	-	-	-	-
Micrococcus nigrofasciens	-	-	-	-	0 24	0 26	0.01	-	-	-	-	-	-
Cordyceps sp.	-	-	-	-	-	0 06	0.01	-	-	-	-	-	-
Beauveria bassiana	-	-	-	-	3 69	1 59	2 83	1 24	-	0 62	0 89	2 73	-
Metarhizium anisopliae	0.43	1 74	-	1 09	2 36	5.26	3.22	3 76	0 57	0 69	1 89	36 98	-
Aspergillus sp.	-	-	-	0.49	0.18	0.59	0 25	0 21	-	-	-	-	-
Fusarium sp.	-	-	-	-	1.93	1 73	0 88	0 23	-	-	-	-	-
Penicillium sp.	-	-	-	-	1 99	0.99	0.29	0 16	-	-	-	-	-
Mikoletzkya aerivora	-	-	-	1 74	2 42	-	7 04	5 40	-	0 31	0 22	-	-
Rhabditid sp.	-	-	-	-	-	0 13	0 01	-	-	-	-	-	-

Table 47 - Continued

Organism	Host stage [c] (number examined)												
	AM (8895)	AF (2647)	E (2525)	L1 (1832)	L2,1 (1652)	L2,2 (1501)	L3,2 (8174)	L3,3 (3771)	PP (175)	PM (1289)	PF (897)	TAM (73)	TAF (37)
Aphelenchoidid sp.	-	-	-	-	-	-	0 01	0 02	-	-	-	-	-
Nematomorpha	-	-	-	-	-	-	-	0 02	0 57	←0 32→		-	2 70
Acarina (15 spp)	34 14	39 21	-	9 44	←42 05→		←54 59→		-	8 76	2 45	31 50	27 02
Parasitic Insecta (10 spp.)	0 02	7 84	-	-	← 0 10→		←0 22→		-	-	0 04	-	-
Predatory Insecta (26 spp.)	-	-	-	1	←32→		←262 →		-	-	-	-	-

[a] Actual number of predated hosts, not percentage (line "Predatory Insecta")

[b] Phyllophaga anxia in over 98% of the cases (see Chapter III)

[c] AM adult male, AF adult female, E egg, L1 first instar grub, L2,1 second instar, first year, L2,2 second instar, second year, L3,2 third instar, second year, L3,3 third instar, third year , PP prepupa, PM pupa male, PF. pupa female, TAM teneral adult male, TAF teneral adult female

[d] Not recovered from this host stage

anxia iridescent virus (Pa IV) This is the first record of an iridescent virus from North American Scarabaeidae. The virus was isolated, purified, and found to kill 37 out of 40 healthy third instars after intrahemocoelic injection The prevalence rate of viral disease in the grub population examined was less than 0 02% No viral disease was found from the other life stages of Phyllophaga spp Pa IV was not regarded as a promising candidate for the biological control of white grubs

A eugregarine protozoan, Actinocephalus sp., was chronically associated with Phyllophaga individuals from 1979 to 1981. Over 4 5% of white grubs were infected with the protozoan but infection rates for the other stages of Phyllophaga were insignificant. The infection appeared to be endemic to the larval stages of Phyllophaga and enzootic in grub populations throughout southern Quebec, without ever gaining epizootic dimensions. The true nature of the Actinocephalus-Phyllophaga association remains to be demonstrated at the host level as does its impact on host populations

Five species of entomogenous bacteria were isolated from diseased and dead Phyllophaga individuals These were: Bacillus cereus Frankland and Frankland, B popilliae Dutky, Pseudomonas aeruginosa (Schroeter) Migula, Serratia marcescens Bizio, and Micrococcus nigrofasciens Northrup. The pathogenicity of all bacterial species for white grubs was demonstrated in the laboratory Bacillus cereus killed 80% of the grubs injected with 2 to 3×10^3 spores Since the bacillus was found causing natural infections of all stages of Phyllophaga except eggs, and was widespread among populations of Phyllophaga in southern Quebec, it

should be further studied as a potential microbial control agent for Phyllophaga spp Topical and soil applications of a local isolate and, of a commercial preparation of B popilliae gave very low control of white grubs The maximum percentage mortality was 7 5% in third instar grubs force-fed high dosages of both isolates of B popilliae The bacterium was, therefore, not considered as a promising biocontrol agent for Phyllophaga spp. However, further testing with a wider range of milky disease isolates should be envisaged

