

BIOSYSTEMATICS OF CONOPHTHORUS HOPKINS (COLEOPTERA: SCOLYTIDAE)
IN EASTERN NORTH AMERICA

by

Peter de Groot

B.Sc.F. (Hons.), Lakehead University, 1981

THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in the Department

of

Biological Sciences

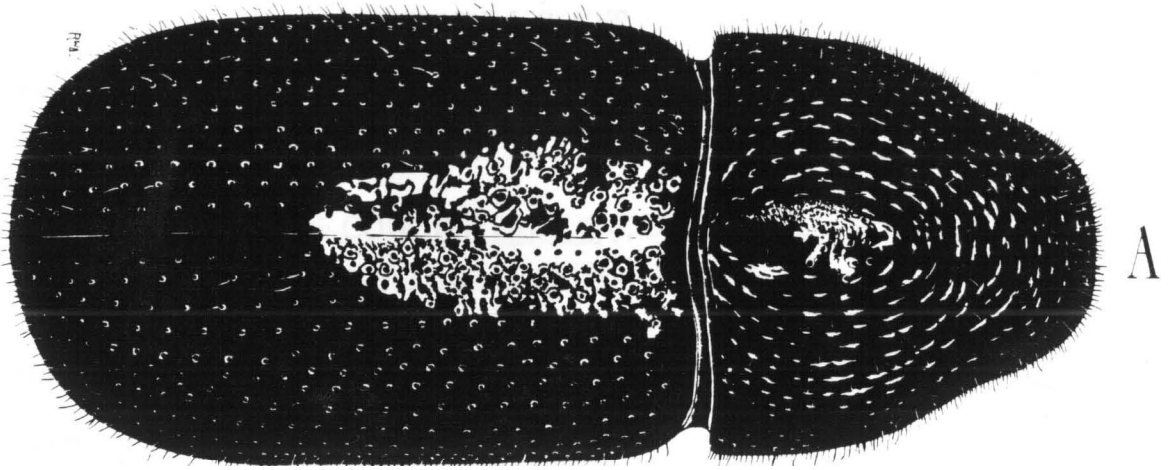
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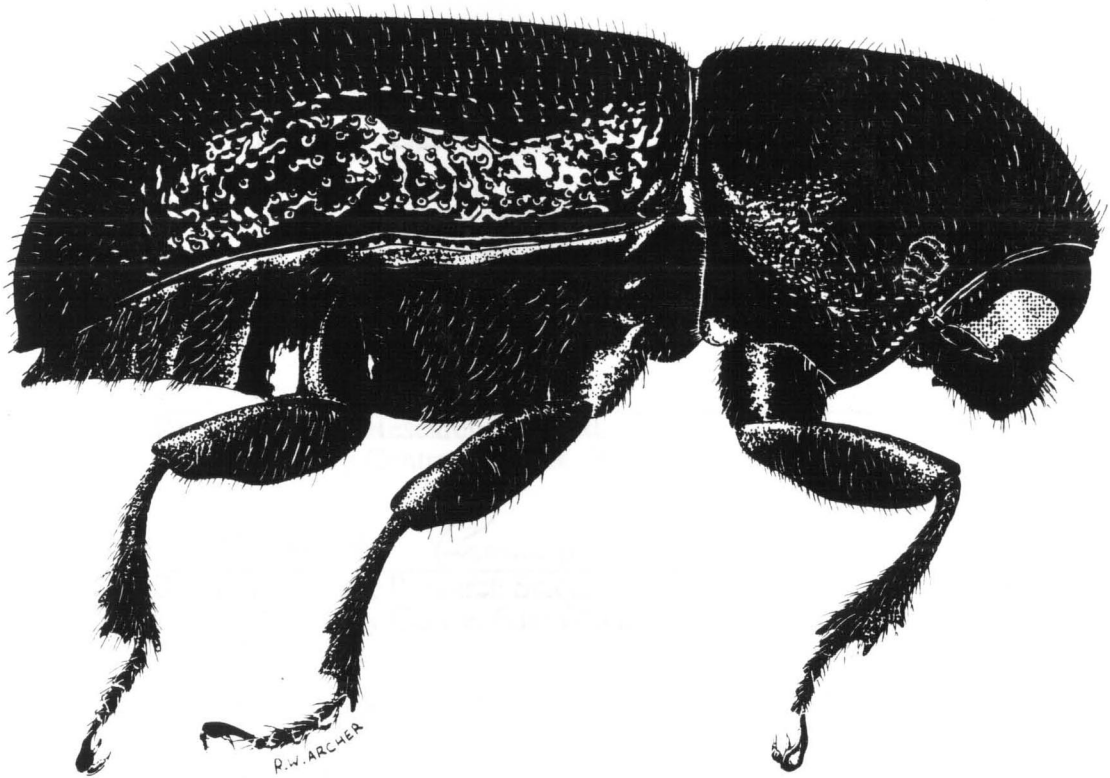
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Frontispiece: Conophthorus resinosae Hopkins (Coleoptera:
Scolytidae) in dorsal (A) and lateral (B) view.



A

1.0 mm



B

APPROVALName: **PETER DE GROOT**Degree: **Doctor of Philosophy**

Title of Thesis:

**BIOSYSTEMATICS OF CONOPHTHORUS (COLEOPTERA: SCOLYTIDAE)
IN EASTERN NORTH AMERICA**

Examining Committee:

Chairman: **Dr. A.S. Harestad, Associate Professor**

**Dr. J.N. Borden, Professor,
Senior Supervisor,
Dept. Biological Sciences, SFU**

**Dr. B.D. Roitberg, Associate Professor,
Dept. Biological Sciences, SFU**

**Dr. J.F.M. Mackauer, Professor,
Dept. Biological Sciences, SFU**

**Dr. G.E. Miller, Research Scientist,
Pacific Forestry Centre, Victoria, B.C.**

**Dr. H.F. Cerezke, Research Scientist,
Northern Forestry Centre, Edmonton, Alberta
Public Examiner**

**Dr. D.E. Bright Jr., Research Scientist,
Agriculture Canada, Ottawa, Ontario
External Examiner**Date Approved 20 December, 1991

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(COLEOPTERA : SCOLYTIDAE) IN EASTERN

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PETER DE GROOT

(name)

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Abstract

In eastern North America, Conophthorus coniperda (Schwarz) and C. resinosae Hopkins, feed on pine cones, while C. banksianae McPherson, feeds on shoots. The latter two species are sibling species, but questions remain about the validity of C. banksianae. The objective of this study was to examine various traits and taxonomic characters to determine if, and how, C. banksianae maintains reproductive isolation from C. resinosae and C. coniperda. A life history analysis showed that C. banksianae is neither bivoltine nor substantially different from C. resinosae in seasonal development of life stages or in shoot attack behaviour, attributes that were used to distinguish the two species. C. coniperda and C. resinosae occupy the entire range of white pine, Pinus strobus L., and red pine, P. resinosa Ait., respectively. C. banksianae is only found on jack pine, P. banksiana Lamb., within the northern range of red pine. Characters on the genitalia failed to distinguish between males of the three Conophthorus species. Karyotyping of first metaphase cells revealed that C. coniperda had one more bivalent and a chromosome morphology different from C. resinosae and C. banksianae, but no karyotypic differences between the two sibling species were found. Similarly, electrophoresis provided diagnostic allozyme characters in two enzymes for distinguishing

C. coniperda from C. resinosae and C. banksianae, but not for distinguishing the sibling species. Mate-finding in all three species was shown to be mediated by two pheromones, "pityol" (a female-produced sex attractant), and "conophthorin" (a male-produced deterrent). Although C. resinosae and C. banksianae differed consistently in their acceptance of red pine cones, the lack of distinct ecological, behavioural, morphological, karyological, and biochemical taxonomic characters in C. banksianae does not support its designation as a species; therefore, it should be placed in synonymy with C. resinosae. The hypothesis that C. resinosae attacks jack pine shoots as a survival strategy to cope with periodic shortages of red pine cones could explain why it seldom persists on jack pine shoots.

Dedication

I dedicate this work to my children, Cathleen and Jonathan, who helped me 'collect cones while we shared those rare 'holidays', and who seemed to understand my long journey, and to my wife, Elizabeth, who quietly sacrificed much, but without whose love, understanding, and patience, this work simply would not have been undertaken -- to her, my utmost gratitude and love.

Acknowledgements

Several people have helped make this study possible. I extend my appreciation and gratitude to Dr. John Borden, my major professor, for his unwavering enthusiasm, guidance and helpful criticism, and to Drs. Bernie Roitberg, Gordon Miller and Manfred Mackauer, my committee members, for their constructive advice, criticism, and questions. I have benefited greatly from my collaboration with Dr. Harold Pierce who showed great patience in teaching a forester about pheromone chemistry. A special thankyou to Dr. Jean Turgeon, a good friend and colleague, for teaching me much about the rigour and fun of entomology. I thank Dr. George Green and Mr. Errol Caldwell for their encouragement and support to take educational leave from Forestry Canada. I greatly appreciate the guidance by Dr. Terry Ennis and Mr. Al Macdonald on chromosome karyotyping, and by Dr. George Harvey and Mrs. Pat Roden who allowed me to use their electrophoresis lab and who taught me much about the art and science of electrophoresis. I am grateful to Drs. Gary DeBarr and Bill Mattson, who helped me with the collections of cone beetles in the United States, and shared freely their knowledege of Conophthorus, and to Miss Beverly Aelick and Messrs. Andrij Obarymskyj and Bert Zylstra who provided invaluable assistance in the laboratory and the field.

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Chapter 1

Introduction

" The first and most fundamental step in the solution of a problem in biology is the accurate identification of the organism under investigation. Without it, all else is meaningless, because effective communication is not possible."

Stephen L. Wood (1982)

The natural world is endowed with a rich diversity of plant and animal species. To comprehend this diversity, it is essential that these organisms be accurately classified and ordered. Clearly, without some system of classification, the understanding of the world fauna would be hopelessly confused. Moreover, naturalists run the risk of seriously misinterpreting biological data if a single species is assumed when in fact two or more species are present. Progress in ecology, indeed all of biology, depends upon accurate identification and sound systematic groundwork (Elton 1947).

The study of the diversity of organisms is systematics (Mayr 1969) or as Simpson (1961) stated, "Systematics is the scientific study of the kinds and diversity of organisms and of any and all relationships among them." Biosystematics, according to Seigler (1974), attempts to study as much as possible of the biology of living populations of organisms and to use these studies to

clarify the taxonomic and phylogenetic relationships of the taxa involved.

Species are a fundamental category in taxonomy. Most species are recognized by external morphological criteria. However, some closely related species differ so little (or not at all) in their external morphology, that their specific status can not be inferred on morphological grounds. Such species are known as cryptic or sibling species (Mayr 1969).

The bark beetles (Coleoptera: Scolytidae) contain several species complexes and sibling species groups (Wood 1982). One group of bark beetles that is important in applied forest management is the cone beetles in the genus Conophthorus Hopkins (de Groot 1990). These beetles, which occur in North America only, feed on the cones or shoots of 13 species of pines, Pinus spp. (Wood 1982; Flores and Bright 1987), and can cause severe destruction of valuable conifer seed crops (Hedlin et al. 1980; de Groot 1986a). In eastern North America, three species of Conophthorus have been recognized: the white pine cone beetle, C. coniperda (Schwarz), and two sibling species, the red pine cone beetle, C. resinosae Hopkins, and the jack pine tip beetle, C. banksianae McPherson (Wood 1982).

Questions have arisen as to whether C. banksianae is a valid

species or a 'biotype' (Diehl and Bush 1984) of C. resinosa that feeds on jack pine shoots. If it is a valid species, how does it maintain reproductive isolation from C. resinosa and C. coniperda? If it is a biotype, why does it attack jack pine shoots, and how has it adapted to feed on jack pine shoots?

This dissertation was undertaken to examine several kinds of taxonomic characters (sensu Mayr 1969) to determine if, and how, C. banksiana maintains reproductive isolation from C. resinosa and C. coniperda.

Historical and taxonomic perspectives

The genus. The first published observations of what eventually became recognized as Conophthorus were in a report by Packard (1890), who listed the observations by W.H. Harrington (in correspondence) of "Dryocoetes affaber (?)" adults and larvae in the cones of red pine, Pinus resinosa Ait. Later, Harrington (1891) published notes under the names D. affaber and D. autographus. Hamilton (1893) reported collecting cone beetles from white pine, P. strobus L., thinking them similar to the species identified by Harrington as Dryocoetes, but questioning their identity as Dyrocoetes.

Schwarz (1895) offered the first taxonomic description of the

cone beetle attacking white pine and placed the species under the genus Pityophthorus Eichhoff. He indicated that this species (P. coniperda) differs from the generic description of Pityophthorus in the structure of the antennae and the anterior tibiae, but thought it premature to erect a new genus for an isolated species. He also noted that superficially this species was at once recognizable by its larger size, less elongate form, and the structure of the elytral declivity.

With the discovery that several other species of pines were infested with cone beetles, A.D. Hopkins (1915a) erected Conophthorus as a new genus, with C. coniperda as the type species. Hopkins (1915a) distinguished Conophthorus from Pityophthorus by the absence of sutural septa in the antennal club.

The concept of Conophthorus as a distinct genus is questionable (Wood 1982). Although the genus does contain a distinctive species group, this may be nothing but a species group or subgenus of Pityophthorus (D.E. Bright, Biosystematics Research Centre, Agriculture Canada, Ottawa, Ontario, pers. comm.). Wood (1982) distinguished Conophthorus from Pityophthorus "... by the larger body size, by the stout body form, by the aseptate antennal club, by the pronotum that has an indefinite summit on the basal third and the asperites continuing to or near

the base in the lateral areas, and by the monogamous cone-infesting habit (with one exception)." However, D.E. Bright (pers. comm.) has found that the aseptate vs septate antennal sutures are not maintained between the two genera, since he has seen species that fill this gap, and that the fine raised line on the lateral margin is present on species in both genera (see also Flores and Bright 1987).

Taxonomic characters for distinguishing species. Hopkins (1915a) appears to have been obsessed with host selection by insects, so much so that it is reflected in his descriptions of new species. Hopkins' host selection principle dictates that a species which breeds in two or more hosts will prefer to continue to breed in the host to which it has become adapted (Hopkins 1916, 1917). Thus, although Hopkins (1915a) described a species by its morphological characters, he named 14 new species of Conophthorus all after their host plants. The number of species of Conophthorus recognized by Hopkins has been reduced by Wood (1977) who, by using external morphological characters, synonymized C. virginiae Hopkins with C. resinosae, declared C. clunicus Hopkins and C. taeda Hopkins synonyms of C. coniperda, and placed six of Hopkins's western species under synonymy with C. ponderosae. Presently 13 species are recognized (Wood 1982; Flores and Bright 1987; Wood 1989).

Separation of some of the species by morphological characters is extremely difficult (Wood 1982). The most recent key to species of Conophthorus uses the structural features of elytra and pronota (Wood 1982). Thomas (1957,1967,1971) examined the external anatomy of larvae and pupae, and the gastric caeca of larvae and adults of several Conophthorus spp., but found no consistent differences that would separate one species from another.

Discovery of C. banksianae. The discovery of C. banksianae began in 1952 when Thomas and Lindquist (1956) found a species of Conophthorus attacking the shoots of jack pine, Pinus banksiana Lamb., in Ontario. Specimens sent by them to specialists for identification were returned with the following descriptions: "Conophthorus coniperda (Sz), Conophthorus possibly coniperda, C. resinosae Hopk. and Conophthorus probably resinosae" (Thomas and Lindquist 1956). Herdy (1963) conducted a thorough study of the external morphology of C. resinosae and of the species on jack pine, which he called Conophthorus "X", and found no morphological character that would separate them. Later, McPherson et al. (1970a,b) distinguished Conophthorus "X" from C. resinosae by body size and ecological characters and called it C. banksianae. Persistent doubts about the validity of C. banksianae (Wood 1982) eventually resulted in its synonymy with C. resinosae (Wood 1989), yet speculation remains that it is valid (Mattson

1989).

The Problem with C. banksianae as a valid species

Although Wood (1982) doubted the validity of C. banksianae, neither he, nor anyone else, has published a critical examination of the evidence and opinion on which McPherson et al. (1970b) recognized C. banksianae.

McPherson et al. (1970b) named the new species C. banksianae McPherson stating as diagnosis: "This species [C. banksianae] is nearly identical to C. resinosa Hopkins but differs in mean size, behavior, and preferred host". Differences in life history parameters were also observed.

Mean size cannot have absolute diagnostic value because the range in size for both insects overlaps (Herdy 1963; McPherson et al. 1970a; Wood 1982). Therefore, it must be used in combination with behavioural or host selection data. More importantly, however, it was not determined by McPherson et al. (1970a) if the differences in body size had a genetic or a nongenetic environmentally induced basis.

McPherson et al. (1970 a,b) found that the immature life stages of C. banksianae were present in the field for a much

longer time than those of C. resinosa. It was also noted that C. banksiana was apparently bivoltine, whereas C. resinosa was not (McPherson et al. 1970a,b). However, the differences in the duration of life stages observed by McPherson et al. (1970a,b) could have been caused by differences in such factors as microclimate or host quality, but alternative explanations were not advanced. McPherson et al. (1970a) suggested that C. banksiana was bivoltine because eggs were not found for at least two weeks in early August and reappeared only when new adults were found, giving sufficient time to complete a second generation. However, they did not check the brood adults to see if they were capable of reproduction, nor did they explain why their data on the numbers of emergent adults and other life stages lacked the bimodality that would be expected for bivoltine insects in temperate climates. Without detailed life history data and a good understanding of the biological factors that influence the seasonal development of C. banksiana, the life history criteria established by McPherson et al. (1970b) for species diagnosis would be impossible to use. Furthermore, correct interpretation of such data would be difficult because the life cycles of both species overlap considerably.

McPherson et al. (1970b) also noted that host acceptance behaviour in C. banksiana was different from that of C. resinosa. They found that field populations of C. banksiana fed

only on shoots of jack pine, red pine, scotch pine, P. sylvestris L., or ponderosa pine, P. ponderosa (Laws.), whereas C. resinosae was known to attack only the cones and shoots of red pine (Lyons 1956; McPherson et al. 1970a). In a series of field cage experiments, McPherson et al. (1970b) found that C. banksianae showed a preference for the shoots of jack pine, and did not attack red pine cones. On the other hand, C. resinosae attacked the cones and shoots of red and jack pine, but showed a preference for red pine cones (McPherson et al. 1970b). When exposed to laboratory concoctions of the resins of jack pine or red pine, C. banksianae was most tolerant of jack pine resin, and C. resinosae showed about equal tolerance for the resins of both trees (McPherson et al. 1970a,b).

Even though some distinct trends in host acceptance are apparent, the reciprocal host tests conducted by McPherson et al. (1970b) should be regarded as inconclusive. First, the tests may have been biased, as the preference of C. banksianae may have been altered because the beetles had fed on jack pine shoots just prior to testing [see Papaj and Prokopy (1986,1989) for a review of the role of feeding experience in insect host preference]. However, the adults of C. resinosae were naive in the sense that they had not had a feeding experience on shoots or cones for several months before the test was conducted. Second, the sample sizes used in the test were small (each host/structure and insect

combination was tested in only 4 cages), thus reducing the probability of rejecting a null hypothesis. Finally, caution must be exercised in using host data for species diagnosis, because both species were able to attack jack pine shoots (in fact C. resinosae attacked more shoots in the tests than did C. banksianae) and both species reproduced in jack pine shoots (McPherson et al. 1970b).

The series of resin toxicity tests conducted by McPherson et al. (1970b) have limited taxonomic value. In the resin toxicity tests, the beetles died when exposed to their own host volatiles (none died in the untreated controls). Furthermore, the test was unrealistic because of the prolonged exposure (72-120 h) to an environment saturated with laboratory concoctions of pine resins. In their own words, the overall results of the resin toxicity test were "...far from conclusive..." (McPherson et al. 1970b).

In my opinion, McPherson et al. (1970a,b) had no firm basis to recognize C. banksianae as a species distinct from C. resinosae. This is, however, just an opinion, and therefore to assess the validity of C. banksianae objectively, additional biosystematic studies are required.

The Species Concept

Species concepts should have a balance between practicality and meaningfulness (Sperling 1987); moreover, operational species definitions should try to be good approximations of theoretically meaningful definitions. To be of practical value to taxonomists and other biologists, one must be able to diagnose a species. A strictly empirical view is embodied in a phenetic approach to taxonomy (Sneath and Sokal 1973), which makes no assumptions about the process that generates species. While a purely phenetic approach to the delineation of species does make the process of distinguishing species mechanically simple (Hull 1968), it does so at the expense of understanding how and why patterns of variation in taxa arise. On the other hand, evolutionary species concepts, while theoretically reasonable, all tend to be difficult to apply strictly. These concepts include: the biological species concept (Mayr 1969), the evolutionary species concepts (Simpson 1961; Wiley 1981), the recognition species concepts (Ghiselin 1974; Patterson 1985), and the cohesion species concept (Templeton 1989). Every one of these concepts has been criticized for a number of different reasons, often on several occasions. The lack of agreement among biologists on the formal species definition constitutes the "species problem", a problem that has for decades received innumerable reviews (Mayr

1982; Otte and Endler 1989) and continues to receive heated, sometimes rancorous, debate (e.g., White et al. 1990). One way out of this dilemma is to subscribe to a philosophy of a pluralistic view of species, i.e., several species concepts are useful (Dobzhansky 1972; Scudder 1974). Another view is that it may be unproductive and misleading to try to apply one species concept to all species, or to answer all questions (Endler 1989).

The biological species concept (Mayr 1969) and the recognition species concept (Patterson 1985), in a sense, are two sides of the same coin (Templeton 1989). Both concepts are applicable to sexually reproducing organisms, particularly when species exist in sympatry, and thus these concepts are applicable to Conophthorus. Both acknowledge that species are genetically cohesive and that they can be recognized by discontinuous characters. A fundamental difference between these species concepts is how discontinuous characters arise. According to the recognition concept, discontinuous characters are a consequence of species-specific, mate recognition systems, and, according to the isolation concept, species exist because of a number of "isolating mechanisms". Practicing taxonomists may have difficulty in a strict application of either of these concepts (Sokal and Crovello 1970); however, advocates for each concept accept that the existence of species can be established by inference without studying directly either species isolation or

recognition, but by discontinuities or gaps in one or more characters.

General Methods

In this study, I examined a number of intrinsic characters (Wiley 1981) to determine if discontinuities existed, which would confirm the validity of C. banksianae. As White et al. (1990) correctly point out, the existence of discontinuities does not make the existence of the species intelligible; therefore, I interpreted my findings in terms of Mayr's (1969) biological species concept. As a working hypothesis, I assigned species identifications by host association, i.e., Conophthorus from jack, red, and white pine, were C. banksianae, C. resinosae and C. coniperda, respectively. This was a necessary 'first-cut' because C. banksianae and C. resinosae were morphologically indistinguishable, but presumably had distinct host affinities, and because the few recognized anatomical characters of C. coniperda (Wood 1982) were extremely difficult and impractical to use.

I re-investigated the life history of C. banksianae to determine the number of generations per year, to document its mating site, and to characterize its shoot attack behaviour. The life histories of C. resinosae and C. coniperda did not require

re-investigation because these have been studied well (Lyons 1956; Godwin and Odell 1965). The baseline data from the life history study were gathered to broaden and clarify understanding of the natural history of this insect. Furthermore, these data could be used to assess the implications of life history differences (if any) between C. banksianae and C. resinosae / C. coniperda for reproductive isolation, and to facilitate formation of hypotheses about species integrity.

The host acceptance patterns of these two species were re-investigated, not only because the study of McPherson et al. (1970b) was inconclusive, but also because host-associated mating can be an important isolating mechanism (Diehl and Bush 1984). I used both laboratory and field experiments to test the null hypothesis that C. banksianae and C. resinosae have the same host acceptance pattern. To further the understanding of plant-insect relationships, and the degree of sympatry among C. banksianae, C. resinosae and C. coniperda, I mapped the distribution of the three species.

The null hypothesis that these three species form a single breeding population was tested by examining the male genitalia, chromosomes, and allozymes. The null hypothesis would be rejected if a fixed genetically-determined difference was found, as it shows that interbreeding is not occurring. For example, a fixed

difference occurs when species fail to share any alleles (diagnostic alleles, sensu Ayala and Powell 1972) at a locus, as determined by allozyme electrophoresis. A fixed difference between populations in chromosome number or morphology, or genital structures would be interpreted similarly.

I also initiated a study to determine if Conophthorus utilize pheromones. Semiochemical-based communication by pheromones predominates in the bark beetles (Borden 1985). Kinzer et al. (1972), and Kinzer and Reeves (1976) demonstrated short range attraction by male C. ponderosae Hopkins to females in cones. Only female beetles initiate cone attack. Males enter the cone after it has been attacked, and mating occurs on or near the cone (de Groot 1986a). These observations led me to erect and test the hypothesis that female cone beetles produce a sex pheromone. Evidence which supports this hypothesis could then lead to the isolation, identification, and synthesis of compounds for further testing.

Specificity of the chemical message may effectively prevent interspecific breeding. Species-specific pheromones could be used as taxonomic characters for distinguishing Conophthorus spp. The discovery, isolation, chemical identification, synthesis, and the formulation and testing of synthesized compounds, is a major enterprise. Such an undertaking requires a multidisciplinary

approach; therefore, I worked in collaboration with Dr. Harold Pierce, Department of Chemistry, Simon Fraser University, to isolate and identify potential pheromones of Conophthorus. The original plan was to identify pheromones for C. banksianae, C. coniperda and C. resinosae. However, populations of C. banksianae collapsed throughout Michigan, Ontario, and Quebec (the only three areas where it is known to occur) soon after the study was initiated in 1988 and have not recovered appreciably since. This collapse limited the work on pheromone identification for C. banksianae, and also forced me to discontinue further studies on host acceptance. Therefore, most of the semiochemical data was collected for C. resinosae and C. coniperda.

In the conclusions of this dissertation, I offer my assessment about the validity of C. banksianae, C. resinosae and C. coniperda and suggest areas for further biological inquiry.

Chapter 2

Life history of the jack pine tip beetle, Conophthorus banksianae McPherson

Introduction

The jack pine tip beetle, C. banksianae, has a number of life history traits rare for the genus. In addition to C. terminalis Flores and Bright (Flores and Bright 1987), it is the only other species of Conophthorus that does not attack and reproduce in cones (McPherson et al. 1970a); instead it attacks shoots only. The shoot attack behaviour is also different from that of its sibling species, C. resinosae (Mattson 1989), and C. terminalis (Flores and Bright 1987). Another fundamental difference is that C. banksianae may be bivoltine (McPherson et al. 1970a), whereas all other species of Conophthorus north of Mexico are univoltine (de Groot 1986a). McPherson et al. (1970a) suggested that C. banksianae may be bivoltine, because there was decline in egg abundance followed by a two-week interval during which they were absent, and because first generation adults were dead by the time eggs re-appeared. McPherson et al. (1970a) also found the immature stages of C. banksianae to be present in the field for a much longer time than those of C. resinosae.

Differences in the number of generations per year, seasonal development, and shoot handling behaviour may have important implications for, or be indicators of, reproductive isolation. However, the conclusions of McPherson et al. (1970a) are questionable, because of the lack of bimodality in other life stages, the fact that the reproductive status of the brood adults was not examined, and because non-genetic explanations for the prolonged seasonal development were not considered. Therefore, I investigated the life history of C. banksianae, specifically to: 1) determine the number of generations per year, 2) document its mating site and seasonal breeding period, and 3) characterize its shoot attack behaviour.

Methods

To determine the number of generations of C. banksianae per year, their breeding period and shoot attack behaviour, insects were collected from two jack pine plantations, located near Aubrey Falls, ON (47°00'W, . 83°00'N). The first plantation was sampled in 1986 only. Trees at this site were 10 years old, with a height of 2.44 ± 0.01 m ($x \pm SE$, $n=30$), a diameter at breast height (dbh, 1.3 m) of 3.0 ± 0.20 cm ($n=30$), and with a density of about 2350 stems per ha. Because access to this plantation was blocked in late April of 1987 and 1988 (time of the first emergence of the adults), another plantation was sampled in those

years. The trees in the second plantation were 10 years old in 1988, with a height of 2.72 ± 0.12 m ($n=30$), a dbh of 3.4 ± 0.25 cm ($n=30$), and a density of about 3350 stems per ha.

Before sampling, the plantations were surveyed to determine the area of infestation by C. banksianae. Infested areas within the plantation were divided into three areas of about equal size. Within each area, a random starting point was selected, from which trees were sampled consecutively along planting rows. All attacked shoots were removed from each tree. The number of trees and planting rows visited in each area depended upon the level of infestation and the time of sampling. Trees were sampled once. As the season progressed and the insect population increased, the number of shoots sampled per visit increased from 30 (time when the first shoots were attacked) to 120. In 1986, weekly sampling of the beetle populations began in June and continued until the beetles had dropped to the ground to overwinter. In 1987, sampling began on 22 April when the beetles began to attack the buds, and was terminated on 23 September when most of the beetles were in the newly-shed buds on the ground. Sampling in 1988 began on 4 May and terminated on 8 June, after three consecutive weekly samples indicated that all overwintered females were mated. Sampling in 1987 and 1988 was preceded by on-site surveys to determine the time of emergence.

