COLD HARDINESS AND SOME ECOLOGICAL OBSERVATIONS OF THE EUROPEAN CORN BORER, *Ostrinia nubilalis* (Hübn.) IN MANITOBA

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by

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ABSTRACT

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Cold-hardiness of diapausing corn borer larvae is correlated with the glycerol concentration. Larvae containing 5.7% glycerol are cold-hardy at -10, -22 and -35°C whereas those containing 5.0% glycerol are cold-hardy only at -10 and -22°C. The larvae containing 2.1% glycerol are not cold-hardy.

Frozen cold-hardy borer larvae survive longer at -22°C than supercooled larvae.

Intermittent chilling caused higher mortality than continuous chilling at -22°C, presumably because larvae experienced frequent lethal freezing-thawing cycles during intermittent chilling.

The protective function of snow layer on the winter survival of the borer larvae was investigated. Larvae removed from corn stalks which were under a snow cover had a survival 15% higher than those from the upper part of the stalk exposed to the air. The under-snow-level population may benefit from the constant and relatively warm surrounding environment provided by the snow layer.

First appearance of borer moths and different cumulative levels of moth flight activity can be predicted by the use of five-year-moth-flight data and heat units.

Five organic acids, citric, succinic, aconitic, malic and tartaric acid were analyzed by gas-liquid chromatography to determine whether different concentrations were present in cold-hardy and non-cold-hardy larvae. The concentration of organic acids in the haemolymph of cold-hardy and non-cold-hardy was similar. Organic acids do not appear to contribute to the development of cold-hardiness in the borer larvae.

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CHAPTER I

INTRODUCTION

The European corn borer, Ostrinia nubilalis (Hübn.) (Lepidoptera: Pyraustidae) was first reported in Manitoba by Mitchener (1948). It has since spread throughout most of the agricultural area of the province with the heaviest and most consistent infestations occurring in the southern regions.

Prior to its discovery in Manitoba, the insect had been known in the United States since 1917 and in Europe, its native home, for several hundred years.

In southern Manitoba, at the northern edge of the corn belt, the borer is greatly influenced by the severe winter conditions. The borer's survival under Manitoba conditions depends greatly on its ability to survive the winter. Our research has been concerned with the identification of the environmental and physiological factors involved in the winter survival.

Hanec (1959) stated that a state of diapause does not in itself enable the borer to survive at winter temperatures. Rather, other physiological changes occur which result in greater endurance of the larvae under freezing conditions. The increasing ability of an insect to survive subzero temperatures is called by Hanec cold-hardening, and the degree of cold-hardening is termed cold-hardiness. According to current concepts of winter survival of insects, there are at least two types of cold-hardiness possessed by them (Hanec, 1959). One type is the avoidance of freezing by supercooling. The insect may be supercooled

many degrees below the freezing point of water without ice formation within its body. This type of insect cannot survive freezing. The extent to which it can be chilled before freezing occurs has been assumed to be an expression of its cold-hardiness; i.e., the undercooling temperature may serve as an index of cold-hardiness.

The second type of insect, to which the European corn borer belongs, has the ability to survive ice formation in its tissues. Since this type of cold-hardiness has been largely neglected in research and the literature concerned contributes little information, it is the object of this study to attempt to explain this problem. The following aspects of cold-hardiness in the mature European corn borer larvae were investigated:

(a) glycerol content and survival of larvae at subzero temperatures.

(b) the effect of contact moisture on survival of the larvae at subzero temperatures.

(c) vertical distribution of the larvae in corn plants and their winter survival in the field.

(d) qualitative and quantitative analyses of some organic acids in the larvae to determine their role in cold-hardiness.

In addition, gamma irradiation of the larvae was also done to determine whether cold-hardy and non-cold-hardy larvae were affected differently by various doses of Co^{60}.

The studies comprising this thesis are organized into two parts. The first part consists of a study of cold-hardiness under laboratory conditions. The second part includes ecological investigations of larvae in the field, irradiation of larvae and analyses of organic acids in the larvae.

In Part I the literature relating to cold-hardiness is reviewed and the results and discussion of the data obtained in these experiments are presented. In Part II, the literature reviews, the results and discussion are incorporated into each chapter separately because of the diversity of the investigations.

CHAPTER II

LITERATURE REVIEW

Since the days of Reaumur (1736) it has been known that some insects could survive freezing to the point of brittleness while other insects could not. It also became common knowledge that all insects could supercool to some extent and so it was correctly deduced that, for those that could not tolerate the freezing of their body fluids, ability to supercool provided a quantitative measure of cold resistance. As for the freezing-tolerant group, to which the European corn borer belongs, no explanation was attempted until Robinson (1927) invoked the bound water theory. According to this theory, bound water was considered to be oriented as layers of hydrogen and hydroxyl ions absorbed alternately on the colloid micelles and hence compressible, whereas free water is practically incompressible. The disruption of the cells by the expansion of water on freezing was regarded as the cause of freezing injury. Robinson was able to show a correlation between bound water and cold-hardiness in three species chosen to represent three levels of cold-hardiness. These were the cold-hardy *Antheraea polyphemus* (Cramer), the moderately cold-hardy *Callosamia promethea* (Drury), and the non-cold-hardy *Sitophilus granarius* (Linnaeus). The distinction between cold-hardy and moderately cold-hardy was vague and was unsupported by survival or mortality data. Furthermore, Robinson (1928) assumed that the free water in his insects was completely frozen at -20°C, when, in fact, his own data show that many of the insects were not frozen at all, but supercooled at this temperature.

Ditman, Voght and Smith (1943) determined the percentage of water frozen in seven species of insects whose cold-hardiness varied greatly. They showed that the percentages of water remaining unfrozen (Robinson's bound water) at various temperatures bore no relation to the cold-hardiness of the insects. Salt (1955) also concluded that bound water had little if any protective value and that the only time when bound water could conceivably protect an insect was before freezing by lowering its undercooling point and this has never been demonstrated experimentally or even theoretically.

