Influence of Epicuticular Waxes From White Spruce And Balsam Fir On Feeding Behaviour of The Eastern Spruce Budworm

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ABSTRACT

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Foliage from eighteen trees, nine white spruce and nine balsam fir, which showed resistance or susceptibility to the eastern spruce budworm (*Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae) in the field was selected. Epicuticular waxes were extracted and comparatively tested in behavioural experiments with fourth instar spruce budworm. Several tree waxes were found to produce significant within-host-species preferences by the budworms. The whole wax was broken down into six fractions by column chromatography. Further behavioural experiments were carried out on these six fractions for those waxes to which the budworms showed significant differences in preferences. These tests indicated that fraction 7 (containing primary alcohols, acids, polyesters, hydroxy esters and diols) and 3 (esters) contained the white spruce chemicals responsible for eliciting a preference or an aversion. In balsam fir, preferences or aversions were shown for fractions 7, 2 (hydrocarbons), and 4 (diesters). Gas liquid chromatographs were produced of the whole wax

and wax fractions from those trees for which differential preferences had been shown. These G.L.C.s confirmed the existence of qualitative differences in both the whole waxes and the fractions concerned. Further work is required to isolate and identify these chemicals.

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LIST OF ABBREVIATIONS

B.F.	balsam fir
C.I.	confidence interval
C.L.	confidence limits
Consum.	consumption
[standard]	standard concentration
dia.	diameter
[equal]	equal or standard concentration
F	female
G.L.C.	gas liquid chromatography
H2O	water (distilled)
M	male
M % Consumption	Mean % Consumption
M.P.C.	mean % consumption
N	numbers of animals
[natural]	natural concentration
%	percent
P	probability value
Pref.	preference
S.E.	standard error
V.	versus
W.S.	white spruce

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INTRODUCTION

The eastern and western spruce budworms *Choristoneura fumiferana* (Clem.) and *Choristoneura occidentalis* (Freeman) (Lepidoptera: Tortricidae) are responsible for significant defoliation of evergreen forests in North America. For example, nearly 60 million acres (24 million hectares) of fir and spruce were visibly defoliated by these insects in 1982. There was significant tree mortality in all locations, especially in eastern North America (Talerico, 1983). An outbreak of the spruce budworm can cause a loss of 1.5 to 10 billion cubic feet of timber. The annual Canadian harvest is 5 billion cubic feet.

Despite the proven success of genetic methods in the development of insect-resistant crop plants (Maxwell and Jennings, 1980), progress in breeding insect-resistant trees has lagged behind. That lag can be partially attributed to the relatively long generation intervals in trees and a lack of knowledge about host physiology and

insect biology (Hanover, 1980). Further, the development of resistance in long rotation hosts such as spruce and fir trees requires an interdisciplinary research effort which has only rarely been put forth (DeHayes, 1983).

Though little is known of the host-pest genetic interactions in an ecosystem sense, some sort of preference-aversion, resistance-susceptibility, or virulence-avirulence polymorphology is known for every system that has been studied (McDonald, 1981). Natural genetic variation in susceptibility of white spruce to eastern spruce gall aphids (Canavera & DiGennaro, 1977) and balsam fir twig aphids (DeHayes, 1981) has been documented. A polymorphic population of Douglas-fir (*Pseudotsuga menziesii*) has been shown to occur in response to feeding damage by hare and deer (Dimock et al., 1976).

FEEDING BEHAVIOUR

Observations of the feeding behaviour of newly hatched larvae indicate that one of the most important factors in the process of searching for food is the wandering movement of larvae until they reach acceptable food. This movement must be of an instinctive nature, perhaps caused by

hunger. Silkworm larvae, especially newly hatched ones, will discover food by chance (Ito & Horie, 1974). Some of the actions of budworms can be inferred from their silk "tracks". McDonald (1983) observed threads hanging directly from hatched eggs of all (three) clusters on one 6-year-old Douglas-fir, apparently indicating a non-preference for this individual host.

Albert (personal communication) filmed the feeding behaviour of sixth instar budworm larvae and observed continuous palpation of the needle surface promptly followed by biting and feeding. This behaviour contrasted with that of other larvae. Presented with needles whose external waxes had been removed by dipping in hexane, larvae spent considerable time palpating the surface without biting (Albert & Parisella, 1983).

Life cycle studies indicate that it is generally the younger larvae that are responsible for selecting a suitable host tree. Due to lack of mobility, well fed adult females probably lay most of their eggs on their own food-plant genotype. Larval host selection is achieved by means of spinning a silk thread and allowing itself to be taken up by the wind (known as ballooning). Measurements of larval dispersal for first and second instar spruce budworms have been made by Regiere and Fletcher (1983) and

Jennings et al. (1983). Older instars are too heavy for such flight and therefore play little or no role in host selection. It is therefore conceivable that older instars have a less acute ability to select the preferred host. To eliminate this possibility, a feeding test for a younger instar was developed.

Herbivores can be categorized into two groups; generalist or specialist feeders. Specifically, generalist herbivores feed on a wide range of plants, not necessarily limited to particular taxa, such as families. In contrast, specialist herbivores generally feed on very closely related host plants and may even be species specific. Though generalists and specialists vary in degree of host specialization, these terms are used to refer to the opposite extremes of the same continuum.

"Recognition and preference of host plants involve the interaction of a complex of neural and metabolic events. These include: the sensing and encoding characteristics of the sense organs, decoding mechanisms in the central nervous system, assessment of across-fiber patterns and deterrent/stimulant ratios, pre- and post-ingestion factors such as level of satiety, nutritional balance, and experimental factors such as induction and aversion-learning" (Dethier, 1982).

The first barrier to be overcome in an insect/host relationship is a behavioural one (Dethier, 1970). Specifically, before nutritional and other factors become operative, the insect must first sense and discriminate. Hence, the single most important attribute in insects is their ability to sense the host tree (Payne, 1983). The idea of plant defence against herbivourous insects by means of chemical weapons is widely accepted (Feeny, 1976; Rhoades & Cates, 1976). That mutualistic interactions, such as those of bees and flowers and many other examples, exist suggests that defensive interactions should likewise not only exist, but be far more common. Many phytophagous insects are restricted in host range because of the presence of naturally occurring distasteful chemical substances in otherwise acceptable plants (Thorsteinson, 1960; Fraenkel, 1969).

Host plants have been shown to have important effects on the biology of insects (Morris, 1971, Morris & Fulton, 1970). According to Feeny (1976) and Futuyma (1976), specialist feeders are more likely to feed on unapparent (short-lived) early-succession plants containing potent toxins, to which they are adapted. Generalist feeders tend to feed on the more mature apparent (long-lived) tissue of woody plants defended primarily by digestibility-reducing

substances (Feeny, 1976; Rhoades & Cates, 1976).

Insects can differentiate acceptable from unacceptable food plants without biting and tasting. By drumming briefly on the leaves with their palps (Bernays & Chapman, 1970), grasshoppers sense the food's palatability. The grasshopper rejecting food after palpation makes contact only with the outer surface of the leaf. Experiments have shown that the epicuticular waxes alone provide the information on which the choice is based (Bernays et al., 1976). Specifically, selection is based on information received from contact with *dry, non-polar* and *non-volatile* compounds. That the sensilla can respond to epicuticular waxes has been shown by means of stimulating them with waxes dispersed by sonication in an electrolyte solution. Results indicate that different response patterns are produced by different waxes (Bernays et al., 1974). Atkin and Hamilton (1982) showed that epicuticular wax from younger *Sorghum bicolor* plants was more deterrent to *Locusta migratoria migratorioides*. Woodhead (1983) found that *Sorghum bicolor* cv. 65D seedling was rejected at palpation by nymphs of *Locusta migratoria* L. unless the surface wax was removed. In contrast, on leaves of mature sorghum, most insects palpated and bit on first contact and started to feed. These deterrent effects were attributed to p-hydroxybenzaldehyde, *n*-alkane, and ester fractions

from the epicuticular wax. Fraenkel et al. (1960) found that the larva of *Bombyx mori* are stimulated to bite by a straight chain primary alcohol of the type occurring in epicuticular waxes of leaves.

PLANT SECONDARY SUBSTANCES

In plants, organic compounds of nearly every conceivable structural class abound (Geissman & Crout, 1969). Plant secondary substances are loosely defined as substances produced by plants and that serve no function in the primary metabolic processes of the plant. Thus, alkaloids, most of the phenolic compounds, terpenes, sugars of unique and specialized structure, and the "rare" amino acids, appear to play no essential role in the cellular economy of plants (Geissman & Crout, 1969). However, Geissman & Crout stress that; "our inability to ascribe a function to these compounds by no means constitutes their existence as 'functionless anomalies' or simply the 'end products' of metabolism."

The distribution of plant secondary substances is enormously varied in both time and space, as well as, according to the morphology, species' ecology and

chemistry, population, or even the individual specimens concerned (Rothschild, 1972). Rothschild also found that alkaloids varied in wild plants within 100 meters of each other. Furthermore, these proportions changed significantly as the season advanced. Jones (1968, 1970, 1972) found that the cyanogenic strains of clover and vetches occur at certain altitudes and not at others. Factors such as temperature, age, or the absence and presence of other plant species can influence or control the occurrence of the precursors of HCN in such plants. Perry and Pitman (1983) reported dynamic changes in Douglas-fir defences to the western spruce budworm in time; in one stand, 9 of 10 trees produced higher larval mortality, and lower pupal mortality in 1982 than 1981. They reported this change in foliage defence as coincidental with the very early stages of a budworm outbreak in the vicinity of the stand, though no budworms were detected in the sampled trees. Perry and Pitman (1983) also demonstrated differences that occurred in the tannin composition as well as the defence strategy employed in different strains of Douglas-fir existing on either side of the Cascade Mountain Crest, Oregon. Variations exist even between the parts of a single plant, as demonstrated by within-plant preferences of *Mamestra configurata* feeding on oilseed rape (Bracken, 1984). An example closer to home is that of the toxic effects associated with the ingestion

of rhubarb leaves by humans. The leaves contain three to four times as much oxalic acid as the stalks (Jeghers & Murphy, 1945).

Plants employ a wide range of defence mechanisms, which can be grouped into avoidance, resistance and tolerance. An example of herbivore avoidance (a form of stress resistance) is the asynchrony between larval hatching and bud-burst, demonstrating the importance of "escape in time". "If the larvae hatch too early they cannot enter the buds; if they hatch too late the young needles are already matured sufficiently that they are no longer a suitable food due to decreasing nitrogen and increasing tannin content" (Rhoades & Cates, 1976). Escape in time has been reported for spruce budworm interactions with both balsam fir (Eidt & Little, 1970) and black spruce (Blais, 1957). The resistance mechanisms by which plant secondary substances function include:

1) plant toxins, and
2) digestibility-reducing substances.

HOST RESISTANCE

Beck (1965) defined host resistance as "the collective heritable characteristics by which a plant species, race, clone, or individual may reduce the probability of successful utilization of that plant by an insect species, race, biotype, or individual."

Lamberti et al. (1983) stated that one of the basic problems in the use of resistance is its frequent lack of durability. Some types of resistance used "break down" after a certain period. The temporary nature of these types of resistance is due to the natural selection for new pest or pathogen strains able to overcome them. Consequently, a continuing effort is required to produce new cultivars with new defences. Such problems brought about the recognition of two types of resistance; horizontal and vertical. Though these terms found their origins in reference to resistance to microbial pathogens, they may also be descriptive of resistance to insect herbivores.

Horizontal (or lateral) resistance is synonymous with biotype-non-specific resistance, defined as resistance spread evenly against all biotypes, and displays greater

durability than does vertical resistance. In contrast, vertical resistance is synonymous with biotype-specific resistance (modified definitions from Van der Plank, 1963), and generally lacks durability. It is thought to work on a gene-for-gene basis (Johnson, 1983). Whether resistance will be vertical or horizontal depends on the sort of defence mechanism incorporated into the host, and the sort of attack mechanism the "herbivore" needs to overcome it (Van der Plank, 1963). Recognition of characteristics of vertical and horizontal resistance would help practical plant breeding.

Durable disease resistance in plants is defined as: "resistance that has remained effective while a cultivar possessing it has been widely cultivated in an environment favoring the disease (Johnson, 1983)." This description is based on observation and does not explain the underlying cause for the durability. Durable resistance is often achieved against diseases that show little or no specialization into biotypes pathogenic to particular cultivars. Resistance may be complete or incomplete in terms of the amount of damage incurred (Johnson, 1983).

Resistance can take on many forms. Qualitative types of defence are exemplified by toxins such as alkaloids, terpenes and cyanide. In contrast, tannins, spines, hair

and foliage toughness are examples of quantitative defenses. Here, the degree of host resistance is proportional to the quantity of the defensive factor in the host. An example is the epicuticular wax of Eucalyptus juvenile leaves which simply prevents the Eucalyptus adult tortoise beetles from gaining a foothold (Edwards, 1982). "The distinction between these two classes of plant defence is arbitrary and not absolute (Talerico, 1983)."

Three classes of plant/herbivore interaction (Rhoades and Cates 1976) are:

I. Negative effect on fitness,

II. Deterrent, or

III. No effect.

Interactions causing decreased fitness of or deterrence to the herbivore are examples of plant resistance. Resistance can also be categorized as physical (thorns, toughness), physiological (toxins, etc.), and behavioural ("escape in time"). Increased mortality, decreased growth rates, and/or decreased fecundity are negative effects on herbivore fitness. These effects are primarily attributed to toxins, though digestibility-reducing substances may have such effects indirectly via starvation.

Negative effect on fitness: Plant Toxins

Plant toxins are defined as: substances which act on metabolic processes that are topologically internal to the herbivore. Toxins are known to be present in all plant groups (Rhoades & Cates, 1976).

"The characteristics of a 'good' toxin, from the plant's point of view, should be that it is cheap to produce, highly effective in small quantities against physiological systems found in animals but not in plants (to minimize autotoxicity problems), have physiochemical properties allowing the toxin easy entry into the herbivore's body, and once there, to be resistant to degradation or deactivation by detoxification mechanisms" (Rhoades & Cates, 1976). Known plant toxins satisfy these criteria well. They are generally present at less than 2% of the plant material by dry weight, and active in small quantities. "Toxins active against metabolic systems found in both plants and animals, i.e. mustard oils, cyanide, protoanemonium are sequestered in the plant as inactive derivatives from which the active toxins are released upon tissue damage" (Rhoades & Cates, 1976). Toxins, as a group, do not readily fall into categories of vertical or horizontal resistance due to their variability in biotype specificity.

Deterrent: Digestibility-Reducing Substances

The second class of plant/herbivore interaction, has a deterrent effect on herbivore grazing activities. The ability of the herbivore to detect the presence of a toxin could affect its grazing activities; however toxins generally aim to kill. Specifically, one would expect the herbivore not to feed, and to search for a new host plant. However, the primary substances responsible for a deterrent effect on herbivore-grazing activities are probably digestibility-reducing substances.

Rhoades & Cates (1976) proposed that toxins represent a "lower level" of chemical defence than do digestibility-reducing substances, in terms of cost in both time and energy to the plant's economy. Digestibility-reducing substances generally represent more than 60 % of the plant's dry weight. Digestibility-reducing substances act within the gut of the herbivore to reduce the availability of plant nutrients. On maceration of the leaves by the herbivore, the digestibility-reducing substance complexes with plant proteins, starch and perhaps digestive enzymes, to form complexes which are refractory to digestion (Rhoades & Cates, 1976).

Digestibility-reducing substances should provide protection against both generalist and specialist herbivores for the following reasons (Rhoades & Cates, 1976):

(1) Chemical reactions begin before the leaves enter the gut, and consequently there are fewer pathways by which the herbivores can adapt to the system, when compared to those available against the toxic system.

(2) The very heterogeneous and nonspecific action of digestibility-reducing substances render adaptations of herbivore digestive enzymes difficult.

Because this defence strategy is aimed at a wide range of herbivore biotypes, it may be thought of as horizontal resistance. Horizontal resistance is constitutive, for example, resistance due to physical characteristics such as tissue toughness is not transient. In contrast, vertical resistance may be injury-dependent and thereby induced.

Rhoades and Cates (1976) suggested that most plants contain several defence systems. They further suggested that the type of defence (toxin versus digestibility-reducing substances) employed by a plant has been naturally selected for due to pressures of the

metabolic economy as a consequence of ephememeral or predictible characteristics of the plant or tissue concerned. Generally, toxic defence systems are characteristic of emphemeral (short-lived) tissue, and digestibility-reducing substances are characteristic of predictable (long-lived) tissue. In turn, the degree of uniqueness of the plant's defence strategy is the driving force that determines the evolutionary outcome of herbivores as specialist versus generalist (Rhoades and Cates, 1976). Specifically, divergent defence strategies (i.e. a unique toxin) select for specialists, and convergent defence strategies (i.e. a common digestibility-reducing substances) select for generalists.

No Effect

This third class of plant/herbivore interaction has no negative consequence on herbivore grazing activities. Plant secondary substances may even cause attraction, and toxins may act as feeding cues and are either rapidly excreted, or metabolized into non-toxic derivatives (Self et al., 1964a, b). Herbivores specialized on a particular host may show this type of interaction. In such cases, toxins may confer no protection against specialists.

Different species of insects store plant secondary substances selectively, or concentrate them in different

proportions and in different body tissues, or may excrete, transform or metabolize all or some of them (Rothschild, 1972). Specialists frequently mirror the toxins present in their food plant. Consequently, insects that sequester plant toxins, in turn, have effects on the prey/predator interaction they have with birds, and are often aposematic.

An analogy can here be made with the information advertised by aposematic insects and the information provided by leaf surfaces (epicuticular waxes) as to the leaf contents. As proposed by Chapman (1977), there are obvious advantages to the plant in making their unsuitable characteristics "visible" rather than keeping their defences hidden until they are damaged by being bitten. This "advertising" of plants is comparable to the aposematic character of the monarch butterfly and other insects.

Resistance And The Spruce Budworm

Outbreaks of the spruce budworm are often the result of several consecutive dry summers (Greenbank, 1956 & 1957; Pilon & Blais, 1961). Prolonged stress apparently reduces resistance of the trees and therefore increases the reproductive success of the spruce budworm. Adult female weights of the eastern spruce budworm were shown to be

greater on artificially stressed trees (Mattson et al., 1983).

By comparison of radial growth patterns between host and nonhost trees growing in eastern Canada, Blais (1954, 1965 & 1968) found that many white spruce and an occasional balsam fir have survived repeated budworm outbreaks. Six white spruce were found that had each survived six outbreaks over the past 300 years in one location in Quebec. Apparently, no further research concerning resistance in these polymorphic-species populations in relation to the spruce budworm has been carried out.

McDonald (1979) reported resistance to the western spruce budworm in Douglas-Fir under laboratory conditions. Later, in 1981, he reported the occurrence of differential defoliation between neighbouring trees. McDonald (1981) reviewed host-insect literature concerning the eastern and western spruce budworms, and provided tentative hypotheses to explain the existence of non-defoliated trees. These hypotheses are: moth escape; physiographic location; moth oviposition preference; hibernacular site selection of first instar larvae; larval feeding preference; pheromone chemistry; parasite and predator effectiveness; and host-insect asynchrony. Perry and Pitman (1983) demonstrated the effects of two defence strategies in

Douglas-fir: increased larval mortality, and increased pupal mortality. Respectively, a toxin, and juvenile hormone analogs (Manville & Rogers, 1977) were implicated.

Mattson et al. (1983) found a negative correlation between weight gain in the eastern spruce budworm and the occurence of several terpenes (alpha-pinene, beta-pinene, camphene, beta-phellandrene, bornyl acetate, and terpinolene) in both white spruce and balsam fir. Further, Cates et al. (1983) found that high concentrations of bornyl acetate and beta-pinene in agar diets reduced the dry weight of budworms.

OBJECTIVES

Unaware of McDonald's work, I began this study after the observation by Mr. Reid (a wood lot owner), that particular trees on Cape Breton Island N.S. survived in areas devastated by the eastern spruce budworm, was brought to my attention. This project therefore began with the search for trees showing variation from their neighbours, in terms of the amount of damage incurred. Subsequently, balsam fir (*Abies balsamea* (L.) Mill.) and white spruce (*Picea glauca* (Moench) (Voss)) were collected in these areas and were the

primary subjects of this study. In addition, foliage from trees in Quebec, south of Mount Laurier, and from the Acadia Forest Experiment Station in New Brunswick were also collected.

Based on the idea that budworm palpation of the needle surface may play a significant role in host selection, the primary focus of this study was on the effects of epicuticular waxes on budworm feeding behaviour. In nature, is the spruce budworm's aversion or preference for a host determined after "sensing" with maxillary palps, but before biting and tasting? Specifically, it was hoped that this study would answer the following questions;

1) can within-host-species resistance be seen in nature?
2) can feeding preference for the epicuticular waxes of individual host trees of the same species be demonstrated in the laboratory?
3) what compounds are responsible for the feeding behaviour?
4) what behavioural responses do these compounds elicit, are they feeding deterrents or stimulants? and
5) can this knowledge be incorporated into some means for control of the spruce budworm?

This study is divided into seven parts; 1) the development of a feeding bioassay for fourth instar larvae, 2) collection of host foliage, 3) testing whole waxes in feeding bioassays, 4) gas liquid chromatographs of the whole waxes, 5) column chromatography and fractionation of the whole wax, 6) testing of wax fractions, using feeding bioassays, and 7) gas liquid chromatographs of the fractions.

MATERIALS AND METHODS

FOLIAGE COLLECTIONS

Two types of tree were sought in forest areas that had been attacked by the spruce budworm: survivor trees appearing to be resistant, and live trees appearing to be susceptible to budworm attack.

Fresh foliage, representing the new growth (1983) as well as the previous years' ("old") growth, from white spruce and balsam fir was collected from branches located at more than five meters above the ground, but below the crown. The foliage was kept on ice until it was frozen ($-20^{\circ}C$) in Montreal. The percent water of both new and "old" foliage was calculated by means of first weighing, and then freeze-drying followed by a second weighing of samples of each tree's foliage. Trees were identified with reference to Hosie's book; "Native Trees of Canada" (1973). Gross approximations of heights, age, defoliation and other details are given in Appendix I (Field Notes). Photographs were also taken to show the conditions of the trees and are presented as Figures 1 through 7. Location of foliage collection sites and the identity of the trees sampled are

given in Table I.

TABLE I

COLLECTION SITE		TREE LABELS: White Spruce	Balsam Fir
Nova Scotia	A	A1	A2
	B	B3	B1-83 B1-84 * B2 B4 *
	C	C4 , C5	C1 C2 C3
	T	T1 * T2 * T3 *	
Quebec	Q	Q1	Q2
New Brunswick	NB	NB(WS)	NB(BF)

Most of the trees were sampled during May 28 and 29, 1983. Those trees sampled during June 14 and 15 are indicated with an *. The tree B1 was the only tree sampled twice. The collection site "A" designates an area near Rural Route #1 and on the west side of MacPhee Cross, Baddeck, N.S., "B" is an area on the hill located northeast of the same intersection, and "C" is in the MacIntyre Mountains, about 12 kilometers south of Judique. These areas were all rather heterogeneous forests. The "T" signifies an area located on the Cabot Trail road-side, 17.5 km for T1 & T2 and 23.7 km for T3, west of the village of Cape North. Trees T1 and T2 were from a very homogeneous forest (i.e. white spruce

alone) where nearly 100% of the host trees were killed by a spruce budworm outbreak.

The trees used as standards for comparing all the other trees and referred to as standards were A1 (see Figure 1) for white spruce and A2 (Figure 2) for balsam fir. Tree A1 was intertwined with a dead white spruce of a very similar stature and was therefore thought to be resistant. The balsam fir A2, was nearby and was largely defoliated between the lower crown and its bottom branches, and therefore was thought of as a susceptible tree. These two trees were chosen as standards for comparision as a consequence of the first experiments performed.

Those trees from area B came from what appeared to be an "oasis" on the west facing bank of a hill. The trees from the immediate surrounding area were apparently all dead and had therefore mostly been logged. The woodlot owner had kept these trees to reseed the area with their potentially resistant characteristics. It was therefore thought that these trees may all have resistant properties. Figure 3 show trees B1 (tallest) and B2 (leaning) in late May 1983. Figure 4 was taken in June 1984, and shows that the same tree B1 had deteriorated in health and that B2 had fallen.

Area C was not in any way remarkable except for the

occurence of some dead and defoliated individuals. The trees here were smaller and presumably younger. Trees from New Brunswick (NB) were healthy individuals planted by man in pure stands. Because the specimens sought were dispersed in an obscure heterogeneous (mixed hardwood/softwood) forest, area Q was not ideal. However, trees were sampled during the search for resistant trees.

Area T possessed the most remarkable and ideal conditions. It was a vast plateau covered with dead white spruce trees. Figures 5 and 6 show trees T1 and T2 and their dead neighbours. In sharp contrast, the tree T3 was sampled from a lush-green-healthy forest a short drive (6.1 km) below this plateau (Figure 7).

FIGURE 1. Here, the white spruce A1 (arrow) is seen standing with a dead spruce to the left.

