EGG PIGMENTATION,

A NEW CRITERION FOR USE IN

DIPRIONID SAWFLY TAXONOMY

by

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Claim of Original Work

Differences in egg colour among European sawfly species were known for many years, but no use of the information in basic taxonomic work was attempted, and there was little application in practical keys or identification tables. The egg colours of North American sawflies were unstudied, except for an occasional, casual reference to the colour in an individual species.

The work reported in this thesis represents the first attempt to study sawfly egg pigmentation in an objective manner, and to apply the findings to taxonomic research, as well as to provide data for use in identification aids. The analytical methods were adapted from other fields, but it was necessary to carry out tests to make certain that confidence could be placed in the results, because the conditions in the present study were not identical to those in the work from which the methods were derived.

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I. ACKNOWLEDGEMENTS

The work reported in this thesis was commenced as a sub-project within the Ontario regional section of the Forest Insect Survey, and has developed into a full-time, independent laboratory project. Contact has been maintained with the Survey through checking all identifications of immature stages of Neodiprion sawflies. Data from Survey files have been used in the preparation of this manuscript. I wish to thank M. L. Prebble and B. M. McGugan, Branch Headquarters, Ottawa, for permission to use departmental information.

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II. INTRODUCTION

Entomologists working in the northern hemisphere soon recognize the actual or potential threat that the conifer sawflies pose to successful management of coniferous forests or plantations. The bionomics of many species are being investigated, yet frequently the taxonomic status of the populations under investigation is not known with certainty, nor can be determined with the information currently available.

The history of taxonomic work on what are now known as the diprionid sawflies dates from the time of Linnaeus, and most European species were described by the end of the 19th century. In addition, a considerable volume of biological work was published in the same period, and thus a fairly stable specific nomenclature had been achieved by the time that Enslin, 1912 to 1917, published his treatise on the central European tenthredinoids.

By contrast, the majority of North American diprionids were not described until after the year 1900, mostly prior to 1935. The early descriptions of North American species and even most of those published since 1900 are very general and not illustrated or accompanied by good biological information. In most instances only a few specimens were examined and males, particularly in the genus Neodiprion, were ignored. Recently there has been a strong tendency to rely heavily upon characteristics of the female genitalia, although Atwood and Peck (1943) showed very clearly the benefits to be gained from a broader biological basis for species recognition of Neodiprion sawflies.

The generic classification in use today has been developed by Rohwer (1918), Enslin (1917), Ross (1937), and Benson (1939, 1945, 1954), while the most recent general revision of the genus Neodiprion is that of Ross (1955) in which he specifically states that his paper points out the need for much more information, particularly of the sort utilized by Atwood and Peck. These authors showed that "if adult characters, larval characters, methods of oviposition, food plant and life histories were known, it was possible to delimit biological units which could be identified" (Atwood, 1961). Smith (1941) was able to use cytological evidence to great advantage in the separation of Gilpinia polytoma (Hartig) and G. hercyniae (Hartig), but the cytology of Neodiprion sawflies is largely unrewarding (S. G. Smith, personal communication). Similarly, parthenogenetic behaviour appears to be uniform throughout the group. Neither the work on internal anatomy of Neodiprion larvae (described as "monotonously similar") nor the cytological work of Maxwell (1955, 1958) show particular promise for taxonomic purposes. Differences in larval rectal tooth patterns referred to by Maxwell (1955) have not been investigated. A number of statements made by Maxwell (1958) appear to be in error and caution must be exercised in applying her findings.

The preliminary report by West et al. (1959) on serological and chromatographic studies of Neodiprion sawflies suggests that these techniques could provide very useful information, and a study by Whittaker and West (1962) on starch gel electrophoresis of hemolymph proteins indicates that this approach could be valuable for investigating infraspecific variation. Griffiths (1953) carried out a quantitative

anatomical study on female adults of five Neodiprion species and found that his samples represented five different statistical populations corresponding to the five species. He also showed, however, that no one of his 30 sets of measurements or 55 sets of ratios would by itself separate all five populations. The quantitative approach to both adult and larval anatomy is one requiring further investigation as it is quite possible that combinations of measurements in discriminant functions might provide a rapid and certain method of identifying museum specimens, and a thorough study of many species would undoubtedly add to our understanding of relationships in the group. Work on behaviour, exemplified by the papers on oviposition patterns by Ghent (1955, 1959), Ghent and Wallace (1958), and Griffiths (1960), also contributes significantly to our appreciation of phylogeny and host selection in addition to assisting in the identification of egg clusters.

The results reported in this paper represent a portion of the author's contribution to a joint project initiated to resolve the taxonomic problems in the genus Neodiprion. In approach, this project is not limited geographically nor restricted with respect to the type of data utilized. We are attempting an integrated study of the group as living organisms, and early in the course of the work it became evident that an extensive collecting program, allowing immediate observation of geographic variation in readily observable characteristics, is one of the most vital parts of the whole schedule. The availability of living material in all stages is important, permitting the accumulation of data on many otherwise neglected characteristics.

Pigmentation, other than that produced by melanin and sclerotin, usually is altered by preservatives and even by drying, and as a consequence cannot be studied with confidence except in living specimens.

During the course of genetic studies, my collegue I. M. Campbell noted that in some instances sister females of Neodiprion swainei Middleton had green and yellow abdominal venters, respectively (Fig. 1) (For convenience, all the coloured illustrations have been grouped as Figs. 1-32 on three pages in Section X). This colour difference was found to be caused by the masses of eggs in the abdomen. Preliminary observations on a few common species showed great promise, and Forsius (1920) had described a wide range of colours in the eggs of 124 species of European sawflies. It is noteworthy, however, that the differences found by Forsius aroused no interest in this character among taxonomists for forty years. The reasons for this are likely that living material was seldom available for study, and also that it was difficult to produce meaningful colour descriptions, although colour differences associated with adult external anatomy were not avoided. The technical problems of objective colour designation became evident at the beginning of the present study on egg pigmentation, and considerable time was spent in developing and testing methods.

The sections that follow deal in turn with a brief general statement on the family Diprionidae, an outline of the chemical basis of egg pigmentation, methods of spectrophotometric examination and spectral curve analysis, methods testing, results of spectral analyses, and a discussion of the method as applied to taxonomic work.

III. GENERAL SYSTEMATICS, HOST ASSOCIATIONS, AND DISTRIBUTION OF THE DIPRIONIDAE

The tenthredinoid family Diprionidae is comprised of 11 genera, totalling about 70 species. Two subfamilies are recognized: the Monocteninae, containing the genera Monoctenus, Augomonoctenus, and Rhipidoctenus; and the Diprioninae, embracing the genera Diprion, Gilpinia, Macrodiprion, Microdiprion, Neodiprion, Nesodiprion, Zadiprion, and Prionomeion.

The genera Augomonoctenus, Rhipidoctenus, Macrodiprion, and Prionomeion are monotypic while only the genera Gilpinia and Neodiprion contain more than ten species, the latter genus accounting for about half the species in the family.

Diprionid sawflies are attached to the Coniferales, and all except Augomonoctenus libocedrii Rohwer, which feeds on cones of Libocedrus decurrens Torrey, defoliate the host trees through larval feeding. Members of the subfamily Monocteninae feed on trees of the families Cupressaceae and Taxodiaceae, while species of the subfamily Diprioninae attack trees belonging to the Pinaceae.

Diprionid sawflies are distributed throughout the coniferous forests of the northern hemisphere, mainly in the Nearctic and Palaearctic Faunal Regions, but also in the Oriental Region and in the Neotropical Region in Mexico and Central America. Although trees of the families Cupressaceae and Taxodiaceae occur in both the Neotropical and Australian Faunal Regions, no Monoctenine sawflies have been found in these areas. Members of the genera Augomonoctenus and Zadiprion are entirely Nearctic

in distribution; Monoctenus and Neodiprion are Holarctic, but the latter genus is represented by only a single species in the Palaearctic Region, being replaced there for the most part by species of Gilpinia. All the other genera are Palaearctic except for four species, Diprion simile (Hartig), Gilpinia hercyniae (Hartig), G. frutetorum (Fabricius), and Neodiprion sertifer (Geoffroy), that have been accidently introduced into North America, probably since 1900. A fifth Palaearctic species, Nesodiprion japonica (Marlatt), was reared from pine imported to the United States from Japan at San Francisco in 1902 (Rohwer, 1911), but apparently the species failed to become established.

IV. THE CHEMICAL BASIS OF EGG COLOUR

The physical and chemical bases of animal colours have been discussed by Fox (1953) and Fox and Vevers (1960), and the pigments found in insects in particular have been treated by Cromartie (1959) and Thomson (1960). Therefore no literature review is included in this section. The objectives are to illustrate in general the types of compounds responsible for the colours of diprionid sawfly eggs, to determine the taxonomic significance of this more purely chemical approach, and to examine the data for evidence of the biological significance of the pigments.

A. METHODS

The components of egg pigmentation were studied by centrifugation in two manners:

1. Ovaries were removed from newly-emerged females and placed in 0.15M sodium chloride solution in a centrifuge tube that had been cooled in a freezer. The head of a Servall Superspeed angle centrifuge was thoroughly cooled in a freezer before use, and centrifugation of the eggs in saline was carried out for 20 minutes at a relative centrifugal force of 20,000xG.

2. Since a microspectrophotometer was not available for examining the layers formed by centrifuging intact eggs, an alternate method was used to obtain material for spectrophotometric examination. Masses of eggs were removed from freshly-emerged females and crushed in a tissue grinder. The resulting viscous material was diluted with 0.15M

saline and centrifuged, allowing separation of the burst chorions and layers of materials analogous to those observed in centrifuged, intact eggs. The chorions were washed in saline and their attenuance spectrum obtained by the opal glass method (Shibata, 1959). Spectra of two additional components were obtained in a similar fashion.

In addition to the centrifugation experiments, extracts of egg pigments in several species were made in order to examine the components in a more purified form. Most of the extraction procedures were patterned after those of Hackman (1952). Eggs from up to 300 females, depending on availability, were homogenized in an all-glass tissue grinder and the homogenate diluted slightly with water for handling ease. Extraction was carried out several times on each sample with a mixture of ethyl alcohol, 1 part, and diethyl ether, 2 parts, made acid to litmus with hydrochloric acid. The ethereal layers were collected, pooled, and extracted with aqueous sodium bicarbonate, yielding two fractions, one ethereal and the other aqueous.

The ethereal solution was dried over sodium sulphate and evaporated under vacuum at room temperature. The oily residue was dissolved in petroleum ether (B. R. 40-60°C) and saponified with 12 per cent by volume of saturated methanolic potassium hydroxide under an inert atmosphere at room temperature with agitation. The mixture after saponification was partitioned between petroleum ether and 90 per cent aqueous methanol. In no case was any pigment transferred to the methanol and this layer was discarded. The petroleum ether extract was washed with water, dried, concentrated, and used for study without additional purification.

The aqueous sodium bicarbonate extract was acidified with acetic acid and the pigments transferred to diethyl ether. This solution was washed, dried, concentrated, and used for study without additional purification.

A crude ethereal extract of Neodiprion swainei eggs was chromatogrammed on aluminium oxide-containing paper (Sleicher and Schüll #667, an experimental batch only) according to the method of Jensen (1959, 1960), and the chromatograms examined under ultraviolet light.

Batches of eggs of N. swainei and N. virginianus complex were burst in a tissue grinder and extracted with 0.1N sodium bicarbonate. Proteinaceous material in the extracts was precipitated by saturation with ammonium sulphate. The precipitate was collected and fractionated by "salting in" with graded concentrations of ammonium sulphate, this process being repeated three times. Selected fractions were dissolved in 0.5M phosphate buffer, pH 7, and dialyzed against buffer for three days in the cold. These solutions were used for testing.

The majority of spectrophotometric tests carried out in conjunction with the chemical studies employed a Beckman DU spectrophotometer, calibrated in wavelength. A few spectra were run on the Unicam SP. 700 recording spectrophotometer, calibrated in wavenumber. The abscissae in illustrations of spectra are presented in linear wavenumber and in wavelength.

B. RESULTS

1. CENTRIFUGATION STUDIES

Centrifugation of sawfly eggs under the conditions outlined resulted in the production of three distinct bands, the central one consisting of clear liquid in all types of eggs (Figs. 9, 10), and varying in size with the efficiency of the treatment. Figure 9 shows a centrifuged egg of N. sertifer, which when intact is pale, brownish purple in colour (Fig. 27). The smaller and more acute end of the crescent-shaped egg appeared as a whitish cap consisting of fatty-appearing globules under microscopic examination. Behaviour in the centrifuge also indicated that this cap consisted of light materials. Below the narrow, central clear zone the egg was filled with purplish-red material that appeared to be relatively heavy. When the bright yellow-green eggs of Gilpinia hercyniae (Fig. 16) were centrifuged, the cap of light materials shown in Fig. 10 was intense orange-yellow, while the heavy materials were green in colour. When the blue eggs of Diprion simile (Fig. 13) were centrifuged, the cap materials were pale, creamy white and the heavy materials were deep blue. The eggs of several species of diprionids of different intensities of yellow in colour were centrifuged, and the fatty caps showed yellow colours, the intensity of which were correlated with the intensity of yellow in the untreated eggs. The larger dense layer was pale yellow in all instances. These results suggest that the basic egg colour is pale yellow and that all other colours result from different combinations of two additional

classes of substances, one consisting of yellow pigments associated with the fatty constituents of the eggs, and the second comprising variously coloured pigments involving a heavy cell component such as protein.

When masses of N. sertifer eggs were crushed, diluted with saline, and centrifuged, clean chorions and two additional fractions were obtained. The bottom of the tube contained a purple sediment while a yellow, fatty layer remained floating on top of the saline. These materials seemed similar to the two main components found when intact eggs were centrifuged. The semi-integral attenuance spectra of the purple sediment, the yellow, fatty layer and the chorions are shown in Fig. 33a, b, and c, respectively. Figure 33d illustrates an attempt to reconstruct the semi-integral attenuance spectrum of intact N. sertifer eggs from the spectra of the three components separated by centrifugation. It was necessary to use empirically selected multiples of the individual attenuance spectra since the concentrations of the materials used in obtaining the spectra bore no specific relationship to the concentrations in the intact eggs. It is evident that the reconstructed spectrum is very similar to that of normal, intact eggs in all major features. There is some deviation in the region 28 to 20 kc/cm and this may be attributable to water soluble substances removed in the preparatory procedures. These findings support the two main-component concept for egg colours differing from the pale yellow basic type. The attenuance spectrum for chorions (Fig. 33c) shows that these structures add little or nothing to the colour complex of the eggs. The spectrum of the yellow layer (Fig. 33b) shows a triple-

banded attenuance maximum in the region 24 to 20 kc/cm (415-500mμ). The colour, the association with lipoidal substances, and the spectrum suggest that the pigment is carotenoid in nature. The spectrum of the purple sediment (Fig. 33a) offers little help in identification of the pigment.

2. EXTRACTION STUDIES

The crude ethereal extracts obtained by the method outlined were yellow when yellow eggs were the source material, and bright yellow-green to greenish blue in all cases where the intact eggs were purple, blue, or green. It was found that the yellow pigment can be extracted with neutral fat solvents such as diethyl ether and petroleum ether, but that the other pigments require the use of hydrochloric acid for their removal and that they are altered in the course of extraction. In the case of D. simile, having blue eggs with a very small amount of fat soluble yellow pigment, the crude extract was bluish. Extraction of the initial ethereal solution with aqueous sodium bicarbonate yielded a yellow ethereal phase and an aqueous phase that was green if the eggs were purple, blue, or green, or colourless if the eggs were yellow.

Residue from evaporation of the yellow ethereal solution gave a strong positive Carr-Price reaction for carotenoids and a negative Gmelin reaction for bile pigments. All the carotenoid pigments were epiphasic when partitioned between petroleum ether and 90 per cent aqueous methanol after saponification, indicating that they were carotenes (i.e., they did not contain hydroxyl groups).

The visible absorption spectra of the carotenoids from seven different sources were obtained and compared with that of a β-carotene sample that had been run through the extraction and separation procedures. The absorption spectra of carotenoids from Neodiprion nanulus nanulus Schedl and Gilpinia hercyniae are compared with that of β-carotene in Fig. 34, and Table I presents a summary of the absorption characteristics of all samples examined. It will be noted that the yellow pigments from N. nanulus, N. abietis complex, N. sertifer, N. swainei, and D. simile are all markedly similar and very much like β-carotene. The wavelengths of maximum absorption, however, show a slight hypsochromic shift from the accepted positions for β-carotene in n-hexane and a more pronounced shift in chloroform and carbon disulphide. It is also evident that the fine structure is not as well developed as in the spectrum for all-trans β-carotene shown by Zechmeister (1962), and that the chromaticity decreased with successive solvent transfers. These are characteristic of the changes involved when the all-trans form is converted to a cis-trans isomerization mixture and it is likely that such a conversion was responsible for the observed discrepancies between my samples and authentic all-trans β-carotene. The blue colour developed in the Carr-Price reaction with a nanulus sample had its wavelength maximum at the same position as obtained with a sample of β-carotene that had been run through the extraction and separation procedures. The chromatograms of crude N. swainei extract showed only a single band of carotene fluorescence under ultraviolet light. It seems safe to conclude that the carotenoid in N. nanulus, N. abietis, N. sertifer, N. swainei, and D. simile is largely

Table I. Absorption characteristics of carotenoids from different sources in three solvents.

Source		Solvent: n-Hexane	Chloroform	Carbon disulphide
β-carotene	Lit.	452,480[1] mμ	466,497[2] mμ	485,520[2] mμ
	Sample	451.5, 478	464,488	483,511
Neodiprion nanulus		450,476	462,485	482,508
Neodiprion abietis		451,477	---	---
Neodiprion sertifer		450,477	---	---
Neodiprion swainei		450,477	---	---
Diprion simile		450,476	---	---
α-carotene	Lit.	445.5, 474[1]	454,485[2]	477,509[2]
Gilpinia hercyniae	a	447,474	456,485	476,505
	b	447,474	---	---
	c	447,475	---	---
Gilpinia frutetorum		447,475	---	---

1. Zechmeister (1962) 2. Karrer and Jucker (1950)

or entirely β-carotene, but more exacting tests should be carried out.

The absorption characteristics of the carotenoid pigment from G. hercyniae (Fig. 34, Table I), and G. frutetorum (Table I) differ from the foregoing and cannot readily be associated with any known carotene, although they are suggestive of α-carotene. It may be that a mixture of epiphasic carotenoids are present in the eggs of these two Gilpinia species. In any case it may be concluded that these two species differ distinctly in carotenoid transfer to the eggs from the Neodiprion and Diprion species that were tested. Apparent also is that the two types of carotenoid in the eggs bear no relationship to the species of tree upon which the sawfly larvae were fed, as at least one sawfly species with each carotenoid type fed on pine and spruce or fir, respectively.

In addition, the characteristics of the spectrum of Monoctenus sp. eggs (Fig. 35) in the carotenoid region are different from those for Neodiprion lecontei (Fitch) and N. pinetum (Norton), species with moderate-to-high and low carotenoid concentrations, respectively, assumed to be β-carotene on the basis of tests with the other species. The form of the curve suggests that the pigment in Monoctenus may be one having an absorption spectrum with a single maximum in the visible region. This suggestion is based on two spectral curves only and will require checking by extraction methods when sufficient material becomes available.

The green colour of the aqueous sodium bicarbonate phase changed to pale blue when the solution was acidified with acetic acid. The pigment in solution was transferred readily to diethyl ether at this

stage, and gave a blue solution. The substance responsible for the blue colour gave a negative Carr-Price reaction for carotenoids and a positive Gmelin reaction for bile pigments. The negative diazo reaction and the spectra of this pigment in ether and methanol - 5% hydrochloric acid (Fig. 36) indicate that the substance is a bilatriene compound similar to biliverdin or mesobiliverdin. The bile pigment extracted from all purple, blue, or green eggs appears to be identical, but the exact identity of the bilatriene pigment has not been determined because of limited material of questionable purity and because of considerable conflict in the literature as to the spectral and other properties of biliverdin and mesobiliverdin. Gray (1962) has shown that other than the IXα isomer of biliverdin is produced in the in vitro conversion of haemin protoporphyrin to biliverdin, as opposed to the production of only the IXα isomer during natural haem catabolism. Perhaps the difference in bilatrienes found in non-haem sources may result from isomeric complexity.

The extracts with sodium bicarbonate indicated that there are some water soluble, non-proteinaceous, yellow chromogens in the eggs of N. swainei and N. virginianus. Proteinaceous materials precipitated from the bicarbonate solutions with ammonium sulphate were yellow when the yellow eggs of N. swainei were the source, green in the case of green eggs from N. swainei, and greyish-purple from the eggs of virginianus (similar to N. sertifer used in the centrifugation studies). The green and purple chromoproteins were soluble in 60% saturated ammonium sulphate, but precipitated at higher concentrations. A pale yellow protein was removed from the extracts of yellow eggs of N. swainei at the same con-

centration. No ether extracts of bile pigment from the green and purple precipitates from swainei and virginianus, respectively, were obtained until the materials were treated with acetic acid. The yellow precipitate from N. swainei yellow eggs did not yield bile pigment even after acidification. The pigments in phosphate buffer were found to be non-dialyzable. The absorption spectra of the green and purple materials are illustrated in Fig. 37. These two spectra are very much like the spectra for intact eggs of their respective species (Fig. 54a, Fig. 62a), with the exception of lacking carotenoid absorption characteristics. The spectrum of the extract from yellow eggs was similar to the two illustrated save for having a less pronounced "shoulder" in the 30-26 kc/cm region and lacking a maximum between 18 and 14 kc/cm. The spectrum levelled in this latter region.

If an opal slide bearing eggs of species such as N. sertifer and N. virginianus, both containing a purple component, is exposed to live steam for 30 seconds, the purple colour is lost and the eggs become bright yellow-green in colour (Fig. 11). The change in attenuance spectrum is shown in Fig. 38 and suggests that the bile compound has been set free by the heat treatment.

C. DISCUSSION

The foregoing results have been interpreted as follows: The basic egg colour among diprionid sawflies is pale yellow resulting from the presence of many weak chromogens. More intense yellows result from the presence of carotenoid pigments in lipid solution. Hues other than

yellows involve bile chromoproteins, and purple, blue, and green compounds of this type have been found. The prosthetic substance in all three pigments is apparently the same bilatriene compound, and the colour differences are the result of differences in protein bonding. In a later section it will be shown that the green bile chromoprotein in closely related taxa may have slightly different but specific spectral characteristics.

Although the carotenoid content of the eggs of some species is very low, no species has been found with eggs devoid of carotenoid. Neither the type of carotenoid nor its concentration is related to the species of host tree, at least in the Diprioninae. On the basis of the literature it is virtually certain that the carotenoid compounds incorporated in various tissues, including eggs, are plant pigments and not synthesized by the insect, although alterations may occur in the course of metabolism. It is apparent that the sawflies have very effective "filter" mechanisms that determine the type and amount of carotenoid incorporated in either somatic or reproductive tissues. The results with the two Gilpinia species suggest that the type of carotenoid in the eggs may be a useful group characteristic among the conifer sawflies.

The bile chromoproteins are probably completely synthesized by the sawflies. They are less variable within taxa and more highly diversified between taxa than the carotenoids. The results suggest that an investigation of egg proteins might yield valuable taxonomic information.

The physiological significance of the carotenoid and bile chromoprotein pigments in sawfly eggs is unknown. There are no obvious

correlations between pigmentation and any readily observable biological functions. The work of Dadd (1961) was the first to demonstrate the need for dietary carotenoid in any insect. His studies showed the importance of egg carotenoid in Locusta migratoria Linnaeus. When hatchlings from eggs deficient in carotene were reared under crowded conditions on an artificial diet lacking carotene, growth was inferior, normal colouration, including melanization, was not developed in either hoppers or adults, and activity was lessened. The viability of carotene-deficient eggs was low also. Perhaps even more significant than these findings, is the recently reported work by Goldsmith and Warner (1964), showing that vitamin A along with retinene participates in the visual processes of the honey bee. They also indicate that preliminary results show that house flies reared on carotenoid-deficient diets are less sensitive to light than normal. The development of suitable artificial or synthetic media for rearing conifer sawflies would permit very useful studies on pigment metabolism.

V. METHODS OF SPECTROPHOTOMETRIC EXAMINATION AND SPECTRAL CURVE ANALYSIS

When it became apparent that even small differences in egg colour might be important taxonomic indicators, it also became obvious that objective colour descriptions would be essential. Initially, attempts were made to obtain ratings by comparisons with standard colour charts in the Munsell Book of Colour (Anonymous, 1942). This approach proved difficult and of limited value because the ratings were still subjective, although they were much superior to arbitrary descriptions. In view of work such as that of Dupraw (1958) on the analysis of egg colour variation in Cyclops vernalis Fischer, a spectrophotometric method of assessment appeared feasible and desirable in that leads to the nature of the pigments would be obtained also. Dupraw obtained absorption spectra of individual eggs with a Pollister-Moses rotating plate type microspectrophotometer and oil immersion. The only instrument immediately available to me, however, was a Beckman DU and a method utilizing this instrument was developed. Several hundred spectra were obtained with the Beckman and the results were good enough to warrant the purchase of a Unicam SP. 700 recording spectrophotometer. The versatility of the latter instrument made it a relatively simple matter to carry out tests proving the reliability of the methods that had been accepted previously without rigorous experimental evidence. The results of these tests are discussed in the section following the outline of actual procedures.

A. PREPARATION OF MATERIAL

The full egg complement was removed from a naturally-emerged female by cutting off the tip of the abdomen just anterior to the genitalia, taking care not to damage the adult unnecessarily. Both portions of the female were retained for anatomical study and as voucher specimens for the spectral data. The mass of eggs was placed in a shallow, open-ended trough on an opal slide (Fig. 39) prepared in the following manner. A rectangular section 40 mm. x 12 mm. was cut from commercial opal glass about 3.4 mm. thick, consisting of a 0.4 mm. opal layer cemented to a clear back 3 mm. thick. Two 20 mm. x 2 mm. pieces of No. 0 cover glass (0.085-0.13 mm. thick) were cemented to the opal surface, spaced about 3 mm. apart, and in a position such that when the opal slide was inserted vertically in the cuvette carrier positioned near the focus of the beam (beam size 10 mm. x 2 mm. in the Unicam SP. 700, the beam passed through the center of the 20 mm. x 3 mm. area between the rectangles of cover glass. The eggs placed on the opal slide were covered with 0.15M sodium chloride and separated from one another using fine forceps. Unwanted materials such as ovariole tissue, immature eggs, and portions of the gut, were removed and the eggs were washed with the saline, employing filter paper to remove the wash solution. The eggs were arranged in a single layer filling the beam area of the trough. A slip of No. 0 cover glass was placed over the layer of eggs and then saline was run in under the cover, filling the voids between eggs by capillary action. Excess saline was drawn off with a piece of filter paper to the extent that the cover glass was drawn down tightly over the eggs, but the space under the cover around the eggs was still completely saline filled. The edges of the cover were sealed with

rubber cement, taking care not to smear the beam area. Drying of the rubber cement tended to draw the cover slip even more tightly over the eggs, flattening them slightly and effectively locking them in position. The slide was placed in the sample carrier of the spectrophotometer, hard cork blocks being used to ensure stability and uniform placement in the carrier.

A saline-filled reference slide was prepared similarly with the eggs omitted.

B. INSTRUMENT CONDITIONS

1. BECKMAN DU MANUAL SPECTROPHOTOMETER WITH PHOTOMULTIPLIER

Optical density readings were taken at 10 mμ intervals over the range 360 to 700 mμ (27.8 to 14.3 kc/cm), utilizing the tungsten source and a constant slit width of 0.78 mm. The beam size was cut down by using a microcell slide adapter with its smallest aperture (0.46 mm. diameter) in position. High electrical gain was required in the near ultraviolet range, and at the red end of the visible spectrum. Each run took about three-quarters of an hour. Readings were taken at more closely spaced intervals in regions of band maxima.

2. UNICAM SP. 700 RECORDING SPECTROPHOTOMETER

Transmission spectra were recorded over the range 30 to 13 kc/cm (333.3 to 769.2 mμ), utilizing a scan speed of 2819 cm.$^{-1}$/min. (Speed 3) and a chart speed of 96 in./hr. This resulted in a running time of six minutes and a chart presentation of 1755 cm.$^{-1}$ per inch of record.

A low level of electrical gain (Resolution 2) and moderate damping (setting 2) were employed. The slit width was variable, automatically holding the reference energy level constant. No beam masking was employed.

C. ANALYSIS OF DATA

1. BECKMAN RECORDS

The semi-integral attenuance (optical density) readings were plotted on log-arithmetic graph paper for visual analysis. Very few of these records are included in this paper since they cannot be treated in a manner like that used for spectra obtained with the Unicam SP. 700.

2. TRANSMISSION SPECTRA MADE WITH THE UNICAM SP. 700

The analysis of these records followed one of two courses, depending upon the presence or absence of bile chromoprotein.

a. Bile Chromoprotein Present (Fig. 40a)

Positions of the distinctive maximum and minimum were taken directly from the transmission spectrum. Then the percentage of transmission was read at these two spectral positions, at 22kc/cm corresponding to the occurrence of the middle (or strongest) maximum of the vibrational fine structure of the carotenoid absorption band, and at 13 kc/cm where maximum transmission was recorded. Each of the four transmission values was corrected by an appropriate factor for differences in the opal slides used for the reference and sample, respectively (this is the same as correcting for unmatched cells). The corrected transmission

values were transformed to $\log_{10}$ reciprocal values graphically, using graphs constructed from the conversion in Table 6c - 3, American Institute of Physics Handbook (McCarthy et al., 1957). The four values were plotted on a single log-axis line of semi-logarithmic graph paper (1 log cycle equaled 5 in.). Considering the value for 13 kc/cm as base, the three heights, base to carotenoid (at 22 kc/cm), base to attenuance minimum, and base to bile chromoprotein maximum, were measured to the closest 0.05 cm. These three values are indicated in Fig. 40a by the bars marked a, b, and c, respectively. For illustrative purposes the entire spectral curve (30 to 13 kc/cm) of a complement of green eggs from Neodiprion swainei was read at 200 cm^{-1} intervals, corrected, transformed and plotted as the log semi-integral attenuance. The arithmetic values of a, b, and c were totalled and each value expressed as a percentage of the total (Fig. 40a). This procedure made it possible to express the shape of the spectral curve as a single point on a triangular co-ordinate graph (Mayr et al., 1953). By plotting points for spectra from different sources, a rapid check for homogeneity was obtained. It must be noted that the triangular co-ordinates found in this manner are comparable in a strict sense only when the spectral positions of b and c were the same in the spectra being compared.

b. Bile Chromoprotein Absent (Fig. 40b)

The "base curve" was interpolated as a straight line under the shoulder produced by carotenoid absorption in the region 25 - 18 kc/cm on the original transmission records. Transmission values were read from the curve at 22 kc/cm, on the interpolated base at 22 kc/cm, at 17 kc/cm, 15 kc/cm, and at 13 kc/cm. These readings were corrected, transformed,

and plotted as described in the foregoing section. Measurements a, b, and c were obtained as indicated in Fig. 40b, which illustrates the complete spectral curve for a complement of orange-yellow eggs from Neodiprion lecontei. These measurements were converted to percentages and plotted as triangular co-ordinates (Fig. 40b).

In cases where intraspecific differences in egg colour were found, the three spectral positions used for analysis in the bile chromoprotein devoid or reduced types were the ones determined for eggs of the type with a strong bile chromoprotein attenuance maximum.

D. PHOTOGRAPHIC RECORDS

A photographic record of egg colour was maintained in co-operation with D. C. Anderson, photographer at the Forest Insect Laboratory. The general features of his equipment and procedures were outlined previously (Anderson, 1957). Eggs to be photographed were dissected from freshly-emerged females, placed in a shallow layer of 0.15M sodium chloride solution in a syracuse watch glass, separated, and spread into a single-layered group. Except for earlier photographs, the colour transparencies were made at 2x magnification using Kodachrome II film and a black background. An early series of transparencies was made using a blue background, but this proved unsatisfactory. All those with blue backgrounds and some of the earlier ones with black backgrounds were made using Kodachrome I film. Fortunately, it was possible to retake most of the series on Kodachrome II film. In addition to the egg photographs, transparencies of mature, feeding larvae and adults of most species and their variants were made.

VI. METHODS TESTING RESULTS AND DISCUSSION

A. SPECTROPHOTOMETRY OF TRANSLUCENT MATERIALS

Plant physiologists have been concerned for many years with the *in vivo* absorption spectra of pigments in plant materials as they relate to energy transfer in photosynthesis. Animal physiologists have had an analogous interest in the visual and respiratory pigments. The problem of obtaining accurate spectra is not a simple one, since intact biological materials are seldom transparent, and direct measurement using conventional spectrophotometric instruments and techniques yield completely unsatisfactory, or at best badly distorted, results. The difficulties are compounded by conflicting evidence from extracts of pigments and *in vivo* measurements because it has become evident that changes in the environment of pigments produced by extraction may alter the absorption spectra.

For example, pigment extracts from the unicellular alga *Chlorella*, containing carotenoids, chlorophyll *a*, and chlorophyll *b*, in organic solvents such as ether and acetone have the chlorophyll *a* red maximum at about 662 mμ, while in measurements on suspensions of intact *Chlorella* cells made by Latimer (1958), using conventional methods, the band position varied from 683 to 691 mμ. He found the band at the longest wavelengths in suspensions of cells in the rapid growth phase. Latimer (1959, cited in Charney and Brackett (1961)), following the work of Barer (1955), found that the absorption peak of suspensions in bovine serum albumin solutions with refractive indices from 1.33 to 1.42 varied from about 685 (689 mμ extrapolated to zero con-

centration) to 668 mμ. Using an Ulbricht or integrating sphere, Charney and Brackett (op. cit.) determined that in Chlorella the in vivo low-frequency absorption of chlorophyll a is at 674 ± 1 mμ. Latimer (op. cit.), employing the opal glass technique of Shibata et al. (1954), consistently found the band to be at 675 mμ despite differences in the growth phase of the cells in the suspension.

In another instance involving plant material, Latimer and Eubanks (1962) located the chlorophyll a low-frequency band in a suspension of spinach chloroplasts at 682 mμ. Using the opal method, the band was found at 679 mμ. In 1958, Latimer (op. cit.) reported that spinach chloroplast (average diameter 5 - 6 mμ) spectra showed changes like those found with Chlorella suspensions when the opal glass was introduced into the system, but that a "clear green 'suspension' of small fragments" of spinach chloroplasts behaved like an extract of plant pigments in that the chlorophyll bands were identical in shape and position with or without the opal insert. Sauer and Calvin (1962) demonstrated the very slight scattering component in the red end of the spectrum of suspensions of spinach chloroplast subunits called quantasomes (lamellar structures, in this case probably less than 1000 Å in diameter and about 160 Å thick. Sauer and Calvin (op. cit.) carried out two sonications on the material used for the spectra, so the subunits were probably in the lowest part of the size range found by Park and Pon (1961)) and found the chlorophyll a red maximum at 678.5 mμ. Quantasomes have been shown to contain the photosynthetic pigment system intact, and can carry out the light reactions of photosynthesis.

Failure to demonstrate the intense Soret band of hemoglobin

in suspensions of intact mammalian erythrocytes has been encountered frequently, even though the band is evident in microspectroscopy of intact, single cells. Shibata et al (op. cit.), using opalescent plates, produced a much improved absorption spectrum of rat erythrocytes in suspension, showing a strong Soret maximum. Barer (1955) suspended human red blood cells in a 35 per cent w/v solution of bovine plasma albumin, fraction V, and again produced an improved absorption spectrum. Latimer and Eubanks (op. cit.) illustrate a spectrum of a human erythrocyte suspension made with conventional methods and instrumentation that is better than the spectrum shown by Barer. The oxyhemoglobin Soret maximum was determined to be at 417 mμ by Latimer and Eubanks under the described conditions. When opal slides were introduced, the band shifted to 415 mμ, and in an extract in 0.85% sodium chloride solution, produced by dissolving the cell wall with 0.1% cutsum, the Soret maximum was found at 414 mμ.

A final example of qualitative differences in absorption spectra dependent upon methods is taken from a paper by Dartnall (1961), dealing with visual pigments before and after extraction from visual cells. He found that the absorption maximum of frog visual pigment in a suspension of visual cells was at 487 mμ and poorly defined when conventional spectrophotometric methods were used. Inserting opal slides produced a more sharply defined maximum at 497 mμ, while extracts of visual pigment in 2 to 4 per cent aqueous digitonin solutions gave spectra with an absorption maximum at 502 mμ. Analogous results were obtained with visual cell suspensions from conger eel and carp retinae, having visual pigment maxima at 487 and 523 mμ, respectively, in digitonin

extracts.