Payne (1929) concluded that cold-hardiness bore a direct relationship to absolute humidity in the case of Popillia japonica Newm. The term cold-hardiness was used to define the lowest temperature at which the insect could survive for an arbitrary short period. In an earlier paper (1927) Payne suggested that hardening was affected by the dehydration that occurs in insects during the fall. Salt (1956) also showed that cold-hardiness of Melanoplus bivittatus (Say) eggs increased as they dried and the same relationship between cold-hardiness and moisture content exhibited in diapausing Cephus cinctus Nort. larvae, and in diapausing Loxostege sticticalis (L.) larvae. However, Salt concluded that moisture content affected cold-hardiness (measured as ability to supercool), only to the extent that it affected the concentration of body fluids and hence their freezing points; only when desiccation was severe did it produce appreciable cold-hardening. In freezing-susceptible insects the cold-hardiness may change with moisture content as the way Salt (1956) suggested. Nevertheless this may not be the case in freezing-tolerant insects; at least not in the corn borer. Hanec and

Beck (1960) tested the cold-hardiness of the borer larvae at subzero temperatures and found that the laboratory reared larvae did show a decrease in the undercooling temperatures due to their water loss but no increase was found in their cold survival at -10°C. Field-collected larvae from August through to December showed quite a constant (52 to 56 per cent) water content while their cold survival at -10 and -15°C increased steadily. It indicated not only that in the European corn borer--maybe in other freezing-tolerant insects as well--water content in the larvae played no major role in the development of their cold-hardiness, but also that the correlation between decreasing undercooling temperature and increasing survival was absent.

Contact moisture was thought to be important in destroying the cold-hardiness of insects at moderate high temperature. Barnes and Hodson (1956) found that at 10 and 20°C and in the absence of free moisture, the undercooling point of the European corn borer larvae rose very little. The authors concluded that it was the effect of temperature and moisture acting together which brought about the rapid and marked rise of the undercooling point level of the cold-hardy larvae. Hanec (1959), however, showed that the borer larvae incubated at 30°C for ten days, those with contact moisture and those without, were essentially equally susceptible to cold at -20°C. It suggested that contact moisture played no role in destroying cold-hardiness, rather, high temperature alone was responsible for that.

Contact moisture was thought to be responsible for the increase of mortality at low temperatures. Hodson (1937) studied the effect of contact moisture on *Cynomya cadaverina* and *Lucilia sericata*. The author

found that these insects had a lower mortality in dry air than in the moist sand and concluded that contact moisture may increase mortality by innoculation of the tissues via the freezing water on the surface of the insect. Similarly, Hanec (1959) stated that the field-collected European corn borer larvae from late fall and winter, when exposed to constant cold at -10°C, only a fraction of the wet chilled survived as successfully as the dry chilled. On this basis, Hanec postulated that under wet field conditions, the winter mortality of borer larvae would be high as compared to dry overwintering conditions. The opposite view was held by Laurence (1967), who claimed that the supercooled state was more injurious to the larvae than was the frozen state. From his survival tests of the borer larvae at -15 and -25°C Laurence found that at -25°C all of the larvae were frozen after 22 days while at -15°C, 100 per cent of the larvae were still in the supercooled state after 40 days, and 70 per cent supercooled after 176 days. The mortality was higher at -15°C than at -25°C, the time to 50 per cent mortality at -15°C being 96 days, at -25°C being about 150 days.

Chilling at constant low temperatures had varied effects on the cold-hardiness of freezing-susceptible insects (Salt, 1956). It was effective in increasing considerable cold-hardiness of Bracon cephi (Gahan) at 5°C, ineffective in Melanoplus bivittatus (Say) and Cephus circtus Nort. at 0 or 5°C, and of doubtful effect in Loxostege sticticalis (L.). Cold-hardening was produced by the variable temperatures of the natural environment in all the species studied. Salt (1956) considered that intermittent periods of varying developmental temperatures were more likely to be responsible for cold-hardiness than steady chilling.

He based his measurements of the degree of cold-hardiness completely on the undercooling temperatures. This concept has since been proven to be invalid.

Salt (1950) proposed that time is an important factor in the freezing of the freezing-susceptible insects. Insects held in an undercooled state froze at irregular intervals often over long periods of time. For a specified degree of cold-hardiness, the probability of freezing depended on chilling temperature and time. When the temperature was fixed, the probability of freezing could be expressed in time units. Undercooling temperature as an index of cold-hardiness lacks practical significance because it merely indicates the degree of maximum supercooling at an unnaturally fast cooling rate at an extremely low temperature. Therefore, in considering survival at subzero temperatures, the time-temperature complex is the important factor rather than the undercooling temperature.

For the freezing-tolerant insects which do not die on freezing, undercooling point was of little significance as an indicator of cold-hardiness (Barnes & Hodson, 1956; Hanec, 1959). Cold-hardy corn borer larvae survived freezing for at least three weeks at -32°C in the laboratory but for the entire winter in the field under snow cover (Barnes & Hodson, 1956). Freezing tolerance of the larvae must be expressed in terms of survival at specific temperature-time exposures (Salt, 1961). Only a few extensive experimental studies on cold-hardiness in the freezing-tolerant insects were expressed in the way Salt suggested. Hanec (1959) described the degree of cold-hardiness of the August corn borer larvae as "survived about 56 days before 50 per cent mortality was

reached at -10°C under dry chilling conditions." The classical concept of cold-hardiness expressed as undercooling temperature has been applied by numerous authors for study of the various aspects of cold-hardiness (Payne, 1927, 1929; Salt, 1956; Barnes & Hodson, 1956).

Free glycerol in insect haemolymph was first reported by Wyatt and Kalf (1956) in the pupae of Hyalophora cecropia. Glycerol has since been found in other diapausing insects. Salt (1957) found glycerol in hibernating Eurosta solidaginis larvae, Antheraea polyphemus pupae and Loxostege sticticalis (Linnaeus) larvae. The diapausing insects synthesize it in time for winter but not necessarily as soon as they enter diapause, i.e., Bracon cephi larvae begin to synthesize glycerol four to six weeks after cocoon-formation under natural conditions (Salt, 1959). Glycerol has been found whenever it has been looked for in freezing-tolerant insects except one freezing-susceptible species, Loxostege sticticalis (Salt, 1961).

The discovery of glycerol in insects naturally promised a better understanding of their freezing-tolerance, since glycerol had, in the preceding decade, come into wide use for preserving spermatozoa, blood cells, etc., in the frozen state. Salt's work on the nucleation, the subsequent formation of ice in the insect tissues, and the effect of time-temperature complex on freezing, indicates that freezing and subsequent mortality is a random occurrence modified by chemical substances within the insect. These substances were suggested by Salt (1957, 1958, 1959) to be glycerol and other solutes. Salt (1959) concluded that "the concentrations of glycerol measured in cold-hardy Bracon cephi (Gahan) larvae leave no doubt that glycerol is the major cause of their phenomenal

cold-hardiness...other solutes must be responsible for the unexplained portion of the melting point depression." These solutes have been suggested by Tanno (1964) to be fructose, glucose and trehalose which he found in high concentrations in overwintering adults of solitary bees. Sømme (1965) also found a concurrent appearance of high sorbitol content and low undercooling points in winter eggs of Panonychus ulmi (Koch). The role of glycerol is at present only indicative and by no means fully explained even in the freezing-susceptible insects. As for the freezing-resistant insects, the role of glycerol has not been investigated at all. Earlier studies have been done on the basis that undercooling temperature was a reliable criterion of cold-hardiness. Unless the role of glycerol is explained in terms of actual survival at specific temperature-time exposures, its effect will still be doubtful.

