FACTORS AFFECTING THE DISTRIBUTION AND SURVIVAL OF AN ENDEMIC AND AN INTRODUCED SPECIES OF OPEROPHTERA (LEPIDOPTERA:GEOMETRIDAE)

by

MARGARET ANGELA HALE
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We accept this thesis as conforming
to the required standard

[redacted]
Dr. Richard A. Ring

[redacted]
Dr. Patrick T. Gregory

[redacted]
Dr. John S. Hayward

[redacted]
Dr. Robert W. Olafson

[redacted]
Dr. Imre S. Otvos

Supervisor: Dr. Richard A. Ring

ABSTRACT

The purpose of this study was to compare the effects of physiological and biochemical factors on the recently introduced European winter moth, Operophtera brumata (L.) (Lepidoptera:Geometridae) with the endemic Bruce spanworm, O. bruceata (Hulst) using breeding experiments, laboratory tests of the effect of temperature and humidity on development and biochemical analysis of protective molecules. The results of these studies were used to make predictions regarding the future spread of the introduced species in North America and to supply evidence that water binding molecules such as glycerol may have a protective role in preventing desiccation during the aestival pupal stage.

O. bruceata males can freely interbreed with females of O. brumata because 94 % of breeding trials resulted in fertile eggs. The reciprocal cross was not as successful due to anatomical incompatibility, with 23 % of trials producing fertile eggs. There is evidence that hybridization occurs in the field.

The results of this study suggest that the introduced O. brumata is at least as cold-hardy and desiccation resistant as the native species. Pupal duration was not affected by relative humidity and the effect of temperature was the same for both species of Operophtera. The highest pupal mortality occurred at the lowest humidity tested (38% RH) and at the high, constant temperature treatment, with slightly higher mortality experienced by O. bruceata pupae. The water content of the pupal stage of both species was measured using the independent methods of freeze drying and heat extraction. Both methods showed that O. bruceata had higher water content (70.7%,69.7%) than O. brumata (65.5%,66.1%) and that the water was more readily extracted from O. bruceata. Supercooling ability increased significantly during the pupal stage of both species and the degree of supercooling ability was greater in O. brumata (Mean scp = -19.6°C). O. brumata probably could expand its range anywhere in temperate North America where O. bruceata is already established.

The sugar fraction of the insect samples was extracted and derivatised along with standard aldoses and alditols for identification and quantification using gas-liquid chromatography.

Analysis of the sugar fraction of the insect samples using gas-liquid chromatography detected glucose, galactose and glycerol. In samples extracted from O. brumata, levels of glycerol increased and levels of glucose decreased in the pupal stage which had the lowest supercooling point.

Examiners:

[redacted]
Dr. Richard A. Ring

[redacted]
Dr. Patrick T. Gregory

[redacted]
Dr. John S. Hayward

[redacted]
Dr. Robert W. Olafson

[redacted]
Dr. Imre S. Otvos

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INTRODUCTION

This study is an attempt to assimilate comparative data on physiological and biogeographical factors that affect two closely related species of moths belonging to the genus Operophtera (Lepidoptera:Geometridae). Both species are severe defoliators of a wide range of deciduous trees and shrubs and are capable of causing extensive economic injury to fruit and shade trees , most notably apple (Malus spp.) and oak (Quercus spp.) (Cuming 1961).

One species, the Bruce spanworm, Operophtera bruceata (Hulst), is native to North America and is known to reach injurious levels only occasionally (Brown 1962). The second species, the European winter moth, Operophtera brumata (L.), is of Palearctic origin and was inadvertantly introduced to North America on at least two separate occasions (Cuming 1961, Gillespie et al.1978). In the absence of natural enemies, this newcomer to North America rapidly expanded its range and populations increased to cause unacceptable levels of injury. The winter moth has had major impact on both apple orchards and oak woods in eastern North America (Embree 1965) and on Vancouver Island (Ferguson 1978)

and has even been reported feeding on young Sitka spruce in Scotland (Stoakely 1985), blueberries in the Fraser Valley (Ring pers. communication) and filberts in Oregon (AliNiazee 1986). O. brumata has been the subject of numerous studies in Eurasia and North America partly due to its availability and importance as a pest species, but mainly because it has been so often cited as a classic example of an insect that has been successfully controlled by introduced natural enemies (e.g. Caltigerone 1981, Embree 1971). O. bruceata has not been the subject of such interest except for reports of occasional outbreaks (see Brown 1962).

Thesis Objectives:

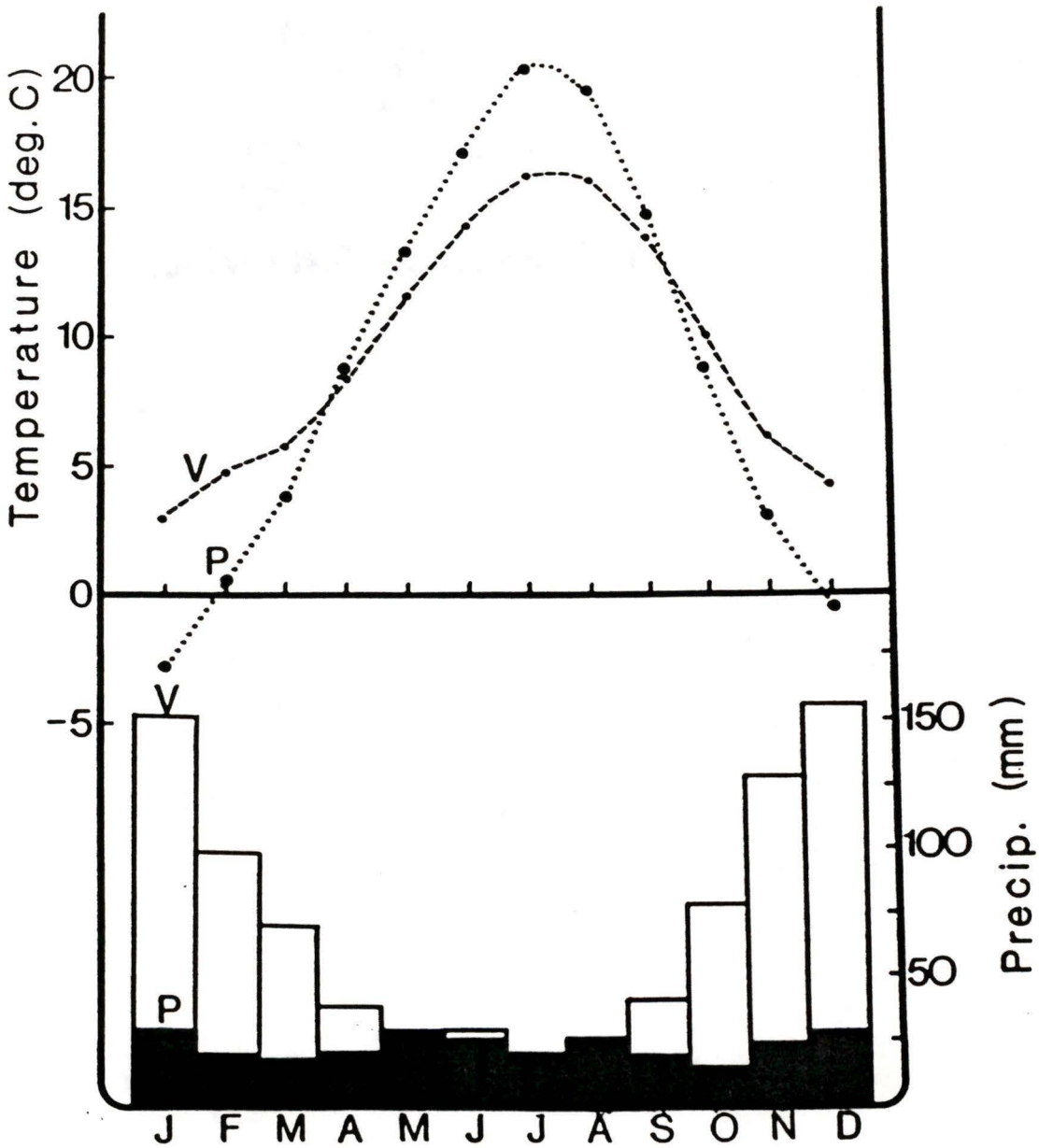
This study was undertaken to answer questions in three major areas which are distinct in themselves, but when viewed as a whole provide valuable information about two interesting and closely related species of Operophtera.

1) How do the native and introduced species compare in terms of their physiology and ecology? The European winter moth is a serious threat to organic apple and cherry growers in British Columbia. If the winter moth

extends its range to the Okanagan region of B.C. will it be able to survive the dry summer climate and cold winters? Figure 1 is a comparison of temperature and precipitation from Victoria, B.C. where the winter moth is well established and Penticton, B.C. where the winter moth has not yet been detected. The climate of southern Vancouver Island is much milder and has considerably more rainfall than the Okanagan region of British Columbia. Since the winter moth is widespread in Europe and Asia in areas of severe climatic conditions it is probably safe to predict that this species would thrive in the Okanagan. However, there are no data available to compare temperature and desiccation tolerance between the native and introduced species.

This was tested by comparing the survival and duration of the long pupal period of each species of Operophtera under a range of temperatures and humidities. Artificial extension or contraction of the pupal stage could affect the phenology of the moths to a degree that would reduce their fitness under certain climatic conditions. These tests could also determine what conditions might induce natural emergence of adults in the field.

Figure 1: Mean monthly temperatures and precipitation for Victoria and Penticton, British Columbia. Data were compiled from records at the Victoria weather office and represents means for the past thirty years ending in 1984. V = Victoria (dashed line, open bars) ;P = Penticton (dotted line, shaded bars).


Temperature (deg.C)
20
15
10
5
0
-5
V
P
V
P
150
100
50
Precip. (mm)
J F M A M J J A S O N D

2) By what biochemical and/or physiological mechanisms do these insects survive extremely stressful conditions of temperature and humidity? What conditions are encountered by each developmental stage and how are they adapted to face those conditions? Cold-hardiness and desiccation resistance are similar mechanisms for survival since water balance is involved in preventing freezing damage and limiting dehydration. Do the desiccation resistant stages of these moths use mechanisms similar to cold hardy species to retain fluids? Is cold-hardiness just one extreme example of a general case? Cryoprotectants may have other physiological roles in addition to those involved in freezing effects. The pupal stage of Operophtera is extremely desiccation resistant and therefore may have a lower supercooling point than the larval stage corresponding to increased levels of a cryoprotectant in the haemolymph as well as reduced body water content. If this is the case, the elevated polyol levels would not be in response to cold acclimation, but would be indicative of a physiological response to protect tissues from dehydration.

These ideas will be tested by measuring the

supercooling ability of each developmental stage of the moths. total water content of each stage was measured, the sugar fraction extracted from insect tissues and the presence and levels of sugars and polyols detected by gas-liquid chromatography. Elevated levels of polyols present in insects capable of an increased ability to supercool would suggest a mechanism for a protective function other than freezing avoidance if the stage with elevated polyols is not an overwintering stage.

3) The final objective is to investigate similarities between the native and introduced species of Operophtera. Allopatric species separated temporarily by geographic barriers may freely interbreed once that barrier is removed (Mayr 1969). The sex pheromone produced by winter moth females is attractive to Bruce spanworm males as well as conspecific males (Roelofs et al. 1982, Underhill et al. 1987). It would be valuable to determine whether or not the winter moth and the Bruce spanworm have evolved mechanisms to isolate them as true species. This will be tested by using breeding experiments to determine if O. brumata and O. bruceata breed true (i.e. only with conspecific individuals) or if they fail to recognize any reproductive barrier. The

offspring of the hybrid crosses will be reared to test for viability and to describe, if interspecific mating does occur.

History of O. brumata in North America:

The winter moth was first established in Nova Scotia around 1930 (Cuming 1961). Its range extended to include Prince Edward Island and New Brunswick by 1964, but introduced parasitoids reduced infestation levels in eastern Canada (Ferguson 1978). The winter moth was first correctly identified in western North America in 1976 from specimens collected in Washington and Oregon (Ferguson 1978) and from Victoria , British Columbia (Gillespie et al. 1978). By this time the moths were well established and were presumably not detected sooner because they had been mistaken for the native Bruce spanworm (Gillespie et al. 1978). In Oregon, insect collections contain specimens of winter moth dating back to 1958 (Ferguson 1978). The range of the winter moth in British Columbia is now at least from Sooke to Nanaimo on Vancouver Island and throughout the greater Vancouver area on the lower mainland including the lower Fraser Valley (Wood and Van Sickle 1985,1986). There have been

several recent studies on the control of winter moth in British Columbia (Otvos and Hunt 1986) and on the success of introduced parasites (Roland 1986a,b).