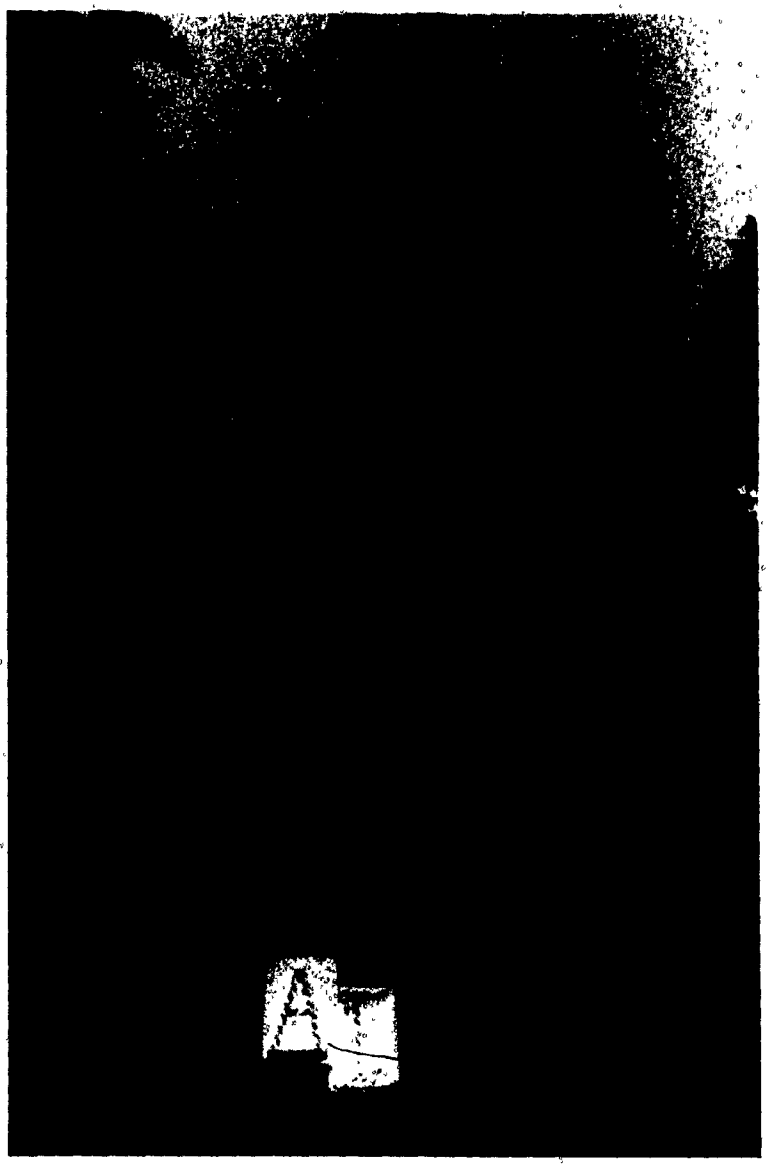


FIGURE 2. Here, the balsam fir A2 is seen to be defoliated between the crown and its lower branches.

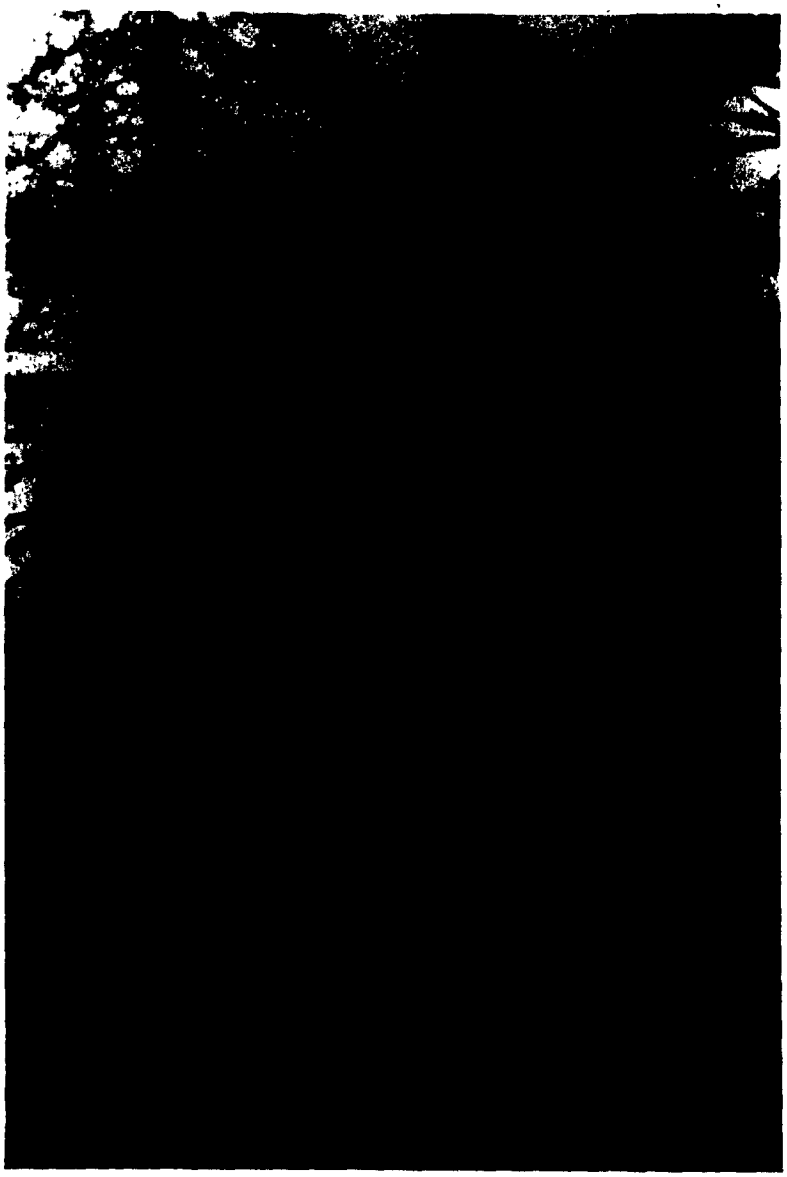


FIGURE 3. The balsam fir B1-83 (tallest) appears quite healthy here. The leaning balsam fir on the right is B2.

FIGURE 4. Again, B1-84 (tallest) was sampled and photographed the following year. B1 has become sparser in foliage and B2 has fallen.

FIGURE 5. The white spruce T1 (arrow) stands out among the dead trees.

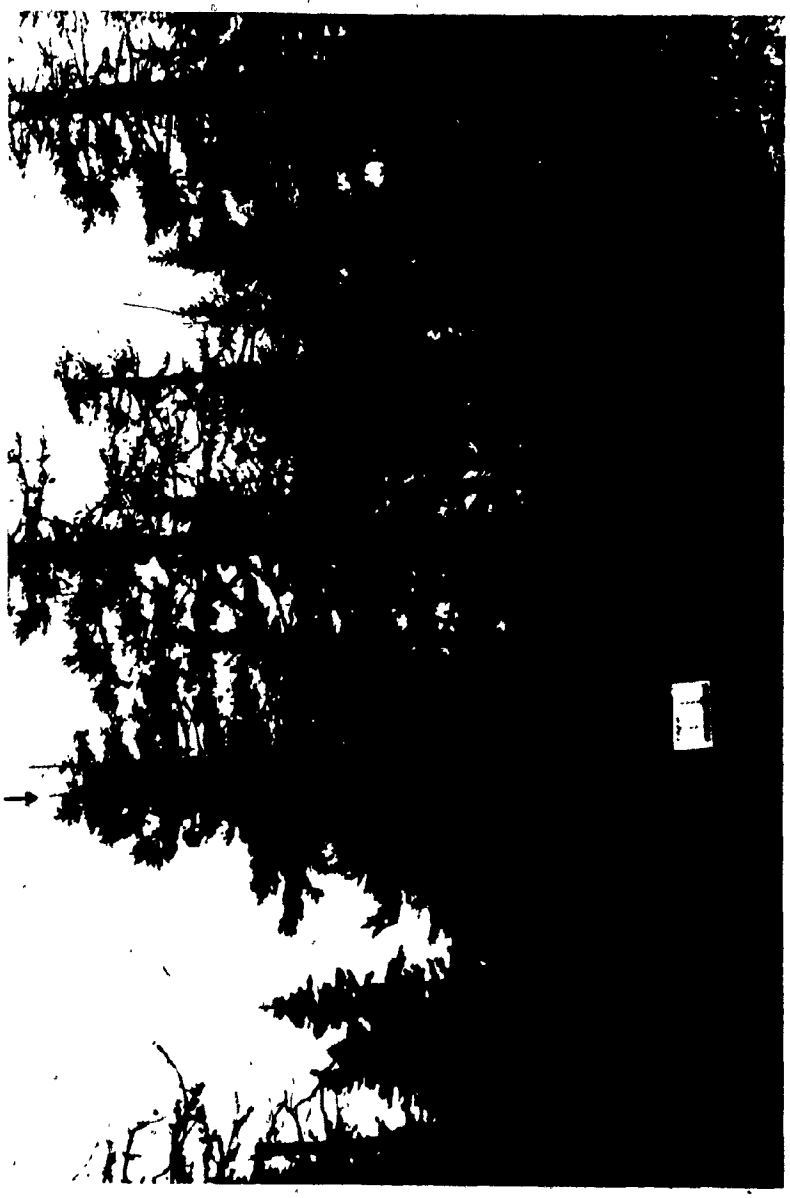


FIGURE 6. Not far from T1 stands the white spruce T2 (arrow), also surrounded by dead trees.

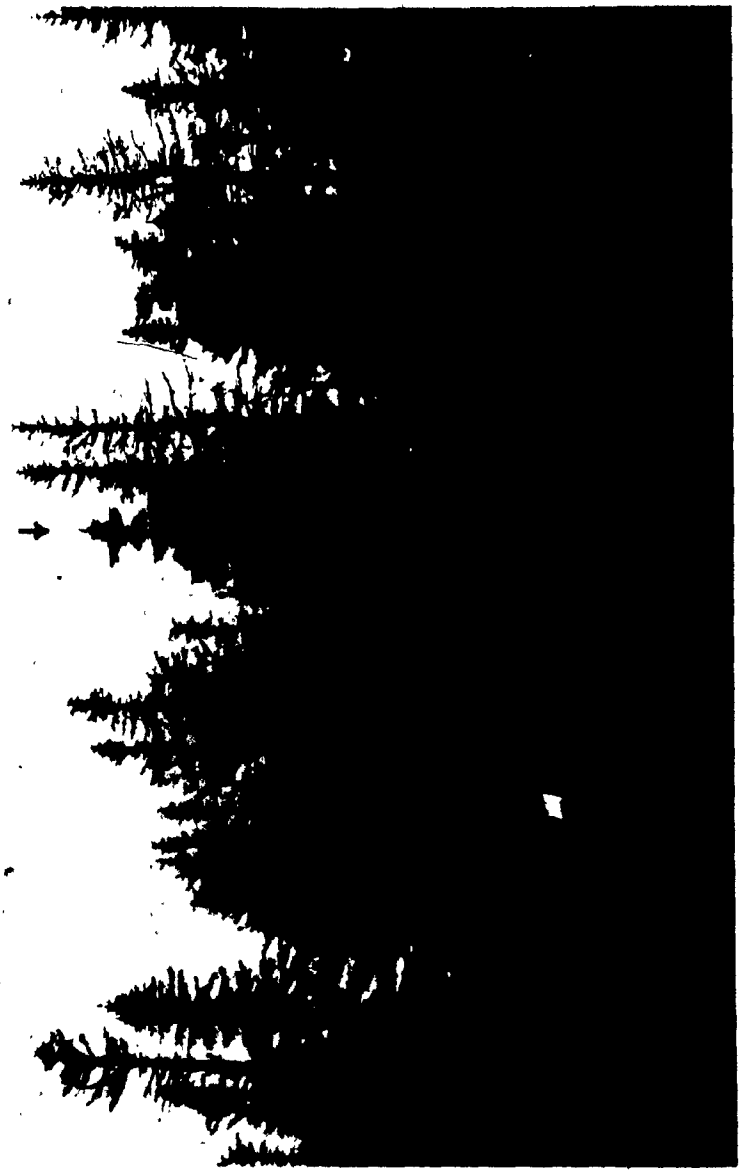


FIGURE 7. Contrasted by the surroundings of T1 and T2 is T3 (6.1 km away) surrounded by a lush-green environment.

EXPERIMENTAL ANIMALS

Unfed second instar larvae were obtained from the FOREST PEST MANAGEMENT INSTITUTE, Sault Ste. Marie, Ontario and were subsequently reared on artificial diet (McMorran, 1965) in an incubator with a 16h:8h light:dark cycle and maintained at 27 degrees Celsius and about 70 % humidity. Fourth instar larvae (determined by head capsule size; McGugan, 1954; Titus, 1977) were starved for twenty-four hours before all experiments.

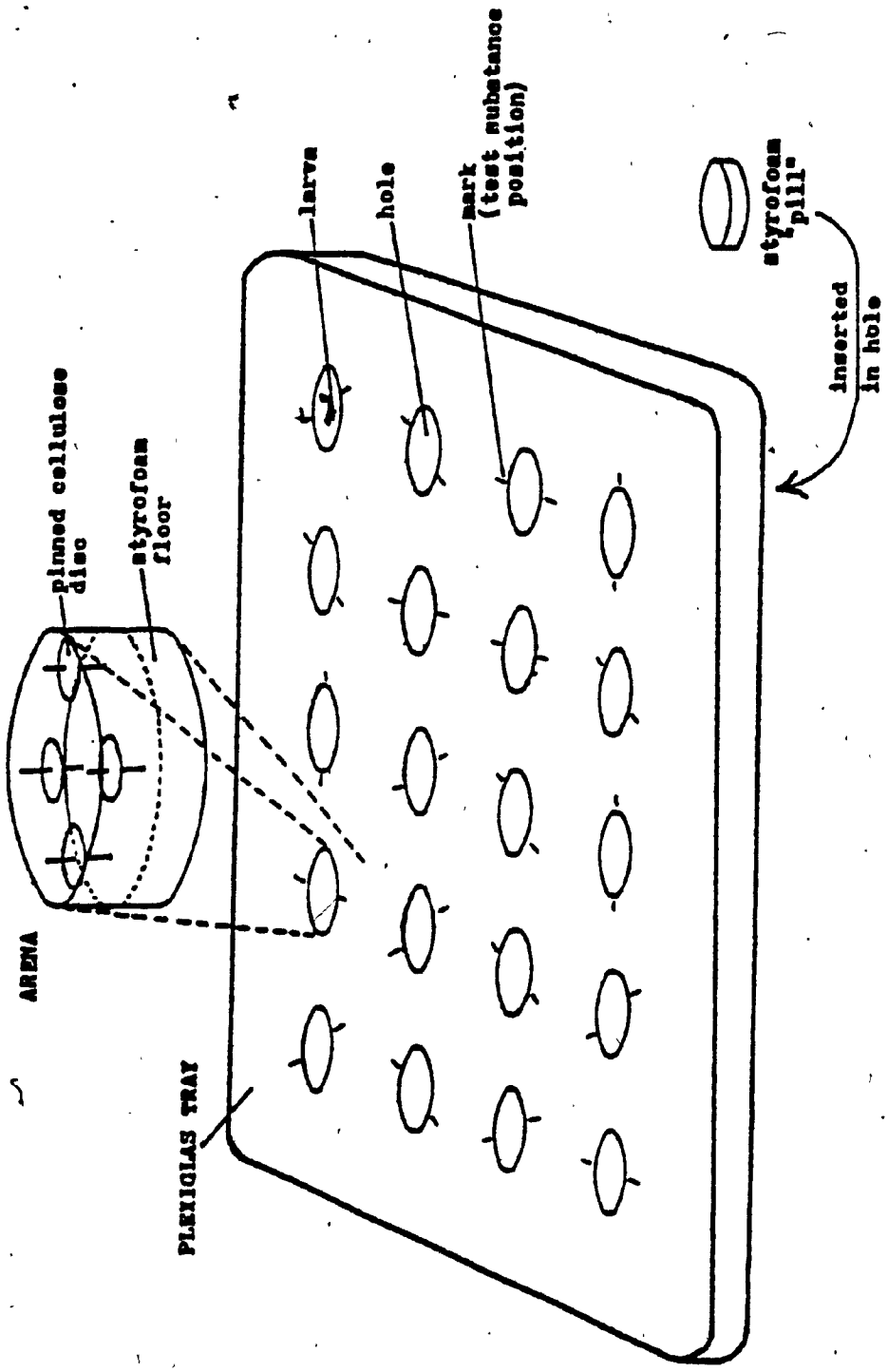
EXPERIMENTAL DESIGN

The primary tool used throughout this study was the experimental tray (Figure 8) wherein all behavioural experiments were carried out. This design was developed at the onset of this study, to accommodate the fourth instar spruce budworm larvae. The larva is contained therein, and is presented with a choice between two "food" substances.

Experimental trays (see Figure 8) were made of a 7 mm thick plexiglas sheet measuring 19.6 cm by 24.8 cm. Twenty test arenas were evenly distributed in the sheet. Each arena consisted of a 1 cm diameter hole, 5 mm deep, with a

styrofoam "pill" (or "plug") fitted at the bottom so as to make a floor in which "minuten" insect pins holding the test materials could be mounted. Each arena was then covered with a square microscope cover slip (22 mm sq.) held in place with distilled water.

FIGURE 8.
EXPERIMENTAL TRAY

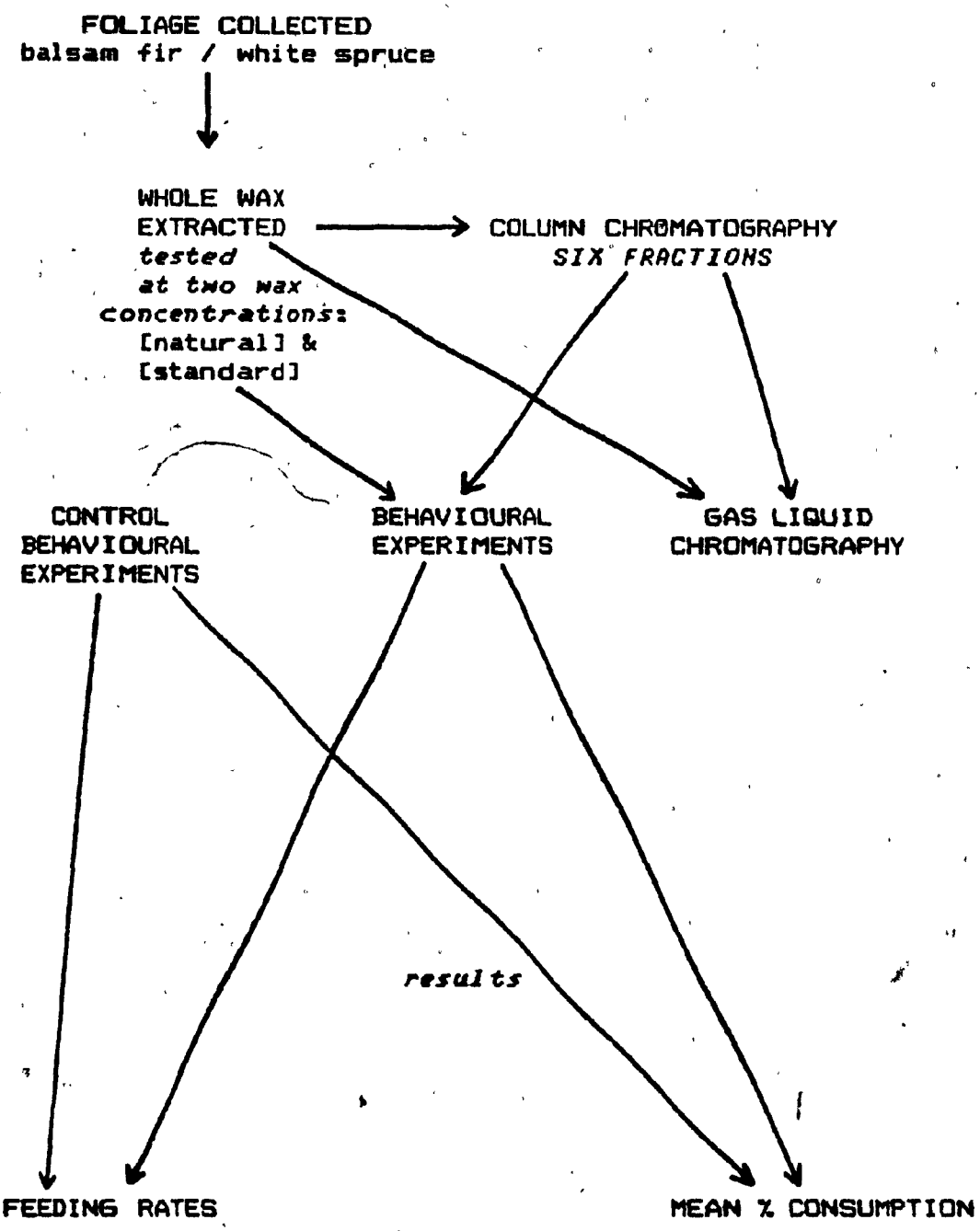


Cellulose discs (3.3 mm diameter) punched out of cellulose filter paper (pore size 0.45 um) were pinned at less than 2 mm above the arena's styrofoam floor, in a circular fashion. Four discs were used in each test arena. Two discs situated opposite each other were impregnated with a standard substance, and the other two represented a test substance. The positions of the discs in each arena were marked at random so as to prevent bias due to a common orientation for all the arenas. The test substance positions were indicated with markings on either side of the arena. Each disc was impregnated with the appropriate host wax (or a fraction thereof) and then wetted with a 3 ul aliquot of distilled water. This experimental design is a modification of the bioassay techniques developed by Jermy et al. (1968), and Albert and Jerrett (1981). The technique allows for the non-biased presentation of two "edible" substances, such that in any direction in which the larva moves, it will always find the same variety of choices.

BEHAVIOURAL EXPERIMENTS

The schematic diagram in Figure 9 illustrates the general procedure followed during this study.

FIGURE 9
SCHEMATIC OF EXPERIMENTAL PROCEDURES



With the exception of a few control experiments (to verify the experimental design), all experiments had forty replicates. However, the N values given in the figures are always much lower than forty. This is due to rejection of molting animals, those feeding insufficiently, and those that died.

Three categories of behavioural experiments were performed:

1) Control Experiments,
2) White Spruce Experiments, and
3) Balsam Fir Experiments.

The first was to test the validity of the behaviour-experimental design that had been developed. Albert et al. (1982) found that 0.025 M sucrose was the strongest of the carbohydrate feeding stimulants for sixth instar larvae. For this reason, 0.025 M sucrose and distilled water were used as standards in these *control experiments*. The results of these control experiments are given in Figures 10 through 13. The effects of four factors were tested. First, the effects of starvation time on feeding behaviour; second, effects due to differences in gender; third, bias in the experimental equipment; and fourth, the effects of the epicuticular waxes, to be used as standards, on the feeding behaviour of the spruce

budworms. For the gender experiments, after experimentation the larvae were reared and sexed as pupae and the data were then divided according to males and females. To remove as many variables among animals as reasonably possible, it was decided that a starvation period before experimentation was desirable.

By extension, from these control experiments, conclusions concerning unknown substances could be made. These unknown substances concern the other two categories of behavioural experiments, namely: epicuticular waxes of *white spruce* and of *balsam fir* needles. In search for differential preferences by the spruce budworm, each tree's wax was compared to the approprate standard (A1 or A2). Then, to check for effects of microsite variation, waxes from neighbouring trees were also compared for preferences by the spruce budworm.

From the data acquired from each behavioural experiment, two parameters were calculated:

1) FEEDING RATE $(FR = (T + S) / H)$,

2) MEAN PERCENT CONSUMPTION $(MPCT = T / (T + S) \times 100)$
$(MPCS = S / (T + S) \times 100)$

where
FR: feeding rate
T: amount eaten of the test discs
S: amount eaten of the standard discs
H: time in hours

MPCT: mean percent consumption test discs
MPCS: mean percent consumption standard discs.

The *feeding rate* is simply an index of the average total consumption per hour of all discs. This may be a measure of the animals' interest in the "foods" presented.

The *mean percent consumption* is a means for comparing feeding preference between substances impregnated into the test and standard discs. These results are graphically displayed as the percent of each disc type consumed.

TEST MATERIALS

The wax used to impregnate the cellulose discs was obtained by dipping 10 grams of needles for 30 seconds in glass distilled hexane. Albert (personal communication) found that epicuticular waxes acquired from an array of different hexane dipping patterns of the foliage did not produce any significant differences in feeding-behaviour experiments from that of wax acquired from a 30 second dip. For these reasons, the 30 second hexane dip was the method used for acquiring the wax. All solvents used throughout this study were of analytical grade. The wax sample from each tree (except the standards A1 & A2) were

applied to the discs and tested at two concentrations:

[natural] &

[standard].

Figures 14 and 15 indicate the "natural" quantities of wax found per area (in millimeters square) for each tree. By knowing the amount of wax naturally occurring on the needles, it was possible to approximately reproduce this amount on the cellulose discs used in the behavioural tests. Experiments performed at *natural* concentrations may yield results reflecting only quantitative differences between tree waxes. To test for qualitative differences, *equal* quantities of waxes had to be compared. Waxes inducing significant differences in preference at equal concentrations are considered more important. Qualitative differences signify a chemical difference between waxes that are significant to the spruce budworm.

Wax from trees A1 (white spruce) and A2 (balsam fir) were used as standards and as references in relation to wax concentrations for other trees. Hence, it can be seen from Figures 14 and 15 that the "standard" concentrations of all other trees represented a decrease in wax quantity so that equal amounts were compared in an experiment. To accomplish this, the extracted wax was redissolved in an appropriate amount of hexane so when applied to individual

discs in 3 ul aliquots, produced batches of discs which resembled the amount of wax found on a particular tree's needles, or that of the standard tree's. These were then kept frozen until required. It should be noted, that all results are labeled with identification codes of trees; natural concentrations are indicated with a capital letter (i.e. B2, C1), and standard concentrations are indicated with a small letter (i.e. b2, c1).