CHAPTER III

MATERIALS AND METHODS

The larvae used in these studies were obtained from corn fields at Morden from 1965 through 1969. During late fall several thousand infested corn stalks were cut and stored outdoors on the university campus. Larvae were removed from the stalks as needed during the course of the work. The larvae were warmed for several hours in the laboratory and then dried with dry compressed air before they were used to determine the undercooling temperature and glycerol content. For determining undercooling temperatures, samples of 20 larvae were used. Only five borers were taken as a sample for glycerol determination because the method is extremely time-consuming and the statistical validity of the results would not be appreciably enhanced by increasing the number of specimens.

The undercooling temperatures of the insects were determined with a 40-gauge copper-constantan thermocouple which was connected to a recording potentiometer. The insect was enclosed in a gelatin capsule and in contact with the measuring junction of the thermocouple. The capsule containing the larva and the thermocouple were placed in a dry 100 ml. test tube. The tube was in turn placed in a large-mouth 500 ml. bottle which was partially immersed in the cooling medium. The large bottle was used to provide an air buffer against the extreme cold of the chilling solution. The chilling bath consisted of 95 per cent ethanol and dry ice held in a large thermos container. The cooling rate of the larva could be regulated by varying the depth of immersion of the bottle.

The chilling bottle was so regulated as to give a temperature of from -30 to -35°C. The cooling rate used in determing undercooling temperatures was 6 to 8°C per minute.

Quantitative data on glycerol content of the borers were obtained by paper chromatography of insect extracts. Larvae were prepared for glycerol analysis by the same way described by Salt (1959), while the chromatographic technique was similar to Sømme's (1964). Larvae were dried to constant weight in a vacuum over calcium chloride at 45°C, and then ground up singly in 80% ethanol and Ontario sand. The mixture was centrifuged, the supernatant removed and the residue washed with more ethanol and centrifuged once more. The two ethanol extractions were combined, dried by air-blowing at room temperature and the resulting residue taken up in a known volume of distilled water varying from 0.3 to 1.0 ml. These preparations were stored in the frozen state until they were analyzed.

For paper chromatography, ascending chromatograms were run on Whatman No. 1 filter paper with n-butanol-acetic acid-water (12:3:5 V/V) as developing solvent. The chromatograms were suspended in the solvent for 16 hours and after drying were sprayed with 0.01 M aqueous potassium periodate solution. After drying, they were oversprayed with a solution of the following composition; 35% saturated sodium tetraborate, 0.8% potassium iodide, 0.9% boric acid and 3.0% soluble starch. With this method, glycerol appeared as distinct white spots on a blue background. It was necessary to trace the outline of the glycerol spots on the paper with a soft pencil immediately as they soon disappeared. Three parallel tests of each extract were run, 10λ (lambda) of solution being used for

each test. Three 5λ, 10λ and 20λ samples of a 0.5% solution of glycerol were used as standards with each chromatogram and a linear relationship was found to exist between the area of the developed spot and the logarithm of the content of glycerol in the spot. The glycerol concentration of each sample was determined by plotting the areas of the developed spots on semi-logarithmic graph paper.

To study the survival of larvae under wet and dry conditions at various subzero temperatures in the laboratory, a sample of 240 larvae was placed on dry corregated paper in Petri dishes at -10°C, and a comparable group was also placed at -10°C but between two discs of wet papers. In the same way larvae were set up at -22 and -35°C. A sample of 20 larvae were removed periodically from the cold cabinets, warmed for 24 hours, and then survival was checked. No examined sample was returned to the freezers.

CHAPTER IV

RESULTS AND DISCUSSION

The relationship between the glycerol content and survival of the borer larvae at subzero temperatures

Three large samples of diapausing larvae (2,000 larvae each) were collected in the field in November, January and April respectively. Glycerol contents were determined on a representative sample of larvae from each of the three groups prior to different subzero temperature incubations. Larvae were removed from the cold cabinets periodically for determining their survival and for glycerol determinations. The number of days required for 50% mortality (T_{50D}) as well as for complete mortality of the three groups of larvae at the different temperatures were recorded. The glycerol content of the November, January and April groups was also obtained. The T_{50D} and glycerol content are shown in Table I.

The results show that the most cold-hardy larvae have the highest glycerol content and can best withstand low temperatures. The winter larvae (containing 5.7% glycerol) had a much higher survival than did the fall larvae (containing 5.0% glycerol) at -35°C. Under dry chilling condition the T_{50D} for the winter larvae was more than 161 days while it was only 40 days for the fall larvae. Under wet chilling condition the T_{50D} was more than 161 days for the winter larvae, whereas it was only 55 days for the fall larvae. At -10 and -22°C, however, the winter larvae showed virtually no higher survival than the fall larvae. Under dry chilling condition the T_{50D} for the winter and for the fall

TABLE I

THE RELATIONSHIP BETWEEN THE GLYCEROL CONTENT AND SURVIVAL OF FIELD LARVAE AT SUBZERO TEMPERATURES

Date sample taken	Mean glycerol conc. (% live wt.)	T_{50D}**								
		-10°C			-22°C			-35°C		
		Wet	Dry	Mean	Wet	Dry	Mean	Wet	Dry	Mean
Nov. 20 1967	5.0 ± 0.35	120	115	118	134	104	119	55	40	48
Jan. 19 1968	5.7 ± 0.68	105	115	108	133	73	103	>161*	>161	>161
Apr. 4 1968	2.1 ± 0.1	70	60	65	60	65	63	30	45	38

** T_{50D} is the exposure time in days required for 50% mortality.

* Per cent survival higher than 50% all through the chilling period of 161 days (Table X).

larvae were both 115 days at -10°C, 73 days and 104 days respectively at -22°C.

Under the wet chilling condition the T_{50D} for the winter and for the fall larvae were 105 days and 120 days respectively at -10°C, 133 days and 134 days respectively at -22°C. The spring larvae (containing 2.1% glycerol) showed a considerably lower survival than did the winter and fall larvae at all three subzero temperatures tested, the T_{50D} being as low as between 30 days and 70 days.