Life History of Operophtera spp.:

The Bruce spanworm, O. bruceata , is a nearctic species that is widespread wherever deciduous trees are found (Brown 1962). The European winter moth, O. brumata, is an introduced pest to North America (Cuming 1961). Both species have univoltine life cycles and are very similar in their ecology and phenology.

Two other species of Operophtera have been reported in North America (Ferguson 1978). They are O. danbyi, Danby's winter moth, and O. occidentalis, the western winter moth. O. occidentalis is now considered to represent an allopatric west coast population of O. bruceata (Pivnick et al. 1988) although the term O. occidentalis is still in use by some American researchers (e.g. Miller and Cronhart 1982, Kimberling et al. 1986).

Adult female moths emerge from the soil from late October to early January. They crawl up the trunks of trees and emit a pheromone which attracts weakly flying

male moths for the purpose of mating (Roelofs et al. 1982, Underhill et al. 1987). Adult moths of both sexes are non-feeding and short lived. Eggs are laid singly in crevices within the bark or under lichens and are the overwintering stage. The eggs are bright green immediately after oviposition and turn orange in ca. 14 days, remaining orange until a few days before larval eclosion when they darken to gray. Larval eclosion takes place in early spring when host plant buds begin to develop. Winter moth larvae develop through five larval instars over a period of four to six weeks. Cessation of feeding occurs when larvae complete development to the prepupal stage after which they drop to the ground where they construct cocoons in the leaf litter or the top few centimeters of soil. The pupal stage lasts four to six months until the first hard frost in the fall, at which time adult emergence takes place (Cuming 1961, Embree 1965). This life cycle is the type described by Masaki (1980) in which there is a summer diapause during the pupal period with activity in the spring and fall. Mating occurs in the fall before a second diapause during the egg stage.

The Bruce spanworm, O. bruceata , has a life cycle

very similar to the winter moth with the only notable differences being that the spanworm develops through four larval instars as opposed to five for winter moth and adult spanworms generally begin to emerge later in the fall (pers. observ.).

Eggs and larvae of the two species of Operophtera are indistinguishable in the field. Although Eidt and Embree (1968) claim to be able to characterize larvae by the alignment of ocelli and the number of ventral prolegs, this is difficult in practice due to overlap between populations. Pupae may be identified by the characteristic shape of the cremaster (Eidt and Embree 1968). Adult females are relatively easy to identify by the length of their vestigial wings, which are much longer in the winter moth (Eidt et al. 1966). It is difficult to discriminate between adult males (Cuming 1961, Brown 1962) and the only reliable method requires dissection and examination of the genitalia (Eidt et al. 1966). Pivnick (1988) found wing color patterns to be diagnostic of species in populations on Vancouver Island.

Insect Cold-Hardiness:

Physiologically, there are two categories of over-wintering insects;

1) Freeze-susceptible (or freeze-intolerant): these insects are killed if ice forms in their tissues. They survive sub-zero temperatures because they have the ability to supercool without ice crystal formation. As the insect cools, water molecules slow down and begin to aggregate, but actual freezing, the supercooling point (scp), does not occur until an aggregate of critical size forms that can become the ice crystal nucleus. At this point, the crystal spreads instantly through the haemolymph (Salt 1961). In many cold-hardy species, the supercooled state is so stable that mechanical shock will not initiate freezing (Asahina 1969). Protective coverings such as cocoons, puparia and egg shells may provide barriers to ice crystal formation (Ring 1980). The waxy layer of the exoskeleton may also prevent freezing by repelling water, thus avoiding ice crystal nucleation in the haemolymph (Bevan and Carter 1980).

2) Freeze-tolerant: These insects can survive extra-cellular ice formation,and the haemolymph generally freezes at relatively high sub-zero

temperatures. Freeze-tolerant species prevent intracellular ice formation by cooling slowly (Miller 1969), by freezing large amounts of body fluid to produce a plasma membrane barrier to ice crystal formation inside the cells (Asahina 1969) or by producing large molecular weight solutes in the haemolymph to promote extracellular ice formation at higher temperatures to inhibit intracellular ice formation at lower temperatures (Duman 1970).

Protective Molecules:

Low molecular weight compounds such as simple sugars and polyhydric alcohols can accumulate in the haemolymph of overwintering insects and contribute to the lowering of their freezing point (Asahina 1969). The protective effect of sugars and sugar alcohols is twofold. These compounds markedly raise the viscosity of water, and at high concentrations in the hemolymph, the rate of ice crystallization (or evaporation during prolonged periods of high temperature) is greatly reduced (Franks 1986). Polyhydroxy compounds also "salt-out" proteins, thus protecting proteins and other labile structures against the effects of desiccation (Gekko and Morikawa 1981).

The most common polyol in cold-hardy insects is glycerol, which is formed from glycogen in response to autumn cooling (Asahina 1969) by the blockage of pyruvate kinase (Storey et al. 1981). Concentrations of glycerol tend to remain constant through the winter and decrease in the spring as glycerol is no longer needed and is converted back to glycogen. There is a correlation between glycerol levels and supercooling ability, although glycerol is not always present in cold-hardy species (Ring 1982). Glycerol is thought to have several roles in cold-hardiness. It acts as a supercooling agent by preventing freezing and increasing viscosity of the haemolymph, which inhibits nucleation, lowering the freezing point even more (Salt 1961). Glycerol also may prevent tissue damage during freezing by binding to membrane proteins (Duman 1979). Because of its relatively small size, the glycerol molecule is able to penetrate most cell membranes, and its ability to prevent freezing damage is generally believed to lie in its colligative properties and its ability to act as a solvent, keeping potentially harmful salts in solution as they are concentrated during ice formation (Meryman 1971).

Excellent recent reviews on the subject of cold tolerance with reference to the association of cold tolerance and dehydration are provided by Cannon and Block (1988), Storey and Storey (1988) and Zachariassen (1985).

MATERIALS AND METHODS

1) Source of insects:

Most of the insects used in this project were collected in the larval stage and reared to the developmental stage required for individual studies. Specimens of winter moth, Operophtera brumata were collected from the vicinity of Victoria, British Columbia, and specimens of the Bruce spanworm, Operophtera bruceata were collected near Keremeos, British Columbia. Since O. bruceata is a minor pest, and most orchardists employ chemical sprays for lepidopterous larvae, it was very difficult to obtain large numbers of Bruce spanworm larvae. This resulted in smaller sample sizes or missing data for some of the studies.

Both species were collected in the larval stage by hand or by beating branches over a beating tray. Larvae were placed in Mason jars with cloth lids held in place by sealing rings. The larvae were provided with a layer of moist peat moss for a pupation medium and fresh, young apple foliage was provided as a food source. After pupation was completed, cocoons were removed from the

peat moss and pupae dissected from the cocoons for immediate study or the pupae were left in their cocoons and reared individually in controlled environment chambers to provide adults and eggs for later studies.

In the first year of study, 49 females of each species of Operophtera were weighed and dissected to count the number of mature oocytes present. The number of mature oocytes per female was related to weight in order to compare the fecundity of the two species. The number of oocytes per mg. body weight was considered an index of fecundity (i.e. ability to produce offspring).

2) Protocol for breeding trials:

Four possible crosses were attempted:

i) male O. brumata with female O. bruceata

ii) male O. bruceata with female O. brumata

iii) both sexes, O. brumata

iv) both sexes, O. bruceata .

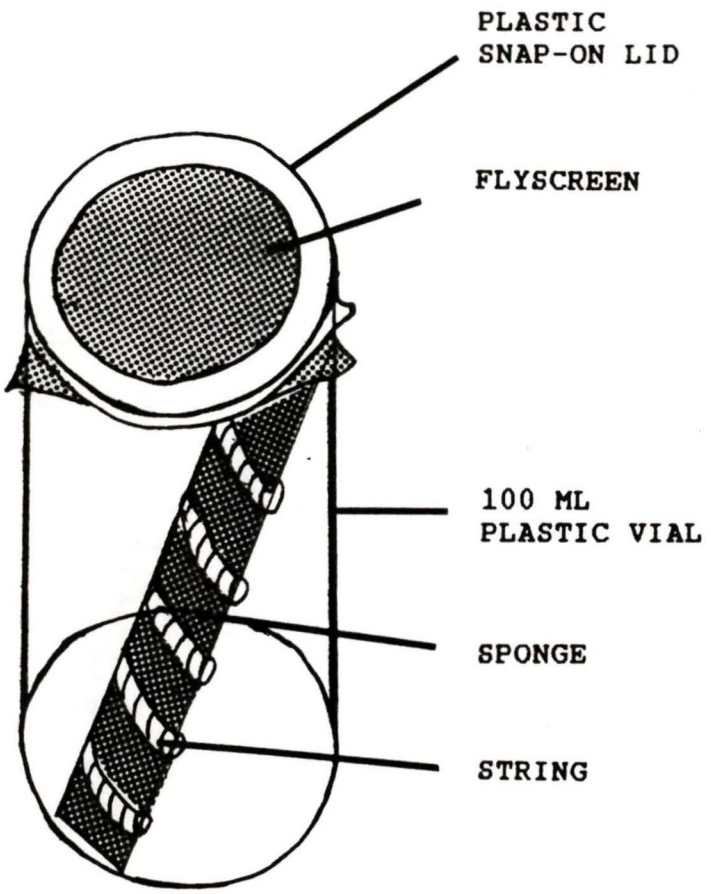
Breeding trials took place in cages constructed from 100 ml plastic vials. Holes were cut through the soft plastic lids and the remaining rim was used to secure pieces of flexible fly screen over the openings. A strip

of synthetic sponge wrapped in twine was placed in each cage to provide an oviposition site (Figure 2). For each trial , an adult male and female were put together in a cage in an outdoor shade house. Moths were used in the trials on the same day that they emerged and remained in the cages until death. Species identification was confirmed by examining the exuviae for shape of the pupal cremaster (Eidt and Embree 1968) and by examining the adult moths for the size and shape of the male genitalia and the size of the vestigial wings of the females (Eidt et al. 1966).

3) Development of hybrids:

After larval eclosion, first-instar larvae were individually transferred to 5 cm diam. plastic culture dishes and each caterpillar was given a fresh, young apple leaf. Foliage was replaced on alternate days. Egg dimensions and larval head capsule widths were measured using a filar micrometer on a dissecting microscope to compare with measurements of parental species. Foliage was removed when the larvae had completed development to the prepupal stage and subsequently ceased feeding. A

Figure 2: Diagram of cages used for breeding trials.


PLASTIC
SNAP-ON LID
FLYSCREEN
100 ML
PLASTIC VIAL
SPONGE
STRING

small amount of peat moss was then put in the culture dishes as a source of material for cocoons. After pupation, pupae were carefully dissected from the cocoons and weighed. Each pupa was examined and the shape of its cremaster described before it was returned to its cocoon. The pupae were reared to the adult stage in individual labelled cages in a controlled environment chamber with the same temperature and light cycle as the low/variable treatment described in the next section. The hybrid adults that emerged from the F1 generation were all placed together in a mason jar with a cloth lid and an oviposition site as described earlier. Any eggs produced (F2 generation) were kept in an outdoor shade house until larval eclosion.

4) Pupal duration, mortality rates and water content:

To determine the approximate summer temperature range to which pupae were exposed in the field, temperatures were measured in an apple orchard in Victoria which was heavily infested with winter moth. Temperatures were measured every four hours using a thermistor with a digital readout. Measurements were made at five locations at each of the four corners and

in the center of a block of 32 trees. The mean temperature for those locations was plotted over a 24 hour period at 1 m above ground (air temp.), at the soil surface and 3 cm. below the soil surface both in an exposed area and a shaded area below the tree canopy.