Where it has been possible to locate the true *in vivo* position of the low-frequency absorption band of chlorophyll *a* with reasonable certainty (under conditions of minimum scattering or by integrating sphere measurements), it has been shown that scattering under standard conditions usually results in a bathochromic shift of the band. A similar shift of the Soret band of human oxyhemoglobin was observed by Latimer and Eubanks (op. cit.), but the spectrum of rat oxyhemoglobin from a suspension of erythrocytes illustrated by Shibata *et al.* (op. cit.) exhibits a definite hypsochromic shift of the Soret band, although the band is poorly defined. It is obvious from the published spectra that Shibata *et al.* used a much more dense suspension, assuming that 1 cm. cuvettes were used in both investigations. The absorbancy at 640 - 680 mμ on the curve shown by Latimer and Eubanks is estimated to be about 0.68 compared with a value of about 1.88 for Shibata *et al.* The respective ratios of absorbancy at 640 -650 mμ to absorbancy at the Soret maximum are about 0.5 and 0.7. The work on visual pigments by Dartnall shows a distinct hypsochromic absorption band shift due to scattering.

In the foregoing, the differences stressed are ones of band positions, but the high level of non-specific absorption and general lack of character shown by spectra of translucent materials produced by standard procedures have been obvious to all investigators.

The grossly distorted spectra produced in a conventional spectrophotometric system such as that illustrated in Fig. 4la result from a number of complex factors. First of all, the general level of

scattering causes a high proportion of the unabsorbed light to miss the detector and erroneously high absorbance values are apparent. Secondly, the heterogeneity of the material produces a complicated, indeterminate path with preferential shading of absorbing molecules in some cells and light passage through interparticle spaces without absorption. This has been termed the "sieve effect" and results in lower apparent absorbance values, particularly at band maxima. These two factors together lead to high, flat spectra such as illustrated in Figs. 42, 44, curve a. The positions of absorption peaks are affected because both scattering and the sieve effect are wavelength dependent. In a non-homogeneous, non-absorbing system, the degree of scattering increases with decreasing wavelength. If the particles are smaller than the wavelength of light, the scattering is inversely proportional to the fourth power of the wavelength, but if the particles are larger, the change with wavelength is not as great. If this type of scattering alone is present in an absorbing system, then the absorbance maxima will be shifted to lower wavelengths (hypsochromic shift). It is known, however, that sharp changes in scattering power occur in the vicinity of absorption bands when pigments are present. This effect is known as anomalous or selective scattering and tends to enhance the long wavelength side of a band, shifting the peak to longer wavelengths (bathochromic shift). The sharper and more intense the absorption band, the greater the effect of anomalous scattering. The spectral curves published by Latimer and Eubanks (op. cit.) suggest that the sieve effect does not alter the position of band maxima to any significant extent, at least with their material and under the conditions of their experiment. Finally,

Shibata (1959) has pointed out that with conventional methods the spectrum of translucent material will not be the same with different makes of instruments because of differences in the arrangement of sample and detector.

Many attempts have been made to minimize or correct for scattering losses. The only experimental technique that eliminates losses through scattering is the use of the Ulbricht or integrating sphere. Latimer and Eubanks (op. cit.) point out that the performance of the sphere must be carefully checked, and that there are many practical problems involved in its use. Thus the method has not had wide application. Sauer and Calvin (op. cit.) believed the spectra they obtained with spinach quantasomes to be free from scattering-produced distortion on the basis of an extremely low value (0.006) for the ratio absorbancy beyond 720 mμ/absorbancy at chlorophyll a red max. There is no question that quantasome preparations yield excellent spectra with conventional instrumentation and technique, but the ratio used to evaluate scattering does not take into account anomalous scattering. In their measurements the chlorophyll a red maximum was located at 678.5 mμ, displaced 1.5 mμ toward longer wavelengths than the position found by Latimer and Eubanks (op. cit.) for whole chloroplasts with full correction for scattering. Possibly anomalous scattering affects the positions of absorption maxima even with very slightly turbid quantasome suspensions. In addition, the technique used to produce chloroplast subunits may not be adaptable to many sorts of material and may be

unavailable to many investigators because of the equipment required.

Barer (op. cit.) found that greatly improved spectra can be obtained with suspensions if the cells are in a protein solution whose refractive index matches that of the cell wall or membrane. He concluded that the main site of scattering is at the cell surface, but noted that with very inhomogeneous cells the degree of clarification is not as great. For example, Charney and Brackett (op. cit.) determined that the refractive index of the cell wall of Chlorella pyrinoidosa was 1.37 ± 0.01, that of the intracellular material exclusive of the chloroplast 1.36 ± 0.005, and that of the chloroplast probably 1.415 - 1.420, but with a lower limit of 1.410. These values refer to the wavelength of the sodium D line. The refractive index of the albumin solution cannot be made to match that of all cell components so there will be residual scattering, and the sharp changes in refractive index in the vicinity of absorption bands cannot be taken into account.

Various other methods of correcting for scattering losses have been used, all with limited success and application. Scattering losses can be reduced by placing the detector against the exit surface of the cuvette, or partially compensated for by measuring scattering outside absorption bands and extrapolating the curve to cover the whole spectrum. Keilin and Hartree (1958, cited in Latimer and Eubanks (op. cit.)) measured absorbance spectra of each sample at a number of different sample-to-detector distances and extrapolated the results to obtain the spectrum that would be observed

if the detector surrounded the sample.

One of the simplest and best methods of partially removing spectrum distortion is through the use of diffusing materials such as opal glass or oil-impregnated filter paper placed against the exit surfaces of both sample and reference cell (Fig. 41b) as described by Shibata et al. (1954) and Shibata (1959). In the case of Chlorella, the position of the red maximum of chlorophyll a at 675 mμ found by Latimer (1958) using the opal technique is very close to the position (674 ± 1 mμ) found by Charney and Brackett (op. cit.), using an integrating sphere. Similarly, Latimer and Eubanks (op. cit.) placed the Soret Band of human oxyhemoglobin at 415 mμ with plates compared to 414 mμ as the accepted position based on other evidence. Dartnall (op. cit.) found a difference of only 5 mμ between opal method spectra of frog visual cells and the pigment in digitonin extracts of the same material. It is interesting to note that the opal method works for both the case where strong anomalous scattering is present (Latimer) and where this effect is absent (Dartnall). Working with the opal plate technique, Dartnall (op. cit.) devised a correction that can be applied to remove the residual scattering effect from opal-method curves; that is, its use with an opal method curve corrects for scattering at all angles. With this correction, the positions of frog visual pigment in cell suspensions and in digitonin extracts coincide at 502 mμ. The same result was obtained for conger eel and carp visual pigments with wavelength maxima at 487 and 523 mμ, respectively. Dartnall felt

that his correction was valid only if the scattering effects were independent of pigmentation, but he failed to consider anomalous scattering that is pigment-dependent. Latimer and Eubanks (op. cit.), apparently unaware of Dartnall's work, developed the same correction method and showed that it is applicable to systems where selective scattering occurs. Latimer and Eubanks found the Soret band of human oxyhemoglobin in fully corrected curves to be at the accepted position of 414 mμ, and the chlorophyll a red maximum in spinach chloroplasts was placed at 677 mμ.

The essential features of the method employed by Dartnall, and Latimer and Eubanks are the measurement of spectra of a sample without opal plates, then with opal plates. The difference between the two is an experimental scattering curve that is scaled by an appropriate factor to reduce the absorbance value of the opal spectrum to zero at a point where the absorption by known constituents is negligible. Then this correction curve is subtracted from the opal curve to give a spectrum free from scattering distortion.

None of the methods discussed so far corrects for the sieve effect. Latimer and Eubanks (op. cit.) claim that microspectrophotometry of single cells, although subject to phase contrast errors, eliminates the sieve effect, but it seems that if this is so, great care must be taken to restrict the area examined to a small one of high homogeneity. Others such as Duysens (1956) have derived equations to correct for the sieve effect.

Latimer and Eubanks (op. cit.) first corrected for

scattering losses and then applied a correction for the sieve effect based on equations of the Duysen's type. This procedure produced excellent qualitative and quantitative agreement between the in vivo spectrum of human oxyhemoglobin and an extract, but was not as successful with spinach chloroplasts and chloroplast extract, probably on account of changes in the pigment spectrum on extraction. There seems little doubt that this work by Latimer and Eubanks has opened the door to much profitable work on pigment spectra in vivo, particularly in plants.

In finishing this section the general complexity of the problem may be emphasized by two quotations from French (1960).

> "The simplest question that can be asked: 'What is the absorption spectrum curve for chlorophyll a in a typical living leaf?' cannot be answered yet, except by a very rough approximation. Of course, the answer to this question, when it is resolved, will not be a single curve, but a family of curves for various organisms."

Again, speaking of extracted pigments, French says

> "The absolute heights of the bands, generally have been established within a few per cent, though the wavelength positions of the peaks vary by a few millimicrons from one laboratory to another. This variation appears to be outside the likely experimental error of the physical measurements. The reasons for the small uncertainty in wavelength of peaks of chlorophyll absorption are not entirely clear;

they probably are due to actual chemical differences in the chlorophyll present in different plants, or to variations in the relative quantities of chlorophyll isomers present in solutions prepared by different methods."

B. THE OPAL GLASS METHOD AND ITS APPLICATION TO SAWFLY EGGS

If sawfly eggs are examined spectrophotometrically in a conventional system (Fig. 41a), employing clear slides, the resulting spectrum is of high general density (Figs. 42, 44, curve a) and with little characteristic form. The insertion of opal slides, as shown in Fig. 41b, results in the production of usable spectra (Figs. 42, 44, curve b). Correcting the opal spectra for all scattering losses according to the scheme of Dartnall does not alter their appearance greatly (Figs. 42, 44, curve c). It may be noted in both instances that the scattering correction curve (Figs. 42, 44, curve d) shows an indication of only a low level of selective scattering by the bile chromoprotein and that scattering intensity apparently decreases toward higher wavenumbers (lower wavelengths). This latter observation is in disagreement with what would be expected.

In the normal sort of egg preparation used in both these tests it is recognized that spaces between the eggs are always present to a greater or lesser degree, thus allowing light to pass

to the detector in a sort of macroscopic sieve effect. In order to determine the effect of this factor, extremely dense egg packs were made using the eggs of more than a single female. The results of these runs are shown in Figs. 42, 44, curves e, f, g, h. It is clearly evident that the flatness shown by Figs. 42, 44, curve a results largely from incident radiation passing through spaces between the eggs. Where such voids have been reduced or perhaps eliminated (Figs. 42, 44, curve e), the general level of apparent absorption is raised and distinct absorption maxima are preserved. Still the bile chromoprotein maxima are significantly flattened in the curves from clear slides by scattering losses, as evidenced by the improvement shown in Figs. 42, 44, curves f, g, for opal spectra and fully corrected spectra, respectively. The scattering correction curves (Figs. 42, 44, curve h) from very dense preparations again fail to show evidence of strong anomalous scattering by the bile chromoprotein, but now the expected increase in scattering intensity with increasing wavenumber is found in Fig. 42, curve h. The absence of this phenomenon in Fig. 44, curve h may reflect the presence of small spaces between the eggs even in this thick preparation.

Microscopic inhomogeneity and its resultant sieve effect have not been assessed directly, but extracts of bile chromoproteins indicate that the absorption bands are neither strong nor particularly sharp and thus the flatness seen in scattering-corrected curves may not be far from an undistorted assessment.

Arithmetic optical density plots of opal or scattering-

corrected spectra from normal and dense preparations show shape of differences that may be related to concentration effects. Plotting the opal spectra on a $\log_{10}$ ordinate scale, a common practice in the study of characteristic spectral form (Hiskey, 1955), results in curves that are of reasonably similar shape (Figs. 43, 45). The scattering-corrected curves have not been plotted on semi-log co-ordinates because of the zero value assigned to the 13 kc/cm terminal reading.

Where it has been possible to assign a position to the bile chromoprotein maximum in spectra from normal density preparations on clear slides, it is evident that these maxima are shifted to a higher wavenumber as well as being lowered in apparent intensity by the light passing between the eggs. Thus the hypsochromic shift shown in Fig. 44, curve e tends to support the opinion that these were small voids in the preparation although it was much denser than normal. In Fig. 42, curve e a slight bathochromic shift is evident in comparison to the opal or the scattering-corrected curve. Within the limitations of the procedures, however, the positions of bile chromoprotein maxima in opal and scattering-corrected curves obtained with normal and dense slides are identical.

The effect of slide density on the triangular co-ordinates was tested in two experiments. In the first of these a fairly dense opal slide was prepared with eggs from a single female. Spectra were recorded for this initial preparation and after each of a number of manipulations designed to reduce the density of the preparation (i. e.,

preparation becoming thinner with increasing space between eggs). Four of the eight spectra are shown in Fig. 46a, plotted on arithmetic co-ordinates. The change in shape with slide density is obvious. Figure 46b shows the same four curves plotted on semi-logarithmic co-ordinates. This treatment compensates for the slide density effects to a high degree. Figure 47a depicts the scatter of triangular co-ordinates from all eight spectra in arithmetic plot. Also illustrated are polygons formed by joining the plotted points in sequence of semi-integral attenuance level at 30 kc/cm for three sequences as follows: all eight points, points for the two least dense runs eliminated, points for the four least dense runs eliminated. These figures show that the triangular co-ordinates are strongly affected by changing density when the attenuance at 30 kc/cm is low, 0.4 or lower. Figure 47b shows the same material as 47a except that the co-ordinates are based on semi-log plots of the spectra. The greater compactness of the scatter is evident at once and only the points for the two least dense preparations are separated from the main group. The area of the polygon with semi-log plotting and removal of the points for the four least dense runs is 40% of that of the comparable polygon with arithmetic plotting and 13% of the area of the polygon formed by all points from semi-log plotting. The advantage of semi-log plotting for the analysis of egg spectral curves is obvious.

In spite of the reduced scatter size shown in Figure 47b, it is evident that there is a slide density limit below which spectra

will not yield valid triangular co-ordinates. Figures 48a, b, and c show plots of each co-ordinate against semi-integral attenuance at 30 kc/cm. The distributions of points on the graphs appear to be well fitted by two intersecting straight lines, the point of intersection indicating the permissible lower limit of slide density. On this basis, spectra were discarded if the semi-integral attenuance at 30 kc/cm was below 0.43 (on the original records, transmission exceeding 40%).

The second experiment was similar, but designed to determine if there is an upper limit to permissible density at 30 kc/cm for obtaining satisfactory triangular co-ordinates. The results showed that the highest density slides conceivably obtainable with the eggs from a single female produce acceptable spectra. Again there was no evidence of slide density affecting the positions of bile chromoprotein maxima.

From the results of all these tests it may be concluded that uncorrected semi-integral attenuance spectra are suitable for the assessment of egg pigmentation.

C. CONSTANCY OF EGG PIGMENTATION WITH RESPECT TO STAGE OF DEVELOPMENT

Normal procedure in the spectrophotometric work was to examine the egg pigmentation at any time from adult emergence until death of the adult (a few days at room temperature, or as long as 4 to 5 weeks at 7°C). Usually the spectra were obtained within a few days of emergence, but occasionally heavy adult eclosion or instrument

difficulties made this impossible. This section deals very briefly with the changes in "egg" pigmentation during oögenesis so that the validity of using egg spectra from both fresh and moribund females may be assessed.

One obvious advantage of using complements of eggs dissected from females is that sufficient material is obtained to fill the beam of the spectrophotometer. This obviates the need for microspectrophotometric examination of a sample of eggs from each female on an individual egg basis, and also the time consuming and painstaking work required to dissect eggs from needles is avoided. Other benefits may also be derived from the method in that once oviposition takes place embryogenesis commences even in those species overwintering in the egg stage (Brygider, (1952)), and it is doubtful if meaningful spectra could be obtained, at least without recourse to uniform sample timing. Still it is possible to distinguish eggs of N. sertifer, N. nanulus nanulus, and N. pratti banksianae Rohwer, for example, at the end of the overwintering period when the eggs contain well segmented embryos, which very shortly will display limb buds (Brygider (op. cit.), Breny (1957)). The evidence strongly suggests that the total egg production of a diprionine female is developed at the time of emergence from the cocoon (oögenesis is not spread out over the adult life span), and on the basis of Smith's (1941) work on Gilpinia hercyniae, it is likely that at the time of emergence the eggs are relatively quiescent, all in a single stage of development, awaiting the stimulus of oviposition to trigger the maturation divisions of the nucleus. Resorption of

eggs over a prolonged adult life span has not been demonstrated. From the foregoing it would be reasonable to expect that egg pigmentation is stable from adult emergence until death or oviposition.

A portion of a sample of Neodiprion swainei cocoons that had been stored to fulfill the normal diapause requirements was dissected and the eonymphal larvae placed individually in short lengths of glass tubing with both ends plugged with cellucotton. This procedure enables observations on development to be made with relative ease. The individuals in tubes and the unopened cocoons were incubated at about 21°C. At the commencement of the incubation period the ovaries were small, transparent, jelly-like masses with no discernible pigmentation (Fig. 2, the bluish cast is due to opacity that developed after dissection). Pupation took place after about ten days of incubation, and at this point individual ovarioles with oocytes could be distinguished readily, but the oocytes were not pigmented. The white pupal stage lasted about five days and during this period oogenesis proceeded rapidly. External pigmentation of the pupae commenced with a darkening of the tips of the mandibles, and at about this time (15 days) the first (most mature) oocytes showed distinct pigmentation, predominantly yellow (Fig. 3). The mature eggs of N. swainei are green (Fig. 4) and semi-integral attenuance spectra indicated that the level of carotenoid concentration attained in the mature egg was already present in the first oocyte of these early dark pupae. The green bile chromoprotein level was much lower than in mature eggs. The pupal skins were shed in

about 20 days in the glass tubes, but emergence from unopened cocoons commenced after 23 days of incubation and terminated on the 29th day. Peak emergence from the cocoons was on the 25th and 26th days of incubation. The adults apparently stay in the cocoons a few days before eclosing. Fresh adults in glass tubes are generally inactive for a time, and then make a great effort to chew their way out through the cellucotton plug. Possibly this latter activity corresponds with normal eclosion from a cocoon. Egg spectra were constant from normal emergence until death of the female, but the development of bile chromoprotein pigmentation in the egg is probably still occurring while an apparently mature female is in the cocoon. Occasionally a few females emerge precociously from cocoons that have been subjected to a variety of conditions during shipment and storage, and these females often have eggs that are deficient in bile chromoprotein.

It may be concluded that spectra of egg complements dissected from normally emerged females are not affected by age of the adult.

D. STABILITY OF PIGMENTS

1. IN SLIDES OF FRESH MATERIAL

Figure 49 shows the log semi-integral attenuance spectrum of a fresh (within 10 minutes of dissection from the female) preparation of green eggs from a female belonging to the *N. swainei* group, and the spectrum of the same preparation after 30 hours

storage at room temperature (21 - 25°C). Two differences are obvious: attenuance in the region 30 - 24 kc/cm is much higher in the 30-hour curve, and the fine structure of the carotenoid band is enhanced. The bile chromoprotein band appears unaltered. Triangular co-ordinates for the two spectra illustrated and for others obtained at four intermediate times are presented in Table II. Here it is

Table II. Triangular co-ordinates for a preparation of N. swainei group eggs during storage at room temperature

	Co-ordinate		
Time (hours)	Carotenoid	Attenuance min.	Bile chromoprotein max.
0	53.1	10.4	36.4
4	50.2	13.7	36.2
6	50.9	12.7	36.4
22.5	51.8	10.9	37.4
26.5	50.5	12.5	36.9
30	51.0	11.7	37.2

notable that there is no significant trend in the co-ordinates for bile chromoprotein or attenuance minimum. On the other hand, the carotenoid co-ordinate shows a distinct decrease by the end of the first four hours. An examination of the whole series of spectra reveals that the most rapid increase in attenuance in the 30 - 24 kc/cm range took place during the first four hours, and following this period of initial rapid change a stable level of attenuance was reached. There was also a marked unmasking of the carotenoid fine

structure during the first four hours, but this change continued unabated, attaining a stable condition some time before 22 hours had elapsed. It appears that the two major pigments are very stable when intact eggs are maintained in saline, and in fact satisfactory spectra have been obtained from preparations 72 hours old, providing that the rubber cement seal prevents desiccation. It may be noted here that water rather than a 0.15M sodium chloride solution was used in early test preparations with the Beckman DU, but was replaced by the saline (which is approximately isotonic with the egg contents) when it was found that the preparations did not yield stable spectra. The instability was particularly serious in view of the prolonged time required to obtain a spectral curve with the DU. In addition, crushing the eggs leads to instability.

In other tests where preparations were scanned twice with a total running time of about 13 minutes (end of run no more than 18 to 20 minutes after dissection from the female) the triangular co-ordinates calculated for the first and second runs differed by 0.2% at most.

The normal operating procedure employed with the Unicam SP. 700 ensured that the egg spectrum was recorded within 15 to 20 minutes of dissection and thus it may be concluded that preparation stability need not be considered in the interpretation of the results.

2. IN EGGS WITHIN DRIED MUSEUM SPECIMENS

Many thousands of sawfly adults were killed, pinned, dried, and stored for later examination before representative egg spectra

were considered an asset to the taxonomic work, and even currently some material may not be readily available in a living state. With this consideration in mind, dried females (4 to 5 1/2 years old), representative of diverse egg colour types, were rehydrated in saline, the eggs removed, and spectra obtained. The four species selected and their general egg pigment types were as follows: a) Neodiprion lecontei, moderate concentration of carotenoid, no bile chromoprotein; b) Neodiprion sertifer, low carotenoid, high purple bile chromoprotein; c) Diprion simile, very low carotenoid, high blue bile chromoprotein; and d) Neodiprion swainei, moderate carotenoid, moderate green bile chromoprotein.

Figure 50a, b, c, and d show the spectra of the rehydrated eggs with the spectra from normal, fresh preparations for comparison. The carotenoid band region of all the spectra of eggs taken from dried females is devoid of any indication that carotenoids had been present. The curves for D. simile (Fig. 50c) indicate that an attenuance shoulder around 24 - 20 kc/cm occurs in rehydrated material even when very little carotenoid could have been present in fresh material. On the other hand, the bile chromoprotein regions of the spectra of N. lecontei, N. sertifer, and N. swainei reveal sufficient detail to determine that these complements of eggs originally contained no bile chromoprotein, purple bile chromoprotein, and green bile chromoprotein, respectively. The spectrum of dried D. simile eggs shows only a very slight indication that blue bile chromoprotein had been present in a high concentration, but with care it can be seen that there is a slight difference between this curve and that for

N. lecontei where bile chromoprotein was absent.

In some instances the general nature of the colour of eggs in a fresh state can be determined by direct visual observation of rehydrated specimens. In other cases instrumental checking is necessary. It is obvious that spectra from rehydrated eggs cannot be analyzed in detail, but it is possible that occasions could arise when knowing even some of the gross features of egg pigmentation in a group of museum specimens would be very valuable. The usefulness of spectrophotometric examination will undoubtedly be affected by factors such as the age and storage conditions of the specimen, and the type of killing agent employed as well as the way in which it was used.

E. PROBLEMS ASSOCIATED WITH SPECTROPHOTOMETER CAPABILITIES AND DESIGN

One of the main drawbacks of the Beckman DU manual spectrophotometer used for the initial trials was the length of time required to run a single sample. This made it impossible to obtain spectra of a satisfactory number of egg complements during the fairly short period that adults of any given type were available. The long observation period was particularly troublesome when spectrum instability was suspected. In addition, the repeated mechanical shifting of the sample positioning carriage, necessary to place the reference and sample slides alternately in the beam, often caused changes in the spacing of eggs during the course of a run. Such

shifts could be detected in most instances by the appearance of sudden, unexpected changes in attenuance, but the danger of not detecting more subtle differences was always present, and consistent rechecking of the attenuance at the starting wavelength was required. The use of beam restriction by the 0.46 mm. aperture of the microcell adapter slide made the problem of shifting eggs more serious and also introduced a sampling error in that only a few of the eggs produced by a female were in the beam and some variation in egg colour within a single complement is obvious (not that the eggs of one female may represent two distinct general types).

The high electrical gain required at the near ultraviolet-violet and red ends of the spectrum hindered obtaining accurate readings. A further drawback became evident when it was found that no satisfactory base level for spectral curve analysis was available because of the limited spectral range that could be examined when the specimens were not transparent as in the case of the sawfly eggs.

The use of the Unicam SP. 700 recording spectrophotometer overcomes all the aforementioned difficulties and by virtue of its versatility provides many additional benefits such as continuous spectral presentation and various scale expansions. Unfortunately, the detailed qualitative analysis of SP. 700 spectra upon which much of this paper is based is meaningless, because the wavenumber calibration in the silica prism range of the SP. 700 series to which our instrument belongs was subject to change with the temperature of the monochromator. The temperature was dependent mainly upon heat

produced by electrical components of the instrument, and stability was not achieved without a warm-up period of about five hours with all components operating, including the scan drive motor (drive in neutral). In addition, day-to-day changes in room temperature produced much smaller, but still significant, changes in calibration. After extensive testing, the problem was referred to Unicam for correction, and a water cooling coil plus insulating baffles were provided. These, in conjunction with a water temperature regulating device built in this laboratory, reduced the warm-up period to about one and one-half hours and eliminated the day-to-day fluctuations in calibration. The flow and temperature regulating device being used at present is of a temporary nature and will be replaced; therefore final testing and wavenumber calibration have not been undertaken. The need for a convenient calibration standard is of extreme importance and the holmium oxide filter described by Vandenbelt (1961) has proven indispensable.

The egg pigmentation work was carried out all through the course of these instrumental difficulties and, while it was possible with care to ensure quantitative accuracy of the spectra, the corrections necessary to make possible qualitative analyses were not practicable. A few qualitative observations have been included for cases where constancy of calibration was known.

The nominal spectral bandwidth (which in essence controls the resolving power of the instrument) for the SP.700, operated according to my standard schedule for sawfly eggs, is approximately as follows:

Spectral position (kc/cm)

30	26	19	16	13
600 cm^{-1} (6.7 mμ)	200 cm^{-1} (3 mμ)	100 cm^{-1} (2.8 mμ)	200 cm^{-1} (7.8 mμ)	1500 cm^{-1} (89 mμ)

From this it can be seen that the resolution of attenuance bands toward the red end of the spectrum was poor, and future studies from which valid quantitative data may be taken must employ modified procedures to improve resolution. It appears that an operating schedule yielding nominal spectral slit widths as follows would be feasible:

Spectral position (kc/cm)

	30	27
RLD3	220 cm^{-1} (2.2 mμ)	94 cm^{-1} (1.4 mμ)

	27	19	16
R3D3	122 cm^{-1} (1.5 mμ)	83 cm^{-1} (2.8 mμ)	117 cm^{-1} (4.0 mμ)

	16	15	14.5	14	13
RLD3	105 cm^{-1} (3.9 mμ)	133 cm^{-1} (5.9 mμ)	160 cm^{-1} (7.1 mμ)	246 cm^{-1} (12.7 mμ)	650 cm^{-1} (39 mμ)

Full scale response 0 - 100%

The high gain (R setting) required to obtain slit widths producing good spectral slit widths is necessitated by the loss of energy through scattering by the opal slide. The problem in the SP. 700 is compounded by the large sample tc detector distance. Perhaps the advent of commercially available fibre optics will make it possible to

develop a device that will collect light scattered at greater angles close to the sample or reference and "pipe" it to the detector. Such a device would result in narrower spectral slit widths without the use of high gain and its concomitant high noise-level with low damping or the need for heavy damping. The use of a modified cuvette carrier suggested by Shibata (1959) was found unnecessary and valueless with the SP. 700, but it is likely that a thinner opal layer on the slide would be feasible and result in narrower slit widths.

An additional instrumental feature of value, particularly with samples such as intact eggs, would be provision for ordinate scale expansion of any say 10 or 20 per cent transmittance portion of the range to full scale.

Finally, it may be noted that currently available instrumentation would record egg spectra ready for measurement without the need for mathematical corrections or conversions. Desirable features of such a system would be (1) zero absorbance (100 per cent transmittance) line compensation by means of plug-in multipot banks, and (2) external recording capability allowing the use of an extinction coefficient (absorptivity) recorder. With these potentialities the specialized requirements of recording egg spectra ready for measurement would be possible while at the same time maintaining the main instrument ready for more commonplace work without major adjustments. Unfortunately, not all of these desirable features are available in one make or model of spectrophotometer.

VII. RESULTS AND DISCUSSION PERTAINING TO INDIVIDUAL SPECIES OR COMPLEXES

In this section the results of spectrophotometric examinations of egg preparations of various diprionid entities are presented with reference to other information required to assess the spectrochemical data meaningfully. No attempt has been made to achieve a full taxonomic coverage. Scientific names of species of pine are according to Gaussen (1960), and the names of other host trees follow Harlow and Harrar (1958).

A. DIPRION SCHRANK

1. D. PINI (LINNAEUS, 1758)

Benson (1951), speaking of D. pini and D. simile, says "The 2 European species are easily distinguished on larval characters, but in the adults are so variable in external features as to be only separable for certain on genitalia structures". Eliescu (1932) quotes De Geer, writing about 1775, who described the colour of D. pini eggs in dissections of females as white-yellowish and then in a subsequent paragraph says that the colour of the ready-to-be-laid egg is yellowish white, and when laid white. Eliescu also says "I can confirm the observation of Müller [Müller, D. G. Über den Afterraupenfrass in den fränkischen Kiefernwaldungen vom Jahr 1819-1820. 1. und 2. Aufl. Aschaffenburg 1821 and 1824] that the differing pigmentation of the females has no influence on the colour of the eggs".

Scheidter (1926) and Sturm (1942) both agree with Eliescu and use the colour of laid eggs in a field diagnosis of diprionid eggs. (data summarized in Table III which also includes the observations of Forsius (1920)). In the present study, the colour of D. pini eggs in females from Pinus sylvestris Linnaeus at one locality in Austria was found to be pale yellow (Fig. 12). Spectrophotometric tests showed no significant absorption by bile chromoproteins, and evidence of only a small amount of carotenoid (Fig. 51). The triangular co-ordinates based on three spectra are as follows:

Carotenoid	17 kc/cm	15 kc/cm
1.9(1.0-3.6)%	73.7(68.6-78.6)%	24.5(17.8-30.4)%

The first figure given is the mean value and the range is shown in parentheses.

2. D. SIMILE (HARTIG, 1834)

Britton and Zappe (1917) describe the colour of D. simile eggs as bluish in material collected shortly after the first discovery of this species in North America at New Haven, Connecticut, in 1914. Forsius (1920), Scheidter (1926), and Sturm (1942) all agree that the eggs in European material are bluish green (Table III). Middleton (1923) says that when the eggs are first laid they "are pale whitish blue, translucent, shining, much like gelatin", and also that several days after being laid "the colour appears bluish green". Figure 13 illustrates the colour commonly observed in eggs of this species from Ontario, Wisconsin, and Minnesota. In some instances

Table III. Egg colours of European species of Diprionidae as described by three European authors.

Species	Author: Scheidter (1926,1934) laid eggs	Sturm (1942) laid eggs	Forsius (1920) unlaid eggs
Diprion			
pini (Linnaeus)*	pure white	white-yellowish white	---
simile (Hartig)*	turquoise	green-bluish green	blue green
Macrodiprion			
nemoralis Enslin	white	white	pale yolk yellow
Microdiprion			
fuscipennis (Forsius)	---	---	whitish yellow
pallipes (Fallen)*	pure white	---	---
Neodiprion			
sertifer (Geoffroy)*	pure white	white	---

Table III (cont'd.)

Species	Scheidter	Sturm	Forsius
Gilpinia			
abieticola (Dalla Torre)	green	---	---
frutetorum (Fabricius)*	white	pure white	---
hercyniae (Hartig)*	sap green	---	---
laricis (Jurine)	dark sap green	dark green	---
pallida (Klug)*	pure white	pure white	grass green
socia (Klug)*	dirty white, grey	dirty yellow	grey
variegata (Hartig)	deep ivory yellow	pure white	---
virens (Klug)	green	light grass green	---
Monoctenus			
juniperi (Linnaeus)	---	---	pale yellow

* species examined by me.

even the unlaid eggs are more turquoise in appearance than those shown in the picture. The spectrum of D. simile eggs (Fig. 51) is indicative of a high concentration of blue bile chromoprotein and very little carotenoid. The more turquoise eggs have a somewhat higher level of carotenoid pigment. The triangular co-ordinates based on five spectra from material collected on P. sylvestris at three Ontario localities are as follows:

Carotenoid	Attenuance Min.	Bile Chromoprotein Max.
33.9(32.5-35.0)%	26.9(22.6-29.3)%	39.3(37.0-42.6)%

It appears from the foregoing evidence that egg colour is a valid criterion for distinguishing between D. pini and D. simile.

B. MICRODIPRION PALLIPES (FALLEN, 1808)

Scheidter (1926) reported that the eggs of this species are pure white when laid. Material collected on P. sylvestris at two localities in Austria was examined and the mature eggs appeared pale yellow (Fig. 14). Spectra (Fig. 51) showed the presence of only a slight amount of carotenoid and no detectable bile chromoprotein. The triangular co-ordinates from four runs are as follows:

Carotenoid	17 kc/cm	15 kc/cm
8.2(2.6-14.1)%	59.8(55.8-64.5)%	32.0(25.0-38.4)%

Although it is impossible to distinguish the egg colours of M. pallipes

and D. pini visually, the spectra and the co-ordinates indicate differences, a slightly higher carotenoid concentration being present in M. pallipes.

C. GILPINIA BENSON

1. G. FRUTETORUM (FABRICIUS, 1793)

Scheidter (1934) and Sturm (1942) give the colour of laid G. frutetorum eggs as white, but the colour of unlaid eggs from Ontario material is strong yellow (Fig. 15), and the spectral curve (Fig. 51) from such specimens indicates a moderate concentration of carotenoid. The Ontario populations appear to be correctly identified and it seems unlikely that an egg colour difference exists between the introduced North American populations and the native European ones. It is conceivable of course that two egg colour types exist in Europe and that only one of them was introduced into North America. More likely the difference noted above stems from the fact that both Scheidter and Sturm examined laid eggs that had lost some of their yellowness. Due to instrumental difficulties, only one of several egg spectra for G. frutetorum was satisfactory for analysis, but all the spectra appeared to be very similar. The triangular co-ordinates for the spectral curve of the single Ontario sample from a female reared on P. sylvestris are as follows:

Carotenoid	17 kc/cm	15 kc/cm
42.4%	37.6%	20.0%

These co-ordinates are sufficiently different from those of D. pini

and M. pallipes, both with pale yellow eggs, to ensure separation of the scatters. It may be noted here that eggs of G. frutetorum, like those of G. hercyniae, contain a different carotenoid from those of D. pini and any Neodiprion species tested.

2. G. HERCYNIAE (HARTIG, 1837)

Eggs of this species dissected from females in Ontario rearings are bright, yellowish green (Fig. 16). Scheidter (1934) says that G. hercyniae eggs that have been laid are sap green. It cannot be determined whether or not Scheidter worked with pure populations of G. hercyniae, pure populations of G. polytoma (Hartig, 1834), or mixtures of the two, and so no meaning can be attached to his description. The spectral curve (Fig. 51) of G. hercyniae eggs shows a very high carotenoid attenuance and a moderately high green bile chromoprotein peak. The triangular co-ordinates of three spectra from Ontario material, two from the first generation on Picea glauca (Moench) Voss, and one from the second generation on Picea abies (Linnaeus) Karsten, are as follows:

Carotenoid	Attenuance Min.	Bile Chromoprotein Max.
50.0(48.0-51.0)%	19.5(18.7-20.4)%	30.6(29.7-31.6)%

As mentioned in the preceding section, G. hercyniae eggs contain a different carotenoid from most species tested by extraction methods.

3. G. SOCIA (KLUG, 1812)

Eggs of G. socia examined in this study had a definite purplish-red cast (Fig. 17), but the colour is very difficult to describe,

and the grey designations of European authors (Table III) probably refer to the same species. The spectrum (Fig. 51) shows evidence of a very low carotenoid level, but a high concentration of purple bile chromoprotein. Triangular co-ordinates from three runs on material from P. sylvestris at one locality in Austria are as follows:

Carotenoid	Attenuance Min.	Bile Chromoprotein Max.
37.1(37.0-37.2)%	29.9(29.8-30.0)%	32.9(32.9-33.0)%

D. NEODIPRION ROHWER

1. N. SWAINEI MIDDLETON, 1931

This species, restricted to jack pine (Wallace, 1961), has a distribution (Fig. 52) conforming in most respects with Type E5 of Munroe (1956), except for being absent from the Maritime Provinces and the northeastern United States. It has been found only rarely in the area north of Lake Superior where very short frost-free seasons are the rule (Wallace, 1959). The cluster of larvae shown on the left in Fig. 8 have the yellow colour typical of N. swainei in the northeastern and central parts of its range with points shown as stars in Fig. 52. The larvae in the cluster to the right in Fig. 8 are whitish by comparison, and are found throughout the southwestern part of the range of N. swainei as shown by the dots in Fig. 52. The circles in Fig. 52, comprising the northwestern portion of the range of N. swainei, represent areas for which the larval colour is not well known. The problems associated with this region will be discussed later.