Several species of entomogenous fungi were found infecting natural populations of Phyllophaga spp They were: Cordyceps sp , Metarhizium anisopliae var anisopliae (Metschnikoff) Sorokin Beauveria bassiana (Balsamo) Vuillemin, Fusarium sp. near F. solani (Martius) Appel and Wollenweber, and unidentified species of Penicillium Link Fries and Aspergillus Micheli : Fries. The latter five fungi killed third instar grubs after contact with petri dish cultures of the pathogens. In another series of laboratory bioassays, 97% of third instar grubs were killed when placed in soil contaminated with 5×10^6 spores per 1.75 kg of soil of a local isolate of M. anisopliae Under similar bioassay procedures, a local isolate of B bassiana was less pathogenic to white grubs than M. anisopliae In field-microplot studies on M. anisopliae, the LD_{50} of the fungus was estimated to be $10\ 2 \times 10^6$ spores per m^2 of soil. The local isolate M. anisopliae 104, applied as a spore powder in talcum, is a highly virulent potential suppressant of Phyllophaga grubs in southern Quebec; this strain should be considered as a valid component of any

future IPM program against white grubs Further field-testing with M. anisopliae 104 and possibly other isolates of hyphomycete fungi is strongly advocated by the author.

The diplogasterid nematode, Mikoletzkya aerivora (Cobb), was an important natural enemy of white grubs in southern Quebec from 1979 to 1981 Behavioral studies demonstrated that juveniles of M. aerivora were attracted to live grubs and grubs' tissues. The survival of M aerivora in the laboratory was found to depend on regular passages through its host. The nematode was infectious to the three larval instars of Phyllophaga when applied topically and to the soil of infection chambers. In field-microplot trials, M. aerivora killed over 68% of third instar grubs in soil application tests at the rate of 2.8×10^5 nematodes per m^2 of soil The field trials demonstrated sufficient tolerance to environmental stress by M. aerivora for it to be considered for field control of white grubs or at least, for longer term field tests. A new simple apparatus was developed to extract large numbers of live M. aerivora from infected hosts; this device proved more efficient than the Baermann funnel and the Carne-Reed apparatus for such extractions. Besides M. aerivora, one species each of Rhabditidae, Aphelenchoididae and Gordiaceae were found from field-collected immatures of Phyllophaga spp.

Fifteen species of mites were found associated with all stages of Phyllophaga spp except for eggs or prepupae. Natural infestation rates were relatively high for most mite species. The nature of relationships with the hosts was not elucidated, although it is likely that the macrochelid, parasitid and

possibly eviphidid mites that were found were active natural enemies of local Phyllophaga populations Most of the species of mites, including a new, undescribed species of Scarabaspis, found during this survey have not previously been reported in Canada from Phyllophaga beetles

At least 36 species of parasitic and predatory insects were found attacking or in close proximity to Phyllophaga spp. These were. Cicindelidae (2 spp), Carabidae (19 spp.), Elateridae (1 sp), Curculionidae (1 sp), Staphylinidae (1 sp.), Scarabaeidae (1 sp.), Tenebrionidae (1 sp.), Asilidae (1 sp.), Pelecinidae (1 sp.), Muscidae (1 sp), Pyrgotidae (1 sp.), Sarcophagidae (1 sp), Tachinidae (4 spp) and Tiphiidae (2 spp.). However, the survey clearly showed that entomophagous insects had little impact on the size of Phyllophaga field populations in southern Quebec, from 1979 to 1981

Field-tests on the attractiveness of 27 chemicals to adults of P. anxia were carried out in 1981 in four localities of southern Quebec. One chemical, hexanoic acid, was consistently more attractive than the other compounds Hexanoic acid did not act as a sex attractant. I recommend further screening of hexanoic acid and other behavior-modifying chemicals to complete this study on the feasibility of autocidal control of Phyllophaga anxia in southern Quebec.