Pupae within the undisturbed cocoons were weighed singly and then caged in containers with separate compartments for each pupa. The containers had 10 compartments each and were constucted from pieces of light fixture and fly screen as described by Humble (1987). The containers were labelled to identify individual pupae for the duration of the experiment. The caged pupae were placed inside small Frigoseal containers (30cm by 15cm by 5cm) and suspended above one of five saturated salt solutions (Table 1) to produce the desired relative humidity at a given temperature (Winston and Bates 1960). The experimental units were maintained in walk-in Conviron controlled environment chambers under constant light conditions (L:D 16:8) and one of three temperature treatments (Table 1). Once adult emergence had commenced, pupae were transferred inside the containers to a cold room at 8°C until adult

Table 1 : Experimental conditions of temperature and humidity for comparing pupal duration and mortality rates between Operophtera brumata and O. bruceata. Listed with each RH is the name of the salt used in saturated solution to produce the desired humidity, as given in Winston and Bates (1960)

Relative Humidity				
99% potassium sulphate	88% potassium chloride	75% sodium chloride	55% glucose	38% sodium iodide

Temperature

1) High/Constant - TC 25°C:20°C - temperature cycle remained constant until the end of October, then moved to 8°C until adult emergence.

2) Variable - TC 20°C:15°C - for 8 weeks
TC 15°C:10°C - for 8 weeks
8°C - until adult emergence

3) Low/Variable - TC 15°C:10°C - for 8 weeks
TC 10°C: 5°C - until adult emergence

emergence was complete.

Operophtera brumata were tested under all fifteen possible conditions and O. bruceata were tested under six: all temperatures with 75% RH and 55% RH. In all treatments, the highest temperature was accompanied by the light cycle except for when pupae were transferred to the cold room at 8°C where they were maintained in total darkness.

To determine rate of weight loss each pupa was weighed bi-weekly from late June until the end of October, a period of four months. Rate of weight loss was calculated only for individuals that completed development to the adult stage. To determine pupal duration and mortality rate, at completion of adult emergence all cocoons were opened and the contents scored as dead, emerged, or parasitized. Pupal duration was defined as the number of days from the first day of the test until adult emergence. Pupal survival was expressed as the proportion of unparasitized pupae successfully emerging as live adult moths.

To compare total water content, 10 individual pupae of each species were suspended, one at a time, in a Kahn electrobalance in an evacuatable glass bottle at 120°C

and dried under nitrogen. Weights were recorded every minute for the first 5 minutes , then every 2.5 minutes until equilibrium was reached.

5) Supercooling point determination:

Insects were placed inside a narrow glass tube within a larger glass tube with a copper-constantan thermocouple held in place with cork stoppers. The thermocouple was either placed against the abdominal wall of the insect (large larvae, pupae and adults) or the specimen was attached directly to the thermocouple using a spot of silicone lubricant (eggs, small larvae). The tube assembly was immersed in the 95% ethanol coolant of an electronic cryobath, programmed to cool at a rate of -1°C per minute. The temporary rise in body temperature, associated with the heat of crystallization released as ice forms, occurs at the supercooling point. This was sensed by the thermocouple and observed as a rebound in the cooling curve displayed on a potentiometric chart recorder (described in Humble and Ring 1985).

6) Separation of lipid and sugar fractions:

This was achieved essentially by the methods of Van Handel (1965), but modified in the manner described by Ring and Tesar (1980). Each sample was weighed, freeze-dried and reweighed to obtain total water content in the fresh tissue. The dried samples were mixed with 3.8 ml of a mixture of saturated solution of sodium sulphate, chloroform and methanol (0.8:1:2), homogenized in a thick-walled glass tube submerged in a bath of melting ice, and centrifuged under refrigeration at 5000rpm for 20 minutes. The supernatant was transferred into a large test tube (A), and the residue extracted once more with distilled water, chloroform and methanol (0.8:1:2); both supernatants were pooled in tube A. To these combined supernatants was added 2 ml of a water-chloroform mixture (1:1) and the contents thoroughly mixed by reversing the tube several times. This combined extract contained the lipids, carbohydrates and amino acids. After formation of the two-phase system, the upper one, containing sugars and amino acids, was removed with a syringe and transferred to test tube B. Test tube A, the lower phase, contained the lipid fraction.

The lipid-free residue remaining after the chloroform extraction was then washed twice with 70% ethanol, centrifuged at approximately 6000rpm, the supernatants being combined with the aqueous portion in test tube B and saved for the determination of total sugars and polyols. The extract was dried *in vacuo* , the residue resuspended in 1 ml distilled water and derivatised to alditol acetates for quantification by gas-liquid chromatography.

7) Determination of polyol concentration:

Preparation of alditol acetate derivatives:

Aldose and polyol standards were derivatised by the following methods modified from Oades (1967). An excess amount (40 mg) of sodium borohydride ($NaBH_4$) was added to 1 ml standard in a round bottomed flask. Reduction was allowed to proceed at room temperature for two hours. Glacial acetic acid was added dropwise until the bubbling stopped, indicating thay any excess $NaBH_4$ had been destroyed. The standard was dried under nitrogen, suspended in 1.0 ml methanolic HCl (0.1 ml HCl in 100 ml methanol), dried, resuspended in 1 ml 95 % ethanol and

again dried. The standard was left overnight over phosphorous pentoxide to remove any traces of water. The standard was then scraped off the sides of the flask and mixed in 2 ml of a 1:1 mixture of acetic anhydride and pyridine, refluxed for one hour and then dried under nitrogen. The resulting alditol acetate was dissolved in chloroform, filtered to remove any unreacted material, then injected onto a GLC column. Insect samples were prepared identically to the standards.

Gas- Liquid Chromatography:

Alditol acetates were separated using a 3% SP2340 on 100/120 Supelcoport column on a Varian Gas Chromatograph. Peaks were detected using a flame ionization detector and xylose was used as an internal standard. The most satisfactory peak separations were obtained using an injector temperature of 180°C, detector temperature of 300°C, and a programmed column temperature ranging from 145°C (held initially for ten minutes) to 220°C (increasing at 7°C per minute then holding at the final temperature for fifteen minutes). Peaks were identified by retention times and concentrations were calculated using peak areas. The

amount of sugar or polyol in the insect samples was expressed as % fresh weight using the formula:

$$IVi/IVs \times PAi/PAs \times 1.25 \text{ mg/ml} / FWi$$

where IVi = injection volume on insect sample

IVs = injection volume of standard

PAi = peak area of insect sample

PAs = peak area of corresponding standard

FWi = fresh weight of insect sample.

8) Statistical analyses:

Statistical analyses followed Zar (1984), and MINITAB (Penn. State Univ. 1982) was used to analyse many of the data. Differences between means were compared using Student's t-test for independent samples. Analysis of variance was used to assess variation between each species of moths and the hybrids of the two species, followed by Duncan's multiple range test to assess individual effects. Effects of temperature and humidity on pupal duration were tested separately using one-way analysis of variance. Chi-square analysis using contingency tables was used to test the effect of temperature and humidity on pupal mortality. A minimum rejection level of alpha=0.05 was used in all statistical tests.

RESULTS

1) Fecundity:

The regressions of fecundity (y - expressed as number of mature oocytes) against fresh weight in mg (x) of female winter moths and Bruce spanworm moths are given in Figure 3. The regression equation for Operophtera brumata is y=9.0-47.3 and for O. bruceata the equation of the line is y=8.8x -70.0. There was no difference between the equations ($t=20.9, p<0.001$), so for moths of comparable size, there is no reproductive advantage in number of eggs produced.

2) Breeding trials:

Observations:

Interspecific matings were observed between both reciprocal crosses of Operophtera brumata and O. bruceata (Figure 4). All pure crosses were successful, i.e. fertile eggs were produced from every trial. Female O. brumata paired with male O. bruceata produced fertile eggs in 15 of 16 trials. Female O. bruceata paired with male O. brumata were not as productive since only 3 of 13 trials resulted in fertile

Figure 3: Number of oocytes vs. weight for Operophtera brumata (solid circles, r = 0.955) and O. bruceata (open circles, r = 0.948). n = 49 for both species.

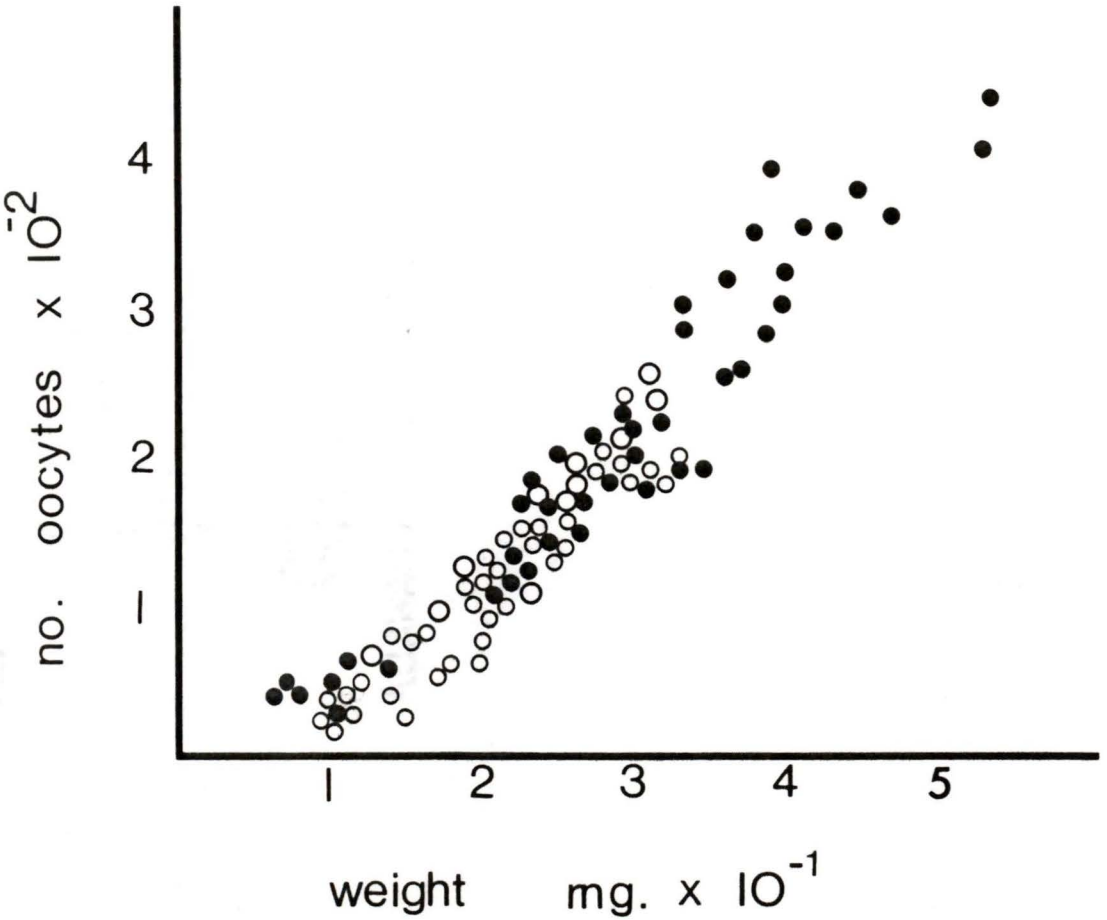
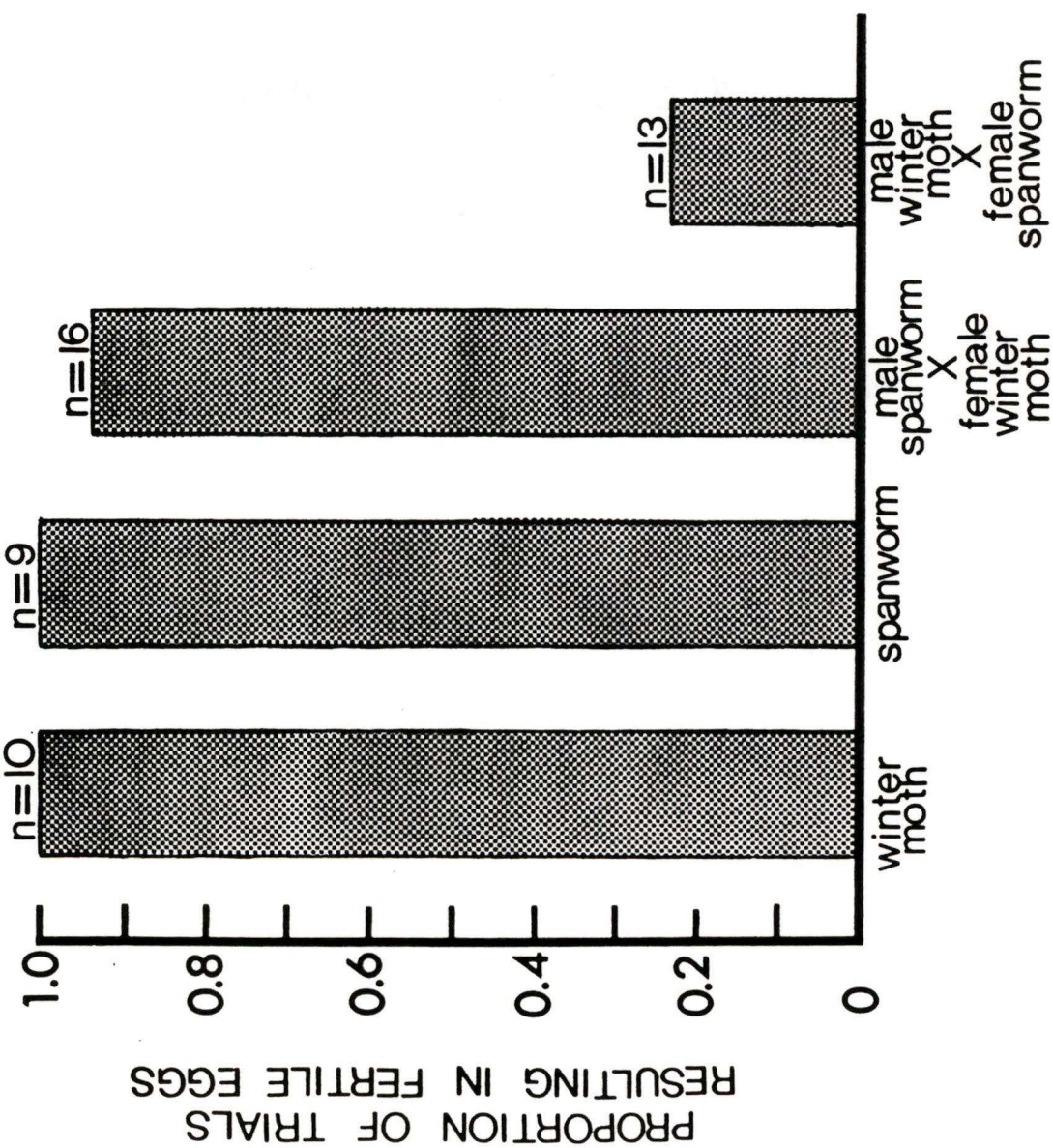