Hemolymph pigmentation is an insignificant factor in the determination of the larval colour type, and similarly food in the gut does not affect the general body colour in the manner observed. It was found, however, that the determining factor is the fat body pigmentation. For the purposes of this discussion the larval fat body may be considered in two parts (Kilby, 1963): The peripheral fat body, consisting of clumps of cells located segmentally on the inner surface of bulges of the body wall; and the deep fat body, comprising a sheath around the organs in the body cavity. The deep fat body is green in both white and yellow larvae and attenuance spectra (Fig. 53a) obtained by the opal technique do not indicate major differences in pigment content. The spectra from both show the presence of carotenoids and green bile chromoprotein. If larvae are dissected so that the interior surface of the body wall can be examined, the peripheral fat body of yellow larvae appears yellow in colour while that of white larvae is pale, creamy white. Attenuance spectra of body wall preparations obtained by the opal technique are shown in Fig. 53b. The significant difference between the spectrum from a white larva and a yellow larva is the presence in the latter of carotenoid absorption in the region around 22 kc/cm. The conclusion is that the concentration of carotenoid pigment in the peripheral fat body is responsible for the larval colour. The difference between the two types is maintained in laboratory stocks reared under one set of conditions on foliage from one source.

The usual egg colour in N. swainei is green (Fig. 4), but spectra of eggs produced by females arising from yellow larvae show

a higher attenuance due to carotene than the eggs of females from white larvae (Fig. 54a). Spectra of both types indicate the presence of a moderate concentration of green bile chromoprotein. It is improbable that the slight difference in egg colour can be detected visually. Figure 54b shows the triangular co-ordinates plotted according to origin from larval colour type, and it is apparent that egg colour on an individual basis will not separate the two types completely. The mean co-ordinates and ranges for the two types based on 25 spectra from Ontario, Upper Michigan, Wisconsin, and Minnesota are as follows:

	Carotenoid	Attenuance Min.	Bile Chromoprotein Max.	N
yellow larval race	48.8(46.8-50.2)%	21.3(20.1-22.2)%	29.9(28.0-31.5)%	14
white larval race	45.2(43.6-47.6)%	22.6(21.3-24.8)%	32.2(30.8-33.6)%	11

On the basis of locality means for the carotenoid co-ordinates from the foregoing information plus the co-ordinate for a single spectrum from the Lake St. John area of Quebec, there appears to be a cline of increasing egg carotenoid concentration from the western range limit on the Mississippi River in Wisconsin and Minnesota to the eastern limit of the species in eastern Quebec north of the St. Lawrence River. This cline is sharply stepped in the area of overlap of white and yellow larval types along the north shore of Lake Michigan and the boundary between Wisconsin and Upper Michigan (Fig. 53).

The problem of this step area and the western Ontario-

Manitoba populations requires comment. Material collected in the step area by H. A. Tripp in 1963 is indicative of a high degree of intermediacy in larval colour in this region, and it seems likely on the basis of these observations and laboratory interbreeding (I. M. Campbell, personal communication) that hybridization of the two types takes place in nature even though laboratory studies show developmental rate differences that should provide some degree of temporal isolation. In recent years, population levels in northern Minnesota and western Ontario have been very low and thus little living material was available for study. The limited number of collections indicate intermediacy in larval colour in the Rainy River-Lake-of-the-Woods area with a tendency to more yellow larvae farther north.

In the introduction it was noted that N. swainei exhibits egg colour dichromatism (Fig. 1). The basis of this green-yellow differentiation is the absence of green bile chromoprotein in the yellow eggs. Campbell (personal communication) found that the dichromatism is genetically controlled, being monogenic and recessive, but lack of knowledge of the sex determining mechanism(s) prevented a thorough investigation of the phenomenon. Figure 54a shows spectra of yellow eggs from females reared from yellow larvae and from white larvae. The higher carotenoid content in the former is obvious, and provides a demonstration of the difference in egg colour between the larval types uncomplicated by the presence of bile chromoprotein. It is my general impression that the yellow egg variant is more common among white larval populations than yellow ones, but extensive samp-

ling and rearing are required to investigate this possibility.

Campbell (personal communication) also found that larval appearance as affected by fat body colour does not differ between families that from breeding history are known to be pure for the yellow-egg type and green-egg type, respectively. The "white" pupae from similar pure families are the same colour when first formed, but as development progresses, the abdomens of pupae leading to adults with green eggs become greener, while the abdomens of pupae leading to adults with yellow eggs lose their green colour (Fig. 7) as the eggs develop. The bile chromoprotein of the pupal fat body apparently is metabolized and none is produced for incorporation in the eggs or transferred from the fat body to the eggs, as the case may be.

It has also been noted that "white" pupae from yellow larval populations (Fig. 5) are distinctly more yellowish-green than those from white larval populations (Fig. 6). The latter are more greyish due to the concentrations of both purple and green bile chromoproteins in the fat body.

The taxonomic status of the two forms of N. swainei is not completely clear. Excepting the problem of northwestern Ontario, it appears that there are two geographical races or subspecies. Further work is required to determine the certainty with which the two can be separated, and the characteristics of the species west of Lake Superior.

It is interesting to speculate about the factors that may

have produced the described racial differentiation. First of all, it is recognized that the evolution and present distribution of North American plants and animals, particularly in Canada and the northern United States, are highly dependent upon conditions during the most recent glacial period, the Wisconsin glaciation, with a first major advance reaching its maximum about 25,000 B.P. It is also evident that, although environmental conditions are never static, the present ones represent only the very young stages of the return to full interglacial conditions, and thus may well be a period of relatively rapid change. According to the work of Löve (1959), Potzger (1952), and Curtis (1959) it seems that the present distribution of jack pine, Pinus banksiana Lambert, has resulted from repopulation from probably three Wisconsin-time refugia: a western one on the approaches to the Rocky Mountains, a central one which may be either the Driftless Area of Wisconsin, Minnesota, Illinois, and Iowa, or some other area south of the present Great Lakes (or both), and an eastern coastal one such as the Pine Barrens area of New Jersey. Stock from the western refuge, possibly carrying characteristics of lodgepole pine, P. contorta v. latifolia Engelmann, with which it may have hybridized (Moss (1949), Löve (1959)), appears to have been involved in the reforestation sequence east into Manitoba and northwestern Ontario, that from the central refugium the area south of the Great Lakes and north into Manitoba and northwestern Ontario, and probably stock from the Atlantic coastal refuge repopulated the Canadian Maritime area, Quebec and Ontario to

west of Lake Superior. It seems highly likely that the jack pine in northwestern Ontario, southeastern Manitoba, and northern Minnesota represents a mixing from all three sources. The known distribution of N. swainei suggests that it was not associated in any way with the western refuge, in addition to the fact that all the species closely related to N. swainei are eastern. The distribution of the white larval type suggests an association with the Driftless Area, and that of the yellow larval type with the eastern refugium. At least, if jack pine existed in a more or less continuous distribution south of the Wisconsin ice from Minnesota to the Atlantic, the distribution of N. swainei probably was broken by the Appalachian Highlands. This is not an unreasonable assumption as even today N. swainei is excluded from the height of land region north of Lake Superior by adverse climatic conditions such as probably existed at higher elevations in the Appalachians during glacial times. Contact of migrating floras in the area west of Lake Superior may not have taken place until near the close of the hypsithermal period about 4,000 B.P. Thus contact of yellow and white larval N. swainei in western Ontario may be a very recent event, and the deteriorating climate (Potzger, 1953) following the hypsithermal is likely responsible for breaking the gene flow across the height of land north of Lake Superior. The work of Lawrence (1958) suggests that this trend reached its most recent maximum about 1750 A.D. and that conditions have been ameliorating since then. Contact of the two types across the St. Mary's River through Upper Michigan has taken place on

a narrow front and also may be recent.

There is no hope of proving that the foregoing suggested course of events took place with respect to the sawfly, but additional paleobotanical studies, particularly north of Lake Superior and Lake Huron, could perhaps strengthen the hypothesis.

2. NEODIPRION SPECIES CLOSELY RELATED TO N. SWAINEI

Ross (1955) includes three species in this group in addition to N. swainei: N. hetricki Ross, 1955; N. excitans Rohwer, 1921; and pinirigidae (Norton, 1869). As far as can be determined, they are distinctly set apart from other species in the genus by their oviposition pattern and by the structure of the scopal pads. The status of Neodiprion merkeli Ross, 1961 is doubtful, but its high, thin scopal pads described by Ross (1961) suggest that it is also a member of the N. swainei group, rather than the N. virginianus complex as Ross believes. The oviposition pattern is unknown.

Material collected at five localities in North and South Carolina in May, 1962, produced females having yellowish green eggs (Fig. 18). The egg spectrum (Fig. 55a) indicates moderate to high concentrations of both carotenoid and green bile chromoprotein, in both instances higher than the concentrations found in N. swainei. The triangular co-ordinates from 21 spectra with mean values and ranges listed below are plotted in Fig. 55b. There is no overlap of this scatter with the one for N. swainei (Fig. 54b).

Carotenoid	Attenuance Min.	Bile Chromoprotein Max.
55.1(53.0-58.1)%	13.2(11.9-15.6)%	31.6(29.7-33.1)%

Attempts to identify this species using Ross (1955) yielded uncertain results and Ross (personal communication) was unable to make a determination. The life history fits that of N. hetricki, but the female adults resembled those of N. pinirigidae and/or N. excitans. Schaffner (1943) gives the head colour of N. pinirigidae larvae as red to dark reddish brown (similar to that of N. swainei) while the larval head colour of the species under discussion is almost black, consistent with the description of N. hetricki by Hetrick (1956). The scopal processes of female adults, however, were not as described by Ross (1955) in that they did not generally have their apices curved mesad. A tendency toward this latter condition was noted in a few instances. The species has been identified as possibly N. hetricki for present purposes.

Material received from Florida and identified as N. excitans also proved difficult. It has been treated as two entities in this work. A small number of females from Welaka were morphologically distinct from the remainder of the females examined. These Welaka adults compared "in all respects with the two female paratypes of excitans" on loan to the Canadian National Collection from the United States National Museum (H. E. Milliron, personal communication). This identification has been accepted and the remainder of the Florida material has been called simply Neodiprion species. Both groups had

green eggs (Fig. 19), but the egg spectra are not identical (Fig. 55a). The triangular co-ordinates of three spectra of excitans and 13 of Neodiprion sp. are as follows:

	Carotenoid	Attenuance Min.	Bile Chromoprotein Max.
N. excitans	45.4(45.0-45.7)%	19.4(19.0-19.9)%	35.2(34.4-36.0)%
Neodiprion sp.	50.6(47.5-53.3)%	12.3(8.8-16.3)%	37.1(35.1-38.7)%

It may be noted that all members of the N. swainei group have green eggs, but with quantitative differences in carotenoid and green bile chromoprotein content. Figure 56 shows the bile chromoprotein attenuance maxima of N. hetricki?, N. excitans, and N. swainei in more detail than previously presented, and it is obvious that the attenuance maximum has a different position in each case, although the colour is green in all three. These spectra were obtained with stable instrumental conditions and the differences were repeatable. Such differences between taxa warrant careful study. At the same time, while broad bile chromoprotein maxima have been found among Neodiprion species, the spectrum of the yellowish green eggs of Pikonema dimmockii (Cresson, 1880) (Family Tenthredinidae, Subfamily Nematinae), shown in Fig. 57, exhibits a sharp attenuance maximum similar in position to that of the green bile chromoproteins of Neodiprion species. This band is also distinctive in having a strong shoulder on its high wavenumber side. Similarity in egg colour cannot be taken to mean that the egg spectra are not quite different.

In summary of this section on the N. swainei group, it is

apparent that the relatives of N. swainei in the eastern United States are very imperfectly known, but egg colour studies combined with other information show promise of great value. In fact, this complex with its egg overwintering and cocoon overwintering species, its univoltine and multivoltine species, and its wide host range provide an excellent testing ground.

3. N. LECONTEI (FITCH, 1858)

This polyphagous, widespread, eastern species (Benjamin, 1955) has larvae that are orange-yellow in ground colour with rows of small black spots on the subdorsal and supraspiracular areas. The head colour is normally reddish orange, but occasionally larvae are found with dark brown to almost-black heads (these are usually males). The eggs are yellow (Fig. 20) and the egg spectrum (Fig. 58a) indicates a moderate-to-high carotenoid content. The scatter of triangular co-ordinates from seven runs on material from Florida is shown in Fig. 58b, and the mean values and ranges are as follows:

Carotenoid	17 kc/cm	15 kc/cm
61.5(38.9-82.9)%	29.7(17.1-38.9)%	8.7(0-22.2)%

Other spectra have been obtained from material collected in Minnesota and Ontario, but the records were not suitable for numerical analysis due to instrumental failures.

4. N. PINETUM (NORTON, 1869)

N. pinetum apparently is closely related to N. lecontei, and resembles it superficially. The two species have been confused

occasionally in the past, but there should be little difficulty in their separation. Eastern white pine, Pinus strobus Linnaeus, is the only tree species upon which N. pinetum oviposits as well as feeds. N. lecontei seldom, if ever, oviposits on white pine, but may feed on the foliage of this tree where high population densities cause larvae to travel in search of food. The larvae of N. pinetum are chalky white with black heads and the subdorsal and supraspiracular spots are usually much larger than those observed in N. lecontei. Finally the eggs of N. pinetum are pale yellow (Fig. 21) and the egg spectrum (Fig. 58a) shows evidence of a low carotenoid concentration. The triangular co-ordinates from seven spectra run on material collected at four localities in Ontario, Nova Scotia, and New Brunswick are as follows:

Carotenoid	17 kc/cm	15 kc/cm
16.5(3.4-25.4)%	60.6(51.6-72.4)%	22.9(19.4-28.2)%

The scatters of triangular co-ordinates for N. lecontei and N. pinetum are shown in Fig. 58b. No overlap is present, but the samples were small. Note that the trends of the scatters are different.

5. N. PRATTI (DYAR, 1899)

This is an eastern and central North American species, probably distributed over most of the combined ranges of Pinus banksiana Lambert, P. rigida Miller, and P. echinata Miller. Actual records show it to be present from western Saskatchewan to Nova

Scotia and south to Clinton, South Carolina. Ross (1955) divided the species into three geographic units: N. pratti pratti (Dyar, 1899), N. pratti banksianae Rohwer, 1925, and N. pratti paradoxicus Ross, 1955.

Neodiprion p. banksianae is known to occur from north central Saskatchewan, through Manitoba, northern Ontario, Minnesota, and into northwestern Wisconsin. It attacks only jack pine. Neodiprion p. banksianae and N. p. paradoxicus intergrade in a narrow zone that crosses central Wisconsin, Upper Michigan, the Manitoulin Island - Bruce Peninsula area of Ontario, and swings northeastward into Quebec where its boundaries have not been traced.

Immediately south and east of this transitional zone, N. p. paradoxicus is found on jack pine in Wisconsin, Lower Michigan, southeastern Ontario, New Brunswick, and Nova Scotia. It is recorded as attacking jack pine in the northeastern United States, but in this area it is found principally on pitch pine, P. rigida, and it appears that N. p. paradoxicus is associated mainly with the latter host species.

Neodiprion p. pratti has been found from Delaware and Maryland west into Indiana and south into South Carolina. A transitional zone between N. p. paradoxicus and N. p. pratti has not been delimited, but appears to embrace Delaware, Maryland, northern Virginia, and West Virginia. In Virginia, Virginia pine, P. virginiana Miller, and shortleaf pine, P. echinata, are the preferred hosts according to Morris, Schroeder, and Bobb (1963). McIntyre (1960)

says "Where pitch pine is abundant in Maryland and northern Virginia, the sawfly favours this species for egg-laying; and adjacent Virginia pines may be only lightly infested. However, Virginia pine is the most generally abundant of the two hosts, and pure stands of it may be subjected to severe and repeated defoliations. Shortleaf pine appears to be the favoured host in southern Virginia and North Carolina". The recent outbreak in Maryland, Virginia, and North Carolina, probably involved both N. p. paradoxicus and N. p. pratti as well as intermediate forms. There is a great need for study of N. p. pratti in the southeast.

All N. pratti larvae are black-headed: those of N. p. banksianae are striped, those of N. p. paradoxicus spotted, and those of N. p. pratti are striped. The egg colour in all three subspecies is yellow. The egg spectra (Fig. 59) indicate the presence of a moderate-to-high concentration of carotenoid. On the basis of a limited number of examinations it appears that eggs of N. p. pratti and N. p. banksianae have similar carotenoid concentrations, but that eggs of N. p. paradoxicus have a higher concentration of yellow pigment. Much additional testing is required. The triangular co-ordinates for 14 spectra of eggs from nine localities in Nova Scotia, Ontario, Maryland, Virginia, and South Carolina are as follows:

Carotenoid	17 kc/cm	15 kc/cm
42.9(26.2-64.1)%	43.7(32.8-55.7)%	13.4(3.1-24.1)%

6. N. MAURUS ROHWER, 1918

Although Atwood and Peck (1943) left little doubt as to the existence of a valid species named N. maurus, and Ross (1955) supported this view, the author was unable to identify N. maurus reasonably well until 1958. This situation resulted in part from rearing failure in what is now recognized as N. maurus and in part from a poor understanding of N. pratti.

Atwood and Peck (1943) record the distribution in Canada as within a "triangle formed by a line from Kenora to Lake Abitibi and the upper St. Maurice Valley, and extending south as far as the north shore of Lake Huron". Other published records and our own collections show that this species is also found in the jack pine areas of Michigan, Wisconsin, and Minnesota, and Forest Insect Survey material shows it to be present through Manitoba, Saskatchewan, and Alberta west of the Swan Hills. Except for a single record from Alberta on lodgepole pine, N. maurus has been found only on jack pine.

Neodiprion maurus supposedly overwinters as cocooned, prepupal larvae, but in some areas in certain years N. maurus larvae may be found in feeding groups with N. p. banksianae and N. nanulus larvae of the same developmental stage. The latter two are known to have hatched from overwintering eggs, and in most instances N. maurus occurs considerably later in the summer than N. p. banksianae. In addition it has been found that under laboratory conditions N. maurus adults may emerge either in the fall or following spring. At a constant high temperature (approx. 21°C) in the

laboratory apparently fully developed adults may remain in the cocoons for almost two months. These observations suggest that this species may overwinter as larvae in cocoons, eggs, or advanced stages of morphogenesis within the cocoons. Additional field and laboratory studies are needed to provide an understanding of this developmental problem. The field identification of overwintering N. maurus eggs should be relatively simple as they are laid very close together on the flat side of the jack pine needles while those of N. p. banksianae, also on the flat side, are more widely spaced (Atwood, 1943), and eggs of N. nanulus are laid widely spaced under the curved surface of the needles.

Larvae of N. maurus from Ontario are characterized by a distinctive pattern of black markings on a strong yellow background, while larvae from Michigan, Wisconsin, and Minnesota have the same dark markings on a white ground colour. Neodiprion maurus may have two geographical races analogous to those found in N. swainei and the existing specific trivial name would apply to the white larval race.

The egg colour in N. maurus is yellow (Fig. 22) and the egg spectrum (Fig. 59) shows moderate-to-high attenuance due to carotenoid pigment. The evidence suggests that eggs from yellow larval females contain more carotenoid than eggs from white larval females. The triangular co-ordinates of three spectra from white larval type females originating at two localities in Michigan and Wisconsin are as follows:

Carotenoid	17 kc/cm	15 kc/cm
22.4(15.8-28.2)%	58.9(55.2-65.8)%	18.6(16.0-21.6)%

No spectra of yellow larval type eggs have been obtained with the Unicam SP. 700 so the triangular co-ordinates are unknown.

7. N. TAEDAE ROSS, 1955

This is an eastern species mainly associated with loblolly pine, P. taeda Linnaeus. Ross considers that there are two subspecies: N. taedae taedae Ross, 1955 (formerly known as N. americanum (Leach)), and N. taedae linearis Ross, 1955. Hetrick (1941, 1956) described the general biology of N. t. taedae and Warren and Coyne (1958) provide similar information on N. t. linearis.

Neodiprion taedae is very similar to N. pratti except that the larvae have reddish brown heads. The body pattern on N. t. taedae larvae is spots while that in N. t. linearis larvae is stripes. The former appears to be associated with the northeastern portion of the loblolly range while the latter is found in the southwestern portion. The egg colour in both subspecies is yellow. The egg spectrum (Fig. 59) of a single run of N. t. taedae from North Carolina shows moderate-to-high carotenoid attenuance. The triangular co-ordinates of this curve are as follows: carotenoid, 27.2%; 17 kc/cm, 51.8%; 15 kc/cm, 20.9%. It may be that N. t. linearis eggs contain less carotenoid, in analogy to what is indicated for N. pratti subspecies.

8. N. VIRGINIANUS COMPLEX AND N. ABBOTI COMPLEX

These two complexes provide the most puzzling group of all the eastern Neodiprion species. Ross (1955) interpreted the group as follows:

affinis Rohwer, 1918 - Virginia rugifrons Middleton, 1933 - Ontario dubiosus Schedl, 1933 - Ontario	= virginianus Rohwer, 1918 W. Virginia
americanus Leach, 1817 - Georgia fabricii Leach, 1817 - Georgia rileyi Cresson, 1880 - Florida eximina Rohwer, 1912 - Wisconsin ferrugineum Middleton, 1933 - Ontario flemingi Peck, 1943 - Quebec	= abboti (Leach, 1817) Florida
	nigroscutum Middleton, 1933 Ontario
lateralis Cresson, 1880 - Georgia lanielensis Peck, 1943 - Ontario	= compar (Leach, 1817) Georgia

Analysis of several years' data and the examination of new material from the southeastern United States now permit some suggestions regarding the species composition of these complexes. All the suggestions are of a preliminary nature and only point to the direction of future work.

There appears to be a natural division based on oviposition behaviour. In the first group, including N. virginianus

and probably N. ferrugineum, the eggs are clustered by the females and the larval feeding groups are large. In the second group, which includes N. compar, N. nigroscutum, and most of N. abboti sensu Ross, the females scatter the eggs, seldom laying on more than a single needle at one place. This results in very small feeding clusters, and late stage larvae are often found feeding individually. There is also a tendency for cocoon spinning to take place on the trees.

Orange-brown headed larvae with either a supraspiracular band or a row of distinct supraspiracular spots on a white background that have been collected in Ontario, Michigan, Wisconsin, and Minnesota appear to be referable to N. rugifrons placed as a synonym of N. virginianus by Ross. Figure 60 shows the distribution of larval pattern types in Ontario and the Lake States. It is noticeable that the spotted type is the more common one with the banded form being represented more strongly in the area of Ontario just northeast of Lake Superior. This species as here recognized is restricted to jack pine and is found across Ontario, west into Manitoba (possibly into Saskatchewan as suggested by Peck, 1943), and east through Quebec. It is also distributed throughout Minnesota, Wisconsin, and Michigan. In Ontario and the Lake States N. rugifrons females generally have black abdominal tergites, but N. dubiosus is considered to be a lighter coloured variant. The eggs of N. rugifrons contain a low concentration of carotenoid and a high concentration of purple bile chromoprotein as indicated by

spectra for material from both spotted and striped larval types (Fig. 62a). The triangular co-ordinates based on 15 runs from material collected at five localities in Ontario and Michigan are as follows:

Carotenoid	Attenuance Min.	Bile Chromoprotein Max.
38.0(36.6-39.5)%	29.6(28.8-30.3)%	32.4(31.2-33.4)%

Material from the eastern United States is required to determine if the names N. affinis and N. virginiana also apply to this species as suggested by Ross.

Orange-brown headed larvae with either a supraspiracular band or a row of distinct supraspiracular spots on a yellow background that have been collected in Ontario, Michigan, Wisconsin, and Minnesota have been referred to tentatively by the name N. ferrugineum. Figure 61 shows the distribution of larval pattern types in Ontario and the Lake States. The striped form is the more common at the northeastern boundary of Ontario, while the spotted form is more common at the western boundary. Only the latter type was collected in the Lake States, but additional work is likely to reveal some striped or intermediate forms. The sympatric relationship of N. rugifrons and N. ferrugineum, both on jack pine, is indicative that they are distinct species. Becker and Benjamin (1963) recognized the two types, but suggest that in Wisconsin the two are allopatric with the white larval type being found in the south only, and the yellow larval type in the north of the State. My observations

indicate that the yellow larval type may be restricted to northern Wisconsin, but that the white larval type occurs throughout the State. In Ontario and the Lake States the females of N. ferrugineum have reddish brown abdominal tergites. The eggs (Fig. 23) of this species in the central North American area under discussion have spectra (Fig. 62a) that indicate a low carotenoid concentration and a high purple bile chromoprotein level. The carotenoid content in N. ferrugineum eggs is somewhat higher than in N. rugifrons. Triangular co-ordinates from 19 specimens originating at 10 localities in Ontario, Michigan, Wisconsin, and Minnesota are as follows:

Carotenoid	Attenuance Min.	Bile Chromoprotein Max.
42.0(39.6-44.2)%	28.1(27.1-29.0)%	29.9(28.5-31.3)%

The scatter of co-ordinates for N. rugifrons and N. ferrugineum eggs shows a slight overlap (Fig. 62b).

The evidence at hand suggests that N. ferrugineum is a widespread, polymorphic, northern species with possibly three more or less distinct geographical units. The information presented here refers to the central unit only.

Forms in which the females spread the egg complement widely may represent at least five species. Material collected in the southeastern United States produced adults so different from members of the N. abboti complex in the north that it seems unlikely only a single species is represented. Different collections of southern material produced females with green eggs (Fig. 24) and

females with purple eggs (Fig. 25). Their egg spectra (Fig. 63) differ accordingly. Dimorphism in bile chromoprotein type has not been observed, so perhaps a pair of sibling species is involved. Triangular co-ordinates for the two based on seven spectra of the green-egg type from two localities in North and South Carolina and four spectra of the purple-egg type from two localities in South Carolina and Georgia are as follows:

Type	Carotenoid	Attenuance Min.	Bile Chromoprotein Max.
green	43.8(42.0-46.6)%	20.0(17.9-21.7)%	36.0(34.9-36.7)%
purple	36.7(36.4-37.3)%	29.9(29.6-30.0)%	33.4(33.0-33.6)%

The names available for the southern taxa are N. abboti, N. americanus, N. fabricii, and N. rileyi.

No living material of N. compar from the south has been studied, but it appears that Ross' placing of N.lanielensis as a synonym is valid. The eggs of N. compar are green (Fig. 26) and the egg spectrum (Fig. 63) indicates a moderate concentration of carotenoid and a high level of green bile chromoprotein. In the north N. compar is associated with jack pine and red pine, P. resinosa Aiton. The host trees in the south have not been documented.

The remainder of the northern forms may represent two species: N. nigroscutum, primarily associated with jack pine; and N. eximina (flemingi), found mainly on red pine. A spectrum of green eggs of the latter type is shown in Fig. 63. Evidence suggests

that N. nigroscutum may have yellow eggs, but there is also a strong possibility of green-yellow dichromatism in one or both of these species.

9. N. SERTIFER (GEOFFROY, 1785)

This species is the only known Palaearctic representative of the genus Neodiprion. A map published by the Commonwealth Institute of Entomology (Anonymous, 1959) shows it to be present throughout most of Europe, Korea, and Honshu (Japan), but does not indicate its presence in Siberia. Pschorn-Walcher (in press) cites a reference by Pavlovskiĭ and Shtakel'berg (1955) to its existence in Siberia as a pest of Pinus cembra Linnaeus (equivalent to P. sibirica Mayr?) reported by Florov, 1951. Neodiprion sertifer was first found in North America near Somerville, New Jersey, in 1925 (Schaffner, 1943), and its current distribution is fairly well represented on the map already cited. A map of N. sertifer distribution in the United States (Anonymous, 1959) does not appear to be completely trustworthy, particularly with respect to the presence of this species in western South Dakota. The history of spread of N. sertifer in eastern America is clearly related to recent introduction.

The altitudinal distribution of N. sertifer is from near sea level to 8,500 - 9,000 feet above sea level in the Japanese Alps, and to 6,000 - 6,500 feet above sea level in the High Swiss Alps (Pschorn-Walcher, in press).

In Europe at low elevations the principal host tree of

N. sertifer is Scots pine, P. sylvestris, according to Pschorn-Walcher, and Austrian pine, P. nigra Arnold, is also heavily attacked. The erect form of mugho pine, P. mughus Scopoli, is infested at lower elevations and its dwarf form at high altitudes. Swiss stone pine, P. cembra, is also a host at high elevations in the central Alps.

In Japan at lower elevations the main host trees are Japanese red pine, P. densiflora Siebold et Zuccarini, and Japanese black pine, P. thunbergii Parlatore, while in alpine habitats dwarf Siberian pine, P. pumila Regel, is the host plant.

The chief native host trees in North America are red pine, jack pine, and pitch pine (Ross, 1955).

The egg colour of European and North American populations is shown in Fig. 27, and the egg spectrum in Fig. 64a. A low carotenoid concentration and a high purple bile chromoprotein concentration are evident. The egg colour of Japanese material appears yellow (Fig. 28) and uniform to the eye. Spectra of eggs of Japanese origin (Fig. 64a) indicate the presence of two types: one, which is called "Japan normal" in analogy with the green eggs of N. swainei, with a low carotenoid content and a low purple bile chromoprotein level; and the other, called "Japan yellow" in analogy with the yellow-egg type of N. swainei, in which bile chromoprotein cannot be detected. The triangular co-ordinates based on 74 spectra representing 19 localities in Austria, Canada, Germany, Japan, and Latvia are as follows:

Source	Carotenoid	Attenuance Min.	Bile Chromoprotein Max.
Europe, N. A.	38.4(35.4-42.2)%	29.6(27.7-31.3)%	32.0(29.7-34.2)%
Japan, normal	51.0(46.2-56.6)%	24.7(23.0-26.9)%	24.3(20.4-28.2)%
Japan, yellow	63.1(62.2-65.0)%	21.9(21.3-22.3)%	15.0(13.7-15.8)%

The co-ordinates are plotted in Fig. 64b and show three distinct groups: one corresponding to material of European-North American origin; and the two others relating to specimens from Japan, the division being dependent upon the presence or absence of bile chromoprotein. Additional spectra obtained recently from material collected in New Jersey appear to be identical with other spectra in the European-North American group, and, while as yet no spectra have been run, the egg colour in specimens from Sofia, Bulgaria, appears to correspond with that in other European collections.

The points in the European-North American group in Fig. 64b represent elevations of under 45 feet above sea level in Latvia to over 5,000 feet in the Grimsel Pass, Swiss Alps. The host trees represented are Scots pine, Austrian pine, mugho pine, red pine, and jack pine. Figure 65 is an expansion of the co-ordinate scatter coded according to source in Europe and North America. There is no tendency for discrete groupings according to country of origin and the variation on the carotenoid axis shown by Canadian material encompasses the spread on this axis exhibited by specimens from all other sources. A similar expansion coded according to host tree is shown in Fig. 66. It may be noted that, while

the small number of points for red pine and for jack pine, respectively, appear to be segregated and at extreme positions in the total scatter, the whole range of variation is covered by specimens collected on Scots pine.

In the material from Japan there do not seem to be differences in egg colour between specimens from the lower elevations mainly on Japanese red pine and those from the high mountain sites on dwarf Siberian pine. Material of the Japanese type has not been reared on European or North American hosts for test purposes. Female adults from Japan key to N. sertifer satisfactorily and appear grossly similar to European specimens (Fig. 29). The male adults from Japan, on the other hand, have all-black abdominal sternites instead of the orange band displayed by European males (Fig. 29). The Japanese type apparently also differs in that it possesses a yellow-egg variant (genetically controlled as in N. swainei?) while a female with yellow eggs has never been seen in the course of handling thousands of living European and North American females.

Two points seem certain: first, N. sertifer in eastern North America is referable to the same species in Europe, and second, that these differ from what is recognized as N. sertifer in Japan. The crucial evidence required is what N. sertifer is like in Korea and across Siberia. It is almost certain that there is a continuous distribution across Siberia on the basis of the single record already mentioned and the fact that Scots pine and dwarf

Siberian pine (a species closely related to Swiss stone pine of the European Alps and the Russian "cedar", P. sibirica Mayr) form a complete host chain.

At low elevations the life history of N. sertifer is constant, including Japan, although in Europe there appear to be differences dependent upon latitude. Seitner (1933), however, described high-alpine populations of N. sertifer that apparently overwinter in the cocoon rather than as eggs. Pschorn-Walcher (in press) supports this view and indicates that alpine populations in Japan may not have the normal low latitude, low elevation, life cycle. If populations having a different breeding season do exist in alpine habitats, then these represent sibling species, since complete reproductive isolation is involved. Very little is known about the factors regulating diapause in sawflies and interpretation of the altitudinal and latitudinal differences in developmental sequence must await additional field studies and laboratory tests such as the ones reported by Wallace and Sullivan (1963).

Ross (1955) suggested that N. sertifer is of western North American origin and has achieved its Eurasian distribution following dispersal across the Bering Land Bridge (Hopkins, 1959). Thus the close relatives of N. sertifer may be expected to exist among the western species of Neodiprion. A colony of larvae collected on ponderosa pine, P. ponderosa Douglas, in southwestern South Dakota in 1963 produced female adults very similar to those of N. sertifer. The egg colour appeared identical to that of

European N. sertifer and, were it not for larvae quite different from those of N. sertifer, confusion in identification possibly could take place. The material can be referred to the N. fulviceps complex. I believe that similar specimens identified in the adult stage only may be responsible for the N. sertifer records indicated for western South Dakota. The most westerly valid records of European N. sertifer dispersal from New Jersey is likely south-eastern Iowa.

10. N. NANULUS SCHEDL, 1933

Ross (1955) divided this transcontinental species into two units, N. nanulus nanulus Schedl, 1933 on jack pine and red pine east of the 100th meridian of longitude and N. nanulus contortae Ross, 1955 on lodgepole pine west of the 100th meridian. Ross believed that the two populations are separated by a wide gap through Alberta, Saskatchewan, and Manitoba. Forest Insect Survey material proves this false.

Neodiprion n. nanulus has been quite thoroughly investigated in Ontario and the Lake States. Atwood (1943) recognized that host preference with respect to jack or red pine varies between areas, but could not detect any morphological differences in adults of the two populations. Kapler and Benjamin (1960), working in Wisconsin, found a strong preference for oviposition on red pine even with adults that had been reared on jack pine. W. Y. Watson (personal communication) suggests that the females from red pine populations are darker than those from jack pine. The egg colour

is green in females from either jack or red pine, but in the case of the red pine type (Fig. 30) the appearance is more greenish. The egg spectra (Fig. 67a) indicate differences between the two types with the red pine form having a lower carotenoid concentration and a higher concentration of green bile chromoprotein. The triangular co-ordinates from 51 spectra of material from jack pine and 33 spectra from red pine material, representing 51 localities in Ontario, New Brunswick, Nova Scotia, Michigan, Wisconsin, and Minnesota are as follows:

Source	Carotenoid	Attenuance Min.	Bile Chromoprotein Max.
jack pine	52.5(48.0-58.3)%	22.9(19.9-24.8)%	24.6(21.7-28.4)%
red pine	48.8(39.2-53.6)%	23.0(20.8-25.9)%	28.2(25.3-29.9)%

When all the points are plotted according to host (Fig. 67b), an overlap in the triangular co-ordinates of egg spectra for the two types is evident. Preliminary studies suggest that the overlap could result from cross feeding. All the available information points to host races in an incipient state of development. The change-over from jack pine to lodgepole pine in Alberta may involve the same sort of differences.

11. N. ABIETIS COMPLEX

Populations assigned to this complex have broad geographical and host ranges, being associated with species of Abies, Picea, Pseudotsuga, and Tsuga; and occurring north and south along the western cordillera, transcontinentally in the north, and probably

along the Appalachians in the east. Very little is known about any part of the complex.. Suggestions have been made that populations on Picea differ from those on Abies, but Ross (1955) states that the adults are indistinguishable.