During each experiment, larvae were allowed to feed on discs for 24 hours, at 27 degrees Celsius and 70% relative humidity with a 16h:8h light dark cycle. The quantity of each disc consumed was then scored visually as a percent of the total area. This type of scoring is only minimally subjective, and discrepancies rarely vary more than 10% for different scorers (Albert, personal communication). Each disc was scored as 100% of the possible feeding area. Data from individuals that consumed a total area exceeding 175 out of 200 (87.5%), or less than 25 out of 200 (12.5%) of either the two standard discs or the two test discs were discarded. The purpose of these limits was to avoid the bias effect of an unequal choice due to excessive feeding, or insufficient feeding activity to show adequate preference. All results were analyzed with the Wilcoxon Ranked Signs Test. The null hypothesis (not significantly different) was rejected at a significance level set at $p >$

0.05 (Sokal and Rohlf, 1969).

COLUMN CHROMATOGRAPHY

Those waxes for which test animals showed significant differences in feeding preference were seperated into six fractions using column chromatography. Wax compounds were separated according to their degree of adsorption on silicic acid. Adsorption of a compound depended on its degree of polarity. The column used measured 2.5 X 40 centimeters and was packed with a hexane slurry of 200 millimeters of Bio-Sil A silicic acid (200-400 mesh) that had previously been kept in an oven at 110 degrees Celsius to ensure activity. The wax sample was then applied to the top of the silicic acid and eluted with the following:

Fraction 1	1 litre hexane
Fraction 2	500 ml hexane
Fraction 3	1 litre hexane + 1% diethyl ether
Fraction 4	1 litre hexane + 2.5% diethyl ether
Fraction 5	1 litre hexane + 2.5% diethyl ether
Fraction 6	1 litre hexane + 5% diethyl ether
Fraction 7	600 ml hexane + 600 ml diethyl ether + 150 ml ethanol

Note: Fractions 4 and 5 were combined and labeled as F4. Hence, F5 is omitted.

The following is a list of compound types that can be expected to be extracted from conifer wax, and are presented in order of increasing "polarity" (Tulloch, personal communication):

FRACTION	
	(lowest polarity)
1 & 2	hydrocarbons
3	esters
4 & 5	diesters (e.g. phthalates)
6	secondary alcohols (e.g. 10-nonacosanol)
7	primary alcohols
7	acids
7	polyesters
7	hydroxy esters
7	diols
	(highest polarity)

Based on materials recovered from the column for each tree's wax, the proportion of each fraction size was calculated. By averaging the fraction sizes for three trees for each white spruce and balsam fir, a standard size for each fraction was calculated. These averages served as standard fraction sizes for comparison in further

qualitative behavioural experiments, and also to prevent excessive deviation from the "normal" concentration range. The standard concentrations (ug./mm.sq.) of A1 and A2 multiplied by the average fraction sizes served as standard-fraction sizes for all the white spruce (Figure 24) and balsam fir (Figure 25) respectively. Specifically, to know the quantity of a fraction required to impregnate a disc, the percent value for that fraction was multiplied by the standard concentration. These standard-fraction quantities were then reproduced on experimental discs in an attempt to further isolate the chemical(s) responsible for causing preferences in whole-wax-behavioural experiments.

In order to increase the number of successful replicates (i.e. consumption greater than 12.5% of the test or standard discs) in the behavioural experiments, all discs were impregnated with 0.025 M sucrose before each respective fraction was applied. Otherwise the N would be too low and the confidence limits unacceptably wide.

GAS LIQUID CHROMATOGRAPHY

Gas Liquid Chromatography (G.L.C.) is an effective means for comparing the chemical composition of one wax versus

another (Tulloch, 1975). Whole waxes and fractions thereof that produced significant differences in feeding preference, in the whole wax behavioural experiments, were then examined for differential patterns in their chemical composition by means of a Hewlett Packard model 402 G.L.C. (courtesy of Dr. Tulloch). The G.L.C. was fitted with a three foot (0.92 meter) by one eighth inch (3.2 millimeter) stainless steel column, packed with 1.5% Dexsil 300 on 80-100 mesh acid-washed silanized chromosorb W. The injector temperature was set at about 275 degrees Celsius. The carrier gas was helium, flowing at a rate of 30-50 millimeters per minute. The oven temperature was programmed to increase from 125 to 375 degrees Celsius at a rate of 3 degrees per minute, and the dector system was a Flame Ionization Gauge (F.I.G.). The chart speed was set at 30 centimeters (12 inches) per hour.

A nuclear magnetic resonance spectrometer was used to verify what appeared to be the presence of large quantities of phthalate in some of the wax samples. Phthalate was confirmed to be present in the whole wax sample A2 (courtesy of A.P. Tulloch), and thereby indicates that the first large peak appearing in most of the whole wax tracings (Figures 19 and 23) are phthalate.

To increase the G.L.C. response to the free acids, resins

and fatty alcohols expected to be found in fractions 7, the samples were first acetylated (courtesy of Dr. Tulloch). Fractions 7 were acetylated by first being methylated with diazomethane, and then acetylated with acetic acid and hydride in a steam bath with a pyridine catalyst. This treatment improves identification of alcohol groups and decreases clinging to the G.L.C. column. Thereby, the volatility of the compound types expected to be present in these fractions 7 is increased.

Other complimentary chemical analyses were also run on the fractions (courtesy of Dr. Tulloch). These include Thin Layer Chromatography and Gas Chromatography-Mass Spectrometry. By comparison with known compounds, some peaks in the G.L.C.s could be identified.

EXPERIMENTS PERFORMED

Control Experiments

Control experiments were performed to verify that the experimental design and technique used were appropriate for measuring differences in feeding preference of the budworm for one wax versus another from individual trees of the same host species. All control experiments performed are given in Table II.

TABLE II
CONTROL EXPERIMENTS

			Figure
1)	Starvation (hrs) sucrose/water choice	Nil 12 20 24	10
2)	Gender sucrose/water choice	M F	11
3)	Test for Bias	H2O versus H2O	12
4)	Tests for Standards	Sucrose/A1+sucrose Sucrose/A2+sucrose	13

For these experiments, the mean percent consumptions are given in the figures indicated, and the feeding rates are given in Table VI.

White Spruce

The following is a list of the whole-wax-white-spruce experiments preformed:

TABLE IIIa
WHITE SPRUCE EXPERIMENTS

	TREES COMPARED [natural]	[equal]	Figures
Comparing test trees to standard A1	B3/A1	b3/A1	16 & 17
	C4/A1	c4/A1	
	C5/A1	c5/A1	
	NB/A1	nb/A1	
	Q1/A1	q1/A1	
	T1/A1	t1/A1	
	T2/A1	t2/A1	
	T3/A1	t3/A1	

TABLE IIIb
WHITE SPRUCE EXPERIMENTS

	TREES COMPARED [natural]	[equal]	Figures
Comparing neighbouring tree waxes	C4/C5	c4/c5	18
	T1/T3	t1/T3	
	T2/T3	t2/T3	

The resulting mean percent consumptions are given in the figures indicated, and the feeding rates are in Table VII. To look for significant differences in feeding preferences for different white spruce waxes was the primary objective of these experiments (Table IIIa). The experiments in Table IIIb were performed to check for site variability of tree

waxes that elicited a preference or an aversion. The tree A1 was sampled because it appeared to be resistant, as shown in Figure 1 (page 26). However, data from the first experiments run in 1983 (b3/A1, c4/A1, nb/A1 and q1/A1) indicated that A1 was not qualitatively (Figure 17) different from the other trees, and therefore was mistakenly thought to be an average "run of the mill" susceptible tree. The project was started with the preconceived assumption that resistance is uncommon, and that susceptability was the norm. Henceforth, A1 was used as a standard against which other trees could be compared.

Balsam Fir

The following is a list of the whole-wax-balsam fir experiments performed:

TABLE IVa
BALSAM FIR EXPERIMENTS

	TREES COMPARED [natural]	[equal]	Figures
Comparing test trees to standard A2	B1-83/A2	b1-83/A2	20 & 21
	B1-84/A2	b1-84/A2	
	B2/A2	b2/A2	
	B4/A2	b4/A2	
	C1/A2	c1/A2	
	C2/A2	c2/A2	
	C3/A2	c3/A2	
	NB/A2	nb/A2	
	Q2/A2	q2/A2	

TABLE IVb
BALSAM FIR EXPERIMENTS

	TREES COMPARED [natural]	[equal]	Figures
Comparing neighbouring tree waxes	B1-83/B1-84	b1-83/B1-84	22
	B1-83/B2	b1-83/b2	
	B1-84/B4	b1-84/B4	
	C1/C2	c1/c2	
	C1/C3	c1/c3	
	C2/C3	c2/c3	

The resulting mean percent consumptions are given in the figures indicated, and the feeding rates are in Table VIII. Again, looking for significant differences in feeding preferences for different balsam fir waxes was the primary

objective of these experiments (Table IVa). The experiments in Table IVb were performed so as to verify that tree waxes eliciting preferences or an aversion were not characteristic of a particular area. The tree wax A2 was sampled because (see Figure 2, page 27) it appeared to be an example of a tree susceptible to budworm attack. As a consequence of the first experiments run, B1-83/A2 and b1-83/A2 (Figures 20 & 21), A2 was subsequently used as a susceptible standard for comparison.

Column Chromatography

Column chromatography was used to fractionate the waxes into several fractions so as to isolate the chemicals eliciting preferences or aversions in the spruce budworm.

Behavioural Experiments With Wax Fractions

The following is a list of the wax fraction experiments performed:

TABLE V
WAX FRACTIONS EXPERIMENTS

	WAXES COMPARED		Figures
	T1/A1	T2/A1	
White Spruce	F1		26
	F2		
	F3		
	F4		
	F6		
	F7	F7	
	WAXES COMPARED		
	B1-83/B1-84	C1/B1-84	
Balsam Fir	F1		27
	F2		
	F3		
	F4		
	F6		
	F7	F7	

The resulting mean percent consumptions are given in the figures indicated, and the feeding rates are in Table IX. With the exception of fraction 7, preliminary behavioural experiments using discs impregnated with wax fractions alone (no sucrose) produced results with few replicates

(N), in turn producing unacceptably large confidence intervals. For this reason, subsequently all discs were first impregnated with 0.025 M sucrose. Consequently, the numbers of successful replicates was very high (see N values in Figures 26 & 27). Due to impurities in a solvent mistakenly used, the standard A2 was here replaced with B1-84.

Gas Liquid Chromatography Of Wax Fractions

Gas liquid chromatographs were made of the waxes and the wax fractions that elicited a preference or an aversion in the behavioural experiments. These were made so that the chemical differences between these waxes or fractions could be determined.

Feeding Rates

Feeding rates (Page 39) were also calculated from the data of the behavioural experiments and were examined for characteristics that may help us in our understanding of budworm feeding behaviour.

RESULTS

CONTROL EXPERIMENTS

1) *Effects of Starvation*

Differences between unstarved and starved (12, 20 and 24 hours without food) individuals was apparent (Figure 10), and the degree of preference was greatest for those starved 24 hours. The mean percent consumption indicated a stronger preference for sucrose by budworms starved 24 hours. The percent consumption of sucrose had the greatest value, and the water consumption the smallest value compared to the other experiments. For this reason (and that of my own nocturnal activities), all experimental animals were subsequently starved for 24 hours before testing.

2) *Effects of Gender*

Females did not eat significantly more than males (Table VI). Further, males and females had essentially the same degree of preference (Figure 11). These data showed that, in this study, gender could be ignored. This test and the

tests for starvation times also confirmed that 0.025 M sucrose is always preferred over water.

3) *Test for Bias*

The third, and most important factor to test for was preferential *bias* built into the experimental design or technique. The results, given in Figure 12, show that the experimental method does not produce any significant bias in preference ($P > 0.05$, page 39). A choice of water versus water indicated no preference for the standard or the test positions of the arena. Experimental bias due to phototaxis or attraction to particular positions in the test chambers were not found. It is of interest to note that the feeding rate in this experiment, water-versus-water (distilled), was not very different from experiments either using sucrose (Table VI) or many of those with wax, to be seen later.

4) *Tests for Standards*

Figure 13 shows that discs impregnated with sucrose plus the epicuticular waxes from tree A1 or A2 were preferred to discs with sucrose alone. These significant preferences indicate the presence of chemicals in these waxes that stimulate the feeding behaviour of spruce budworms.

FIGURE 10. STARVATION TIME VERSUS MEAN PERCENT CONSUMPTION

Mean percent consumption of spruce budworms starved for 0, 12, 20 and 24 hours. Distilled water was used as the standard substance, and 0.025 M sucrose served as the test substance. The solid line on each bar here indicates the standard error. Probability (P) values less than 0.05 (page 39) indicate significant differences in the M.P.C. of the standard (water) and the test substance (sucrose). "N" indicates the number of replicates for each experiment.

Figure 10.

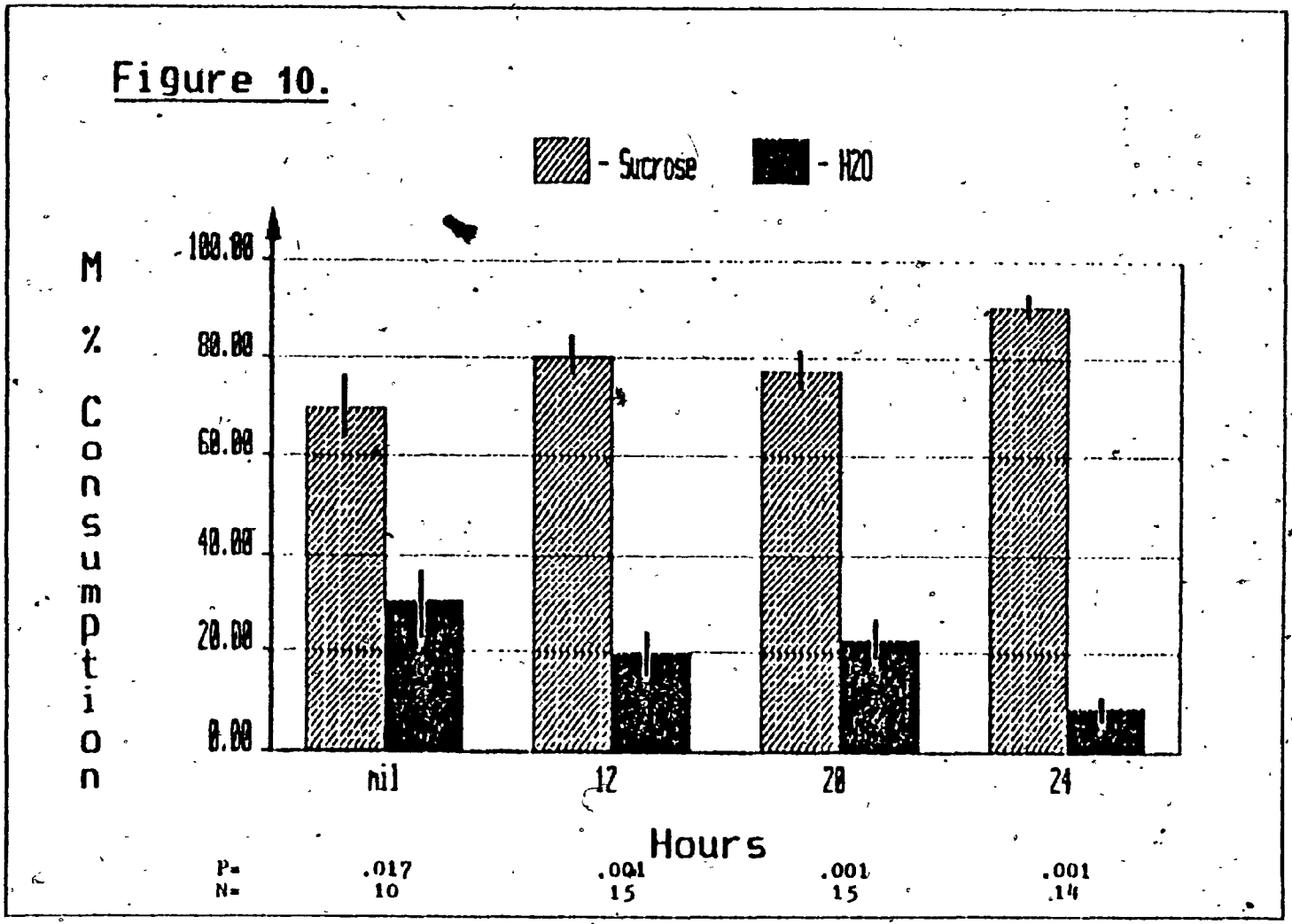

- Sucrose
- H2O
M % Consumption
100.00
80.00
60.00
40.00
20.00
0.00
nil
12
20
24
Hours
P=
N=
.017
10
.001
15
.001
15
.001
14

FIGURE 11. GENDER VERSUS MEAN PERCENT CONSUMPTION

Mean percent consumption by males and females. Distilled water was used as the standard substance, and 0.025 M sucrose served as the test substance. The solid line on each bar indicates the standard error. Probability (P) values less than 0.05 (page 39) indicate significant differences for the M.P.C. of the standard versus the test substance. "N" indicates the number of replicates for each experiment.

Figure 11.

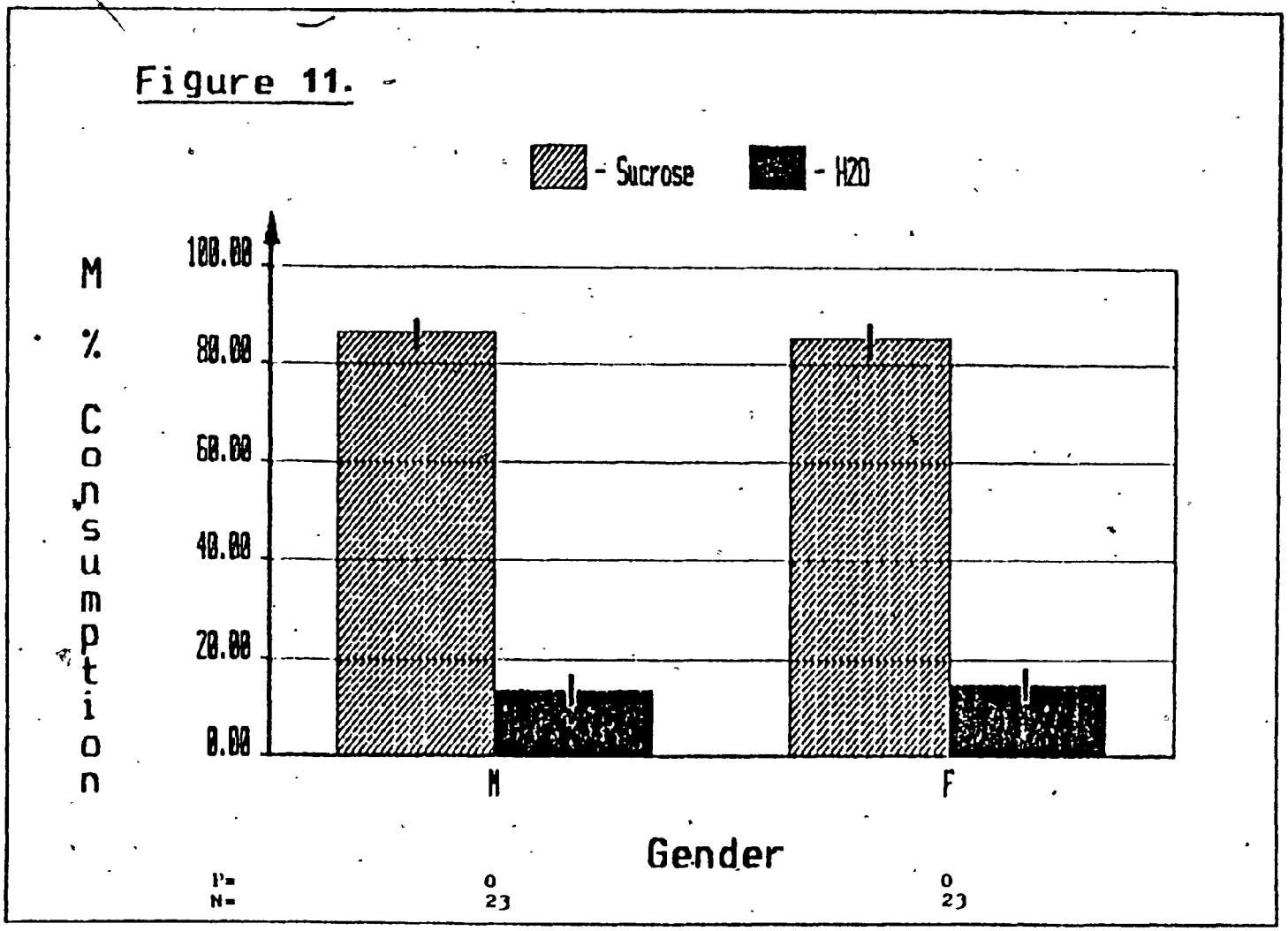

- Sucrose
- H20
M % Consumption
100.00
80.00
60.00
40.00
20.00
0.00
M
F
Gender
P= 0 0
N= 23 23

FIGURE 12. MEAN PERCENT CONSUMPTION: WATER VERSUS WATER

Distilled water was used as both the standard and the test substance. The solid line on each bar here indicates the standard error. Probability (P) values less than 0.05 (page 39) indicate significant differences between the M.P.C. of the standard and the test substance. "N" indicates the number of replicates for this experiment.

Figure 12.

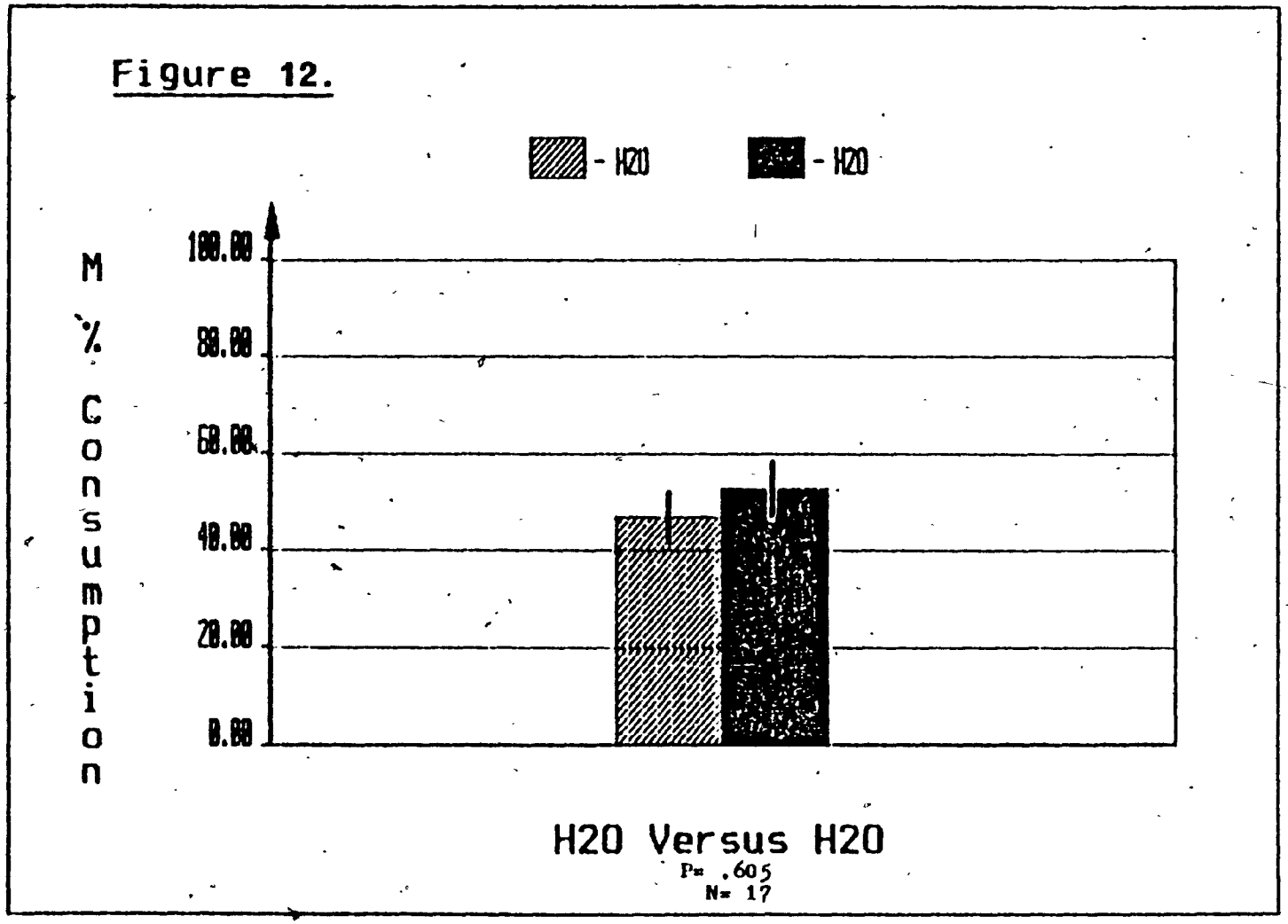
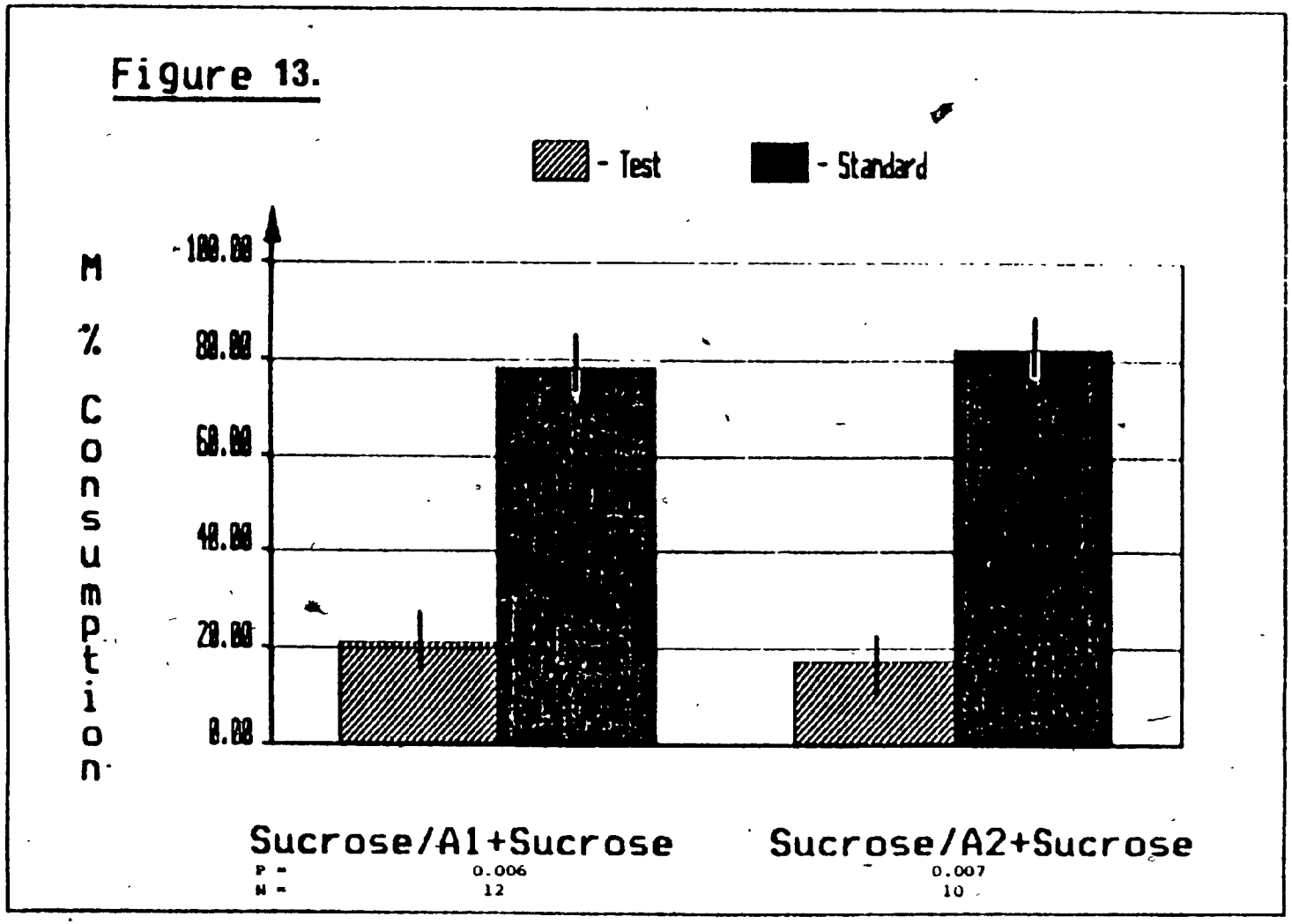