no. oocytes x 10^{-2}
4
3
2
1
1
2
3
4
5
weight mg. x 10^{-1}

Figure 4: Results of breeding trials between Operophtera brumata and O. bruceata . Bars represent the proportion of mating pairs which produced fertile eggs in each of the four attempted crosses.


PROPORTION OF TRIALS
RESULTING IN FERTILE EGGS
1.0
0.8
0.6
0.4
0.2
0
n=10
n=9
n=16
n=13
winter moth
spanworm
male spanworm X female winter moth
male winter moth X female spanworm

eggs. In addition, the genitalia of the copulating pair became locked, preventing post-copulatory disengagment often resulting in death of the male due to tearing of the posterior segments of the abdomen during separation from the female.

Description of hybrids and pure species:
All described hybrids resulted from the crosses of O. bruceata males with O. brumata females.

Egg dimensions: O. brumata and hybrid moth eggs were not significantly different from each other in either length or width, but O. bruceata eggs were larger in both dimensions than both other types (Table 2).

Larval characters: manipulation of larvae was minimized to prevent mortality by handling. This made it difficult to determine the number of instars in hybrid development. The number of hybrid larval instars varied between four and five; however, the proportion of each could not be determined because it was not always possible to recover the exuviae. The range in head capsule width was larger in the hybrid group than in either parent group and the size frequency distribution of head capsule width had a bimodal distribution

Table 2: A comparison of measurements of eggs from parent and hybrid crosses of Operophtera brumata and O. bruceata (n = 20).

	Mean length (SD) range in mm	Mean width (SD) range in mm
O. brumata	0.67(0.02) a 0.64 - 0.71	0.43(0.01) a 0.41 - 0.46
Hybrid cross	0.70(0.03) a 0.65 - 0.77	0.41(0.02) a 0.37 - 0.46
O. bruceata	0.89(0.03) b 0.83 - 0.93	0.56(0.02) b 0.53 - 0.59

Means vertically followed by the same letter are not significantly different from each other. ($p < 0.05$, one-way analysis of variance followed by Duncan's Multiple Range test).

(Figure 5). Hybrid prepupae weighed more and had wider head capsules than either parent group, but mortality in the prepupal stage was much higher (Table 3). Those hybrids which completed development to the pupal stage were actually smaller than the parent group pupae.

Pupal characters: cremaster shape was variable. Figure 6 provides a description of the variation in the cremaster. Pupae had cremasters which were indistinguishable from those of the parent groups (type C and D) or were combinations of both parents (type A and B).

Adult characters: adult O. brumata and O. bruceata may be distinguished by differences in the male genitalia and by the length of the vestigial wings of the female (Eidt et al.1966). Characteristics of both parent species were seen in the F1 offspring. Adult hybrid females had morphological characteristics of either one, but not both, parents. Adult hybrid males were difficult to assign to either of the parent groups since intermediate characteristics were noted in the size and shape of the clasper and uncus. Three of four males closely resembled males of O. brumata and the fourth resembled O. bruceata with the large, sickle

Figure 5: Size frequency histograms of head capsule widths of the prepupal stage of Operophtera bruceata, O. brumata and hybrids. Size classes are in increments of 0.05 mm.

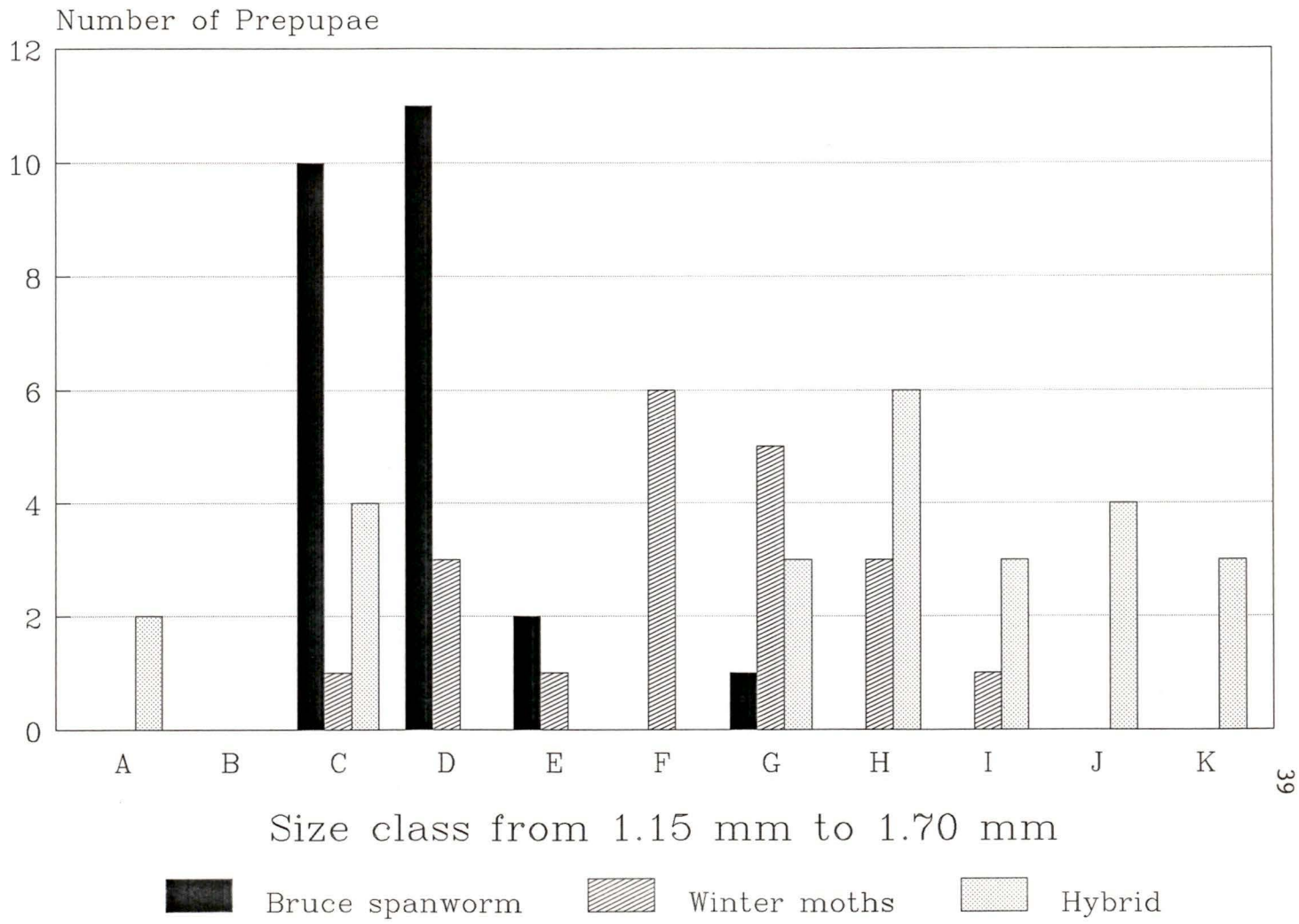
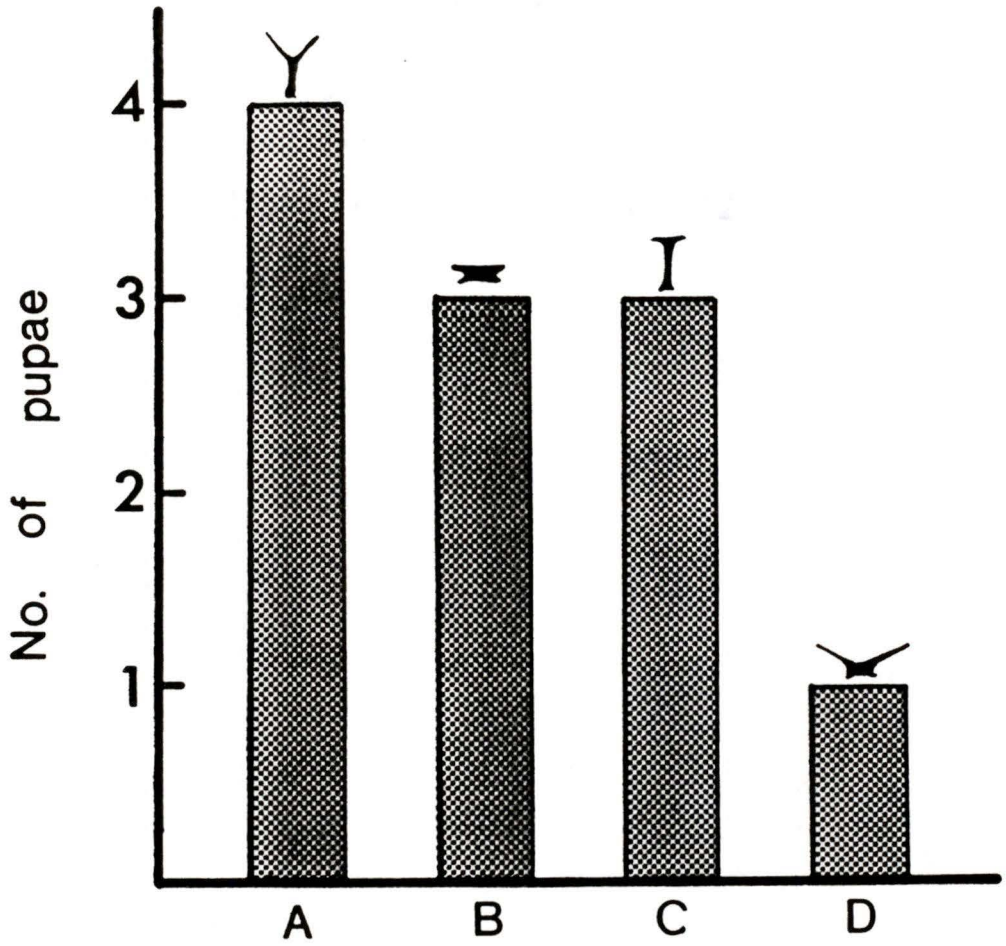

Number of Prepupae
12
10
8
6
4
2
0
A
B
C
D
E
F
G
H
I
J
K
Size class from 1.15 mm to 1.70 mm
Bruce spanworm
Winter moths
Hybrid

Table 3. Means (± SD) and ranges of weights of prepupae and pupae, widths of head capsules of prepupae and mortality during the pupal stage of Operophtera brumata ,O. bruceata and O. brumata female / O. bruceata male hybrids.