Cold-hardiness is a relative term used to express the degree of resistance an insect possesses at a specific subzero temperature (Hanec & Beck, 1960; Salt, 1961). Presumably the April larvae were not cold-hardy but still survived under wet and dry chilling conditions for an average of 65 days at -10°C. Hanec (1959) found that mature diapause larvae collected in August also survived for 56 days at -10°C before T_{50D} was reached. On this basis and for the convenience of discussion the cold-hardy larvae will be defined as those whose T_{50D} were no less than 70 days. Thus the larvae with 5.7% glycerol were cold-hardy at all three subzero temperatures. The larvae with 5.0% glycerol were not cold-hardy at -35°C but were cold-hardy at -22 and -10°C. The larvae with 2.1% glycerol were not cold-hardy even at a mild -10°C level. It is concluded, therefore, that the cold-hardiness of the larvae in diapause is related to the level of glycerol which is produced under the natural environment. The most cold-hardy larvae contained the highest glycerol concentration. The fact that at -10 and -22°C "the 5.7% glycerol content group" showed virtually no longer survival than "the 5.0% group," suggests that 5.0% of glycerol is sufficient for the protection against

freezing or chilling injury at -10 and -22°C. It may be that smaller concentrations are adequate for this protection and that higher concentrations are not wholly necessary.

For this quantitative relation study it would be ideal to determine the glycerol content of each individual larva before its freezing survival was investigated. But for the glycerol analysis it was necessary to grind the whole larva. Therefore it is impossible to obtain the data of both glycerol content and freezing survival out of the same larva. However, the low standard deviation values of the representative glycerol samples (Table I) showed that the glycerol content was quite uniform within each group. The average values of the glycerol samples were therefore used to represent their respective groups.

Salt (1950) showed that any subfreezing temperature can become lethal in time. In order to find out whether the glycerol content in larvae decreased as the survival of larvae dropped with the increasing exposure time, the glycerol samples were taken regularly from the -22°C-incubated larvae when survival was examined (Table II) during the course of the survival studies. The observed value for correlation between the per cent survival and the average glycerol concentration was found to not be statistically significant at 5% level (Table II). Although the glycerol concentration varied from 4.3% to 6.6% during the 23 week chilling period there was no significant decline as survival of the larvae decreased. The conclusion is drawn, therefore, that survival would inevitably drop with the chilling time even when sufficient glycerol was maintained by the larvae. In other words, the decline of survival was not due to a short supply of glycerol.

TABLE II

GLYCEROL CONCENTRATIONS OF LARVAE AT -22°C CHILLED DRY LARVAE BROUGHT IN FROM OUTSIDE ON JANUARY 19, 1968

Time Weeks	% survival	Mean glycerol conc. (% live weight)	S.D. of glycerol
0	100	5.7	0.7
5	95	6.0	1.2
9	65	4.5	0.9
13	45	6.0	0.4
15	35	6.6	1.1
17	5	5.0	3.1
19	10	4.3	1.9
23	5	5.1	2.0

* 20 larvae as a sample.

r value for correlation between the per cent survival and the average glycerol content was calculated: r = 0.382 obtained from this sample of size 8. $r_{(.05)} = 0.707$; d.f. = 6. The observed r is not statistically significant.

Glycerol samples were taken from -22°C-incubated larvae, both wet and dry chilled, which showed considerable differences in their survival (Table III) in order to investigate whether the two treatments had any effects on glycerol concentration. At the end of 19 weeks of chilling at -22°C the mortality of the wet larvae was 50%, whereas 90% of the dry larvae died. In spite of the large difference in survival between the wet and dry chilled larvae, their glycerol contents showed no significant difference at 5% level at the end of 17 and 19 weeks. At the end of 17 weeks the glycerol content of the wet and dry larvae was 5.4% and 5.0% respectively and at the end of 19 weeks 5.6% and 4.3% respectively. It was concluded that the difference in survival between the wet and dry groups was not due to the difference in glycerol content because their glycerol contents were virtually the same. Rather, contact moisture was more probably responsible for this difference in survival. This aspect will be discussed later in this chapter.

Hanec (1959) in his studies of cold-hardiness of the European corn borer stated that a state of diapause did not in itself enable the borer to survive at winter temperatures. Larvae reared in incubators under diapause inducing conditions and larvae collected from the field during August and September were undoubtedly in diapause, but the mortality data showed conclusively that their cold-hardiness was very low. Hanec concluded that other physiological changes occurred which resulted in greater endurance of the larvae under freezing conditions. He investigated the possible role of water and fat content on the cold-hardening and found that in field larvae both water and fat content were not responsible for cold-hardiness. The high concentrations of glycerol, more than 5M,

TABLE III

GLYCEROL CONCENTRATIONS OF LARVAE AT -22°C CHILLED WET AND DRY LARVAE BROUGHT IN FROM OUTSIDE ON JANUARY 19, 1968

	Dry chilled*		Wet chilled	
Time (weeks)	Mean glycerol conc. (% live weight)	% survival	Mean glycerol conc. (% live weight)	% survival
17	5.0 ± 3.1	5	5.4 ± 1.7	80
19	4.3 ± 1.9	10	5.6 ± 2.9	50

* T-test for the glycerol content of dry chilled and wet chilled larvae after 17, 19 weeks at -22°C are both

$$t = 0.31,$$

$$t_{(.05)} = 2.13$$

Neither of them is significant at 5% level.

in overwintering larvae of Bracon cephi was reported (Salt, 1959) to be the factor responsible for cold-hardiness in that insect. Salt (1959) concluded that "glycerol is directly responsible for the cold-hardening of the larvae B. cephi in two ways; by increasing supercooling, it increases the ability of the larvae to avoid freezing and by its protective action it allows the larvae to survive even if they do freeze." Here the author worked with a freezing-susceptible insect. For the corn borer, a freezing-tolerant insect, freezing is not necessarily more injurious than supercooling (Hanec, 1959; Laurence, 1967). Since the borer does not rely on supercooling for survival, glycerol may protect those larvae that freeze during the winter.