- H20
- H20
M % Consumption
100.00
80.00
60.00
40.00
20.00
0.00
H20 Versus H20
P= .605
N= 17

FIGURE 13. MEAN PERCENT CONSUMPTION: WAX STANDARDS

Mean percent consumption for two experiments. Sucrose (0.025 M) and then wax were impregnated into the standard discs. The test discs contained sucrose alone. The solid line on each bar indicates the standard error. Probability (P) values less than 0.05 (page 39) indicate significant differences between the M.P.C. of the standard and the test substance. "N" indicates the number of replicates for these experiments.

Figure 13.


- Test
- Standard
M % Consumption
100.00
80.00
60.00
40.00
20.00
0.00
Sucrose/A1+Sucrose
P = 0.006
N = 12
Sucrose/A2+Sucrose
0.007
10

EPICUTICULAR WAX

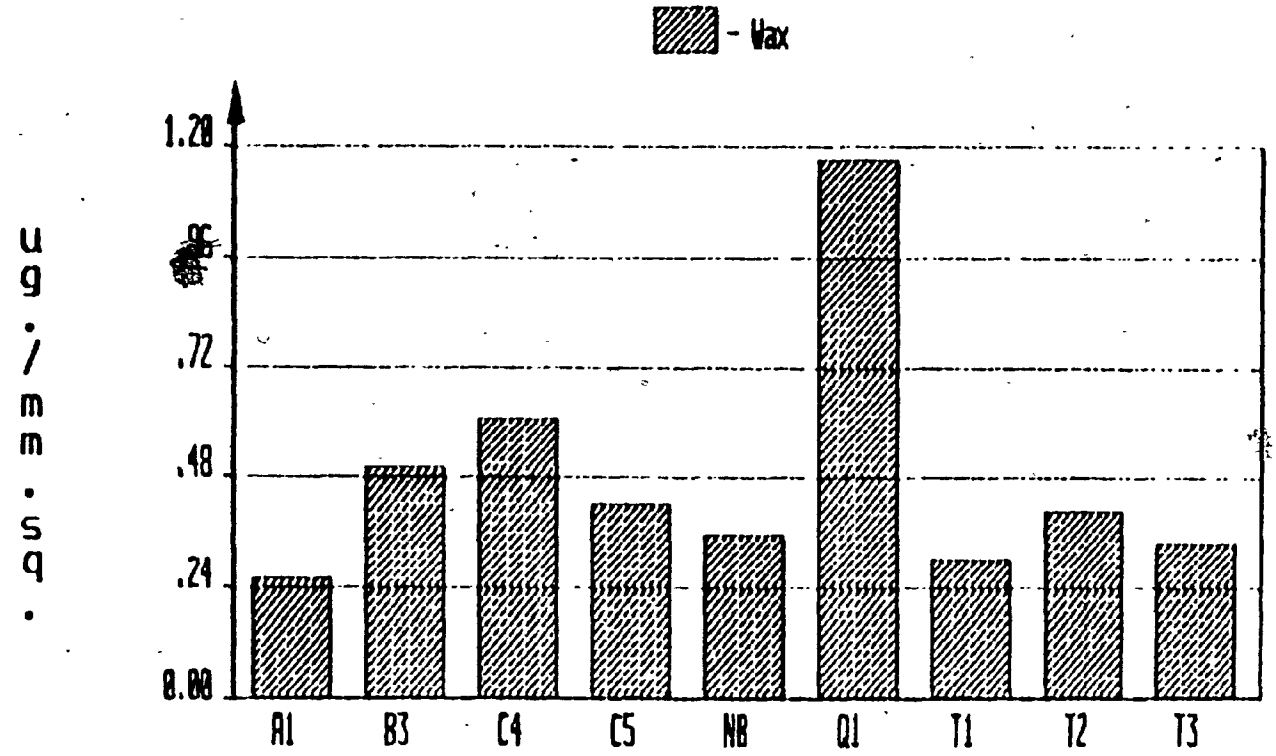
Various characteristics of balsam fir and white spruce were quantitatively determined. Field notes concerning the approximate ages, sizes, defoliation, and environment of the trees tested are contained in Appendix I. Data concerning dimensions, weights, wax quantities and water content of the needles of each tree are given in the tables contained in Appendix II. The quantities of epicuticular wax extracted from each needle sample (10 grams) are represented as micrograms of wax per millimeter square of needle area and are plotted in Figure 14 for white spruce and Figure 15 for balsam fir. These values are referred to as "natural concentrations". The white spruce A1 had the thinnest layer of wax on its needle surfaces, while Q1 had approximately four times that amount (Figure 14). Figure 15 shows that the balsam fir A2 has the smallest quantity, while B2 and C1 have approximately twice that amount.

FIGURE 14. RELATIVE WAX QUANTITIES ON WHITE SPRUCE NEEDLES

Graphic representation of the relative amounts of epicuticular wax extracted from 10 grams of needles by means of one 30 second dip in glass distilled hexane. The wax quantity from tree A1 is used as a standard concentration in many of the experiments.

Note: A1 has the lowest amount of wax per unit area. Hence, when wax concentrations are adjusted from these "natural" concentrations to "standard" concentrations, this represents a dilution in all cases.

Figure 14.



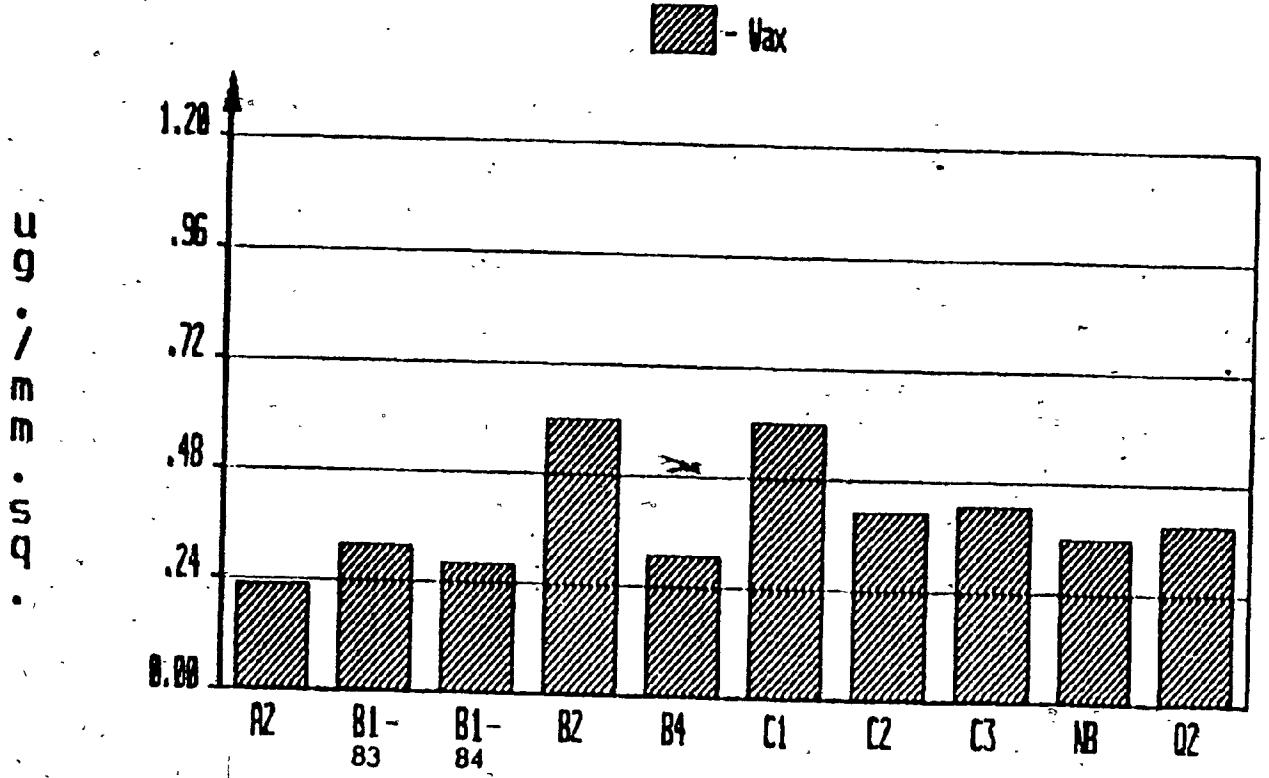
Epicuticular wax from new growth.

FIGURE 15. RELATIVE WAX QUANTITIES ON BALSAM FIR NEEDLES

Graphic representation of the relative amounts of epicuticular wax extracted from 10 grams of needles by means of one 30 second dip in glass distilled hexane. The wax quantity from tree A2 is used as a standard concentration in many of the experiments.

Note: A2 has the least amount of wax per unit area. Hence, when wax concentrations are ajusted from these "natural" concentrations to "standard" concentrations, this represents a dilution in all cases.

Figure 15.



Epicuticular wax from new growth.

The natural wax concentrations calculated for C5 (Figure 14) is based on needle dimensions in Table 1, Appendix II. Mistakenly, only 19 needles were weighed and measured, but erroneously averaged on 20. Therefore the natural wax concentrations used for C5 are slightly higher than they should be. However, because C5 at natural concentrations elicits feeding responses similar to the standard A1, this error is of no consequence.

WHITE SPRUCE

Mean Percent Consumption

The *mean percent consumption* gives a visual representation of the amount of each diet type consumed by the spruce budworms. The white spruce which were significantly different from the standard (A1) at natural concentrations were B3, NB, Q1, T1, T2 and T3 (Figure 16). These results suggest that either or both quantitative or qualitative differences of the waxes are responsible for the behaviour. At standard concentrations (i.e.[A1]) C5,

t1, t2 and t3 (Figure 17) were significantly different from the standard A1. This confirms that the significant differences in preference with waxes B3, NB and 01 result from quantitative differences in the amounts of each wax presented. Therefore, at standard concentrations, waxes b3, nb, q1 and A1 may be thought of as similar with respect to the budworm behaviour elicited. On the other hand, percent consumption of T1, T2 and T3 was higher than consumption for A1 at both concentrations, indicating that these waxes do differ qualitatively from A1. Among the waxes at standard concentration that proved to be significantly different, c5 (Figure 17) stands out as different from t1, t2 and t3 in that A1 is preferred to c5.

Results of experiments comparing feeding on waxes from closely located trees show that, to the spruce budworm, the tree waxes t1 and t2 are not qualitatively different from T3 (Figure 18). Quantitatively, T2 and T3 are different.

Gas Liquid Chromatographs

Figure 19 shows the *gas liquid chromatographs* of waxes from four trees that produced diferential preferences in the previous experiments. The traces are presented in order of increasing preference by the budworms, going from bottom upwards in the figure. Differences in the traces of A1 versus those of T1, T2 and T3 can be seen. The arrows point to peaks that seems to follow the desired patterns. Specifically, the first arrow (trace T1) shows a peak that is larger in T3. When T3 was diluted in the behavioural experiments (Figures 16 & 17) the preference is seen to increase, as though an optimal concentration exists. This peak is absent from trace A1, which was found to be significantly different from the rest. The arrow in trace A1 indicates a peak that could be a factor in making A1 the least preferred to the budworms. More work is required so as to isolate these chemicals and, thereby, confirm that these peaks do influence the budworm's feeding behaviour.

FIGURE 16. MEAN PERCENT CONSUMPTION ON WHITE SPRUCE WAXES AT NATURAL CONCENTRATIONS

Mean percent consumption (M.P.C.) during 8 experiments, comparing waxes at their natural concentrations (indicated by the capitalized letter(s) of the wax's label). The standard A1 is common to all experiments here. The solid line on each bar indicates the standard error. Probability (P) values less than 0.05 (page 39) indicate significant differences between the M.P.C. of the standard A1 and the test tree. "N" indicates the number of replicates for each experiment.

Figure 16.

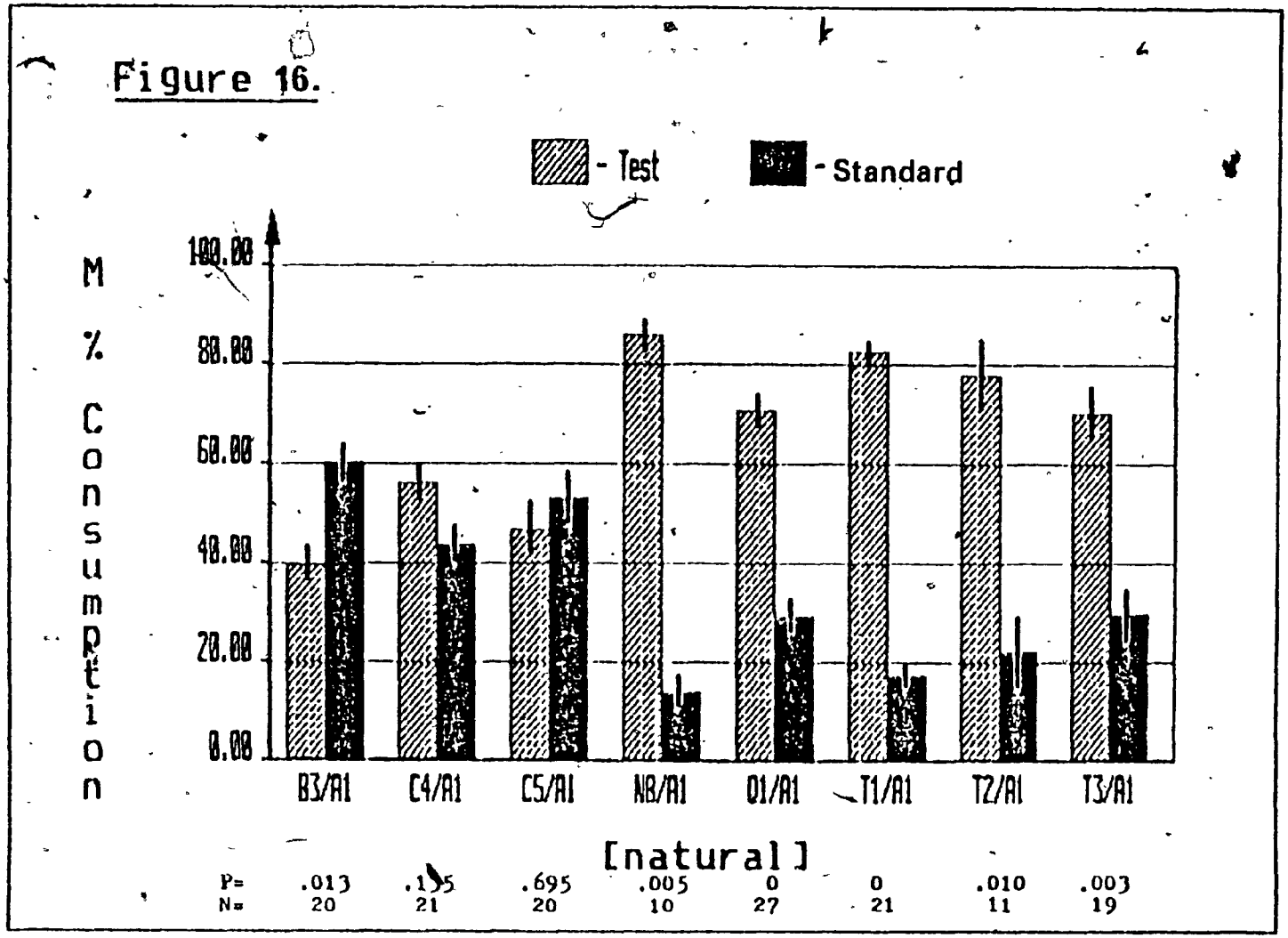

- Test
- Standard
M % Consumption
100.00
80.00
60.00
40.00
20.00
0.00
B3/A1
C4/A1
C5/A1
NB/A1
Q1/A1
T1/A1
T2/A1
T3/A1
[natural]
P= .013 .155 .695 .005 0 0 .010 .003
N= 20 21 20 10 27 21 11 19

FIGURE 17. MEAN PERCENT CONSUMPTION ON WHITE SPRUCE WAXES AT STANDARD CONCENTRATIONS

Mean percent consumption (M.P.C.) during 8 experiments, comparing waxes at concentrations equal to that found on the standard tree (indicated by the small letter(s) of the test wax's label). The solid line on each bar here indicates the standard error interval. Probability (P) values less than 0.05 (page 39) indicate significant differences between the M.P.C. of the standard A1 and the test tree. N indicates the number of replicates for each experiment.

Figure 17.

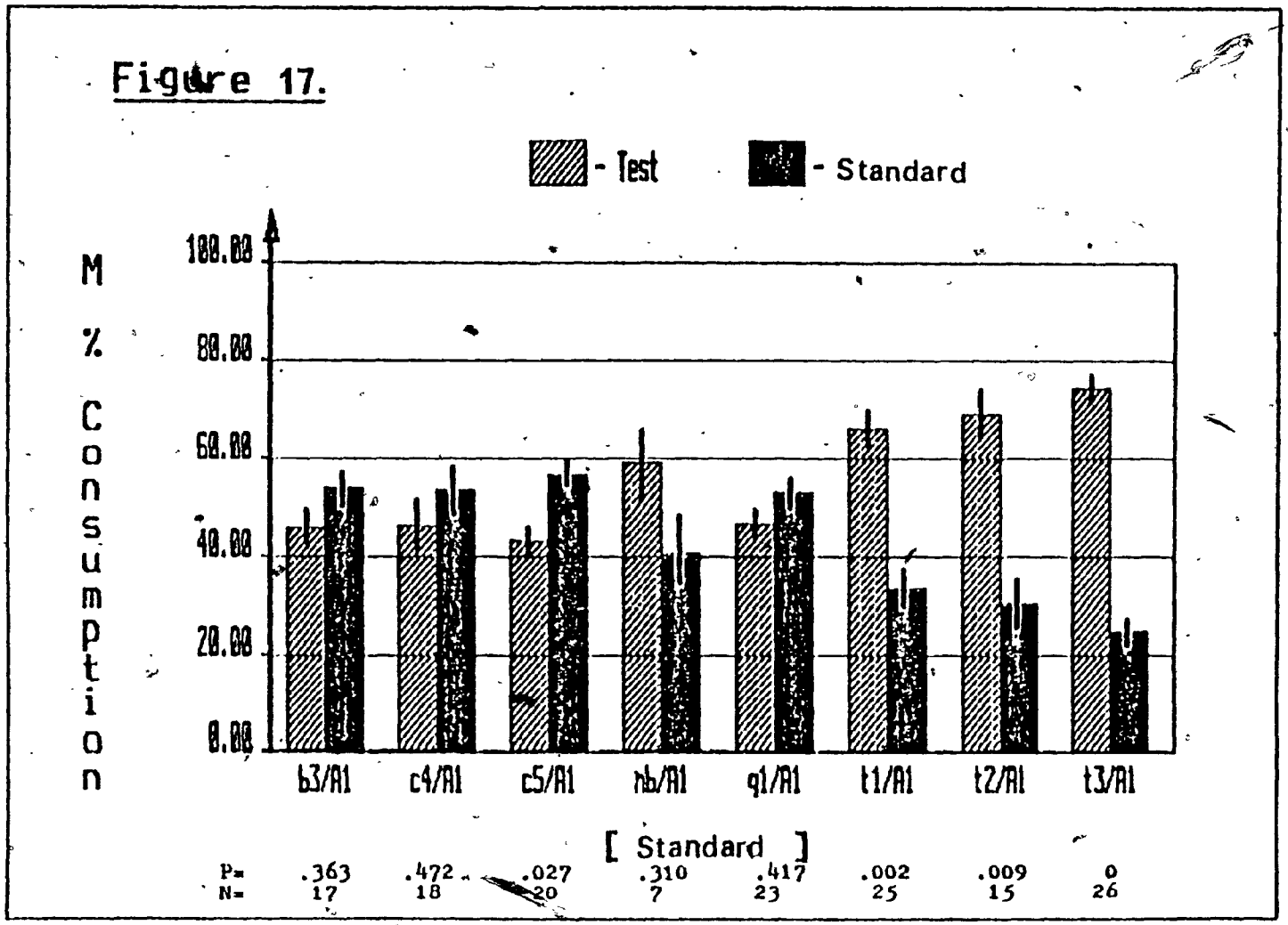

- Test
- Standard
M % Consumption
100.00
80.00
60.00
40.00
20.00
0.00
b3/A1
c4/A1
c5/A1
nb/A1
q1/A1
t1/A1
t2/A1
t3/A1
[Standard]
P= .363 .472 .027 .310 .417 .002 .009 0
N= 17 18 20 7 23 25 15 26

FIGURE 18. MEAN PERCENT CONSUMPTION ON WAXES FROM NEIGHBOURING WHITE SPRUCE TREES

Mean percent consumption (M.P.C.) during 6 experiments, comparing trees from the same area, generally within 50 meters of each other (except T1 & T2 are 6.1 kilometers from T3). The experiment labels are always presented in this format: TEST/STANDARD. Experiments alternate from natural wax concentrations (capital letters for both test and standard wax labels) to standard concentrations (small letter for the test tree's label). The solid line on each bar here indicates the standard error interval. Probability (P) values less than 0.05 (page 39) indicate significant differences between the M.P.C. of the standard tree's wax and the test tree's wax. "N" indicates the number of replicates for each experiment.

Figure 18.