Each infested shoot was placed separately in a vial, and stored < 24 h at 4°C until examined. For each shoot, the numbers of eggs, larvae, pupae, and adult males and females were recorded. Completed oviposition galleries collected in June 1986 were measured to determine gallery length in old and new wood, shoot diameter at the entrance hole, and distance between egg niches. The lengths of galleries used for feeding and mating only were measured in 1987. The incidence of galleries containing eggs with entrance holes in old and new shoots was recorded in 1987. Head capsule widths of larvae preserved in 70% ethanol were measured with a calibrated ocular micrometer. Head capsule widths >0.435 mm indicated the second of two instars (Herdy and Thomas 1961). The sex of adults was determined by examination of the terminal abdominal tergites (Herdy 1959). To estimate the seasonal period of breeding, spermathecae were examined in all females collected from the field samples. Spermathecae were removed from the females which had been preserved in ethanol-acetic acid (3:1), and stored at 4°C. The spermathecae were stained with 45% aceto-carmine for 1-2 min, mounted under cover slips on glass slides, and examined under phase-contrast microscopy for the presence of spermatozoa.

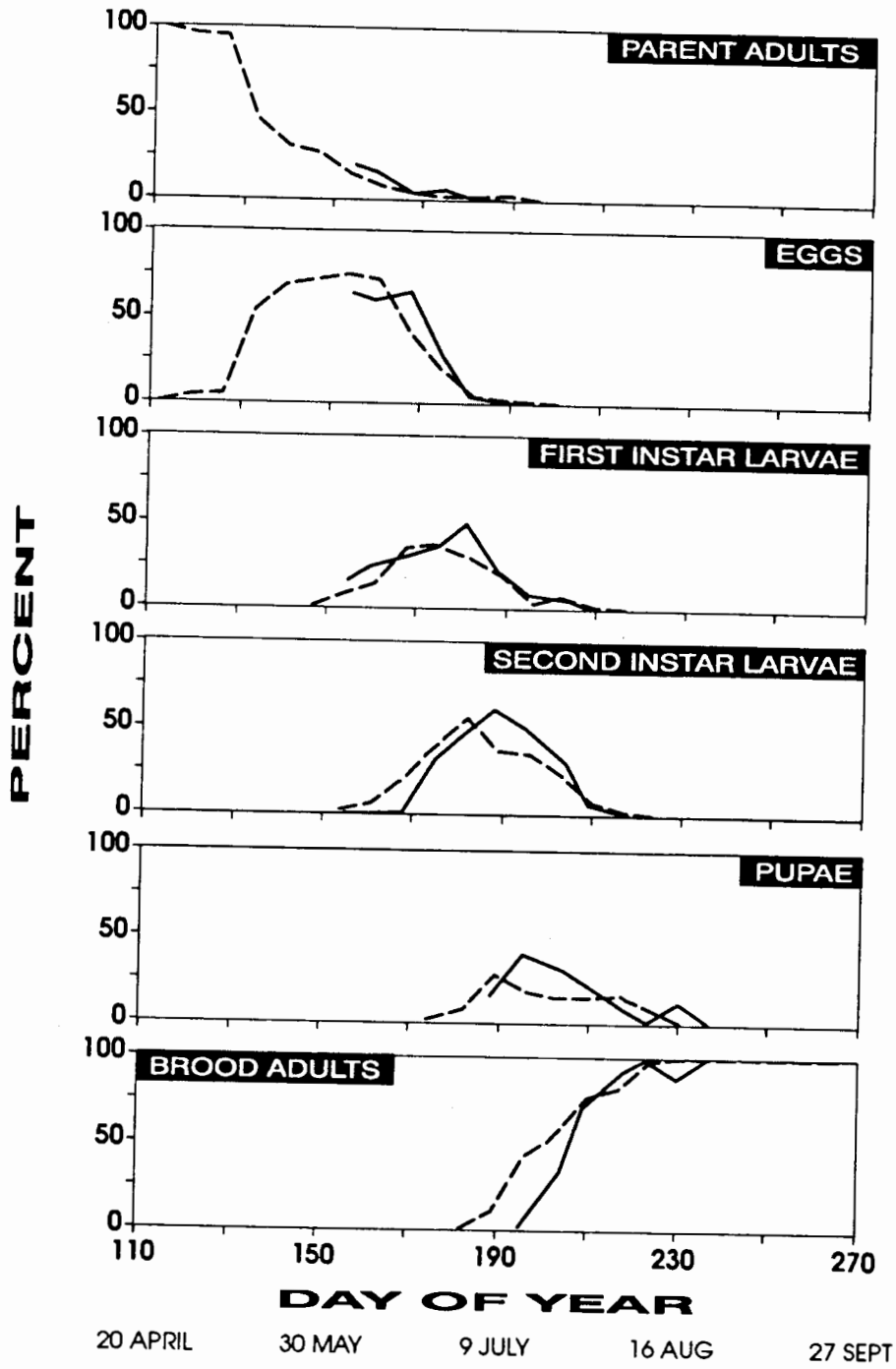
Following the discoveries that there was a female-biased sex ratio in C. banksianae and that some females were mated before overwintering, I examined C. resinosae for these specific traits.

Adults of C. resinosa were collected from infested buds in October 1986 and in 1987 from a red pine, P. resinosa Ait., plantation, near Thessalon, ON (83°30'W, 46°15'N). The plantation was 53 years old in 1986 and the trees were approximately 19 m tall. Living and dead adults were removed from the buds to calculate the sex ratio. Spermathecae were examined as above.

Results

Overwintered adults appeared at the end of April and were last collected in the 1st week of July (Fig. 2.1). Eggs were found about 1 week after the first adults appeared, and were present until mid-July. Unmated females from the previous years' brood were never found in the presence of eggs in the spring. Larvae were present from late May to early August, and pupae were found for 6-7 weeks after the 3rd week of June. It appears that it takes about 70 days for C. banksianae to develop from eggs into adults (Fig. 2.1). Brood (teneral) adults first appeared in early July, slightly before the last overwintered adults were seen. The overwintered beetles were black and found exclusively in freshly-attacked shoots, often in the presence of eggs. They were easily distinguished from the teneral adults, which remained brown for about 1 week and were in the sealed brood gallery. After emergence from the brood gallery, the new

Fig. 2.1 Temporal frequency of stages of Conophthorus banksianae during 1986 (solid line) and 1987 (dotted line), Aubrey Falls, Ontario.



adults mined the current year's shoots and fed inside the buds. These buds fell to the ground beginning in September and the beetles remained within them until the following spring.

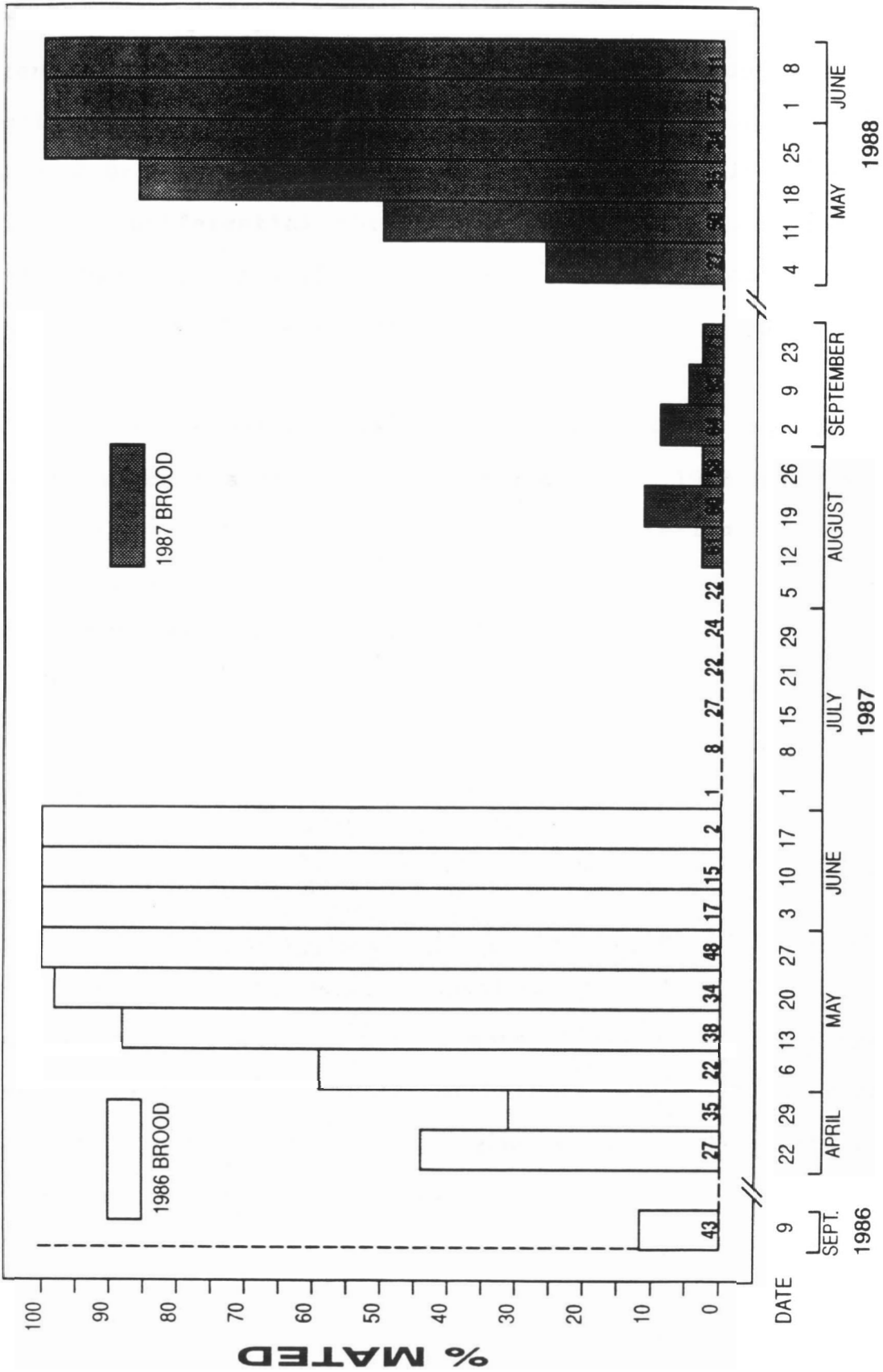
Beetles started a gallery for feeding, mating, or both, in the previous year's (old) shoot when the new shoots of jack pine began to elongate in late April or early May. These single galleries were bored axially along the centre of the shoot and averaged 8.3 mm (SE = 0.24, n = 127) in length. Galleries used for oviposition averaged 16.1 mm (SE = 0.49, n = 131) in length, of which 11.1 mm (SE = 0.33, range 1.6-23.0 mm) was in the old shoot and 5.0 mm (SE = .45) in the new shoot. Until mid-June of 1987, all beetle galleries originated in the old shoots; thereafter, most attacks started in the new shoots. Although the percentage of maximum annual shoot elongation was not measured, it appeared that beetles completed their attacks before shoot elongation was completed. Eggs were deposited singly in shallow niches cut along the gallery. The first egg niche was 5.9 mm from the gallery entrance (SE = 0.21, n = 131) with egg niches irregularly spaced apart (\bar{x} = 3.2 mm, SE = 0.14, range 0.4 to 17.9 mm, n = 260). Egg niches extended into the new shoot about 49% of the time (n = 131). Each gallery contained an average of 2.67 eggs (SE = 0.07, n = 381). After oviposition, the female sealed the gallery entrance with resin and boring debris, unlike the galleries used for feeding only, which were left open.

Gallery construction was done solely by the females. Males removed debris produced by the females. Males were almost always found in an enlarged area, possibly a turning niche, a nuptial gallery, or both, just inside the gallery entrance. Mating was observed in the gallery, but also with the male on the outside of the shoot and the female's abdomen protruding from the gallery entrance. Some males stayed with the female for the entire oviposition period in the shoot.

Mated females were not found for 6 weeks after the 1st of July in 1987 (Fig. 2.2). Some C. banksianae adult females had mated by September before overwintering: 12% (n=43) in 1986, and 4% (n=63) in 1987 (Fig. 2.2). These females were found only in the overwintering buds, about 5 weeks after eggs were no longer found in the shoots. The remaining females mated the following spring and were mated by the end of May in both years. Virgin females were found in the shoots until about 3-4 weeks after the first adults had appeared in the spring. Pre-overwintering mating also occurred in C. resinosae, about 5% of the overwintering females (n=56) in 1986 and 6% (n=30) in 1987.

A female-biased sex ratio was found in two generations of C. banksianae throughout the period of adult occurrence. In September 1986, the ratio of overwintering females to males was 2.9:1 (n=482). Although this ratio decreased to 2.1:1 (n=406) the

Fig. 2.2 Proportion of Conophthorus banksianae females from 1986 and 1987 broods mated by specific sampling dates. Numbers below each bar indicate sample size. Data for 1987 brood begin 01 July 1987; no brood female was mated until 12 August 1987.



following spring, the sex ratio of their progeny was also female-biased; 2.7:1 (n=167) for the teneral adults in the brood gallery, 1.8:1 (n= 712) for the adults in the buds in September, and 2.0:1 (n=307) for the newly-emerged adults the following spring. Differential mortality of teneral males and females was not observed. None of the females (n=106) examined from the brood galleries in 1987 was mated.

A female-biased sex ratio also was observed in the overwintering adults of C. resinosa. In 1986 the ratio of C. resinosa females to males in overwintering buds was 3.5:1 (n=314); in 1987 this ratio was 2.9:1 (n=246). All ratios for both species were significantly different from 1:1 (Chi-square test, $P < 0.001$).

Discussion

The pattern of seasonal abundance of the life stages lead me to reject the hypothesis that C. banksiana is bivoltine. I did not observe a temporal bimodality in insect abundance nor a discernable absence in one or more of the insect life stages that would suggest bivoltinism. Furthermore, there is no evidence from the examination of the spermathecae and life history data to indicate that the teneral adults mated and laid eggs in the year they were born as suggested by McPherson et al. (1970a).

Specifically, the life cycle data do not show a bimodality in egg abundance, the only evidence upon which McPherson et al. (1970a) suggested that C. banksianae was bivoltine. The life cycle data from the sampling sites show that C. banksianae is univoltine, a finding that is in agreement with the preliminary study of Herdy and Thomas (1961), and the observation that all other species of Conophthorus north of Mexico are univoltine (Hedlin et al. 1980; de Groot 1986a).

The length of time that parent adults of C. banksianae attacked shoots (79 days, 1987 data) was in close agreement with the time observed by McPherson et al. (1970a) (81 days). Similarly, the time intervals between the appearance of the parent adults and the brood adults were 72 and 75 days, respectively. My estimate of 70 days for C. banksianae to develop from eggs to adults agrees well with the estimate of 71 days for C. resinosae (Lyons 1956); however it appears from McPherson's et al. (1970a) data that new adults appeared about 30 days after eggs were first noticed. Pupae were present about 3 weeks longer in McPherson's et al. (1970a) study, than in mine. Also I found eggs within 5 days after adult feeding began; McPherson et al. (1970a) did not find eggs until about 6 weeks after the adults emerged. Reasons for the differences are not known, although both studies were based on weekly samples and a similar number of shoots. In northern Ontario, Herdy and Thomas (1961) found C. banksianae

eggs within 2-3 weeks of emergence. Mattson (1989) sampled jack pine in Michigan in the same locality as McPherson et al. (1970a) and concluded that oviposition occurs in the early attacks.

It is possible that the few mated C. banksianae females found in the samples in September, were females overwintering for a 2nd year. However, the sampling protocol required that the entire tree be surveyed, thus ensuring that all females on trees (teneral and those that may overwinter for a second time) would be collected for examination. Furthermore, I did not find mated females in our weekly samples for 6 weeks in 1987 before September. Also, the 1987 samples revealed that males and females do pair in buds before overwintering, and in some of these pairs, the female was mated.

Female-biased sex ratios in Conophthorus and other scolytids are commonly found during the attack phase (Godwin and Odell 1965; Dale and Schenk 1979; Cook et al. 1983). Reasons for the biased sex ratios of the brood adults of C. banksianae that begin overwintering are not known. I found no evidence of all-female broods as in some species of Scolytidae (Lanier and Oliver 1966). A sex ratio bias due to inbreeding (Hamilton 1967) appears unlikely because none of the females examined from the brood galleries was mated.

Both McPherson et al. (1970a) and Mattson (1989) observed in northern lower Michigan that beetles attacked the old shoots for feeding, but used the current year's shoots for oviposition. In Ontario, my study and that of Herdy and Thomas (1961) found that oviposition begins in the old shoots, and only after mid-June are the current year's shoots used exclusively. The difference between the studies probably is related to the time when peak oviposition occurred and may be due to differences in weather. McPherson (1968) noted that the spring of 1967 (the year most data were collected) was colder than normal and thus may have delayed the development of the beetles. McPherson et al. (1970a) and Mattson (1989) observed that peak oviposition for C. banksianae began in late June when most new shoots had fully elongated; peak oviposition in our study occurred in early June before shoots had completed elongation.

Because C. banksianae is univoltine there is no potential for at least one generation in a year to be reproductively isolated from C. resinosae. There are, however, no direct comparisons between the breeding periods of the two species. McPherson et al. (1970a) indicated that overwintering and brood adults of C. resinosae and C. banksianae appeared at about the same time of year. If both species have similar prereproductive requirements and mating behaviour, brood females of both species should be receptive to mating at about the same time for 1-2 months before

overwintering. In the spring, the amount of temporal isolation between the two species is more difficult to estimate because there may be differences in the onset of emergence and mating due to microclimatic differences between sites (Henson 1967; Stark 1982). These differences may be of less significance if females are receptive to, and use the spermatazoa of, more than one male, thus effectively extending the spring mating period. Complete reproductive isolation may not occur if there is a temporal overlap of the spring breeding period of the two species.

Mattson (1989) compared the shoot attack behaviour of C. banksianae and C. resinosae and noted that most C. banksianae entered shoots about 6 mm below the base of the bud. On the other hand, C. resinosae entrance holes appeared randomly along the shoots but were absent very close to the bud. There was a tendency for C. resinosae to attack new shoots near the base early in the growing season and near the top later (Mattson 1989). It appears, however, that the shoot handling behaviour of C. resinosae near the end of the oviposition period parallels the behaviour of C. banksianae at the beginning of oviposition. A striking similarity between both species is that they need to feed on buds before overwintering, unlike all other Conophthorus spp. (de Groot 1986a). Although the feeding behaviours of C. banksianae and C. resinosae are similar in many ways, differences do remain (McPherson et al. 1970b; Mattson 1989). As Mattson

(1989) indicated, the shoot attack behaviour of C. banksianae on jack pine appears to be a canalized subset of the behaviour of C. resinosae, which accepts both cones and shoots of red and jack pine and cones of white pine, P. strobus L., (Tabashnik et al. 1985). Clearly, the insect-plant relationships of C. banksianae and C. resinosae need to be investigated further.

Chapter 3

Geographic distribution of Conophthorus in eastern North America

Introduction

The distributions of plants and animals are produced by historical and ecological events, but, it is difficult to separate the effects of these two factors and to estimate their relative roles (Endler 1982). Describing the spatial and temporal distribution of organisms, and understanding how these patterns came about, is an aim of biogeography (Darlington 1957). However, before biogeographic hypotheses can be proposed, baseline data on ranges, habitat requirements, and hosts are needed.

In this chapter, I collate the published and unpublished data on the range of C. banksianae, C. coniperda and C. resinosae, and discuss these distributions in relation to their hosts.

Methods

Distribution of hosts. Range maps for jack, white, and red pine were obtained from Little (1971).

Distribution of Conophthorus. Maps showing collection localities were produced for C. banksianae, C. coniperda, and C. resinosae from: 1) published records (Bright 1976; Wood 1982), 2) published reports and unpublished data from the Forest Insect and Disease Survey (FIDS), Forestry Canada, and 3) from requests to 37 museums and institutions in North America known to have a collection of Coleoptera.

Distribution data used to compile the maps were subjected to the following limitations and assumptions:

1) Only host records for C. banksianae, C. coniperda and C. resinosae on jack, white, and red pine, respectively, were used for this treatment. There are a few records of C. banksianae and C. resinosae from Scotch pine, P. sylvestris L., an introduced species that is widely used for Christmas trees. The range of Scotch pine has not been documented, nor are there sufficient data to determine if it has become a significant additional host for Conophthorus. In the original treatment of the genus, Hopkins (1915a) named C. virginianae as a species found on virginia pine, P. virginiana Mill. Wood (1977) placed this species in synonymy with C. resinosae; because no other records for virginia pine are known to me, I have not included it here. The few records of C. coniperda on other hosts are regarded as accidental (Wood 1982).

2) Host species were correctly identified. This appears to be a reasonable assumption as the cones and shoots of red, jack and white pine are easy to distinguish by a number of features.

3) Insect species were correctly identified. Wood (1982) personally examined all specimens listed in his taxonomic monograph; these specimens are indicated by an asterisk in the list below. Specimens collected by FIDS are routinely verified by FIDS taxonomists, and therefore are assumed to be correct.

4) The three species are specific to one host (see number 1 above). Both C. banksianae and C. resinosae are always distinguished on the basis of their hosts (Wood 1982), and this practice is often used for C. coniperda as well (D.E. Bright, Biosystematics Research Centre, Ottawa, Ontario, pers. comm.). Therefore, there is no way to distinguish a jack pine shoot attacked by C. resinosae from one attacked by C. banksianae. In my karyotype and allozyme analyses of field-collected specimens, I never found any evidence that C. coniperda attacked red pine, nor that C. resinosae attacked white pine, even in forest stands where red and white pine were interspersed (see Chapters 6 and 7).

To facilitate any future taxonomic study of these three species, a list of locality records from museum specimens, the

museums where they are stored, and their present curators, was compiled. Acronyms for the institutions were obtained from Heppner and Lamas (1982) and Arnett and Samulenson (1970). Specimens verified by Wood (1982) are marked with an asterisk.

American Museum of Natural History, New York, New York (AMNH),

Lee Herman

Canadian National Collection of Insects, Ottawa, Ontario (CNC),

Donald E. Bright

Connecticut Agricultural Experiment Station (CAES),

Kenneth A. Welch

Cornell University, Ithaca, New York (CU),

James K. Liebherr

Department of Agriculture, Bureau of Plant Industry, Harrisburg,
Pennsylvania (PADA), Karl Valley

Forest Insect and Disease Survey, Sault Ste. Marie, Ontario
(FIDS-SSM), Paul Syme

Forest Insect and Disease Survey, Fredericton, New Brunswick
(FRLC), Edward Hurley

Michigan State University, East Lansing, Michigan (MSUE), Gregory
A. Dahlem

New York State Museum, Albany, New York (NYSM),

Tim L. McCabe

North Carolina State University, Raleigh, North Carolina (NCSR),
Robert C. Blinn

Ohio State University, Columbus, Ohio (OSU),

Charles A. Triplehorn

Purdue University, West Lafayette, Indiana (PUL),

Arwin Provnsa

Southern Illinois University at Carbondale, Carbondale, Illinois,

(SIUC), J.E. McPherson

United States Museum of Natural History, Washington, D.C.

(USNM), Donald M. Anderson

University of Connecticut, Storrs, Connecticut (UCS),

Jane E. O'Donnell

University of Georgia, Athens, Georgia (UGA),

Cecil L. Smith

University of Massachusetts, Amherst, Massachusetts (UMA),

T. Michael Peters

University of Michigan, Ann Arbor, Michigan (UMAA),

Mark F. O'Brien

University of Minnesota, St Paul, Minnesota (UMSP),

Philip J. Clausen

University of Wisconsin-Madison, Madison, Wisconsin (UWM),

Steven Krauth

Virginia Polytechnic Institute and State University, Blacksburg,

Virginia (VPI), Mary Roades

Yale University, New Haven, Connecticut (PMY),

David Furth

Results

Distribution of hosts. The botanical ranges of jack, white, and red pine are shown in Figs. 3.1-3.3, respectively.

Distribution of Conophthorus. The collection localities for C. banksianae, C. coniperda and C. resinosae are shown in Figs. 3.4-3.6, respectively. Lists of curated specimens are presented below.

Distribution of C. banksianae. CANADA. Ontario: Black Sturgeon Lake* (CNC), Chapleau, (CNC, FIDS-SSM), Dryden (CNC, FIDS-SSM), Franz* (CNC), Gogama (CNC, FIDS-SSM), Hurkett (CNC), Oba* (CNC), Sault Ste. Marie* (FIDS-SSM). UNITED STATES. Michigan: Cadillac* (CNC, MSUE, USNM), Christensen Nursery, Fife Lake* (CNC, MSUE, USNM), Grand Traverse Co* (CNC, MSUE, USNM), Kalkaska Co.* (CNC, MSUE, USNM), Wexford Co.* (MSUE, CNC USNM). Minnesota: Cass Lake, Ithasca Co.* Wisconsin: Three Lakes.*

Fig. 3.1 Botanical range of jack pine, Pinus banksiana Lamb., in North America, from Little (1971).



Fig. 3.2 Botanical range of eastern white pine, Pinus strobus L.,
in North America, from Little (1971).



Fig. 3.3 Botanical range of red pine, Pinus resinosa Ait. in North America, from Little (1971).



Fig. 3.4 Geographic range of Conophthorus banksianae McPherson in
North America

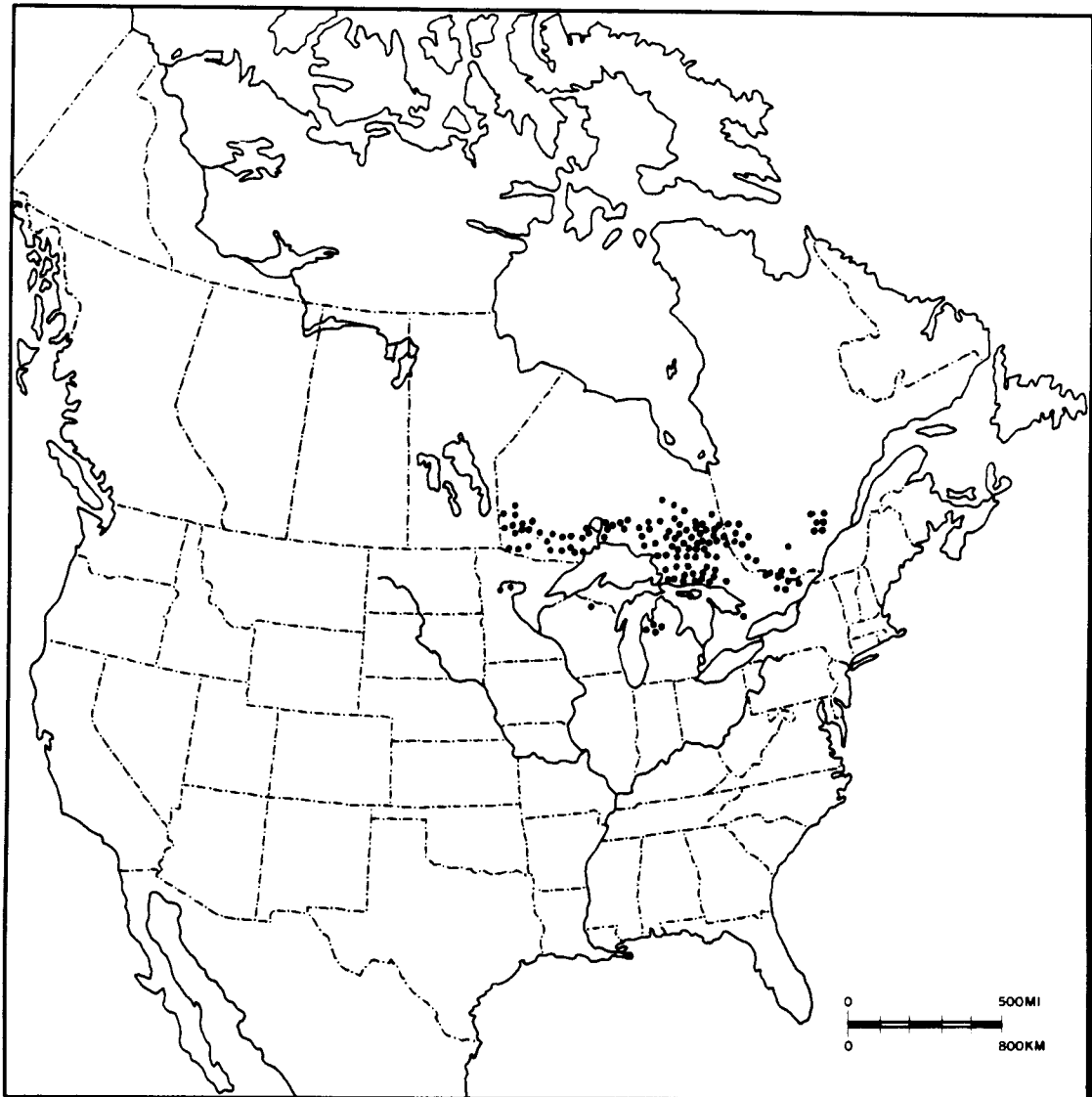


Fig. 3.5 Geographic range of Conophthorus coniperda (Schwarz) in
North America



Fig. 3.6 Geographic range of Conophthorus resinosae Hopkins in
North America



Distribution of C. coniperda. CANADA. Ontario: Algonquin Park* (USNM), Angus* (CNC,FIDS-SSM), Blind River, (CNC, FIDS-SSM), Chalk River (CNC,FIDS-SSM), Dorset (CNC, FIDS-SSM), Fort Francis (CNC, FIDS-SSM), Haley Station* (CNC), Hurkett*, Lindsay, (CNC, FIDS-SSM), Ottawa*, Simcoe* (CNC,FIDS-SSM), Thessalon (CNC, FIDS-SSM),. Quebec: Berthierville (CNC), Lanoraie,* (CNC), Quebec City* (CNC) New Brunswick: Hillsboro (CNC, FRLC), Little River (CNC, FRLC), Pollett River (CNC, FRLC), Queens Co. (CNC, FRLC) Upper Gagetown (CNC FRLC), Nova Scotia: Hant Co.*, Kentville (CNC, FRLC), Lawrencetown (CNC, FRLC).