This study of natural enemies of Phyllophaga spp. in southern Quebec inventoried numerous species of invertebrate organisms found associated with Phyllophaga individuals. Most of the organisms had a detrimental effect on the individual host.

Rates of incidence in the host population levels were relatively low, but these diverse organisms exerted a constant pressure upon the host populations. Laboratory and field evaluation of selected natural enemies of Phyllophaga spp has identified a fungus, a nematode and possibly a bacterium as the most promising candidates for the biological control of white grubs I hope that the results of this study will provide a basis for future management strategies against the pest by supplementing present local crop protection with microbial control agents and, in a forseeable future, with behavior-modifying chemicals

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APPENDIX

List of manuscripts and oral presentations derived from this thesis (as of April 1985)

Publications

Poprawski, T J and W N. Yule 1982 Prédateurs, parasites, et pathogènes du hanneton commun, Phyllophaga anxia (Le Conte), au Québec méridional Phytoprotection, 63 · 37.

Poprawski, T J. and W N Yule 1982 Pathogens of June beetles (Coleoptera Scarabaeidae) in Quebec, Canada. Proc Int Colloq. Invertebr Pathol , 3 121

Yule, W.N and T J Poprawski 1983 Microbial control of white grubs in soil Proc Can Pest Manag. Soc., 30: 64-65.

Manuscripts in preparation

Poprawski, T J and W.N Yule A new small iridescent virus (PaIV) found infecting white grubs, Phyllophaga anxia (Le Conte) (Coleoptera: Scarabaeidae) in southern Quebec.

Poprawski, T J. and W N. Yule Observations on Actinocephalus sp., a eugregarine protozoan chronically associated with Phyllophaga spp in southern Quebec

Poprawski, T J. and W.N Yule. Bacillus popilliae Dutky and other bacterial pathogens of Phyllophaga spp. in southern Quebec their potential as control agents of white grubs.

Poprawski, T J and W.N Yule Entomogenous fungi associated with Phyllophaga spp in southern Quebec: laboratory and field evaluation of muscardine diseases as suppressants of white grubs

Poprawski, T\J and W.N Yule Susceptibility of white grubs, Phyllophaga spp. (Coleoptera. Scarabaeidae) to the diplogasterid nematode, Mikoletzkya aerivora (Cobb).

Poprawski, T.J and W.N Yule Acari from the Phyllophaga (Coleoptera: Scarabaeidae) fauna of southern Quebec

Poprawski, T J and W N Yule. Field survey of entomophagous insects asoociated with Phyllophaga spp (Coleoptera Scarabaeidae) in southern Quebec

Poprawski, T J and W N Yule Trapping the common June beetle, Phyllophaga anxia (LeConte) (Coleoptera Scarabaeidae) with chemical attractants

Poprawski, T J , W N. Yule and C. Vincent Biotic regulation of Phyllophaga spp. (Coleoptera Scarabaeidae) in southern Quebec a multivariate analysis

Oral presentations

Poprawski, T.J. 1982. Trapping the common June beetle, Phyllophaga anxia (LeConte) with synthetic chemical attractants. Paper presented at the Joint Meeting of the Entomological Societies of America, Canada and Ontario, Nov 29-Dec 3, Toronto, Ont

Poprawski, T J. and W.N Yule 1980 Pathogens of June beetles and white grubs in Quebec (Phyllophaga spp , Coleoptera Scarabaeidae). Display presented at the Beltsville Symposia in Agricultural Research V Biological Control in Crop Production, May 18-21, Beltsville, Md

Poprawski, T J and W.N Yule 1980 Pathogens of June beetles and white grubs in Quebec Display presented at the Joint Meeting of the Entomological Societies of Canada and Quebec, Oct. 5-8, Quebec City, Quebec.