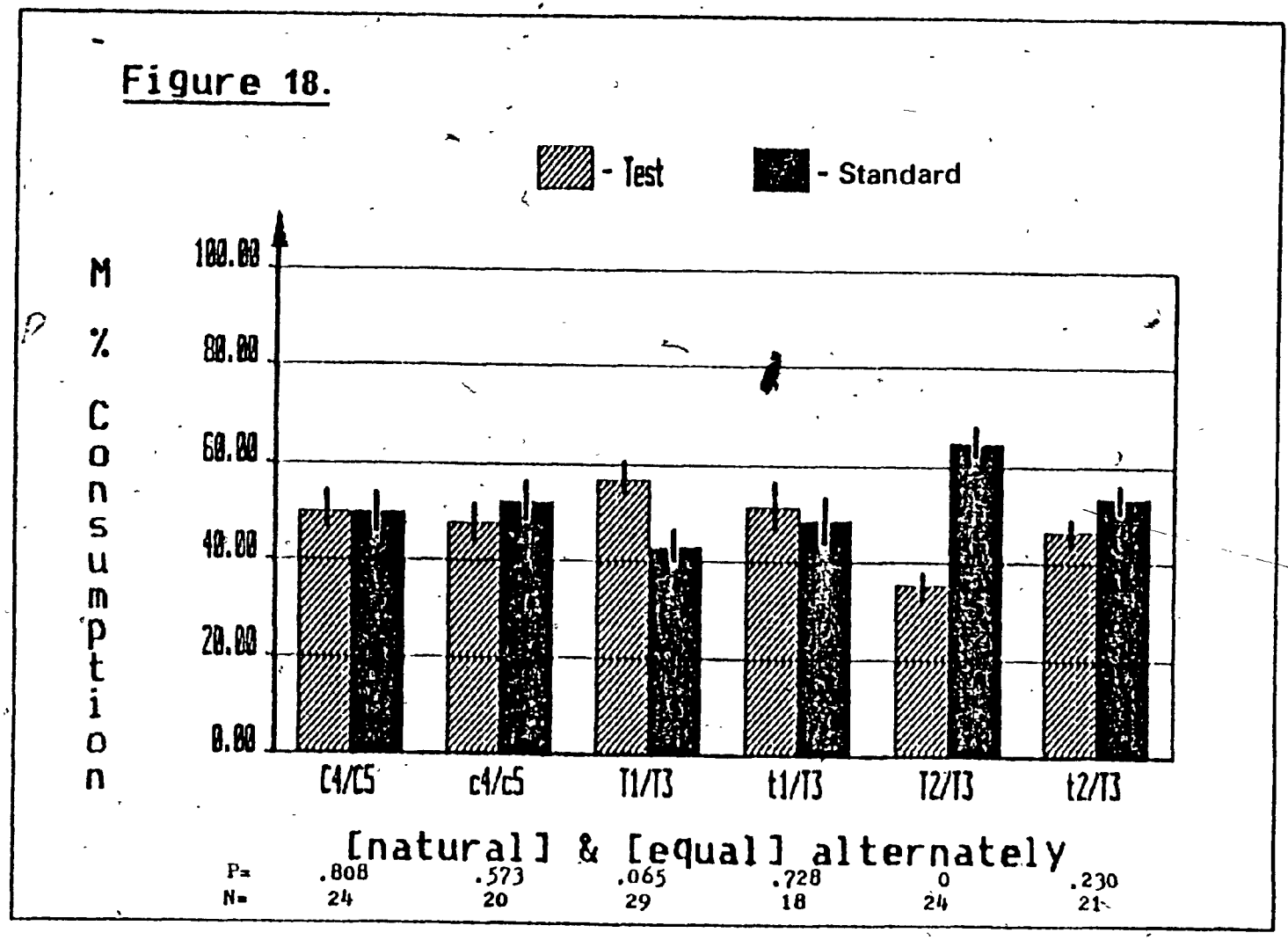

- Test
- Standard
M % Consumption
100.00
80.00
60.00
40.00
20.00
0.00
C4/C5
c4/c5
T1/T3
t1/T3
T2/T3
t2/T3
[natural] & [equal] alternately
P=
.808
.573
.065
.728
0
.230
N=
24
20
29
18
24
21

FIGURE 19. GAS LIQUID CHROMATOGRAPHS OF WHOLE WAXES FROM WHITE SPRUCE

The trace for A1 whole wax at the bottom is the least preferred of those presented here. Feeding behaviour on A1 is significantly different from T1, T2 and T3. The G.L.C.s are given in the order of increasing preference going upward. The solvent peak in each trace is indicated with an "s". Two tall peaks are common to all four traces, the first of which is thought to be phthalate. The two arrows point to peaks present only in the waxes eliciting a preference or an aversion respectively.

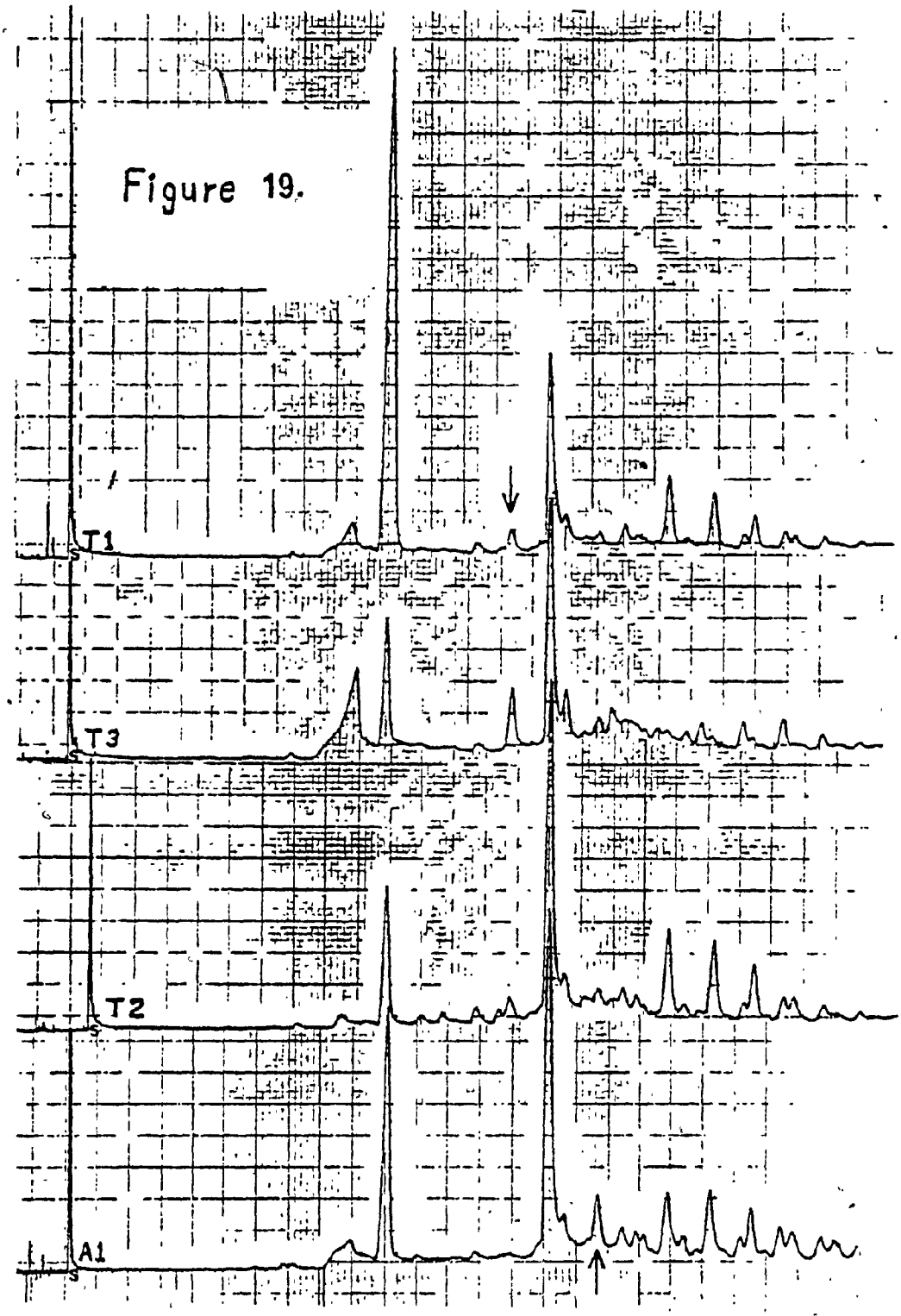


Figure 19.

BALSAM FIR

Mean Percent Consumption

At natural wax concentrations, a preference for B1-84 and B4, and an aversion for B1-83 and C1, relative to wax A2 was found (Figure 20). Similar experiments at equal ([standard]) wax quantities (Figure 21) suggest that, for these waxes, these differences reflect qualitative differences. Interestingly, in this same figure, the dilution of waxes C3 and NB to the concentration of A2 resulted in an aversion by the budworm to waxes C3 and NB.

When preferences for waxes from neighbouring trees were tested, significantly different preferences for the waxes tested (Figure 22) were found in all but three cases. These results are consistent with what would be expected by extrapolation of previous results. Preference for the waxes B1-83 and B1-84 when compared to A2, fall in the following order: B1-84 > A2 > B1-83 (Figures 20 & 21). Therefore, when comparing B1-83/B1-84, B1-84 is expected to be preferred as is the case (Figure 22). Furthermore, waxes

such as B2 that proved to be similar to A2 (Figures 20 & 21), continued to produce similar behaviour in later experiments. The results of B1-83/B2 and b1-83/b2 (Figure 22) resemble those of B1-83/A2 and b1-83/A2 (Figures 20 & 21). Consequently, considering the previous results and the waxes being compared, it is of little suprise that significant differences occur in so many experiments illustrated in Figure 22.

Gas Liquid Chromatographs

In Figure 23, *gas liquid chromatographs* of four epicuticular waxes, for which behavioural data suggested qualitative differences, are displayed. The preferred waxes are placed at the top of the page, the least preferred at the bottom. Patterns relating to preferances were looked for in the traces. The arrow in trace B1-84 indicates a peak which appears smaller in waxes B1-84 and A2, which are both expected to be significantly different from B1-83 and C1 in preference experiments. Again, the arrow in trace C1 indicates a peak that appears greater in waxes C1 and B1-83. These peaks may represent chemicals that elicit preferences in the spruce budworm.

FIGURE 20. MEAN PERCENT CONSUMPTION ON BALSAM FIR WAXES AT NATURAL CONCENTRATIONS

Mean percent consumption (M.P.C.) during 9 experiments. Comparisons are all at natural wax concentrations (capitalized wax labels) and to the same standard A2. The solid line on each bar indicates the standard error. Probability (P) values less than 0.05 (page 39) indicate significant differences in the M.P.C. of the standard A2 and the test substance. "N" indicates the number of replicates for each experiment.

Figure 20.

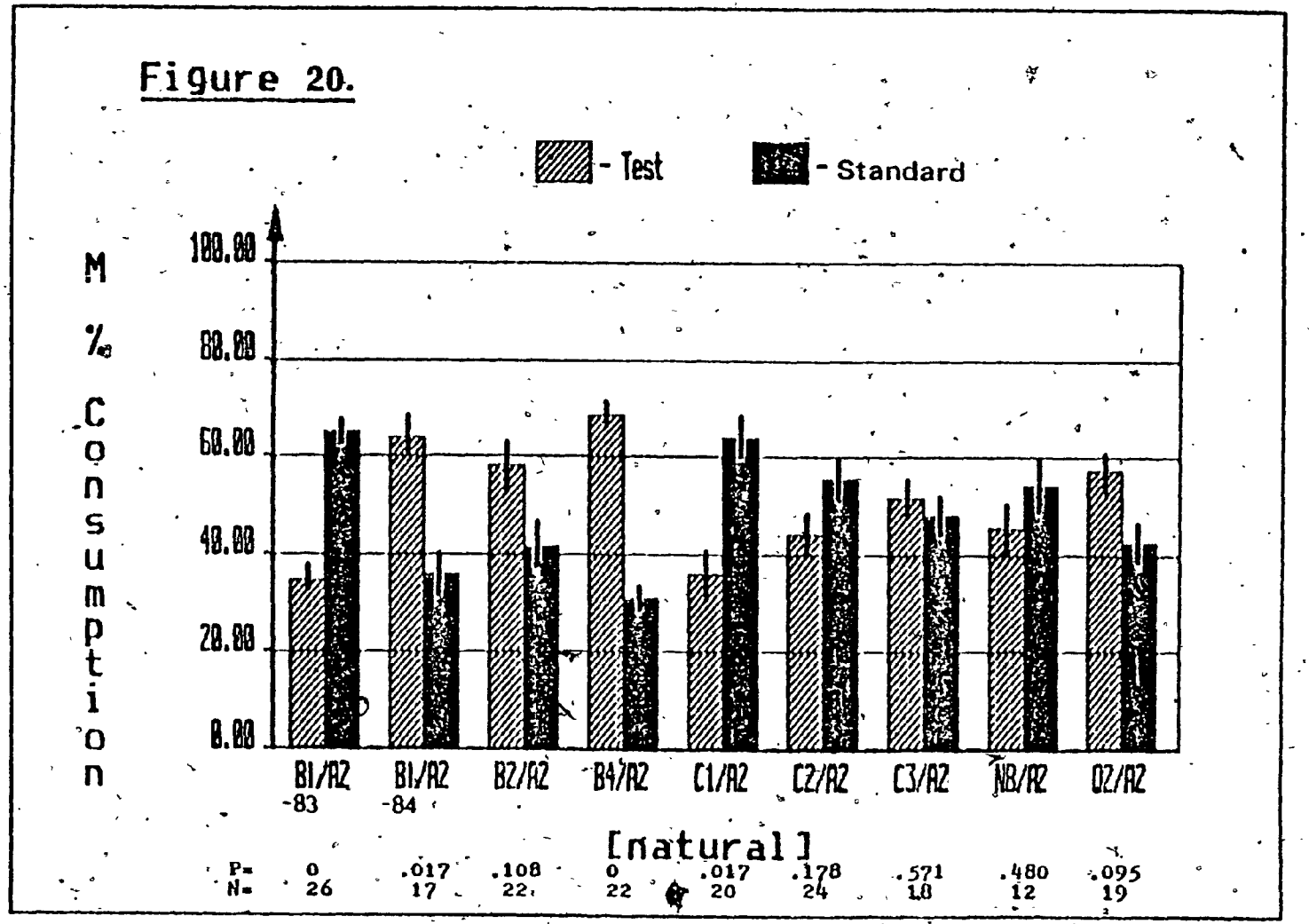

- Test
- Standard
M % Consumption
100.00
80.00
60.00
40.00
20.00
0.00
B1/A2 -83
B1/A2 -84
B2/A2
B4/A2
C1/A2
C2/A2
C3/A2
NB/A2
O2/A2
[natural]
P= 0 .017 .108 0 .017 .178 .571 .480 .095
N= 26 17 22 22 20 24 18 12 19

FIGURE 21. MEAN PERCENT CONSUMPTION ON BALSAM FIR WAXES AT STANDARD CONCENTRATIONS

Mean percent consumption (M.P.C.) during 9 experiments. Comparisons are all at standard wax concentrations (small letters for wax labels) and to the same standard A2. The solid line on each bar indicates the standard error. Probability (P) values less than 0.05 (page 39) indicate significant differences between the M.P.C. of the standard and the test tree's wax. "N" indicates the number of replicates for each experiment.

Figure 21.

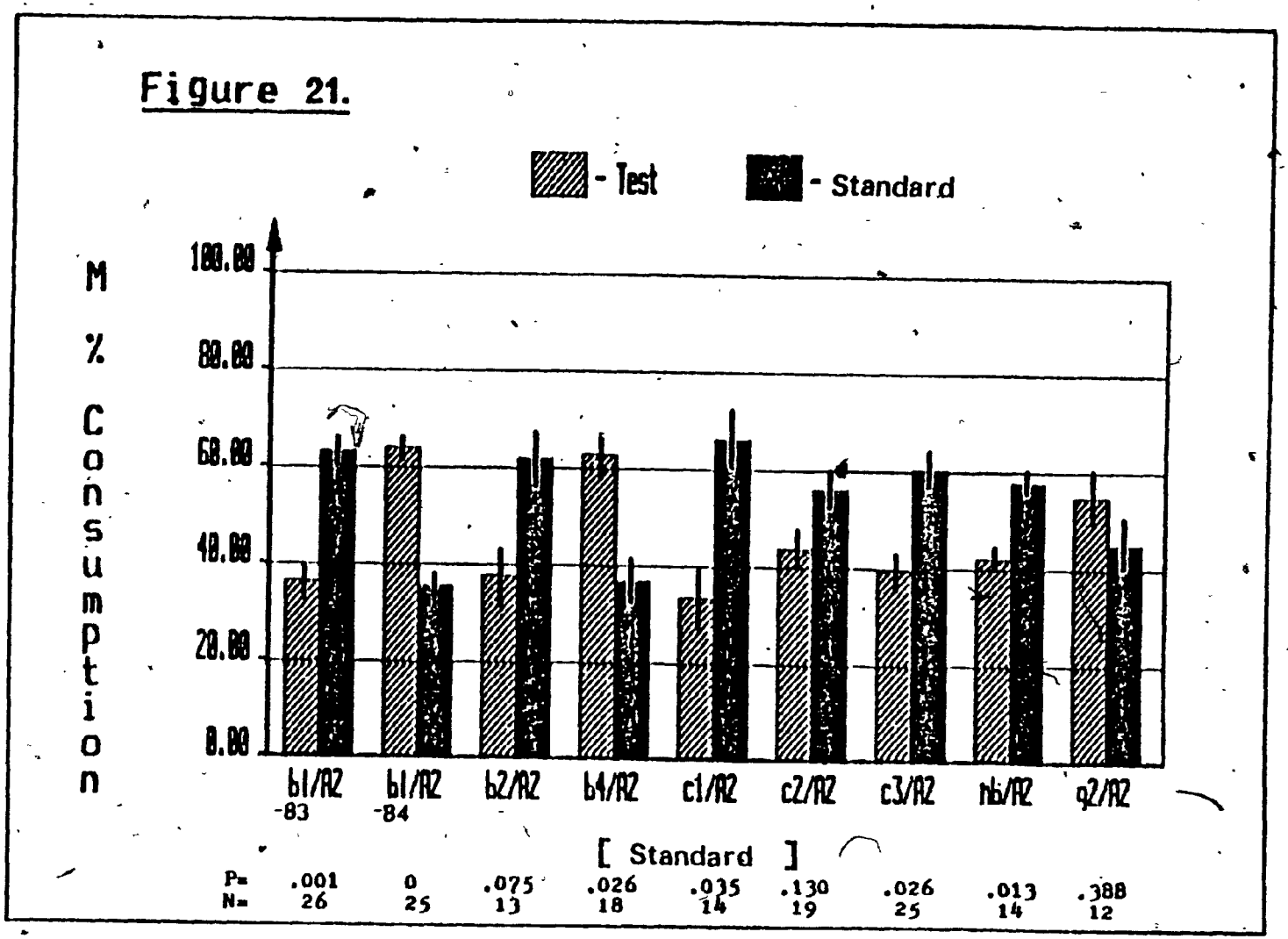

- Test
- Standard
M % Consumption
100.00
80.00
60.00
40.00
20.00
0.00
b1/R2 -83
b1/R2 -84
b2/R2
b4/R2
c1/R2
c2/R2
c3/R2
nb/R2
q2/R2
[Standard]
P= .001 0 .075 .026 .035 .130 .026 .013 .388
N= 26 25 13 18 14 19 25 14 12

FIGURE 22. MEAN PERCENT CONSUMPTION ON WAXES FROM NEIGHBOURING BALSAM FIR TREES

Mean percent consumption (M.P.C.) during 12 experiments, comparing waxes of trees from the same areas, within 50 meters of each other. The experiment labels are always presented in this format: TEST/STANDARD. Experiments alternate from natural wax concentrations (capital letters for both test and standard wax labels) to standard or equal concentrations (small letter for the test wax's label). The solid line on each bar indicates the standard error. Probability (P) values less than 0.05 (page 39) indicate significant differences between the M.P.C. of the standard and the test wax. "N" indicates the number of replicates for each experiment.

Figure 22.

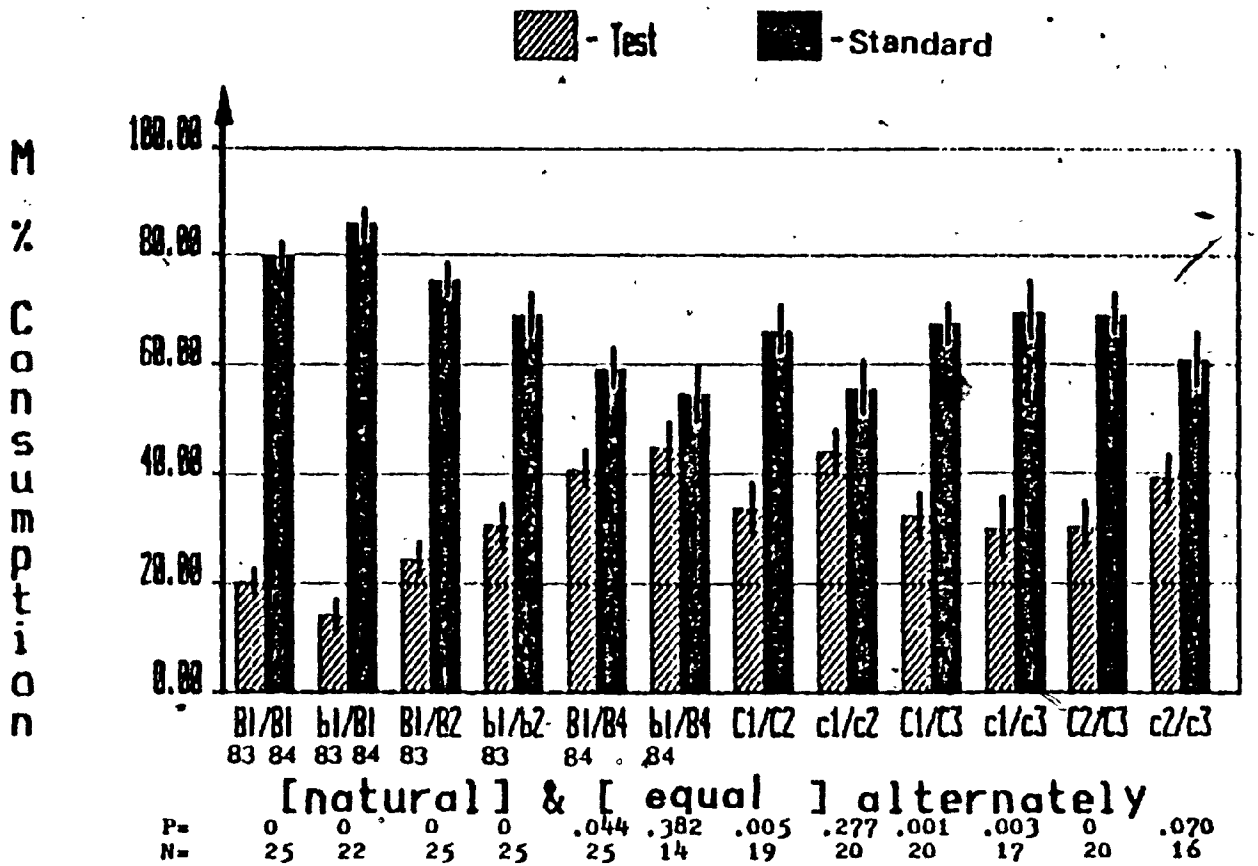
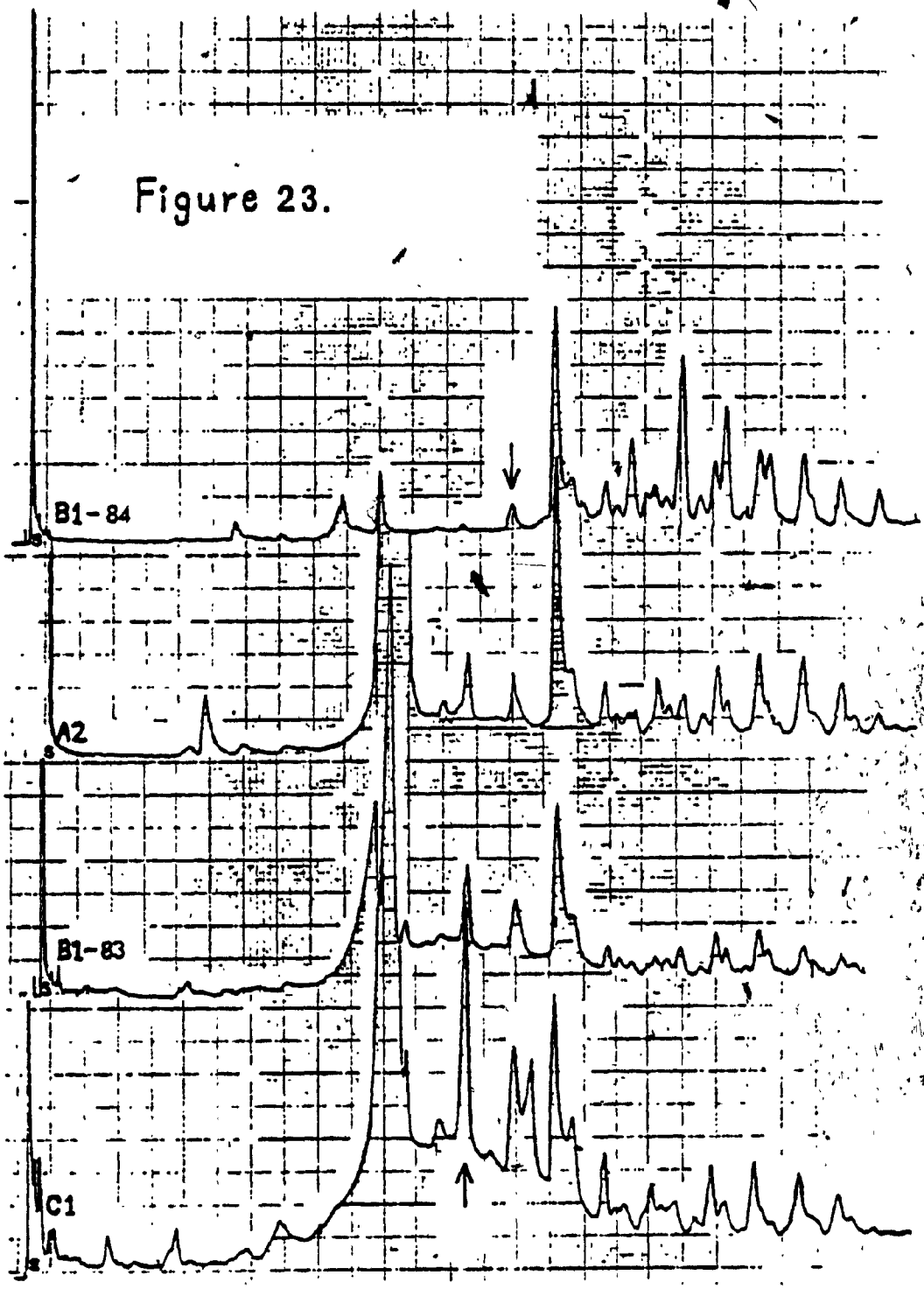

- Test
- Standard
M % Consumption
100.00
80.00
60.00
40.00
20.00
0.00
B1/B1 b1/B1 B1/B2 b1/b2 B1/B4 b1/B4 C1/C2 c1/c2 C1/C3 c1/c3 C2/C3 c2/c3
83 84 83 84 83 83 84 84
[natural] & [equal] alternately
P= 0 0 0 0 .044 .382 .005 .277 .001 .003 0 .070
N= 25 22 25 25 25 14 19 20 20 17 20 16

FIGURE 23. GAS LIQUID CHROMATOGRAPHS OF WHOLE WAXES FROM BALSAM FIR

The least preferred wax, C1 and B1-83 are at the bottom of the figure and are significantly different from both A2 and probably B1-84 as well, in their effects on feeding behaviour. The G.L.C. traces are given in sequence, with the most preferred at the top. The solvent peak in each trace is indicated with an "s". The two arrows point to peaks that follow the pattern of preference. Both peaks are small in the preferred waxes, and larger in the least preferred waxes. The first arrow indicates a peak that is also common to the white spruce G.L.C.s in Figure 19. The phthalate peak is seen to go off scale in both A2 and C1. The A2 phthalate peak was confirmed by NMR Spectrometry.