	O. brumata (n = 20) Mean (SD) Range	O. bruceata (n = 24) Mean (SD) Range	Hybrids (n = 24) Mean (SD) Range
Head capsule width of prepupae(mm)	1.43(0.08) 1.25-1.58	1.27(0.22) 1.25-1.46	1.49(0.17) 1.17-1.67
Weight of prepupae(mg)	47.3(13.3) 23.4-83.0	39.9(5.5) 29.8-50.2	55.0(21.1) 24.3-100.9
Weight of pupae(mg)	43.7(7.9) 30.7-57.5	37.8(4.3) 30.0-47.2	31.1(11.0) 20.8-51.0
Prepupal mortality (proportion dying)	0.10	0.08	0.58

Figure 6: Observed morphological variation in the cremaster of hybrid pupae (n = 11). A and B are intermediate types while C = typical winter moth cremaster and D = typical Bruce spanworm cremaster.


No. of pupae
4
3
2
1
A
B
C
D

shaped uncus, but with the smaller clasper and saccus characteristic of O. brumata (Figure 7).

Total number of hybrid adults was insufficient for paired breeding trials, so all seven adults were caged together to increase the likelihood of successful mating. Fertile F2 eggs were produced but they hatched prematurely before any suitable foliage was available. To date I have had no success rearing Operophtera spp. on artificial diets and although the F2 offspring were provided with a standard Choristoneura diet they did not feed and soon starved. Premature larval eclosion was most likely due to an unusually warm spell rather than early development of the eggs, but this has not been tested.

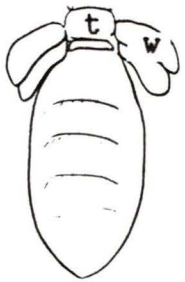
Field Observations: in November and December 1986, O. bruceata accounted for less than two percent of female Operophtera spp. collected in traps in an apple orchard in Victoria B.C.. On three separate occasions (Nov.29, Dec.6 and Dec.9, 1986) interspecific matings were observed between male O. brumata and female O. bruceata . The mating pairs were observed in traps while still engaged in copulation. Identification of the males was confirmed by examination of the genitalia.

Figure 7: Observed variation of morphology of hybrid adults. The number on the left of the drawing represents the number of individual moths assigned to each category of parent type. a= hybrid females with O. brumata morphology, b= hybrid females with O. bruceata morphology, c= hybrid males with O. brumata morphology, d= hybrid males with genitalia showing morphological characteristics of both O. bruceata and O. brumata. w= wing, t= thorax, un= uncus, cl= clasper, aed= aedegus, sac= saccus.

♀

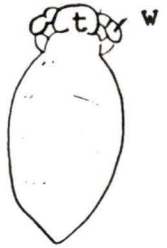
a)

n = 2



b)

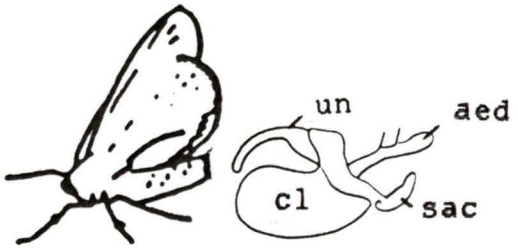
n = 1



♂

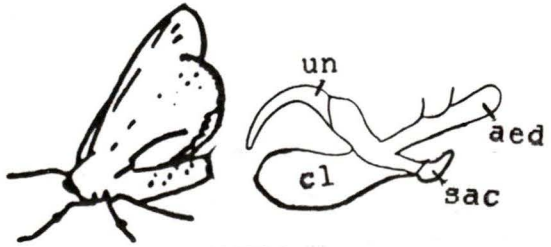
c)

n = 3



d)

n = 1



3) Pupal duration and mortality:

Field temperatures: Figure 8 shows the range of temperatures in an apple orchard in Victoria over a 24 hour period during August 1985. The purpose of this figure is to show the extreme range of temperatures expected to be encountered by the pupal stage of Operophtera spp., the majority of which are located between the soil surface and 3 cm. soil depth.

Duration of pupal period:

The length of time (in days) required to complete development and emerge as an adult O. brumata moth varied from a mean of 142.8 days under the low, variable temperature treatment to 161.2 days under the variable treatment to 172.4 days under the high, constant temperature treatment (Table 4). The effect of temperature was significant in both species ($F=144.22$, $p<0.0005$, O. brumata ; $F=47.88$, $p<0.0005$, O. bruceata). For O. brumata pupae which survived until adult emergence, the level of relative humidity did not have any effect on the duration of the pupal period except under the low variable treatment ($F=7.50$, $p<0.0005$). The variance under this treatment was mostly accounted for

Figure 8: Air, soil surface and below ground temperatures in an apple orchard during mid-summer over a 24 hour period. Points represent mean values for 5 locations. Open circles = exposed sites; Solid circles = shaded sites.

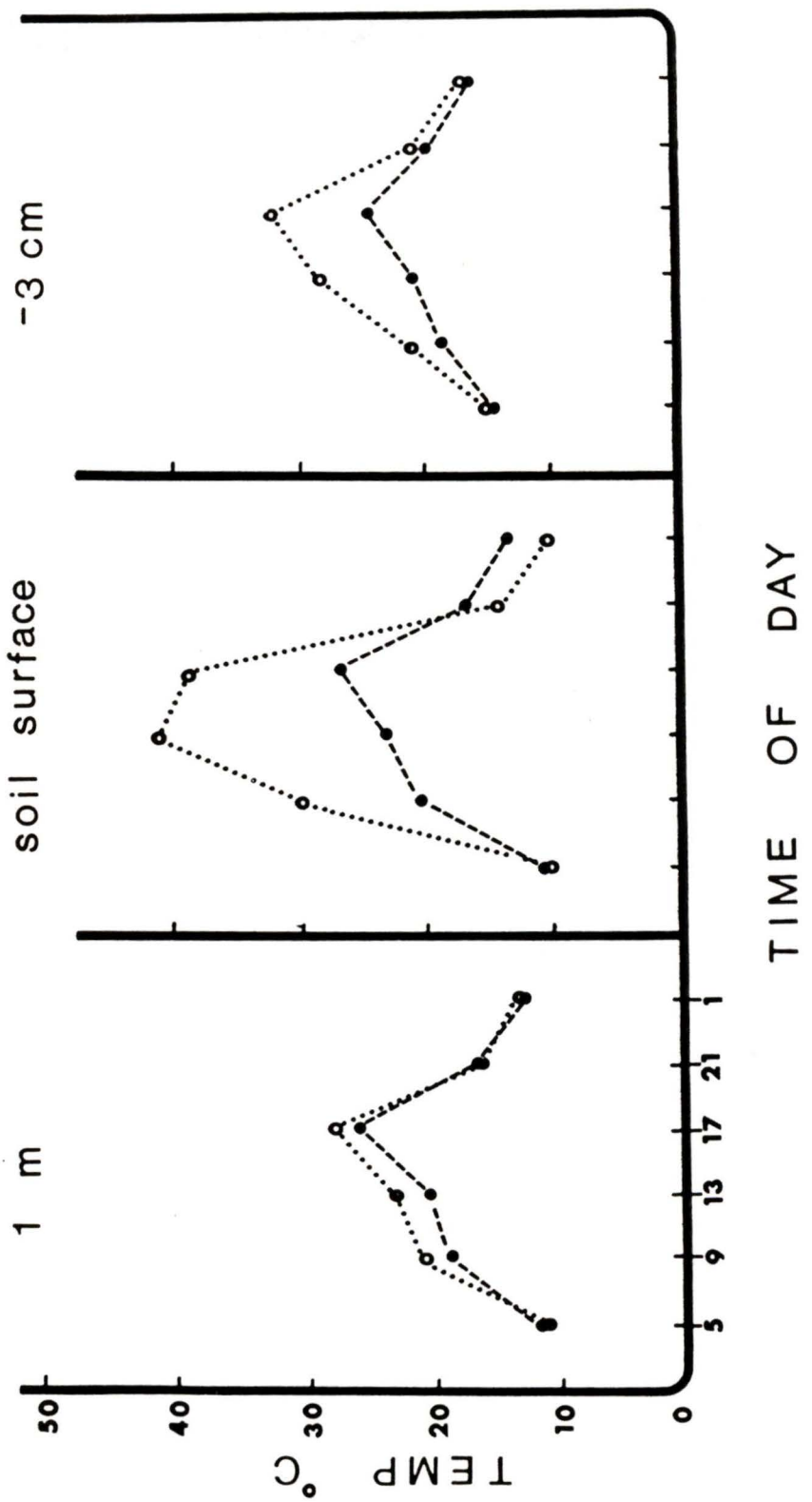

1 m
soil surface
-3 cm
TEMP °C
50
40
30
20
10
0
5
9
13
17
21
1
TIME OF DAY

Table 4: A comparison of the length of time required to complete pupal development under three temperature treatments.

Temperature treatment	O. brumata n	O. brumata Days Mean(SD)	O. bruceata n	O. bruceata Days Mean(SD)
Variable				
99% RH	12	161(5.6)NS	--	--
88% RH	11	163(4.6)NS	--	--
75% RH	12	161(7.0)NS	5	148(3.0)*
55% RH	8	161(4.0)NS	4	141(4.0)*
38% RH	7	160(2.0)NS	--	--
Mean	50	161.2 **	9	144.5 **
High, constant				
99% RH	6	172(6.0)NS	--	--
88% RH	8	170(5.0)NS	--	--
75% RH	6	173(3.0)NS	5	155(9.0)NS
55% RH	9	171(7.0)NS	7	155(12.0)NS
38% RH	1	176(3.6)NS	--	--
Mean	30	172.4 **	12	155.6 **
Low, variable				
99% RH	9	143(5.0) *	--	--
88% RH	8	148(16.0)*	--	--
75% RH	7	136(5.0) *	5	125(7.0)NS
55% RH	9	144(8.0) *	5	123(7.0)NS
38% RH	2	143(11.0)*	--	--
Mean	35	142.8 **	10	124.0 **

NS: No significant effect of humidity on O. brumata, variable temp. ($F=0.41$, $p>0.25$) and high, constant temp. ($F=0.34$, $p>0.25$)or on O. bruceata, high, constant temp. ($F=0.02$, $p>0.25$) and low, variable temp.($F=0.24$,$p>0.25$).
*: Significant effect of humidity on O. brumata, low, variable temp. ($F=7.50$, $p<0.0005$) and O. bruceata, variable temp. ($F=13.23$, $0.01<p<0.05$).
**: Significant effect of temperature on O. brumata ($F=47.88$, $p<0.0005$) and O. bruceata ($F=47.88$, $p<0.0005$).

by a single pupa which required 186 days to complete development, which deviated far from the mean pupal duration of that group.

Mortality:

In all treatments, the bulk of adult emergence occurred during October, November and December although three adults emerged during the first week of January. The proportion of pupae completing development to the adult stage under a range of temperatures and relative humidities varied from 0.10 to 1.00 (Table 5).

There was a significant effect of humidity on the mortality of O. brumata pupae ($\pi^2=31.18$, $p<0.001$). Treating temperature and humidity effects separately, the largest proportion of winter moths to complete development was reared under the moderate humidites, 88% RH, 75% RH and 55% RH (0.85, 0.81 and 0.80 respectively). The lowest survival occurred in the group maintained at 38% RH (0.30), the lowest humidity tested. The lowest survival due to temperature effects alone was 0.66 for O. brumata and 0.60 for O. bruceata. Both groups with the highest mortality were maintained under the high, constant temperature treatment until one month before adult emergence. O. bruceata was more

Table 5a: Effect of temperature and humidity on the proportion of pupae completing development to the adult stage.