In the present work, two egg colour types were detected by the triangular plot method (Fig. 68a). Figure 31 shows the more yellow or B type, the spectrum (Fig. 68b) of which indicates a moderate carotenoid content and a low-to-moderate purple bile chromoprotein level. Type A eggs have a higher concentration of bile chromoprotein as shown by their spectrum (Fig. 68b). Seventy-three spectra from specimens fed on white spruce, Picea glauca, (Moench) Voss, black spruce, P. mariana (Miller) Britton, Sterns and Poggenberg, red spruce, P. rubens Sargent, and balsam fir, Abies balsamea (Linnaeus) Miller, collected at 23 localities in Nova Scotia, Ontario, and Upper Michigan give triangular co-ordinates as follows:

	Carotenoid	Attenuance Min.	Bile Chromoprotein Max.
Type A (N=55)	49.8(45.0-55.4)%	24.4(21.6-26.8)%	25.8(22.6-28.4)%
Type B (N=18)	41.4(38.5-43.0)%	28.1(26.6-29.5)%	30.5(29.1-32.0)%

Type A egg pigmentation occurs in populations on both spruce and fir, while Type B pigmentation is strongly associated with balsam fir. Insufficient samples were tested to determine the relative regional abundance of the two types. W. Y. Watson (personal communication) found a morphological separation of the female adults that follows the division suggested by egg colour.

Host races are clearly indicated by the following observations. Eggs collected on balsam fir on Manitoulin Is. (Ontario) were allowed to hatch and then each colony of young larvae was divided into three parts, one to be fed on balsam fir, one on white spruce, and the last on black spruce. Shortly after the transfers were made it was apparent that the larvae on white spruce were doing much better than those on either balsam fir or black spruce, showing much better development. Larval survival on white spruce was about 85 per cent and on black spruce and balsam fir it was about 30 per cent. The adults from the white spruce rearings were larger than those from the other two lots. Finally, the egg spectrum was found to be of Type A, normally associated with either spruce or fir. The second observation was made in an N. abietis outbreak on balsam fir near Manistique, Michigan, where a young balsam fir was found growing up through the lower branches of a larger white spruce. The balsam fir had been heavily attacked by N. abietis yet there was no evidence of any attack on the white spruce. The egg spectrum of material collected on the balsam fir was of the B type.

12. N. VENTRALIS ROSS, 1955

Five spectra from specimens collected as larvae on ponderosa pine at Boulder, Colorado, give triangular co-ordinates as follows:

Carotenoid	Attenuance Min.	Bile Chromoprotein Max.
56.9(55.9-58.3)%	11.5(10.7-13.9)%	31.6(29.9-33.3)%

The eggs are green in colour (Fig. 32) and the spectrum (Fig. 69a) indicates moderate-to-high levels of both carotenoid and bile chromoprotein. The scatter for the five runs is shown in Fig. 69b with data for two other western species.

13. N. BURKEI MIDDLETON, 1931

Four spectra (Fig. 69a) for the green eggs of this species were obtained from material collected on lodgepole pine at one locality in British Columbia.

Carotenoid	Attenuance Min.	Bile Chromoprotein Max.
47.6(47.2-48.0)%	21.2(20.8-21.5)%	31.2(31.0-31.4)%

14. N. TSUGAE MIDDLETON, 1933

Spectra (Fig. 69a) from the green eggs of two females, one each from western hemlock, Tsuga heterophylla Rafinesque-Schmaltz) Sargent, at two localities in British Columbia give the following triangular co-ordinates:

Carotenoid	Attenuance Min.	Chromoprotein Max.
45.3(44.9-45.7)%	22.4(21.7-23.1)%	32.2(31.9-32.6)%

VIII. CONCLUSIONS AND GENERAL DISCUSSION

The material presented in the foregoing sections allows the following conclusions to be drawn.

1. A relatively simple spectrophotometric method provides reliable, objective data for egg and fat body pigmentation studies.
2. Egg pigmentation is a characteristic of conifer sawflies valuable for taxonomic purposes.

The data suggest that egg colour variation is a particularly strong indicator of intraspecific categories, which often show quantitative differences in the pigments deposited in the eggs. For example, the two forms of Neodiprion abietis complex in eastern Canada, and the red pine and jack pine forms of Neodiprion nanulus nanulus are both instances where obvious quantitative differences in egg pigments provide the clearest evidence discovered to date that the suspicions of taxonomic complexity voiced by earlier workers were likely well founded. In the case of Neodiprion lecontei and Neodiprion pinetum, the knowledge of constancy in fat body pigmentation allows a rapid identification of the two species in the larval stages, and the two also show distinct quantitative differences in the carotenoid content of the eggs.

Among the European species that were studied, it is interesting to consider the case of Diprion simile and Diprion pini.

Although the larvae of these two species are readily distinguished, the adults cannot be separated on the basis of gross appearance. Suppose that one wishes to determine the proportion of females of each species in cocoon collections from mixed infestations (a commonplace occurrence in central Europe). Unless the egg colour difference between the two species is known, a genitalic preparation for each female would have to be made. Determination of the two species employing egg colour does not involve a complicated, time consuming procedure, or any instrumentation, and may be achieved without even killing the female adults in this instance where the abdominal sternites are not strongly pigmented. At most, only a slit in the abdomen is required.

Qualitative differences in the egg carotenoids or bile chromoproteins appear to be more indicative of specific separation, but instrumental problems prevented a detailed investigation. No instance was found, however, where two distinct bile chromoprotein types were present in a single, well delimited species. The preliminary tests on the in vivo position of the green bile chromoprotein maximum in different taxa of the Neodiprion swainei group also shows promise.

The colours of laid eggs, while not as distinctive in general as those of unlaid eggs, may be used in conjunction with information such as the species of tree upon which the eggs are laid, the seasonal occurrence and state of development of the eggs, and the pattern of eggs in the needles (Ghent, 1955, 1959, and

and Ghent and Wallace, 1958) to produce readily usable field diagnoses such as those of Scheidter (1926, 1934) and Sturm (1942) for central European diprionid species. Knowledge of the colours of unlaid eggs in a gross sense may be used in combination with information on the general biology of taxa to produce an easy means of identifying the females of the majority of species obtained in laboratory or field rearing programmes without the use of difficult techniques or elaborate equipment.

The foregoing discussion emphasizes the use of egg colour data at the field level and has little application to museum determinations where, although it would be possible to have adequate equipment available, the specimens are usually unsatisfactory for detailed colour analyses of the eggs. The purpose of this study in any case is not to show that the sawfly taxonomist who has to make identifications on a routine basis should be expected to throw over his classical approach and methods in favour of spectrophotometric pigment analyses. The ultimate objective is by means of thorough fundamental studies to provide a sound basis for species segregation, and to find the simplest and most certain means of making routine identifications of as many life stages and forms of material as possible. Perhaps it may not be feasible to recognize all the intraspecific entities in a formal taxonomic sense, but the recognition of the existence of the different biological entities is very important to other avenues of research.

It would be misleading to create the impression that egg

pigmentation will solve all the taxonomic problems that become apparent in the Diprionidae. Very dissimilar species may have identical egg colours, and in this respect egg pigmentation is no different from other single characters suggested in taxonomic studies. Spectral measurements of egg pigments, however, provide a simple, rapid assessment of population homogeneity, suggesting areas requiring detailed investigation by other methods. The equipment necessary to provide a basic record need be little more expensive or difficult to operate than, for example, a modern, research microscope equipped with phase contrast optics. The preparation of eggs for spectrophotometric examination is not difficult and requires less time and skill than making many whole mounts used in microscopic work.

The idea of applying knowledge of chemical differences to systematics is not new, but recent advances in biochemical technique have fostered rapid growth along this line to such an extent that we have just seen the almost simultaneous publication of two books (Alston and Turner, 1963, and Swain, 1963) dealing primarily with biochemical systematics of plants. With respect to the present work, it is obvious that the studies reported here are only a modest beginning of what may be achieved by extending the range of spectrophotometric studies and by applying other techniques such as may be indicated by the initial results. I believe that in the sawfly studies, as in all other applications of chemical differentiation to systematics, the aim should be to complement rather than replace

other perhaps older approaches, in order to give a better impression of the whole, living animal. Egg pigmentation is a very useful criterion, but it must be assessed in relation to a wide array of other characteristics. Many of the failures of the past may be traced to reliance upon a single trait.

IX. REFERENCES

Alston, R. E., and B. L. Turner

1963 Biochemical Systematics. 404 p. Prentice-Hall, Inc. Englewood Cliffs, New Jersey.

Anderson, D. C.

1957 Speedlights in the colour photomacrography of living insects. J. biol. photogr. Ass. 25: 136-139.

Anonymous

1942 Munsell Book of Colour. Pocket Edition. Munsell Color Company, Inc. Baltimore, Maryland.

1959 Commonwealth Institute of Entomology. Distribution Maps of Insect Pests. Series A, Map No. 98, Neodiprion sertifer (Geoffr.). 2 p. London.

1959 Status of some important insects in the United States. European Pine Sawfly (Neodiprion sertifer (Geoff.)). U. S. Dept. Agric. Co-op. econ. Insect Rep. 9: 497-498.

Atwood, C. E.

1961 Present status of the sawfly family Diprionidae (Hymenoptera) in Ontario. Proc. ent. Soc. Ont. (1960) 91: 205-215.

---, and O. Peck

1943 Some native sawflies of the genus Neodiprion attacking pines in eastern Canada. Canad. J. Res. "D", 21: 107-144.

Barer, R.

1955 Spectrophotometry of clarified cell suspensions. Science 121: 709-715.

Becker, G. C., and D. M. Benjamin

1963 A new look at the N. virginianus complex in Wisconsin. Ent. Soc. Amer. Proc. N. Cent. Br. 18: 62.

Benjamin, D. M.

1955 The biology and ecology of the red-headed pine sawfly. U. S. Dept. Agric. Tech. Bull. 1118, 57 p.

Benson, R. B.

1939 On the genera of the Diprionidae (Hymenoptera Symphyta). Bull. ent. Res. 30: 339-342.

1945 Further note on the classification of the Diprionidae (Hymenoptera, Symphyta). Bull. ent. Res. 36: 163, 164.

1951 Hymenoptera 2. Symphyta. Section (a). Handbooks for the Identification of British Insects 6 (2a), 49 p. R. ent. Soc. Lond.

1954 A new sawfly genus of the Monocteninae (Hym., Diprionidae) in Morocco. Proc. R. ent. Soc. Lond. "B" 23: 115-118.

Breny, R.

1957 Contribution à l'étude de la diapause chez Neodiprion sertifer Geoffr. dans la nature. Mém. Acad. R. Belg. cl. Sci. 30: 1-86.

Britton, W. E., and M. P. Zappe

1917 The Imported Pine Sawfly, Diprion (Lophyrus) simile Hartig. Conn. Agric. Expt. Sta. Bull. 203: 273-290.

Brygider, W.

1952 In what embryonic stage do the eggs of Neodiprion enter the winter diapause? Canad. J. Zool. 30: 99-108.

Charney, E., and F. S. Brackett

1961 The spectral dependence of scattering from a spherical alga and its implications for the state of the light-accepting pigments. Arch. Biochem. Biophys. 92: 1-12.

Cromartie, R. I. T.

1959 Insect pigments. In Steinhaus, E. A., and R. F. Smith (Eds.), Ann. Rev. Ent. 4: 59-76.

Curtis, J. T.

1959 The Vegetation of Wisconsin. 657 p. Univ. Wisc. Press, Madison, Wisc.

Dadd, R. H.

1961 Observations on the effects of carotene on the growth and pigmentation of locusts. Bull. ent. Res. 52: 63-81.

Dartnall, H. J. A.

1961 Visual pigments before and after extraction from visual cells. Proc. roy. Soc. "B" 154: 250-266

DuPraw, E. J., Jr.

1958 Analysis of egg colour variation in Cyclops vernalis. J. Morph. 103: 31-64.

Duysens, L. N. M.

1956 The flattening of the absorption spectrum of suspensions, as compared to that of solutions. Biochim. biophys. Acta 19: 1-12.

Eliescu, G.

1932 Beitrage zur Kenntnis der Morphologie, Anatomie und Biologie von Lophyrus pini L. Z. angew. Ent. 19: 22-67, 188-206.

Enslin, E.

1917 Die Tenthredinoidea Mitteleuropas, VI. Dtsch. ent. Z. 1937, Beiheft: 539-565.

Forsius, R.

1920 Kleinere Beitrage zur Kenntnis der Tenthredinoiden - Eier I. Medd. Soc. Fauna Fl. fenn. 45: 169-184.

Fox, D. L.

1953 Animal Biochromes and Structural Colours. 379 p. Cambridge University Press, Cambridge.

Fox, H. M., and G. Vevers

1960 The Nature of Animal Colours. 246 p. Sidgwick and Jackson Limited, London.

French, C. S.

1960 The chlorophylls *in vivo* and *in vitro*. In Ruhland, W. (Ed.), Encyclopedia of Plant Physiology, Vol. 5 The Assimilation of Carbon Dioxide Part 1, 252-297. Springer-Verlag, Berlin.

Gaussen, H.

1960 Les Gymnospermes. Actuelles et Fossiles. Fascicule VI, Chapitre XI, Généralités, Genre *Pinus*, 272 p. Toulouse, Faculté des Science, Allées Jules-Guesde.

Ghent, A. W.

1955 Oviposition behaviour of the jack-pine sawfly, *Neodiprion americanus banksianae* Roh., as indicated by an analysis of egg clusters. Canad. Ent. 87: 229-238.

1959 Row-type oviposition in *Neodiprion* sawflies as exemplified by the European pine sawfly, *N. sertifer* (Geoff.). Canad. J. Zool. 37: 267-281.

---, and D. R. Wallace

1958 Oviposition behaviour of the Swaine jack-pine sawfly. For. Sci. 4: 264-272.

Goldsmith, T. H., and L. T. Warner

1964 Vitamin A in the vision of insects. J. gen. Physiol. 47: 433-441.

Gray, C. H.

1962 Isomeric bile pigments as products of the *in vitro* fission of haemin. Nature 194: 1047,1048.

Griffiths, K. J.

1953 Variations in Morphological Characters of Some Sawflies of the Family Diprionidae. 47 p. M. A. Thesis, Univ. of Toronto. Unpublished.

1960 Oviposition of the red-headed pine sawfly, Neodiprion lecontei (Fitch). Canad. Ent. 92: 430-435.

Hackman, R. H.

1952 Green pigments of the hemolymph of insects. Arch. Biochem. Biophys. 41: 166-174.

Harlow, W. M., and E. S. Harrar

1958 Textbook of Dendrology. 4th Ed. 561 p. McGraw-Hill Book Co., New York.

Hetrick, L. A.

1941 Life history of Neodiprion americanum (Leach). J. econ. Ent. 34: 373-377.

1956 Life history studies of five species of Neodiprion sawflies. For. Sci. 1: 181-185.

Hiskey, C. F.

1955 Absorption Spectroscopy. In Oster, G., and A. W. Pollister (Eds.), Physical Techniques in Biological Research I: 73-130. Academic Press Inc., New York.

Hopkins, D. M.

1959 Cenozoic history of the Bering Land Bridge. Science 129: 1519-1528.

Jensen, A.

1960 Chromatographic separation of carotenes and other chloroplast pigments on aluminium oxide-containing paper. Acta chem. scand. 14: 2051.

---, and S. L. Jensen

1959 Quantitative paper chromatography of carotenoids. Acta chem. scand. 13: 1863-1868.

Kapler, J. E., and D. M. Benjamin

1960 The biology and ecology of the red-pine sawfly in Wisconsin. For. Sci. 6: 253-268.

Karrer, P., and E. Jucker

1950 Carotenoids (Translated and Revised by Ernest A. Braude). 384 p. Elsevier Publishing Company, Inc., Amsterdam.

Kilby, B. A.

1963 The biochemistry of the insect fat body. In Beament, J. W. L., Treherne, J. E., and V. B. Wigglesworth (Eds.), Advances in Insect Physiology 1: 111-174. Academic Press, London and New York.

Latimer, P.

1958 Apparent shifts of absorption bands of cell suspensions and selective light scattering. Science 127: 29,30.

---------, and C. A. Holmes Eubanks

1962 Absorption spectrophotometry of turbid suspensions: A method of correcting for large systematic distortions. Archiv. Biochem. Biophys. 98: 274-285.

Lawrence, D. B.

1958 Glaciers and vegetation in southeastern Alaska. Amer. Scient. 46: 89-122.

"
Love, D.

1959 The postglacial development of the flora of Manitoba: A discussion. Canad. J. Bot. 37: 547-585.

Maxwell, D. E.

1955 The comparative internal larval anatomy of sawflies (Hymenoptera: Symphyta). Canad. Ent. 87, Supplement 1, 132 p.

1958 Sawfly cytology with emphasis upon the Diprionidae (Hymenoptera: Symphyta). Proc. X. Int. Cong. Ent. (1956) 2: 961-978.

Mayr, E., Linsley, E. G., and R. L. Usinger

1953 Methods and Principles of Systematic Zoology. 328 p. McGraw-Hill Book Company, Inc., New York.

McCarthy, K. A., and S. S. Ballard

1957 6c. Absorption and Transmission, 6-36 to 6-40. In Gray, D. E. (Ed.), American Institute of Physics Handbook. McGraw-Hill Book Co., New York.

McIntyre, T.

1960 The Virginia pine sawfly outbreak 1955-1959. Northeastern For. Expt. Sta., For. Res. Note 99, 4 p.

Middleton, W.

1923 The imported pine sawfly. U. S. Dept. Agric. Bull. 1182, 21 p.

Morris, C. L., Schroeder, W. J., and M. L. Bobb

1963 A pine sawfly Neodiprion pratti pratti (Dyar) in Virginia. 42 p. Virginia Div. of Forestry.

Moss, E. H.

1949 Natural pine hybrids in Alberta. Canad. J. Res. "C" 27: 218-229.

Munroe, E.

1956 Canada as an environment for insect life. Canad. Ent. 88: 372-476.

Park, R. B., and N. G. Pon

1961 Correlation of structure with function in Spinacea oleracea chloroplasts. J. mol. Biol. 3: 1-10.

Pavlovskiĭ, E. N., and A. A. Shtakel'berg (Eds.)

1955 Vrediteli lesa: spravochnik. 2 vols., 1097 p. Izdatel'stvo Akademii Nauk SSSR, Moskva i Leningrad.

Potzger, J. E.

1952 What can be inferred from pollen profiles of bogs in the New Jersey Pine Barrens. Bartonia 26: 20-27.

1953 History of forests in the Quetico-Superior country from fossil pollen studies. J. For. 51: 560-565.

Pschorn-Walcher, H.

1964 The ecology of Neodiprion sertifer (Geoffr.) (Hym.: Diprionidae) and a review of its parasite complex in Europe. In press.

Rohwer, S. A.

1911 Japanese sawflies in the collection of the United States National Museum. Proc. U. S. Nat. Mus. 39: 99-120.

1918 New sawflies of the subfamily Diprioninae (Hym.). Proc. ent. Soc. Wash. 20: 79-90.

Ross, H. H.

1937 A generic classification of the Nearctic sawflies (Hymenoptera, Symphyta). Illinois biol. Monogr. 15(2): 173 p.

1955 The taxonomy and evolution of the sawfly genus Neodiprion. For. Sci. 1: 196-209.

1960 Two new species of Neodiprion from southeastern North America (Hymenoptera: Diprionidae). Ann. ent. Soc. Amer. 54: 451-453.

Sauer, K., and M. Calvin

1962 Absorption spectra of spinach quantasomes and bleaching of the pigments. Biochim. biophys. Acta 64: 324-339.

Schaffner, J. V., Jr.

1943 Sawflies injurious to conifers in the northeastern States. J. For. 41: 580-588.

Scheidter, F.

1926 Forstentomologische Beiträge 8. Über die Art der Eiablage der gesellig lebenden Buschhornblattwespen. Z. Pflkrankh. 36: 193-202.

1934 Forstentomologische Beiträge 30. Die Art der Eiablage der einzelnfressenden Lophyrus-Arten. Z. Pflkrankh. 44: 497-500.

Seitner, M.

1933 Lophyrus rufus Ratz. (=sertifer Geoffr.) an der Zirbe im Kampfgürtel des Waldes. Zentralbl. f. d. ges. Forstwesen 59: 129-131.

Shibata, K.

1959 Spectrophotometry of translucent biological materials - Opal glass transmission method. In Glick, D. (Ed.), Methods of Biochemical Analysis, Vol. 7, 77-109. Interscience Publishers, Inc., New York.

---, Benson, A. A., and M. Calvin

1954 The absorption spectra of suspensions of living micro-organisms. Biochim. biophys. Acta 15: 461-470.

Smith, S. G.

1941 A new form of spruce sawfly identified by means of its cytology and parthenogenesis. Sci. Agric. 21: 245-305.

Sturm, H.

1942 Untersuchungen über Buschhornblattwespen (Diprionidae). Z. angew. Ent. 29: 412-442, 601-635.

Swain, T. (Ed.)

1963 Chemical Plant Taxonomy. 500 p. Academic Press Inc., New York.

Thomson, R. H.

1960 Insect pigments. XI. Int. Kongress f. Entomologie. Verh. B. III (Symp. 3, 4): 21-43.

Wallace, D. R.

1959 Occurrence of the Swaine jack-pine sawfly and external anatomy of the mature, feeding larvae. 173 p. M. Sc. Thesis, McGill University, Montreal. Unpublished.

1961 Red pine as a host for Neodiprion swainei Midd. Can. Dept. For., Ent. and Path. Br., Bi-Mo. prog. Rpt. 17 (2): 1.

---, and C. R. Sullivan

1963 Laboratory and field investigations of the effect of temperature on the development of Neodiprion sertifer (Geoff.) in the cocoon. Canad. Ent. 95: 1051-1066.

Warren, L. O., and J. F. Coyne

1958 The pine sawfly -- Neodiprion taedae linearis Ross -- in Arkansas. Agric. Expt. Sta., Univ. of Arkansas, Bull. 602, 23 p.

West, A. S., Horwood, R. H., Bourns, T. K. R., and A. Hudson

1959 Systematics of Neodiprion sawflies. I. Preliminary report on serological and chromatographic studies. Rpt. ent. Soc. Ont. (1958) 89: 59-65.

Whittaker, J. R., and A. S. West

1962 A starch gel electrophoretic study of insect hemolymph proteins. Canad. J. Zool. 40: 655-671.

Zechmeister, L.

1962 Cis-Trans Isomeric carotenoids Vitamins A and Arylpolyenes. 251 p. Academic Press Inc., New York.

X. ILLUSTRATIONS

Fig. 1. Neodiprion swainei, ventral aspect of females: upper left, green type; lower right, yellow type.

2. N. swainei, ovary at the end of diapause.

3. N. swainei, ovary from young, white pupa.

4. N. swainei, normal, green eggs.

5. N. swainei, dorsal aspect of female white pupa, Quebec-Ontario race.

6. N. swainei, dorsal aspect of female white pupa, Wisconsin race.

7. N. swainei, dorsal aspect of female white pupae, Quebec-Ontario race, showing loss of green colour during development to produce yellow eggs.

8. N. swainei, larval types: left, Quebec-Ontario populations; right, Wisconsin populations.

9. N. sertifer, centrifuged egg.

10. Gilpinia hercyniae, centrifuged egg.

11. N. sertifer, upper group, normal eggs; lower group, heated eggs.

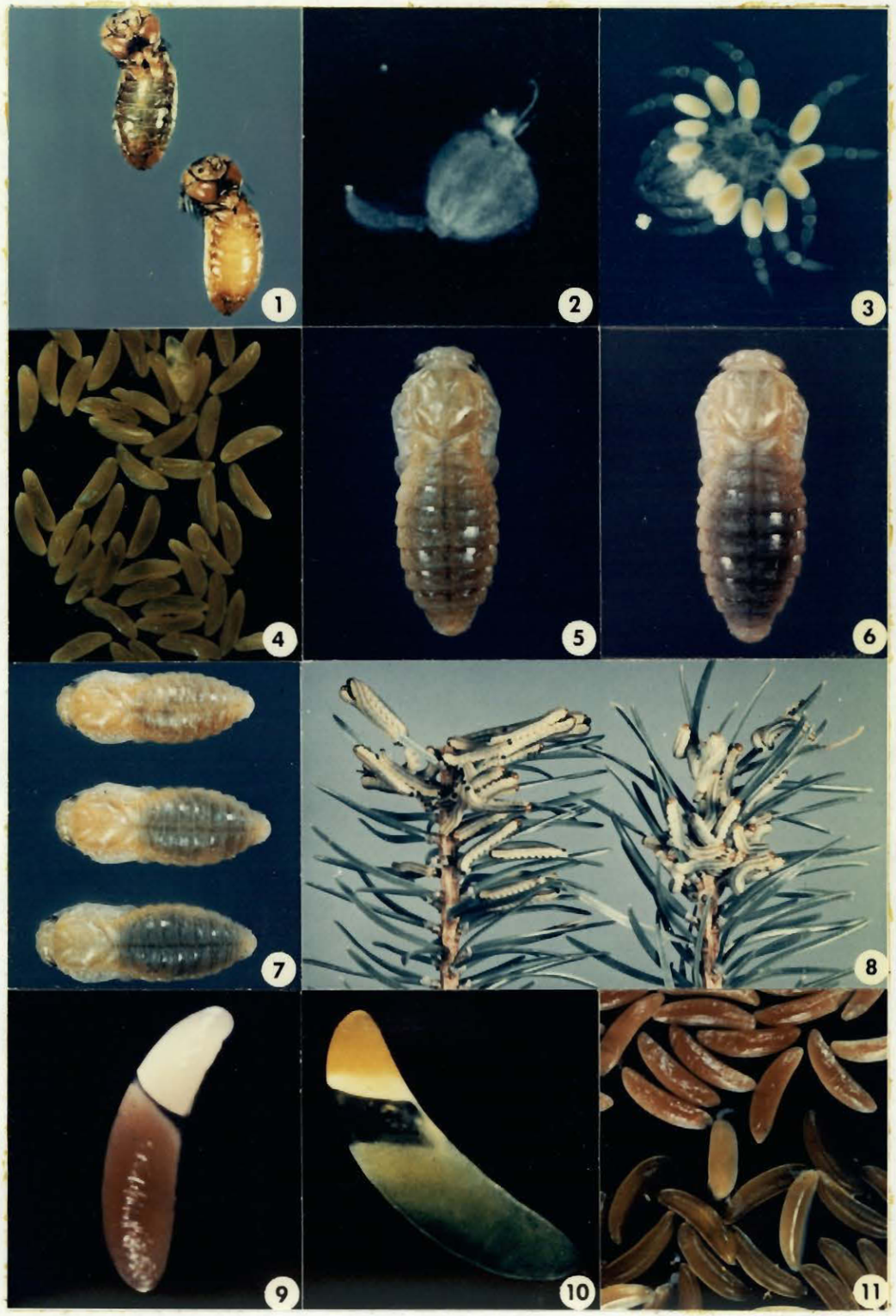
1
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Fig. 12. Diprion pini, normal eggs.

13. D. simile, normal eggs.

14. Microdiprion pallipes, normal eggs.

15. Gilpinia frutetorum, normal eggs.

16. G. hercyniae, normal eggs.

17. G. socia, normal eggs.

18. Neodiprion hetricki?, normal eggs.

19. N. excitans, normal eggs.

20. N. lecontei, normal eggs.

21. N. pinetum, normal eggs.

22. N. maurus, normal eggs.

23. N. ferrugineum, normal eggs.


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Fig. 24. Neodiprion abboti, green egg form.

25. N. abboti, purple egg form.

26. N. compar, normal eggs.

27. N. sertifer, normal eggs, Europe and North America.

28. N. sertifer, normal eggs, Japan, bile chromoprotein present.

29. N. sertifer, ventral aspect of adults: upper left, European female; upper right, European male; lower left, Japanese females with eggs containing bile chromoprotein (upper) and with yellow eggs (lower); lower right, Japanese male.

30. N. nanulus nanulus, normal eggs, red pine form.

31. N. abietis, normal eggs.

32. N. ventralis, normal eggs.


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25
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31
32

Fig. 33. Semi-integral attenuance spectra of fractions obtained in the centrifugation of diluted homogenate of N. sertifer eggs.

a. Heavy, purple sediment.

b. Yellow, fatty layer.

c. Chorions.

d. Reconstructed log semi-integral attenuance spectrum of N. sertifer eggs derived by combining the spectra shown in a, b, and c, compared with the spectrum for normal, intact eggs.

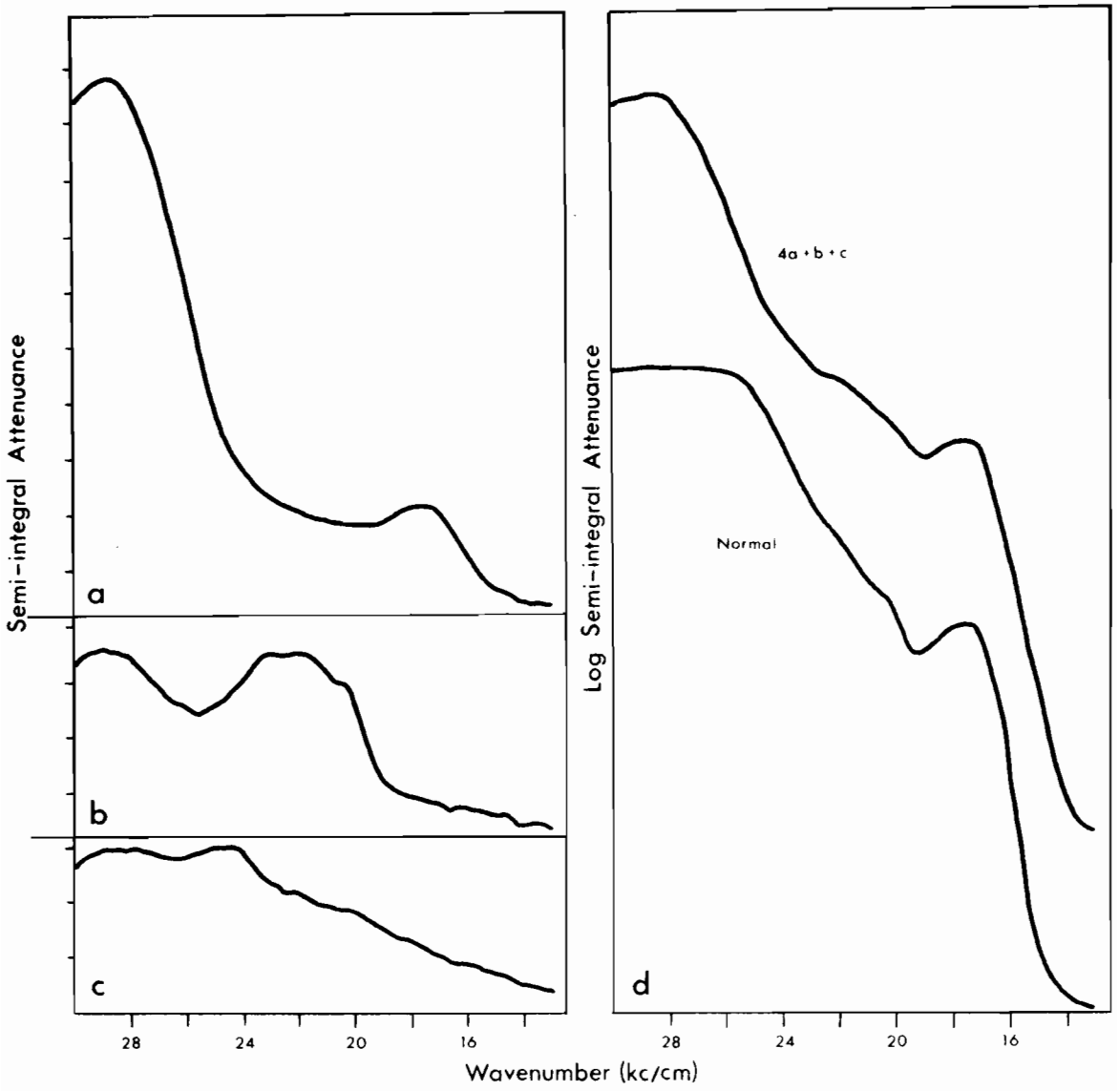

Semi-integral Attenuance
a
b
c
28
24
20
16
Wavenumber (kc/cm)
Log Semi-integral Attenuance
4a + b + c
Normal
d
28
24
20
16

Fig. 34. Log absorbance spectra of n-hexane solutions of β-carotene and carotenoids extracted from eggs of Neodiprion nanulus and Gilpinia hercyniae.

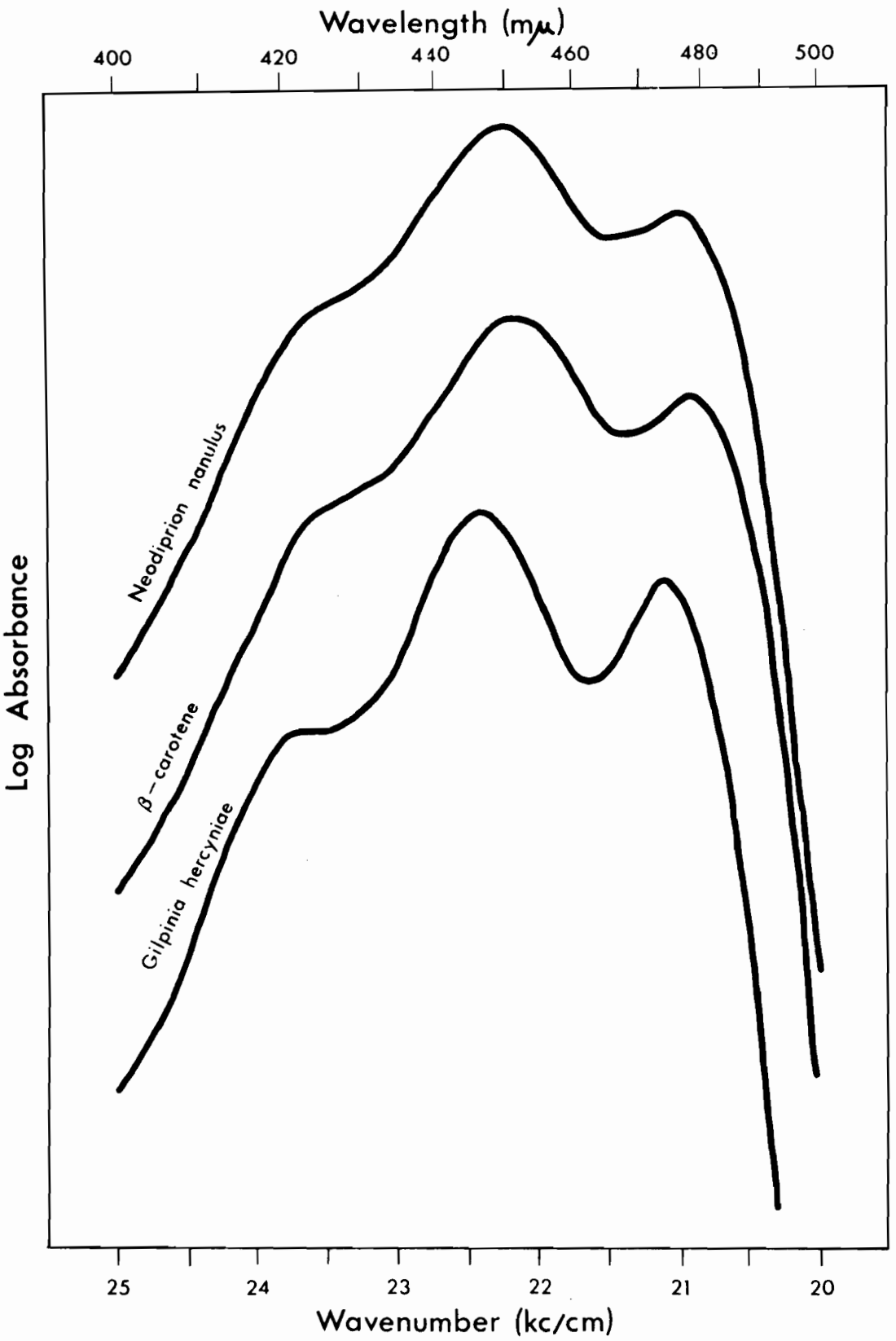

Wavelength (mμ)
400
420
440
460
480
500
Log Absorbance
Neodiprion nanulus
β-carotene
Gilpinia hercyniae
25
24
23
22
21
20
Wavenumber (kc/cm)

Fig. 35. Log semi-integral attenuance spectra of eggs of *Monoctenus* sp., *Neodiprion lecontei*, and *N. pinetum*.