Figure 23.



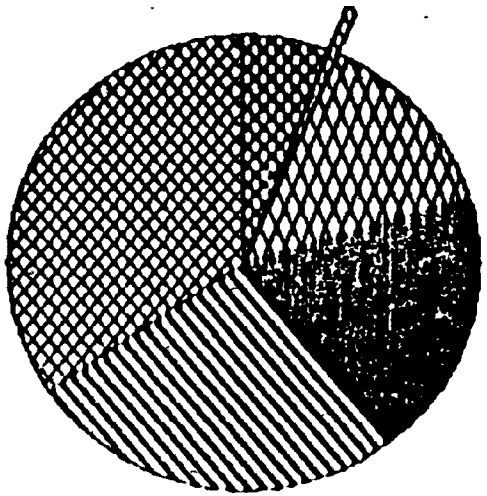
COLUMN CHROMATOGRAPHY

Figures 24 and 25 represent the average fraction sizes of waxes from white spruce and balsam fir respectively. The circle represents the whole wax in dry weight, and each wedge of the pie is an average fraction based on waxes extracted from three trees for both white spruce and balsam fir. The data used to calculate these values are given in Table 5 of Appendix II. These fraction concentrations were measured and calculated for the purpose of testing these fractions in further behavioural experiments. Discs could then be impregnated with standard fraction concentrations.

FIGURE 24. AVERAGE FRACTION SIZES OF WAXES FROM THREE WHITE SPRUCE TREES

Column chromatography was used to produce six fractions from the "whole" wax of three trees which produced significant preferences in the behavioural experiments. Dry weights of the common fractions from each of the three trees (A1, T1 and T2) were averaged, and are presented as a percent of the total in this pie chart. The fractions are designated by "F" and its corresponding number. , Note that there is an F7, but no F5. The percentage of each fraction was then used to calculate the required dilution of each sample so that the fractions could be tested at concentrations equal to that of A1 in the previous whole wax experiments, and specifically at the same fraction proportions thereof. The actual concentrations applied to the discs are given. The "total" of the six fractions is equal to that of the natural whole wax concentration of A1.

Figure 24.



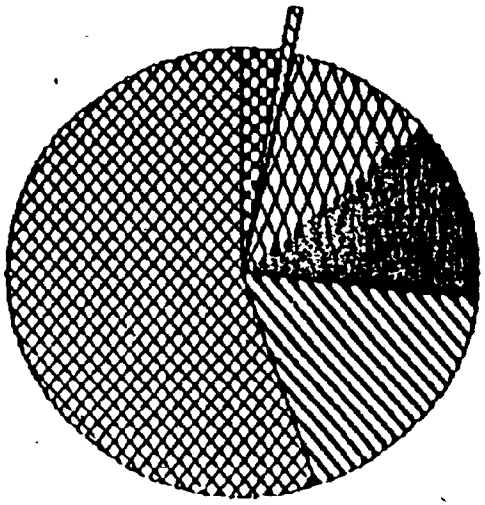
- F1 = 0.01534 ug./mm.sq. (5.9%)
- F2 = 0.00234 ug./mm.sq. (.9%)
- F3 = 0.03518 ug./mm.sq. (13.5%)
- F4 = 0.04888 ug./mm.sq. (18.8%)
- F6 = 0.06864 ug./mm.sq. (26.4%)
- F7 = 0.08970 ug./mm.sq. (34.5%)

TOTAL: 0.26 ug./mm.sq. (100%)

FIGURE 25. AVERAGE FRACTION SIZES OF WAXES FROM THREE BALSAM FIR TREES

Column chromatography was used to produce six fractions from the "whole" wax of three trees which produced significant preferences in the behavioural experiments. Dry weights of the common fractions from each of the three trees (A2, B1-83 and C1) were averaged, and are presented as a percent of the total in this pie chart. The fractions are designated by "F" and its corresponding number. Note that there is an F7, but no F5. The percentage of each fraction was then used to calculate the required dilution of each sample so that the fractions could be tested at concentrations equal to that of A2 in the previous whole wax experiments, and specifically at the same fraction proportions thereof. The actual concentrations applied to the discs are given. The "total" of the six fractions is equal to that of the natural whole wax concentration of A2.

Figure 25.



- F1 = 0.00667 ug./mm.sq. (2.9%)
- F2 = 0.00207 ug./mm.sq. (.9%)
- F3 = 0.02392 ug./mm.sq. (10.4%)
- F4 = 0.02998 ug./mm.sq. (13.0%)
- F6 = 0.04163 ug./mm.sq. (18.1%)
- F7 = 0.12581 ug./mm.sq. (54.7%)

TOTAL: 0.23 ug./mm.sq. (100%)

BEHAVIOURAL EXPERIMENTS WITH WAX FRACTIONS

Mean Percent Consumptions

The *mean percent consumptions* (Figure 26) of these qualitative experiments indicate that fractions 7 of both waxes T1 and T2 give positive feeding preference in relation to the same fractions from wax A1. When the feeding preference index (PI) formula (PI = [T − C] / H) was used, the fraction 3 of white spruce was also found to be significantly different. The PI value for experiment T1 F3 versus A1 F3 is 0.57, and the confidence limits are 0.11 and 1.03. For the balsam fir results, Figure 27 indicates that three fractions (2, 4 & 7) contain chemicals that elicit spruce budworm preference for wax B1-84.

FIGURE 26. MEAN PERCENT CONSUMPTION ON WAX FRACTIONS FROM WHITE SPRUCE

Mean percent consumption (M.P.C.) of wax fractions during 7 experiments, qualitatively comparing wax fractions at concentrations proportional to the given average fraction sizes (Figure 24) of the standard (A1). All six fractions of T1 were tested, and only fraction 7 of T2 was tested. The standard A1 is common to all experiments here. The solid line on each bar indicates the standard error. Probability (P) values less than 0.05 indicate significant differences between the M.P.C. of the standard fraction of wax A1 and the test fraction. All discs were first impregnated with 0.025 M sucrose in order to increase the number of successful replicates (N).

Figure 26.

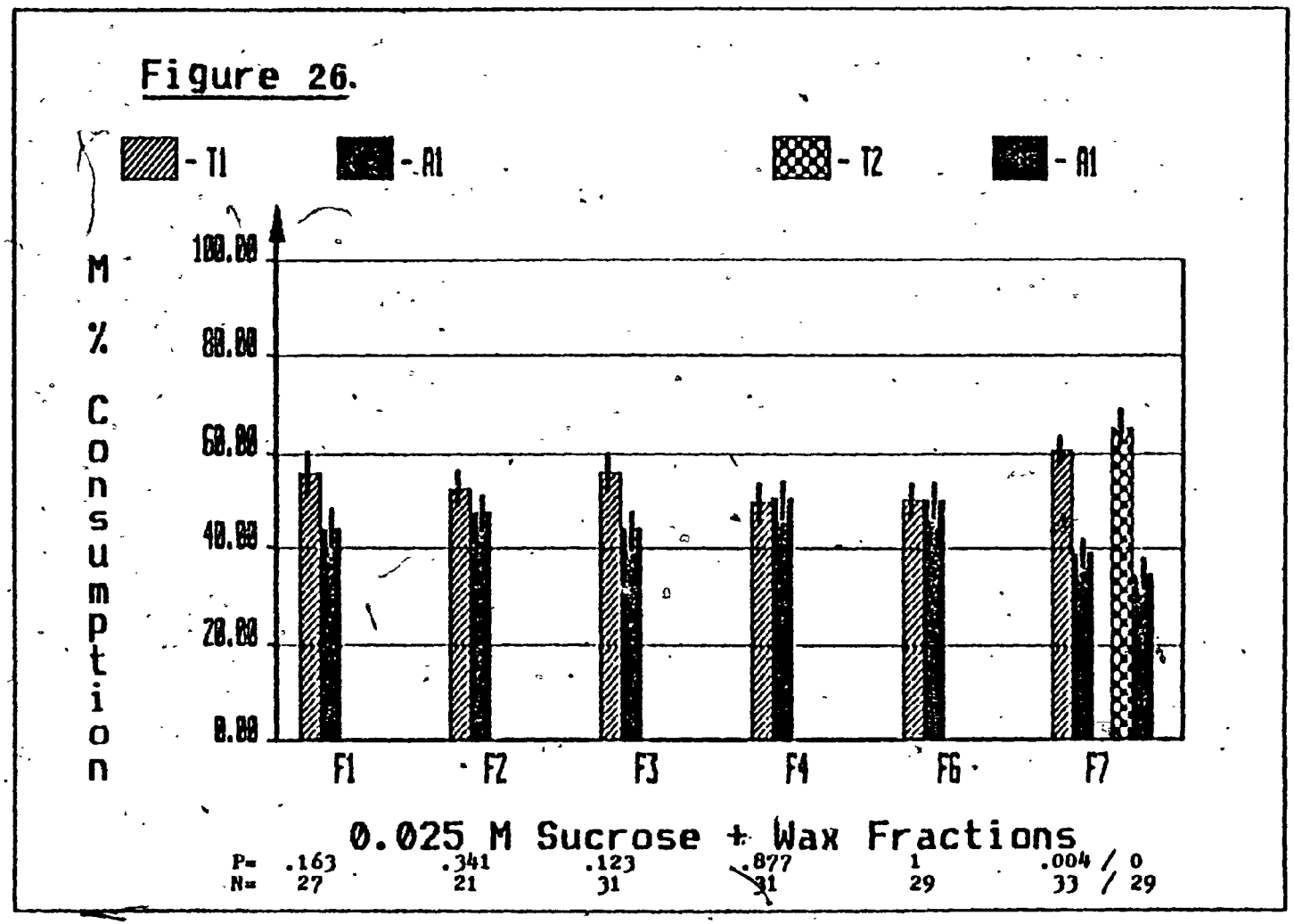
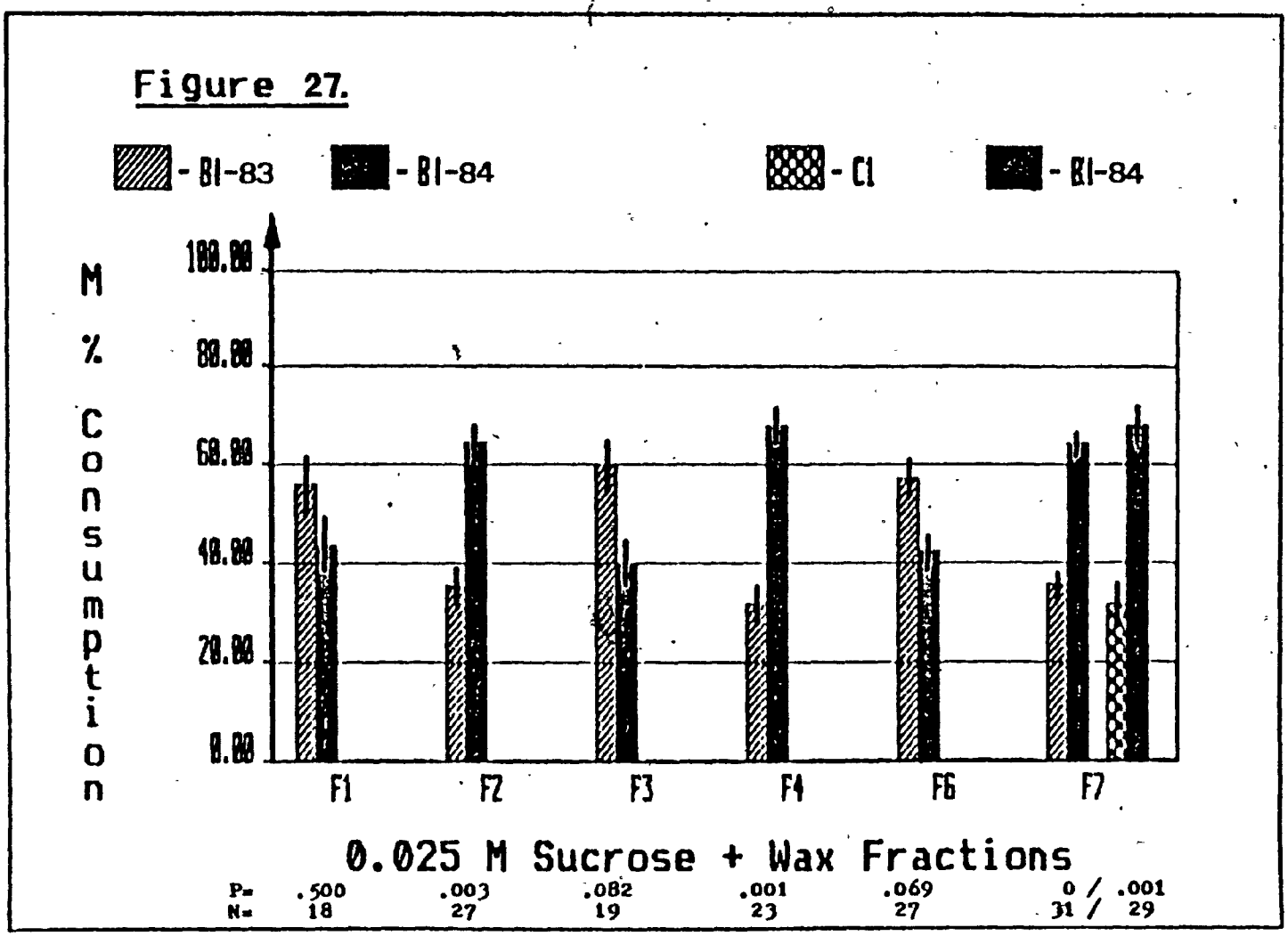

T1
A1
T2
A1
M % Consumption
100.00
80.00
60.00
40.00
20.00
0.00
F1
F2
F3
F4
F6
F7
0.025 M Sucrose + Wax Fractions
P= .163 .341 .123 .877 1 .004 / 0
N= 27 21 31 31 29 33 / 29

FIGURE 27. MEAN PERCENT CONSUMPTION ON WAX FRACTIONS FROM BALSAM FIR

Mean percent consumption (M.P.C.) of wax fractions during 7 experiments, qualitatively comparing wax fractions at concentrations proportional to the given average fraction sizes (Figure 25) of the standard (A2). All six fractions of B1-83 were tested, and only fraction 7 of C1 was tested. The standard B1-84 is common to all experiments here. The solid line on each bar indicates the standard error. Probability (P) values less than 0.05 indicate significant differences between the M.P.C. of the standard fraction of wax B1-84 and the test fraction. All discs were first impregnated with 0.025 M sucrose in order to increase the number of successful replicates (N).

Figure 27.


- BI-83
- BI-84
- C1
- BI-84
M % Consumption
100.00
80.00
60.00
40.00
20.00
0.00
F1
F2
F3
F4
F6
F7
0.025 M Sucrose + Wax Fractions
P= .500 .003 .082 .001 .069 0 / .001
N= 18 27 19 23 27 31 / 29

GAS LIQUID CHROMATOGRAPHY OF WAX FRACTIONS

The G.L.C.s for wax fractions for which significant preferences against standard-wax fractions were demonstrated are presented in Figures 28 and 29 for white spruce, and 30, 31 and 32 for balsam fir. The most preferred waxes (based on whole wax experiments) are presented at the top and the least preferred at the bottom of these figures. The labeled peaks were identified by Dr. Tulloch, by means of complimentary tests including Thin Layer Chromatography, Gas Chromatography-Mass Spectrometry and Nuclear Magnetic Resonance Spectrometry. Some obvious components as well as many minor ones are unidentified. Some of the major ones are labeled "Acid B", "C", "D" and "E".

The bottom trace in Figure 28 represents the retention time on the column of some known compounds. Due to limited space, this trace was not included in Figure 30. In Figure 28, F7 of wax A1 was previously shown to differ, in the behavioural experiments, from both F7s of waxes T1 and T2. The complexity of these traces makes it difficult to distinguish patterns or peaks that make A1 different. Speculating, the peak labeled "Acid B", as well as the peak (labeled "?") between the "Phthalate" and the "C24 Fatty

acid" on the T2 trace are candidates. However, the results from balsam fir in Figure 30 do not confirm the importance of these differences. Because these trees are of different species, these findings need not be complementary. In Figure 30, the peaks labeled "Diols?" do differ substantially not only between those eliciting an aversion (B1-83 & C1) and the preferred (A2 & B1-84), but also between B1-84 and A2 which were also shown to be significally different.

In Figure 29, fraction 3 of wax T1 has been established as being significantly preferred, by the budworm, to A1 F3. "Methyl triacontanoate" apparently occurs as a larger proportion of A1 F3 in comparison to T1 F3 and T2 F3. As well, A1's very first peak, after the vertical solvent (chloroform) peak at the beginning of the trace, stands out as different from the above two traces. The arrows in trace A1 F3 indicate two other peaks making A1 F3 different from the others shown.

Figure 31 indicates two obvious differences. Specifically, the peak labeled "?" in trace B1-84, and the peak labeled "E" in trace B1-83. Many other smaller peaks also differ.

The peaks labeled "?" in Figure 32 in trace B1-83, and

also apparent in trace C1, stand out as being different from the other traces. These appear to be the only candidates in this fraction that may be responsible for the feeding preferences.

FIGURE 28. GAS LIQUID CHROMATOGRAPHS OF FRACTION 7 FROM WAXES EXTRACTED FROM WHITE SPRUCE FOLIAGE

Gas Liquid Chromatographs of three white spruce-wax fractions obtained from column chromatography. Fraction 7 contains primary alcohols, acids, polyesters, hydroxy esters and diols. The bottom trace represents the retention time of several known compounds. The labeled peaks were identified by means of complimentary tests including Thin Layer Chromatography, Gas Chromatography-Mass Spectrometry and Nuclear Magnetic Resonance Spectrometry. To increase the response of the free acids in Fractions 7 in these G.L.C.s, the samples were first acetylated. This treatment increases the volitility of the compound types expected to be present in Fractions 7. The least preferred waxes are at the bottom, and the most preferred are at the top. T1 F7 and T2 F7 have been shown in behavioural tests to be significiantly different from A1 F7. The solvent peak in each trace is indicated with an "s".

Figure 28.

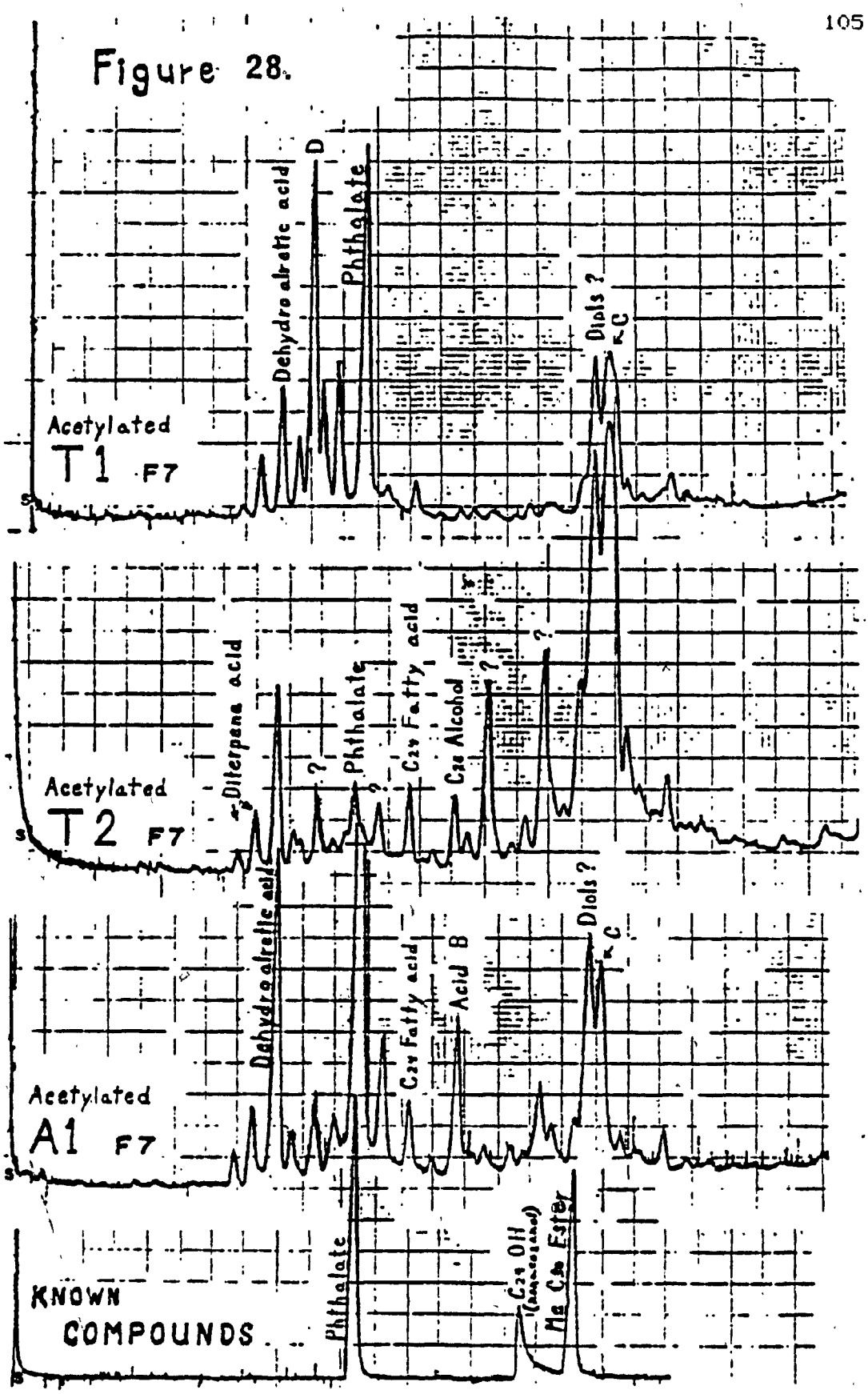

Acetylated
T1 F7
Dehydroabietic acid
D
Phthalate
Diols ?
C
Acetylated
T2 F7
Diterpene acid
Dehydroabietic acid
Phthalate
C29 Fatty acid
C28 Alcohol
Diols ?
C
Acetylated
A1 F7
Dehydroabietic acid
C29 Fatty acid
Acid B
Diols ?
C
KNOWN
COMPOUNDS
Phthalate
C29 OH
Me C16 Ester

FIGURE 29. GAS LIQUID CHROMATOGRAPHS OF FRACTION 3 FROM WAXES EXTRACTED FROM WHITE SPRUCE FOLIAGE

Gas Liquid Chromatographs of three white spruce wax fractions obtained from column chromatography. Fraction 3 is expected to contain esters. The labeled peaks were identified by means of complimentary tests including Thin Layer Chromatography, Gas Chromatography-Mass Spectrometry and Nuclear Magnetic Resonance Spectrometry. The least preferred waxes are at the bottom, and the most preferred are at the top. T1 F3 has been shown in behavioural tests to be significiantly different from A1 F3, and by extrapolation, T2 is expected to be similar to T1. The solvent peak in each trace is indicated with an "s".

Figure 29.