Hanec (1959) suggested that cold-hardiness should be used to describe the resistance to cold of a particular insect which had undergone cold-hardening under defined environmenal conditions and that these environmental conditions had to be described. Salt (1961) further suggested that in order to establish a quantitative relation between freezing-tolerance and the glycerol content, freezing tolerance should be expressed in terms of survival of specific temperature-time exposures and these related to the concentrations of glycerol. In this study cold-hardiness of the European corn borer has been shown quantitatively related to the glycerol content in the way the above authors suggested. A clue to the interpretation of the present results is given by the work of Lovelock (1954) on the freezing of human red blood cells. Lovelock observed the protective action of 15 neutral solutes, including mono-, di- and poly-hydric alcohols, amides, and sugars against the haemolysis of human red blood cells by freezing and

thawing. On the basis of his results, Lovelock suggested the criteria for an ideal agent for protection against damage during freezing-thawing as follows: (1) high solubility in aqueous salt solutions at temperatures from +40 to -40°; (2) ability to permeate the cell rapidly and completely; (3) low molecular weight, and (4) absence of toxicity. Also he found that of all the 15 substances tested, glycerol alone was capable of fulfilling all these requirements. He concluded, "damage on freezing is a consequence of the increase in concentration of electrolytes within and without the cell. In the presence of glycerol the electrolyte concentration at temperatures below the freezing point is greatly diminished, by an amount sufficient to explain the protection afforded by glycerol." Meryman (1956) in his study on freezing of animal tissues also stated, "The lethal factor, as a direct result of crystal formation, is the exceedingly high concentration of electrolyte resulting from the removal of water from solution." In other words, since the concentrating of electrolytes during ice formation is reduced by the presence of neutral solutes, mainly glycerol, the injuries allegedly caused are proportionately reduced.

Rate of loss of glycerol at various temperatures

Post-diapause, cold-hardy corn borer larvae were incubated at 4.4, 10, 15, 20 and 27°C and after different periods of time glycerol analyses were done. Samples of 40 larvae each were used. The results are shown in Table IV.

The glycerol concentration decreased most rapidly at 27°C and the least at 4.4°C. After 20 days at 27°C the glycerol concentration

TABLE IV

RATE OF LOSS OF GLYCEROL AT VARIOUS TEMPERATURES. LARVAE BROUGHT IN FROM OUTSIDE ON MARCH 5, 1969 FOLLOWED BY VARIOUS TEMPERATURE TREATMENTS

Temperatures (°C)	Time (days)	Mean undercooling points (°C)	Mean % glycerol (Live wt. basis)
	0	-22.6	5.4
	8	-18.0	1.4
27	20	-17.5	0.3
	33	-20.2	0.1
	52	-19.4	< 0.1
	0	-22.6	5.4
	20	-20.6	0.4
20	33	-18.9	0.2
	52	-19.2	0.1
	65	-18.1	< 0.1
	0	-22.6	5.4
	20	-19.6	1.0
15	33	-19.1	0.2
	52	-17.5	0.1
	65	-20.8	< 0.1
	0	-22.6	5.4
10	20	-20.6	2.6
	33	-20.7	0.3
	0	-22.6	5.4
	8	-20.2	3.9
4.4	20	-21.1	3.4
	33	-21.9	1.9
	52	-22.2	1.7
	65	-20.8	1.6

decreased from 5.4 to 0.3%. At 4.4°C it decreased from 5.4 to 3.4%. There appears to be no critical temperature between 4.4°C and 27°C at which glycerol catabolism is drastically increased or reduced. Rather, the process is directly related to temperature, the lower the temperature, the slower the process of glycerol breakdown. There was no apparent relationship between the rate of loss of glycerol and the rate of increase in undercooling temperatures. The former showed a steady decrease while the latter fluctuated slightly. The data in Table IV confirm Hanec (1959) that the undercooling temperatures are not reliable criteria of cold-hardiness in the corn borer. If it were a reliable criterion the undercooling temperature should have increased steadily as the cold-hardiness declined.

The effect of temperature on the survival of cold-hardy European corn borer larvae

It was shown previously that the larvae with 5.7% glycerol were cold-hardy at all three subzero temperature levels--35, -22 and -10°C; the larvae with 5.0% glycerol were cold-hardy except at -35°C; the larvae with 2.1% glycerol were not cold-hardy. The survivals at -10 and -22°C were essentially the same; T_{50D} were 118, 119 respectively with the fall larvae; 108, 103 respectively for the winter larvae and 65, 63 respectively for the spring larvae (Table I). The survivals were shorter at -35°C than at -10 and -22°C except for the winter larvae. The decrease in T_{50D} in the fall larvae from 119 (at -22°C) to 48 (at -35°C) was an indication that at -35°C they are not as cold-hardy as at -22°C. The long T_{50D} (161) of the winter larvae at -35°C is consistent with the high concentration (5.7%) of glycerol in the borers. Besides the

factor of glycerol protection, other unknown factor or factors which favor the survival must have played a role in the prolonging of the survival because the T_{50D} at -35°C was shown considerably higher than those at -22 and -10°C instead of being kept at about the same level as at -22 and -10°C. Laurence's (1967) result in a similar survival experiment at -15 and -25°C also showed that the borer larvae at -25°C had a higher survival. Nevertheless his conclusion that the supercooled state is more injurious to the larvae than the frozen state has been shown inconsistent with our data as will be discussed later. Indeed more data are needed before a satisfactory answer as to "why the cold-hardy winter larvae can survive longer at -35°C than at the higher subzero temperatures" can be given.

The effect of contact moisture on the survival of cold-hardy European corn borer larvae

The borer larvae frequently overwinter in wet locations inside the corn stalks. The effect of wet and dry surroundings under subzero temperatures on the survival of cold-hardy borer was investigated. Three groups of cold-hardy larvae were used as mentioned in Chapter I. Those larvae collected in the winter were most cold-hardy while those collected in the spring were the least hardy. Their survivals at -10, -22 and -35°C were studied. Statistical analyses showed that the survival data from all samples were better fitted to linear regression lines than to regression lines of the second degree polynomial type. For the general purpose of comparing the slope of the lines of each pair (the dry and the wet chilled survival at the same temperature), it was assumed that a linear regression of survival on days of chilling existed in all samples. The

regression lines for wet and dry chilled larvae are shown in Figures 1 - 3. They show that steeper slopes (faster decline in survival) exist in the dry chilled larvae with the exceptions of the fall and the winter larvae at -10°C. To test whether the difference in survival in each pair was significant, T-tests for paired observations were made (Tables V to XIII).

The results show that the survival of wet or dry overwintering borer larvae did not differ at -35°C or at -10°C [$T_{(.01)} = 2.718$]. However, at -22°C the wet chilled cold-hardy larvae had a significantly higher survival rate than the dry chilled ones. A possible explanation of the survival rates may be made on the basis of the degree of cold-hardiness possessed by the larvae, the chilling temperature and the chilling regime, i.e., wet or dry. The spring larvae were not cold-hardy and survival due to cold-hardiness was not significant even at -10°C. There were no more cold-hardy than summer larvae placed at similar temperature (Hanec, 1959).