UNITED STATES. Connecticut: Stamford (UMA), Storrs (UCS). Indiana: Radioville, Pulaski Co. (PUL). Maine: Bangor* (USNM), Orono* (USNM). Massachusetts: Amherst (UMA), Branford (PMY), Cambridge* (USNM), Framingham* (USNM), Marion* (USNM), Martha's Vineyard* (UMA,UMSP,USNM), Petersham* (UMSP,USNM, PMY), So. Harwich (UMA). Maryland: Ashton* (USNM). Michigan: Bear Lake*, Eagle Harbor*, Marquette*. Minnesota: Cass Lake (UMSP), Chippewa Falls*, Duluth (UMSP), Filmor Co.*, St Anthony*. New Hampshire: Durham* (USNM), Pike* (MSUE,USNM). New Jersey Clemonton* (USNM), Greenwood*, Lakehurst*, Orange Mt.*. New York: Cranberry Lake (UMA,AMNH,OSU,USNM), Ithaca* (USNM), Lackawacki (UMA), Long Island (NYSM), Olcott* (CU), Saratoga (NYSM), Schenectady (UMA,CU), South Hampton (USNM), Upper Jay* (USNM), Warrensburg (NYSM,UMSP), Westbury* (USNM), West Point* (USNM). North Carolina: Asheville (UGA), Alleghany Co. (NCSR), Avery Co.

(NCSR), Buncombe Co. (USNM), Hendersonville* (USNM), McDowell Co (SIUC). **Pennsylvania:** Caledonia (OSU,PADA), Cook Forest* (USNM), Dauphin Co. (PADA), Monroe Co. (PADA), Mt. Alto* (OSU,USNM). **Virginia:** Blacksburg (VPI), Culpepper* (USNM), Monroe*, Montgomery Co. (VPI), Norfolk*. **Wisconsin:** Clintonville, Wood Co.* (UWM).

Distribution of C. resinosa. **CANADA.** Ontario: Algoma Mills (CNC, FIDS-SSM), Burnt River* (CNC, FIDS-SSM), Camp Borden* (CNC), Carnarvon (CNC, FIDS-SSM), Carp*, Chalk River* (CNC, FIDS-SSM), Dorset* (CNC), Haley Station (CNC), Manitoulin Island (CNC, FIDS-SSM), Midhurst (CNC), Midland* (CNC, FIDS-SSM), North Bay (CNC, FIDS-SSM), Parry Sound* (CNC), Sault Ste. Marie* (CNC, FIDS-SSM), Temagami* (CNC), Tobermory (FIDS-SSM). **Quebec:** Laniel*, St. Chrysostome (CNC), Kazubazua Park Reserve*. **New Brunswick:** Albert Co. (CNC, FRLC), Sunbury Co. (CNC), York Co. (CNC,FRLC). **Nova Scotia:** Kentville* (CNC, FRLC). **Prince Edward Island:** Murray Harbor, Kings Co. (CNC,FRLC). **UNITED STATES.** **Indiana:** Underwood, Clark Co. (PUL). **Michigan:** Cheboygan Co. (UMAA), Harrison (UMSP), Keweenaw Co.* (USNM), Marquette (UMSP), Racoon* (UWM), Rapid River (UMSP), Wexford Co. (UMSP). **Minnesota:** Brainerd (UMSP), Cass Lake* (USNM), Itasca Co.* (USNM). **New Hampshire:** Effingham* (USNM). **New Jersey:** Greenwood Lake*. **New York:** East Hampton* (USNM), Farmingdale* (USNM). **North Carolina:** McDowell Co. (SIUC). **Pennsylvania:** Troy (OSU). **West Virginia:**

Huttonsville* (USNM). Wisconsin: Chippewa Falls*, Dane Co. (UWM), Lincoln Co. (UWM), Oneida Co. (UWM), Three Lakes (UMSP), Lake du Flambeau (UMSP), Vilas Co. (UWM), Wood Co. (UWM).

Discussion

The distribution of C. coniperda and C. resinosae (Figs. 3.5, 3.6) suggests that the species are found throughout the range of white pine and red pine, Figs. 3.2 and 3.3, respectively. In contrast, the geographic distribution of C. banksianae (Fig. 3.4) suggests that it is confined to isolated and local parts of northern Michigan, and verified in a few locations elsewhere in the Lake States, and to localized parts of the provinces of Ontario and Quebec.

Conophthorus banksianae is totally absent from the vast tracts of jack pine west of Ontario (Fig. 3.1). The possibility that C. banksianae occurs west of Ontario appears remote, because 30-35 years of forest insect surveys have never recorded C. banksianae in jack pine in the Praire provinces (Forest Insect and Disease Survey, Forestry Canada, unpublished data; B. Moody, pers. comm., Forestry Canada, Ottawa, Ontario). The distribution of C. banksianae does not appear to follow the known genetic and phenotypic variability in jack pine (Schoenike 1976; Rudolf and Yeatman 1982). Jack pine populations have been divided into the

Great Lakes and Western geographic regions (Critchfield 1985), and C. banksianae occurs in both of them. Much of the variation in jack pine occurs in the Great Lakes region (Schoenike 1976; Hyun 1979). In fact, Wright (1972) proposed that the populations of jack pine in the upper and the lower peninsulas of Michigan USA represent distinct races. Outside this region, jack pine is fairly uniform from eastern Alberta to Quebec, with increased variation in the Maritime provinces and in New England where the distribution is fragmented (Schoenike 1976). However, the distribution of C. banksianae follows the northern distribution of red pine (Fig. 3.3), and it is not found outside the range of red pine. Furthermore, C. banksianae has not been found in the most southern and eastern parts of the range of C. resinosa (Fig. 3.6). Therefore, in northern Michigan, Ontario and Quebec, it appears that C. banksianae and C. resinosa (and C. coniperda) are sympatric (at least at a broad level),

The apparently confined distribution of C. banksianae presents an interesting puzzle. If C. banksianae is an invalid species, then all twig attacks on jack pine are by C. resinosa, which thus explains why jack pine is only attacked within the distribution of red pine. The circumstances under which C. resinosa attacks jack pine twigs (i.e. switches hosts) need to be explored, as well as the apparent low incidence of attacks on jack pine cones (de Groot 1986a, 1986b). If C. banksianae is a

valid species, then the reasons why it only attacks jack pine within the range of red pine must be explained. McPherson et al. (1970b) indicated that C. banksianae and C. resinosae have different host preferences; these differences may be significant to the maintenance of reproductive isolation between them. However, both species under no choice field cage conditions, can attack jack pine shoots; their behaviour under choice conditions is unknown (McPherson et al. 1970b). Therefore, further investigation of the host range of these species is warranted and is the subject of the next inquiry.

Chapter 4

Host selection by C. banksianae and C. resinosae

Introduction

Studies of host selection by insects have led to the recognition of host races and sibling species (e.g Diehl and Prokopy 1986; Prokopy et al. 1988; Pashley 1988). Differences in host preference, particularly when mate choice is directly coupled to host selection, may maintain full or partial reproductive isolation between sympatric sibling species or host races, respectively (Diehl and Bush 1984).

Different host selection behaviours, combined with differences in their life history and body size, led McPherson et al. (1970a, 1970b) to describe the jack pine tip beetle, C. banksianae, as a sibling species of C. resinosae. McPherson et al. (1970b) found that field-caged C. resinosae attacked the cones and twigs of red and jack pines, they most preferred red pine cones and least preferred jack pine cones. On the other hand, C. banksianae would not attack cones of either species, even when they were the only hosts available, but readily attacked the twigs of both pines, preferring those of jack pine. Host choice may be critical in maintaining reproductive isolation

between C. banksianae and C. resinosae because mating occurs on or near the host after females have initiated feeding (Lyons 1956; Chapter 2). However, the experiments of Mcpherson et. al. (1970b) should be considered inconclusive because of the small sample sizes used, and because the prior feeding experience of C. banksianae may have influenced their host-choice 'decisions' (see Papaj and Prokopy 1989).

To assess the potential importance of host choice in maintaining reproductive isolation between C. banksianae and C. resinosae, I examined the feeding and oviposition behaviours of females under 'choice' and 'nochoice' in the laboratory and under semi-natural field conditions.

Methods and Materials

General methods. Beetles were collected in September and October as overwintering adults in buds. Conophthorus banksianae came from a jack pine plantation near Aubrey Falls, Ontario, and C. resinosae came from a red pine plantation near Thessalon, Ontario. Fall-collected adults in host buds were stored in paper bags in a dark room, at 2-4°C and 80% RH, for ca 9 months. In the field, I observed that beetles feed inside buds before emergence. To minimize this feeding, buds were removed from storage, held at room temperature for < 1 day, and then placed under the heat of a

60 W incandescent light to compel beetles to emerge. Emerged beetles were quickly removed from the heat and placed in petri dishes lined with moist filter paper. The sex of the adults was determined by examining the terminal abdominal tergites (Herdy 1959). Beetles were maintained in groups of 20-30 on moist filter paper in petri dishes for 1-7 days at 4°C. All experiments used beetles that had no previous exposure to hosts after overwintering. Feeding assays tested the response of females because only females initiate attacks (Lyons 1956; de Groot 1986a; Chapter 2).

Both laboratory and field experiments were conducted in May and early June when cones and twigs are normally attacked by Conophthorus. Field experiments were conducted near Thessalon, Ontario, on sites containing a mixture of red and jack pine and where both C. resinosae and C. banksianae have been recorded. All single-host experiments used 45 X 12 cm (length by diam.), white polyester, sleeve cages placed over branches 2-8 m above the ground on the south side of the trees. Beetles were scored as accepting a test host when they had bored into the host at least one body length within a specified time (see below); otherwise they were scored as rejecting the host.

The proportion of test beetles that accepted the test hosts was compared statistically by G- tests, alpha = 0.05 (Sokal and

Rohlf 1981). The sample size required when the power of the test is set at $1 - \beta = 0.9$, and p_1 , p_2 , n_1 , and n_2 are set at 0.8, 0.5, 30, and 30, respectively is 29 samples (Sokal and Rohlf 1981). The null hypothesis was that C. banksianae and C. resinosa do not differ in their acceptance of cones and shoots of red and jack pine for feeding and for oviposition.

Acceptance of hosts for feeding by females in the laboratory. Host acceptance was assessed in 25 x 100 mm vented plastic petri dishes lined with moist filter paper and a 5 mm layer of plastic foam at the bottom (modified from Jermy et al. 1968). Three 0.2-0.3 g disks of each of 2 hosts were placed 60° apart in an alternate fashion around the circumference of the petri dish. Cone and shoot disks were cut from red or jack pine branches that had been collected the previous day from trees near Thessalon, Ontario. They were placed temporarily in distilled water to reduce deterioration until the required number of disks for the assay were obtained. For each species, single females were released in the center of each of 25 dishes (replicates), and the dishes were held for 3 days at $20-22 \pm 2^\circ\text{C}$, $70 \pm 5\%$ RH and 16L:8D photoperiod. Females were exposed to the following choices: Exp. 1) red or jack pine cone, Exp. 2) red or jack pine shoot, Exp. 3) red pine cone or jack pine shoot, and Exp. 4) red pine shoot or jack pine cone. Dishes were inspected daily, and host acceptance recorded.

Acceptance of hosts for feeding by females in the field. In single host, single stimulus experiments, individual females were placed inside sleeve cages that enclosed either a cone or shoot of red or jack pine (Exp. 1-4). In single-host, dual stimuli choice experiments, females were given a choice between red pine cones or shoots (Exp. 5) or jack pine cones or shoots (Exp. 6). In a mixed-host experiment with shoots (Exp. 7), 1 m X 45 cm sleeve cages were suspended between and enclosed the branch tips of red and jack pine trees. Cages were orientated in an east-west direction with each species in the east position for half of the replicates.

Since mixed-host assays with cones were not possible in situ, the final two experiments used branches removed from trees on the site. Branches were cut underwater and the cut ends placed in tap water in plastic bags wrapped in aluminum foil to reduce solar heating of the water. In these experiments, branches were placed inside 1 m X 45 cm sleeve cages, and oriented as in the mixed-host, shoot assays, but on the ground. Beetles were given a choice between red pine and jack pine cones in Exp. 8. Exp. 9, comprised a choice of four feeding sites: a red pine cone and shoot on the same branch, and a jack pine cone and shoot on the same branch. All experiments used single twigs and/or cones, with surplus cones and shoots removed, and the wounds sealed with

white glue, 1-2 days before the experiment. For each assay, 30 cages were assigned at random to either C. resinosa or C. banksiana. Cages were inspected periodically for a 2-week duration. When two host structures were attacked, the first attack was used for analysis.

Oviposition in field-cages. Males and females were paired at random and placed inside 45 X 12 cm sleeve cages on trees that enclosed two cones and shoots of either red pine (Exp. 1) or jack pine (Exp. 2). For each experiment, a total of 30 mating pairs of C. resinosa and C. banksiana were assigned to each host, one pair per cage, at random. The host structures were examined 7 weeks later to determine where oviposition occurred.

Results

Acceptance of hosts for feeding by females in the laboratory. There was no difference in response between C. resinosa and C. banksiana to cone slices of red and jack pine that were offered together (Table 4.1, Exp. 1). When red and jack pine shoots were offered, C. resinosa accepted significantly more red pine shoots than C. banksiana, but the response of both species to jack pine shoots did not differ significantly. Similarly, beetles of both species did not differ in their acceptance of red pine cones and jack pine shoots when these two were provided simultaneously (Table 4.1, Exp. 3), but when red pine shoots and jack pine cones

Table. 4.1. Feeding responses by individual female Conophthorus resinosa and C. banksiana in the laboratory in mixed host assays with disks of red pine or jack pine cones, shoots, or both

Exp. no.	Choice	Percent accepting choice ^a	
		<u>C. resinosa</u>	<u>C. banksiana</u>
1.	red pine cone	52 a	40 a
	jack pine cone	48 a	36 a
	no choice	0 a	24 b
2.	red pine shoot	64 a	36 b
	jack pine shoot	36 a	44 a
	no choice	0 a	20 b
3.	red pine cone	76 a	80 a
	jack pine shoot	24 a	8 a
	no choice	0 a	12 b
4.	red pine shoot	32 a	8 b
	jack pine cone	68 a	84 a
	no choice	0 a	8 a

^a Values in each row followed by the same letter are not significantly different ($P < 0.05$; G test).

were presented, C. resinosae accepted significantly more red pine shoots than C. banksianae (Table 4.1. Exp. 4). In all experiments, except Exp. 4, C. banksianae, had a significantly higher propensity to reject host pairs than C. resinosae (Table 4.1).

Acceptance of hosts for feeding by females in the field. In all experiments with red pine cones (Table 4.2, Exp. 1; Table 4.3, Exp. 5,8,9), C. banksianae accepted significantly fewer cones than did C. resinosae. When red pine shoots were offered in single host assays (Table 4.2, Exp. 2), or with red pine cones (Table 4.3, Exp.5,9), C. resinosae attacked significantly more red pine shoots than C. banksianae. When jack pine shoots were presented together with red pine shoots (Table 4.3, Exp. 7,9) the proportion of either species accepting red pine shoots did not differ significantly. In all experiments where jack pine cones were offered, there were no significant differences in host acceptance between the two species (Table 4.2, Exp. 3; Table 4.3, Exp. 6,8,9) In experiments where jack pine shoots were provided (Table 4.2, Exp. 4; Table 4.3, Exp. 6,7,9), C. resinosae and C. banksianae did not differ in their acceptance, except in experiment 6. The single and multiple host experiments suggested C. resinosae had a preference for red pine cones and C. banksianae had a preference for jack pine shoots. This trend was observed in Exp. 9 (Table 4.3) where all four hosts were offered.

Table 4.2. Feeding responses by individual female Conophthorus resinosa and C. banksiana in the field in single host assays with cones or shoots of red pine or jack pine

Exp. no.	Choice	Percent accepting choice ^a	
		<u>C. resinosa</u>	<u>C. banksiana</u>
1.	red pine cone	80 a	7 b
	no choice	20 a	93 b
2.	red pine shoot	70 a	7 b
	no choice	30 a	93 b
3.	jack pine cone	30 a	27 a
	no choice	70 a	73 a
4.	jack pine shoot	70 a	77 a
	no choice	30 a	23 a

^a Values in each row followed by the same letter are not significantly different ($P < 0.05$; G test).

Table 4.3. Feeding responses by individual female Conophthorus resinosae and C. banksianae in the field in single and mixed host assays with cones, shoots, or both, of red pine and jack pine

Exp. no.	Choice	Percent accepting choice ^a	
		<u>C. resinosae</u>	<u>C. banksianae</u>
5.	red pine cone	57 a	10 b
	red pine shoot	30 a	0 b
	no choice	13 a	90 b
6.	jack pine cone	7 a	10 a
	jack pine shoot	67 a	40 b
	no choice	26 a	50 a
7.	red pine shoot	27 a	13 a
	jack pine shoot	37 a	53 a
	no choice	36 a	34 a
8.	red pine cone	43 a	3 b
	jack pine cone	3 a	13 a
	no choice	54 a	84 b
9.	red pine cone	53 a	0 b
	red pine shoot	10 a	0 b
	jack pine cone	0 a	3 a
	jack pine shoot	13 a	33 a
	no choice	24 a	64 b

^a Values in each row followed by the same letter are not significantly different ($P < 0.05$; G test).

Interestingly, C. banksianae had a significantly higher propensity to reject hosts, when red pine cones and shoots were offered (Table 4.2, Exp.1,2; Table 4.3, Exp. 5,8,9, except Exp.7).

Oviposition in field-cages. In field cages containing red pine, C. resinosae accepted significantly more red pine cones for oviposition than C. banksianae (Table 4.4, Exp. 1). Neither species utilized red pine shoots exclusively for oviposition, but significantly more C. resinosae used both red pine cones and shoots for oviposition than C. banksianae. When jack pine cones and shoots were offered, C. resinosae accepted significantly more jack pine cones than C. banksianae, and conversely, C. banksianae accepted significantly more jack pine shoots than C. resinosae (Table 4.4, Exp. 2). In a few cages, C. resinosae used both jack pine cones and shoots for oviposition.

Discussion

The field experiments demonstrated that C. resinosae and C. banksianae have a similar propensity to accept jack pine cones and shoots for feeding, but differ consistently in their acceptance of red pine cones, and variably in their acceptance of red pine shoots. However, the laboratory experiments did not indicate a difference between the two species in their propensity

Table 4.4. Oviposition responses by individual female Conophthorus resinosae and C. banksianae in the field where offered a choice of red pine cones and shoots or jack pine cones and shoots

Exp. no.	Choice	Percent accepting choice ^a	
		<u>C. resinosae</u>	<u>C. banksianae</u>
1.	red pine cone	27 a	7 b
	red pine shoot	0 a	0 a
	red pine cone and shoot	23 a	0 b
	no choice	50 a	93 b
2.	jack pine cone	27 a	0 b
	jack pine shoot	0 a	23 b
	jack pine cone and shoot	3 a	0 a
	no choice	70 a	77 a

^a Values in each row followed by the same letter are not significantly different ($P < 0.05$; G test).

to accept red pine cones for feeding. The differences between laboratory and field results support the claim that the results of insect preference experiments can be method-specific (Risch 1985; Jones and Coleman 1988). It is likely that cues that beetles use for host discrimination and choice were not available under the laboratory conditions.

A clear trend in the data, which is supported by the observations of McPherson et al. (1970b) and Mattson (1989), is that C. resinosa is more a generalist feeder while C. banksiana is more specialized. Furthermore, C. resinosa seems to prefer red pine cones while C. banksiana seems to prefer jack pine shoots. Although C. banksiana is able to feed and oviposit in red pine cones (contrary to the conclusions of McPherson et al. 1970b), few females 'decide' to do so. This suggests that there may be physiological and, or, behavioural restraints imposed on C. banksiana in accepting red pine cones as feeding and oviposition sites. One interesting phenomena that appears to be quite consistent for C. banksiana was the 'decision' to reject hosts, including jack pine shoots, when red pine cones were present in the field cages. This may suggest that red pine cones produce volatile substances which are toxic, repellent, or both, to C. banksiana. It would be worthwhile to determine a dose-response relationship for red pine cone volatiles and to determine the relationship of this response to the physiological

and physical (body size) condition of this beetle.

The lack of host fidelity, particularly by C. resinosa, suggests that some movement between hosts with consequent intermating may occur where both species are within cruising range of each other's host. The capability of C. resinosa to attack other hosts in nature when cones are scarce is evident from several reports of red pine shoot attacks (e.g., Lyons 1956; Mattson 1978, 1980). The movement of C. resinosa would be most likely when red pine cone crops have failed, which occur frequently (Mattson 1980), or when red pine has been removed from the area. Tabashnik et al. (1985) suggested the host discrimination behaviour of C. resinosa is modified by a prolonged absence of cues from red pine cones. Consequently, in the absence of the preferred host (red pine cones), C. resinosa may become less specific in its acceptance of other hosts such as jack pine or white pine (Tabashnik et al. 1985). Conophthorus resinosa is also known to attack virginia pine (Hopkins 1915a; de Groot and DeBarr, unpublished data), and pitch pine (de Groot and DeBarr, unpublished data). These results clearly indicate that host-selection behaviour is a permeable barrier to gene flow. Thus, other barriers are needed to ensure complete or partial reproductive isolation.

The differences in the host selection behaviours can be

indicative of (1) distinct species (McPherson et al. 1970b), (2) host races (see Diehl and Bush 1984), or (3) intraspecific variation. To resolve if the host-selection behaviours of C. resinosae and C. banksianae are 'properties' or 'characters' of species or host races, further studies should first be directed to determine if host preference (sensu Singer 1986) can vary among populations (e.g., Wiklund 1981,1982; Wasserman and Futuyma 1981; Tavormina 1982; Rausher and Papaj 1983), and within populations (e.g., Tabashnik et al. 1981; Jaenike and Grimaldi 1983; Thompson 1988). Moreover, it should be determined if intraspecific variation (if present) is genetically-based or induced by environmental or behavioural components (Fox and Morrow 1981; Futuyma and Peterson 1985). In addition, studies of individual behaviour should be pursued because host preferences by an individual are not necessarily fixed and can vary in response to changes in the internal physiological state (Miller and Strickler 1984), such as caused by a previous experience with hosts (i.e. host-associative learning) (Papaj and Prokopy 1986), or by a shortage of preferred hosts (Roitberg and Prokopy 1983). Finally, studies should be conducted to assess host selection behaviour in relation to the ebb and flow of available high-quality food resources over time and space (Prokopy et al. 1988). To facilitate studies on the behavioural ecology of C. resinosae and C. banksianae, additional taxonomic characters are needed foremost to verify that sibling species, or host races

exist, and secondly to provide a means of correct identification of populations.

Chapter 5

Taxonomic characters on the male genitalia of Conophthorus banksianae, C. coniperda and C. resinosae.

Introduction

Male genitalia can undergo rapid and divergent evolution (Eberhard 1985), and their structures have provided useful taxonomic characters for many groups of insects (Tuxen 1970). Features on the male genitalia are often used for distinguishing species where external morphological characters are unreliable, non-apparent, or non-existent (Erwin 1970; Clark 1978; Smetana 1982). In the Scolytidae, genitalia have provided reliable characters to separate species of Dendroctonus Erichson (Wood 1963; Lanier et al. 1988) and Pseudohylesinus Swaine (Bright 1969). Herdy (1963) provided a general description of the male genitalia of Conophthorus based on single populations, but did not disclose if genitalic characters could be used to delimit species.

In this study, I examined the male genitalia of C. coniperda, C. resinosae and C. banksianae from different geographic sites to assess intraspecific variation and to determine if characters were present that could be used to distinguish these species.

Methods

Twenty-five males of each species, 5 specimens from each of 5 sites throughout the range of the species (Table 5.1), were examined. Adult males were either preserved in 70% alcohol or in 3:1 ethanol-glacial acetic acid, or dried. Dried specimens were softened in Barber's fluid for 3 h before dissection. Specimens were immersed in distilled water, and the male genitalia removed from the body by pulling on the 7th and 8th abdominal tergites with fine forceps. Genitalia were cleared by placing them in 0.5 mL cell plate wells containing 5% cold potassium hydroxide for 6-8 h at room temperature, at 20-22°C. After washing the genitalia 3-4 times with freshly-distilled water, they were transferred to depression slides containing glycerin. In glycerin, the internal accessory apparatus was teased away from the median lobe with fine forceps and number 000 insect pins. Sclerotized genitalic structures were examined at 40X with a dissecting microscope, and some specimens were examined with a scanning electron microscope at various magnifications. After examination, the genitalic parts from each specimen were placed in microvials containing glycerin. Genitalia were photographed through light and scanning electron microscopy, but because not

Table 5.1. Collection sites for Conophthorus banksianae, C. coniperda and C. resinosae used in the analysis of characters on the male genitalia

Species	Collection Sites
<u>C. banksianae</u>	Wardle Twp., ON Lane Twp., ON Bellecombe Twp., PQ Emard Twp., PQ Kalkaska Co., MI
<u>C. coniperda</u>	Burke Co., NC Cumberland Co., NS Clairfield Co. PA Moose Lk., North West Angle Prov. Pk. MN Mackinac Co., MI
<u>C. resinosae</u>	Tosorontio Twp., ON Wylie Twp., ON Cumberland Co., NS Strathcona Twp., ON Kirkwood Twp., ON

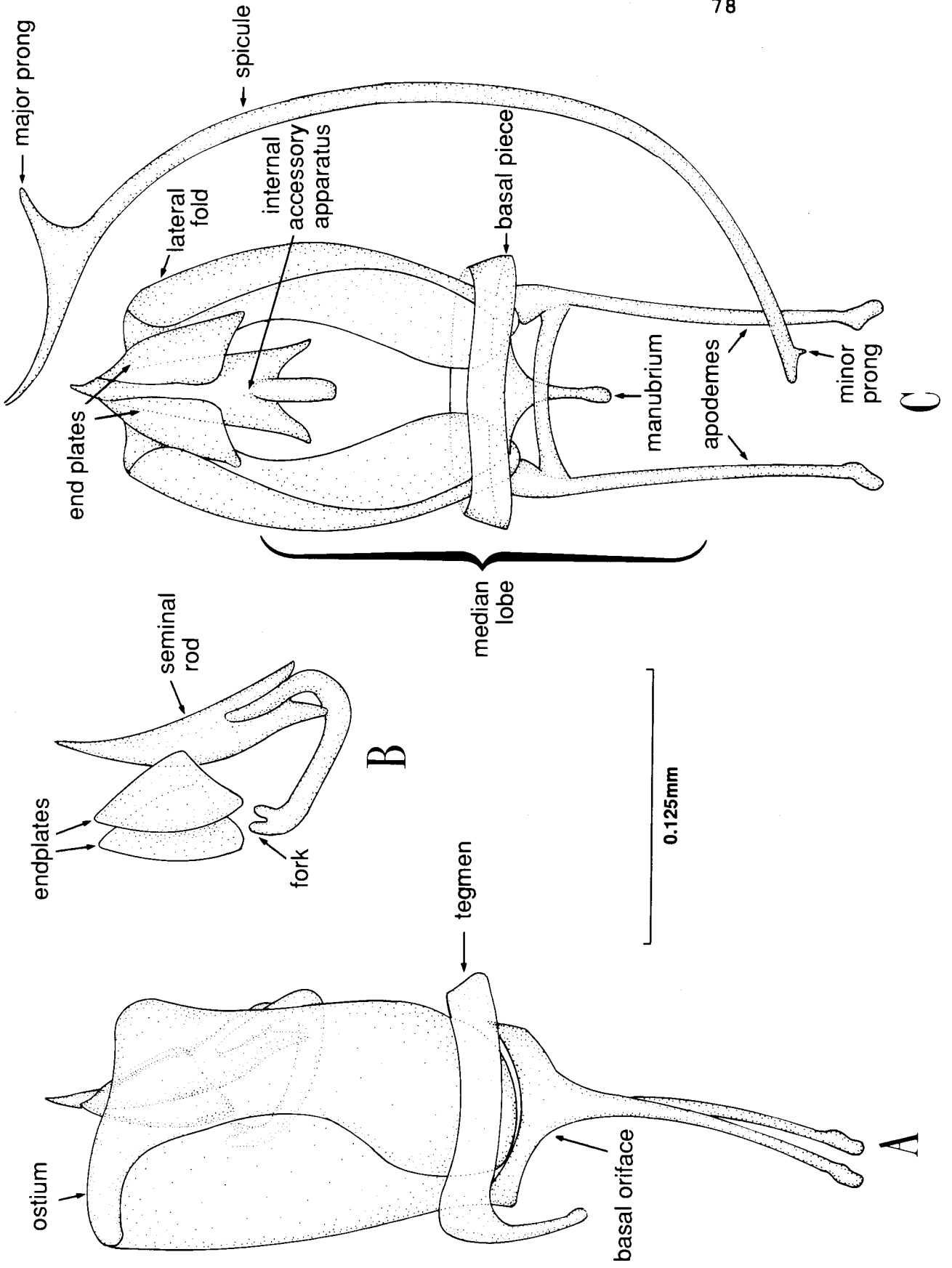
all genitalic structures could be clearly illustrated by these means, hand-drawings were made.