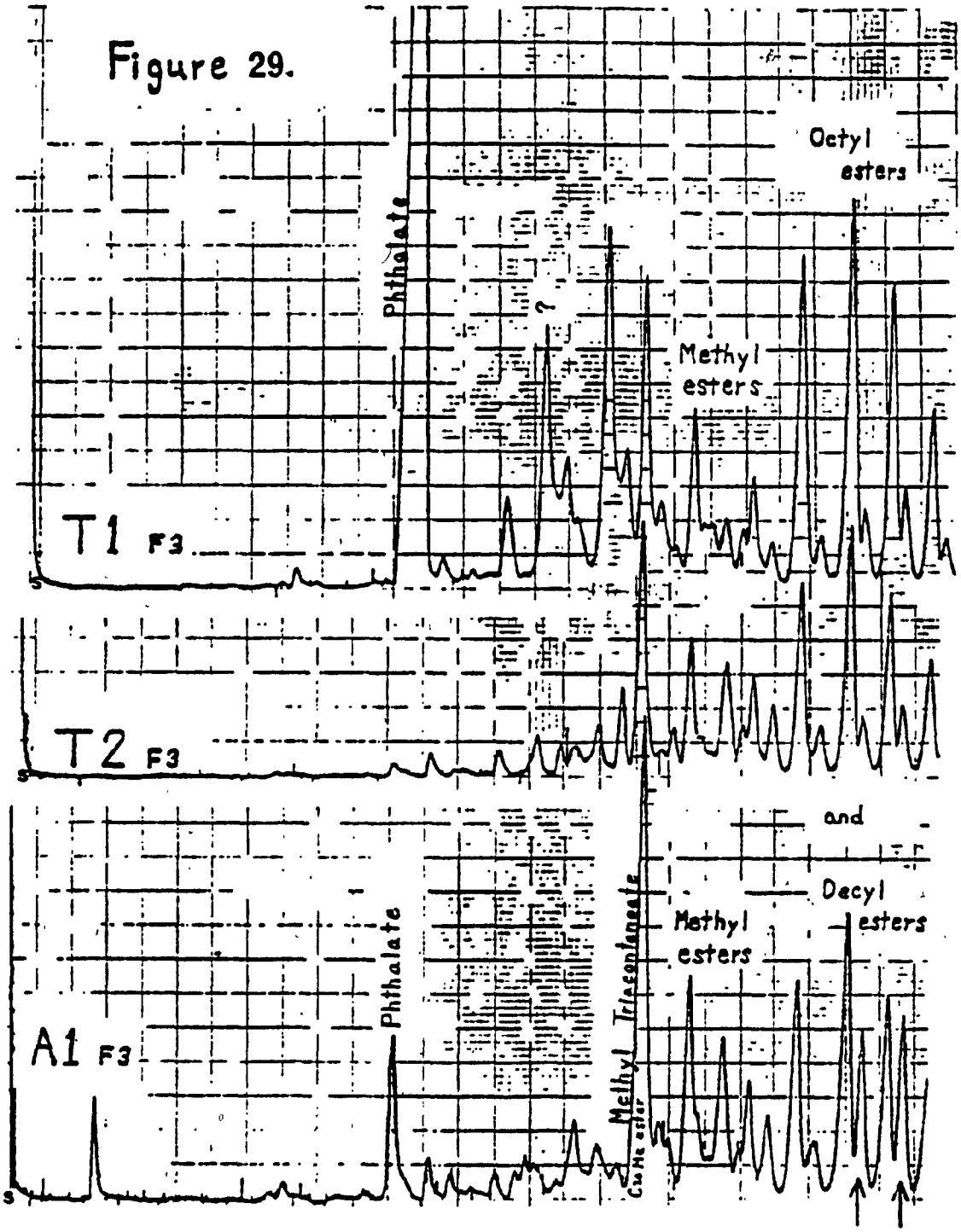

Octyl
esters
Phthalate
?
Methyl
esters
T1 F3
T2 F3
and
Decyl
esters
Methyl
esters
Phthalate
Methyl Triacontanoate
A1 F3

FIGURE 30. GAS LIQUID CHROMATOGRAPHS OF FRACTION 7 FROM WAXES EXTRACTED FROM BALSAM FIR FOLIAGE

Gas Liquid Chromatographs of fraction 7 contains primary alcohols, acids, polyesters, hydroxy esters and diols. The labeled peaks were identified by means of complimentary tests including Thin Layer Chromatography, Gas Chromatography-Mass Spectrometry and Nuclear Magnetic Resonance Spectrometry. To increase the response of the free acids in fractions 7 in the G.L.C.s, the samples were first acetylated. This treatment increases the volitility of the compound types expected to be present in these fractions 7. The least preferred waxes are at the bottom, and the most preferred are at the top. B1-84 F7 and A2 F7 have been shown in behavioural tests to be significiantly different from B1-83 F7 and C1 F7. By extrapolation, B1-84 is also expected to be different from A2. The solvent peak in each trace is indicated with an "s".

Figure 30.

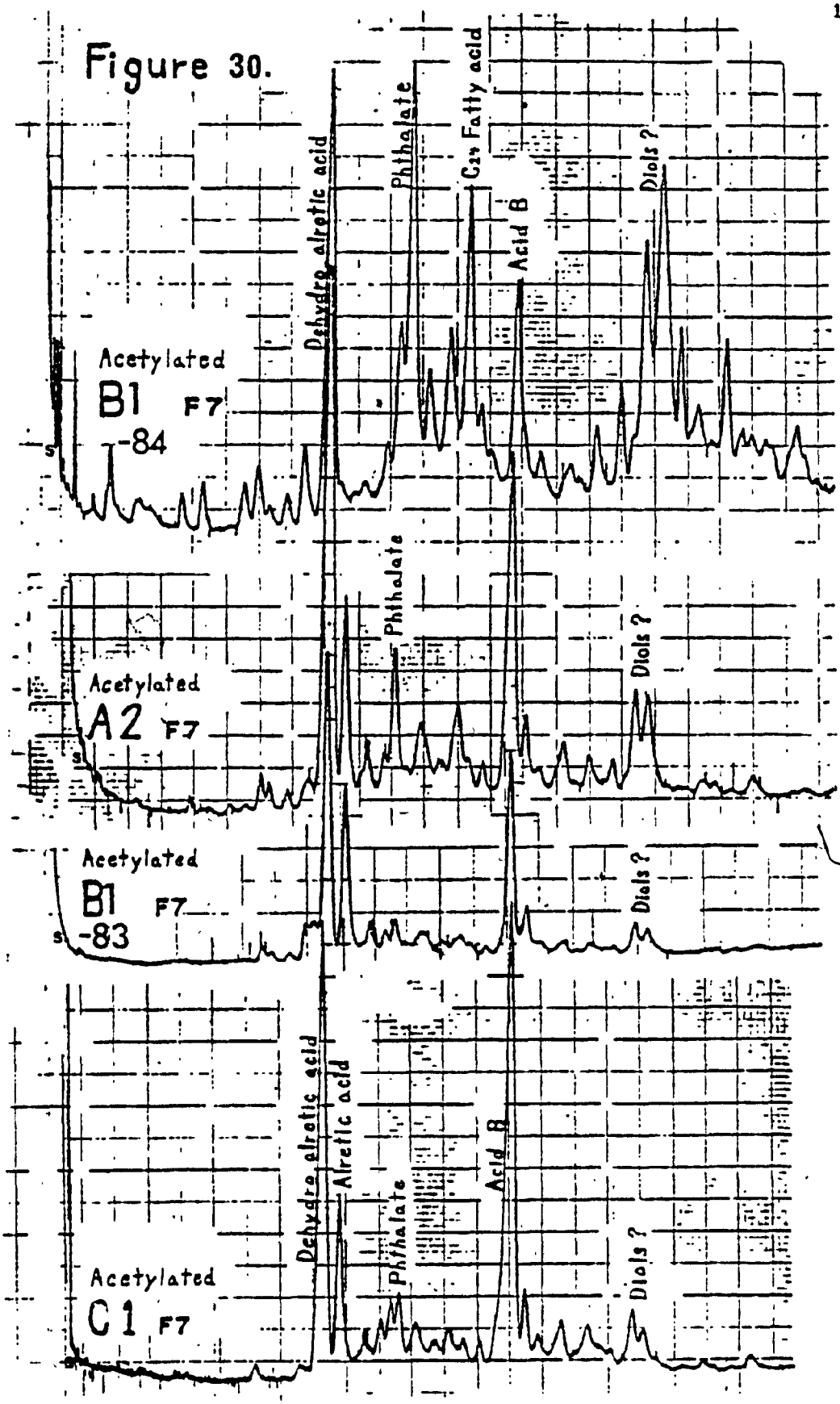

Dehydro alretic acid
Phthalate
C24 Fatty acid
Acid B
Diols ?
Acetylated
B1 F7
-84
S
Phthalate
Diols ?
Acetylated
A2 F7
S
Acetylated
B1 F7
-83
S
Diols ?
Dehydro alretic acid
Alretic acid
Phthalate
Acid B
Diols ?
Acetylated
C1 F7

FIGURE 31. GAS LIQUID CHROMATOGRAPHS OF FRACTION 4 FROM WAXES EXTRACTED FROM BALSAM FIR FOLIAGE

Gas Liquid Chromatographs of two wax fractions. Fractions 4 is expected to contain diesters. The labeled peaks were identified by means of complimentary tests including Thin Layer Chromatography, Gas Chromatography-Mass Spectrometry and Nuclear Magnetic Resonance Spectrometry. The least preferred wax is at the bottom, and the most preferred wax is at the top. B1-84 F4 has been shown in behavioural tests to be significiantly different from B1-83 F4. The solvent peak in each trace is indicated with an "s".

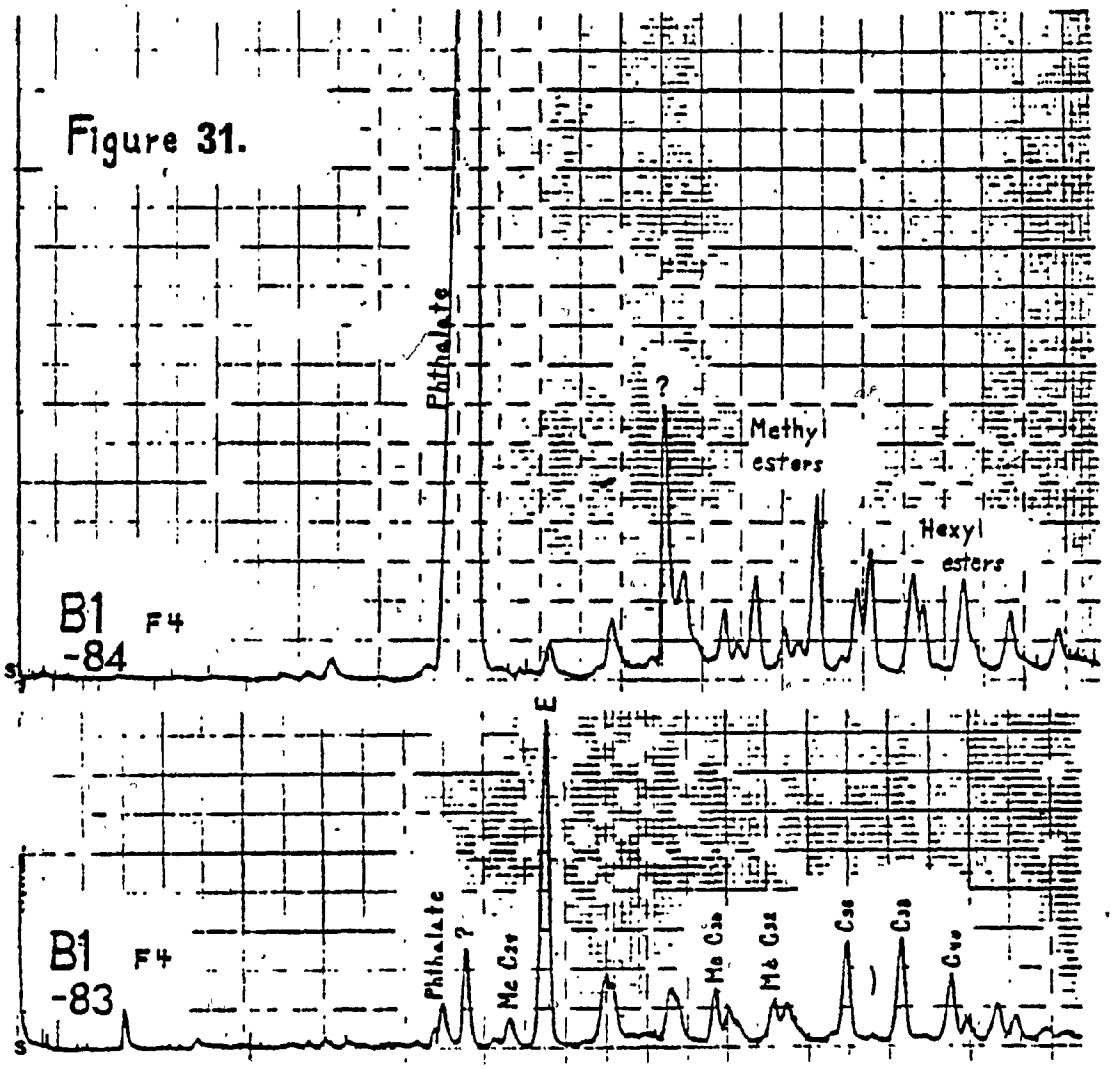

Phthalate
?
Methyl
esters
Hexyl
esters
B1 F4
-84
S
E
Phthalate
?
Me C₂₇
Me C₂₈
Me & C₂₉
C₂₈
C₂₉
C₃₀
B1 F4
-83
S

Figure 31.

FIGURE 32. GAS LIQUID CHROMATOGRAPHS OF FRACTION 2 FROM WAXES EXTRACTED FROM BALSAM FIR FOLIAGE

Gas Liquid Chromatographs of four wax fractions. Fraction 2 is expected to contain hydrocarbons. Gas Liquid Chromatographs of The labeled peaks were identified by means of complimentary tests including Thin Layer Chromatography, Gas Chromatography-Mass Spectrometry and Nuclear Magnetic Resonance Spectrometry. The least preferred waxes are at the bottom, and the most preferred are at the top. B1-84 F2 has been shown in behavioural tests to be significiantly different from B1-83 F2, and by extrapolation A2 is expected to be similar to B1-84, and B1-83 is expected to be similar to C1. The solvent peak in each trace is indicated with an "s".

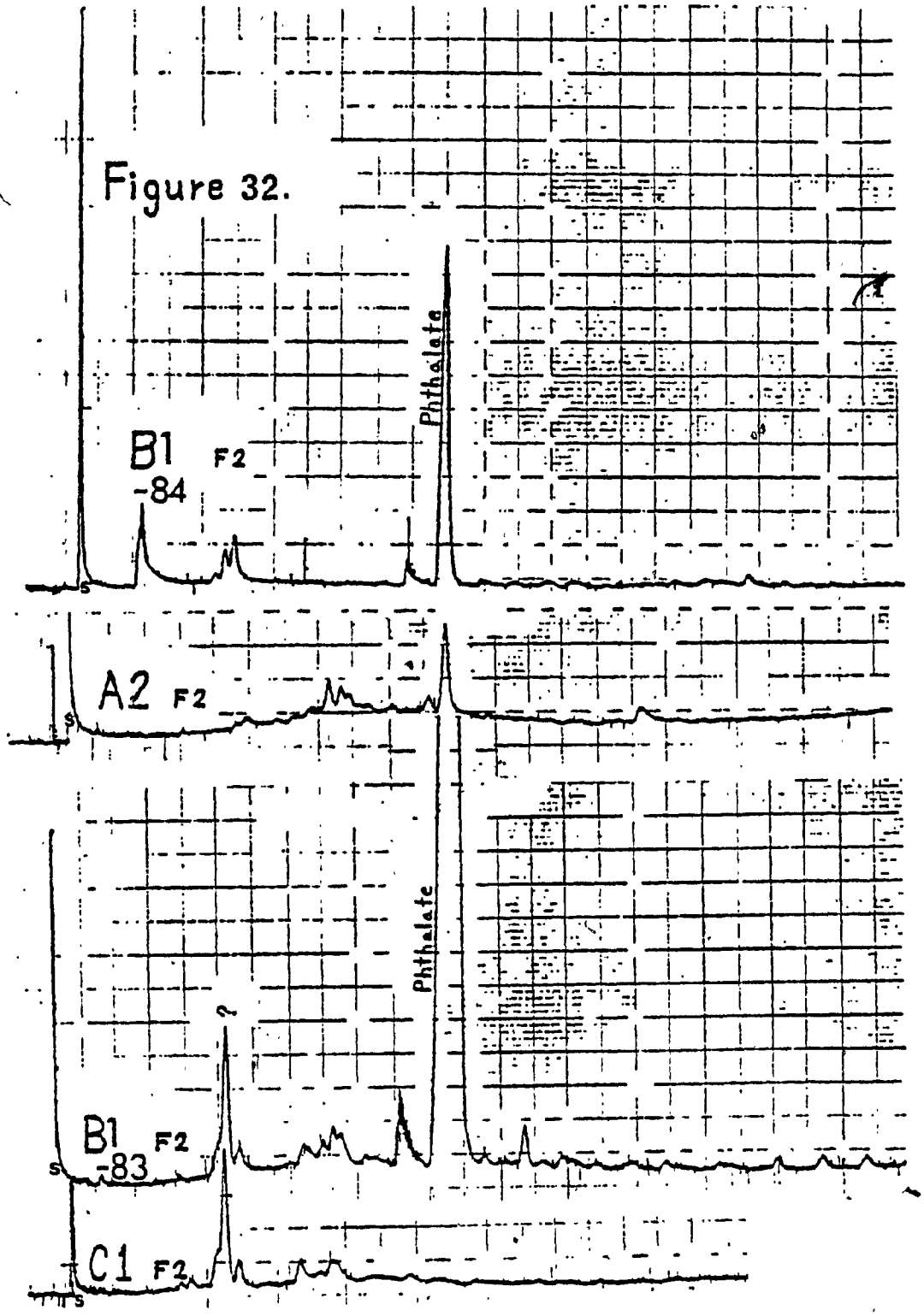


Figure 32.

FEEDING RATES

The *Feeding rates* (page 39) in the following tables can be cross-compared between experiments within this study, and generally the confidence limits overlap, including those using water alone (Table VI).

TABLE VI

FEEDING RATES OF THE CONTROL EXPERIMENTS

	Experiment	F.R.	C.L.
1) Starvation (hrs) sucrose/water choice	Nil	3.83	2.03 - 5.62
	12	3.01	1.38 - 4.64
	20	4.10	3.03 - 5.18
	24	2.67	1.78 - 3.56
2) Gender sucrose/water choice	M	2.46	1.81 - 3.10
	F	2.87	2.08 - 3.66
3) Test for Bias	H2O versus H2O	3.15	1.97 - 4.33
4) Tests for Standards	Sucrose/A1+sucrose	2.86	1.71 - 4.00
	Sucrose/A2+sucrose	2.92	1.72 - 4.13

The confidence limits of experiment T1/A1 (Table VII) do not overlap those of C4/A1 and C5/A1 at natural wax concentrations. However, at equal wax concentrations only the experiments t2/A1 and t3/A1 are shown to differ from c5/A1. Most feeding rates are similar. The feeding rates on the whole wax from white spruce samples are only remarkable for their constancy.

TABLE VII

FEEDING RATES ON WHITE SPRUCE WAXES

	Experiment	F.R.	C.L.
[natural]	B3/A1	2.84	2.17 - 3.51
	C4/A1	3.88	2.91 - 4.86
	C5/A1	3.54	2.54 - 4.54
	NB/A1	2.26	1.58 - 2.94
	Q1/A1	3.05	2.52 - 3.58
	T1/A1	1.86	1.27 - 2.46
	T2/A1	2.32	1.72 - 2.93
	T3/A1	2.39	1.87 - 2.92
[standard]	b3/A1	3.65	2.56 - 4.75
	c4/A1	2.89	1.97 - 3.82
	c5/A1	4.58	3.05 - 6.10
	nb/A1	3.06	1.62 - 4.50
	q1/A1	3.99	2.84 - 5.13
	t1/A1	2.75	2.24 - 3.26
	t2/A1	2.26	1.71 - 2.81
	t3/A1	2.37	1.96 - 2.79
Neighbours	C4/C5	3.08	2.40 - 3.77
	c4/c5	2.48	2.03 - 2.92
	T1/T3	2.65	2.17 - 3.14
	t1/T3	2.66	2.01 - 3.30
	T2/T3	2.56	2.18 - 2.93
	t2/T3	2.89	2.27 - 3.51

Feeding Rates on balsam fir waxes, like those on white spruce, are rather constant in value. That in B1-84/A2 (Table VIII) is different from C3/A2 and NB/A2 in that their confidence limits do not overlap. The feeding rate obtained in experiment nb/A2 is greater than all the others, and has confidence limits that do not overlap most of the others. What is particularly remarkable is that the higher concentration of NB (experiment NB/A2, Table VIII) produced less feeding. This change in the budworms feeding rate suggests that either a feeding deterrent or a stimulant was diluted to within an optimal range to trigger this behaviour. The confidence interval in this experiment nb/A2 (Table VIII) overlaps only those of experiments b1-83/A2 and c1/A2. A remarkably constant range of values for feeding rates is seen for experiments comparing neighbouring trees (bottom of Table VIII).

TABLE VIII

FEEDING RATES ON BALSAM FIR WAXES

	Experiment	F.R.	C.L.
[natural]	B1-83/A2	3.61	2.99 - 4.23
	B1-84/A2	2.31	1.90 - 2.72
	B2/A2	3.02	2.03 - 4.02
	B4/A2	3.17	2.26 - 4.08
	C1/A2	3.20	2.26 - 4.15
	C2/A2	3.24	2.43 - 4.05
	C3/A2	4.74	3.47 - 6.01
	NB/A2	4.83	3.66 - 6.01
	Q2/A2	3.10	2.46 - 3.74
[standard]	b1-83/A2	4.42	3.31 - 5.52
	b1-84/A2	2.39	2.01 - 2.78
	b2/A2	2.05	1.54 - 2.56
	b4/A2	2.40	1.92 - 2.89
	c1/A2	3.52	2.50 - 4.53
	c2/A2	2.30	1.90 - 2.71
	c3/A2	2.82	2.08 - 3.55
	nb/A2	5.99	4.39 - 7.59
	q2/A2	2.26	1.75 - 2.76
Neighbours	B1-83/B1-84	2.50	2.05 - 2.96
	b1-83/B1-84	1.94	1.54 - 2.34
	B1-83/B2	2.29	1.82 - 2.76
	b1-83/b2	2.41	1.92 - 2.91
	B1-84/B4	2.39	2.07 - 2.71
	b1-84/B4	2.94	1.82 - 4.05
	C1/C2	2.43	2.05 - 2.80
	c1/c2	1.95	1.73 - 2.17
	C1/C3	2.63	1.98 - 3.28
	c1/c3	2.19	1.61 - 2.77
	C2/C3	2.52	1.86 - 3.18
	c2/c3	2.05	1.74 - 2.36

TABLE IX

FEEDING RATES ON WAX FRACTIONS PLUS SUCROSE

Experiment		F.R.	C.L.
White Spruce			
T1/A1	F1	3.86	3.02 - 4.70
	F2	3.88	2.74 - 5.02
	F3	3.19	2.51 - 3.87
	F4	3.37	2.79 - 3.96
	F6	4.30	3.47 - 5.12
	F7	3.42	2.87 - 3.97
T2/A1	F7	2.81	2.24 - 3.38
Balsam Fir			
B1-83/B1-84	F1	3.09	2.10 - 4.08
	F2	3.30	2.66 - 3.95
	F3	2.78	1.80 - 3.76
	F4	2.45	2.00 - 2.90
	F6	3.51	2.75 - 4.27
	F7	3.15	2.61 - 3.70
C1/B1-84	F7	3.44	2.71 - 4.18

On average (Table X), the *feeding rates* of budworms was greatest on the wax fractions plus sucrose (Table IX).

TABLE X
AVERAGE FEEDING RATES

TABLE		SOURCE
VI	3.16 *	Control Experiments
VII	2.90	White Spruce Experiments
VIII	2.92	Balsam Fir Experiments
IX	3.33	Fractions + Sucrose Exp.
	3.08 *	Average of total.

* (does not include F.R. for the 2 Standard Exp.)

However, on examination by means of an Anova Table, a

greater variance was found to exist within a group than between the groups (i.e. Tables VI to IX). Therefore, the different treatments in the above groups did not cause any significant difference in the feeding rates.

DISCUSSION

CONTROL EXPERIMENTS

In some cases, hunger may be a biting stimulant in itself, and therefore the effects of such chemicals in the waxes may be masked. Blaney and Chapman (1970) found that after prolonged starvation biting becomes indiscriminate. The control experiments determined that:

1) starvation does not interfere with the budworms preference choice,
2) use of both males and females does not bias the results,
3) no bias, due to this new design of experimental tray for testing fourth instar budworms, was apparent, and
4) the waxes used as standards enhanced feeding.

To improve the efficiency of this experimental tray for future work, modifications are suggested in Appendix III.

These experiments provided the basis on which all subsequent behavioural experiments could be substantiated.

HOST TREES AND WHOLE WAX BEHAVIOURAL EXPERIMENTS

Whole-wax-behavioural experiments served to show that:

1) a feeding preference, for epicuticular waxes from different individual host trees of the same species, could be demonstrated in the laboratory, and
2) in several cases, a correlation could be made between epicuticular waxes that elicit an aversion and trees selected from a forest for resistance, or conversely preferred waxes and susceptibile trees.

An ideal *resistant tree* was expected to be found unharmed while intertwined with neighbours showing significant defoliation or death due to budworm attack. Such a situation would indicate that preferential feeding by the budworms was occurring. The ideal *susceptible tree*, for comparison of feeding behaviour in laboratory experiments,

would be a neighbour presently under attack, thus indicating palatability. However, these ideals were in fact not found.