Temperature treatments	n	Relative humidity 99%	88%	75%	55%	38%
Variable						
O. brumata	73	1.00	0.85	0.86	0.57	0.41
O. bruceata	14	--	--	0.71	1.00	--
High, constant						
O. brumata	50	0.54	0.89	0.75	1.00	0.10
O. bruceata	15	--	--	0.62	0.57	--
Low, variable						
O. brumata	51	0.77	0.80	0.82	0.82	0.40
O. bruceata	14	--	--	0.71	1.00	--

Table 5b: Mean survival of pupae at all temperatures and all relative humidities.

		n	O.brumata	n	O.bruceata
Mean survival over all temperatures	99% RH	42	0.77*		--
	88% RH	32	0.85*		--
	75% RH	33	0.81*	22	0.68NS
	55% RH	35	0.80*	21	0.86NS
	38% RH	32	0.30*		--
Mean survival over all RH	Variable	73	0.74NS	14	0.86NS
	High/Constant	50	0.66NS	15	0.60NS
	Low/Variable	51	0.72NS	14	0.86NS

NS: No significant effect of humidity on mortality of O. bruceata (π^2=1.84, 0.10<p<0.25), temperature on O.brumata (π^2=2.03, 0.25<p<0.50), temperature on O. bruceata (π^2=3.65, 0.10<p<0.25).
*: Significant effect of humidity (π^2=31.18, p<0.001).

sensitive to high constant temperature than O. brumata under the two humidities tested. The proportion of both species surviving to the adult stage was similar for the variable and low, variable temperature treatments, suggesting that high temperatures early in development do not have as profound an effect as those closer to adult emergence.

Rate of weight loss:

The rate of weight loss during pupal development was compared for O. brumata and O. bruceata under the three temperature treatments at 75% RH (Figure 9). Weights used in the regressions were mean weights of pupae within their cocoons measured immediately prior to treatment, near the halfway point in pupal development and finally close to adult emergence. Slopes were similar for the variable temperature treatment. Under the high, constant treatment and the low, variable treatment, proportional weight loss and rate of weight loss appeared to be higher for O. bruceata. Total water content was also higher in O. bruceata than O. brumata when comparing results of weight loss from thermogravimetry (Figure 10) and freeze drying

Figure 9: Rate of weight loss of Operophtera brumata and O. bruceata pupae under three temperature conditions at 75% RH. a=high, variable; b=high, constant; c=low, variable. Solid circles = winter moth; Open circles = Bruce spanworm. See text for explanation of temperature treatments.

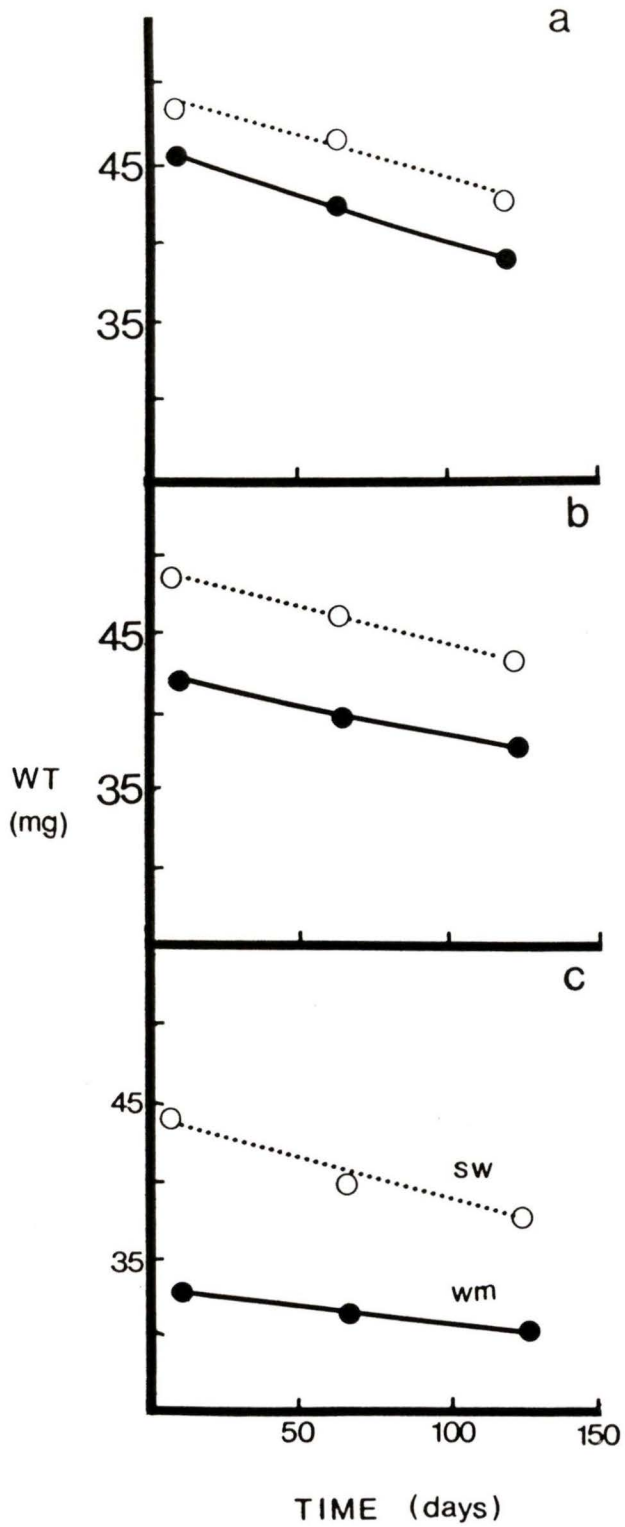
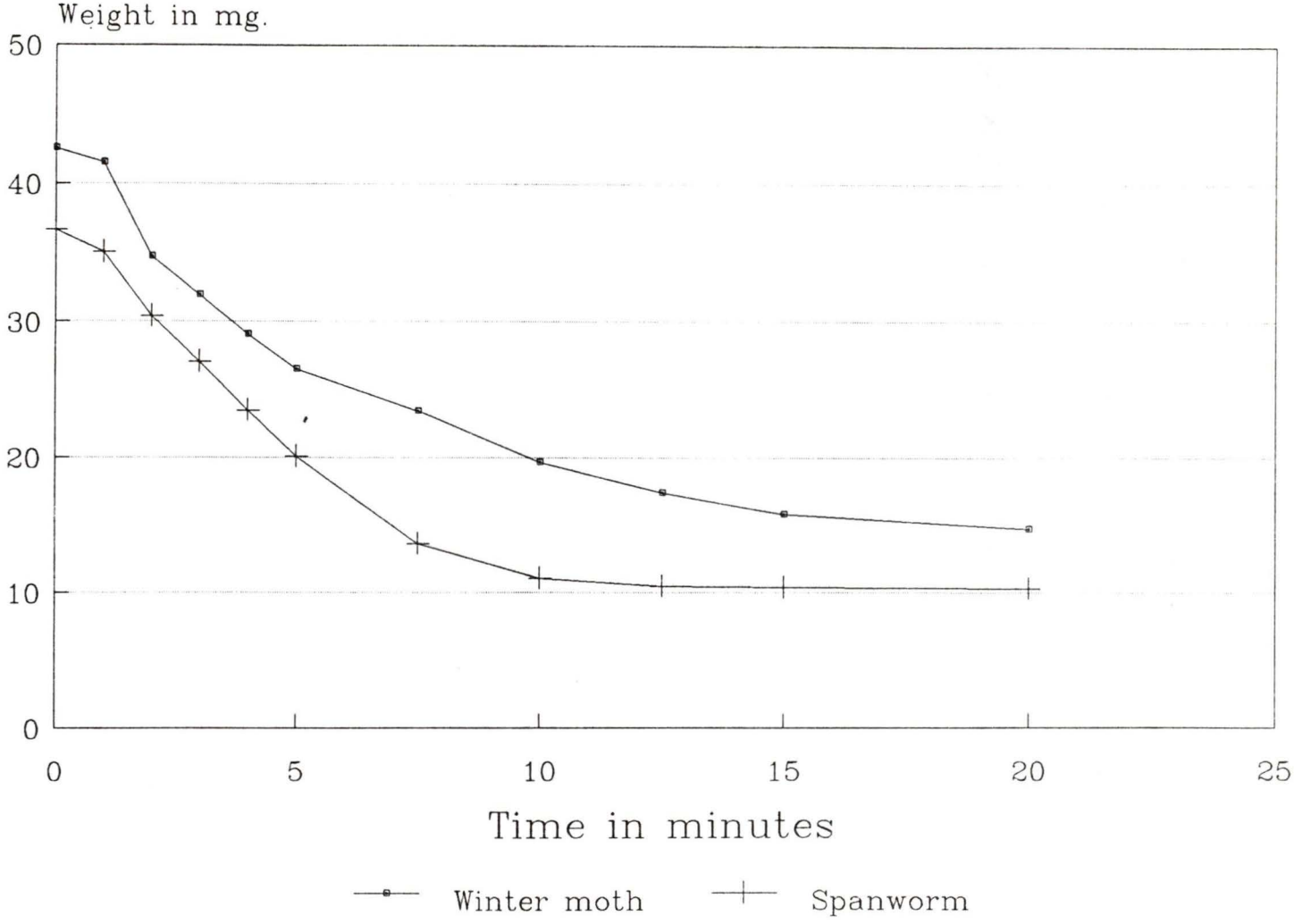

a
45
35
b
45
35
WT
(mg)
c
45
35
sw
wm
50
100
150
TIME (days)

Figure 10: Mean weight loss over time for pupae heated in a thermogravimetric balance. n = 10 for both species.


Weight in mg.
50
40
30
20
10
0
0
5
10
15
20
25
Time in minutes
Winter moth
Spanworm

(Table 6).

4) Supercooling ability:

The supercooling ability was similar in the larval, prepupal and male adult stages in development of O. brumata and O. bruceata (Table 7). The pupal stage of O. brumata could be cooled to a significantly lower temperature (mean=-19.6°C) than O. bruceata (mean=-16.7°C) before freezing occurred. Conversely, the egg stage of O. bruceata was able to withstand significantly lower temperatures (mean=-37.3°C) than O. brumata (mean=-34.3°C). Since relatively large numbers of eggs of both species were available, a frequency histogram was produced of the supercooling points of the eggs (Figure 11). Both species had a wide range of egg supercooling points although O. bruceata values were more tightly clustered about the mean.

After tests were completed, all insects were re-warmed and freezing was lethal in all cases. Therefore, all stages in development of both species of Operophtera were freezing intolerant.

5) Biochemical analysis:

After determination of supercooling points, insect

Table 6: A comparison of water content of pupae of O. brumata and O. bruceata using two methods of water extraction.

	n	O. brumata Water Content (%) Mean (SD)	O. bruceata Water Content(%) Mean (SD)
Method of Extraction:			
Freeze Dried	10	65.5(1.2)a	70.7(2.3)b
Heat Extraction (thermogravimetry)	10	66.1(0.4)a	69.7(0.6)b

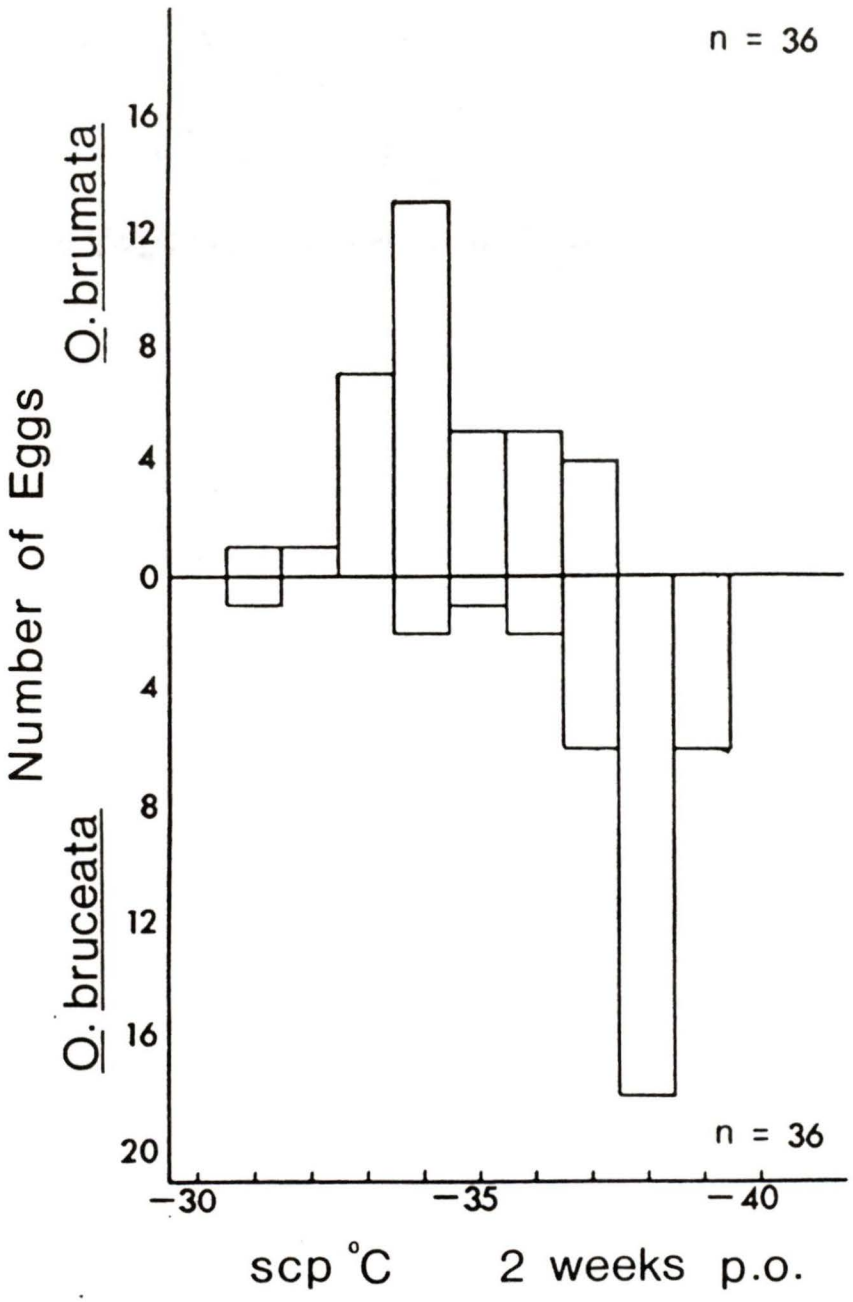
Means followed by the same letter are not significantly different. ($p<0.05$, Students t-test)