As there is no standard set of taxonomic terms for the structures of the male genitalia of the Scolytidae, I used the general terminology of Lindroth and Palmén (1970) to describe most structures. Probable synonyms used by several, but not all, other Coleopterists are provided. Where Lindroth and Palmén (1970) did not supply terms for some of the structures in the Scolytidae, I employed terms used by various scolytid taxonomists.

Results and Discussion

The sclerotized structures of the male genitalia in Conophthorus consist of the median lobe, internal accessory apparatus, tegmen and spicule (Fig. 5.1a - 5.1c). The median lobe (Sharp and Muir 1912; Sharp 1918; Lindroth and Palmén 1970) also termed the body or stem (Hopkins 1915b) or penis, (Lindroth and Palmén 1970; Cerezke 1964), is bilaterally symmetrical and resembles a tube (Fig. 5.1a,c). It has a basal orifice at the anterior end and an ostium at the posterior end (Lindroth and Palmén 1970) (Fig. 5.1a). There are a number of small sensory pores (Hopkins 1915b) or sensory areas (Cerezke 1964) on the

Fig. 5.1. Structures of the male genitalia of Conophthorus resinosa, (A) genitalia in lateral view, (B) internal accessory apparatus in lateral view, and (C) genitalia in dorsal view.



surface of the median lobe of Conophthorus, which can be clearly seen by electron microscopy. Two lateral folds (Hopkins 1915b) (Fig. 5.1c) or lateral lobes (Cerezke 1964), of the median lobe are seen in dorsal view (genitalia in repose). Extending anteriorly from the median lobe are two prominent apodemes (Cerezke 1964), body apodemes or femora (Hopkins 1915b), or struts or temones (Sharp 1918). Attached within the median lobe is the internal accessory apparatus (Cerezke 1964), also called the transfer apparatus (Sharp 1918) or accessory parts (Hopkins 1915b) (Fig. 5.1c), consisting of two end plates and a seminal rod (Hopkins 1915b) (Fig. 5.1b). Cerezke (1964) suggested that the internal accessory apparatus provides posterior support for the ejaculatory duct, orientates the gonophore (seminal rod) with the exterior median lobe, and guides the seminal fluid. Enclosing the internal accessory apparatus is the membranous internal sac (Cerezke 1964; Lindroth and Palmén 1970).

Encircling the median lobe near the posterior end of the apodemes is the tegmen (Sharp and Muir 1912; Sharp 1918; Hopkins 1915b; Lindroth and Palmén 1970) consisting of a ring-like structure called the basal piece and a long ventral apodeme called the manubrium (Lindroth and Palmén 1970) or apodemal process (Hopkins 1915b) (Fig. 5.1a,c). The tegmen functions as an apodeme for the attachment of muscles to permit posterior extension of the genital tube during copulation (Hopkins 1915b;

Cerezke 1964). Lying along the right side of the median lobe, in dorsal aspect, is a long slender curved rod called the spicule or spiculum gastrale (Sharp and Muir 1912; Hopkins 1915b; Sharp 1918; Lindroth and Palmén 1970) (Fig. 5.1c). On the posterior end of the spicule is a prominent fork or major prong (posterior section, Hopkins 1915b) and on the anterior end is a small, or minor, prong (Fig. 5.1c). The spicule provides the major support for the male genitalia (Cerezke 1964).

None of the structures examined on the male genitalia of the three Conophthorus species provides useful taxonomic characters. Unlike Dendroctonus spp. (Wood 1963; Vité et al. 1974,1975; Lanier et al. 1988), the shapes of the seminal rods of C. banksianae, C. coniperda and C. resinosae were indistinguishable from each other in lateral, dorsal and ventral view, within and outside the median lobe. Slight variation in width and separation of the prongs of the posterior "fork" of the seminal rod (where the seminal rod is anchored to the end plates, Fig. 5.1b) was observed, but was not consistent within species. There were no noticeable differences in the shape of the end plates. Ratios of apodeme length to median lobe length (ca. 1:1.2) and of manubrium length to basal piece width (ca. 1:2) were about the same for the three species. The manubrium and basal piece (Fig. 5.1a,c) were examined for differences in shape or size, but unlike in some Hylobius spp. (Warren 1960), differences in these structures were

not observed in Conophthorus. Some specimens of all 3 species, have 2 minute parameres (Lindroth and Palmén 1970) on the basal piece in ventral aspect, but in most specimens they were absent. Consistent differences in the shape of the lateral folds and apodemes between the three species could not be found. Minute variations in the bluntness and angle of the major and minor prongs of the spicule (Fig. 5.1c) were observed, but again consistent differences between the three species were absent.

The lack of taxonomic characters on the male genitalia frustrates the attempt to separate C. coniperda from C. resinosae, two species, that at present, are extremely difficult to separate on the basis of external morphology. Furthermore, the lack of genitalic characters between these two distinct species and C. banksianae confounds any interpretation about the validity of the latter as a valid sibling species. Interestingly, and perhaps coincidentally, the lack of useful male genitalic characters has also been noted for Pityophthorus Eichhoff (Bright 1981) the closely allied, or perhaps the same genus (Wood 1982; Bright and Flores 1987). Other intrinsic characters (Wiley 1981) need to be examined to assess the validity of C. banksianae, and to provide additional taxonomic characters to separate C. coniperda and C. resinosae.

Chapter 6

Karyotypes of Conophthorus banksianae, C. resinosae and C. coniperda

Introduction

Closely related species may have different chromosome numbers and differences in chromosome architecture (e.g. shape, structure, or banding pattern), because speciation is often associated with chromosomal rearrangements in one of the species involved (White 1978). As with external anatomical characters, the appearance of the chromosomes (the karyotype) can provide useful taxonomic characters to distinguish species. Karyotype data have been particularly useful in taxonomy for the recognition of sibling species in insects, e.g. black flies (Rothfels 1979) and bark beetles (Lanier and Wood 1968; Lanier 1981; Lanier et al. 1988).

Smith and Virkki (1978) list the karyotype formula of C. resinosae as 8AA + XY, but offered neither a morphological description of the chromosomes nor any indication that the karyotype was constant for the species across its range. They also list C. coniperda with the karyotypic formula of 9AA + neoXY but, inexplicably, the collection site of C. coniperda is listed

as California, whereas the species is found only in eastern North America. An examination of the remaining records of S.G Smith (on file at Forestry Canada, Sault Ste. Marie, ON.), reveals that the California locality is not a transcriptual error. Therefore, it cannot be ascertained if the species identification or the locality is incorrect. The karyotype of C. banksianae was not investigated.

The purpose of this study was to examine the karyotypes of C. banksianae, C. resinosae and C. coniperda to determine if taxonomic characters were present.

Materials and Methods

Larvae, pupae, and teneral adults of the three species of Conophthorus were collected in the field from their hosts (Wood 1982) in 1986 and 1987 from populations in Manitoba, New Brunswick, Nova Scotia, Ontario, and Quebec, Canada, and Michigan, U.S.A. (Table 6.1). Larvae were reared in their host plants until pupation. Pupae were removed from their hosts, and placed on filter paper moistened with distilled water. Upon eclosion, teneral adult males were maintained, without food, until they were 4 days old. A preliminary study of 1-7 day-old males indicated that 4-day old males had the greatest number of

Table 6.1. Collection sites, number of specimens, and number of metaphase cells examined of Conophthorus males for karyotype determinations

Species	Collection site, nearest locality	No. specimens examined	No. cells examined
<u>C. banksianae</u>	Aubrey Falls, ON	6	31
	Cobalt, ON	5	36
	Dubois, PQ	5	26
	Grayling, MI	4	40
	La Verendrye Pk., PQ	1	9
<u>C. coniperda</u>	Chalk River, ON	6	47
	Chignecto Pk., NS	1	7
	Desbarats, ON	6	40
	NW Angle Pk., MB	4	16
	St. Ignace, MI	1	2
<u>C. resinosae</u>	Minto, NB	3	10
	Chignecto Pk., NS	1	1
	Thessalon, ON	6	60
	Pembroke, ON	1	10

well-spread chromosomes in meiotic metaphase. Insects were reared at $20-22 \pm 2^{\circ}\text{C}$, $70 \pm 5\%$ R.H., and under a photoperiod of 16L:8D. Teneral adult males, <1 week old, obtained from the field collections also were used for chromosomal analysis. The sex of adults was determined by examining the abdominal tergites (Herdy 1959).

Adult males were placed in ethanol-glacial acetic acid (3:1) for ca. 20 min at room temperature and stored in the fixative at 4°C until processing 1-10 days later. Testes were excised from each male, placed immediately in a drop of 45% aceto-carmine on a glass slide, separated and teased apart with fine needles before covering with a cover slip. After gently warming the slide for 2-3 sec over an alcohol flame, the cover slip was covered with filter paper and the testes were squashed with gentle pressure.

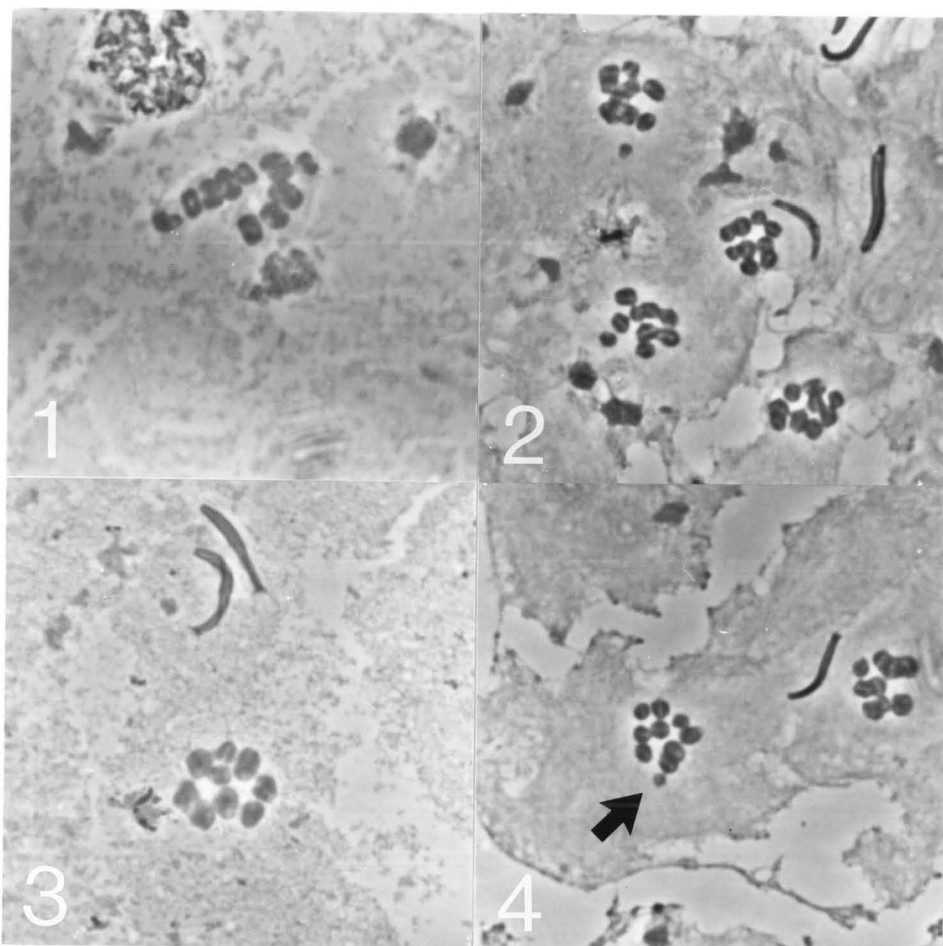
The slides were examined by phase contrast microscopy; only those with clear chromosomal divisions were used and these slides were held at 4°C for 0.5-3.0 h before making permanent mounts using the dry-ice technique of Conger and Fairchild (1953). Karyotype determinations were made on intact first metaphase cells and from early spermatogonial metaphases. Up to 10 intact first metaphase cells were examined for each specimen and up to six specimens with first metaphase divisions were examined from each collection site (Table 6.1). Well-spread cells observed

under immersion oil (100X) with phase contrast and a green filter were photographed on Kodak^R technical pan film (ASA 25 pushed to 100), with a Nikon Microflex HFX-II^R camera system mounted on a Nikon Optiphot^R microscope. Voucher specimens and chromosome preparations of the populations for which the karyotypic formulae were determined are deposited in the Insect Collection of Forestry Canada, Ontario Region, Sault Ste. Marie, ON.

Results and Discussion

The diploid number of C. coniperda is 20 and the male meiotic formula is 9 AA + XY (Fig. 6.1). Based on one specimen, Smith and Virkki (1978) listed C. coniperda as neoXY (centric fusion of the X chromosome with an autosome); however, the regularity in pairing in all specimens examined clearly indicates XY. Specimens from various populations did not show any differences in chromosome number or general morphology. Supernumerary chromosomes never were observed in C. coniperda. Typically at first metaphase, chromosomes 1 and 2 are large, almost round, bivalents; chromosomes 3-8 are more oval, all about the same size, but detectably smaller than chromosomes 1 and 2; chromosome 9 is slightly more rounded and smaller than chromosomes 3-8 but noticeably larger than chromosome 10, which is the smallest.

Fig. 6.1- 6.4. Meiotic metaphase I of Conophthorus: (1) C. coniperda 9AA + XY; (2) C. banksianae 8AA + XY; (3) C. resinosae 8AA + XY; (4) C. banksianae with supernumerary chromosome indicated by arrow.



The diploid number of C. banksianae (Fig. 6.2) and C. resinosae (Fig. 6.3) is 18 with the male meiotic formula of 8AA + XY, which was consistent for all the populations examined. Occasionally, a small supernumerary chromosome (Fig. 6.4) was found in C. banksianae (Grayling MI and Aubrey Falls ON) and C. resinosae (Thessalon ON and Pembroke ON). In both species, the typical first metaphase configuration consisted of oval bivalents with chromosome 1 as the largest, chromosomes 2-7 all about the same size, chromosome 9 clearly the smallest, and chromosome 8 intermediate between 7 and 9.

Spermatagonial divisions were seen in all three species, and confirmed the chromosome numbers observed in first metaphase. Several other phases of cell division (e.g. pachytene, diakinesis) were examined in the specimens; however, it was not possible to ascertain additional structural attributes of the chromosomes with confidence.

With a karyotypic formula of 9AA + XY, specimens of C. coniperda can be distinguished easily from C. resinosae and C. banksianae (with a karyotypic formulae of 8AA + XY). The distinct karyotype thus supports the validity of C. coniperda (Hopkins 1915a; Wood 1982), and provides a useful taxonomic character for this species. I was unable to distinguish C. banksianae from C. resinosae on the basis of chromosome number or shape at first

metaphase. Chromosome banding techniques, e.g. C-, G-, or Q-banding (Ennis 1974), might disclose structural differences in the chromosomes between the two sibling species; however, the small size of the chromosomes of Conophthorus may preclude further cytotaxonomic analysis. Another genetic approach to detecting sibling species is to examine allozymes by electrophoresis (Ayala and Powell 1972).

Chapter 7

Allozyme characters and genetic divergence of Conophthorus banksianae, C. coniperda, and C. resinosae

Introduction

Biochemical character analysis by gel electrophoresis has proven to be a powerful method to detect sibling species (Avisé 1974; Berlocher 1984; Hillis and Moritz 1990). Frequently, the analysis of allozymes, variant proteins produced by allelic forms of the same gene locus (Prakash et al. 1969), have provided diagnostic characters (Ayala and Powell 1972) in studies of sibling species groups (e.g. Berlocher 1980; Narang et al. 1989).

In this study I used allozyme gel electrophoresis to determine the extent of genetic divergence among populations of C. banksianae, C. coniperda, and C. resinosae, and to identify diagnostic loci (Ayala and Powell 1972; Richardson et al. 1986) for the discrimination of species.

Materials and Methods

Adult C. banksianae, C. resinosae, and C. coniperda were collected from jack, red, and white pine, respectively, from 25 sites throughout much of the geographical range of these putative

species (Table 7.1). Most collections were made in August and September of 1986-1988. Species identifications were verified by karyological evidence for several sites (Chapter 6). Samples were drawn from several trees scattered throughout each site in order to sample genetic diversity. Populations of C. coniperda and C. resinosa were sampled from adjacent trees within the same forest stand near St. Ignace, MI, and from plantations in close proximity at Petawawa, ON. Populations of C. banksianae and C. resinosa sampled for 3 consecutive years at the same site (Table 7.1) were treated as separate populations. Voucher specimens from each site were deposited in the collection of the Forest Insect and Disease Survey, Forestry Canada, Sault Ste. Marie, ON. Live adults were removed from their cones or overwintering buds, sexed by the method of Herdy (1959), and placed in glass vials at -80°C until electrophoresis, 1 to 5 months later.

Individual beetles were homogenized whole in 0.05 mL New Bridge buffer (Ayala et al. 1972). Supernatants of individual beetles were absorbed onto filter paper wicks and inserted into slots in a horizontal 13% hydrolyzed potato starch gel. Each gel

Table 7.1. Collection sites of Conophthorus banksianae, C. resinosae and C. coniperda used for allozyme analyses

Species	Pop. No.	Host	Collection Sites
<u>C. banksianae</u>	1	Jack pine	Grayling, Crawford Co., MI
	2		Kalkaska, Kalkaska Co., MI
	3		Aubrey Falls, Lane Twp., ON (1986)
	4		Aubrey Falls, Lane Twp., ON (1987)
	5		Aubrey Falls, Lane Twp., ON (1988)
	6		Aubrey Falls, Wardle Twp., ON
	7		Dubois, Emard Twp., PQ
	8		Laverendrye Prov. Park, PQ
	9		Stanton Lake, Quetico Prov. Park, ON
<u>C. resinosae</u>	10	Red pine	Gaylord, Otsego Co., MI
	11		St. Ignace, Mackinac Co., MI
	12		Thessalon, Kirkwood Twp., ON (1986)
	13		Thessalon, Kirkwood Twp., ON (1987)
	14		Thessalon, Kirkwood Twp., ON (1988)
	15		Angus, Tosorontio Twp., ON
	16		Chalk River, Wylie Twp., ON
	17		Temagami, Strathcona Twp., ON
	18		Chignecto, Cumberland Co., NS
<u>C. coniperda</u>	19	White pine	Murphy, Cherokee Co., NC
	20		Morganton, Burke Co., NC
	21		Pickett State For., Pickett Co., TN
	22		Litchfield Co., CT
	23		Clearfield Co., PA
	24		St. Ignace, Mackinac Co., MI
	25		Northwest Angle Prov. Forest, MB
	26		Agawa Prov. Park, ON
	27		Bradford, West Gwillimbury Twp., ON
	28		Chalk River, Wylie Twp., ON
29	Chignecto, Cumberland Co., NS		

usually held an equal number of wicks from each Conophthorus species and four wicks with extracts of spruce budworm, Choristoneura fumiferana Clem., larvae as a standard reference. Gels were run at 50 mA in a refrigerator until a red food dye marker had migrated 5.5-6.0 cm (5-6 h) toward the anode (Harvey and Sohi 1985).

After electrophoresis, gels were cut into slices and each slice was stained for one of the following enzymes (EC numbers from the Nomenclature Committee of the International Union of Biochemistry 1984): Aspartate amino transferase (AAT, 2.6.1.1), Diaphorase (DIA, 1.8.1.4), Isocitrate dehydrogenase (IDH, 1.1.1.42), Lactate dehydrogenase (LDH, 1.1.1.27), Malate dehydrogenase (MDH, 1.1.1.37), Malic enzyme (ME, 1.1.1.40), Phosphoglucose isomerase (PGI, 5.3.1.9), and Phosphoglucomutase (PGM, 5.4.2.2). A Poulik buffer, pH 8.0 (Poulik 1957), was used for AAT, and LDH, and a Tris-citrate buffer, pH 7.1 (Ayala et al. 1972), for the remaining enzymes. An additional 11 enzymes were surveyed, but they all gave inconsistent or uninterpretable banding patterns, including Acid phosphatase (3.1.3.2), Adenylate kinase (2.7.4.3), Alcohol dehydrogenase (1.1.1.1), Alkaline phosphatase (3.1.3.1), Glycerol-3-phosphate dehydrogenase (1.1.1.8), Esterase, Glucose-6-phosphate dehydrogenase (1.1.1.49), Hexokinase (2.7.1.1), Cytosol aminopeptidase (3.4.11.1), Phosphogluconate dehydrogenase (1.1.1.44), and

Superoxide dismutase (1.15.1.1). Staining methods followed or were modified from Ayala et al. (1972), Shaw and Prasad (1970), and Selander et al. (1971). After staining, the gels were fixed in a solution of glacial acetic acid, absolute methanol, and water (1:5:5), wrapped in plastic film, and stored at 2°C.

Putative loci were scored on the basis of resolution and consistency, and numbered in order of decreasing anodal mobility. Similarly, electromorphs (presumed alleles) were designated alphabetically. For each allele, a mobility ratio was calculated as the ratio between its migration distance and that of the front of the dye marker. Statistical analyses of the allozyme data were performed by BIOSYS-1 (Swofford and Selander 1981) with individual genotypes used as input. The assessment of genetic variation excluded the Dia loci because they were not sampled in all populations. The genetic variation was estimated for each population by computing mean heterozygosity (H) per locus per individual (direct count), and the percentage of polymorphic loci (0.99 criterion, i.e. frequency of the most common allele is < 0.99). Deviations from the Hardy-Weinberg equilibrium for each population were computed, for males and females separately and together, and Wright's inbreeding coefficient (F_{IS}) was calculated to assess heterozygosity. Fixation index (F_{ST}) values (Wright 1978) were derived from allele frequencies to estimate the amount of genetic divergence among populations and species.

Rogers' and Nei's genetic similarity and distance coefficients (nine in total) were calculated for pairwise comparisons, and phenograms of each coefficient were constructed with the UPGMA method (Sneath and Sokal 1973).

Failure to share alleles at a locus, or loci, was interpreted as evidence for no gene flow (reproductive isolation), and thus rejection of the null hypothesis that these species form a single breeding population (Ayala 1975; Richardson et al. 1986).

Results

Of the loci examined, all except Dia-1, were polymorphic; Mdh-2 was monomorphic in all but one population (Table 7.2). Only Pgi was sex-linked. The proportion of polymorphic loci ranged from 25.0 to 87.5 for populations (Table 7.2). Mean heterozygosity in populations of C. banksianae and C. resinosae was nearly the same (0.038 ± 0.019 (SE) vs 0.040 ± 0.015), respectively and less than half of that found in C. coniperda (0.098 ± 0.064).

Geographic variation in allele frequencies was limited. Within each species, the most common allele at a polymorphic locus was the same even when populations were separated by hundreds of kilometers (Tables 7.1 and 7.2).

Table 7.2. Allele frequencies, mean number of alleles per locus (A), percent polymorphic loci (P), and mean individual heterozygosity (H) for populations of *Conophthorus banksianae*, *C. resinosa*, and *C. coniperda* from Canada and the United States^a

		<i>C. banksianae</i>							
Locus ^b	Allele ^c	1	2	3	4	5	6	7	
Aat	(n)	(42)	(47)	(70)	(43)	(48)	(76)	(42) ^d	
	A							0.012	
	B			0.007					
	C							0.012	
	D	0.952	0.926	0.993	0.953	0.990	1.000	0.976	
	E	0.048	0.074		0.047	0.010			
Dia-1	(n)	(25)	(31)			(30)			
	A	1.000	1.000			1.000			
Dia-2	(n)	(10)	(27)			(29)			
	A								
Idh-1	(n)	(41) ^d	(42)	(70)	(43) ^d	(48)	(76)	(42)	
	A	0.061		0.007					
	B	0.012							
	C	0.610	0.786	0.929	0.919	0.948	0.947	0.952	
	D	0.012							
	E	0.305	0.214	0.064	0.081	0.052	0.053	0.048	
Idh-2	(n)	(41)	(30)	(68)	(43) ^d	(36)	(74) ^d	(29) ^d	
	A								
	B		0.017		0.023		0.068		
	C	1.000	0.983	1.000	0.954	1.000	0.932	0.931	
Mdh-1	(n)	(39)	(49)	(70)	(43)	(48)	(76)	(42)	
	A	1.000	1.000	1.000	1.000	1.000	0.993	1.000	
	B						0.007		
	Mdh-2	(n)	(46)	(49)	(68)	(43)	(48)	(72)	(42) ^d
		A							0.024
		B	1.000	1.000	1.000	1.000	1.000	1.000	0.976
Me	(n)	(38)	(46)	(70)	(43)	(50) ^d	(76)	(42)	
	A								
	B	1.000	0.989	0.986	1.000	0.880	1.000	0.893	
	C		0.011	0.014		0.120		0.107	
Pgi	(n)	(45)	(49)	(70)	(39)	(51)	(70)	(42)	
	A								
	B	0.944	1.000	1.000	1.000	1.000	1.000	1.000	
	C	0.056							
	D								
	E								
Pgm	(n)	(44)	(49)	(67)	(36)	(48)	(73) ^d	(42)	
	A						0.041		
	B	1.000	1.000	1.000	1.000	1.000	0.959	1.000	
	C								
A		1.8	1.5	1.5	1.5	1.4	1.5	1.8	
	P ^e	37.5	50.0	37.5	37.5	37.5	50.0	62.5	
H ^f		0.068	0.067	0.023	0.026	0.016	0.018	0.042	

Table 7.2 (cont.). Allele frequencies, mean number of alleles per locus (A), percent polymorphic loci (P), and mean individual heterozygosity (H) for populations of *Conophthorus banksianae*, *C. resinosa*, and *C. coniperda* from Canada and the United States

Locus ^b	Allele ^c	<i>C. banksianae</i>		<i>C. resinosa</i>				
		8	9	10	11	12	13	14
Aat	(n)	(42)	(49)	(55)	(55)	(70)	(41)	(44)
	A						0.012	
	B			0.010	0.009	0.014	0.012	
	C							
	D	0.964	0.990	0.945	0.918	0.964	0.878	0.966
	F	0.036	0.010	0.045	0.073	0.022	0.098	0.034
Dia-1	(n)			(33)	(25)			(41)
	A			1.000	1.000			1.000
Dia-2	(n)			(28)	(28)			(38)
	B			1.000	1.000			1.000
Idh-1	(n)	(42)	(42)	(48) ^d	(57) ^d	(70) ^d	(41)	(44)
	A	0.012		0.083	0.026	0.043	0.037	0.011
	B				0.018			
	C	0.952	0.845	0.823	0.956	0.957	0.963	0.978
	D							
	F	0.036	0.155	0.094				0.011
Idh-2	(n)	(36) ^d	(25) ^d	(38)	(52)	(70)	(40) ^d	(37) ^d
	A		0.020					
	B		0.040	0.053				0.054
	D	0.944	0.940	0.947	1.000	1.000	0.975	0.946
		0.056				0.025		
Mdh-1	(n)	(42)	(39)	(55)	(51)	(67)	(41)	(48)
	A	0.988	1.000	1.000	1.000	1.000	1.000	1.000
	B	0.012						
Mdh-2	(n)	(40)	(32)	(55)	(58)	(67)	(41)	(44)
	B	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Me	(n)	(42)	(42)	(55)	(51)	(70)	(41)	(48)
	A							
	B	0.952	0.917	0.927	0.931	1.000	0.976	1.000
	D	0.048	0.083	0.073	0.069		0.024	
Pgi	(n)	(42)	(42)	(55)	(57)	(70)	(41)	(48)
	A				0.035	0.064	0.012	0.021
	B	1.000	1.000	1.000	0.956	0.936	0.988	0.979
	C				0.009			
	D							
	F							
Pgm	(n)	(42) ^d	(42)	(55)	(53)	(64)	(35)	(48)
	A							
	B	0.810	0.964	1.000	1.000	1.000	1.000	1.000
	D	0.190	0.012					
		0.124						
A	1.9	1.9	1.8	1.9	1.5	1.9	1.6	
P ^e	75.0	62.5	50.0	50.0	37.5	62.5	50.0	
H ^f	0.042	0.070	0.058	0.051	0.032	0.049	0.019	

Table 7.2 (cont.). Allele frequencies, mean number of alleles per locus (A), percent polymorphic loci (P), and mean individual heterozygosity (H) for populations of *Conophthorus banksianae*, *C. resinosa*, and *C. coniperda* from Canada and the United States

Locus ^b	Allele ^c	<i>C. resinosa</i>				<i>C. coniperda</i>			
		15	16	17	18	19	20	21	22
Aat	(n)	(86)	(42)	(42)	(46)	(52)	(45)	(42)	(49)
	A								
	B	0.017							0.010
	C								
	D	0.890	0.976	0.964	1.000	1.000	0.978	1.000	0.970
	F	0.093	0.024	0.036			0.022		0.020
Dia-1	(n)					(37)	(26)	(16)	(34)
	A					1.000	1.000	1.000	1.000
Dia-2	(n)					(39)	(12)	(24)	(34)
	A					1.000	1.000	1.000	1.000
Idh-1	(n)	(86)	(42)	(42) ^d	(39)	(52)	(45)	(42)	(42)
	A	0.058	0.095	0.036					0.012
	B			0.024					
	C	0.942	0.905	0.940	1.000	0.952	1.000	1.000	0.976
	D								
	F					0.038			0.012
Idh-2	(n)	(84) ^d	(35) ^d	(42)	(39)	(42) ^d	(37) ^d	(36)	(26) ^d
	A								
	B	0.036				0.048	0.270		0.038
	C	0.964	0.886	1.000	1.000	0.952	0.730	1.000	0.962
Mdh-1	(n)	(83)	(42)	(42)	(37)	(52)	(40)	(42)	(49)
	A	0.988	0.988	1.000	1.000	1.000	0.988	0.976	1.000
	B	0.012	0.012				0.012	0.024	
Mdh-2	(n)	(83)	(42)	(41)	(32)	(52)	(47)	(42)	(49)
	A								
Me	(n)	(86)	(42) ^d	(42)	(39)	(43)	(40)	(50)	(49)
	A								
	B	1.000	0.952	0.988	0.988	0.942	0.975	0.990	1.000
	C		0.024	0.012	0.012	0.058	0.025	0.010	
Pgi	(n)	(80)	(42)	(42)	(39)	(52) ^d	(45) ^d	(50) ^d	(49) ^d
	A	0.013							
	B	0.981	1.000	1.000	1.000	0.221	0.289	0.210	0.255
	C	0.006							
	D					0.010			
	F					0.769	0.711	0.790	0.745
Pgm	(n)	(81)	(42)	(42) ^d	(39)	(52)	(45)	(50)	(49)
	A	0.056							
	B	0.944	1.000	0.881	0.988	1.000	1.000	1.000	1.000
	C			0.119	0.012				
A	2.0	1.8	1.6	1.3	1.8	1.6	1.4	1.8	
P ^e	75.0	62.5	50.0	25.0	50.0	62.5	37.5	50.0	
H ^f	0.061	0.039	0.021	0.006	0.070	0.087	0.061	0.077	