Minus 35°C was much below the undercooling temperature of all three groups of larvae and consequently all larvae froze rapidly at this temperature. Both wet and dry larvae were frozen after 24 days at -35°C. Therefore, irrespective of whether they were wet or dry complete freezing occurred rapidly at -35°C and the larvae remained frozen during the chilling period. Supercooling would not be a major factor in survival. At -22°C all the wet larvae froze within 24 hours after exposure but few of the dry chilled larvae froze initially. Only 5 per cent of the fall and winter larvae froze after eight days of exposure, 50 per cent after 24 days and all did not freeze until the completion of the experiment. (This temperature is within the range of the undercooling temperatures

FIGURE 1

Regression lines of survival of European corn borer larvae on incubation periods in days; larvae collected on November 29, 1967.

Dotted line: wet chilled (w.c.)

Solid line: dry chilled (d.c.)

Regression lines calculated;

at -10°C:	w.c.	$y = 104.8468 - 0.5583x$
	d.c.	$y = 111.2005 - 0.5507x$
at -22°C:	w.c.	$y = 118.5721 - 0.5103x$
	d.c.	$y = 102.1756 - 0.6086x$
at -35°C:	w.c.	$y = 70.6221 - 0.3103x$
	d.c.	$y = 68.6729 - 0.3551x$

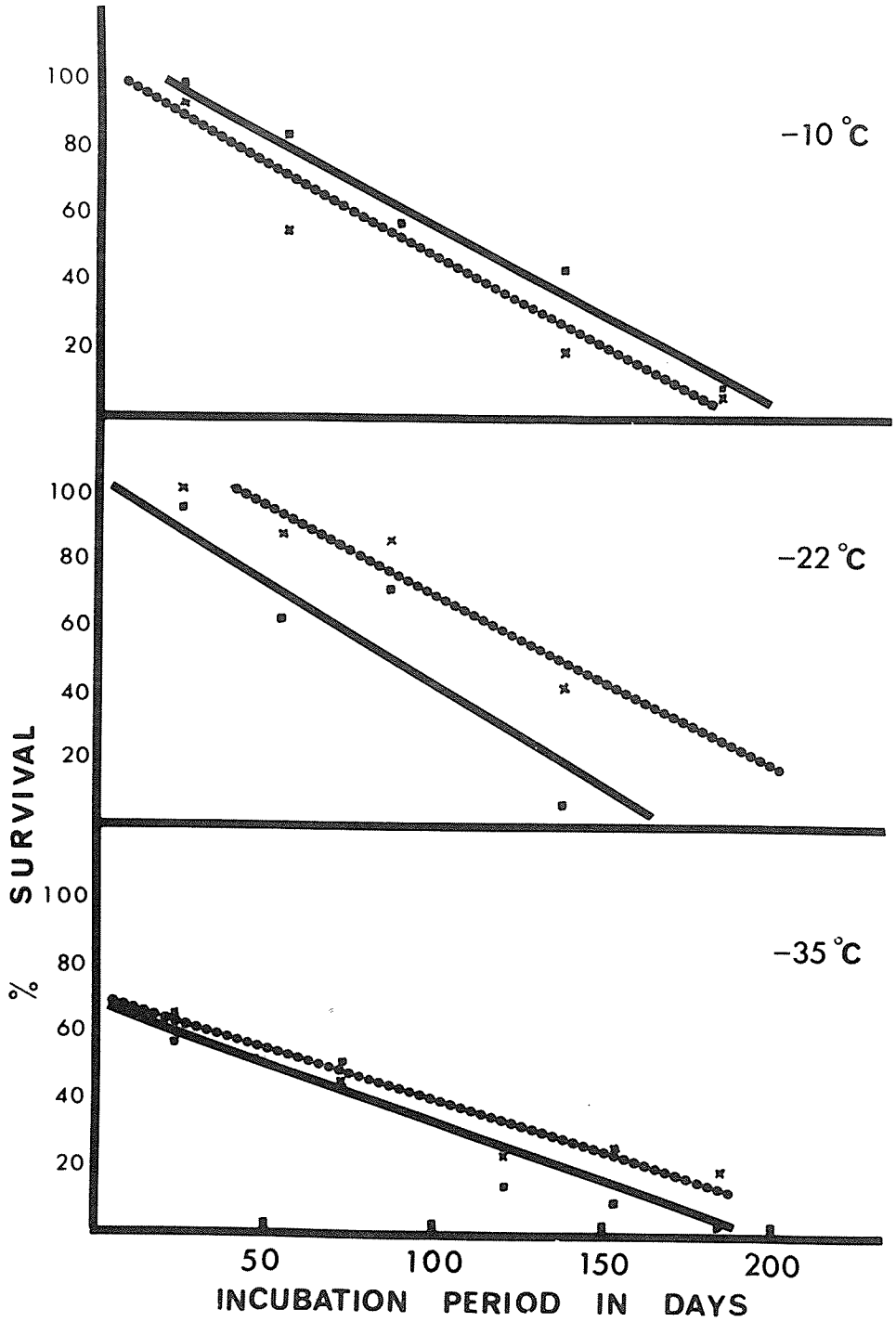

100
80
60
40
20
-10 °C
100
80
60
40
20
-22 °C
% SURVIVAL
100
80
60
40
20
-35 °C
50
100
150
200
INCUBATION PERIOD IN DAYS

FIGURE 2

Regression lines of survival of European corn borer larvae on incubation periods in days; larvae collected on January 19, 1968.

Dotted line: wet chilled (w.c.)

Solid line: dry chilled (d.c.)