Table 7: Supercooling ability of each developmental stage of Operophtera brumata and O. bruceata . SCP = Supercooling Point.

Stage	n	O. brumata SCP °C Mean(SD) Range	O. bruceata SCP °C Mean(SD) Range
Egg	36	-34.3(1.4) -31.4 to -37.0	-37.3(1.6) ** -31.2 to -39.2
Larvae I-III	20	-9.5(0.6) -8.5 to -10.0	----
Larvae IV	10	----	-9.1(1.6) -7.8 to -12.0
Larvae V	10	-8.9(2.5) -7.5 to -11.2	----
Prepupae	10	-9.6(2.7) -8.0 to -13.0	-10.4(2.1) -7.0 to -12.5
Pupae	10	-19.6(4.6) -13.5 to -29.0	-16.7(2.3) ** -14.3 to -19.5
Adult Male	10	-19.7(3.4) -12.2 to -23.5	-19.5(3.3) -11.4 to -21.5
Adult Female	10	-20.6(4.3) -12.5 to -24.0	----

** Significant difference between O. brumata and O. bruceata ($p < 0.05$; Student's t-test).

Figure 11: Frequency histogram showing difference in supercooling points of eggs of Operophtera brumata and O. bruceata , measured two weeks after oviposition. Mean supercooling point = 34.3±1.4°C for O. brumata , 37.3±1.6°C for O. bruceata . The difference in mean supercooling point was significant at $p < 0.05$, $t = 7.25$.


n = 36
16
12
8
4
0
4
8
12
16
20
O. brumata
O. bruceata
Number of Eggs
n = 36
−30
−35
−40
scp °C
2 weeks p.o.

samples were freeze-dried before tissue extraction. Wet and dry weights and percentage of weight accounted for by water are listed in Table 8. Egg and adult stages are not included in this table because comparative data were lacking and the larval, prepupal and pupal stages were the focus of the investigation of the accumulation of protective molecules. The highest percentage of water was detected in the feeding larval stage (77% of O. brumata larvae; 75% of O. bruceata larvae). Winter moth pupae had less water in their tissues (65.5%) than Bruce spanworm larvae (70.7%) which is consistent with the results from the long-term tests of weight loss during pupal development.

Gas-liquid Chromatography:

A mixture of alditol acetate standards was run to obtain retention times and peak areas (Table 9) for identification and quantification of the corresponding aldoses and polyols in the derivatised insect samples. Xylose, with a retention time of 22.48 minutes, was added to the insect samples as an internal standard. The results of the separation of aldoses and polyols contained in the sugar fraction of the insect tissue are given in Table 10. Sugars and polyols detected in

Table 8: Wet and dry weights and water content (%) of larvae, prepupae and pupae of Operophtera brumata and O. bruceata . Values given are mean weights for insect samples that were then extracted, derivatised and separated by GLC.

Developmental Stage	Wet wt. (mg)	Dry wt. (mg)	Water content (%)
Larval			
O. brumata	163.9	37.9	77.0
O. bruceata	186.5	46.7	75.0
Prepupal			
O. brumata	118.6	34.4	71.0
O. bruceata	175.9	57.5	67.0
Pupal			
O. brumata	140.2	48.3	65.5
O. bruceata	147.6	44.0	70.7

Table 9: Alditol acetate standards used in the qualitative and quantitative analyses of sugar extracts of insect samples. Retention times are for the injector, detector and column temperatures given below and discussed in the text.

Injector Temperature	180 °C
Detector Temperature	300 °C
Column Temperature	145 °C initially for 10 minutes
(programmed)	145 °C to 220 °C @ 7 °C/min
	220 °C for 15 minutes

7μl of standard mixture was injected onto SP2340 column.

Standard (Alditol Acetate)	Retention Time (minutes)	Peak Area
glycerol	7.01	260,000
xylose(internal standard)	22.48	700,000
mannitol	24.74	470,000
galactitol	25.68	620,000
glucitol	26.90	615,000
inositol	28.61	680,000

Table 10: Results of separation of simple sugars and polyols using GLC. Amounts of sugar or polyol present in insect tissues are given as percent of fresh weight where it was possible to calculate the amount from peak areas. Retention times were corrected using the internal standard (xylose).

Developmental Stage	Sugar or Polyol			
	glycerol	glucose	galactose	unknown
Larval				
O. brumata	trace	0.510	---	0.130
O. bruceata	0.070	0.126	0.217	---
Prepupal				
O. brumata	---	0.452	0.257	---
O. bruceata	***	0.238	---	---
Pupal				
O. brumata	0.701	0.220	---	0.600
O. bruceata	0.262	0.254	0.195	0.111
Adult female				
O. brumata	***	---	---	trace
Eggs				
O. brumata	0.201	---	0.991	trace

** Peaks that came off the column early in separation could not be detected due to large solvent peak.

the insect samples were glycerol, glucose and galactose. In five of the insect samples, unknown peaks were detected with four of these having retention times intermediate between those of galactose and glucose.

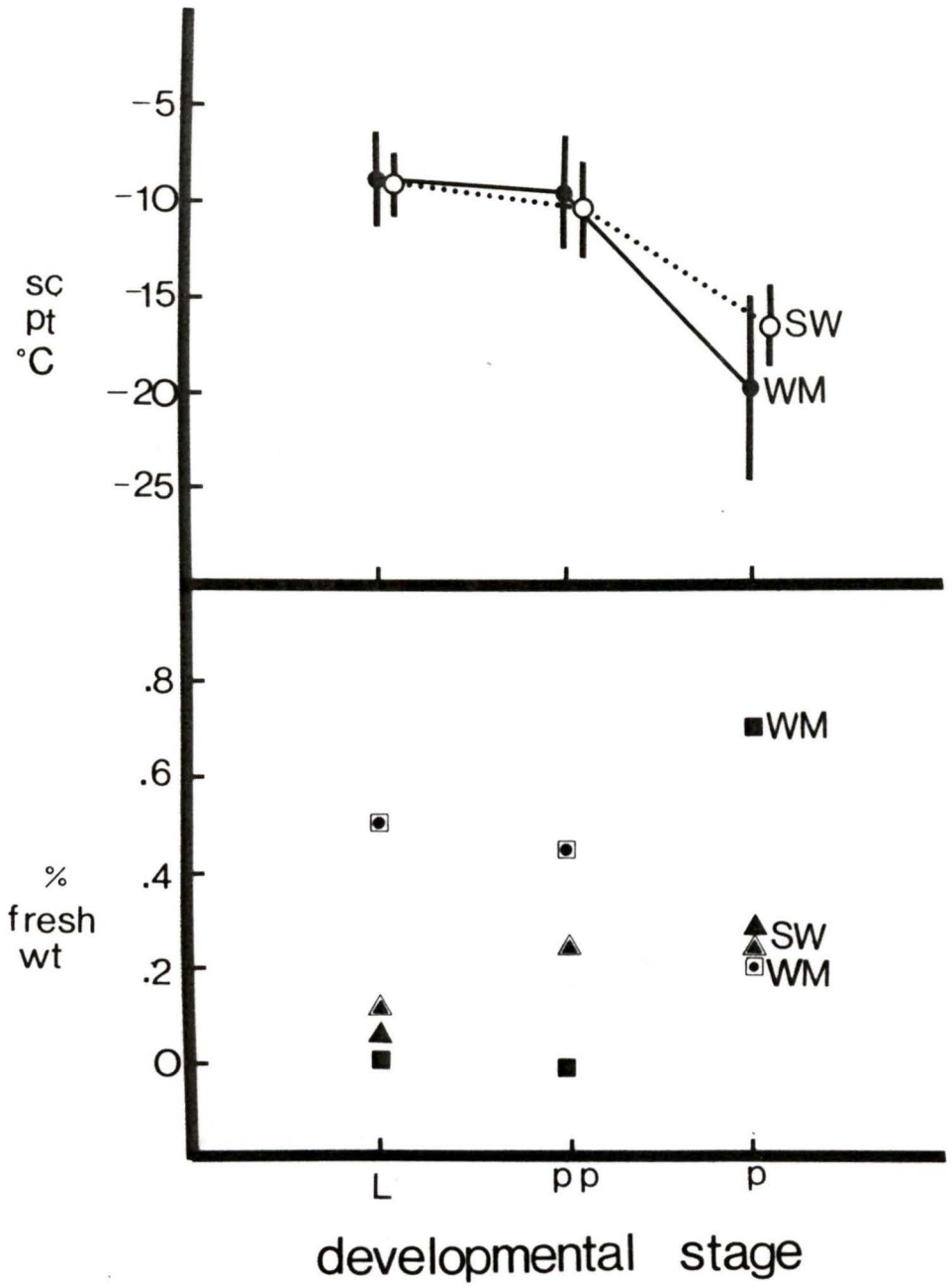
Glycerol was detected in small quantities in the larval stages of both species of Operophtera , in relatively large amounts in the pupal stage of both species and in the eggs of O. brumata. The prepupal stage of O. bruceata and the adult female stage of O. brumata showed the tip of a peak corresponding to the retention time for glycerol, but these peaks were masked by a large solvent peak and therefore quantification or even positive identification was not possible.

Glucose was detected in quantifiable levels in the larval, prepupal and pupal samples of both species of Operophtera. Levels of glucose decreased markedly from 0.51% fresh wt. in the larval stage to 0.22% fresh wt. in the pupal stage of O. brumata. In O. bruceata, however, levels of glucose increased slightly from 0.126% fresh wt. of the larval sample to 0.254% fresh wt. of the pupal sample. There was no evident pattern to the presence of galactose, but this sugar was found in the egg sample of O. brumata in the amount of 0.991%

fresh weight, the highest concentration of sugar found in this analysis.

The freezing points of the larvae, prepupae and pupae of each species and the levels of sugars or polyols contained within each developmental stage were charted together (Figure 12) in order to visualize the relationship between supercooling ability and sugar or polyol content. Although patterns are similar for both species, the most distinctive patterns are seen in the data for the winter moth, O. brumata. During the pupal stage there is a marked increase in the supercooling ability accompanied by a marked increase in glycerol levels and a corresponding decrease in glucose levels.

Figure 12: Supercooling points (a), and sugar and polyol levels (b) of larvae (L), prepupae (PP), and pupae (P) of Operophtera brumata and O. bruceata. ■ = winter moth, glycerol levels; ⊙ = winter moth, glucose levels; ▲ = spanworm, glycerol levels; ◬ = spanworm, glucose levels. Bars in the upper figure represent two standard deviations from the mean.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DISCUSSION

1) Fecundity:

The regressions of fecundity against fresh weight of female moths showed no differences between O. brumata ($y=9.0x-47.3$) and O. bruceata ($y=8.8x-70$) ($t=20.9, p<0.001$). This suggests that neither species is able to produce more eggs than a congeneric individual of similar size. Holliday (1977) found a very similar relationship for winter moth in Great Britain in 1972-3. When he calculated the regression of fecundity against fresh weight of female moths he obtained the equation $y=9.54x-67.68$. In Great Britain the regressions were similar in form for each year, but differed significantly in slope and intercept (Holliday 1977). This relationship would have to be tested for more than one year and include data on success of larval eclosion to reach meaningful conclusions about fecundity.