Table 7.2 (cont.). Allele frequencies, mean number of alleles per locus (A), percent polymorphic loci (P), and mean individual heterozygosity (H) for populations of *Conophthorus banksianae*, *C. resinosa*, and *C. coniperda* from Canada and the United States^a

		<i>C. coniperda</i>						
Locus ^b	Allele ^c	23	24	25	26	27	28	29
Aat	(n)	(52)	(43)	(47)	(41)	(79)	(41)	(43)
	A							
	B		0.012				0.012	
	C							
	D	0.961	0.988	1.000	0.951	0.994	0.988	0.977
	E	0.029						
Dia-1	(n)	(36)	(15)					
	A	1.000	1.000					
	B							
	C							
	D							
	E							
Dia-2	(n)	(29)	(18)					
	A	1.000	1.000					
	B							
	C							
	D							
	E							
Idh-1	(n)	(52)	(42)	(41)	(41)	(79)	(41)	(43)
	A	0.029				0.025		
	B							
	C	0.923	0.881	0.634	0.854	0.918	0.634	0.860
	D						0.012	
	E	0.048	0.119	0.366	0.146	0.057	0.354	0.140
Idh-2	(n)	(38) ^d	(22)	(31)	(41) ^d	(69) ^d	(29)	(37)
	A							
	B	0.026			0.024	0.449		
	C	0.974	1.000	1.000	0.976	0.551	1.000	1.000
	D							
	E							
Mdh-1	(n)	(52)	(42)	(37)	(41)	(79)	(41)	(43)
	A	1.000	1.000	1.000	0.988	1.000	1.000	1.000
	B				0.012			
	C							
	D							
	E							
Mdh-2	(n)	(52)	(42)	(34)	(41)	(77)	(39)	(41)
	A							
	B	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	C							
	D							
	E							
Me	(n)	(43)	(49)	(41)	(41)	(78)	(41) ^d	(43) ^d
	A					0.006		
	B	0.977	0.949	0.976	0.963	0.994	0.963	0.895
	C	0.023	0.051	0.024	0.037		0.037	0.093
	D							0.012
	E							
Pgi	(n)	(52) ^d	(49) ^d	(41) ^d	(41) ^d	(73) ^d	(41) ^d	(43) ^d
	A	0.019						
	B	0.231	0.224	0.244	0.244	0.301	0.268	0.256
	C							
	D							
	E	0.740	0.776	0.756	0.756	0.685	0.671	0.709
Pgm	(n)	(52)	(49)	(41)	(34)	(75)	(41)	(43) ^d
	A							
	B	1.000	1.000	0.988	0.941	1.000	1.000	0.837
	C			0.012				0.163
	D				0.059			
	E							
A		2.1	1.5	1.5	1.9	1.9	1.8	1.9
P ^e		62.5	50.0	50.0	87.5	62.5	50.0	62.5
H ^f		0.097	0.102	0.131	0.131	0.103	0.134	0.105

(The following appear at the bottom of the Table 7.2)

- ^a Localities corresponding to population numbers are given in Table 7.1.
- ^b Designations for codes of electrophoretic loci are given in text.
- ^c (n) Refers to the number of individuals sampled at each locus from each population.
- ^d Loci deviating from Hardy-Weinberg equilibrium expectations.
- ^e A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.99.
- ^f Determined by direct count.

Diagnostic loci were represented by Dia-2 and Pgi, and were consistent in all populations sampled (Table 7.2). At Dia-2, populations of C. coniperda were fixed for allele A, whereas C. banksianae and C. resinosae were fixed for allele B. At the Pgi locus, all C. coniperda males exhibited a triple band (typical for a dimeric enzyme), most commonly for alleles BE, and very rarely for alleles AE, BD, and BF (Fig. 7.1). Most males of C. banksianae and C. resinosae were homozygous for allele B; however, a few rare heterozygotes for alleles AB in C. resinosae were encountered in Ontario and Michigan. Almost all female C. coniperda were homozygous for allele E at the Pgi locus (Fig. 7.1); but a few females heterozygous for alleles EF were found in Nova Scotia and two sites in Ontario. Like the males, most females of C. banksianae and C. resinosae were homozygous for allele B, but a few were heterozygous for alleles BC, and some C. resinosae females for alleles AB.

Of the several hundred insects examined over a wide geographic and ecologically diverse area, not one specimen of C. coniperda (as indicated by the diagnostic loci) was found on red pine or jack pine, nor was one specimen of C. resinosae found on white pine.

Fig. 7.1 Representative genotypes at the Pgi locus for Conophthorus banksianae, C. coniperda and C. resinosae.

Genotype frequencies of polymorphic loci generally met Hardy-Weinberg expectations (Chi-square test with pooling); departures are indicated in Table 7.2. Wright's F_{IS} indicated that a deficiency of heterozygotes caused these departures at all loci, except at Pgi. An excess of heterozygotes at Pgi was noted only for males of C. coniperda, which, as noted above, were always heterozygous at this locus.

F_{ST} values combined across all loci for C. banksianae, C. coniperda, and C. resinosae, were 0.070, 0.087, and 0.037, respectively. These values indicated that genetic differences among conspecific populations were relatively low to moderate (Hartl 1980), i.e., more than 90% of the total genetic variation was found within populations. The mean F_{ST} value for all populations combined was 0.289, indicating very great (sensu Hartl 1980) genetic divergence among species. When populations of C. resinosae and C. banksianae were combined and compared with populations of C. coniperda, the mean F_{ST} value was 0.284.

Phenetic pairwise comparisons between species indicated C. banksianae and C. resinosae had genetic distance values of 0.032 and 0.001, for Rogers' modified distance (Wright 1978) and Nei's (1978) unbiased genetic distance coefficients, respectively. In comparison, populations of C. coniperda were considerably more distant from the other two, having Rogers' and Nei's values of

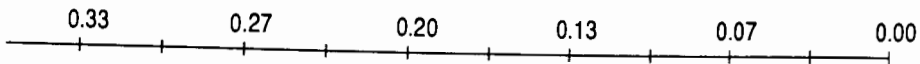
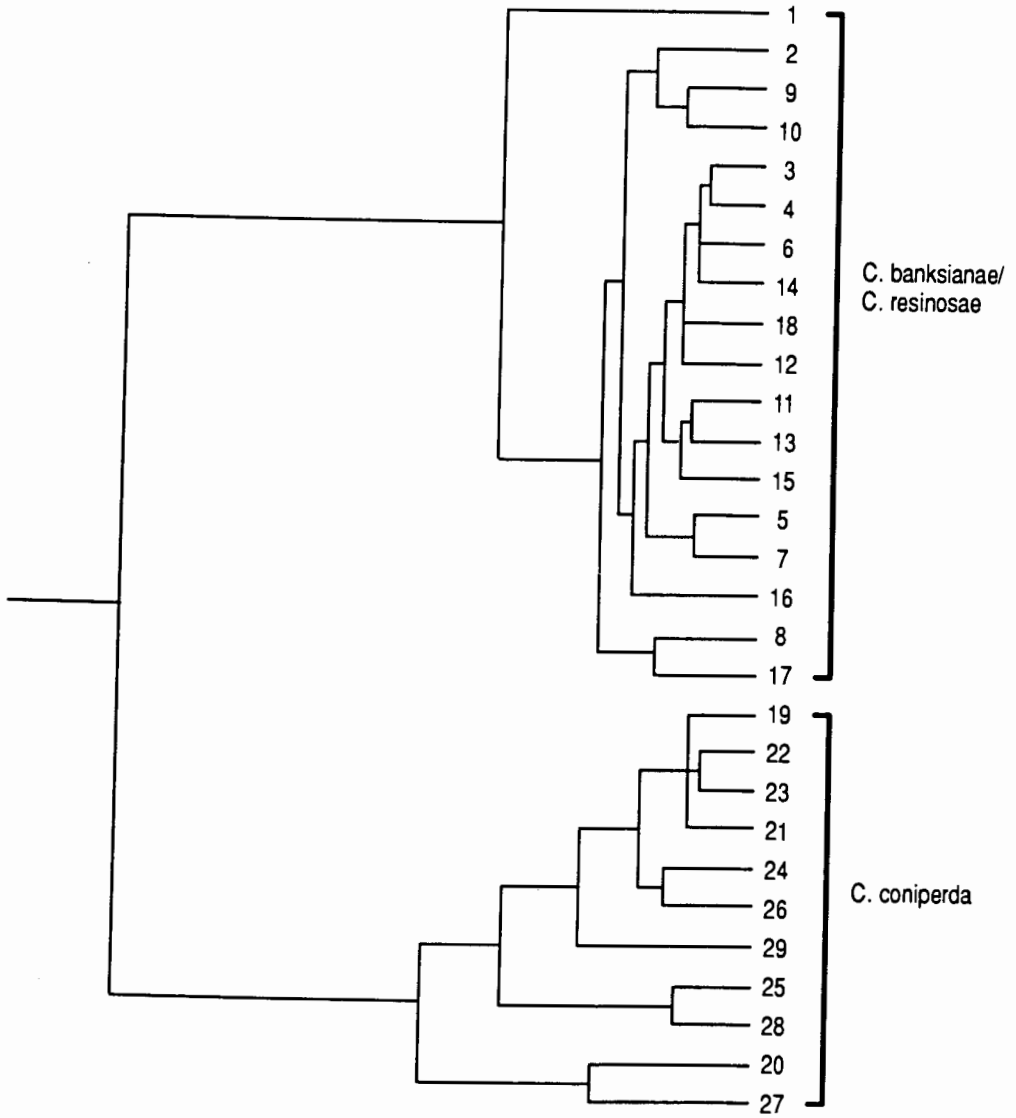
0.263 and 0.078-0.079, respectively.

Phenograms of Rogers' and Nei's genetic similarity and distance coefficients were identical in topology for populations of C. coniperda, and nearly identical in the clustering of populations of C. banksianae and C. resinosae. Cophenetic correlation coefficients for the nine Rogers' and Nei's similarity and distance coefficients ranged from 0.962 (Rogers' genetic similarity) to 0.993 (Rogers' modified distance). Rogers' modified distance coefficients (Fig. 7.2) clustered all populations of C. coniperda into one well-separated group, whereas populations of C. banksianae and C. resinosae were intermixed in the other primary cluster. Phenetic cluster analyses failed to group populations by geographic proximity.

Discussion

The fixed allelic differences found between C. coniperda and C. resinosae, and between C. coniperda and C. banksianae adults at the Dia-2 and Pgi loci unequivocally supports the separation of C. coniperda as a distinct species. This evidence is consistent with the taxonomy, based on morphological (Hopkins 1915a; Wood 1989), karyological (Chapter 6), and chemical (Page et. al 1990) characters. Therefore, these loci can be used as

Fig. 7.2 Phenogram of genetic relationships (UPGMA) clustering of Rogers's (modified) distance coefficients) among populations of Conophthorus banksianae, C. coniperda and C. resinosae. (Localities corresponding to population numbers are given in Table 7.1).



diagnostic characters to distinguish between adults of C. coniperda, and C. resinosae and C. banksianae.

No diagnostic loci were found to distinguish C. banksianae from C. resinosae. Pairwise comparisons of Nei's and Rogers' genetic distance coefficients for eight loci indicate very little genetic divergence between these two taxa. The lack of significant genetic divergence is clearly exemplified in the phenogram (Fig. 7.2) which shows interspersions of both species in the cluster of the populations. Furthermore, cluster analysis of the distance coefficients showed a remarkable similarity between some pairs of C. banksianae and C. resinosae populations despite considerable geographic distance between them. Although similar allozyme frequencies between populations does not necessarily indicate conspecificity (Avice and Aquadro 1982), I interpret these data as failing to reject the null hypothesis that populations of C. banksianae and C. resinosae form a single species.

The allele frequencies in C. coniperda at the Pgi locus are particularly noteworthy because Pgi_b is absent in the females, males are always heterozygous, and females are rarely so. It is unlikely that the rare heterozygous females represent wrongly sexed males, because the sexing of beetles by the method of Herdy (1959) is unequivocal. Although several genetic explanations are

possible, the most parsimonious is that Pgi_b allele has been translocated on the Y chromosome, and thus is sex-linked. This allele appears to be autosomal in C. banksianae and C. resinosae.

The high incidence of heterozygote deficiencies at the Idh-2, is also noteworthy. There are three frequent causes of deviation from Hardy-Weinberg expectations observed in electrophoretic studies (Pasteur et al. 1988): 1) sex-linkage, 2) "Wahlund effect", that is, apparent panmictic populations were actually composed of several subpopulations between which breeding was restricted, and (3) failure to detect a silent recessive (null) allele. Idh-2 is not sex-linked because heterozygotes occurred in both sexes. The occurrence of the Wahlund effect appears unlikely because heterozygote deficiencies were not present in the same geographic population in different years (Table 7.2), and because there is no evidence from other loci of genetically distinct subpopulations. There is no indirect evidence for null alleles as examination of the gels did not reveal beetles with no enzyme activity, which may be assumed to indicate individuals homozygous for one or more null alleles. Inbreeding can result in heterozygote deficiencies; however, there is no evidence for this since it would affect all loci. I suggest that the rarity of heterozygous genotypes at the Idh-2 locus may indicate a strong selection against them, but for reasons yet unknown.

The absence of C. resinosae in the collections from white pine cones strongly suggests that this host is rarely, if ever, used by this beetle. Similarly, C. coniperda does not appear to attack red pine in nature. The apparent host specificity of C. coniperda and C. resinosae may have an important role in maintaining reproductive isolation between these sympatric species. Nevertheless, host affinity may break down for C. resinosae after a prolonged absence of cues from red pine cones (Tabashnik et al. 1985). The availability of the two diagnostic loci as taxonomic characters can now facilitate further experimentation on the host switching behaviour of C. resinosae.

Host races are usually considered to be genetically different forms of the same species that are partially reproductively isolated (Jaenike 1981; Diehl and Bush 1984; Futuyma and Peterson 1985). In the Scolytidae, genetic substructuring of populations along host lines appears to have occurred in the mountain pine beetle, Dendroctonus ponderosae Hopkins, and may be indicative of host races (Sturgeon and Mitton 1986; but see Langor and Spence 1991). However, my genetic data did not disclose host-associated genetic substructuring between populations of C. banksianae and C. resinosae. Although host races may not necessarily exhibit variation in allozyme frequency (Diehl and Bush 1984), these data do not provide any evidence that populations of Conophthorus on jack pine are a host race.

Chapter 8

Evidence for a female-produced sex pheromone in Conophthorus

Introduction

After a female cone beetle initiates attack, and begins to construct a gallery in the cones, a male will join her (Lyons 1956; Godwin and Odell 1965; de Groot 1986a). Many scolytids use pheromones to find suitable hosts and mates (Borden 1985). Kinzer et al. (1972) observed attraction of male ponderosae pine cone beetles, C. ponderosae Hopkins, to females feeding in conelets and to ethanol extracts of frass and tunnel shavings in a laboratory olfactometer, and suggested that sex pheromones were involved. These observations led me to hypothesize that female C. coniperda and C. resinosae in eastern North America also utilize a sex pheromone. Specificity of the sex pheromone may effectively prevent interspecific mating, and the chemical structure of these sex pheromones may be useful as a taxonomic character.

The objective of this investigation was to assess the response of males and females of C. coniperda and C. resinosae to host- and beetle-produced volatiles, and to determine if there was evidence for a sex pheromone.

Materials and Methods

Collection and handling of insects. Adult C. coniperda were obtained from cones collected during September and October in a natural white pine stand near Chalk River, Ontario. Buds infested with C. resinosae were collected during September and October from red pine plantations near Thessalon, Ontario. The two diagnostic loci at Dia-2 and Pgi (Chapter 7), verified the species identifications in both collection and field study sites.

Infested cones and buds were stored in the dark at 1-2° C and 70-80% RH. To induce emergence of adult C. coniperda, infested cones were placed in boxes lined with moist paper and fitted with clear lids. The boxes were held under a 16L:8D photoperiod at 20-22 °C. Emerged beetles were collected 2-3 times daily. Adult C. resinosae were induced to emerge by gently warming the infested buds under a 60 W incandescent light bulb. Sex of adults was determined by examining the 7th and 8th tergites (Herdy 1959) and held at 4-5°C on moist filter paper in Petri dishes.

Frass Production and Collection. Frass was collected from groups of 20-30 male or female C. coniperda feeding beetles on white pine conelets and held in Petri dishes at 20-22°C under a 16L:8D photoperiod. Similarly, frass was collected from groups of C. resinosae feeding on red pine conelets. Frass was removed from

the petri dishes daily, pooled with previous collections, and stored at -40°C . Beetles were removed once a week from the hosts and given fresh material. Uninfested red and white pine cones, collected during November from Thessalon, ON., were shredded into frass-sized pieces which were placed in Petri dishes, usually collected daily, and stored in the same way as the frass.

Collection of Beetle and Host Volatiles. Small white pine branches bearing conelets were stripped of needles, cut 2-3 cm below the terminal bud, and equally distributed between two 2-piece aeration chambers. Male or female C. coniperda were introduced into their own chambers and allowed to attack host material. The same procedures were followed for C. resinosae feeding on red pine conelets. For the control, each cone was punctured 3-4 times with the tang of a small file before branches were placed in the aeration chamber. Humidified and charcoal-filtered air was drawn at 1.65 L per min through vertically oriented chambers and traps containing Porapak Q. Volatiles were recovered by overnight extraction of the Porapak Q with pentane in a Soxhlet extractor. The pentane solution of volatiles was concentrated (1-5 mL) under a stream of helium and then diluted with an equal volume of hexane. The concentration of the volatiles in the solution was expressed as beetle-min (bm) where 1 beetle-min is the amount of volatiles collected from 1 beetle for one min.

Laboratory experiments. The responses of walking C. coniperda and C. resinosae to frass and Porapak Q extracts were tested in four experiments (Table 8.1) in an olfactometer (Payne et al. 1976). For experiments 1-2, frass and uninfested cone shreds were placed in a 25 mL flask connected between a flowmeter and a 1 cm diam glass outlet tube by teflon tubing. For experiments 3 and 4, extracts of Porapak Q-trapped volatiles were diluted in distilled pentane:hexane (1:1) and delivered at 1 μ L per min from a 10 μ L syringe mounted on a syringe pump. The tip of the syringe needle was placed through a 1 mm hole cut in the side of a plastic outlet tube that was connected to the air supply. Bottled medical air (Grade F) was passed over the stimuli at 1.5 L per min and through the outlet tube to the olfactometer arena. Air was exhausted from the arena by a variable speed fan connected to a duct that led to the outside of the building.

Before each bioassay, beetles were held for at least 30 min in darkness at ca. 20°C. Beetles were divided into five paired groups, of 12 males or 12 females, and held in plastic vials lined with filter paper. Stimuli were presented in random order to each group. Each replicate of 12 beetles was released 10 cm downwind from the outlet tube on a 28 X 21.5 cm letter-paper floor. Beetles that walked within 2 cm of the outlet tube within 5 min were considered to be positive responders and were removed

from the arena. Beetles that left the airstream were returned to the release point up to three times. At least 1 h was allowed to elapse before any beetles were retested against a different stimulus. Beetles were not reused for subsequent experiments. After each replicate, the letter-paper was removed from the arena and discarded outside the bioassay room. An independent set of flasks or syringes, and outlet tubes was used for each stimulus. The bioassays were conducted in a windowless room at 20°C and 65% RH, with diffuse overhead light, ca. 80 lux. Barometric pressure was monitored continuously.

Percent positive response data were transformed by arcsine and analyzed by ANOVA, using the Tukey test to separate means (BMDP program 7d, 1990 version).

Field experiments. Field experiments for C. coniperda were conducted in a natural white pine forest in Pancake Provincial Park, 80 km north of Sault Ste. Marie, ON (Table 8.2). Traps were made from yellow Japanese beetle trap tops (Trécé Inc., Salinas, California) and Mason jar bottoms containing 25 mL of ethylene glycol. Baits consisted of 10 C. coniperda females feeding on four white pine cones (from Thessalon, ON), or four intact white pine cones. These were put in white, polyester, mesh bags tied to one of the trap vanes. Cones were left intact on branches to reduce desiccation. Control traps were unbaited. Traps were hung

ca. 10-15 m high in the upper third of white pine crowns, one trap per tree, and were spaced 15-20 m apart, in a 3 x 7 (treatments x replicates) randomized block design. This experiment began 19 May 1989, and was repeated twice every 10 days until 19 June, each time with fresh baits and newly randomized treatment locations. Captured beetles were preserved in 70% alcohol, identified, and their sex determined.

A field experiment for C. resinosa, similar to the above was set-up; however, because catches were too low to permit analyses, the results are not presented.

The first two replicates for the C. coniperda experiment caught too few beetles, and therefore were not used for analyses. Trap catch data were analyzed by the Kruskal-Wallis test with nonparametric multiple comparisons (BMDP program 3s, 1990 version).

Results

Laboratory experiments. Response of female C. coniperda to volatiles of females or males feeding on cones, or their frass, did not differ significantly from their response to cone-produced volatiles (Exp. 1,3; Table 8.1). Similarly, female C. resinosa were not significantly different in their response to frass or

Table 8.1. Response of walking C. coniperda and C. resinosae in the laboratory to frass and extracts of volatiles from aerations of beetles feeding in cones

Exp. No.	Species	Stimulus	Amount ^a	% Responders ($x \pm SE$) ^b	
				Females	Males ^c
1	<u>C. coniperda</u>	Female frass	0.1 g	62 \pm 3 a	60 \pm 8 a
		Male frass	0.1 g	52 \pm 4 a	40 \pm 7 a
		Cone shreds	0.1 g	47 \pm 6 a	37 \pm 4 a
		Airstream	1.5 L/min	5 \pm 2 b	5 \pm 2 b
2	<u>C. resinosae</u>	Female frass	0.1 g	47 \pm 10a	53 \pm 6 a
		Male frass	0.1 g	48 \pm 9 a	32 \pm 5 ab
		Cone shreds	0.1 g	40 \pm 10a	23 \pm 8 b
		Airstream	1.5 L/min	20 \pm 9 a	13 \pm 6 b
3	<u>C. coniperda</u>	Females in cones,	600 bm	57 \pm 5 a	60 \pm 3 a
		vol. extr.	60 bm	45 \pm 6 ab	48 \pm 3 ab
			6 bm	42 \pm 5 ab	45 \pm 7 ab
		Males in cones,	600 bm	33 \pm 3 b	35 \pm 12ab
		vol. extr.	60 bm	43 \pm 5 ab	32 \pm 6 ab
			6 bm	33 \pm 3 b	23 \pm 3 bc
		Cones vol. extr.	60 cm	55 \pm 2 ab	32 \pm 3 ab
			6 cm	52 \pm 3 ab	33 \pm 7 ab
		Pentane:hexane	1.0 μ L/min	7 \pm 3 c	5 \pm 2 c
		4	<u>C. resinosae</u>	Females in cones,	600 bm
vol. extr.	60 bm			64 \pm 5 a	94 \pm 2 a
	6 bm			56 \pm 7 a	64 \pm 8 b
Males in cones,	600 bm			50 \pm 8 a	60 \pm 3 b
vol. extr.	60 bm			52 \pm 8 a	42 \pm 8 bc
	6 bm			34 \pm 2 ab	34 \pm 11bc
Cones vol. extr.	60 cm			60 \pm 0 a	64 \pm 7 b
Pentane:hexane	1.0 μ L/min			14 \pm 4 b	18 \pm 4 c

^a One bm = the volatiles produced by one beetle in a cone during one min. One cm = the volatiles released by one cone in one min.

^b Means in a column within experiments followed by the same letter are not significantly different, $P < 0.05$, Tukey's test (arcsine transformed data), BMDP program 7d (1990 version).

^c Sixty beetles of each sex used were tested.

captured volatiles of females on cones, males on cones, or cones (Exp. 2,4; Table 8.1).

Male C. resinosa responded significantly more to volatiles from females on cones than to other stimuli in some experiments (Exp. 2,4; Table 8.1). More than 90% of the C. resinosa males responded to the volatile extract of females on cones at 600 and 60 bm (Exp. 4), a response significantly greater than to volatiles from males on cones, or those of cones alone. Male C. coniperda responded significantly more to females than to males at 6 bm, but this response was not significantly different at 600 and 60 bm (Exp. 3; Table 8.1).

In laboratory bioassays, aggressive behaviour was noted for males of both species. In the presence of volatiles from females on cones, males would often bunt other males and chase after each other. This behaviour was most pronounced near the stimulus outlet. There was also sexual variability in the locomotory behaviour. Most of the responsive beetles would move slowly and steadily to an odour source. Some beetles, mostly males, would stop, sit up and move their antennae before moving on. In Exp. 4, male C. resinosa walked quickly in an almost straight line to the outlets releasing volatiles of females on cones at 600 and 60 bm.

Field experiments. Traps baited with female C. coniperda caught significantly more males than traps baited with cones only (Table 8.2). The responses by female C. coniperda to cones infested with females and to uninfested cones also differed significantly.

Discussion

The data provide the first evidence that C. coniperda and C. resinosae use semiochemicals to locate each other. The most distinct result was pronounced response by males in the laboratory to extracts of the volatiles from females in cones. This result provides strong evidence that mate-finding is mediated by female-produced sex pheromones. A good response by C. coniperda males was also noted in the field experiments. The laboratory and field data agree well with the results of Kinzer et al. (1972), which showed a strong positive response of C. ponderosae males to live females boring in ponderosa pine cones. Furthermore these data agree with similar studies conducted in North Carolina on C. coniperda (de Groot et al. 1991). A lack of difference in the response by females to cones infested with males or females suggests that their response is not inhibited by beetles of either sex. The response by females to infested cones in the field (Table 8.2) may indicate that the females are attracted to the volatiles released from damaged cones.

Table 8.2. Numbers of C. coniperda captured in modified Japanese beetle traps, 9 - 19 June, 1989, Pancake Bay Provincial Park, Ontario

Treatment ^a	No. of beetles captured per trap ($\bar{x} \pm SE$) ^b	
	Females	Males
females in cones	4.1 \pm 1.6 a	5.1 \pm 2.0 a
cones	0.1 \pm 0.1 b	0.3 \pm 0.3 b
unbaited	0.4 \pm 0.4 b	0.0 \pm 0.0 b

^a Four females in 10 cones, or 10 cones in each trap; 7 traps per treatment in a randomized block design.

^b Means in a column within an experiment followed by the same letter are not significantly different, $P < 0.05$, Kruskal-Wallis test.

I hypothesize that females do not exploit the female-produced sex pheromone to find cones. If they did, a clumped distribution of cone attacks either within or among cone-bearing trees could be expected, i.e., cone damage would not be proportional to cone abundance. Although their evidence was largely circumstantial, Mattson et al. (1984) found that cone damage by C. resinosa was proportional to cone abundance both within and among trees. They tentatively rejected the hypothesis that beetles in flight are directed by cues from cones, and proposed that beetles alight on trees at random.

Further studies are needed to establish that the response by males is due to attractive volatiles produced by females, i.e. pheromones, and to identify, synthesize, and test the volatiles. This work will also provide a foundation for testing hypotheses about the role of chemical-based communication in maintaining reproductive isolation among Conophthorus species, and furthermore, it may lead to the provision of additional taxonomic characters.

Chapter 9

Isolation, identification, synthesis and bioassay
of pheromones of three species of Conophthorus

Many scolytids use pheromones to locate suitable mates and hosts (Borden 1985). Although different species may share certain semiochemicals, most appear to use unique, yet variable, blends of more than one compound (Lanier and Wood 1975; West-Eberhard 1984; Miller et al. 1989). In addition to facilitating species recognition, species-specific sex pheromone systems may also mediate reproductive isolation between sympatric species (Lanier and Burkholder 1974; Roelofs and Cardé 1974). The specificity of the chemical message may be achieved by different semiochemicals, by different structural and optical isomers of shared semiochemicals, or by different ratios and release rates of identical shared components.

In chapter 8, I provided evidence for a female-produced sex pheromone in C. coniperda and C. resinosae, and suggested that knowledge of the chemistry and bioactivity of beetle-produced volatiles might disclose additional taxonomic characters and lead to a greater understanding of reproductive isolation. Therefore, in collaboration with H.D. Pierce Jr., Dept. of Chemistry, Simon Fraser University (SFU), I undertook a study to isolate,

identify, synthesize, and bioassay compounds produced by male and female Conophthorus to determine their activity and function. My role in this research was to collect and prepare the insects and host materials, to prepare crushed beetle extracts, and to conduct all laboratory assays and field experiments.