Regression lines calculated:

at -10°C:	w.c.	$y = 117.1737 - 0.6856x$
	d.c.	$y = 117.6769 - 0.6469x$
at -22°C:	w.c.	$y - 109.9172 - 0.4008x$
	d.c.	$y = 108.8587 - 0.7255x$
at -35°C:	w.c.	$y = 85.1108 - 0.0162x$
	d.c.	$y = 95.7665 - 0.2373x$

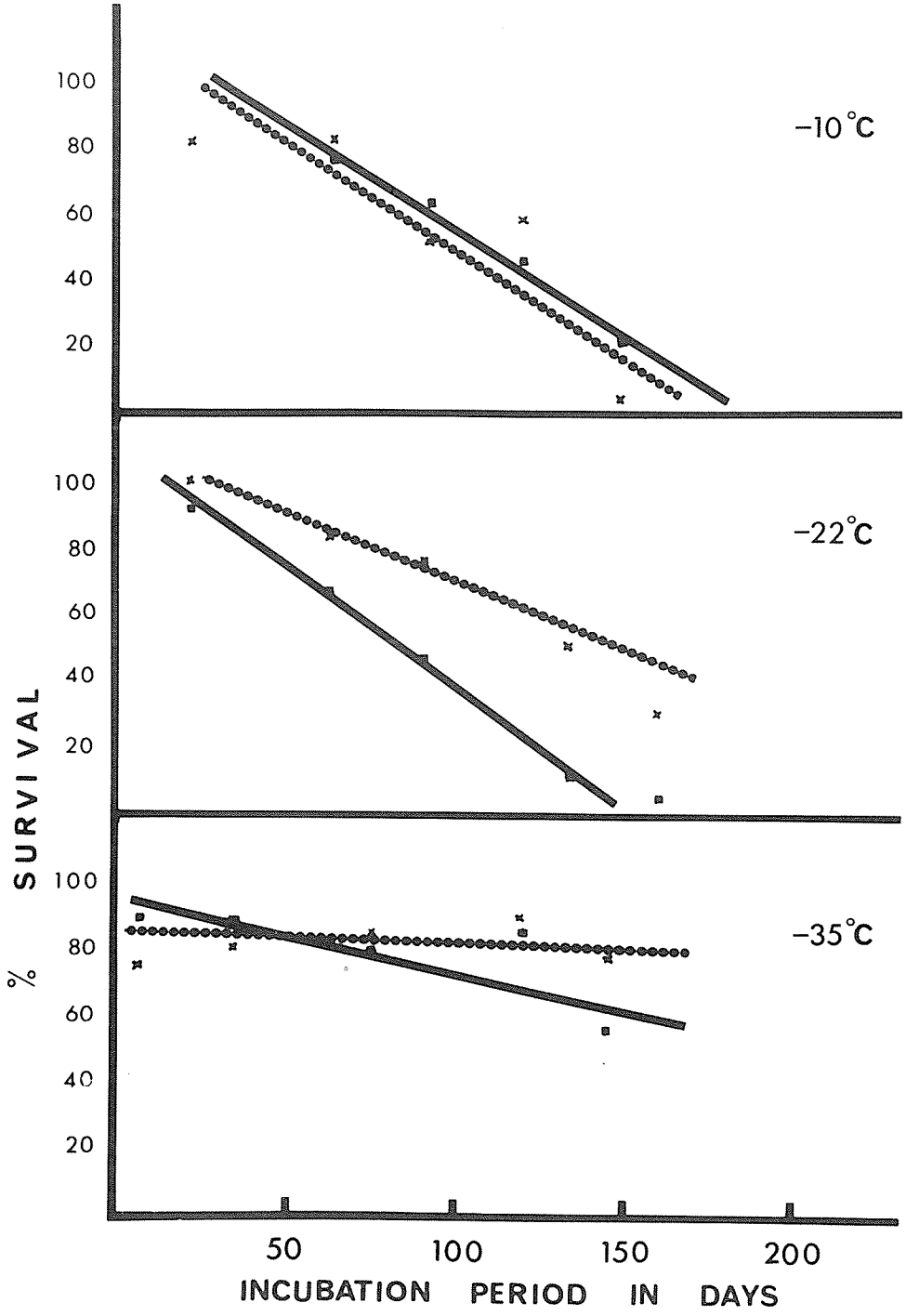

% SURVIVAL
100
80
60
40
20
-10°C
-22°C
-35°C
50
100
150
200
INCUBATION PERIOD IN DAYS

FIGURE 3

Regression lines of survival of European corn borer larvae on incubation periods in days; larvae collected on April 4, 1968.

Dotted line: wet chilled (w.c.)

Solid line: dry chilled (d.c.)

Regression lines calculated;

at -10°C:	w.c.	$y = 102.5730 - 0.6693x$
	d.c.	$y = 97.4246 - 0.7043x$
at -22°C:	w.c.	$y = 86.3654 - 0.6194x$
	d.c.	$y = 92.9571 - 0.6781x$
at -35°C:	w.c.	$y = 59.0726 - 0.3149x$
	d.c.	$y = 76.3620 - 0.4820x$