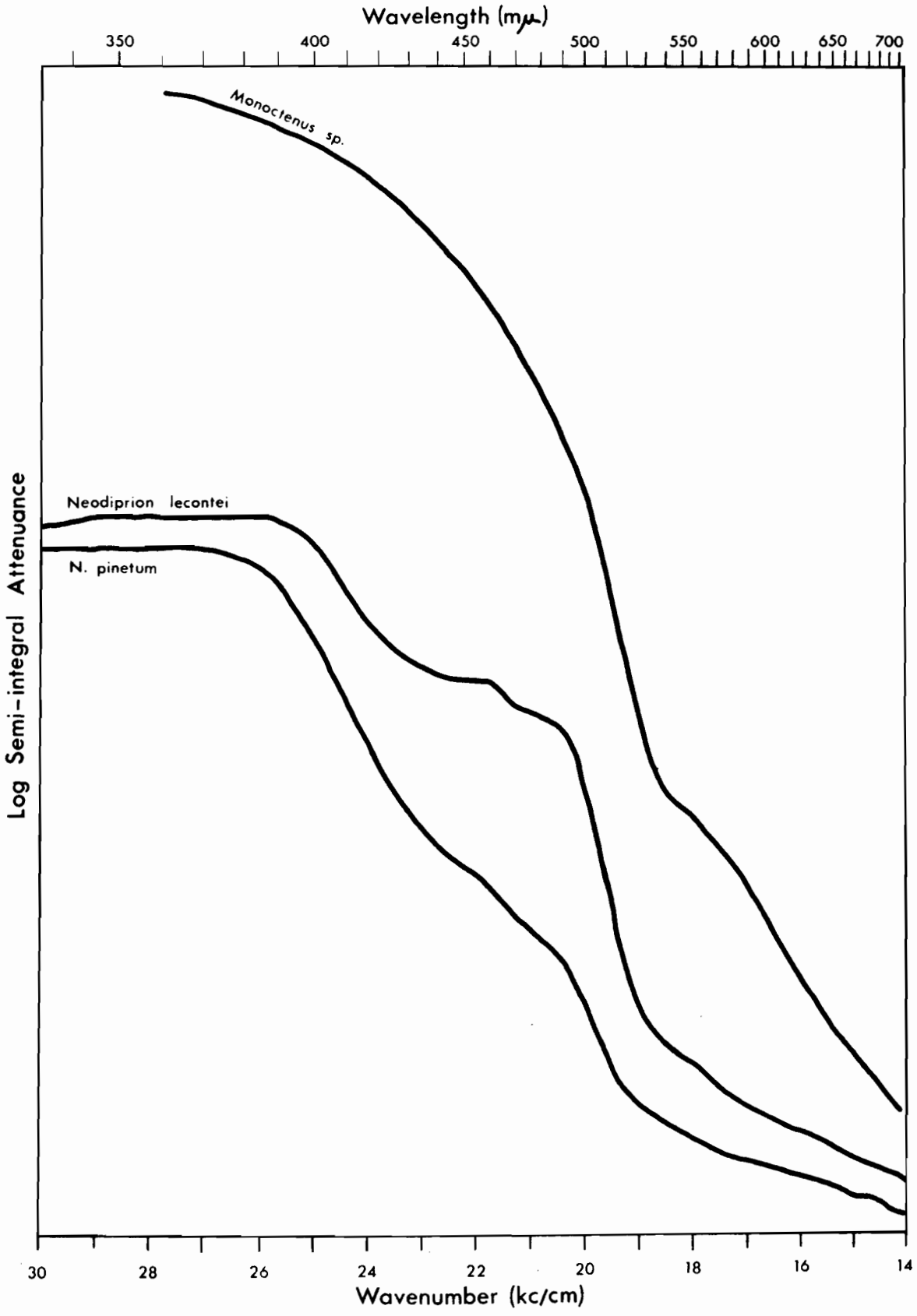

Wavelength (mμ)
350
400
450
500
550
600
650
700
Monoctenus sp.
Neodiprion lecontei
N. pinetum
Log Semi-integral Attenuance
30
28
26
24
22
20
18
16
14
Wavenumber (kc/cm)

Fig. 36. Log absorbance spectra of bilatriene compounds extracted from eggs of five species.

Solid lines - in methanol/5% HCl solution
N. sertifer, *N. "virginianus"*, *D. simile*, *N. nanulus*, *G. hercyniae*.

Dotted lines - in diethyl ether solution
N. nanulus, *G. hercyniae*.

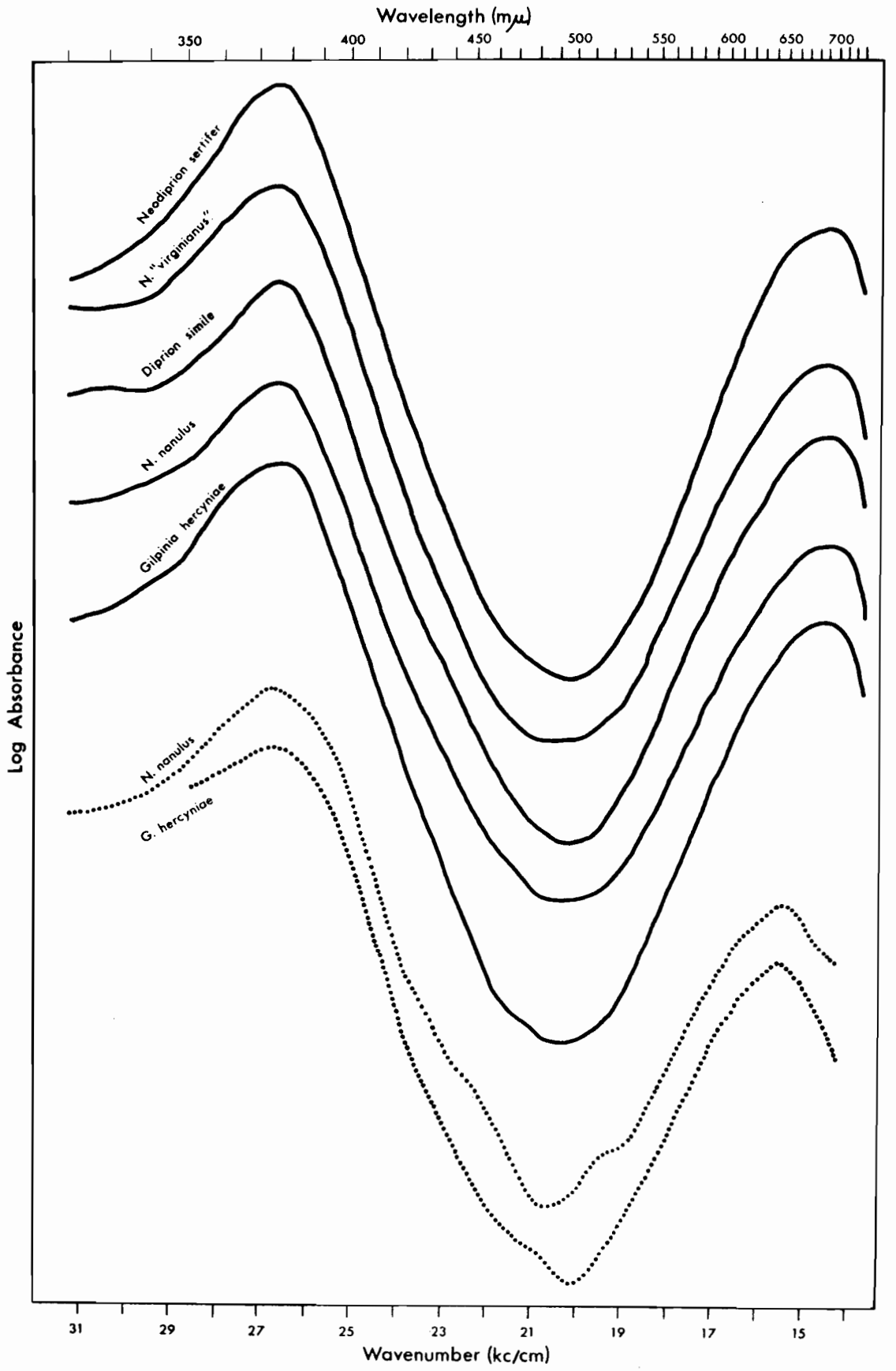

Wavelength (mμ)
350
400
450
500
550
600
650
700
Neodiprion sertifer
N. "virginianus"
Diprion similis
N. nanulus
Gilpinia hercyniae
N. nanulus
G. hercyniae
Log Absorbance
31
29
27
25
23
21
19
17
15
Wavenumber (kc/cm)

Fig. 37. Log absorbance spectra of bile chromoproteins from eggs of N. swainei and N. "virginianus" in 0.5M, pH 7, phosphate buffer.


Wavelength (mμ)
250
300
350
400
450
500
550
600
650
700
Neodiprion swainei - green
N. "virginianus" - purple
Log Absorbance
42
38
34
30
26
22
18
14
Wavenumber (kc/cm)

Fig. 38. Log semi-integral attenuance spectra of eggs of N. sertifer before and after heating.

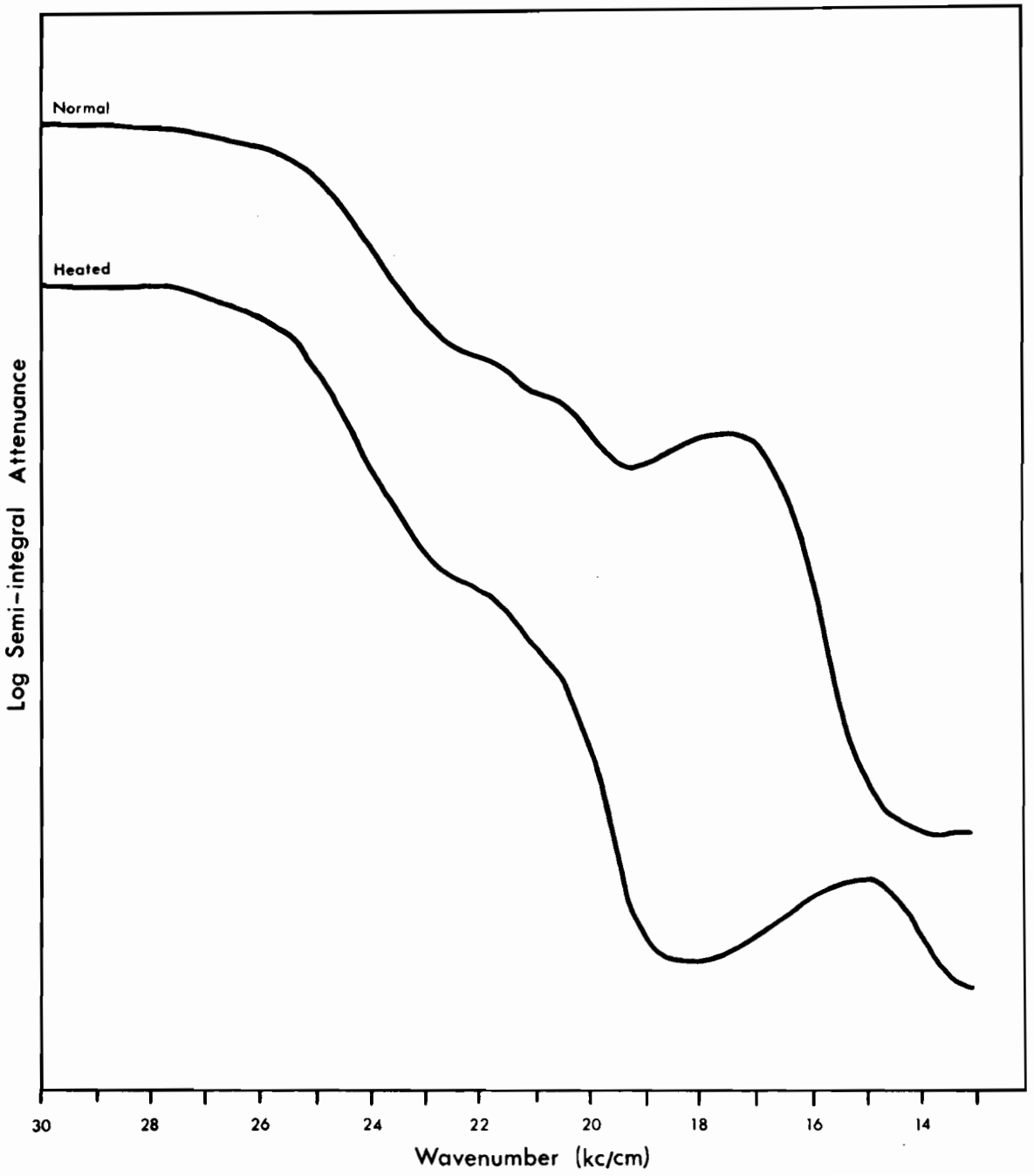
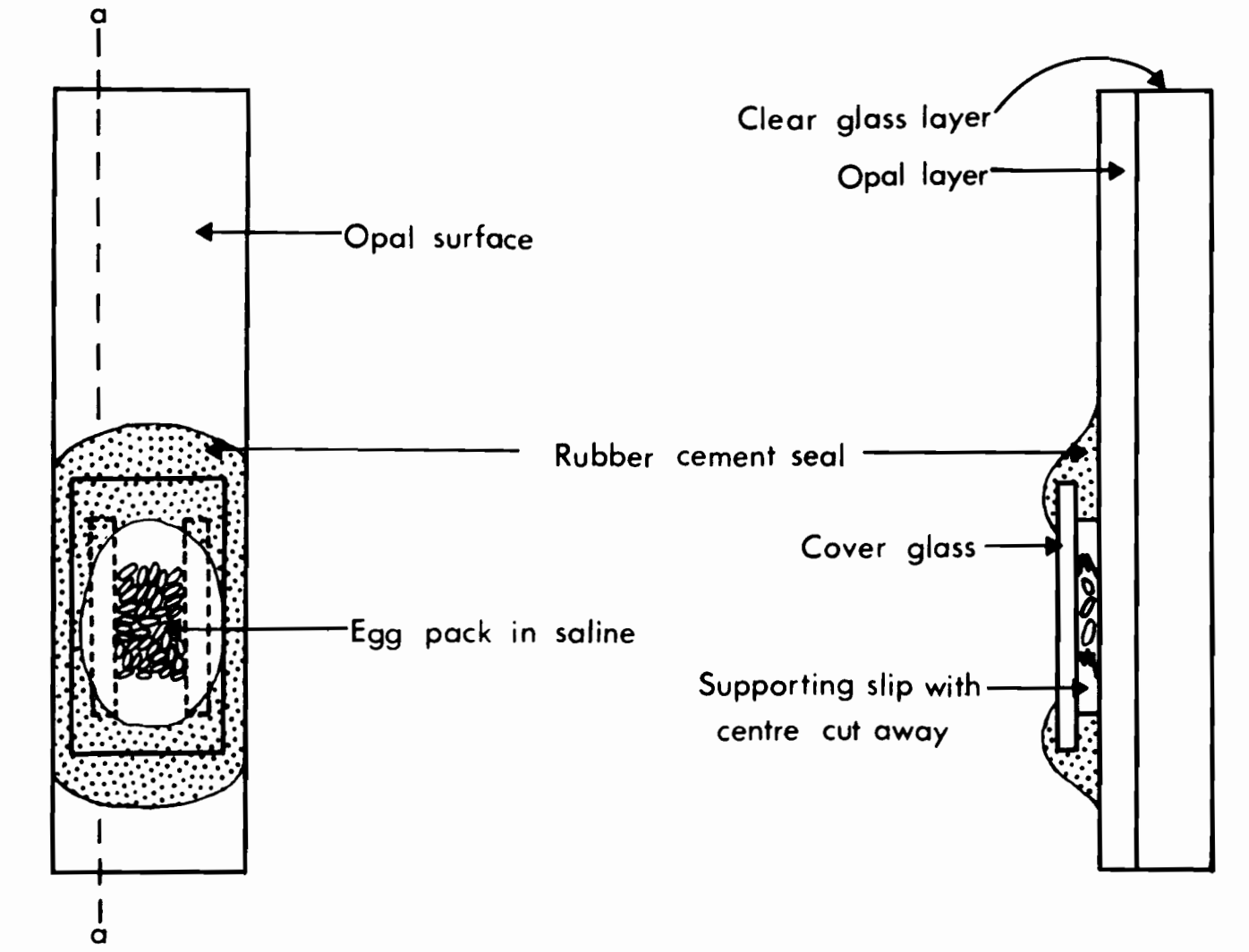

Normal
Heated
Log Semi-integral Attenuance
30
28
26
24
22
20
18
16
14
Wavenumber (kc/cm)

Fig. 39. Diagrams of the surfacial aspect and sectional view of an opal slide bearing sawfly eggs.

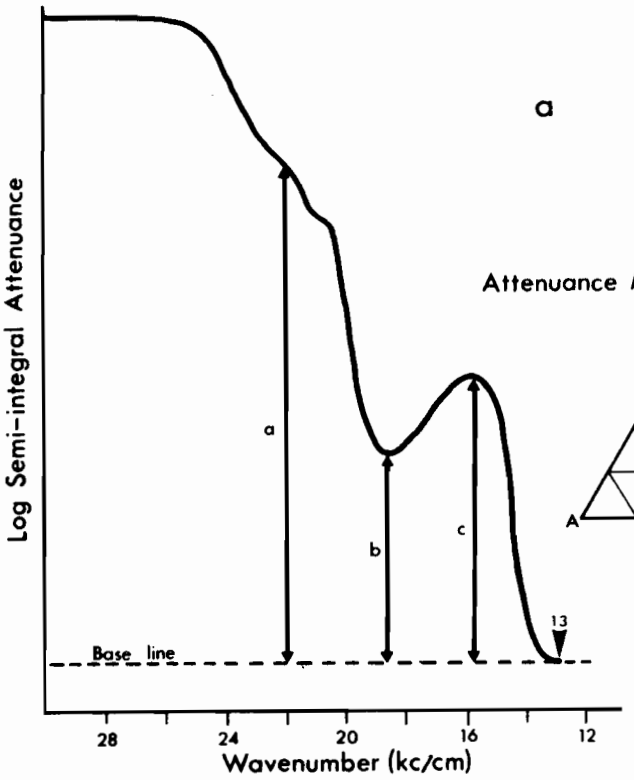


Surfacial aspect

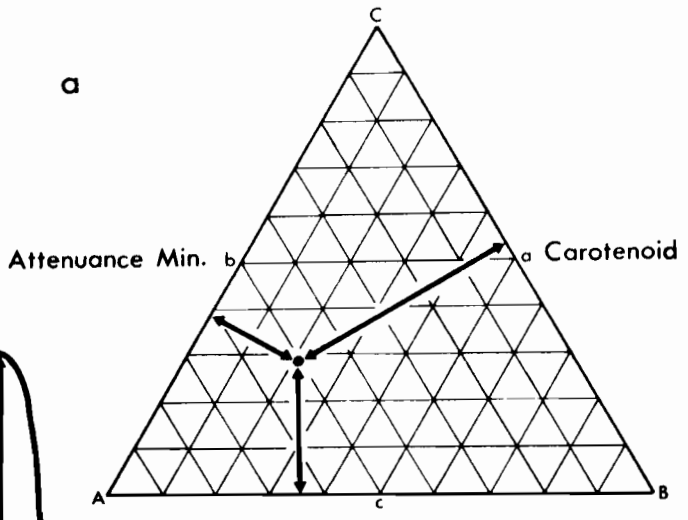
Section through a – a

Fig. 40 a. Log semi-integral attenuance spectrum of a complement of green eggs from an Ontario specimen of N. swainei, illustrating the method of calculating the triangular co-ordinates shown on the accompanying graph.

b. Log semi-integral attenuance spectrum of a complement of yellow eggs from a Minnesota specimen of N. lecontei, illustrating the method of calculating the triangular co-ordinates shown on the accompanying graph.



a

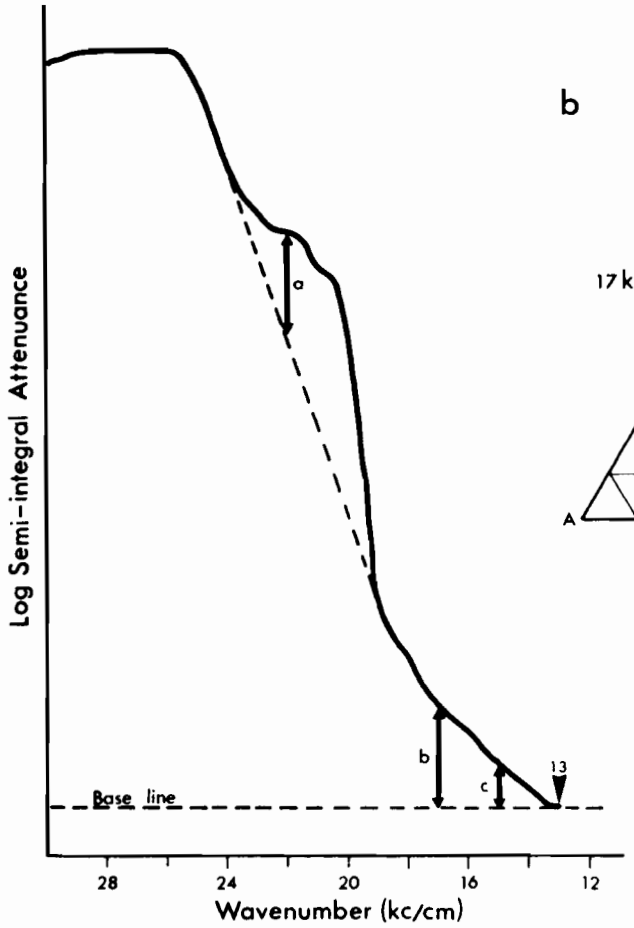


a. Carotenoid =10.85 cm. = 50.2%

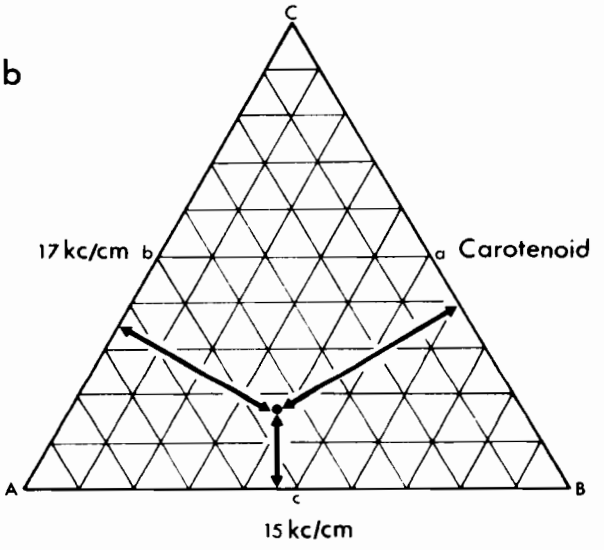
b. Attenuance Min. =4.55 cm. = 21.1%

c. Bile Chromoprotein =6.20 cm. = 28.7%

21.60 cm. 100.0%



b



a. Carotenoid = 2.45 cm. = 45.0%

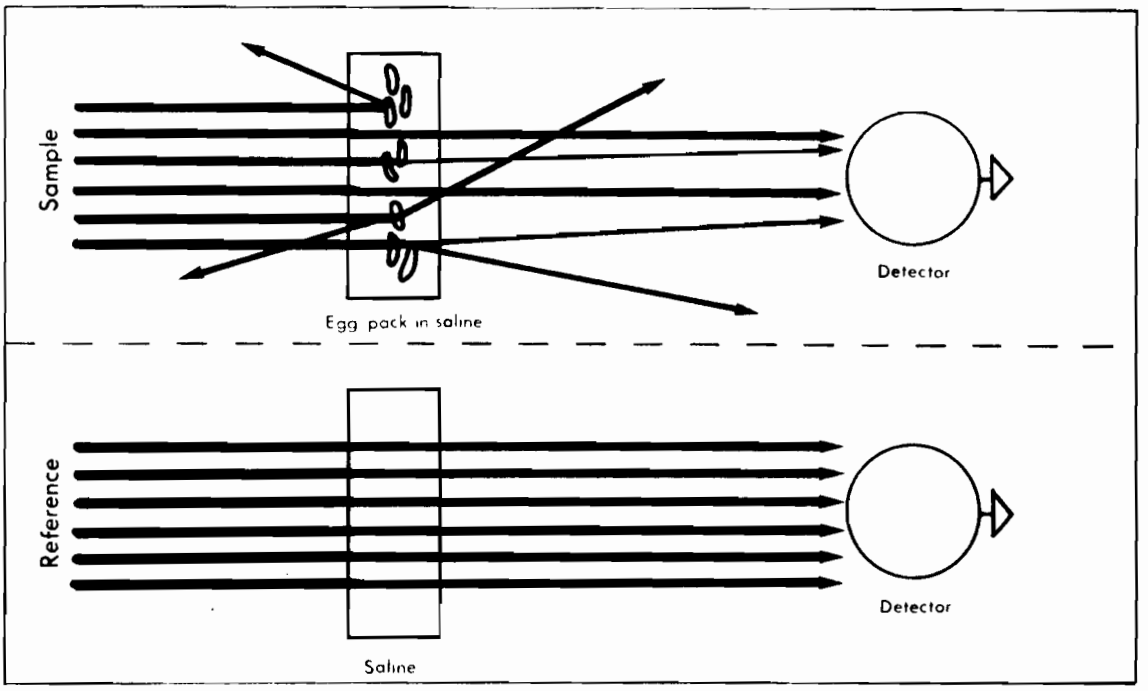
b. 17 kc/cm = 2.10 cm. = 38.5%

c. 15 kc/cm = 0.90 cm. = 16.5%

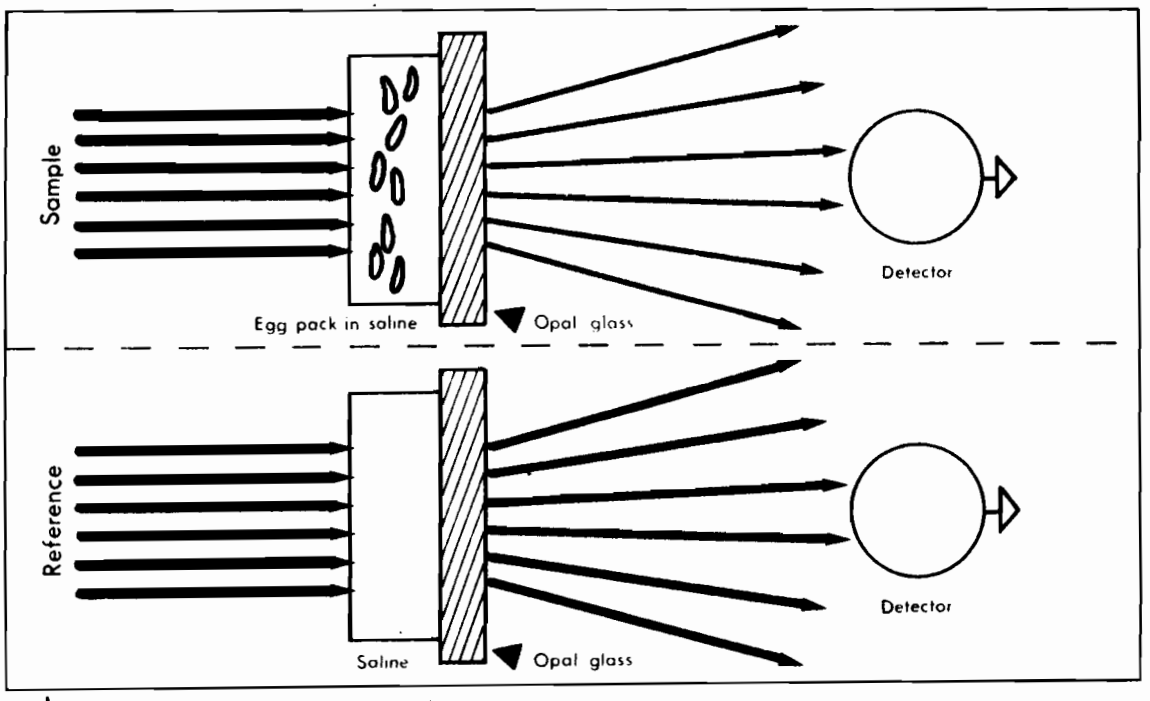
5.45 cm. 100.0%

Fig. 41. Diagrammatic representation of the spectrum of translucent, biological material being recorded in
a) a conventional spectrophotometric system.
b) a system with opal inserts.

(after Shibata, 1959)


Sample
Egg pack in saline
Detector
Reference
Saline
Detector

a


Sample
Egg pack in saline
Opal glass
Detector
Reference
Saline
Opal glass
Detector

b

Fig. 42. Semi-integral attenuance spectra of N. sertifer eggs.

a) Normal density slide, without opal inserts.

b) Normal density slide, with opal inserts.

c) Spectrum (b) corrected for residual scattering losses according to the method of Dartnall (1961), and Latimer and Eubanks (1962).

d) Quasi scattering spectrum with normal density slide.

e) High density slide, without opal inserts.

f) High density slide, with opal inserts.

g) Spectrum (f) corrected for residual scattering losses.

h) Scattering spectrum with high density slide.

Fig. 43. Log semi-integral attenuance spectra of high density and normal density slides of N. sertifer eggs with opal inserts.

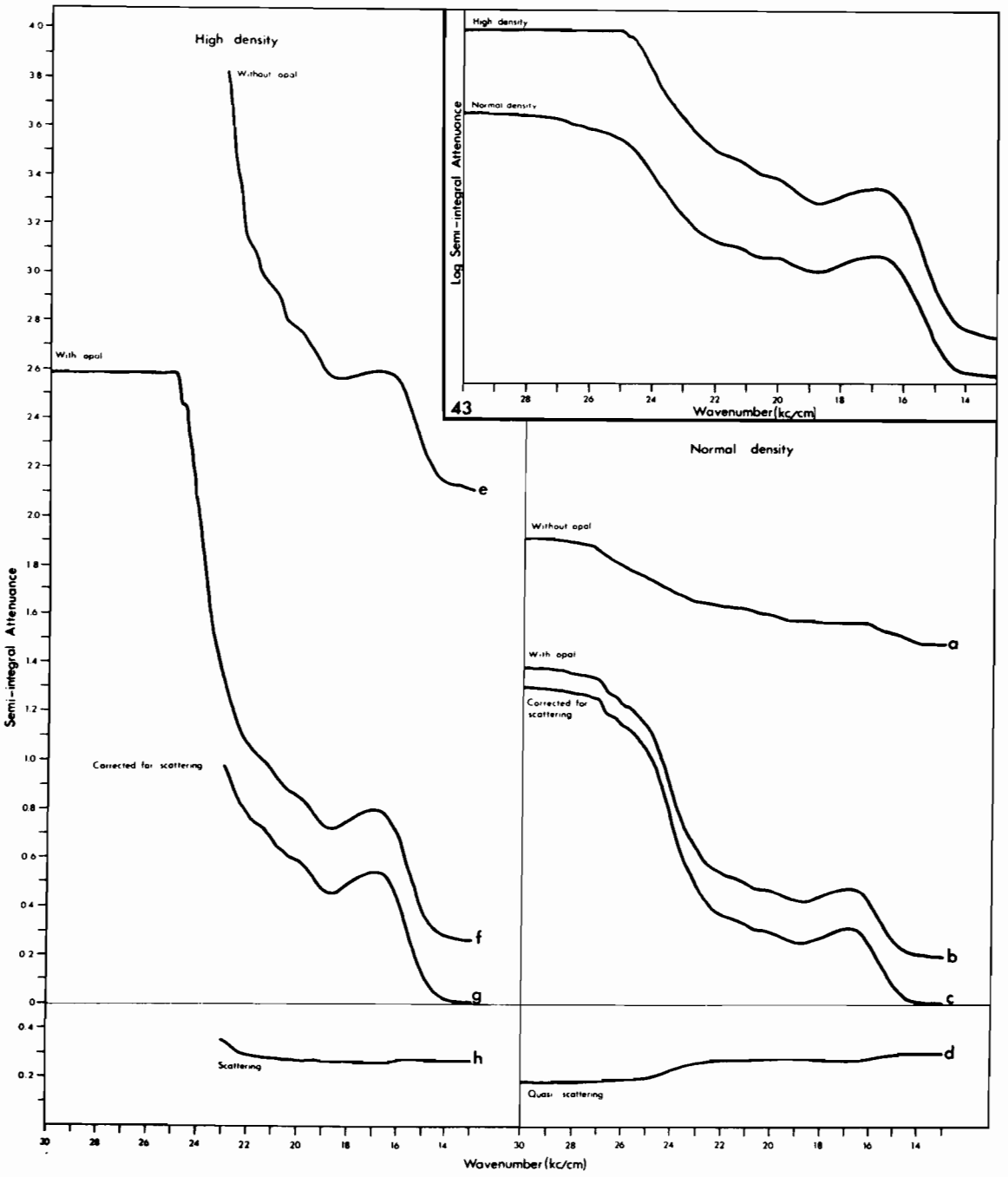

High density
Without opal
With opal
Corrected for scattering
Scattering
Quasi scattering
Normal density
Without opal
With opal
Corrected for scattering
Semi-integral Attenuance
Wavenumber (kc/cm)
High density
Normal density
Log Semi-integral Attenuance
Wavenumber (kc/cm)
43
a
b
c
d
e
f
g
h

Fig. 44. Semi-integral attenuance spectra of D. simile eggs.

a) Normal density slide, without opal inserts.

b) Normal density slide, with opal inserts.

c) Spectrum (b) corrected for residual scattering losses according to the method of Dartnall (1961), and Latimer and Eubanks (1962).

d) Quasi scattering spectrum with normal density slide.

e) High density slide, without opal inserts.

f) High density slide, with opal inserts.

g) Spectrum (f) corrected for residual scattering losses.

h) Scattering spectrum with high density slide.

Fig. 45. Log semi-integral attenuance spectra of high density and normal density slides of D. simile eggs with opal inserts.

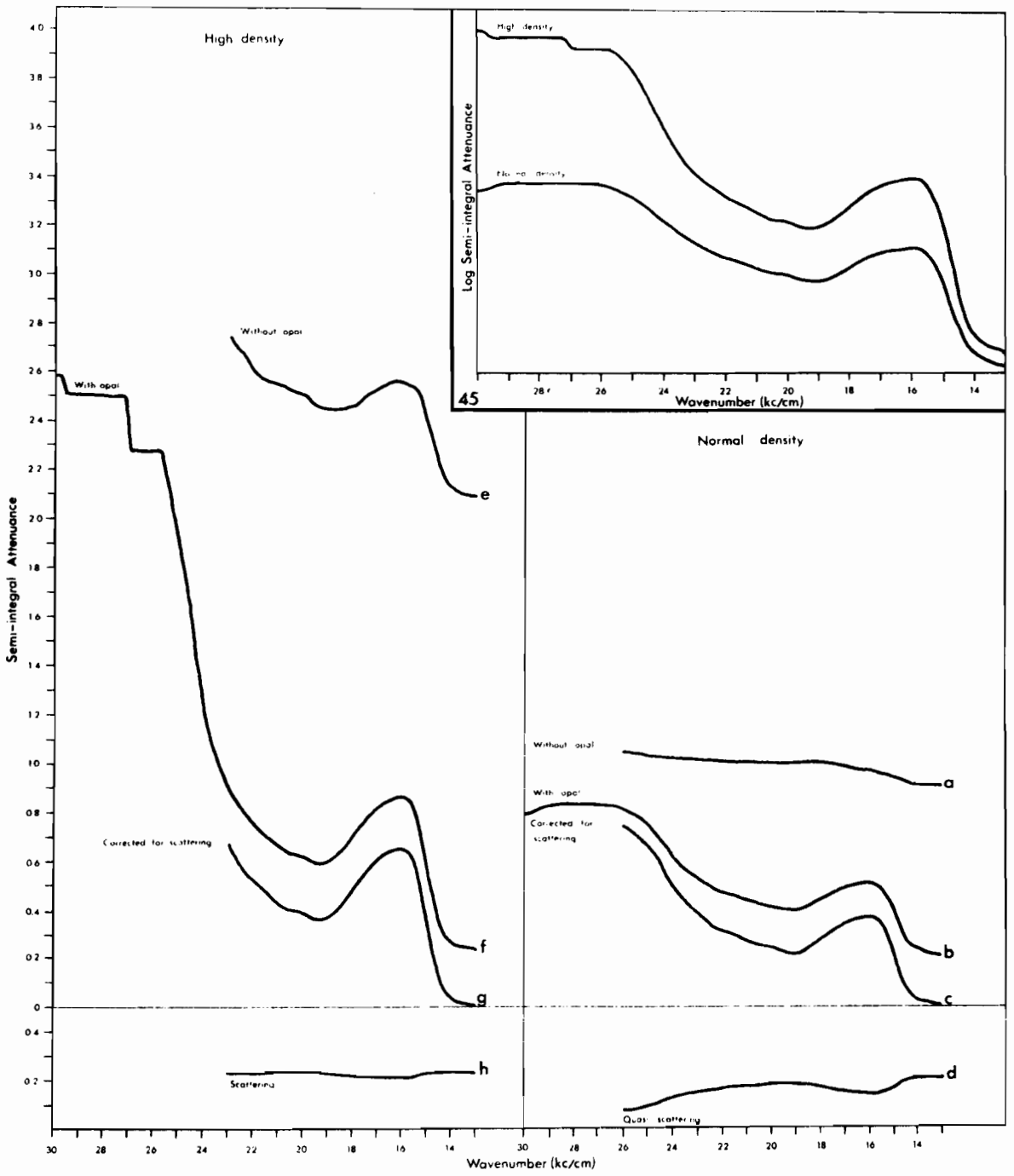

High density
Normal density
Log Semi-integral Attenuance
Wavenumber (kc/cm)
45
Semi-integral Attenuance
Wavenumber (kc/cm)
Without opal
With opal
Corrected for scattering
Scattering
Quasi scattering
a
b
c
d
e
f
g
h

Fig. 46 a. Semi-integral attenuance spectra of a complement of N. swainei eggs at four different slide densities. The attenuance value at 30 kc/cm is indicated under each curve.

b. Log semi-integral attenuance spectra as in (a).