Materials and Methods

Collection and Handling of Insects. Adult C. banksianae, C. coniperda and C. resinosae were collected near Aubrey Falls, Chalk River, and Thessalon, Ontario, respectively, during September and October. Uninfested jack pine shoots and cones of red or white pines were collected from Thessalon in October. Infested and uninfested cones and shoots were placed in paper bags and burlap bags, respectively, and stored in the dark at 1-2°C and 70-80% RH.

To induce emergence of C. coniperda, infested cones were placed in plastic boxes lined with moist paper and fitted with clear plastic lids. The boxes were held under a 16L:8D photoperiod at 20-22°C. Emerged beetles were collected 2-3 times daily. Adult C. banksianae and C. resinosae were induced to emerge by gently warming infested buds under a 60 W incandescent light bulb after being held in paper bags at 20-22°C for 2-3 days. Beetles were sexed by examining the 7th and 8th abdominal

tergites (Herdy 1959) and held at 4-5°C on moist filter paper in petri dishes.

Collection of Beetle and Host Volatiles. Small branches bearing conelets of white or red pine were stripped of needles, cut 2-3 cm below the terminal bud, and equally distributed between 2 aeration chambers (Pierce et al. 1984). Male or female C. coniperda, or C. resinosa, were introduced into separate chambers and allowed to attack host material. For the control, each cone was punctured 3-4 times with the tang of a small file before placement of cone-bearing branches into the aeration chamber. Humidified and charcoal-filtered air was drawn at 1.65 L/min through the vertically-oriented chambers and traps (ca. 200 mm x 14 mm OD) containing Porapak Q for 1-5 weeks. Volatiles were recovered by overnight extraction of the Porapak Q with pentane in a Soxhlet extractor. The pentane solution of the volatiles was concentrated (1-5 mL) either by distilling off the pentane through a Dufton column or by evaporation under a stream of helium. The concentration of the volatiles in the solution was expressed as beetle-min where 1 beetle-min is the amount of volatiles collected from 1 beetle for one min. Samples of extracts were diluted with an equal volume of hexane and shipped from SFU to Sault Ste. Marie, ON., for laboratory and field experiments.

For C. banksianae, volatiles were collected from separated males and females feeding on jack pine shoots on analytical traps (6 mm OD) packed with ca. 30 mm length of Porapak Q. Collection durations ranged from 2 to 7 days. Volatiles were recovered by eluting the traps with 1 mL of anhydrous ether. Several collections with C. coniperda and C. resinosae were also conducted in this manner.

Isolation by Extraction and Analysis of Beetle Volatiles. After removal from the host, beetles were placed in a vial containing pentane which was set in dry ice. The frozen beetles were crushed to a fine powder. The vial was removed from the dry ice and allowed to warm to room temperature. A small aliquot of the supernatant was then analyzed by gas chromatography (GC). Samples which were not immediately analyzed were stored in a freezer. For several samples of C. banksianae volatiles, the extract was placed on glass wool in the upper portion of an analytical Porapak Q trap. The volatiles were transferred from the glass wool to the Porapak Q with nitrogen (ca. 200 mL/min for 0.5 h) and recovered by eluting the Porapak Q with pentane-ether (1:1, v/v). These samples were analyzed by GC for determination of chirality and enantiomeric enhancement (ee).

Isolation of Beetle Volatiles by Steam Distillation. A micro steam distillation-continuous extraction apparatus (Godefroot et

al. 1985) was used for the isolation of volatiles from the pentane extracts of crushed beetles. Pentane was used for extraction. The solutions of the volatiles were stored in the freezer until analysis by GC.

Isolation of Volatile Oils by Steam Distillation. A simultaneous steam distillation-continuous extraction still head (Flath and Forrey 1977) was used for the isolation of the volatile oils from macerated (Waring blender) cones, buds, or needles of red pine, cones of white pine, and shoots of jack pine. The steam distillation was conducted for 4 h after boil-up, and pentane was used as the extracting solvent. The bulk of the pentane was removed under a stream of argon or nitrogen. Residual solvents were removed by brief vacuum pumping. The oils were stored in a freezer until use.

Instrumental Methods. Hewlett-Packard 5830, 5880, and 5890 gas chromatographs equipped with capillary inlet systems and flame-ionization detectors were used for analyses by gas-liquid partition chromatography. Glass columns (30 m x 0.5 mm ID) coated with SP-1000 or Carbowax 20M (Supelco Canada Ltd., Oakville, ON) and a fused silica column (15 m x 0.25 mm ID) coated with DB-1 (J & W Scientific Inc., Folsom, CA) were used. A fused silica column (25 m x 0.25 mm ID) coated with Chirasil-Dex (8) (V. Schurig, University of Tübingen, Germany) was used for chirality

determinations. The injection port and detector temperatures were 260 and 270° C, respectively.

A Hewlett-Packard 5895B GC/MS/DS was employed for coupled gas chromatography-mass spectroscopy (GC-MS). Fused silica columns (0.32 mm ID) coated with DB-1 (30 m), DB-WAX (60 m) or SP-1000 (30 m) (J & W Scientific Inc., Folsom, Calif.) were coupled directly into the ion source. The injection port, transfer line, and ion source were 260, 250, and 200° C, respectively. Helium was the carrier gas for GC and GC-MS.

A Varian 1200 GC equipped with 10:1 effluent splitter and thermal gradient collector (Brownlee and Silverstein 1968) was used for micropreparative isolation of pheromones from the Porapak Q-trapped volatiles for chirality determinations. The column was a 3 m x 3.17 mm OD stainless tube packed with a 10% SP-1000 on Supelcoport (100/120 mesh) (Supelco, Bellafonte, PA).

Synthetic pheromones. (E)-(±)-and (E)-(+) pityol were prepared from (±)-sulcatol and (S)-(+)-sulcatol (97% optically pure, Phero Tech, Inc., Delta, B.C.) by the method of Mori and Paupoomchareon (1987). Distilled (E)-(±)-pityol (84%) containing ca. 2.3% of the Z isomer and 5% sulcatol was further purified by flash chromatography on silica gel with pentane-ether (4:1) as eluent to give a (E)-(±)-pityol (98.3%) which contained 0.3% of the Z

isomer and no sulcatol. The distilled (E)-(+)-pityol containing 3.1% of the Z isomer and no sulcatol was 86.6% chemically pure and 98.8% enantiomerically enhanced.

The enantiomers and racemate of 7(E)-methyl-1,6-dioxaspiro [4.5] decane were synthesized by procedures recently developed by Ramaswamy and Oehschlager (1990). The racemic compound was 99.4% pure and contained 0.3% of the Z isomer. The (5_R,7_R)-(+) and (5_S,7_S)-(-) isomers were each ca. 93% chemically pure and contained 1.6 and 1.2% of the Z isomer, respectively. Both enantiomers were ca. 97% ee.

Samples of (+)-7(E)-methyl-1,6-dioxaspiro [4.5] decane and (+)-pityol (E/Z, 1:2) were gifts from W. Francke, Univ. of Hamburg, Germany.

Laboratory bioassays. The responses of walking C. coniperda and C. resinosae to stimuli were tested in 6 experiments (Tables 9.2-9.3) in an olfactometer (Payne et al. 1976). Pityol and conophthorin (Table 9.1) were diluted to 0.01 $\mu\text{L}/\text{mL}$ and cone oils to 10 $\mu\text{L}/\text{mL}$ in a pentane:hexane (1:1) solvent mix for experiments 1 and 3, and all were diluted to 0.1 $\mu\text{L}/\text{mL}$ for experiments 2,4,5 and 6. Volatiles were emitted at 1 $\mu\text{L}/\text{min}$ from a 10 μL syringe mounted on a syringe pump. The tip of the syringe needle was

Table 9.1. Purity and release rates for pityol, and conophthorin tested for attractancy to Conophthorus in field experiments

Compound	% Purity		Exp. No.	Release ^a rate, mg/24 h ($\bar{x} \pm SE$)
	\bar{E}	\bar{Z}		
(±)- <u>trans</u> -pityol	91.8	1.4	1-3, 6-11	0.61 ± 0.03
(±)- <u>trans</u> -pityol	98.3	0.3	4,5	--
(±)- <u>trans</u> -pityol	99.7	0.3	2,7,10	0.66 ± 0.04
(±)- <u>trans</u> -pityol	95.0	5.0	2,7,10	0.70 ± 0.03
(+)- <u>trans</u> -pityol	86.6	3.1	1,4,6,9	0.59 ± 0.03
(±)-conophthorin	87.8	1.0	5	--
(±)-conophthorin	99.4	0.3	3,8,11	0.95 ± 0.03
(+)-conophthorin	93.0	1.6	3,8,11	1.00 ± 0.03
(-)-conophthorin	93.0	1.2	3,8,11	0.93 ± 0.02

^a Release rate determined from 10 capillary tubes at 24 °C.

placed through a 1 mm hole cut in the side of a plastic outlet tube that was connected to the air supply. Bottled medical air (Grade F) was passed over the stimuli at 1.5 L/min and through the outlet tube to the olfactometer arena. Air was exhausted from the arena by a variable-speed fan connected to a duct that led to the outside of the building.

Before each 5-min bioassay, beetles were held for at least 30 min in darkness at ca. 20°C. Beetles were divided into 5 paired groups, of 12 males or 12 females held in plastic vials lined with filter paper. stimuli were presented in random order to each group. Each replicate of 12 beetles was released 10 cm downwind from the outlet tube on a 28 by 21.5 cm letter-paper floor. Beetles that walked within 2 cm of the outlet tube were considered to be positive responders and were removed from the arena. Beetles that left the airstream were returned to the release point. At least 1 h was allowed to elapse before any beetles were retested. Beetles were not reused for subsequent experiments. After each replicate, the letter-paper was removed from the arena and discarded outside the bioassay room. An independent set of flasks or syringes, and outlet tubes was used for each stimulus. The bioassays were conducted in a windowless room at 20°C and 65% RH, with diffuse overhead light. Percent positive response data were transformed by arcsine and analyzed by ANOVA, using the Tukey test to separate means (BMDP program

7d, 1990 version).

Field experiments. Field experiments were conducted to determine the attractiveness of pityol, conophthorin and host oils, either alone, or in combination, to C. banksianae, C. coniperda and C. resinosae. Experiments for C. banksianae were located in a jack pine plantation in Hurlbert Twp. ca. 60 km northeast of Sault Ste. Marie, ON. A red pine plantation near Thessalon, ON was used for the experiments with C. resinosae. Field tests for C. coniperda were conducted in the US Forest Service's, Beech Creek Seed Orchard, near Murphy North Carolina, and in a natural white pine forest in Pancake Provincial Park, ca. 80 km north of Sault Ste. Marie.

Traps were made from yellow Japanese beetle trap tops (Trécé Inc., Salinas, CA) and Mason jar bottoms containing 50 mL of ethylene glycol. Pityol and conophthorin (Table 9.1) were released from glass capillary tubes. Release rates from capillary tubes were calculated by determining volume loss at 24°C (Table 9.1). Capillary tubes were placed inside 1.5 mL plastic centrifuge tubes in which four 1 mm holes were made to facilitate volatiles release. Host oils were released from plastic centrifuge tubes each with two 0.5 mm holes. Centrifuge tubes containing baits were taped to one vane of the trap. Control traps were unbaited.

The experiments were laid out as complete randomized blocks, with traps hung in the upper third of the tree crown, one trap per tree, spaced 15-20 m apart. Captured beetles were preserved in 70% ethanol, identified, and their sex determined. In all experiments, except at Beech Creek, baits were replaced weekly and treatment locations re-randomized. The dates and number of replicates for each experiment are shown in Tables 9.4-9.6. Trap catch data were analyzed by the Kruskal-Wallis test with nonparametric multiple comparisons (BMDP program 3s,1990 version).

Results

Pheromone identification. Analyses of the Porapak Q-trapped volatiles from female and male C. banksianae feeding on jack pine by GC established that beetles of each sex produced at least one unique compound (Fig. 9.1). Sex specific compounds were also present in pentane extracts of the feeding beetles (Fig. 9.2). The mass spectra of these compounds were obtained by analyses of these pentane extracts and the Porapak Q-trapped female volatiles by GC-MS. The spectrum of the female-produced compound exhibited a base peak at m/z 59, suggesting a tertiary alcohol, and prominent peaks at m/z 85, 102 and 129 but lacked a molecule ion peak. A literature search revealed that the spectrum of the unknown compound was similar to that of (E)-pityol, an

Fig. 9.1 Gas chromatograms of the Porapak Q-trapped volatiles from female and male Conophthorus banksianae feeding on jack pine.

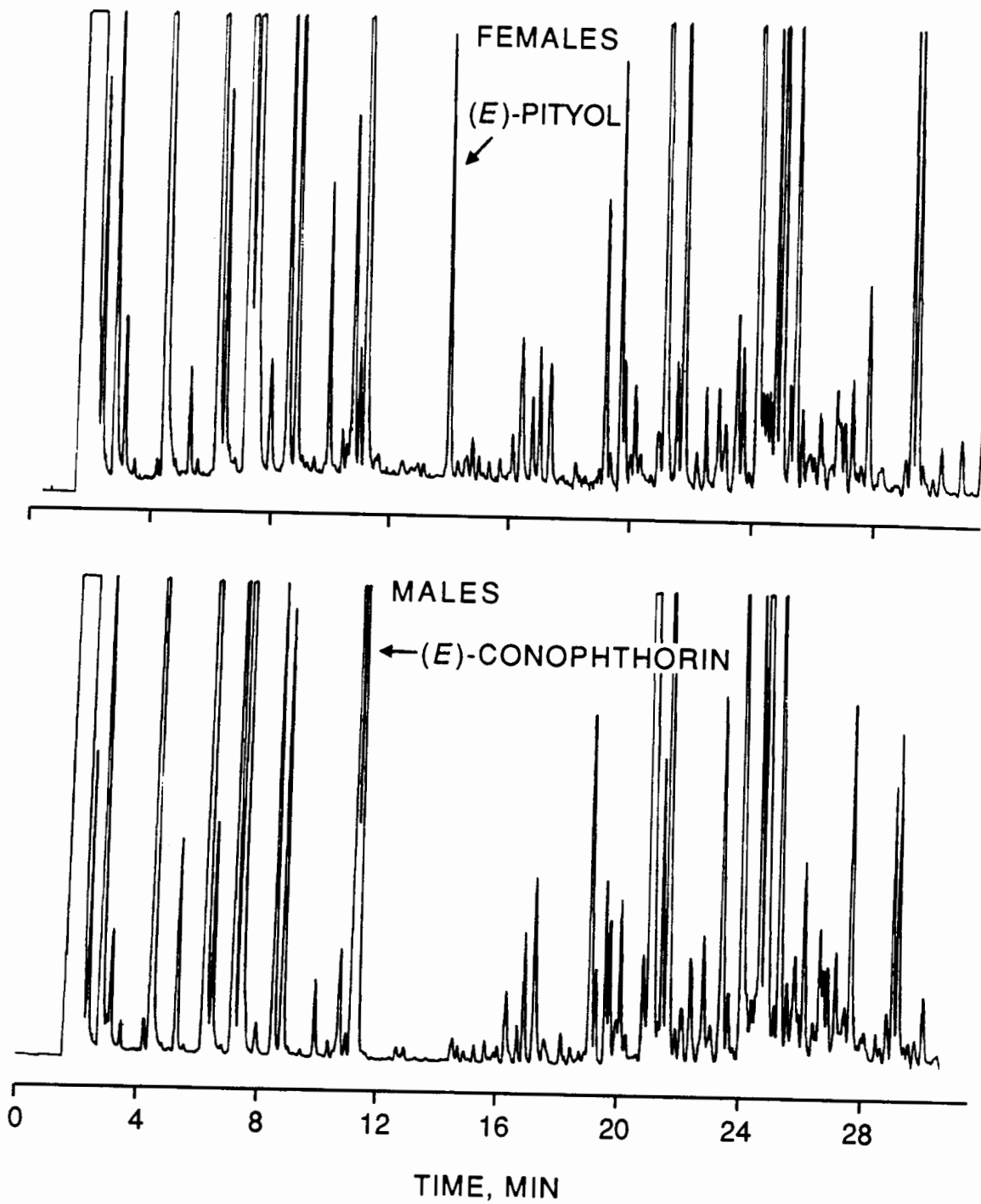
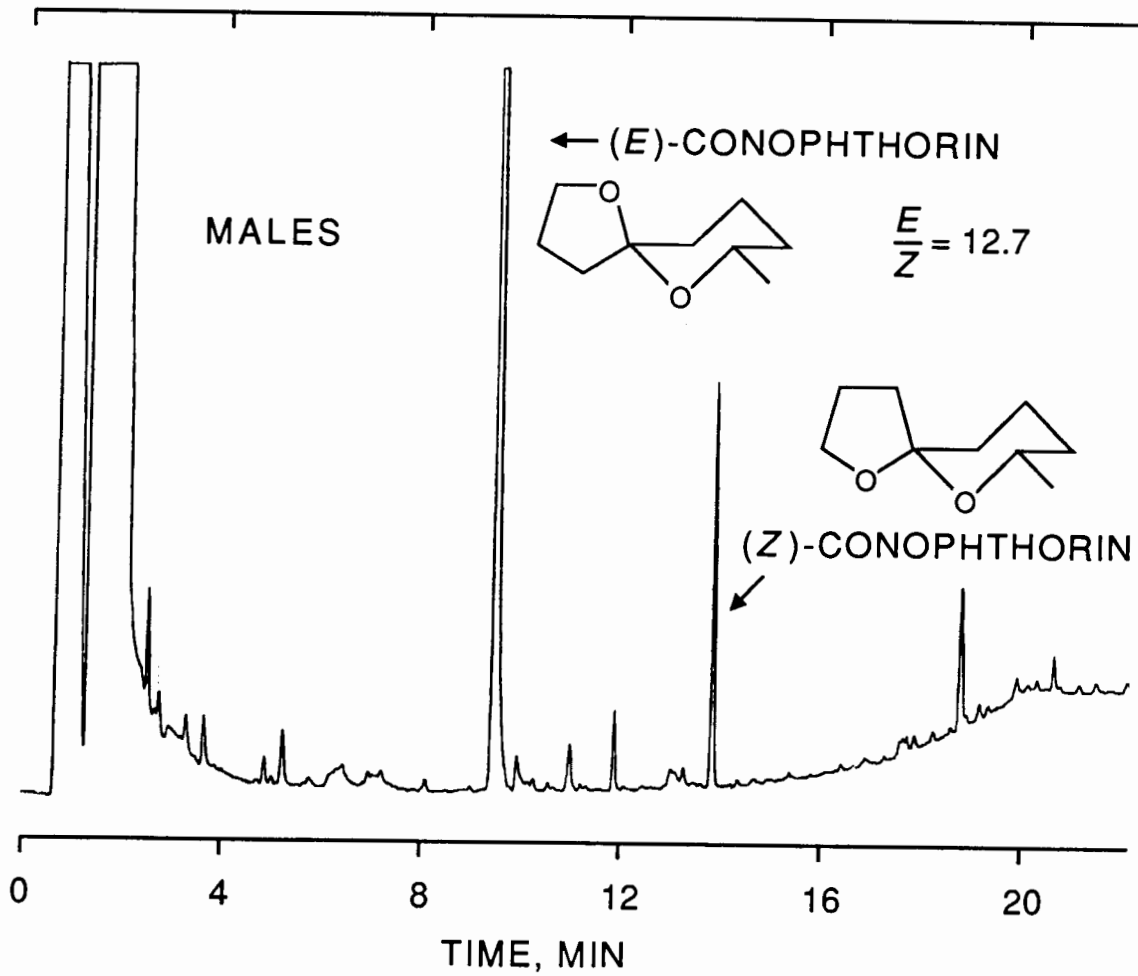
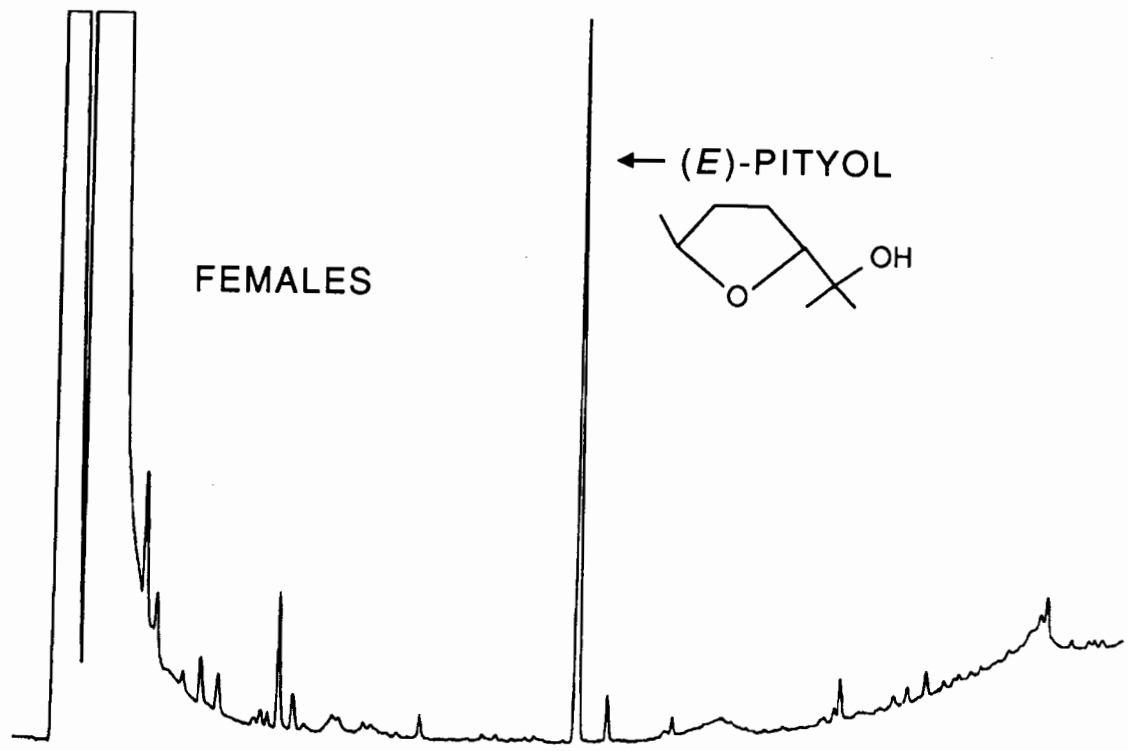


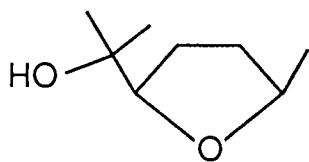
Fig. 9.2 Gas chromatograms of volatiles in pentane extracts of feeding female and male Conophthorus banksianae excised from jack pine.



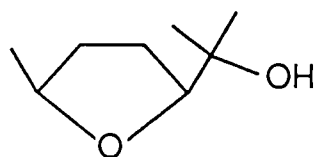
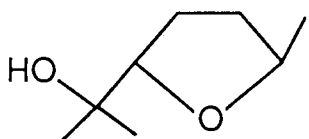
aggregation pheromone produced by male Pityophthorus pityographus (Franke et al. 1987). Comparison of the mass spectrum and GC retention times of the unknown compound to those of authentic pityol (E/Z, 1:2) established that female C. banksianae had produced only the E isomer of pityol (Fig. 9.3).

The mass spectra of the major and minor unknown compounds in the pentane extract of the males were identical and exhibited a molecule ion at m/z 156, but were completely different from those of exo- and endo-brevicommin (MW = 156). The retention time of the major unknown was longer than frontaline, but just slightly shorter than exo-brevicommin. Frontaline and brevicommins were not present in the pentane extracts of the males. Because Conophthorus spp. are scolytids, numerous species of which produce bicyclo and spiroketal pheromones (Borden 1985), it was hypothesized that the unknown compounds were related to these types of compounds. A literature search revealed that mass spectra of the unknowns matched the partial spectrum reconstructed from data tabulated for (E)-7-methyl-1,6-dioxaspiro[4.5] decane (Francke et al. 1979a), the principal volatile component in pentane extracts of the workers of the wasps Paravespula vulgaris L., P. germanica (F.), and Dolichoybespula saxonica (F.) (Francke et al. 1979b). The mass spectrum and GC retention time of the major male-produced unknown compound were identical to those observed from an authentic

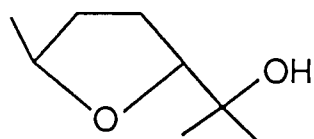
Fig. 9.3 Stereoisomers of Conophthorus pheromones.

*(2R,5R)*

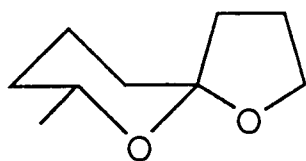
Z ISOMERS

*(2S,5S)**(2S,5R)*

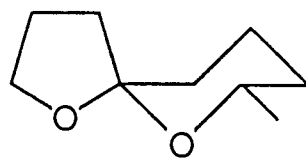
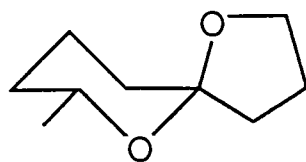
E ISOMERS

*(2R,5S)*
(MAJOR)

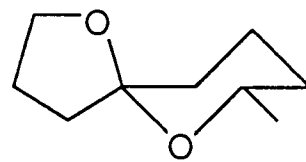
PITYOL

*(5S,7R)*

Z ISOMERS

*(5R,7S)*
(MINOR)*(5R,7R)*

E ISOMERS

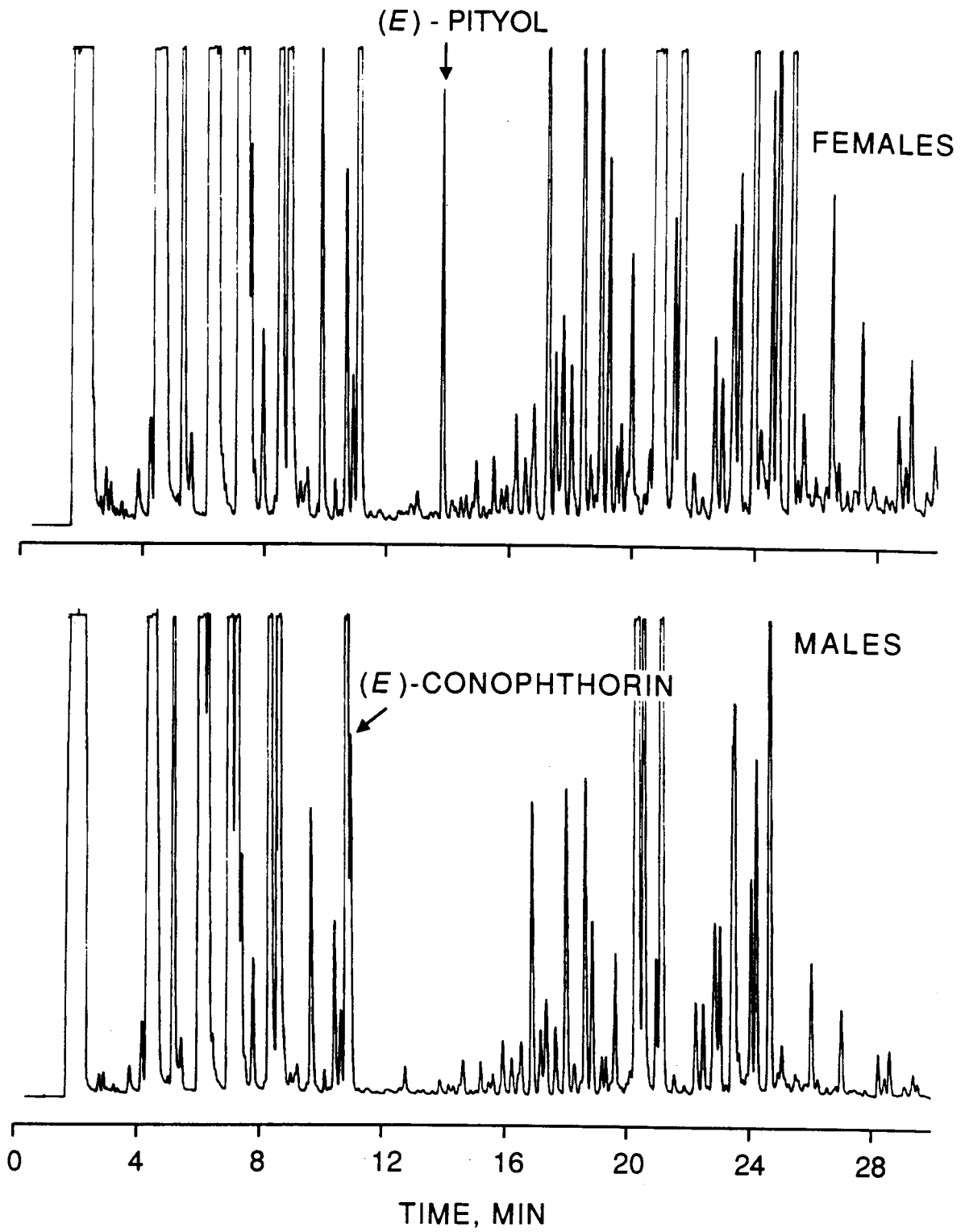
*(5S,7S)*
(MAJOR)

CONOPHTHORIN

sample of (E)-7-methyl-1,6-dioxaspiro[4.5]decane (87% pure), which contained ca. 1% of the longer-eluting Z isomer. The structure of the minor male compound was assigned as the Z isomer because its mass spectrum and retention time in GC were, respectively, identical to and considerably longer than that of the E isomer (Franke et al. 1979b). The isomer ratio (E/Z) was 9.4 and 12.4 in two pentane extracts, and 10.5 and 27.0 in two samples of Porapak-Q trapped volatiles. Although 7-methyl-1,6-dioxaspiro[4.5]decane was first found in the aforementioned wasp species and males of the ash bark beetle, Leperisinus varius (F.) (Franke et al. 1979b), and later in some plants (W. Francke, pers. communication), it has not been given a trivial name. "Conophthorin" is suggested as a name for this spiroketal.