The drastic change in the budworm's preferences, for the epicuticular wax from the same tree B1 (Figure 22) over the period of about one year, emphasises that this change is due to a dynamic, chemical character in these host trees. It seems highly likely that some trees survive within areas that have been devastated by the spruce budworm primarily due to the genetic disposition of these trees to "call upon" a deleterious principle under particular conditions. The foliage collection site closest to the ideal conditions sought were those of Area T. However, at standard concentrations, those trees sampled as good examples of resistant trees (T1 & T2) failed in this respect to elicit an aversion in the laboratory. This may be explained as a relaxing of host defence either due to excessive stress, or the perception (see Brownlee, 1983 & Maugh II, 1982) by the host trees of no imminent danger from budworm feeding. Some observations suggest that stressed trees may produce waxes that are preferred by the budworms. Photographs of B1 in 1983 and 1984 (Figures 3 & 4) show a deterioration in the trees stamina that correlates with the change in the budworm's preference in the experiments. The photograph of A2 (Figure 2) also provides an example.

As stressed by McDonald (1981), "very subtle variations in the chemical message system between plant and insect could account for these survivor trees." "Resistance to disease is widely distributed and often substantial in amount. We are inclined to overlook this fact until it is brought to our notice (Van der Plank, 1968)." Studies that fail to find correlations between plant secondary substances and their herbivores in terms of toxicity or deterrence may at times overlook as yet unimagined modes of action, as well as those such as parasite and predator effectiveness (for example see Elzen et al., 1983); host-insect phenologic asynchrony, and other influences described by McDonald. Mitchell and Sutcliffe (1984) suggest, based on electrophysiological work, "that the more common mechanisms of deterrence are based on inhibition of cells that respond to phagostimulants, and evocation of high frequency or bursting trains of action potentials." Such plant secondary substances may lack any toxic effects. Most research on the responses of insects to plant secondary substances seems to concern their effects in terms of insect mortality and fecundity. "Since the acceptance or rejection of a plant depends in the final analysis on the ability of the herbivore to assess some characteristic of the plant, the fundamental question is whether the compounds under discussion affect individual

behaviour in any way" (Dethier, 1982). The importance of the behavioural response must be stressed. Initially, the failure of DDT to control house flies and mosquitoes, was considered to be due to "physiological resistance" (biochemical processing of toxicants within the body), and DDT was thought to have no repellent effect (Buxton, 1945). Only later, reports of behavioural resistance affecting control with DDT appeared (see Pluthero & Singh, 1984). In conclusion, given the general importance of the role of behaviour in adaptation (Mayr, 1974), behavioural adaptations are as likely to arise as physiological ones (Pluthero & Singh, 1984).

That chemicals in the epicuticular wax provide information to the herbivore is of particular interest. Woodhead (1983) reported that starved locusts would take an experimental bite of an unpalatable leaf even after palpation, but would then reject the leaf presumably on encountering the internal deterrents. On subsequent contact, these individuals usually rejected the plant at palpation. Woodhead (1983) proposes that insects may detect surface chemicals by short-range olfaction. Rejection of the plant occurs after sensing, but before biting. This imparts a more effective defence to the plant. Host trees of the spruce budworm can be thought of as having dynamic, aposematic-surface waxes (page 17), in

the sense that communication occurs before damage is incurred by the host.

Woodhead further suggests that this phenomenon may resemble the learning by association in locusts, demonstrated by Blaney and Winstanley (1982). They showed that the insects associated the chemical characteristics of its surface wax with the presence of the internal deterrents.

Feeding Preference

Important factors concerning the stability of resistance in a plant to an insect species are:

1) the genotype of the plant,
2) the genotype of the insect, and
3) the genetic interaction between the plant and insect under different environmental conditions (Pathak & Olela, 1983).

Consequently, all results in this study must be examined in the light of the fact that these behavioural results were

obtained from a laboratory budworm strain and do not necessarily represent those of the native budworm bio-types from the local areas sampled.

Waxes tested against the standards A1 and A2 in the first series of experiments (Figures 16 to 17 and 20 to 21, pages 73 & 83) are consistent with what would logically be expected when retested against neighbouring trees (Figures 18 & 22, pages 77 & 87). For example the effects of b1-83 and b1-84 versus A2 in Figure 21 can be used to predict the results in Figure 22, when b1-83 and B1-84 are compared. Because A2 is preferred to b1-83, and b1-84 is preferred to A2, they can thus be categorized: b1-84 > A2 > b1-83. Therefore, when comparing b1-83/B1-84, the preference is expected to be for B1-84 as is the case. Experiments in Figures 18 and 22 also serve to show that a preference or an aversion for epicuticular waxes is not common to particular sampling sites, and that neighbouring trees do differ.

Resistance within these host species has been previously observed. During an outbreak of budworms that runs its full course, few understory seedlings of balsam fir are killed (Ghent, 1958), while typically ninety to ninety-seven percent of merchantable balsam fir are destroyed (Blais, 1954; Ghent, 1958). Between-host-species

preference is also demonstrated during an outbreak. White spruce intermingled with balsam fir are much less subject to mortality than the balsam fir (Ghent et al., 1957). McDonald (1981) emphasizes that there is a striking difference in mortality levels between white spruce and balsam fir.

The importance of epicuticular wax has been discussed, and results have shown feeding preferences that do in several cases correlate with survival and susceptability in the forest. Therefore, trees possessing waxes that elicit an aversion can be thought of as resistant. By extension, though only A1, C5, B1-83 and C1 were shown to have deterrent properties at standard concentrations, the trees can thus be categorized:

TABLE XIa

[STANDARD] RESISTANT	SUSCEPTIBLE
W.S.	
a1	t1
b3	t2
c4	t3
c5	
nb	
q1	
66:33 %	
B.F.	
b1-83	a2
c1	b1-84
c3	b2
nb	b4
	c2
	q2
40:60 %	
Totals 53:47 %	

TABLE XIb

[NATURAL] RESISTANT	SUSCEPTIBLE
	W.S.
B3	T1
* A1 C4 C5	T2
T2(T3)	T3
	NB
	Q1
44:56 %	
	B.F.
B1-83	
A2(B1-84)	* A2 B2 NB Q2
C1	C3
B4	C2
	B1-84
30:70	
Totals 37:63 %	

At natural concentrations (Table XIb), those tree waxes appearing together on the same line (*) in the above table produce similar feeding preference in the spruce budworm. Preference is relative to what is being compared. For this reason, T2 and A2 appear on both sides in the above table. Specifically, T2 appears "resistant" (to elicit an aversion) when compared to T3 at natural concentrations. The same applies to A2 when compared to B1-84. It is of interest to note that the percentage values for resistance/susceptibility for each host species in the above tables is in accord with Ghent's (1957) observations that balsam fir are more susceptible.

G.L.C.s OF WHOLE WAX

These gas liquid chromatographs indicated (Figures 19 & 23) that differences do exist in the chemical compositions of whole-wax samples from different host trees of the same species. Some peaks in these G.L.C.s correlated with preference. Whole-wax G.L.C.s also showed that another experimental approach was required so that chemicals eliciting preferences in the spruce budworms could be isolated.

COLUMN CHROMATOGRAPHY

In order to further isolate the chemicals eliciting preferences, column chromatography was used to divide the wax into six fractions. Those fractions producing no preferences in the budworms could be eliminated, thereby decreasing the numbers of compounds and possibly increasing the resolution in further G.L.C.s of selected fractions.

WAX FRACTIONS BEHAVIOURAL EXPERIMENTS

Behavioural experiments with wax fractions indicated that fractions 3 and 7 from white spruce, and fractions 2, 4 and 7 from balsam fir contained chemicals that produced preferences in the budworms. In these experiments, the use of sucrose with both test and standard fractions being compared served to increase the numbers of budworms feeding, without interfering with their preference. These fraction results (Figures 26 & 27, page 97) are in accord with the preferences of the whole wax experiments (Figures 17 & 21, pages 75 & 85).

G.L.C.s OF WAX FRACTIONS

Gas liquid chromatographs of fractions 3 and 7 for white spruce, and fractions 2, 4 and 7 for balsam fir were made (Figures 28 to 32). For each of the fraction types (2, 3, 4 & 7s), a visual comparision was made between the G.L.C. traces of the fractions eliciting a preference versus those eliciting an aversion. These G.L.C.s of fractions did produce greater resolution as compared to those of the whole waxes. Several peaks have been recognized as possible candidates for chemicals eliciting preferences in

the spruce budworm (see RESULTS, page 101), but this study falls short of the its objective of isolating and identifying these chemicals. As well, the nature of these chemicals in relation to the budworm's feeding behaviour, deterrents or stimulants, cannot as yet be ascertained. The control experiments with the wax standards (Figure 13) indicate that the discs with waxes are preferred, regardless of how they scored (preferred or not) in the preference experiments between other waxes. A more sophisticated, quantitative approach to analysis of these G.L.C.s versus preference is required. Relevant peaks or compounds would be more readily made apparent, by means of a computerized correlation analysis between numerical data for the G.L.C.s and feeding preference.

Lack of any correlation between the phthalate peaks, in the gas liquid chromatographs, and feeding preference eliminates phthalate as a candidate influencing spruce budworm feeding preference.

FEEDING BEHAVIOUR

Larvae of the eastern spruce budworm are oligophagous insects which feed on buds, needles, and flowers (Miller,

1963) of a variety of coniferous host plants in Eastern Canada and the United States. The spruce budworm is specialized to the ephemeral (short-lived) tissue of its hosts. According to the arguments presented by Rhoades and Cates (1976), the biological economics of this system would select for toxic or highly specific defence systems. Accordingly, vertical resistance (page 11) would be the expected character of this competitive interaction. The small quantities of materials used in these experiments (Figures 14, 15, 24 & 25) indicate that these chemicals do occur in small amounts, characteristic of vertical resistance.

The results of feeding responses of budworms to changes in test-wax concentrations, by dilution, can be divided into three catagories:

1) decreased preference,
2) increased preference, or
3) no effect on preference.

Logically, 1) must indicate the dilution of a stimulant, while 2) must indicate the dilution of a feeding deterrent. However, this is speculation. The interactions of the budworm's nervous system with a complex array of compounds found in the epicuticular waxes is far more

intricate than this simplistic analogy. Certain compounds may have narrow optimal concentration ranges wherein a behaviour is triggered. Specifically, the natural concentration of wax NB (Figure 14, page 66) is not much greater than that of the standard A1; yet, this small dilution (Figures 16 & 17, page 73) produced a remarkable change in feeding preference. This may indicate a narrow concentration range wherein the stimulant is "active" or effective. Therefore, though interpreted from the above three categories as a dilution of a deterrent, it could well be the dilution of a stimulant to its optimal range for triggering the behaviour. In any case, the above three categories will serve as a starting point until more research can bring about a better understanding.

From the figures indicated Table XII, changes in the degree of the spruce budworm's preference, when whole wax samples were tested at natural and then standard concentrations, suggest the following effects:

TABLE XII

	Dilution of a STIMULANT	Figures		Dilution of a DETERRENT	Figures
white spruce					
	NB/A1	16 & 17		B3/A1	16 & 17
	Q1/A1	16 & 17		T3/A1	16 & 17
	T1/A1	16 & 17	*	T2/T3	18
	* T2/A1	16 & 17			
balsam fir					
	B1-B4/A2	20 & 21		B1-B3/A2	20 & 21
	B4/A2	20 & 21		B1-B3/B2	22
	* C1/A2	20 & 21		B1-B4/B4	22
	C3/A2	20 & 21	*	C1/C2	22
	NB/A2	20 & 21		C2/C3	22
	B1-B3/B1-B4	22			
	C1/C3	22			

This table includes only those experiments where one or both of the experimental concentrations produced a significant preference. Waxes T2 and C1 appear on both sides (*) of this table. This is because of differential effects depending on which waxes these were compared with.

Feeding Rate

An interesting pointd I observed when scoring the experiments was, first, the fact that individual larvae appeared to be either stimulated to feed, or not stimulated

to feed. Disinterest in the "foods" provided resulted in a lower N value (replicates). Second, if they were stimulated to feed, a fairly constant rate was maintained (Tables VI to IX, page 114). For example, when presented with choices of wax fractions without sucrose (data not included), the number of individuals that consumed more than 6.25 % (25 out of 400) of the food provided was very low, as though most of the larvae were turned "off". Yet, the feeding rates of these animals that did feed were similar to those in other experiments (with sucrose) where many individuals fed. From observation, it appears that the numbers of budworms turned "on" or "off" to feeding is dependent on the kind of food presented.

Preference will cause differential feeding rates when budworms are given a choice, however, preference does not increase the total feeding rate. In search for the causative factor for the remarkable feeding rate of experiment nb/A2 (Table VIII, page 117), wax nb would be suspected to contain a stimulating chemical because wax nb is the only factor making this experiment different from the others using wax A2 as a standard. Without looking at the feeding preference (Figure 21, page 85), nb would be expected to be the preferred choice of the budworm, because it contains a feeding stimulant. Surprisingly, wax A2 is the preferred "food". This therefore demonstrates the

independence of the total-feeding rate and feeding preferences.

Blaney and Chapman (1970) state that: "biting is not a non-specific reaction as is commonly believed." These results seem to lend support to the idea that at least three different behavioural substances exist; namely,

1) biting stimulants (makes food acceptable or not acceptable for a test bite),
2) feeding stimulants (maintain feeding on a food), and
3) deterrents (arrest either or both biting and feeding.

Waxes provide biting stimulants and induce feeding, but do not maintain feeding as sucrose does. Albert (personal communication) also searched for correlations between feeding and preference. Because of the difficulties in correlating feeding rate to preference, and their apparrent independence as indicated above, these results are considered to have no relation.

CONTROL OF THE SPRUCE BUDWORM

More research will be required before these epicuticular wax eliciting an aversion can be incoporated into control programs for the spruce budworm. However, possible future methods of application may include:

1) Variability in both budworms (Lorimer, 1982; Willhite & Stock, 1983) and their hosts (Hunt & von Rudloff, 1974; von Rudloff, 1973) populations exist. Therefore, control can be achieved by manipulation of the spruce budworm population in order to maintain unfit-budworm phenotypes in local areas where the natural host defence is effective. Thereby, the effectiveness of the host's natural defences can be prolonged by preventing the rapid natural selection of "fit" budworms.

2) Reforestation with resistant host strains. These experimental methods show promise as tools for selection of "resistant" host trees in nurseries for the purpose of reforestration. However, caution must be exercised in order to maintain genetic diversity. Adoption of a few highly productive varieties over broad areas increases

vulnerability to widespread disease. The consequence of widespread use of genetically uniform plants was demonstrated in 1970 when a single leaf blight destroyed 25 per cent of the U.S. corn crop (Tisdall 1984).

3) Extraction or synthesis of the deterrent compound(s) for application in control programs.

Suggestions for further studies include the following:

I) Identify these "active" compounds.

II) Find the mode of action by which these chemicals act on the spruce budworm: i.e. increased mortality, decreased growth rates, effects on behaviour, etc.. If a deterrent is implicated, did it act exclusively as a feeding suppressor or have some physiologically toxic effect or other mode of action?

III) Sampling epicuticular wax in different areas over a period of time may indicate a correlation between environmental factors such as budworm population and the chemical "mood" of the trees, and may be related to outbreaks of the spruce budworm.

More questions have been raised than have been answered.

These include the following:

a) Do environmental factors such as budworm feeding within the vicinity or directly on a host tree elicit chemical changes in the epicuticular waxes similar to that of wax B1 (Figure 21)?

b) Would imitating budworm feeding by pinching needles elicit a chemical change?

c) Can a preference be shown for wax samples taken from different branches of the same tree? Are there within-plant preferences?

CONCLUSION

This study has shown that:

1) After a series of control experiments (Figures 10 to 13), the new experimental tray developed for evaluating fourth-instar-budworm preference at the onset of this study proved useful as an experimental tool. Experiments comparing the effects of starvation time indicated that 24 hours produced the most acute preference. Gender was shown to play no role in differences in preferences. Given a choice test with identical "foods" indicated no difference in preference for the test substance versus the standard substance positions in the arenas. The final control experiments indicated a strong preference for disks treated with epicuticular waxes, indicating the presence of stimulants.

2) The photographs in Figures 1, 5 and 6 show examples of survivor trees. These findings as well as those of other authors (MacDonald, 1979 & 1981; Blais, 1954, 1965 & 1968) substantiate the existence of these resistant trees.

3) Feeding behaviour of the spruce budworm is affected by the epicuticular waxes of both white spruce and balsam fir (Figures 17 & 21), and that these effects change in time within the same individual (B1). Specifically, a very dynamic defence mechanism with the characteristics of vertical resistance is apparent.

4) Column chromatography and feeding experiments, show that the chemicals responsible for the feeding preferences occur in fractions 3 and 7 for white spruce, and fractions 2, 4 and 7 in white spruce (Figures 26 & 27).

5) Gas liquid chromatographs show that waxes and their fractions do differ among trees of the same species, and the occurrence of several peaks (Figures 28 to 32) could tentatively be correlated with preference. Much work however, is needed to further isolate, verify and identify these chemicals.

6) Behavioural experiments show definite activity in terms of preference by the spruce budworm for waxes from different trees of the same species. The control experiment (Figure 13) comparing budworm preference for discs with wax versus discs without waxes indicates that the waxes are preferred, including wax A1 which was shown to be less preferred than several other waxes. As a result

of the methods used in this study, waxes could only be categorised in order of the degree of preference. This degree of preference between waxes could not definitely be attributed to the presence of more feeding (or biting) stimulants, or the absence of deterrents to the spruce budworm. More work is needed to examine correlations between budworm behaviour, neurology of the budworm, and wax composition.

7) Feeding rates indicated that fourth instar budworms fed at a constant rate, and, from observation from data not included, the use of sucrose increased the numbers that would feed.

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FIELD NOTES

Note: The following are field estimates and are therefore only approximate.

TREE	height (meters)	age (years)	budworm damage	condition
A1	13+	40	2nd instars found in buds	healthy
A2	17+	60	more than 60% defoliated	weak
B1	17+	50+	some defoliation	healthy
B2	15	35 - 40	well foliated	leaning
B3	10	30		healthy
B4	10	30		healthy
C1	10+	35	very little	healthy
C2	5	15	nil defoliation	healthy
C3	7	20+	25% defoliation	weak
C4	10	30		healthy
C5	10	40		healthy
NBWS				
NBBF				
Q1	20	45	some defoliation, found budworm	healthy
Q2	12	30 - 35		healthy
T1	12		budworm devastated area	healthy?
T2	13		budworm devastated area	healthy?
T3	17		near above devastated area	healthy

FOLIAGE DATA

TABLE 1

WHITE SPRUCE NEEDLE DIMENSIONS

	Average measurements of 20 (*19) Needles (mm)			
	length	width		0.95 C.L.
TREES		greatest	smallest	
A1	11.52			10.91 - 12.13
		0.96		0.87 - 1.05
			0.76	0.71 - 0.81
B3	8.38			7.62 - 9.14
		0.70		0.67 - 0.73
			0.51	0.49 - 0.53
C4	7.70			7.00 - 8.40
		0.90		0.88 - 0.92
			0.60	0.57 - 0.63
C5 *	7.32			7.27 - 8.13
		0.85		0.81 - 0.89
			0.57	0.51 - 0.61
NB	12.74			12.04 - 13.43
		1.01		0.95 - 1.07
			0.81	0.76 - 0.86
Q1	9.41			8.61 - 10.21
		1.16		1.10 - 1.22
			0.91	0.82 - 1.00
T1	15.05			14.27 - 15.81
		1.06		1.02 - 1.10
			0.82	0.78 - 0.86
T2	12.76			12.31 - 13.21
		0.79		0.75 - 0.83
			0.61	0.59 - 0.63
T3	12.46			12.36 - 12.92
		0.99		0.96 - 1.02
			0.80	0.77 - 0.83

TABLE 2

WHITE SPRUCE FOLIAGE DATA

TREES	area / needle (mm sq.)	wt./20 needles (grams)	wax/10 gr. needles (grams)	% H2O NEW:OLD
A1	19.738	0.0738	0.0140	80.16 55.45
B3	10.136	0.0375	0.0268	77.08 37.09
C4	11.5271	0.0404	0.0346	75.22 47.32
C5	10.349	0.0311	0.0288	75.9 50.71
NB	23.164	0.1111	0.0148	80.52
Q1	19.4493	0.0861	0.0529	78.51 30.51
T1	28.40	0.1437	0.0117	78.93 42.70
T2	17.93	0.0733	0.0203	80.64 37.15
T3	22.66	0.1132	0.0134	76.68 48.33

TABLE 3

BALSAM FIR NEEDLE DIMENSIONS

	Average measurements of 20 Needles (mm)			
	length	width		0.95 C.L.
TREES		greatest	smallest	
A2	7.76			7.46 - 8.06
		1.13		1.09 - 1.17
			0.79	0.74 - 0.84
B1-83	4.84			4.45 - 5.23
		1.05		1.00 - 1.08
			0.66	0.61 - 0.71
B1-84	9.34			8.61 - 10.07
		1.26		1.23 - 1.29
			0.88	0.84 - 0.92
B2	6.16			5.54 - 6.78
		1.11		1.03 - 1.19
			0.79	0.73 - 0.85
B4	10.26			9.55 - 10.97
		1.51		1.47 - 1.55
			1.10	1.06 - 1.14
C1	5.04			4.75 - 5.33
		1.10		1.06 - 1.14
			0.70	0.65 - 0.75
C2	7.84			7.22 - 8.46
		1.24		1.20 - 1.28
			0.78	0.75 - 0.81
C3	7.07			6.61 - 7.53
		1.10		1.08 - 1.12
			0.66	0.63 - 0.69
Q2	10.17			9.78 - 10.56
		1.27		1.24 - 1.30
			0.90	0.87 - 0.93
NB	10.40			9.60 - 11.20
		1.26		1.22 - 1.30
			0.73	0.71 - 0.75

TABLE 4

BALSAM FIR FOLIAGE DATA

TREES	area / needle (mm sq.)	wt./20 needles (grams)	wax/10 gr. needles (grams)	% H2O NEW:OLD
A2	14.941	0.0248	0.0278	70.0 41.84
B1-83	8.26	0.0143	0.0365	70.7 56.21
B1-84	19.99	0.0558	0.0202	73.04 45.11
B2	11.759	0.0334	0.0420	67.5 47.82
B4	26.77	0.0879	0.0190	75.25 48.53
C1	9.072	0.0225	0.0488	63.6 52.94
C2	15.84	0.0381	0.0338	67.6 44.76
C3	12.3920	0.0299	0.0358	69.26 47.15
D2	22.0642	0.0696	0.0250	63.02 37.09
NB	20.6250	0.0590	0.0248	-

TABLE 5

COLUMN CHROMATOGRAPHY FRACTIONS

Fraction weights used for producing average fraction sizes.

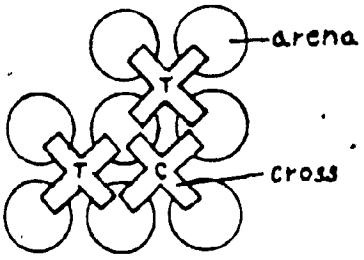
	WHITE SPRUCE			BALSAM FIR		
TREE	A1	T1	T2	A2	B1-8C	C1
FRACTIONS (grams)						
F1	.01736	.00195	.00203	.00490	.00413	.00749
F2	.00021	.00133	.00190	.00290	.00085	.00014
F3	.01936	.02556	.00411	.01490	.00064	.04862
F4	.00067	.05024	.01933	.02285	.02332	.02747
F6	.04260	.00735	.05033	.02990	.01800	.05758
F7	.03846	.03521	.05676	.05277	.17848	.10919
Initial weights:	.1569	.1360	.1461	.2436	.2356	.4508

A BETTER EXPERIMENTAL DESIGN

The following experimental design is proposed to increase the efficiency of the behavioural tests used throughout this study. Such behavioural tests are a valuable tool, however considerable work is required for preparation. Though this proposed design has not been tested, it is not expected to be sufficiently different from the test animals' perspective and should therefore prove quite functional.

EXPERIMENTAL SUBSTANCE

The round discs would be replaced by cellulose crosses, the dimensions of which would be such that the tip of each arm would fill four squares of the disecting microscope grid (about 3 mm) when on low magnification. Each arm would be of a length (perhaps 0.7 cm) so as to allow the tip of each of the four arms to enter a different, but closely associated arena. A special punch would be made to produce these crosses. To treat the tips of each arm, the cross would be rotated on a pin so as to allow each arm to be momentarily dipped in the test solution. The test solution would be at an appropriate concentration so as to pick up the desired quantity of test substance on the cellulose once the solvent has evaporated. Specifically, as the arms are rotated through, the amount of test solution picked up by this dipping method should be known. The following diagram may help visualize the structure:



The "T" and "C" on the crosses represent the test and control respectively.

EXPERIMENTAL TRAYS

Three plexiglas sheets of equal width and length would be hinged like the pages of a book. The bottom sheet would be thickest, and would have a pattern of 40 pits or wells (1 cm dia. X 3 mm deep) to form the floors of the test arenas. The surface of this sheet between four pits would be carved so as to accept one cellulose cross in such a manner as to allow the square tip of each arm to extend into four different test arenas. Positions for crosses containing test or control substances can be marked. The next plexiglas sheet would close down onto the first so as to secure the cellulose crosses in place. This sheet would be drilled with holes complementing those of the first sheet, and forming the upper part of each test chamber or arena. The next sheet would cover the holes and hold water so as to provide humidity as did the cover slips used in the design used in this study. To overcome the problem of trying to close the cover on 40 budworms at once without squashing them, the cover could be cut into strips to accommodate only a few arenas per strip.

The expected advantages of this design over that described in the materials and methods, is that:

1) rotating and dipping is easier and faster than pinning and pipetting,
2) fewer pins, test materials and parts to handle, as well as the greater size of the test materials increase the ease of handling,
3) faster preparation of experiments, and
4) smaller and more sturdy experimental trays.