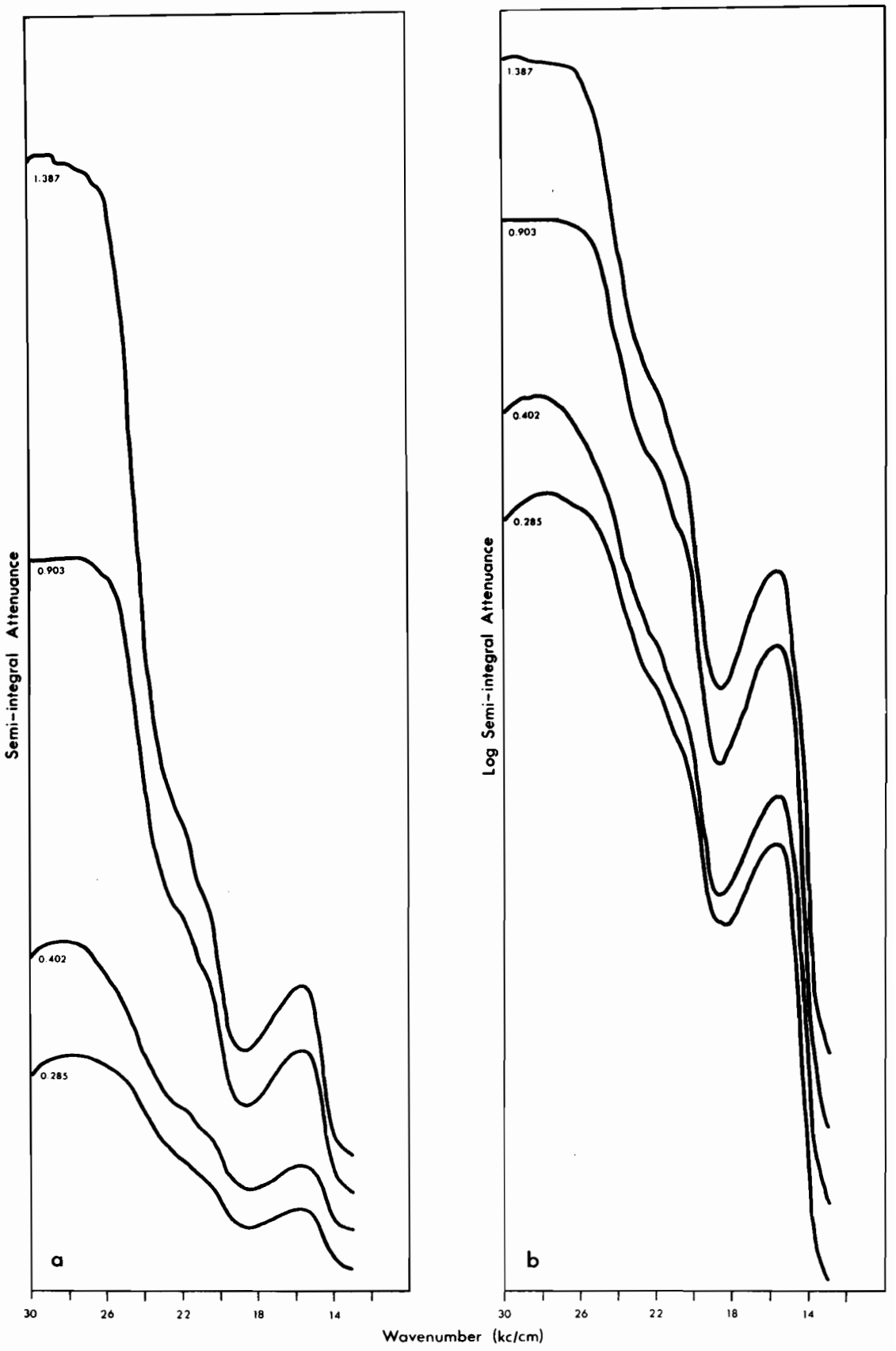

1.387
0.903
0.402
0.285
Semi-integral Attenuance
a
30
26
22
18
14
1.387
0.903
0.402
0.285
Log Semi-integral Attenuance
b
30
26
22
18
14
Wavenumber (kc/cm)

Fig. 47 a. Triangular co-ordinate plot based on semi-integral attenuance values for spectra of a complement of N. swainei eggs at different slide densities (attenuance at 30 kc/cm indicated), and polygons formed by joining three sequences of triangular co-ordinates in order of attenuance level at 30 kc/cm, all eight points included, points for six most dense preparations included, points for four most dense preparations included. The areas of the polygons (in the original plots) are given in square inches and the areal relationships are indicated.

b. As in (a), but based on log semi-integral attenuance values.

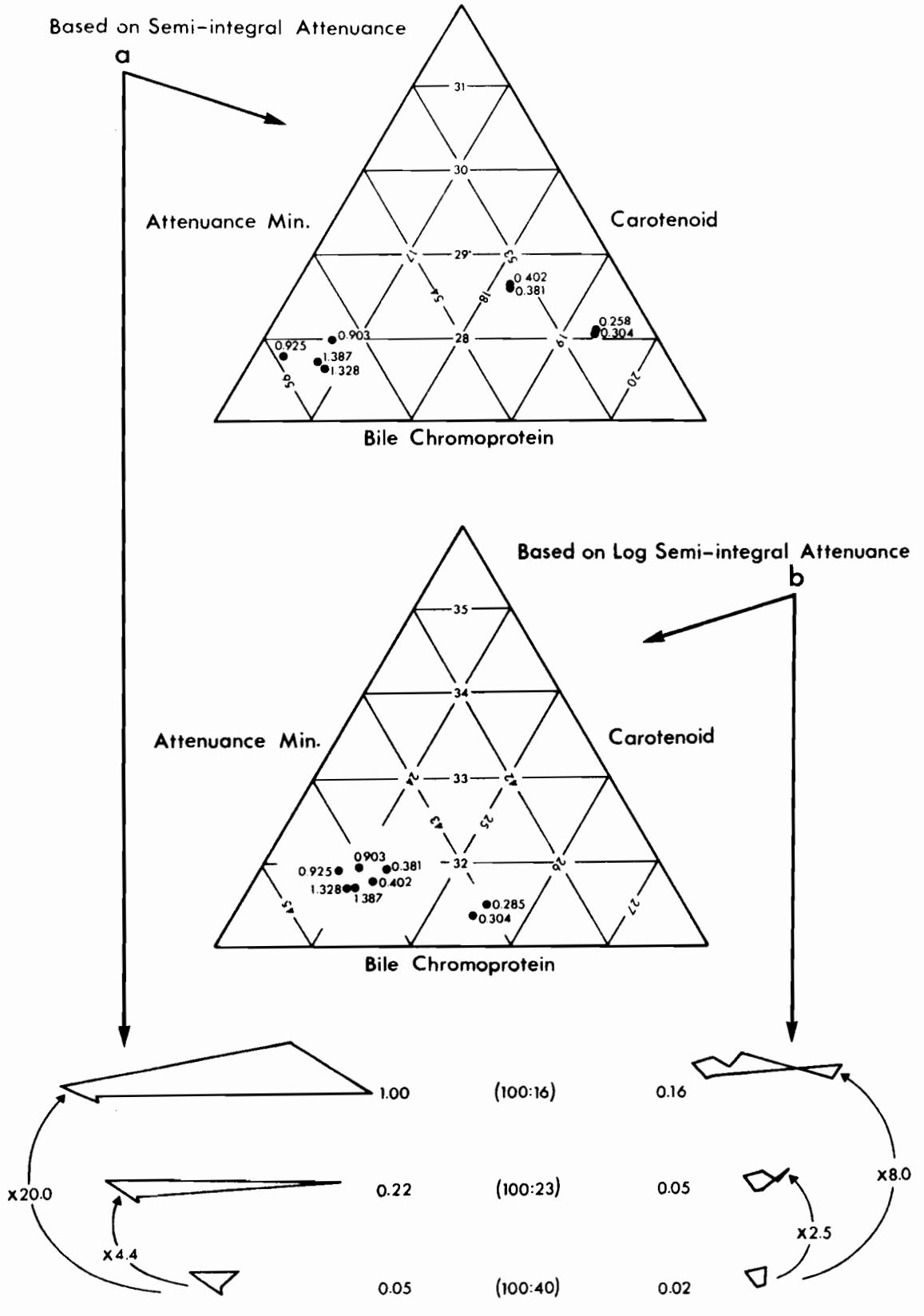

Based on Semi-integral Attenuance
a
Attenuance Min.
Carotenoid
Bile Chromoprotein
31
30
29
28
17
25
18
19
20
26
0.402
0.381
0.258
0.304
0.903
0.925
1.387
1.328
Based on Log Semi-integral Attenuance
b
Attenuance Min.
Carotenoid
Bile Chromoprotein
35
34
33
32
24
23
25
26
27
0.903
0.925
0.381
0.402
1.328
1.387
0.285
0.304
1.00
(100:16)
0.16
0.22
(100:23)
0.05
0.05
(100:40)
0.02
x20.0
x4.4
x8.0
x2.5

Fig. 48. Triangular co-ordinate values (based on log semi-integral attenuance curves) for a complement of N. swainei eggs at different slide densities plotted against attenuance at 30 kc/cm.

a. Carotenoid co-ordinate.

b. Attenuance minimum co-ordinate.

c. Bile chromoprotein co-ordinate.

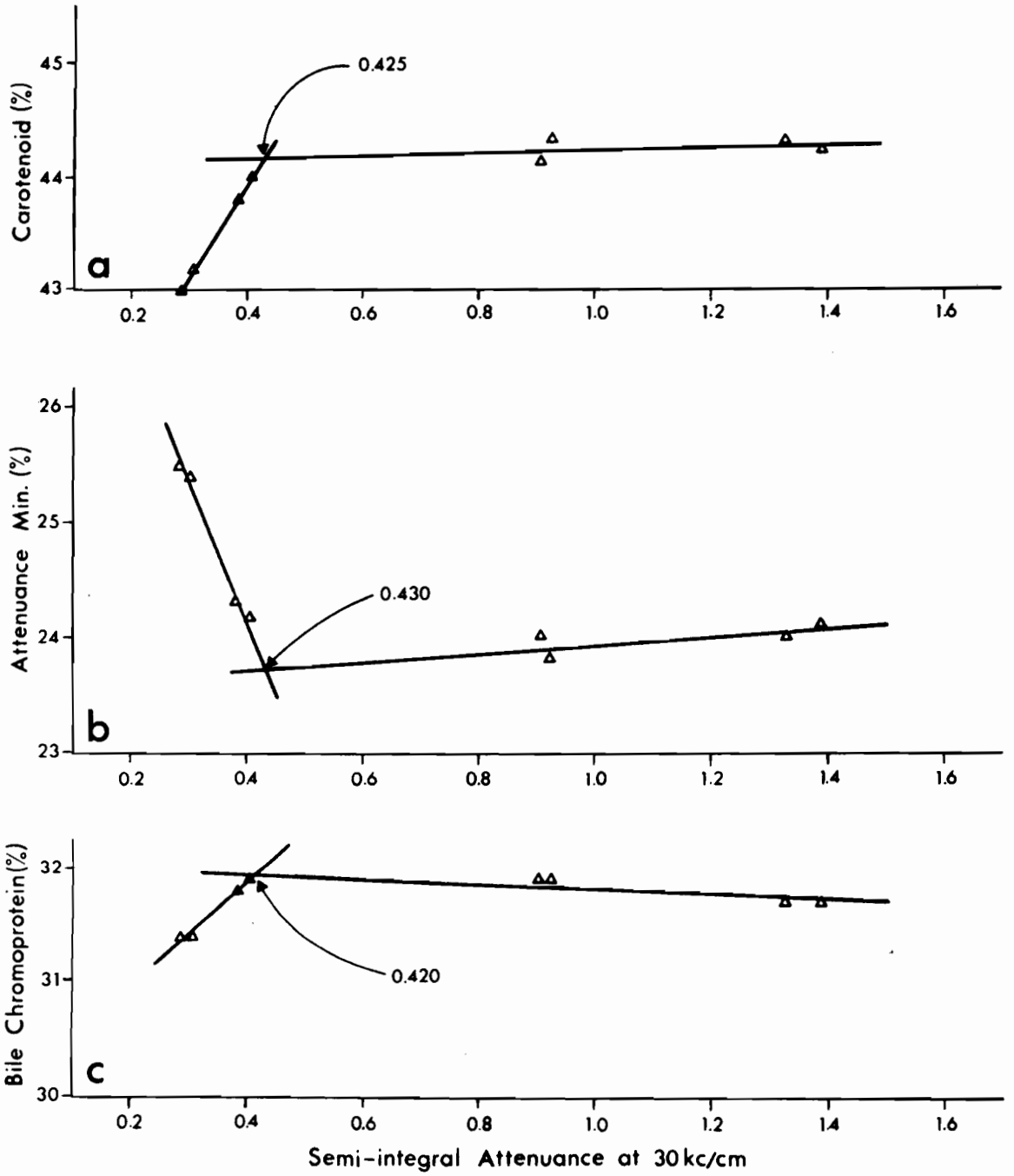

Carotenoid (%)
45
44
43
0.425
a
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
Attenuance Min. (%)
26
25
24
23
0.430
b
Bile Chromoprotein (%)
32
31
30
0.420
c
Semi-integral Attenuance at 30 kc/cm

Fig. 49. Log semi-integral attenuance spectrum of a freshly prepared slide of Neodiprion sp. eggs (solid line) compared with the spectrum of the same preparation after 30 hours at room temperature (dotted line).

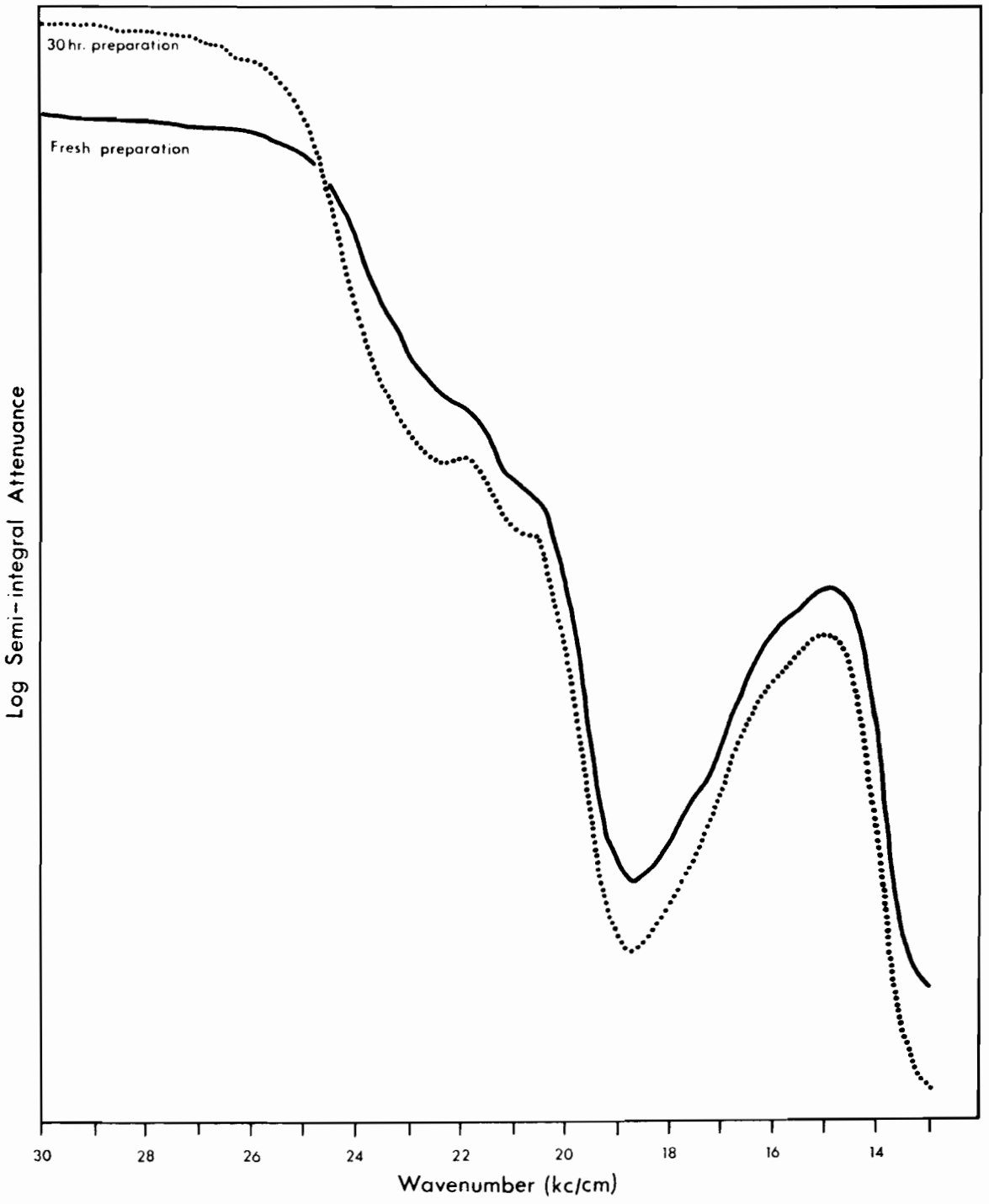

30 hr. preparation
Fresh preparation
Log Semi-integral Attenuance
30
28
26
24
22
20
18
16
14
Wavenumber (kc/cm)

Fig. 50. Log semi-integral attenuance spectra of preparations of eggs removed from freshly emerged females compared with rehydrated eggs of similar females that had been killed, pinned, dried, and stored for periods of four to five and one-half years.

a. Neodiprion lecontei, yellow eggs.

b. Neodiprion sertifer, "purple" eggs.

c. Diprion simile, blue eggs.

d. Neodiprion swainei, green eggs.

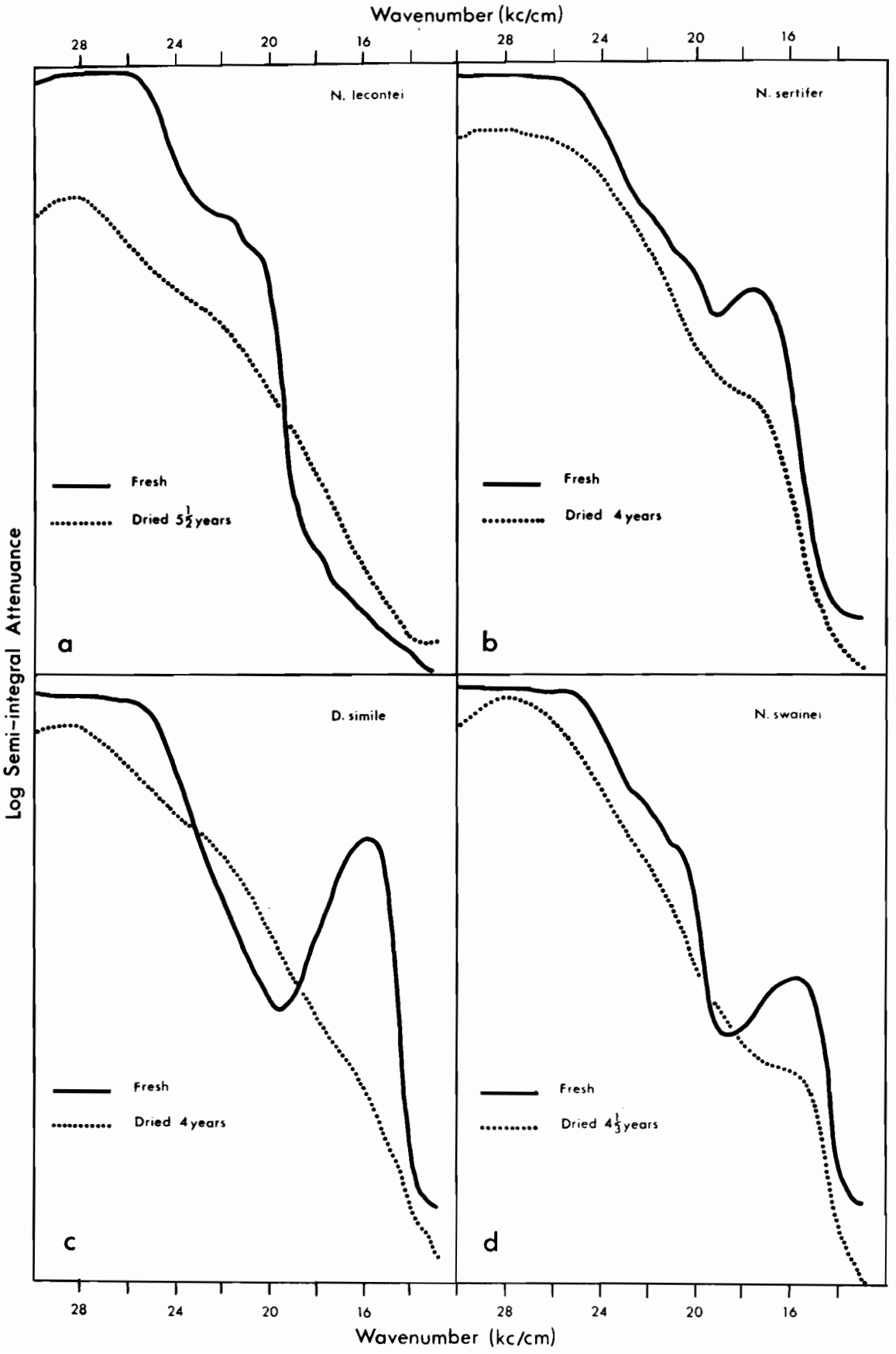

Wavenumber (kc/cm)
28
24
20
16
N. lecontei
Fresh
Dried 5½ years
a
N. sertifer
Fresh
Dried 4 years
b
D. simile
Fresh
Dried 4 years
c
N. swainei
Fresh
Dried 4⅓ years
d
Log Semi-integral Attenuance
Wavenumber (kc/cm)

Fig. 51. Log semi-integral attenuance spectra of eggs of six European diprionid species: Diprion simile, D. pini, Gilpinia hercyniae, G. socia, G. frutetorum, and Microdiprion pallipes.

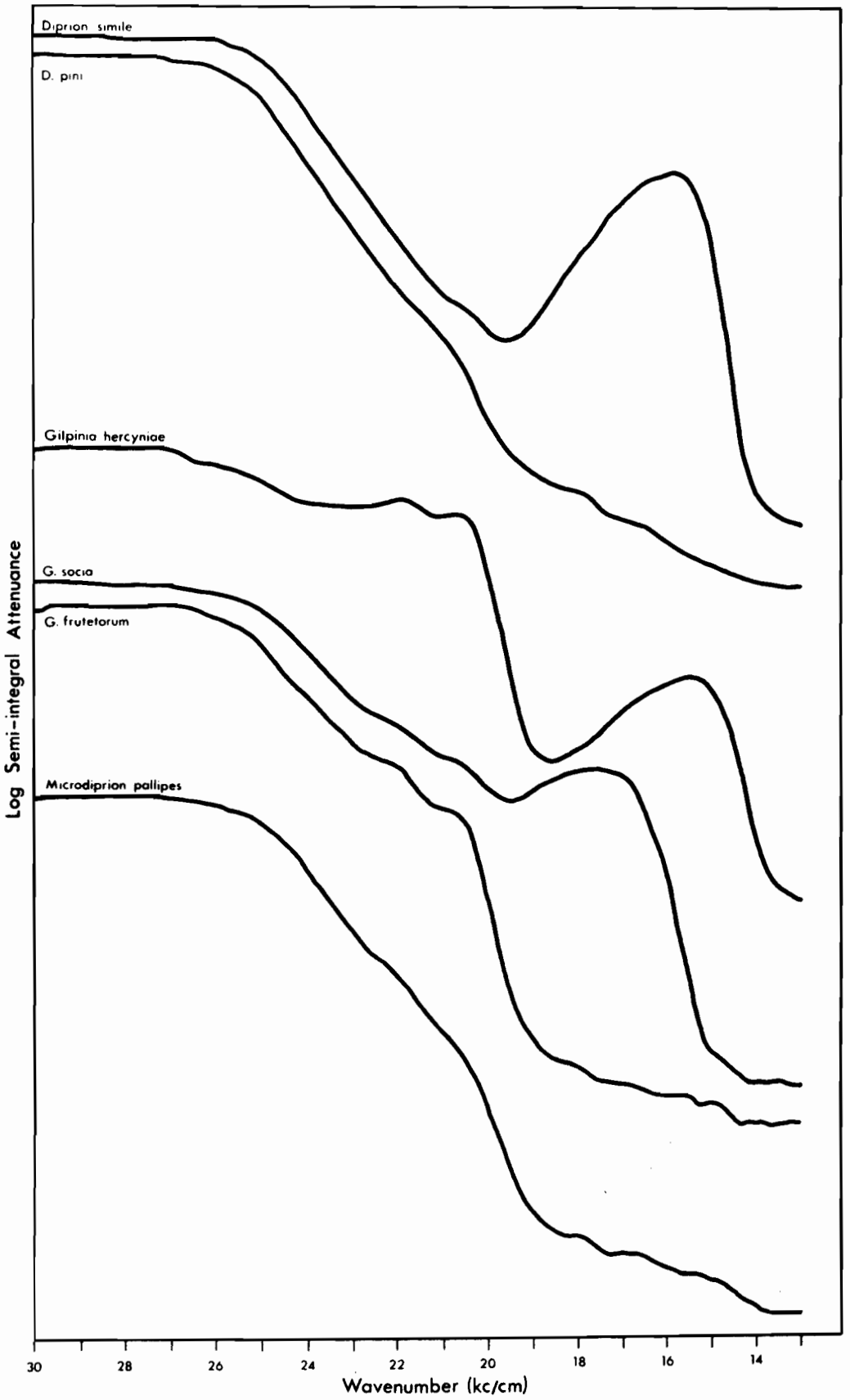

Diprion simile
D. pini
Gilpinia hercyniae
G. socia
G. frutetorum
Microdiprion pallipes
Log Semi-integral Attenuance
30
28
26
24
22
20
18
16
14
Wavenumber (kc/cm)

Fig. 52. The distribution of Neodiprion swainei:

Stars - yellow larval populations

Dots - white larval populations

Circles - populations of uncertain status.

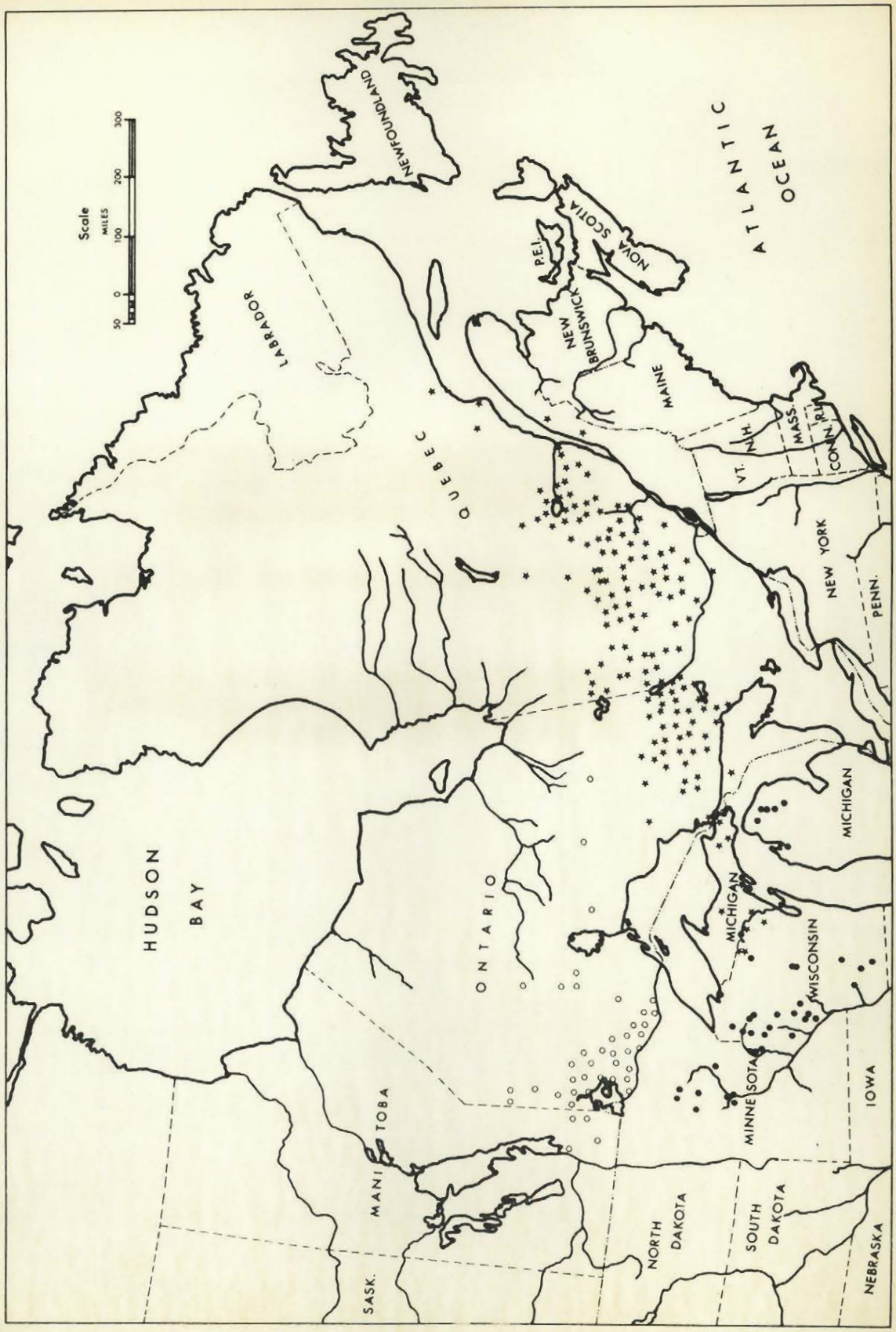

Scale
MILES
300
200
100
0
50
NEWFOUNDLAND
LABRADOR
QUEBEC
P.E.I.
NEW BRUNSWICK
NOVA SCOTIA
ATLANTIC OCEAN
MAINE
VT.
N.H.
MASS.
CONN.
R.I.
NEW YORK
PENN.
HUDSON BAY
ONTARIO
MICHIGAN
MICHIGAN
WISCONSIN
MINNESOTA
IOWA
MANITOBA
SASK.
NORTH DAKOTA
SOUTH DAKOTA
NEBRASKA

Fig. 53. Log semi-integral attenuance spectra of fat body preparations from mature, feeding, female larvae of Neodiprion swainei.

a. Deep fat bodies of yellow and white larval races.

b. Peripheral fat bodies (in association with the integument and body wall musculature) of yellow and white larval races.

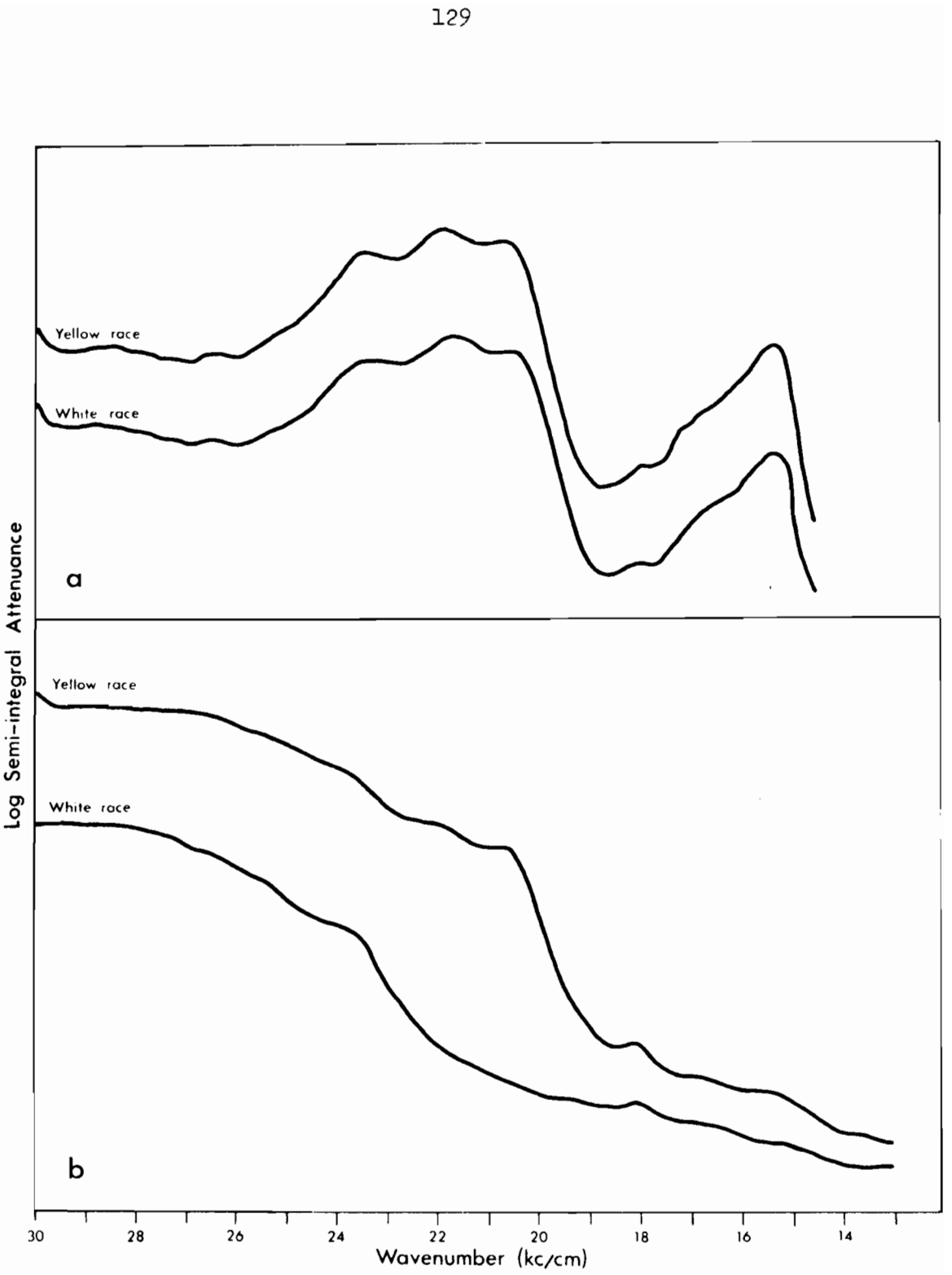
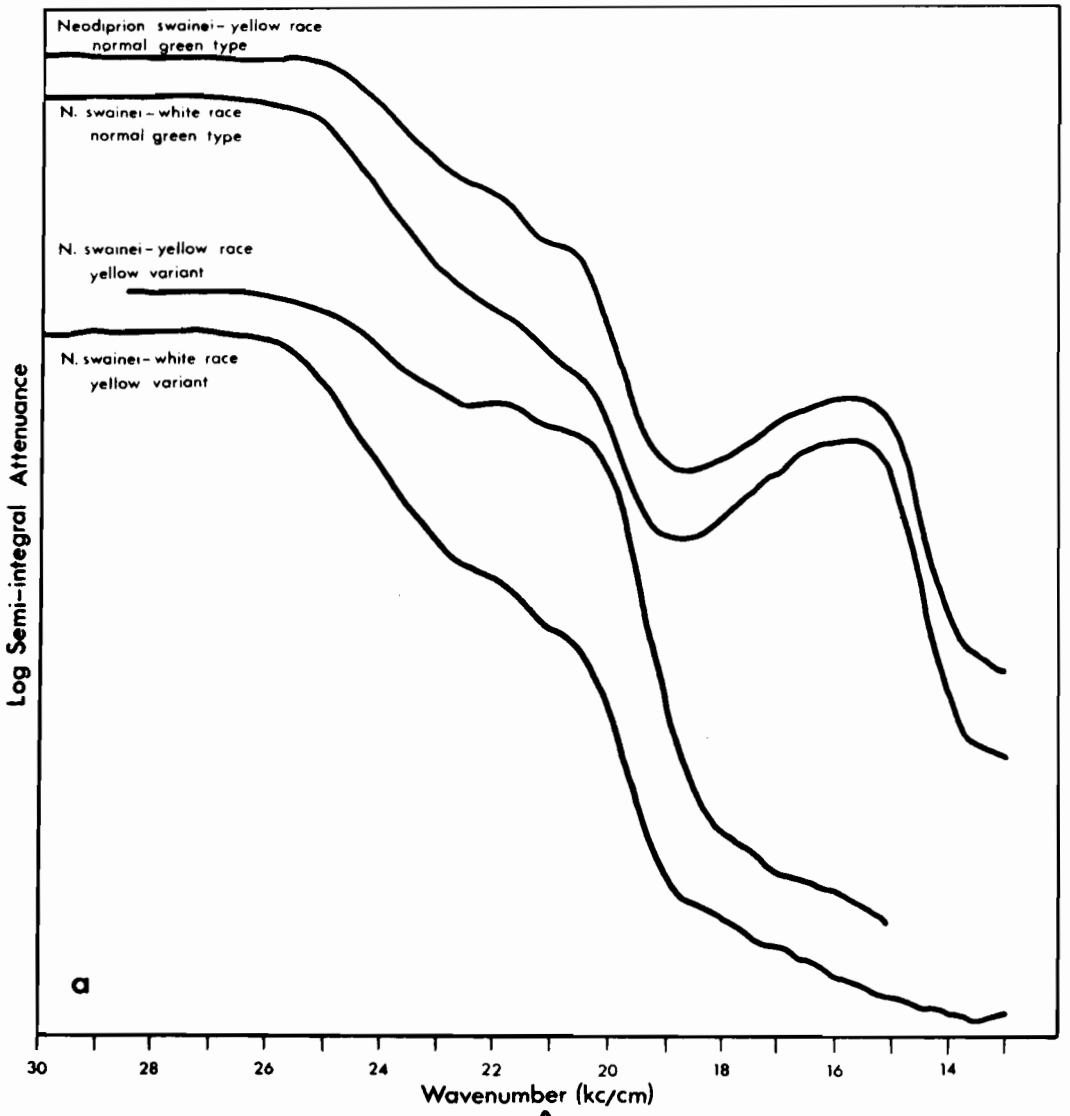

Yellow race
White race
a
Yellow race
White race
b
Log Semi-integral Attenuance
30
28
26
24
22
20
18
16
14
Wavenumber (kc/cm)

Fig. 54 a. Log semi-integral attenuance spectra for Neodiprion swainei eggs: yellow race, normal green type; white race, normal green type; yellow race, yellow variant; white race, yellow variant.

b. Triangular co-ordinate plot of Neodiprion swainei egg spectrum data for normal green eggs.