Analyses of the Porapak Q-trapped volatiles (Fig. 9.4) from feeding female and male C. resinosae by GC, and comparison of the chromatograms to those in Fig. 9.1, indicated that they had emitted the same major sex-specific compounds as had female and male C. banksianae. Analysis of the female volatiles by GC-MS not only confirmed identity of this compound as (E)-pityol but also revealed the presence of a small amount of (Z)-pityol. The ratio of the E and Z isomers was ca. 150:1. The identity of the male compound in a sample of volatiles isolated by steam distillation was confirmed by GC-MS. The ratio of the E and Z isomers of

Fig. 9.4 Gas chromatograms of the Porapak Q-trapped volatiles from female and male Conophthorus resinosae feeding on red pine.



conophthorin in this sample was 49:1. In the Porapak Q-trapped volatiles the E/Z isomer ratio was variable and ranged from 19-39 (n=5).

(E)-Pityol was not detected in pentane extracts of female C. resinosa (Fig. 9.5). The chromatogram of the pentane extract of the males (Fig. 9.5) was quite similar to that obtained from male C. banksiana (Fig. 9.2) as was the ratio (9.4) of the E and Z isomers of conophthorin. Comparison of the chromatograms in Fig. 9.5 indicated that no other sex-specific compound, which might not have been discernable in the complex mixture of Porapak Q-trapped volatiles, was present in these extracts.

Analysis of Porapak Q-trapped volatiles showed that female C. coniperda feeding on second year cones emitted both (E)-pityol and (E)-conophthorin (Fig. 9.6). The ratio of the E and Z isomers of pityol in this sample was ca. 19 as determined by GC-MS. Although female C. coniperda always contained and emitted (E)-conophthorin, they did not always emit (E)-pityol when feeding on first-year conelets. The Porapak Q-trapped volatiles from male C. coniperda also contained (E)-conophthorin (chromatogram not shown). (E)- and (Z)-conophthorin were present in the pentane extracts of both female (E/Z =15.6) and male C. coniperda (E/Z = 13.7) (Fig. 9.7). In other pentane extracts of females and males the E/Z isomer ratio was 10 and 11,

Fig. 9.5 Gas chromatograms of volatiles in pentane extracts of feeding female and male Conophthorus resinosa excised from red pine.

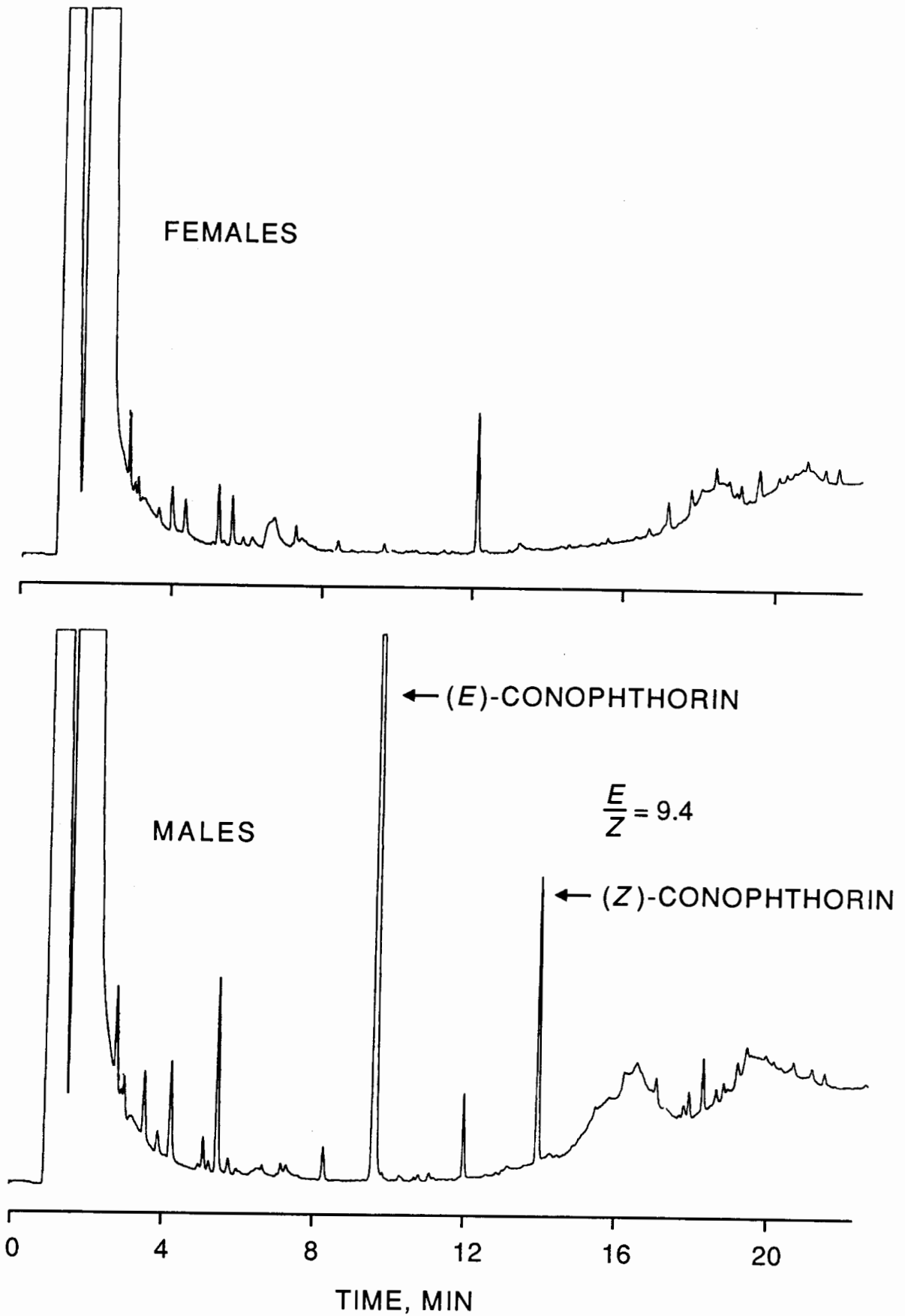


Fig. 9.6 Gas chromatogram of Porapak Q-trapped volatiles from female Conophthorus coniperda feeding on white pine.

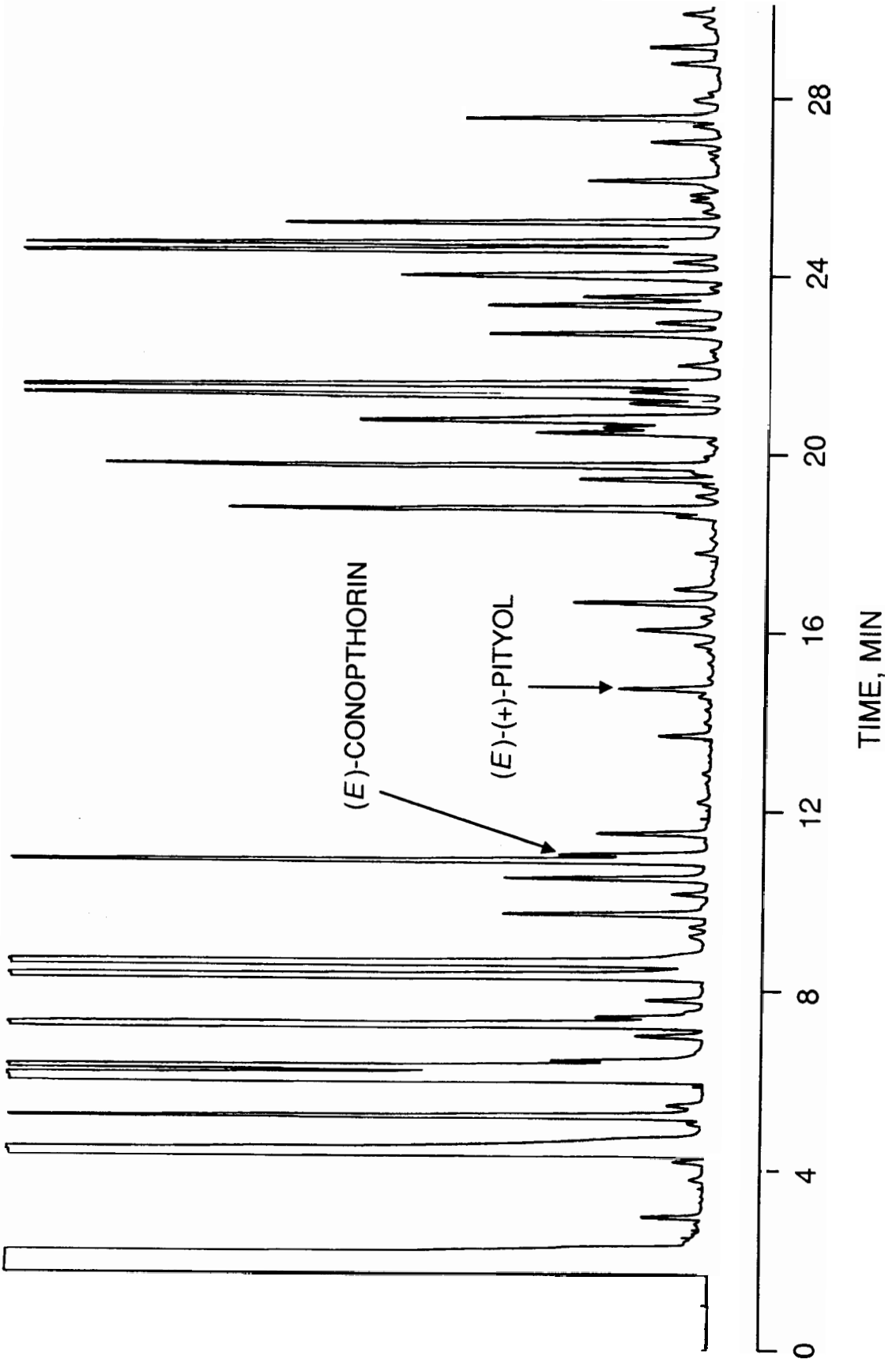
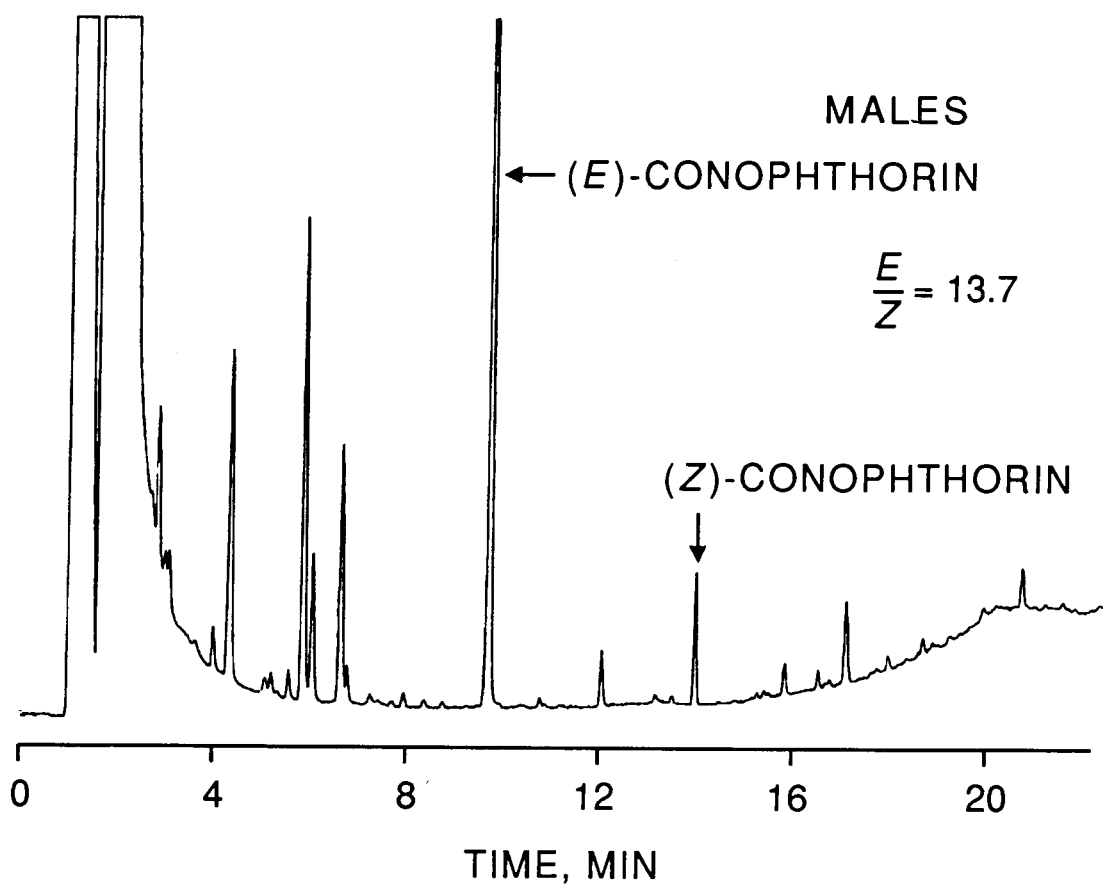
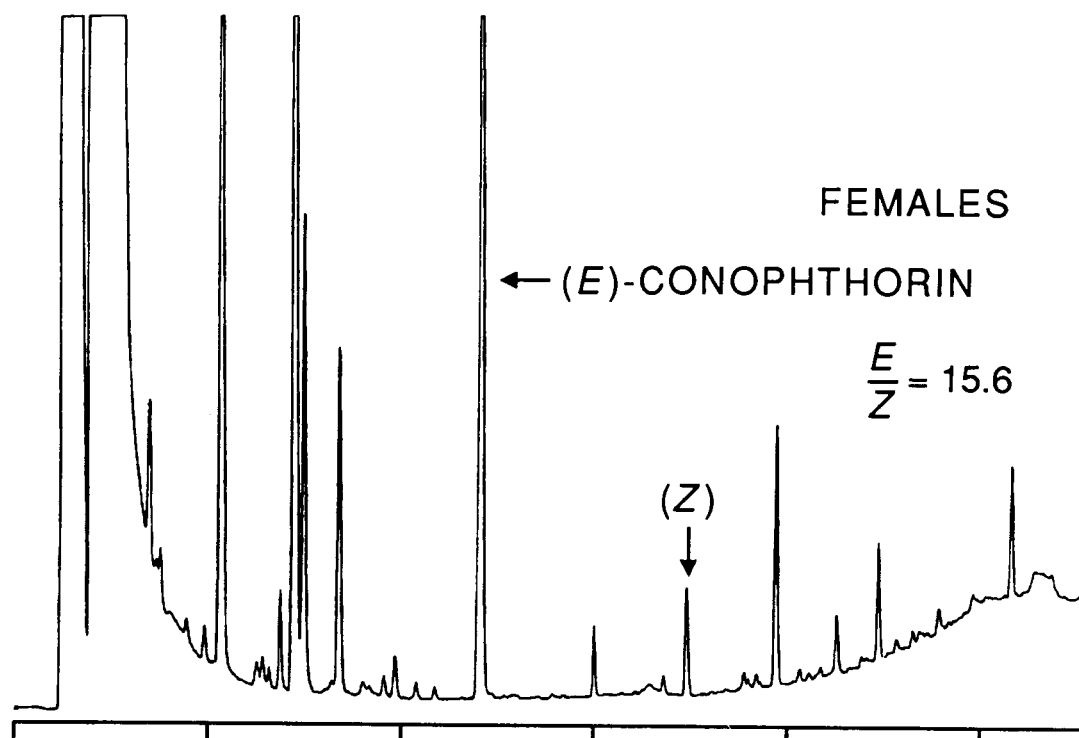


Fig. 9.7 Gas chromatograms of volatiles in pentane extracts of feeding female and male Conophthorus coniperda excised from white pine.



respectively. The E/Z isomer ratio of conophthorin in the Porapak Q-trapped volatiles from females (n=5) or from males (n=3) ranged from 21-34, but in one instance for each sex it was 13.7 and 11, respectively.

Chirality. Analysis of the volatiles in the pentane extract of female C. banksianae (Fig 9.3) on a capillary column coated Chirasil-Dex(8) revealed that the females had produced optically pure (E)-(+)-pityol (Fig. 9.8) as had female C. resinosa (Fig. 9.9). These determinations were made difficult by the presence in these samples of several compounds with retention times quite close to that of (E)-(-)-pityol. As the lower panels in Figs. 9.8 and 9.9 show, a small amount of the (E)-(-) isomer could have been detected if it had been present in the volatiles. Female C. coniperda also produced optically pure (E)-(+)-pityol. The absolute configuration of (E)-(+)-pityol is (2R,5S) (Fig. 9.3).

The (E)-conophthorin from the males of the three species and female C. coniperda had the (5S,7S) configuration and ee's of ca. 96% as determined by analyses on the Chirasil-Dex(8) column. Fig. 9.10 shows chromatograms of the (E)-spiroketals isolated from the Porapak Q-trapped volatiles from feeding male C. resinosa and female C. coniperda by micropreparative GC. Since the isomers were not completely resolved, it was fortunate the (5S,7R) isomer eluted first. Determination of the area of a small peak on the

tail of a large peak is often quite difficult and not particularly accurate.

Laboratory experiments. The response by males and females of C. coniperda and C. resinosae to (E)-(±)- and (E)-(+) - pityol, (±)-conophthorin, cone oils, and their various combinations, generally was significantly greater than the response to the pentane:hexane solvent blank (Exp.1-6; Tables 9.2-9.3). There was no significant difference in the response by males or females to (+)- and (±)- pityol (Exp. 1,4), and the addition of cone oils did not significantly increase the response, except by male C. resinosae in Exp. 4. There was no significant difference in the response by female C. resinosae when (±)-conophthorin was added alone, or with cone oils, to either (+)- or (±)- pityol (Exp. 5,6), but in one of 2 experiments response by female C. coniperda was enhanced (Exp. 2,3). Although the responses by male C. coniperda and C. resinosae were more variable than those by females, they were essentially similar.

Field experiments. In field experiments neither female C. banksianae, C. coniperda, nor C. resinosae responded to any stimulus (Tables 9.4-9.7). Males of all three species responded to (+)- and (±)- pityol, except for C. banksianae males in Exp. 2

Fig. 9.8 Gas chromatograms of volatiles in pentane extracts of female Conophthorus banksianae excised from jack pine (top), and spiked with (E)-(+)-pityol (bottom).

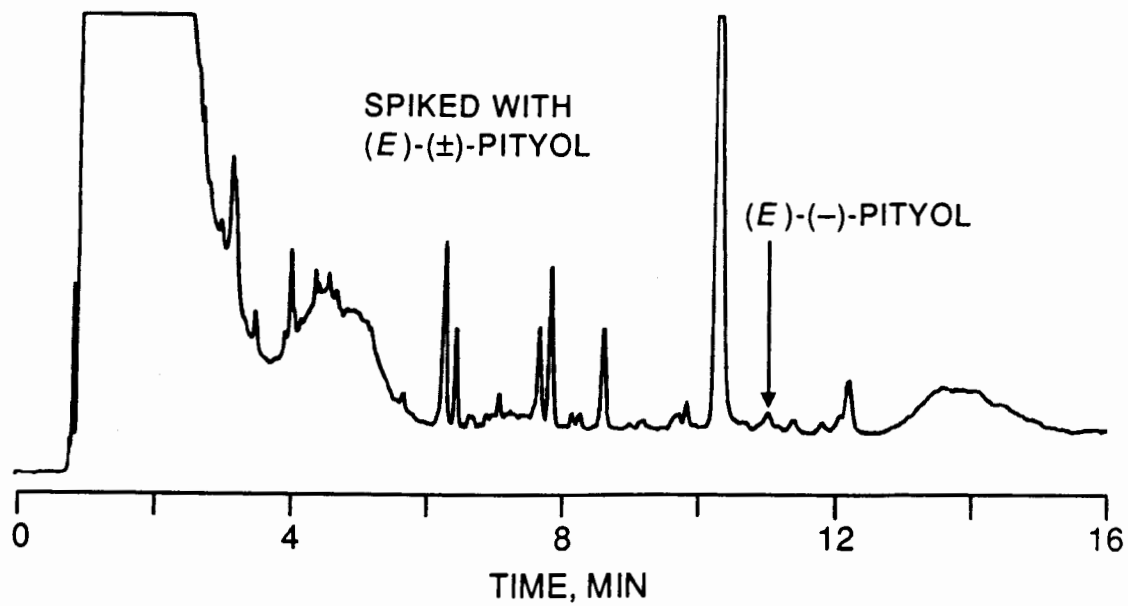
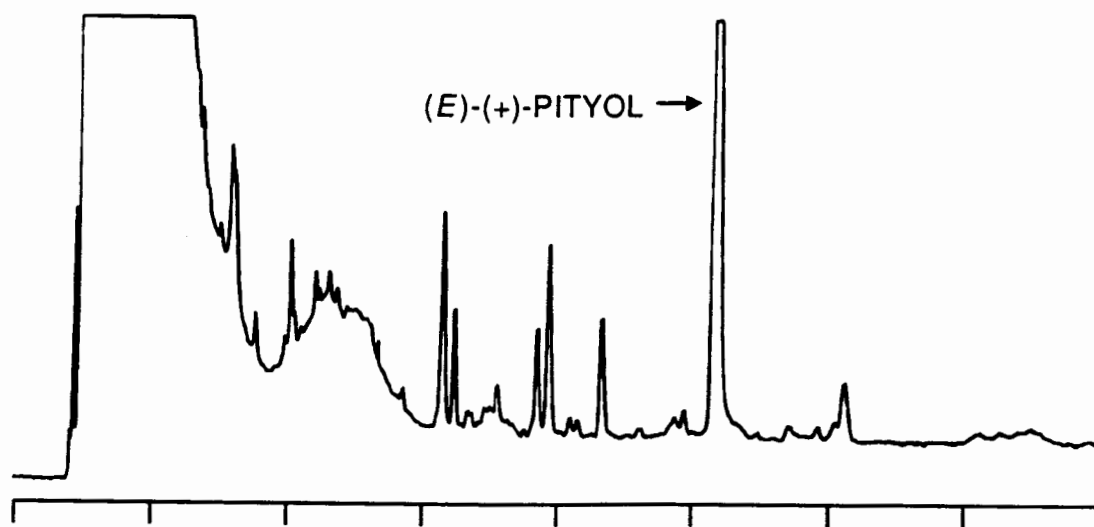


Fig. 9.9 Gas chromatograms of (E)-pityol isolated from Porapak Q-trapped volatiles from female Conophthorus resinosae feeding on red pine (top) and spiked with (E)-(±)-pityol (bottom).

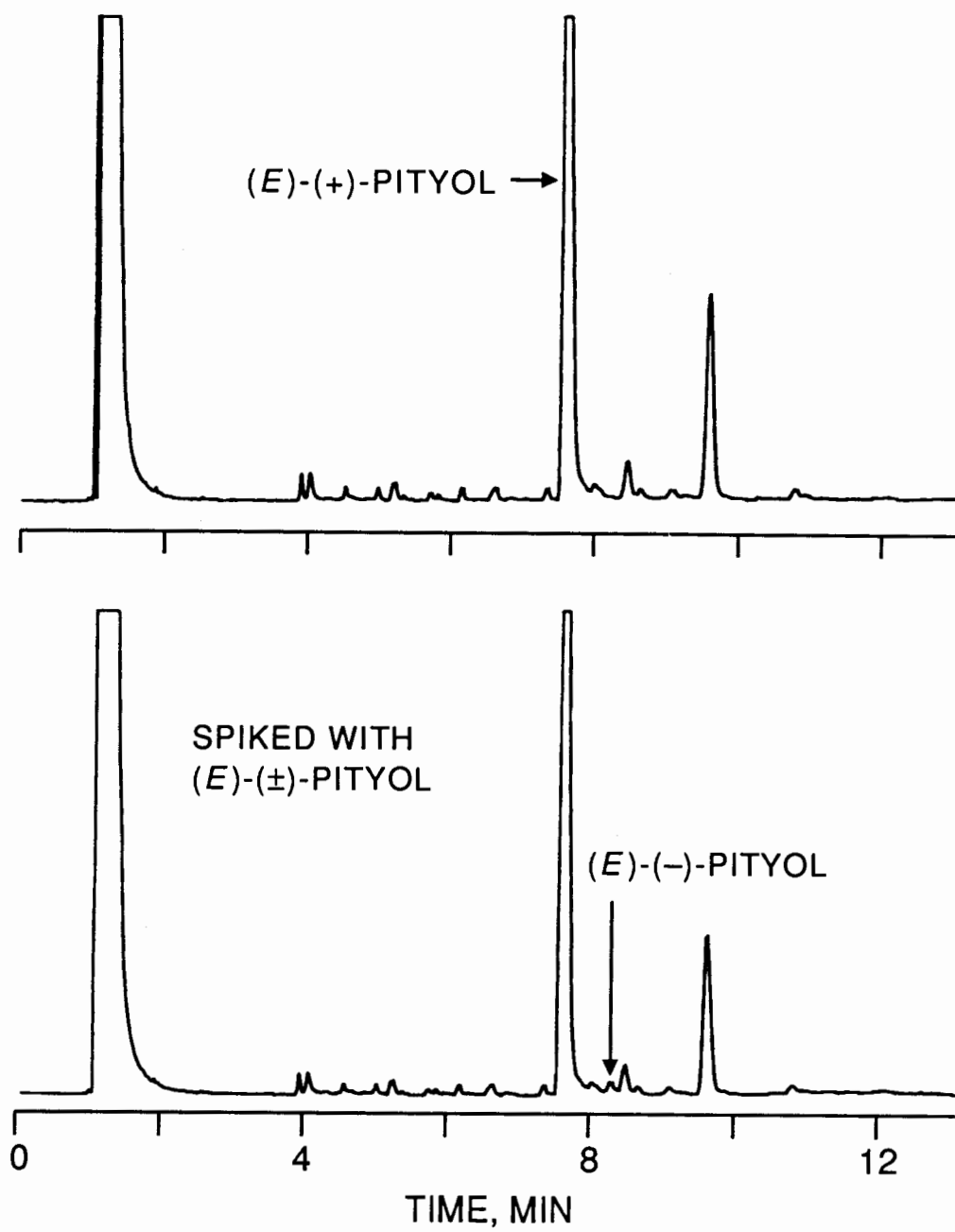


Fig. 9.10 Gas chromatograms of (E)-conophthorin isolated from the Porapak Q-trapped volatiles from male Conophthorus resinosae and female C. coniperda feeding on red and white pine, respectively.