2) Breeding trials:

These tests demonstrated that Operophtera brumata and O. bruceata are capable of interbreeding. This would

imply that since O. brumata has only recently been introduced to North America, there has not been adequate time for behavioral or chemical isolation mechanisms to evolve to allow the male moths to distinguish between congeneric females. Male Bruce spanworm moths can copulate and produce offspring with female winter moths with no apparent detrimental effects to the male. Future fitness may be reduced because of higher hybrid mortality. The reciprocal cross is not as successful and often results in death of the male. The inability of winter moth males and Bruce spanworm females to disengage after copulation is evidence that divergence has occurred and that an anatomical barrier prevents interbreeding in this combination. The energetic cost involved in this type of post-mating isolation mechanism is high. Males are lost to the population and females are either unable to oviposit or produce many fewer eggs than when mated with a conspecific individual. Selective pressure is likely to cause winter moth males to distinguish between females of each species.

Mortality of hybrid larvae was much higher than expected based on previous success in rearing each of

the parent species. Conclusions based on the data must be taken as speculative , however, due to the small sample sizes of hybrid larvae, pupae and adults. Hybrid offspring were more variable in weight, dimensions and morphology than either of the parent species in all developmental stages. Some hybrid larvae were extremely large and robust, as much as double the weight of the average winter moth larvae. These giant individuals did not survive to the pupal stage. The reason for the high mortality of the hybrid larvae during the prepupal stage is not known , but perhaps they lack some trigger (hormone?) to cause cessation of feeding and to commence pupal development (see Wigglesworth 1972).

Approximately 5 % of Operophtera pupae reared from samples collected in Victoria, British Columbia had atypical cremasters exhibiting a combination of characteristics of both O. brumata and O. bruceata. A proportion (0.17) of male moths caught in pheromone traps had genitalia intermediate between the two species of Operophtera (Pivnick et al.1988). These unusual individuals could very possibly represent hybrids appearing where both parent species are sympatric.

It is impossible to reproduce all the variables involved in breeding in the field in laboratory encounters. Confining two different species in a small cage may artificially allow an encounter to take place which may not have occurred in the field because of differing emergence dates, chemical signals or complex courtship behavior. Similarly, it is very difficult to test for interspecific mating in the field. By simply designing a test, variables have been manipulated that may be crucial to encounters between sexes. For example, if traps are baited with females, the movement of the females has been restricted and the microhabitat in which she is normally found has been altered. Because male winter moths cannot detach themselves from female spanworms, interspecific mating in the field was observed, albeit in one direction only. This observation occurred even when conspecific females accounted for 98 % of the available mates.

Operophtera brumata and O. bruceata are present at the same time and place and the males are capable of locating and mating with females of both species. Since male winter moths have a choice of females and the wrong decision is fatal, there should be high selection for

distinguishing between females of the two species. The ease with which hybridization occurs may influence the rate of range expansion of the introduced species. The present situation on southern Vancouver Island requires a detailed study of the development of male winter moth discrimination between females of the two species of Operophtera.

In these species, a comparison between British Columbia, where the introduction of winter moth is recent, and Nova Scotia, where winter moths have been established for more than fifty years (Cuming 1961), may show differences in pre-copulatory behavior. It would also be informative to repeat the breeding trials and to mass-rear the hybrid offspring for the purpose of compiling a detailed description of the F1 generation. The F2 generation should be reared through to the adult stage, if possible, and crossed with other F2 individuals and also back-crossed with the parent species to determine if gene flow exists between the hybrid and pure populations.

3) Pupal duration and mortality:

Under each temperature treatment, pupal duration

was not affected by humidity in either species; however, pupal duration did differ between temperature treatments. The shortest pupal period occurred under the low, variable temperature treatment and the timing of adult emergence under this treatment coincided with the peak in adult emergence in the field. The longest pupal period corresponded to the high, constant temperature treatment. This treatment subjected the pupae to high temperatures throughout most of the pupal period.

The mean pupal period was 142.8 days for winter moth under low, variable conditions. This compares to 125 days found by Kozanchikov (1950) in Leningrad, 160 days in Paris (Bonnemaison 1971) and 180 days found by Holliday (1983) in England. The length of time until adult emergence in this study represents the duration of the treatment, not the entire duration of the pupal period since tests were initiated late in June after pupae were well formed. No effect of treatments was experienced by the prepupae, which may be more susceptible to weather effects than the diapausing pupae. For these reasons, the duration of the pupal period in this study was likely to be much closer to the length found by Holliday (1983).

The earlier emergence of Bruce spanworm does not mean that this species has a much shorter pupal period since the larvae were at a more advanced stage in development in the Okanagan than their winter moth counterparts in Victoria at the same time of the year. The entire life cycle of Bruce spanworm is shifted to allow for earlier larval eclosion and earlier adult emergence as fall temperatures decrease sooner and to a greater degree than in Victoria.

The only clear effect of humidity on pupal mortality was that high mortality occurred in both species of Operophtera under the lowest humidity tested (38%). The proportion of O. brumata that did not complete development at the highest humidity tested (99%) may have been influenced by increased fungal growth that was encouraged by the moisture. Survival of both species was similar at both the variable and low, variable temperature treatments but highest at the high, constant temperature treatment.

Winter moth pupae were more susceptible to high temperatures late in development and, if not fatal, exposure to high temperatures late in development delayed adult emergence. Otherwise, pupae appear to

be fairly insensitive to climatic factors, a conclusion reached earlier by Speyer (1938), Bonnemaison (1971) and Holliday (1983). Survival of pupae was higher in this study than that found by Holliday (1983) for winter moth pupae exposed to high temperatures late in the pupal period (66% in this study, 25 - 60% in Holliday 1983). This may be because Holliday (1983) used naked pupae while in this study the pupae remained in cocoons which could protect the pupae from temperature effects to some extent. This study shows that O. bruceata may be more susceptible to high temperatures late in development than O. brumata.

Operophtera brumata and O. bruceata were found to differ significantly in both the total water content of pupal tissues and in the rate of water loss (see Table 6, Figure 8 and Figure 9). This result was consistent for live pupae in the long term temperature and humidity treatments, dead pupae that were freeze-dried before tissue extraction and for live pupae which were dried at high temperatures using the method of thermogravimetry. Thermogravimetry is a very precise method used to remove all traces of unbound water. Since the amount of water was the same as found for the freeze-dried samples, it

confirms that freeze drying is an accurate method for determining water content in insect tissues. These results show that O. brumata pupae have 7% less water in the haemolymph and tissues than O. bruceata pupae and that water is more difficult to extract. This may be due to structural differences in the waxy layer of the exoskeleton, which is of great importance in restriction of water loss (Edney 1977), or to differences in biochemical properties of the tissues or haemolymph. O. brumata may have a higher proportion of bound water than O. bruceata.

4) Supercooling Ability:

Accumulated evidence indicates that the majority of insects in temperate, southern Canada overwinter in diapause and in a supercooled state (Ring and Tesar 1980). The limit to supercooling ability in insects is uncertain, but Salt (1961) places the practical limit in the region of -30° to -35°C. If temperatures much lower than -30°C are to be tolerated for long periods of time, it seems likely that insects would survive in a frozen state rather than a supercooled one (Ring and Tesar 1980).

The mean supercooling points of each developmental

stage were similar for both species of Operophtera with two notable exceptions. The first is the egg stage of O. bruceata which had a lower supercooling point than O. brumata. The mean and range of egg supercooling points for O. brumata were comparable with data collected in Nova Scotia (MacPhee 1967). Although the difference in supercooling point of the eggs of the two species was statistically significant it is unlikely that the difference is biologically significant since the range of supercooling points overlap. The second exception is the supercooling ability of the pupal stage of O. brumata, which was significantly lower than for O. bruceata. The pupal stage would never encounter low sub-zero temperatures in the field since it is not the overwintering stage, but this difference may reflect an advantage to O. brumata unrelated to cold-hardiness. This idea will be discussed further in conjunction with the discussion of sugar analysis.

To elaborate further on the results of the supercooling analysis, the discussion will focus on one species, O. brumata. What is the explanation for the sudden drop in supercooling point during the pupal stage which is not cold-acclimated? Sub-zero supercooling

points for the larval and prepupal stages are not unusual since electrolytes in the haemolymph and tissues lower the temperature at which both freezing and supercooling occur (Wyatt 1961). Freezing in active stages of insect development is often initiated by food particles in the gut that act as ice nucleation sites (Salt 1961). The prepupal stage is non-feeding, so would have no food particles present to initiate ice formation; however, this stage had only a slight and non-significant drop in mean supercooling point, although the lower limit to the range of supercooling points (-13.0°C) was substantially lower than that of the larvae (-11.2°C). The large drop in supercooling point during the pupal stage cannot be explained by cessation of feeding alone.

5) Biochemical analysis:

Using gas-liquid chromatography for the separation of simple sugars and polyols it was possible to identify and, in some samples, to quantify the presence of glycerol, glucose and galactose in the tissues of Operophtera brumata and O. bruceata. Since sugars are non-volatile, it was necessary to convert the samples to their alditol acetate derivatives for detection on the

GLC column. Although many diverse and somewhat unusual polyols have been detected in this manner, threitol and sorbitol in combination (Miller and Smith 1975) and erythritol (Baust and Edwards 1979) for example, only the most common sugars and polyols were sought in this study since a very general situation was being investigated. The insect blood sugar, trehalose, may have an important role in membrane stability at low water activities (Crowe et al.1984).Since trehalose is a non-reducing disaccharide it was not derivatised and detected as were the aldoses and polyols in the insect samples. Galactose has not been reported as an important insect blood sugar and it is possible that the peaks detected may represent a sugar with a retention time very similar to that of galactose. Alternatively, the peak may be an artifact produced by the breakdown products of a disaccharide during the derivatisation process.

The most informative and interesting results of the biochemical analysis are the patterns shown by the winter moth, O. brumata, by comparing the mean supercooling points with the levels of glycerol and glucose present in the larval, prepupal and pupal

stages. The large and significant drop in mean supercooling point during the pupal stage is paralleled by an increase in glycerol levels and a decrease in glucose. This observation is not meant to imply causation. There is no evidence that the increase in supercooling ability is brought about by increased levels of glycerol, or that stored glucose was used to produce the glycerol. There does appear to be an association between the independent observations and it is plausible that if glycerol levels increase to help protect the insect from desiccation then this may coincidentally result in a lower freezing point due to biochemical changes within the hemolymph of the insect. There may well be other important molecules that have a protective function but which were not detected in this study.

6) Summary:

Operophtera brumata and O. bruceata are very closely related species. O. bruceata males can freely interbreed with females of O. brumata, although the reciprocal cross is not completely successful. There is evidence that hybridization is occurring in the field. All information gathered in this study suggests that

O. brumata is at least as hardy as the native species and could probably expand its range anywhere in temperate North America. Biochemical analyses detected quantifiable levels of glucose, galactose and glycerol in the insect tissues. Levels of glycerol increased in the pupal stage which had the lowest supercooling points. In this species glycerol may have a protective role to prevent desiccation of tissues during the estival diapausing pupal period.

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VITA

Surname: HALE Given Names: MARGARET ANGELA

Place of Birth: SUSSEX, ENGLAND

Date of Birth: July 10, 1960

Educational Institutes Attended, with Dates of Entering and Leaving:

UNIVERSITY OF VICTORIA, B.C.	1978	TO	1983
UNIVERSITY OF VICTORIA, B.C.	1983	TO	1989

Degrees, Diplomas, Etc., Awarded, with Dates and Names of Institutions:

B.Sc.(with distinction) 1983 University of Victoria,B.C.

Publications:

Ovaska, K. and M.A. Smith. 1988. Predatory behavior of two species of ground beetles (Coleoptera : Carabidae) towards juvenile salamanders (Plethodon vehiculum). Can. J. Zool. 66:599-604.

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Author [redacted]

MARGARET ANGELA HALE

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