Neodiprion swainei - yellow race
normal green type
N. swainei - white race
normal green type
N. swainei - yellow race
yellow variant
N. swainei - white race
yellow variant
Log Semi-integral Attenuance
a
30
28
26
24
22
20
18
16
14
Wavenumber (kc/cm)

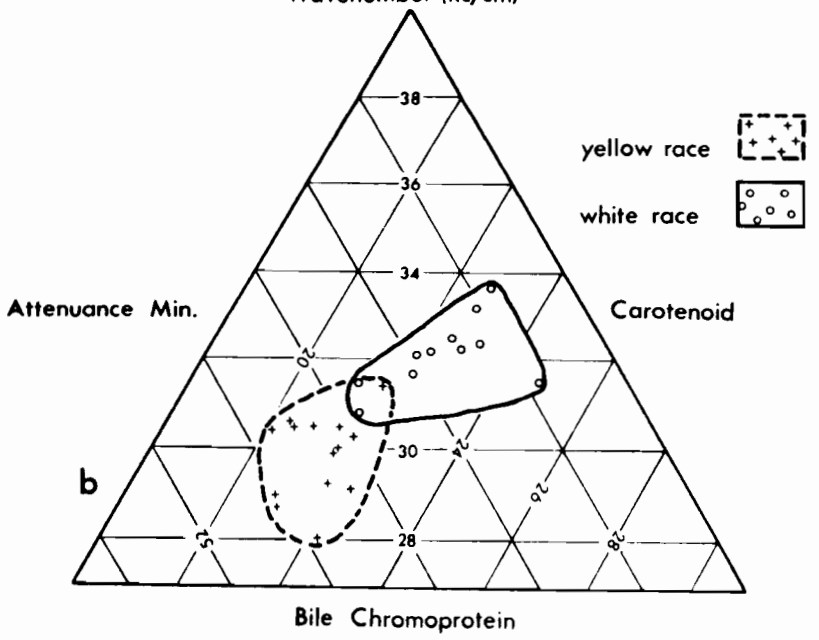
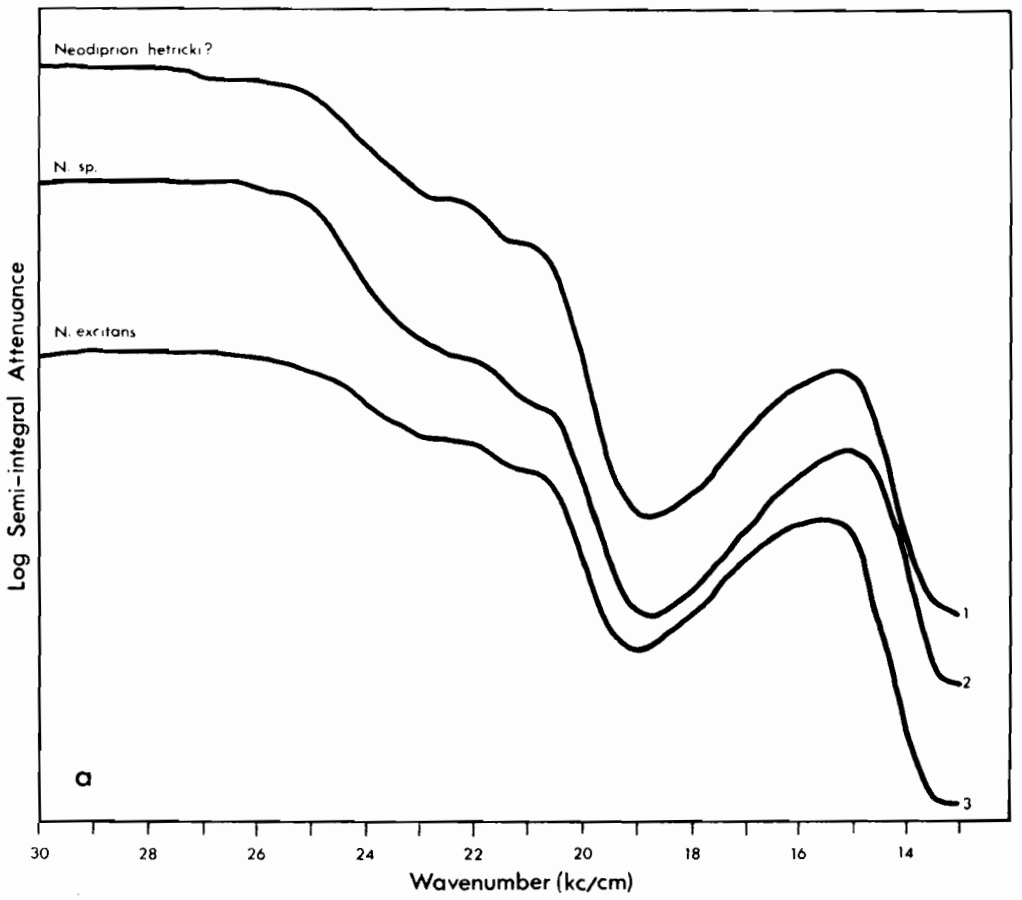

38
36
34
32
30
28
26
24
22
20
yellow race
white race
Attenuance Min.
Carotenoid
b
Bile Chromoprotein

Fig. 55 a. Log semi-integral attenuance spectra for eggs of species in the *swainei* group other than *Neodiprion swainei*: 1. *Neodiprion hetricki*?, 2. *Neodiprion* sp., 3. *Neodiprion excitans*.

b. Triangular co-ordinate plot of the egg spectrum data for species in (a).


Neodiprion hetrickı?
N. sp.
N. excitans
Log Semi-integral Attenuance
a
1
2
3
30
28
26
24
22
20
18
16
14
Wavenumber (kc/cm)

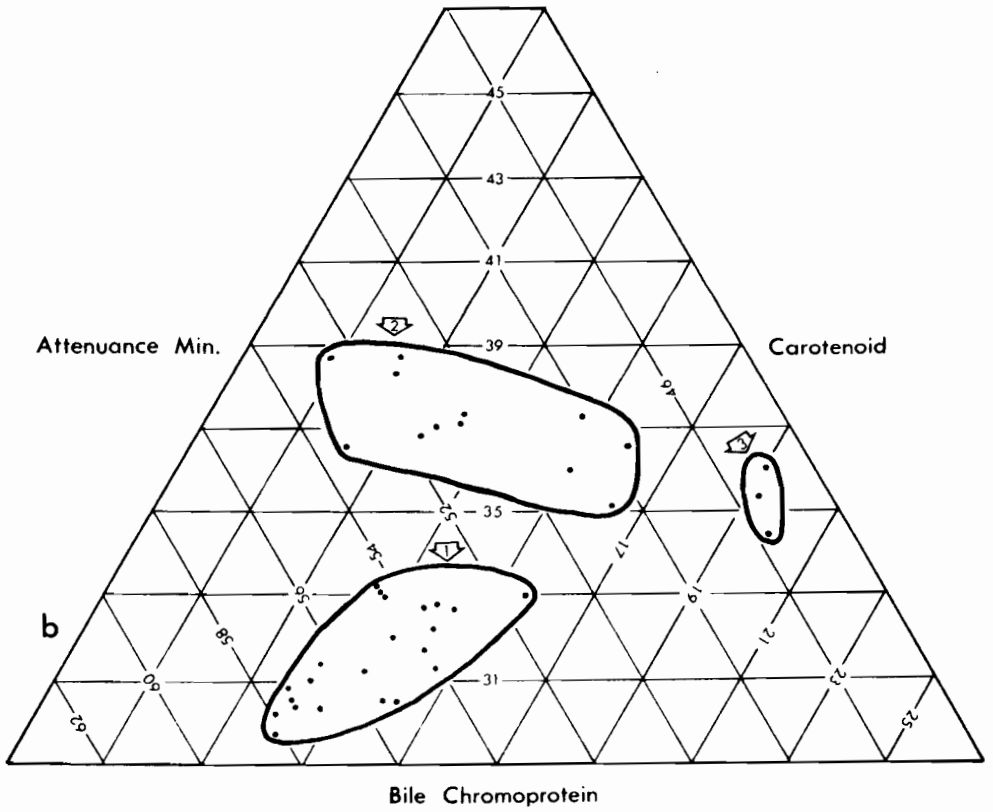
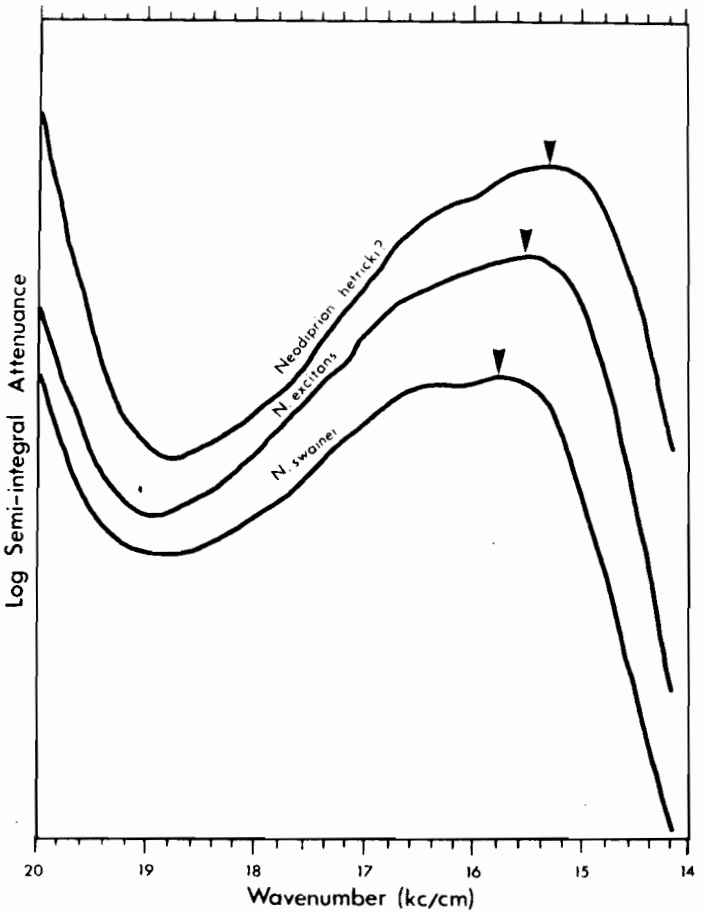

Attenuance Min.
Carotenoid
Bile Chromoprotein
b
45
43
41
39
35
31
46
17
19
21
23
25
54
58
62
60
55
53
1
2
3

Fig. 56. The bile chromoprotein region of the log semi-integral attenuance spectra of the green eggs of Neodiprion hetricki?, Neodiprion excitans, and Neodiprion swainei, showing the differences in position of the bile chromoprotein absorption maximum.

Fig. 57. The log semi-integral attenuance spectrum of the yellowish green eggs of Pikonema dimmockii, showing the distinctive form of the bile chromoprotein absorption band.


Log Semi-integral Attenuance
Neodiprion hetrickii
N. excitans
N. swainei
20
19
18
17
16
15
14
Wavenumber (kc/cm)

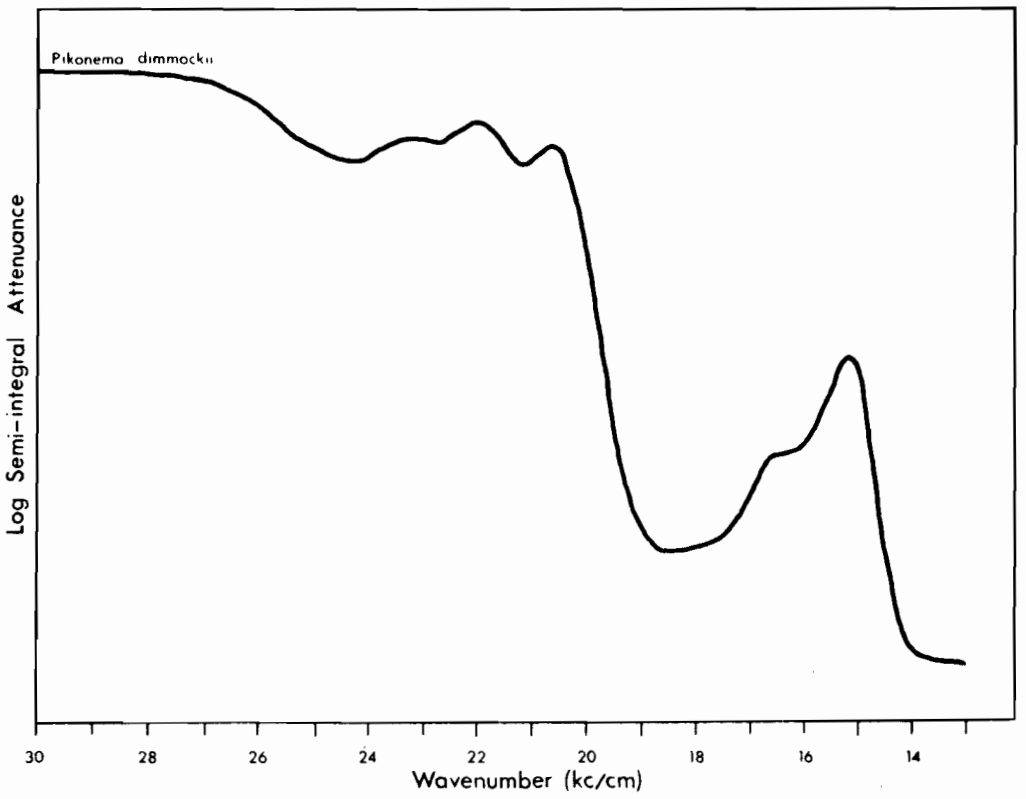
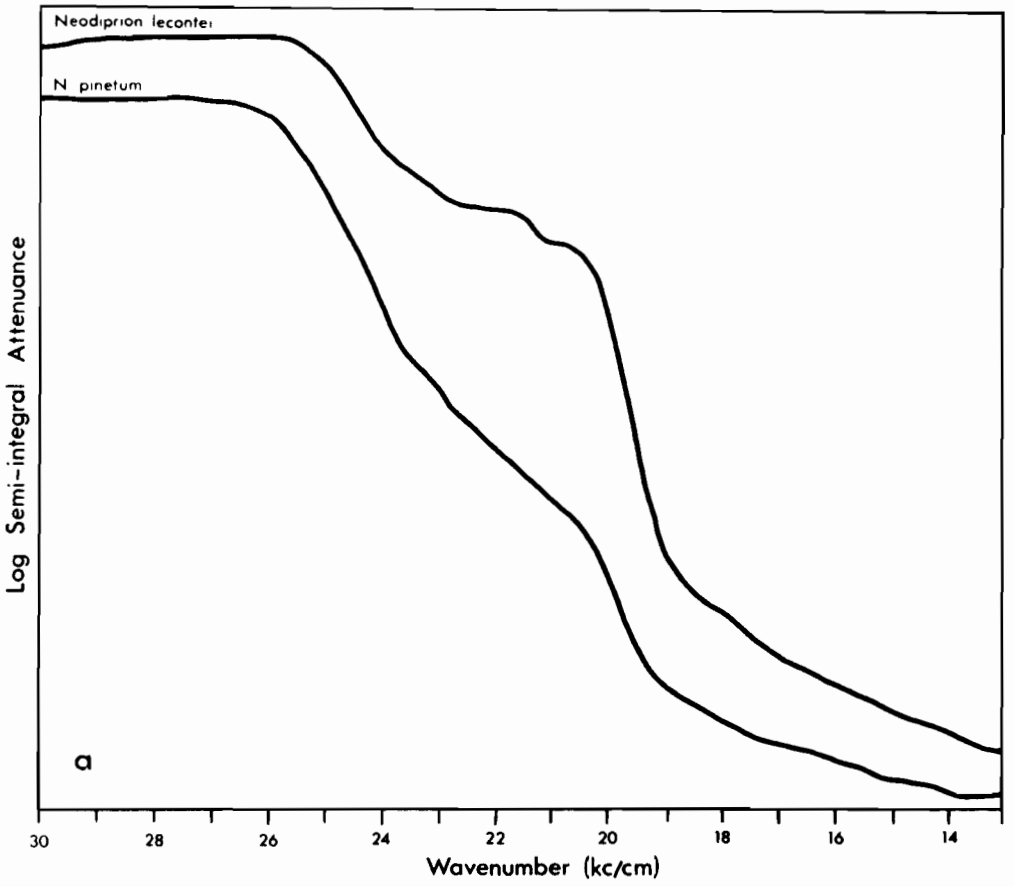

Pikonema dimmocki
Log Semi-integral Attenuance
30
28
26
24
22
20
18
16
14
Wavenumber (kc/cm)

Fig. 58 a. Log semi-integral attenuance spectra of *Neodiprion lecontei* and *Neodiprion pinetum*.

b. Triangular co-ordinate plot of the egg spectrum data for species in (a).


Neodiprion leconteı
N. pinetum
Log Semi-integral Attenuance
a
30
28
26
24
22
20
18
16
14
Wavenumber (kc/cm)

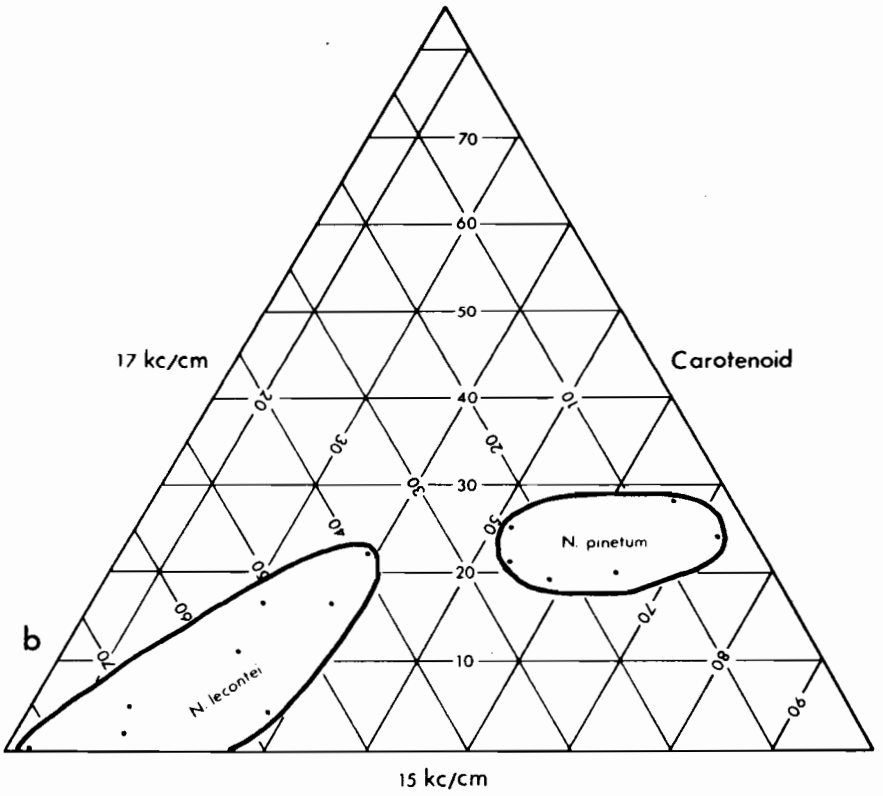

17 kc/cm
Carotenoid
15 kc/cm
70
60
50
40
30
20
10
0
10
20
30
40
50
60
70
80
90
N. pinetum
N. leconteı
b

Fig. 59. Log semi-integral attenuance spectra of eggs of Neodiprion pratti pratti, Neodiprion pratti paradoxicus, Neodiprion pratti banksianae, Neodiprion maurus (yellow), Neodiprion maurus (white), and Neodiprion taedae taedae.

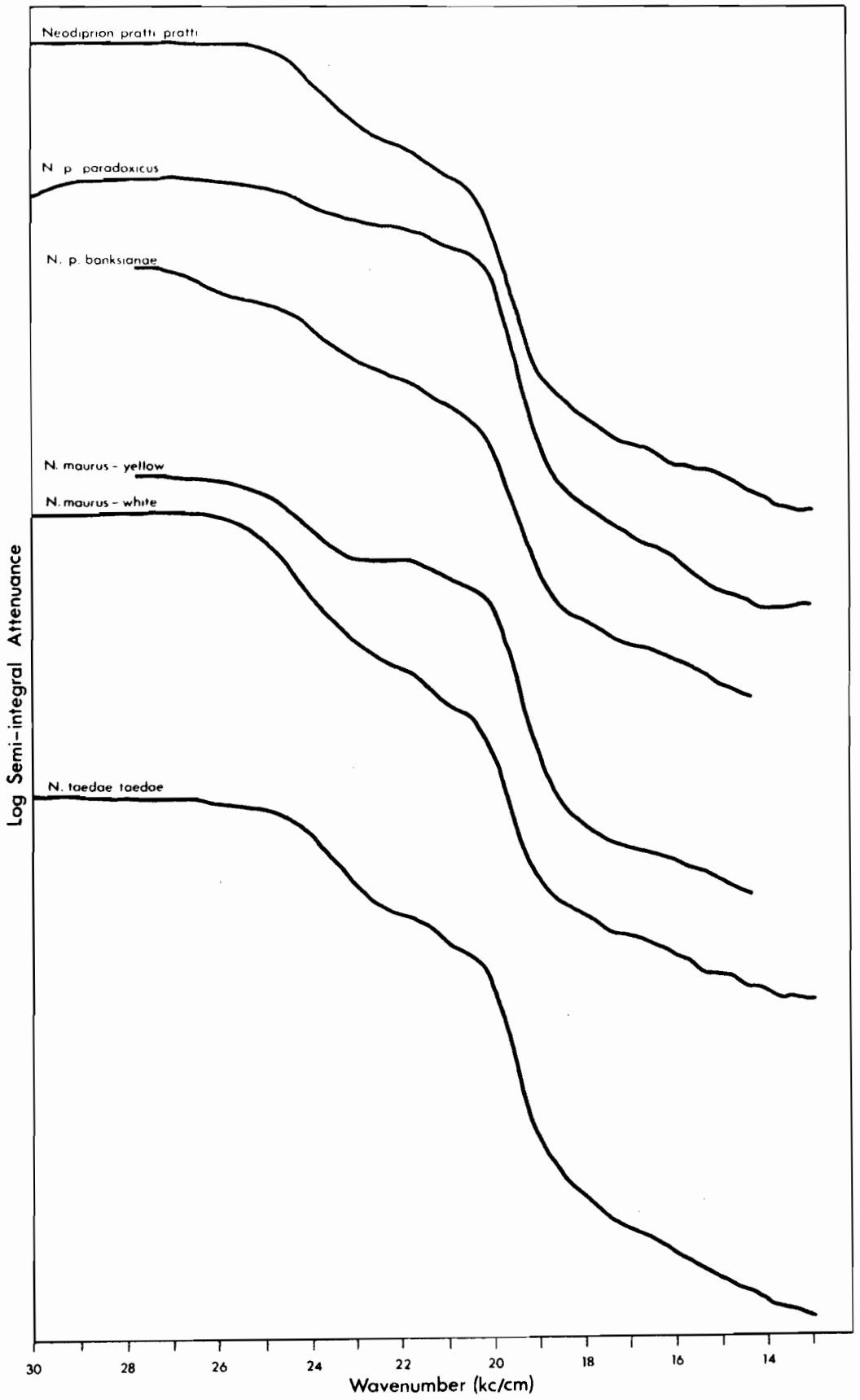
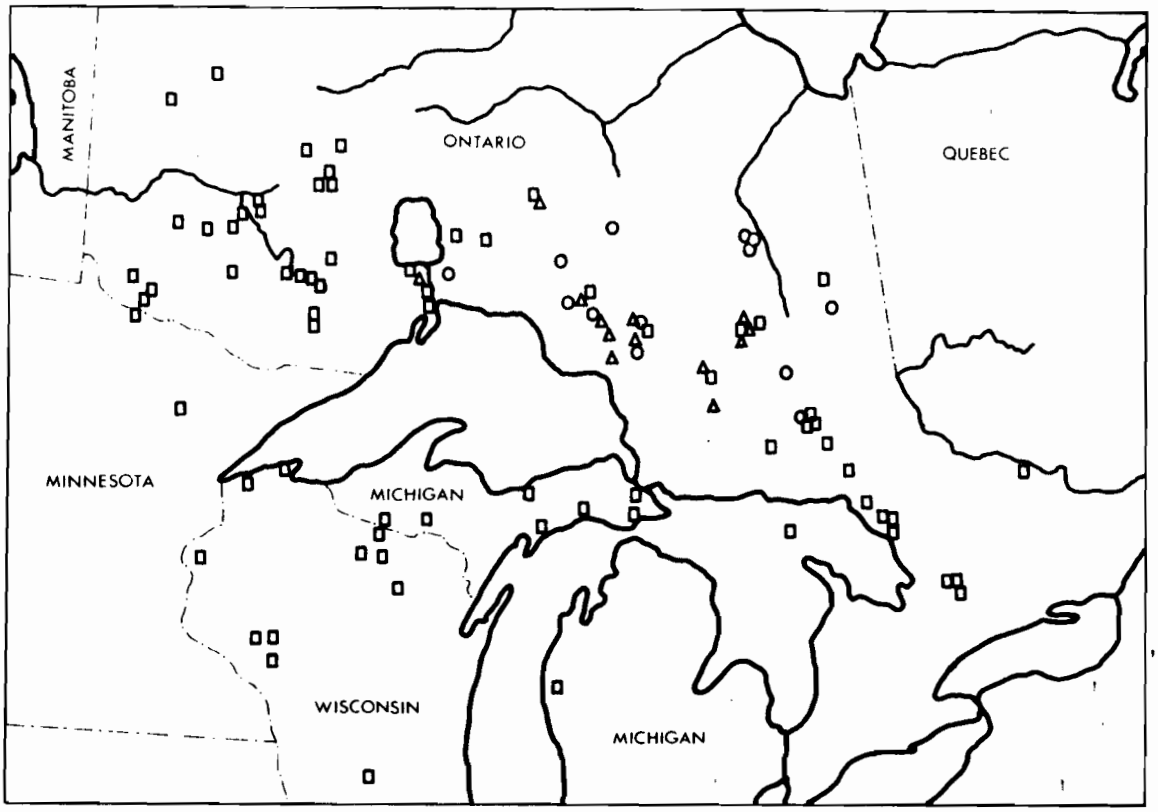

Neodiprion pratti pratti
N. p. paradoxicus
N. p. banksianae
N. maurus - yellow
N. maurus - white
N. taedae taedae
Log Semi-integral Attenuance
30
28
26
24
22
20
18
16
14
Wavenumber (kc/cm)

Fig. 60. Distribution of larval types of Neodiprion rugifrons in Ontario and the Lake States (based on collections for 1960-1962 in Ontario; 1959-1961 in the Lake States).
Squares - spotted larval colonies
Triangles - intermediate larval colonies
Circles - striped larval colonies

Fig. 61. Distribution of larval types of Neodiprion ferrugineum in Ontario and the Lake States (based on collections for 1960-1962 in Ontario; 1959-1961 in the Lake States).
Squares - spotted larval colonies
Triangles - intermediate larval colonies
Circles - striped larval colonies


MANITOBA
ONTARIO
QUEBEC
MINNESOTA
MICHIGAN
WISCONSIN
MICHIGAN

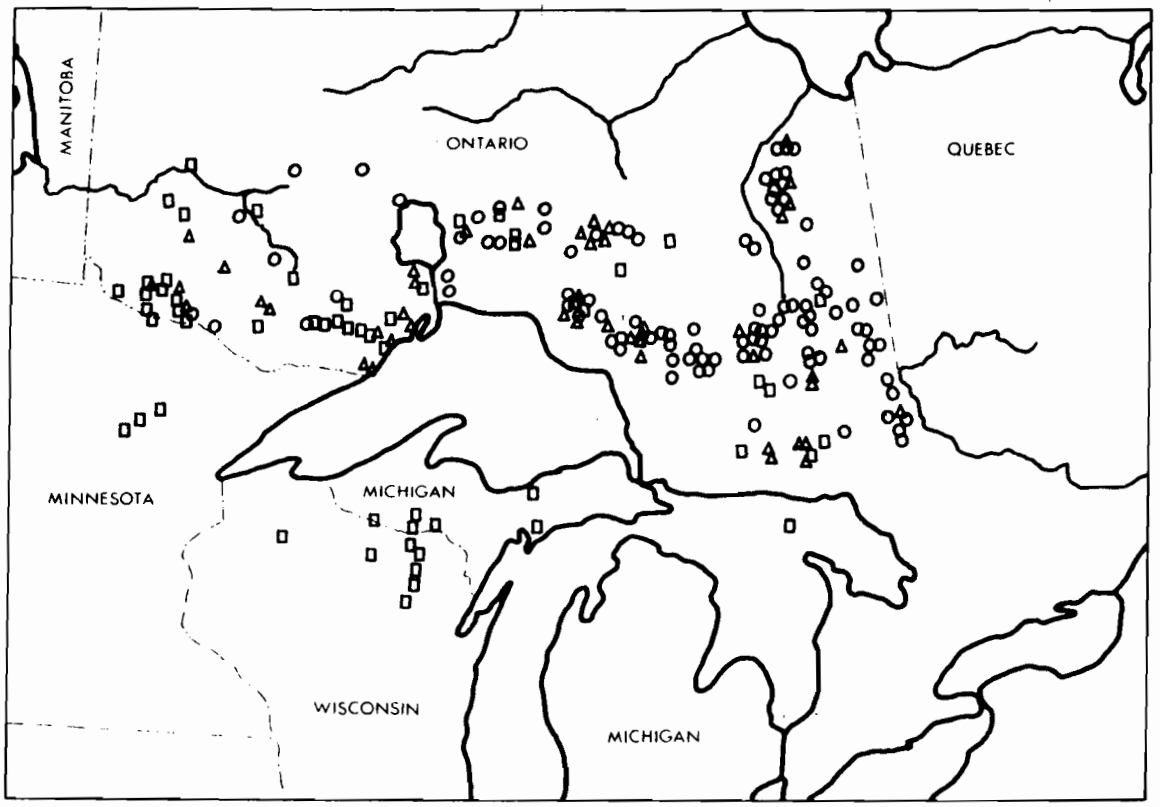
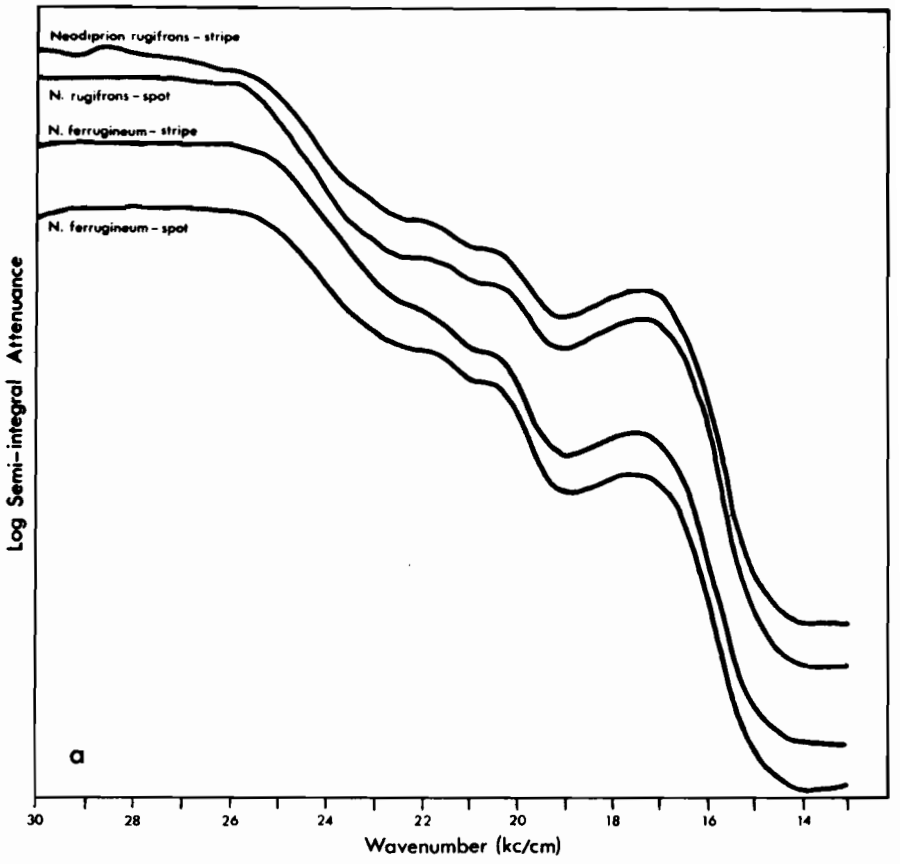
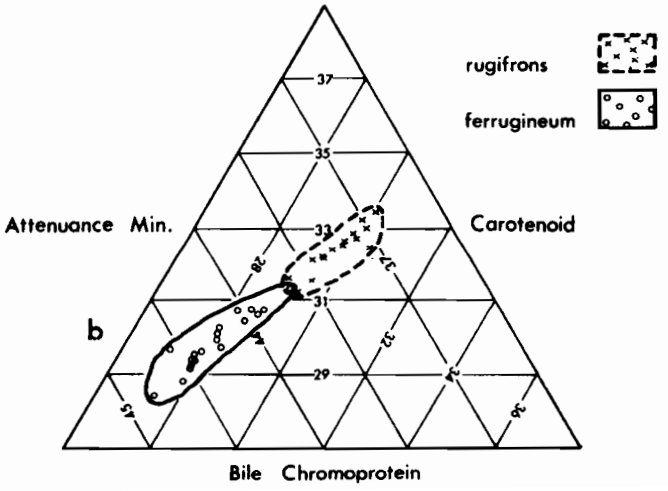

MANITOBA
ONTARIO
QUEBEC
MINNESOTA
MICHIGAN
WISCONSIN
MICHIGAN

Fig. 62 a. Log semi-integral attenuance spectra of eggs of Neodiprion rugifrons, striped and spotted larval types, and Neodiprion ferrugineum, striped and spotted larval types.

b. Triangular co-ordinate plot of the egg spectrum data for species in (a).