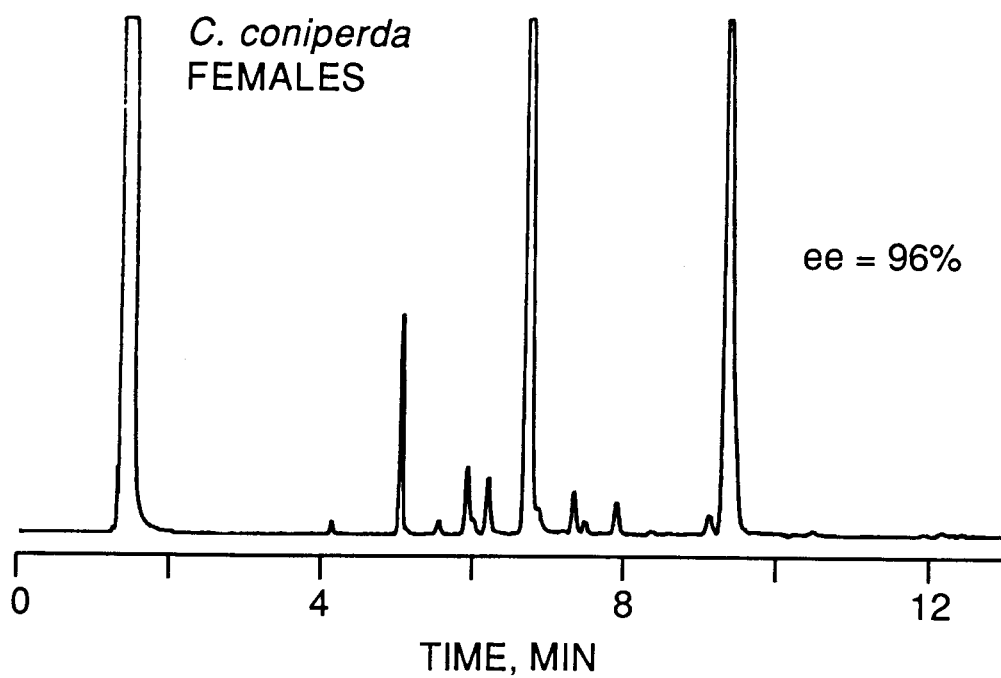
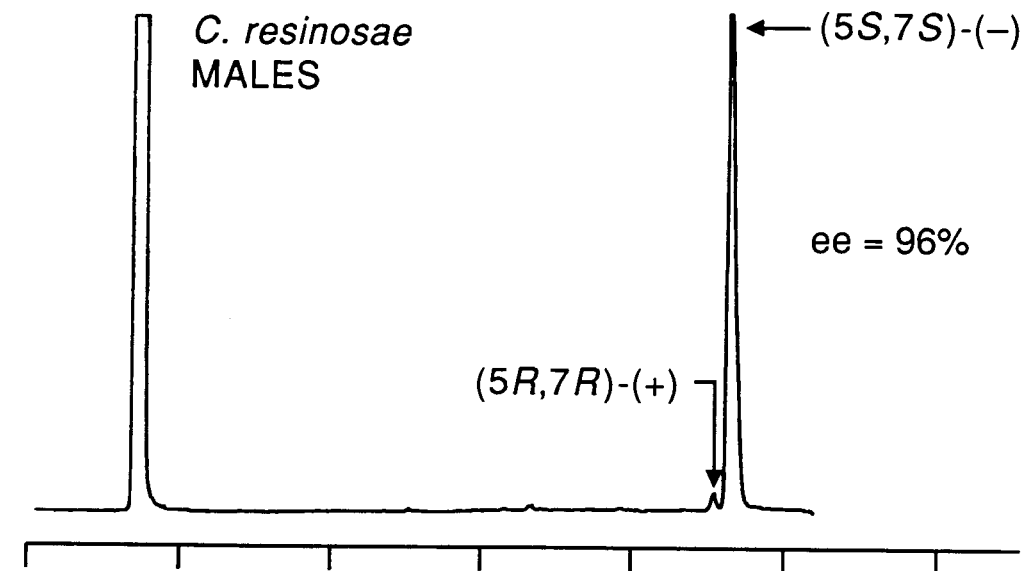


Table 9.2. Responses of walking *C. coniperda* in laboratory bioassays to pityol, conophthorin and host oils

Exp. No.	Stimulus	Percent response ($\bar{x} \pm SE$) ^a			
		Females	Males		
1	(+)-pityol	45 ± 7	a	38 ± 6	a
	(±)-pityol	43 ± 3	a	40 ± 7	a
	(+)-pityol & white pine cone oil	27 ± 8	ab	43 ± 6	a
	(±)-pityol & white pine cone oil	42 ± 5	a	35 ± 7	a
	white pine cone oil	25 ± 4	ab	43 ± 3	a
	pentane:hexane (1:1)	8 ± 3	b	7 ± 3	b
2	(+)-pityol	50 ± 5	b	37 ± 8	ab
	(+)-pityol & (±)-conophthorin	43 ± 4	b	45 ± 3	ab
	(+)-pityol & (±)-conophthorin & white pine cone oil	73 ± 4	a	55 ± 4	a
	(±)-conophthorin	48 ± 5	b	33 ± 0	ab
	white pine cone oil	53 ± 4	ab	32 ± 6	b
	pentane:hexane (1:1)	7 ± 3	c	7 ± 2	c
3	(±)-pityol	48 ± 3	ab	28 ± 4	a
	(±)-pityol & (±)-conophthorin	57 ± 8	ab	40 ± 5	a
	(±)-pityol & (±)-conophthorin & white pine cone oil	63 ± 4	a	30 ± 4	a
	(±)-conophthorin	53 ± 8	ab	33 ± 6	a
	white pine cone oil	35 ± 8	b	28 ± 7	a
	pentane:hexane (1:1)	10 ± 2	c	3 ± 2	b

^a Means in a column within an experiment followed by the same letter are not significantly different, Tukey's test, $P < 0.05$.

Table 9.3. Responses of walking *C. resinosa* in laboratory bioassays to pityol, conophthorin and host oils

Exp. No.	Stimulus	Percent response ($\bar{x} \pm SE$) ^a			
		Females	Males		
4	(+)-pityol	15 ± 7	a	7 ± 3	b
	(±)-pityol	5 ± 3	a	5 ± 2	b
	(+)-pityol & red pine cone oil	10 ± 3	a	27 ± 5	a
	(±)-pityol & red pine cone oil	20 ± 11	a	28 ± 4	a
	red pine cone oil	10 ± 5	a	12 ± 3	ab
	pentane:hexane (1:1)	0 ± 0	a	2 ± 2	b
5	(+)-pityol	43 ± 3	a	32 ± 3	b
	(+)-pityol & (±)-conophthorin	27 ± 3	a	42 ± 5	ab
	(+)-pityol & (±)-conophthorin & red pine cone oil	32 ± 4	a	32 ± 9	b
	(±)-conophthorin	30 ± 3	a	30 ± 6	b
	red pine cone oil	42 ± 6	a	55 ± 2	a
	pentane:hexane (1:1)	8 ± 4	b	8 ± 0	c
6	(±)-pityol	33 ± 3	a	30 ± 7	ab
	(±)-pityol & (±)-conophthorin	53 ± 7	a	20 ± 4	bc
	(±)-pityol & (±)-conophthorin & red pine cone oil	43 ± 8	a	40 ± 7	ab
	(±)-conophthorin	37 ± 5	a	25 ± 4	ab
	red pine cone oil	42 ± 9	a	48 ± 3	a
	pentane:hexane (1:1)	5 ± 3	b	7 ± 2	c

^a Means in a column within an experiment followed by the same letter are not significantly different, Tukey's test, $P < 0.05$.

Table 9.4. Numbers of *C. banksianae* captured in barrier traps baited with pityol, conophthorin and host oils in a jack pine plantation, Hulbert Twp. ca 60 km northeast of Sault Ste. Marie Ontario

Exp. No.	Treatment ^a	No. of beetles captured per trap ($\bar{x} \pm SE$) ^b	
		Females	Males
1	(+)-pityol	0.0 ± 0.0 a	2.6 ± 0.7 a
	(+)-pityol	0.0 ± 0.0 a	2.1 ± 0.5 a
	(+)-pityol & jack pine shoot oil	0.0 ± 0.0 a	2.4 ± 0.7 a
	(+)-pityol & jack pine shoot oil	0.0 ± 0.0 a	5.0 ± 1.6 a
	jack pine shoot oil	0.0 ± 0.0 a	0.0 ± 0.0 b
	blank	0.0 ± 0.0 a	0.0 ± 0.0 b
2	(+)-pityol (E:Z, 19:1) & jack pine shoot oil	0.0 ± 0.0 a	0.1 ± 0.1 a
	(+)-pityol ($\bar{E}:\bar{Z}$, 66:1) & jack pine shoot oil	0.0 ± 0.0 a	0.2 ± 0.1 a
	(+)-pityol ($\bar{E}:\bar{Z}$, 364:1) & jack pine shoot oil	0.0 ± 0.0 a	0.3 ± 0.2 a
	blank	0.0 ± 0.0 a	0.0 ± 0.0 a
3	(+)-pityol	0.0 ± 0.0 a	1.9 ± 0.5 a
	(+)-pityol & (+)-conophthorin	0.1 ± 0.1 a	0.3 ± 0.2 b
	(+)-pityol & (+)-conophthorin	0.0 ± 0.0 a	0.3 ± 0.2 b
	(+)-pityol & (-)-conophthorin	0.0 ± 0.0 a	0.1 ± 0.1 b
	blank	0.0 ± 0.0 a	0.0 ± 0.0 b

^a Exp. 1, 20-27 June, 10 traps/treatment; Exp. 2, 4-11 July, 10 traps/treatment; Exp. 3, 27 June - 4 July, 7 traps/treatment.

^b Means in a column within an experiment followed by the same letter are not significantly different, Kruskal-Wallis test, $P < 0.05$.

Table 9.5. Numbers of *C. coniperda* captured in barrier traps baited with pityol, conophthorin and host oils in the USFS Beech Creek white pine seed orchard, near Murphy, North Carolina

Exp. No.	Treatment ^a	No. of beetles captured per trap ($\bar{x} \pm SE$) ^b	
		Females	Males
4	(±)-pityol	0.0 ± 0.0 a	1.7 ± 0.8 ab
	(+)-pityol	0.0 ± 0.0 a	0.2 ± 0.2 ab
	(±)-pityol & white pine cone oil	0.3 ± 0.2 a	4.5 ± 1.7 a
	(+)-pityol & white pine cone oil	0.3 ± 0.2 a	6.3 ± 2.7 a
	white pine cone oil	0.2 ± 0.2 a	0.0 ± 0.0 b
5	blank	0.0 ± 0.0 a	0.0 ± 0.0 b
	(±)-pityol	0.0 ± 0.0 a	0.3 ± 0.2 ab
	(±)-pityol & (±)-conophthorin	0.0 ± 0.0 a	1.0 ± 0.7 ab
	(±)-pityol & (±)-conophthorin & white pine cone oil	0.3 ± 0.2 a	4.0 ± 1.7 a
	(±)-conophthorin	0.0 ± 0.0 a	0.0 ± 0.0 b
	white pine cone oil	0.0 ± 0.0 a	0.5 ± 0.3 ab
	blank	0.0 ± 0.0 a	0.0 ± 0.0 b

^a Exp.4 and 5, 23 April- 10 May, 6 traps/treatment, rebaited 26 April, traps positions rerandomized 3 May

^b Means in a column within an experiment followed by the same letter are not significantly different, Kruskal-Wallis test, $P < 0.05$.

Table 9.6. Numbers of *C. coniperda* captured in barrier traps baited with pityol, conophthorin and host oils in a natural white pine forest, Pancake Bay Provincial Park, ca 80 north of Sault Ste. Marie, Ontario

Exp. No.	Treatment ^a	No. of beetles captured per trap ($\bar{x} \pm SE$) ^b	
		Females	Males
6	(+)-pityol	0.1 ± 0.1 ab	5.9 ± 1.7 a
	(+)-pityol	0.1 ± 0.1 ab	6.1 ± 1.8 a
	(+)-pityol & white pine cone oil	0.4 ± 0.2 a	10.1 ± 2.0 a
	(+)-pityol & white pine cone oil	0.1 ± 0.1 ab	8.4 ± 2.1 a
	white pine cone oil	0.0 ± 0.0 b	0.1 ± 0.1 b
	blank	0.0 ± 0.0 b	0.0 ± 0.0 b
7	(+)-pityol (E:Z, 19:1) & white pine cone oil	0.0 ± 0.0 a	1.8 ± 1.0 ab
	(+)-pityol ($\bar{E}:\bar{Z}$, 66:1) & white pine cone oil	0.0 ± 0.0 a	2.2 ± 0.7 a
	(+)-pityol ($\bar{E}:\bar{Z}$, 364:1) & white pine cone oil	0.0 ± 0.0 a	1.2 ± 0.4 ab
	blank	0.0 ± 0.0 a	0.2 ± 0.1 b
8	(+)-pityol	0.1 ± 0.1 a	8.2 ± 3.7 a
	(+)-pityol & (+)-conophthorin	0.1 ± 0.1 a	0.4 ± 0.3 b
	(+)-pityol & (+)-conophthorin	0.0 ± 0.0 a	1.2 ± 0.7 b
	(+)-pityol & (-)-conophthorin	0.2 ± 0.2 a	0.2 ± 0.1 b
	blank	0.1 ± 0.1 a	0.0 ± 0.0 b

^a Exp. 1, 28 May-4 June, and 4-11 June, 10 traps/treatment; Exp. 2, 1-8 July, 10 traps/treatment; Exp. 3, 11-18 June and 18-25 June, 7 traps/treatment.

^b Means in a column within an experiment followed by the same letter are not significantly different, Kruskal-Wallis test, $P < 0.05$.

Table 9.7. Numbers of *C. resinosae* captured in barrier traps baited with pityol, conophthorin and host oils in a red pine plantation, Thessalon, Ontario

Exp. No.	Treatment ^a	No. of beetles captured per trap ($\bar{x} \pm SE$) ^b		
		Females	Males	
9	(±)-pityol	0.0 ± 0.0 a	19.8 ± 2.1 a	
	(+)-pityol	0.0 ± 0.0 a	25.1 ± 3.0 a	
	(±)-pityol & red pine cone oil	0.0 ± 0.0 a	18.8 ± 2.4 a	
	(+)-pityol & red pine cone oil	0.0 ± 0.0 a	28.2 ± 2.2 a	
	red pine cone oil	0.1 ± 0.1 a	0.1 ± 0.1 b	
	blank	0.1 ± 0.1 a	0.1 ± 0.1 b	
10	(±)-pityol (E:Z, 19:1) & red pine cone oil	0.1 ± 0.1 a	11.3 ± 2.2 a	
	(±)-pityol ($\bar{E}:\bar{Z}$, 66:1) & red pine cone oil	0.0 ± 0.0 a	16.1 ± 2.6 a	
	(±)-pityol ($\underline{E}:\underline{Z}$, 364:1) & red pine cone oil	0.0 ± 0.0 a	15.3 ± 2.7 a	
			0.1 ± 0.1 a	0.1 ± 0.1 b
	blank			
11	(±)-pityol	0.0 ± 0.0 a	17.9 ± 3.2 a	
	(±)-pityol & (±)-conophthorin	0.0 ± 0.0 a	0.1 ± 0.1 b	
	(±)-pityol & (+)-conophthorin	0.0 ± 0.0 a	2.1 ± 0.8 b	
	(±)-pityol & (-)-conophthorin	0.0 ± 0.0 a	0.4 ± 0.2 b	
	blank	0.3 ± 0.2 a	0.1 ± 0.1 b	

^a Exp. 1, 29 May-5 June, and 5-12 June, 10 traps/treatment; Exp. 2, 3-10 July, 10 traps/treatment; Exp. 3, 12-19 June and 19-26 June, 7 traps/treatment.

^b Means in a column within an experiment followed by the same letter are not significantly different, Kruskal-Wallis test, $P < 0.05$.

where very low trap catches were obtained. Addition of the host oils did not appear to significantly enhance their response (Exps. 1,4,6,9). Male trap catches were not significantly different between the three E/Z ratios of (±)-pityol for C. coniperda and C. resinosa (Exp. 7,10). Addition of (±)-, (+)-, or (-)-conophthorin to (±)-pityol significantly inhibited the response of males in all 3 species; however, there was no such effect for the North Carolina population of C. coniperda males (Table 9.5).

Discussion

The discovery of (E)-(+)-pityol in female Conophthorus constitutes the first record of occurrence in this genus. (E)-(+)-pityol was first found by Francke et al. (1987) in male Pityophthorus pityographus Ratz., which also contained grandisol. In field tests (E)-(+)-pityol and racemic grandisol acted synergistically as aggregation pheromones for P. pityographus, whereas (E)-(-)-pityol and grandisol were inactive. (E)-(±)-pityol exhibited only slight attraction for P. pityographus. Recently, Klimetzek et al. (1989) found that females and males of the elm bark beetle, Pteleobius vittatus (F.) produced the aggregation pheromones 2-methyl-3-buten-2-ol, (Z)-pityol and 3 (Z)-vittatol (3(Z)-hydroxy-2,2,6-trimethyltetrahydropyran). In field tests the combination of all three compounds was far

superior to single compounds or two-component mixtures. The ternary mixture containing (Z)-(-)-vittatol was ca. 7 times more attractive than that containing the (+)-enantiomer. Unlike the responses of P. pityographus and P. vittatus, only male Conophthorus spp. appear to aggregate to (E)-(+)-pityol, which functions as a female-produced sex pheromone for all three species tested.

As for males, there was no discernable trend with respect to species in the structural or optical isomerism of conophthorin released by male Conophthorus spp. Males of the ash bark beetle, Leperisinus varius (F.), were found to produce both (E)-conophthorin and the aggregation pheromone exo-brevicommin (Francke et al. 1979b). In field experiments, (E)-conophthorin released with exo or endo brevicomin greatly reduced trap catch of both males and females, and thus functioned as an antiaggregation pheromone for L. varius (Kohnle 1985). (E)-conophthorin is also known to exhibit repellent effects for worker wasps of several species (Francke et al. 1979b). The repellency of (E)-conophthorin to male Conophthorus spp. is consistent with these results, although its effects on females are unknown. Production of conophthorin by males likely functions as a semiochemical message to conceal receptive females from other rival males, thereby reducing the risks of take-over and ensuring the reproductive success of the male. Males may also

physically protect females from intruders by remaining near the entrance hole between matings and during oviposition (Chapter 2).

The release of (E)-conophthorin, a repellent pheromone, by virgin female C. coniperda, but not by female C. banksianae and C. resinosae is consistent with other differences that separate the latter two species from C. coniperda. Female C. coniperda are known to cease production of pityol and begin production of conophthorin after mating (G.O. Birgersson, Dept. of Chemical Ecology, Göteborg Univ., Göteborg, Sweden, pers. comm.). It is possible that the sample of C. coniperda contained mated females, even though, great care was taken to prevent mating by removing emerged adults from the cones as quickly as possible. Mating by C. coniperda occurs after a period of feeding in the cones (Godwin and Odell 1965). Henson (1961) noted that ovarian development reached its peak several weeks after the females emerged. I hypothesize that females produce conophthorin during the period of feeding until they reach reproductive maturity, then produce pityol to attract a mate (signalling sexual receptivity), and release conophthorin again after mating to repel further males. Alternatively, female production of conophthorin may never cease, but its repellent effects may be overcome when high amounts of pityol are released.

Species specificity in pityol and conophthorin production was

not found between C. banksianae, and C. resinosae. It is possible that the ratio of (E)-conophthorin to (E)-pityol determines whether the blend is repellent or attractive to males and that this ratio is specific for each species. Additional field work would be required to test this hypothesis.

Host volatiles do not appear to synergize the attraction to pityol by Conophthorus species. Moreover, it is unlikely that host terpenes confer specificity to the semiochemical system in Conophthorus for at least two reasons. Firstly, terpene emissions from pine cones and foliage are highly variable both within and between trees especially during the growing season (von Rudloff 1975; Mattson and Strauss 1986). It is doubtful that Conophthorus can rely on such a variable message for highly specific information needed for consistent host finding (Mattson and Strauss 1986) or mate location. Secondly, C. resinosae is not monophagous, suggesting that either C. resinosae does not discriminate among different host 'messages' or that differences are detected but not meaningful. In spite of the apparent vagueness of the chemical message produced by host odours, they may, nevertheless, play a role in host finding, particularly when coupled with visual cues such as tree silhouettes (Henson 1967) and cone colour (Jenkins 1983). Therefore additional studies of the constituents of pine host volatiles (e.g. alpha- and beta-pinene, myrcene, and limonene) and autoxidation products in

'primary' and 'secondary' attraction of Conophthorus should be pursued.

Further studies on the species specificity of semiochemical communication in Conophthorus should await the development of a coupled gas chromatographic electroantennogram detector (GC-EAD) for bark beetles. The use of a highly sensitive GC-EAD system may disclose additional 'minor' but, biologically significant components of the chemical message that may confer species-specificity to the pheromone channels of communication (Cardé and Baker 1984) of sympatric species of Conophthorus.

Chapter 10

Concluding discussion

" Systematists are drawn to study groups of organisms whose members are either pleasing to the eye and thus a delight to examine, or whose included taxa, though the members are plain, are numerous and difficult to distinguish, and thus present a challenge to both one's discriminatory ability and to one's perseverance. "

G.E. Ball (1978)

Scientific research should not only endeavor to answer specific questions but also should pose questions for further investigation. This dissertation was directed primarily to answer the question: "Is Conophthorus banksianae McPherson a species". On the basis of the data presented in this study, I do not consider it to be a valid species. Extensive investigation into ecological, morphological, behavioural, karyological, allozyme, and biochemical characters has not provided a single taxonomic character from which it could be inferred that it is reproductively isolated from C. resinosae and thus is a distinct species. These studies support the conclusion of Wood (1989) that the name of C. banksianae be placed in synonymy with C. resinosae. Therefore in the remainder of this discussion, C. banksianae will be referred to as C. resinosae on jack pine.

Three questions persist about C. resinosa on jack pine:

Why does C. resinosa attack jack pine shoots?

Why hasn't C. resinosa attacked jack pine outside the range of red pine?

Could C. resinosa on jack pine take a separate evolutionary path from C. resinosa on red pine because of human intervention in the forest ecosystem?

Why does C. resinosa attack jack pine shoots?

I postulate that C. resinosa attacks jack pine shoots as a survival strategy, but in order for a change in hosts to occur, a large-scale reduction of red pine trees in an area is required. Without such a catastrophic (disturbance) event to reduce the availability of the red pine resource, C. resinosa will more than likely switch their attack from cones to shoots, and not switch hosts (Chapter 4; Lyons 1956; McPherson et al. 1970b; Mattson 1980). Attacks on shoots following depletion of cone resources have also been observed in other species of Conophthorus, (Harrington 1902; Struble 1947; Godwin and Odell 1965). Disturbance and forest succession favouring jack pine can

occur naturally by fire, or artificially by clear-cutting and planting. In my opinion, the increased incidence of C. resinosa on jack pine is due, in large measure, to the forest harvesting and reforestation methods currently being practiced.

Red pine in the Boreal Forest Region, and in the transition zone between the Boreal Forest and Great Lakes-St. Lawrence Forest Regions (Rowe 1972), usually occurs in small and isolated stands, largely restricted to lake landscapes or rough topography (Haddow 1948; Horton and Bedell 1960; Van Wagner 1971; Butson et al. 1987; Bergeron and Brisson 1990). Horton and Bedell (1960) suggested that many marginal stands of red pine extending into the Boreal Region are relict on warm sites with a favourable soil or fire regime. Haddow (1948) described red pine at the northern limit of its range in Ontario as a decadent and retreating species, and provides several historical accounts of red pine stands fast disappearing from the northern boundary because of cutting and subsequent fires. As a result of extensive logging and changes in fire frequency, large areas that were once occupied by red pine have converted to other species (Chapeskie et al. 1989). Following fires, red pine must re-establish by seeding from trees that have escaped fire (Horton and Bedell 1960; Van Wagner 1971; Ahlgren 1976). Red pine is well adapted to surface fires of light to moderate density, but in the boreal forest the fire regime is dominated by crown fires or

high-intensity surface fires (Van Wagner 1971; Bergeron and Brisson 1990). The fire regime in the boreal forest favors black spruce, Picea mariana (Mill.) BSP and jack pine.

Jack pine, the prodigal son of Ontario forestry (Galloway 1986) has clearly captured the hearts and reforestation minds of foresters (Smith and Brown 1984). Utilization and reforestation of this species has increased dramatically since World War II (Moore 1984). The silvics of jack pine require a type of harvesting that creates an after-effect similar to fire; therefore, jack pine is managed exclusively under the clear-cut system (Galloway 1986). Forest managers tend to plant jack pine after site preparation especially on better sites (Smith and Brown 1984), often as single species plantations.

Clear-cutting of the boreal forest followed by planting of jack pine has probably led to an increase in the incidence of jack pine feeding by C. resinosa. Characteristically, C. resinosa is found in young trees, often in plantations (Herdy and Thomas 1961; McPherson et al. 1970a; Hall and Wilson 1974), and seldom on mature trees (personal observations). My personal observations in northern Ontario of jack pine plantations infested with C. resinosa indicate that almost all of these areas formerly had a small component of red pine. Typically, red pine, which would be a minor component of the forest, would not

be replaced on its former site because it would not be available as nursery stock for the site region, and because the financial and technical capability for its natural replacement (i.e. seed tree method) in the Boreal Forest was lacking (F. Pinto, Ontario Ministry of Natural Resources, North Bay, pers. comm.). Most forest management decisions are made by the unit forester, and published evidence of small-scale conversions of red to jack pine in forest management plans, or elsewhere, would be incomplete or absent (F. Pinto, pers. comm.). Cone beetles left at these cut-over sites face three response options or 'decisions': 1) migrate to find other red pines, 2) die, or 3) feed on the new hosts on the site. It may be adaptive for C. resinosae to 'choose' to attack jack pine shoots rather than to search unsuccessfully for red pine cones (a superior host) because total reproductive success is likely to be higher (Roitberg 1990).

The red pine cone beetle may have used infestation of jack pine as a strategy to survive natural catastrophic disturbances in the forest before the arrival of commercial logging. No doubt, the awareness of jack pine shoot attack is a result of the substantial increase in forest insect and disease surveys conducted after World War II. However, at the same time an increase in the incidence of jack pine shoot attacks could have been caused by an increase in artificial forest regeneration. Jack pine silviculture tends to do two things differently than natural

forest fires to favour populations of C. resinosae on jack pine: 1) silviculture would not decimate cone beetle populations, whereas fires would likely destroy most, but not necessarily all, of the population (less than 10% of the jack pine sites are prepared by prescribed burning (Galloway 1986)), and 2) silviculture provides cone beetles with only one host (jack pine), whereas fires in red pine-jack pine forests can leave unburned areas of red pine (Bergeron and Brisson 1990) and thus some refuge for cone beetles to feed on red pine cones.

Resource availability and suitability may explain why C. resinosae typically attack jack pine shoots and seldom attack jack pine cones. Attacks by C. resinosae on jack pine are almost always found on young trees (Herdy and Thomas 1960; McPherson et al 1970a, 1970b; Hall and Wilson 1974). Even though jack pine has the biological potential of cone production before 10 years of age (Rudolf and Yeatman 1982), clearly the most abundant resource available to populations of C. resinosae is the vegetative shoots. Cone attacks on immature and mature jack pine seldom occur, as extensive studies by de Groot (1986b), de Groot and Fleming (unpublished manuscript), Rauf et al. (1985), and surveys by FIDS, have shown. It is quite possible that the chemical and physical structure of jack pine cones makes them unsuitable for Conophthorus. Further study should be directed at the nutritional ecology and fitness of Conophthorus on jack pine cones. Such a

study may not only uncover why jack pine cones are seldom attacked, but may also lead to a further understanding why cones of southern pines are also not attacked by cone beetles.

**Why hasn't C. resinosa attacked jack pine
outside the range of red pine?**

The confined geographical distribution of attacks by C. resinosa on jack pine suggests at least three hypotheses: C. resinosa may occur west of Ontario, but has simply been overlooked, 2) the distribution of C. resinosa is coincident with the botanical and ecological features of jack pine in the northern range of red pine, or 3) jack pine may be unsuitable to sustain populations of C. resinosa. The first two possibilities have been discussed in Chapter 3. The third hypothesis appears to be valid.

The response of C. resinosa to oviposit in jack pine shoots, is in some ways analogous to superparasitism when hosts are rare, and when the probability of progeny surviving is greater than zero (Charnov and Skinner 1985; Roitberg 1990). That is, in some situations, it is adaptive for C. resinosa to 'choose' to attack an inferior host such as red or jack pine shoots rather than to search unsuccessfully for red pine cones (superior host) because total reproductive success is likely to be higher (Roitberg

1990).

Red pine shoots seem to be an inferior resource to C. resinosa, as Mattson (1980) noted few beetle progeny were produced in them. Although C. resinosa reproduces in jack pine (Chapter 2), jack pine shoots may also be an inferior resource over the long-term. My personal observations (1986 to 1990) of C. resinosa on jack pine shoots indicate that populations are seldom maintained at a site for more than 4-5 years. Conversely, C. resinosa attacks persist considerably longer (Lyons 1956; Mattson 1978), and stable populations may remain in an area, because C. resinosa regulates cone production (Mattson 1978). If jack pine shoots are unable to sustain a population of C. resinosa, then it follows that C. resinosa will not persist in areas of jack pine where cone-producing red pine are not present. During the course of the life history study (Chapter 2) major causes of beetle mortality were not evident. Just what factors lead to the decline of C. resinosa populations in jack pine is not known. Host-related adverse effects on life history traits have been widely studied for a number of insects. The most readily apparent effect of jack pine shoots on C. resinosa is the reduction in body size (Herdy and Thomas 1960; McPherson et al. 1970a; Wood 1982), which may be correlated to adult fecundity and progeny fitness. Undoubtedly, additional studies on various fitness parameters, such as adult fecundity and survival, and

larval survival, are required to determine the suitability of jack pine shoots for the long-term survival of C. resinosa.

Could C. resinosa on jack pine take a separate evolutionary path from C. resinosa on red pine because of human intervention in the forest ecosystem?

An imaginable path for speciation of Conophthorus on jack pine would be through sympatric speciation via host race formation. Sympatric speciation is not a new concept (Bush 1975), but it has remained controversial (Futuyma and Mayer 1980; Diehl and Bush 1984). Before sympatric speciation could occur, several stringent criteria are required to be met (Futuyma and Mayer 1980; Jaenike 1981; Kondrashov and Mina 1986). If there is to be speciation via host race formation, there must be evolution of genes both for host preference (opportunities for assortative mating) and for performance on the new host (Felsenstein 1981). Moreover, opportunities for the sympatric evolution of host races do not necessarily lead to reproductive isolation. Although host races are partially reproductively isolated from other conspecific populations as a direct consequence of adaptation to a specific host, host races seldom, if ever, rely on host preference alone as a basis for their differentiation (Diehl and Bush 1984). Many selective forces simultaneously act on each host

race (Diehl and Bush 1984).

It appears doubtful that silvicultural manipulation of a forest ecosystem would be a powerful selective force that would encourage biotype formation in C. resinosa. Considering the long association of red pine and jack pine, the periodic natural ecological disturbances of the forests that have 'forced' C. resinosa to attack jack pine shoots, and the apparent unsuitability of jack pine shoots to sustain populations of C. resinosa, it appears unlikely that host races would develop. Furthermore considering the current trend of diminishing the association of red pine and jack pine in forest ecosystems in the Boreal Forest Region, the 'opportunities' for C. resinosa to switch to jack pine will decrease over time. Fortunately, the reduction of red pine is limited, as red pine is a widely planted tree in the Great Lakes-St. Lawrence forest and artificial planting has extended its botanical range further south.

Much remains to be discovered about cone beetles and their host associations, but unquestionably members of the genus Conophthorus, although difficult to distinguish and plain to the eye, have been a delight to examine.

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