Neodiprion rugifrons – stripe
N. rugifrons – spot
N. ferrugineum – stripe
N. ferrugineum – spot
Log Semi-integral Attenuance
a
30
28
26
24
22
20
18
16
14
Wavenumber (kc/cm)


rugifrons
ferrugineum
Attenuance Min.
Carotenoid
Bile Chromoprotein
b
37
35
33
31
29
28
32
34
36
45

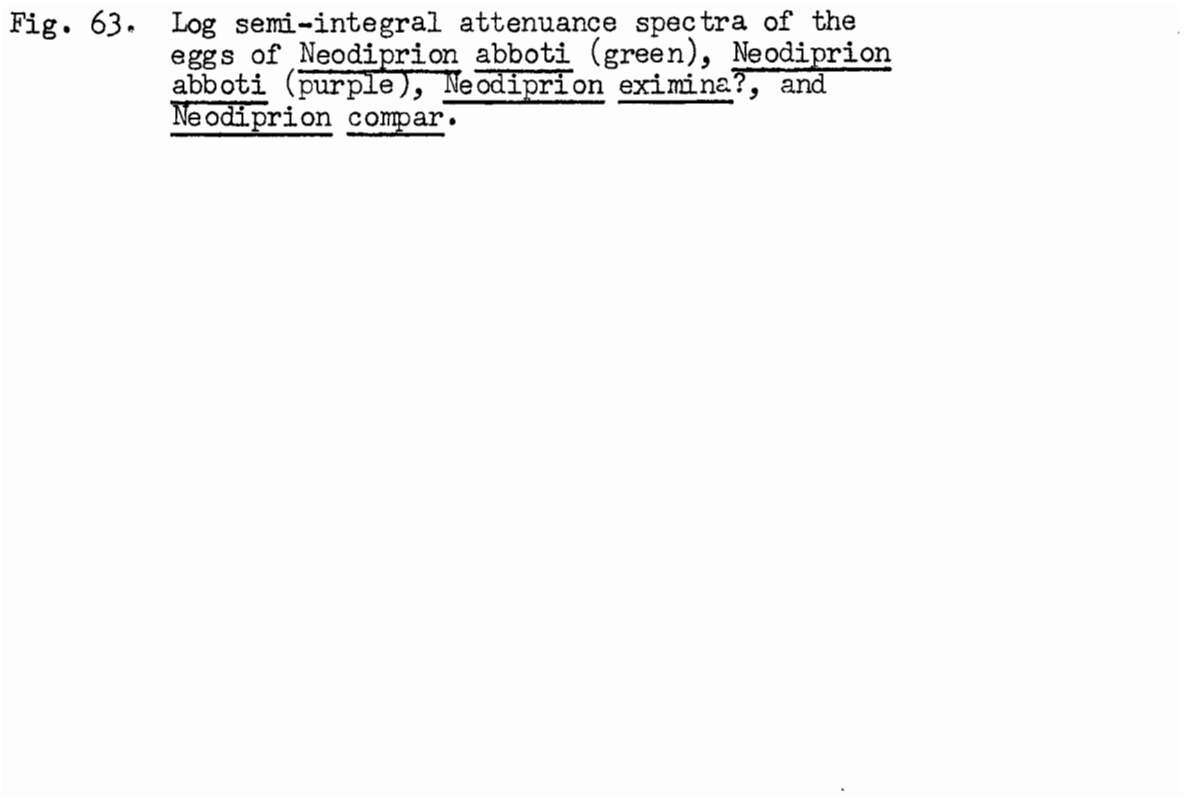


Fig. 63. Log semi-integral attenuance spectra of the eggs of Neodiprion abboti (green), Neodiprion abboti (purple), Neodiprion eximina?, and Neodiprion compar.

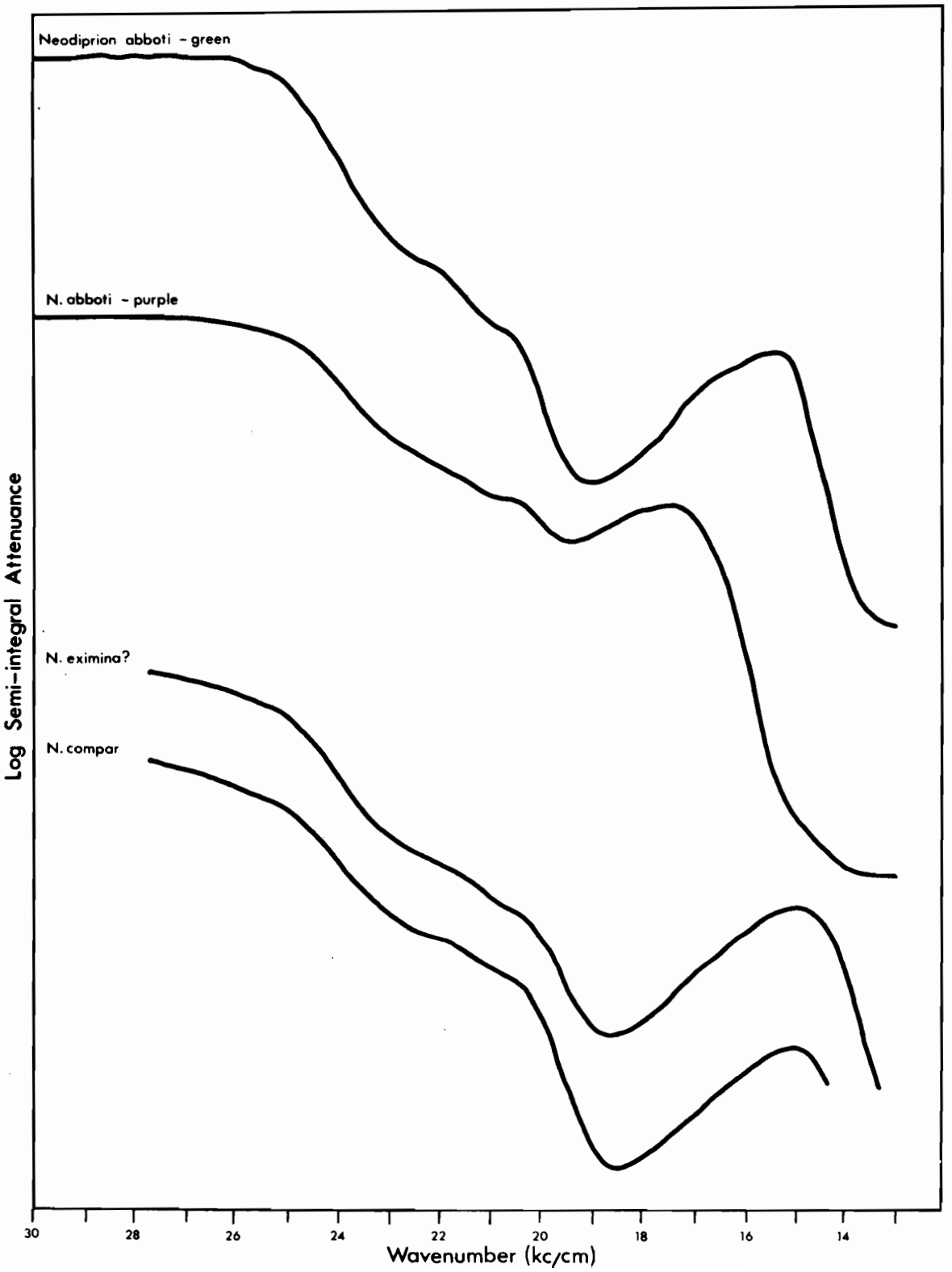
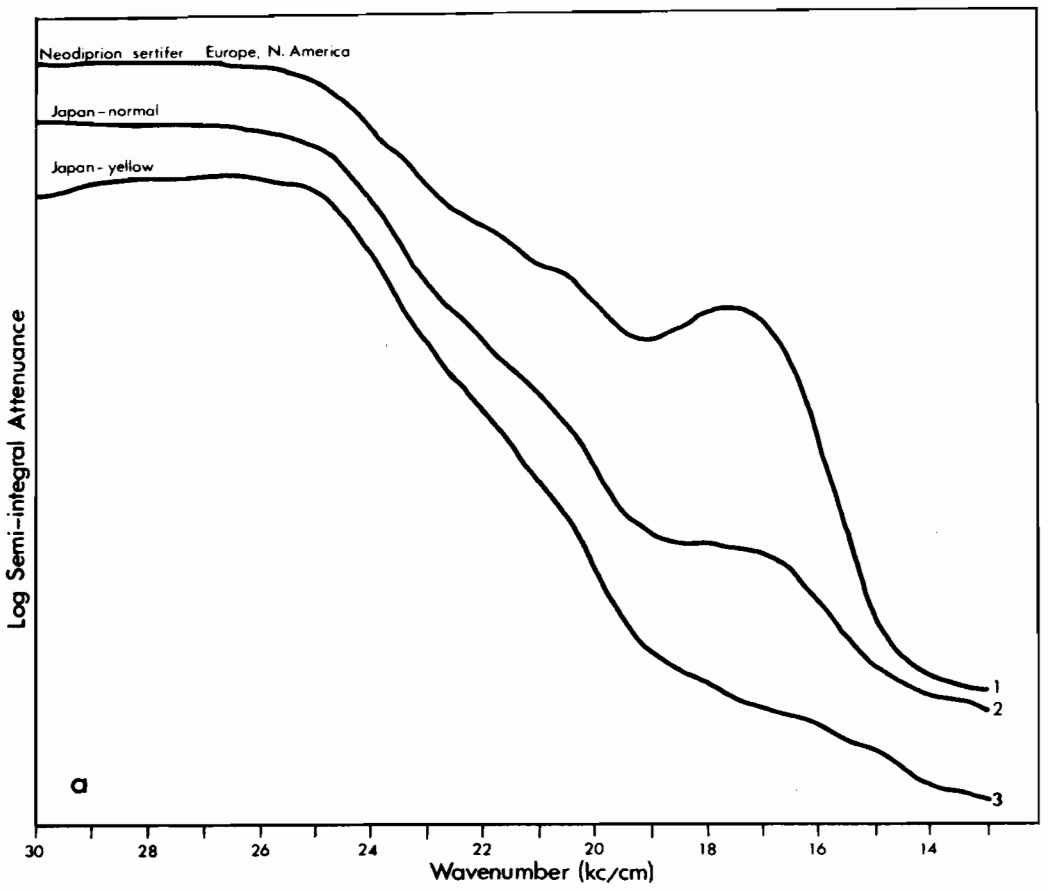

Neodiprion abboti - green
N. abboti - purple
N. eximina?
N. compar
Log Semi-integral Attenuance
30
28
26
24
22
20
18
16
14
Wavenumber (kc/cm)

Fig. 64 a. Log semi-integral attenuance spectra of eggs of Neodiprion sertifer, 1. Europe, North America, 2. Japan - normal, and 3. Japan - yellow.

b. Triangular co-ordinate plot of Neodiprion sertifer egg spectrum data.


Neodiprion sertifer Europe, N. America
Japan - normal
Japan - yellow
Log Semi-integral Attenuance
a
1
2
3
30
28
26
24
22
20
18
16
14
Wavenumber (kc/cm)

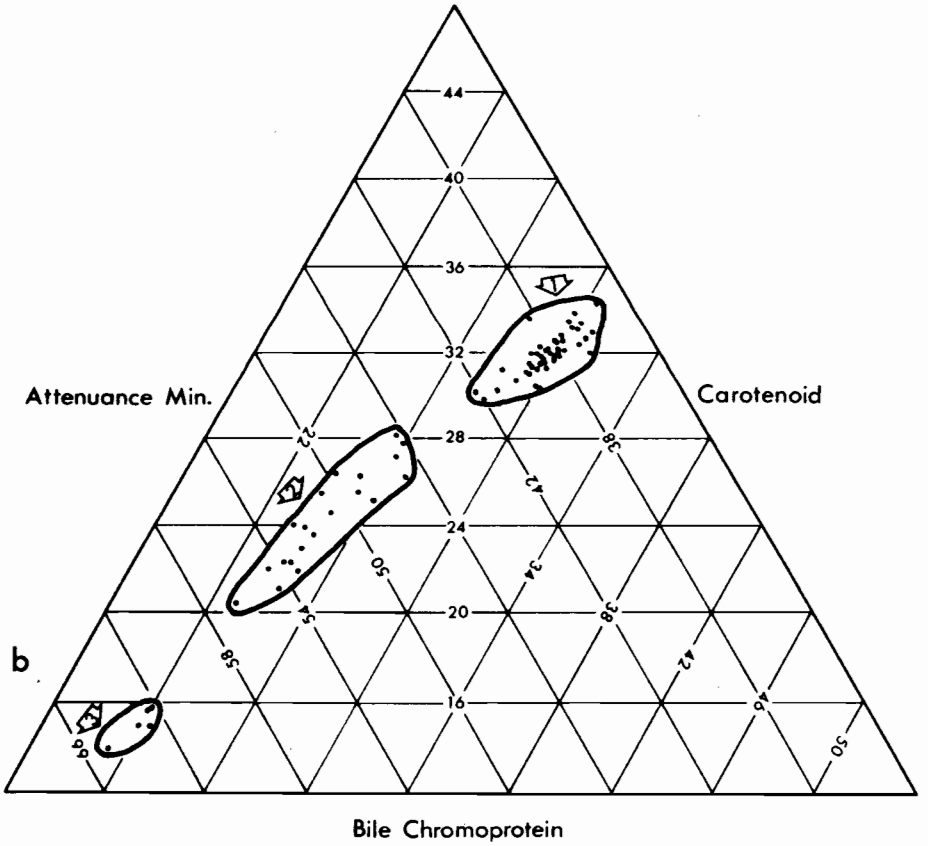

Attenuance Min.
Carotenoid
Bile Chromoprotein
b
44
40
36
32
28
24
20
16
22
58
50
54
42
34
46

Fig. 65. Triangular co-ordinate plot of egg spectrum data for European and North American Neodiprion sertifer specimens coded according to country of origin.

Fig. 66. Triangular co-ordinate plot of egg spectrum data for European and North American Neodiprion sertifer specimens coded according to host origin.

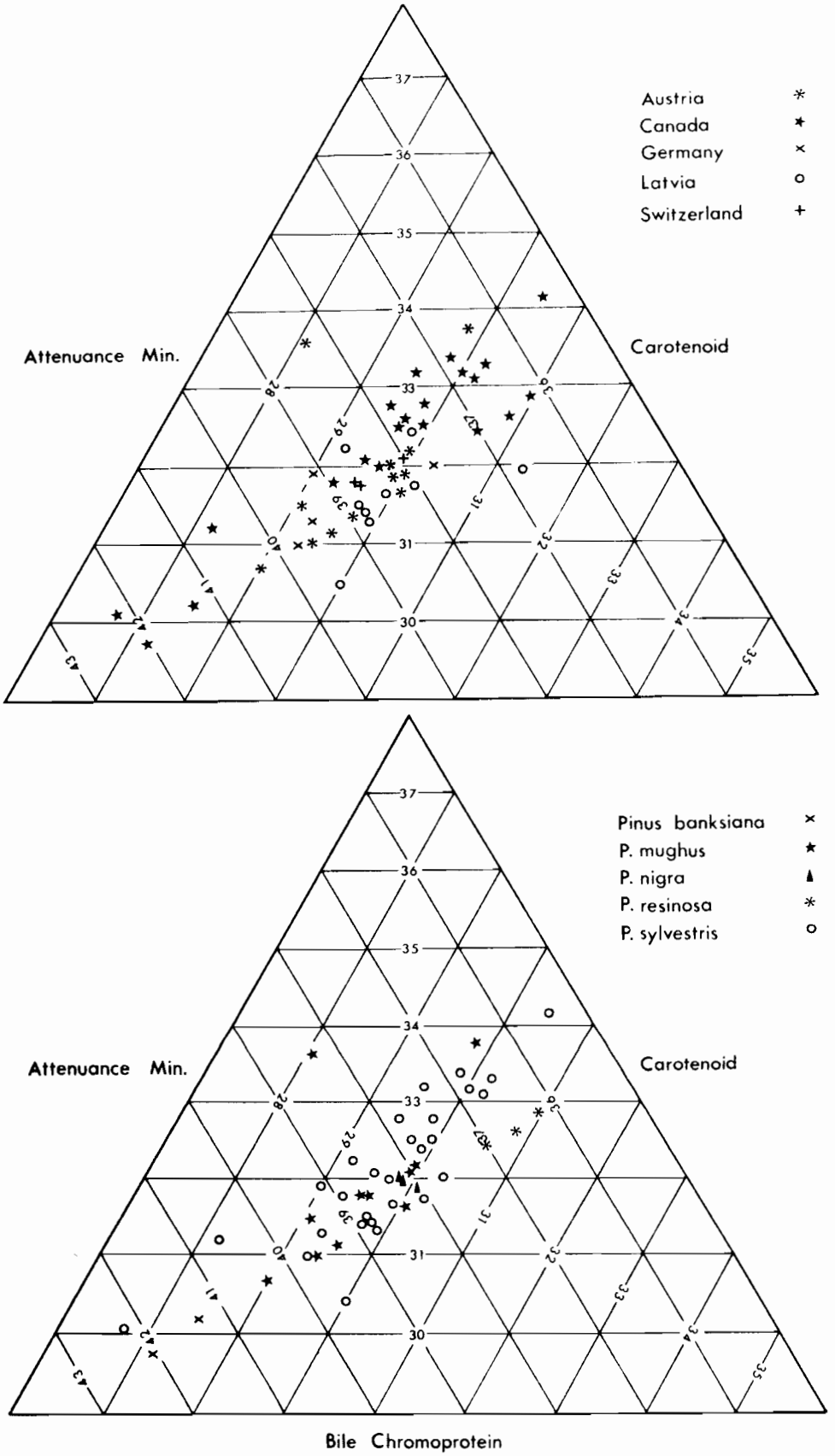
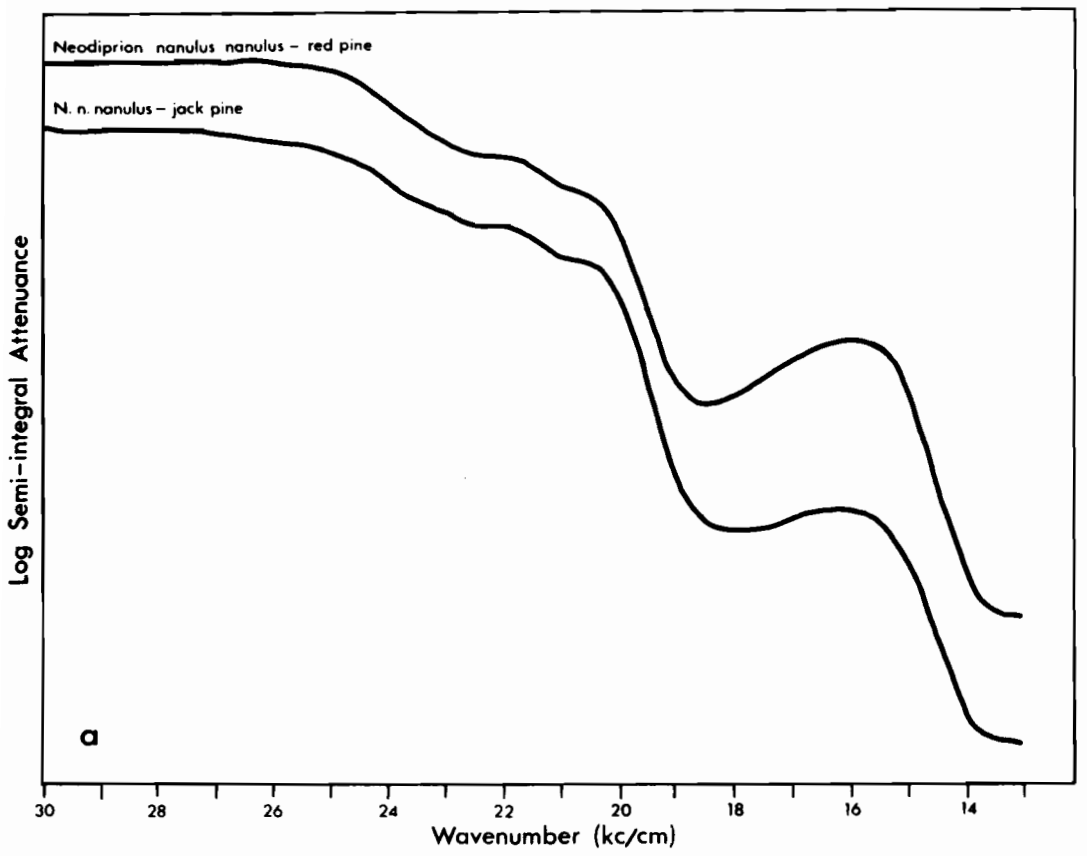

Austria
Canada
Germany
Latvia
Switzerland
Attenuance Min.
Carotenoid
37
36
35
34
33
31
30
28
29
27
32
39
40
41
42
43
Pinus banksiana
P. mughus
P. nigra
P. resinosa
P. sylvestris
Attenuance Min.
Carotenoid
Bile Chromoprotein

Fig. 67 a. Log semi-integral attenuance spectra of eggs of Neodiprion nanulus nanulus from red pine and jack pine.

b. Triangular co-ordinate plot of egg spectrum data for red pine and jack pine forms of Neodiprion nanulus nanulus.


Neodiprion nanulus nanulus – red pine
N. n. nanulus – jack pine
Log Semi-integral Attenuance
a
30
28
26
24
22
20
18
16
14
Wavenumber (kc/cm)

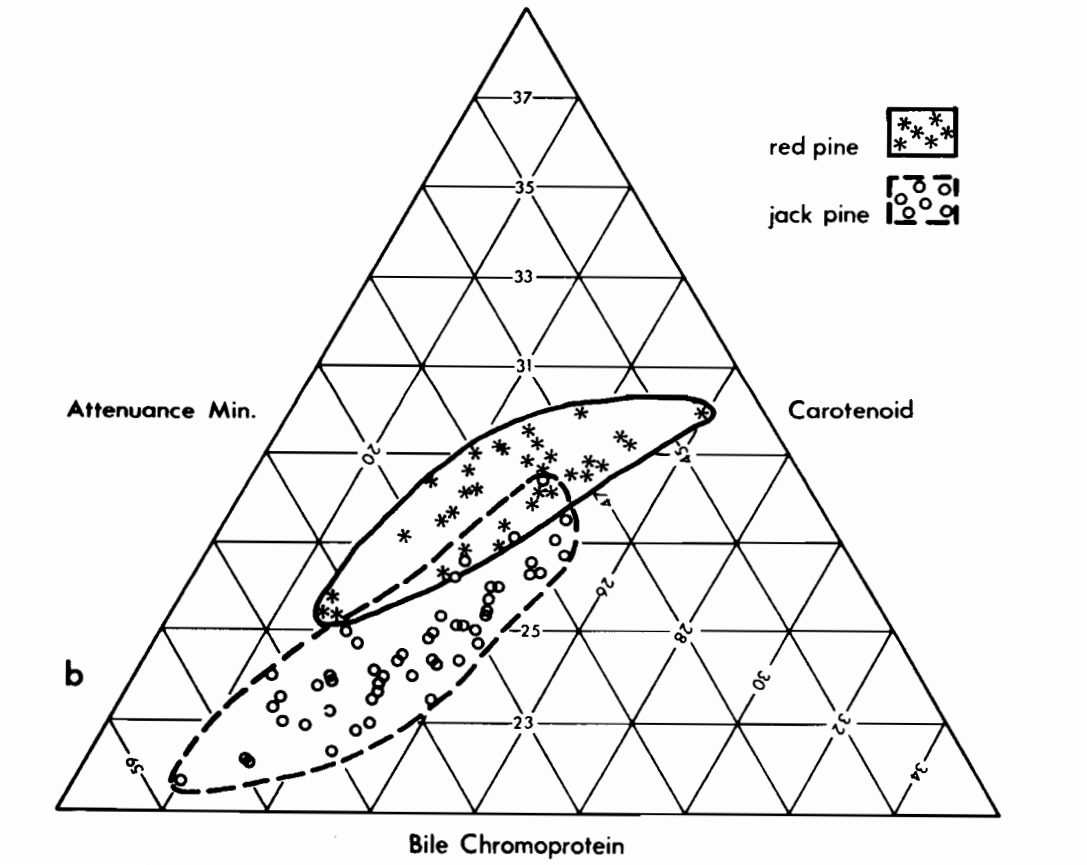
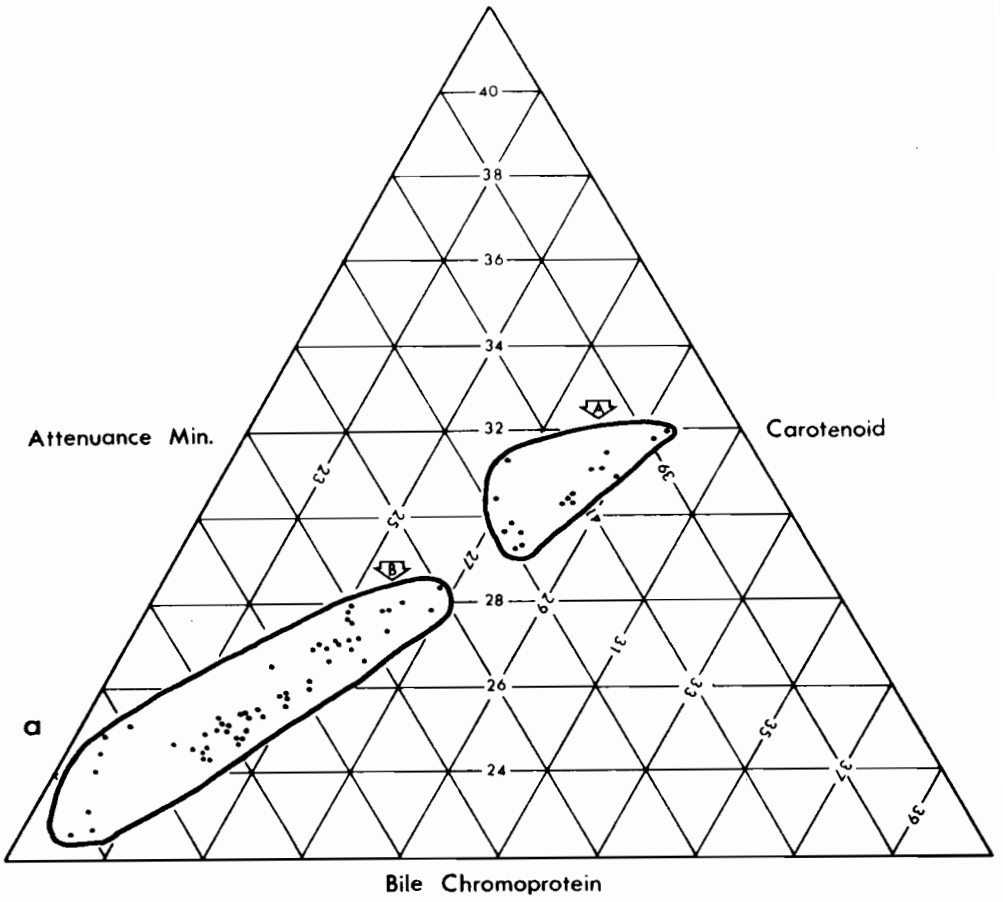

red pine
jack pine
Attenuance Min.
Carotenoid
Bile Chromoprotein
b
37
35
33
31
20
25
23

Fig. 68 a. Triangular co-ordinate plot of egg spectrum data for Neodiprion abietis complex.

b. Log semi-integral attenuance spectra of Neodiprion abietis complex eggs, Type A, Type B.


Attenuance Min.
Carotenoid
Bile Chromoprotein
40
38
36
34
32
28
26
24
23
25
27
29
31
33
35
37
39
A
B
a

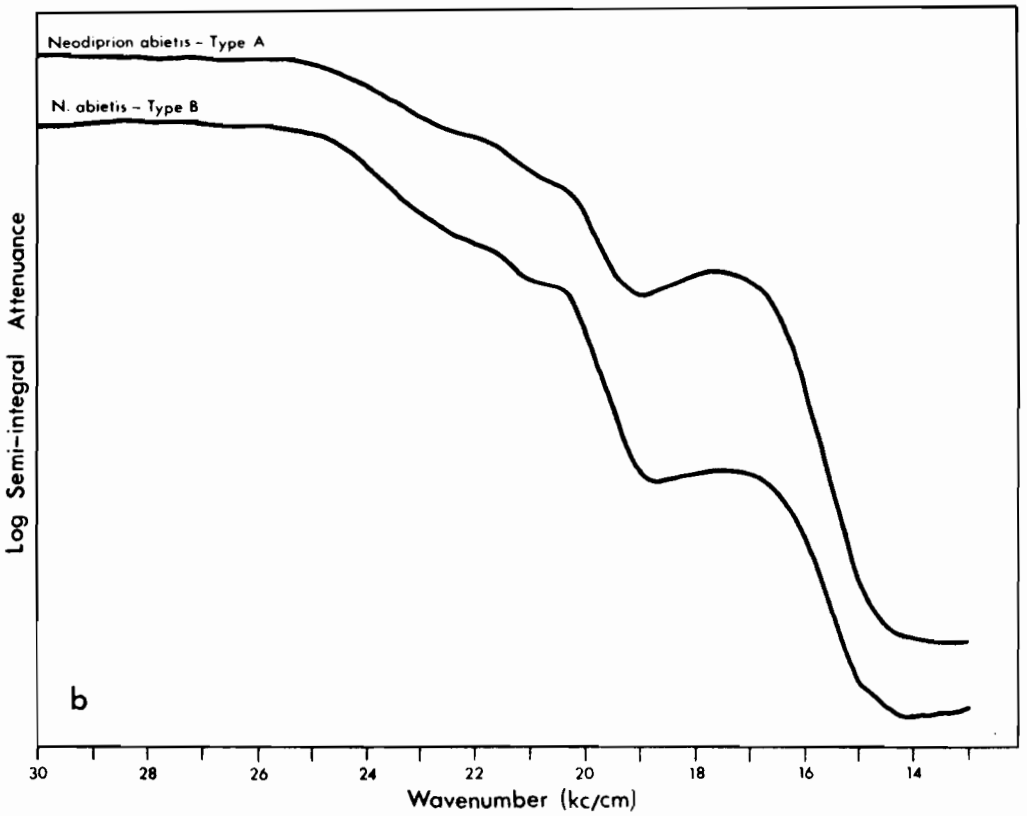
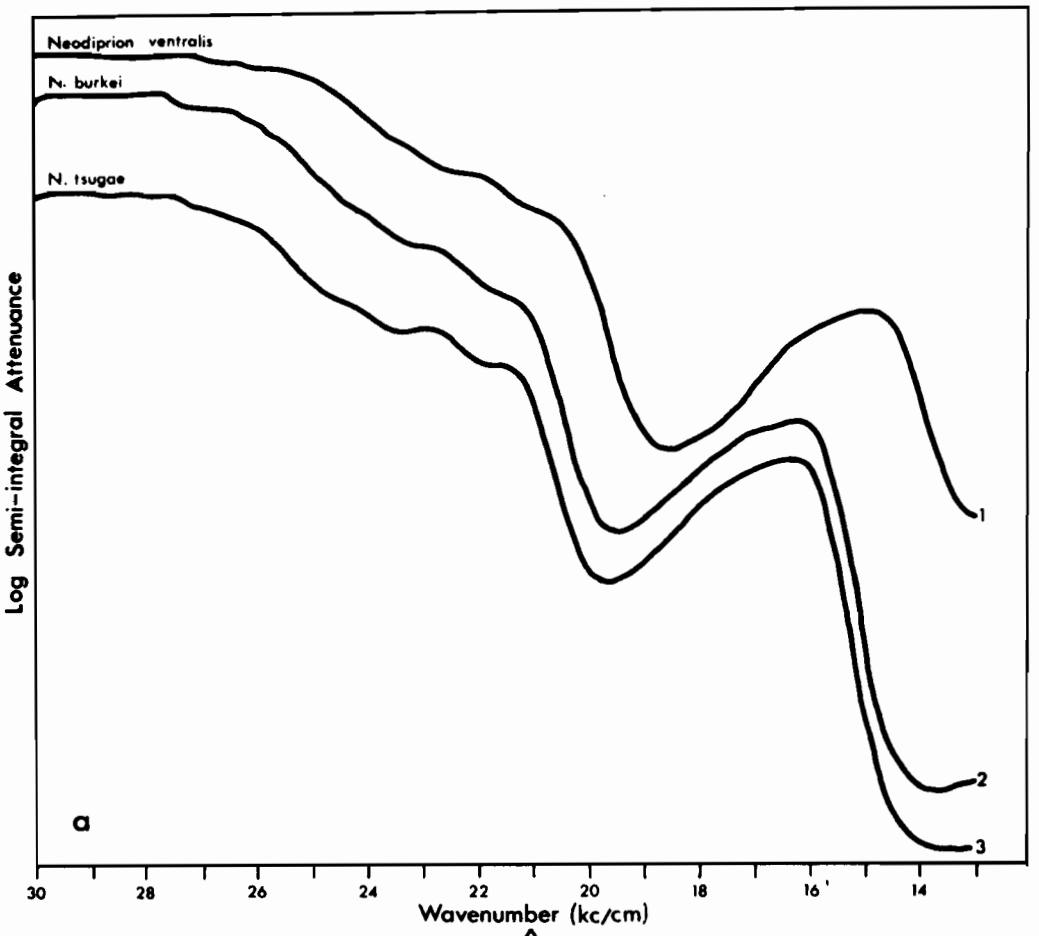
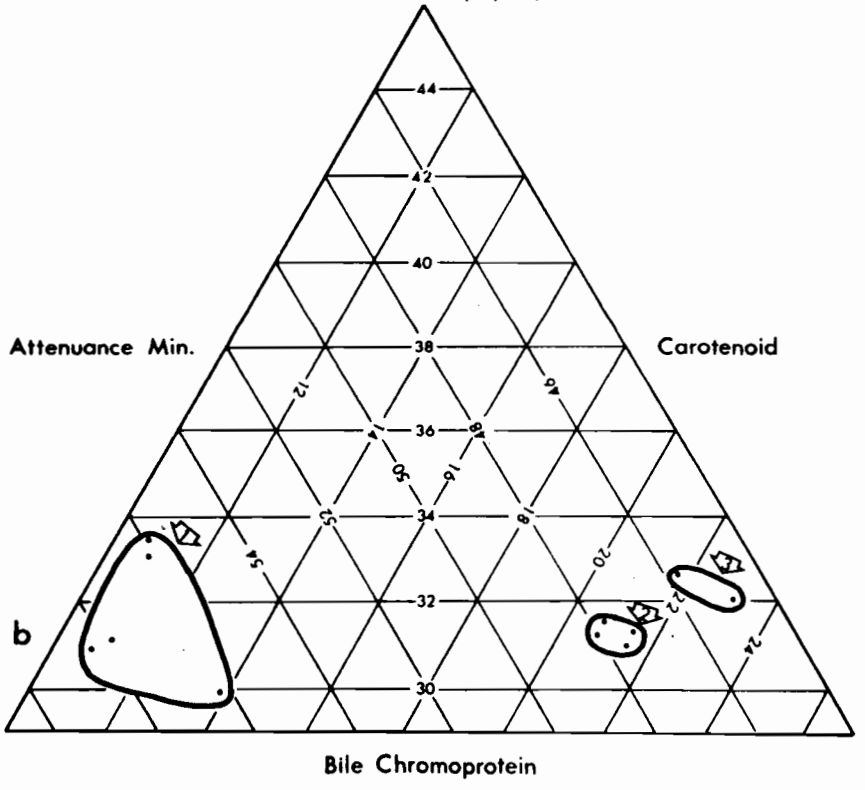

Neodiprion abietis - Type A
N. abietis - Type B
Log Semi-integral Attenuance
b
30
28
26
24
22
20
18
16
14
Wavenumber (kc/cm)

Fig. 69 a. Log semi-integral attenuance spectra of eggs of Neodiprion ventralis, Neodiprion burkei, and Neodiprion tsugae.

b. Triangular co-ordinate plot of the egg spectrum data for species in (a).


Neodiprion ventralis
N. burkei
N. tsugae
Log Semi-integral Attenuance
a
1
2
3
30
28
26
24
22
20
18
16
14
Wavenumber (kc/cm)


Attenuance Min.
Carotenoid
Bile Chromoprotein
b
44
42
40
38
36
34
32
30
12
14
50
52
54
46
48
16
18
20
22
24