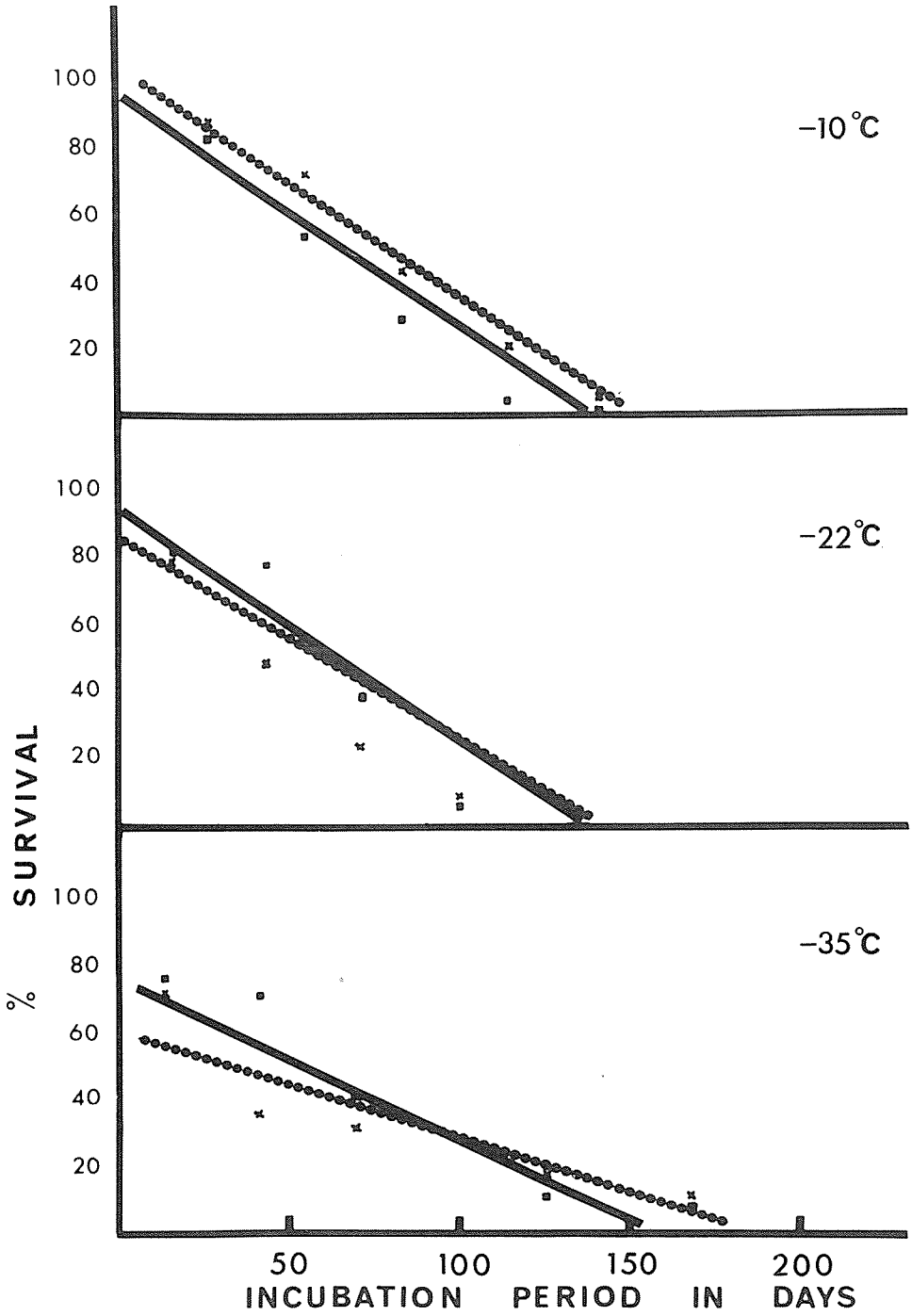

100
80
60
40
20
-10°C
100
80
60
40
20
-22°C
100
80
60
40
20
-35°C
% SURVIVAL
50
100
150
200
INCUBATION PERIOD IN DAYS

TABLE V

SURVIVAL OF EUROPEAN CORN BORER LARVAE AT -10°C., CHILLED DRY AND WET.
COLLECTED FROM FIELD ON NOVEMBER 29, 1967.
AVERAGE OF GLYCEROL CONTENT, 5.0%

Accumulated days at -10°C	(240 Larvae) dry chilled		(240 Larvae) wet chilled	
	No. healthy	% survival	No. healthy	% survival
8	19	95	17	85
24	20	100	19	95
40	18	90	18	90
56	17	85	11	55
72	16	80	18	90
88	11	55	11	55
104	14	70	11	55
120	8	40	11	55
136	9	45	4	20
152	2	10	1	5
168	4	20	0	0
184	2	10	2	10

1. 20 larvae removed at random each time for survival determination.
2. t-test for paired observations (survivals from dry and wet chill) from above 12 items was computed:

   $t = 1.809 \qquad T_{(.01)} = 2.718$

   The difference in survival between the dry and wet chilled larvae was insignificant at the 1% level.

TABLE VI

SURVIVAL OF EUROPEAN CORN BORER LARVAE AT -22°C., CHILLED DRY AND WET.
COLLECTED FROM FIELD ON NOVEMBER 29, 1967.
AVERAGE OF GLYCEROL CONTENT, 5.0%

Accumulated days at -22°C	(240 Larvae) dry chilled		(240 Larvae) wet chilled	
	No. healthy	% survival	No. healthy	% survival
8	19	95	19	95
24	19	95	20	100
40	14	70	20	100
56	12	60	17	85
72	13	65	20	100
88	14	70	17	85
104	10	50	18	90
120	1	5	13	65
136	1	5	8	40
152	1	5	7	35
168	1	5	5	25
184	0	0	3	15

1. 20 larvae removed at random each time for survival determination.
2. t-test for paired observations (survivals from dry and wet chill) from above 12 items was computed:

   $t = 5.471; \quad T_{(.01)} = 2.718$

   The wet chilled larvae had a significantly higher survival at the 1% level.

TABLE VII

SURVIVAL OF EUROPEAN CORN BORER LARVAE AT -35°C., CHILLED DRY AND WET.
COLLECTED FROM FIELD ON NOVEMBER 29, 1967.
AVERAGE OF GLYCEROL CONTENT, 5.0%

Accumulated days at -35°C	(240 Larvae) dry chilled		(240 Larvae) wet chilled	
	No. healthy	% survival	No. healthy	% survival
8	17	75	17	85
24	12	60	13	65
40	5	25	12	60
56	9	45	9	45
72	10	50	9	45
88	14	70	7	35
104	7	35	5	25
120	3	15	5	25
136	3	15	6	30
152	2	10	5	25
168	3	15	6	30
184	0	0	4	20

1. 20 larvae removed at random each time for survival determination.
2. t-test for paired observations (survivals from dry and wet chill) from above 12 items was computed:

   $t = 1.059; \quad T_{(.01)} = 2.718$

   The difference in survival between the dry and wet chilled larvae was insignificant at the 1% level.

TABLE VIII

SURVIVAL OF EUROPEAN CORN BORER LARVAE AT -10°C., CHILLED DRY AND WET.
COLLECTED FROM FIELD ON JANUARY 19, 1968.
AVERAGE OF GLYCEROL CONTENT, 5.7%

Accumulated days at -10°C	(240 Larvae) dry chilled		(240 Larvae) wet chilled	
	No. healthy	% survival	No. healthy	% survival
7	19	95	20	100
21	20	100	17	85
35	20	100	20	100
49	19	95	18	80
63	16	80	19	95
77	17	75	16	80
91	13	65	11	55
105	12	60	10	50
119	9	45	12	60
133	4	20	1	5
147	4	20	1	5
161	1	5	0	0

1. 20 larvae removed at random each time for survival determination.
2. t-test for paired observations (survivals from dry and wet chill) from above 12 items was computed:

   $t = 1.233; \quad T_{(.01)} = 2.718$

   The difference in survival between the dry and wet chilled larvae was insignificant at the 1% level.

TABLE IX

SURVIVAL OF EUROPEAN CORN BORER LARVAE AT -22°C., CHILLED DRY AND WET.
COLLECTED FROM FIELD ON JANUARY 19, 1968.
AVERAGE OF GLYCEROL CONTENT, 5.7%

Accumulated days at -22°C	(240 Larvae) dry chilled		(240 Larvae) Wet Chilled	
	No. healthy	% survival	No. healthy	% survival
7	19	95	19	95
21	18	90	20	100
35	19	95	19	95
49	18	90	19	95
63	13	65	17	85
77	8	40	16	80
91	9	45	15	75
105	7	35	17	85
119	1	5	16	80
133	2	10	10	50
147	0	0	9	45
161	1	5	6	30

1. 20 larvae removed at random each time for survival determination.
2. t-test for paired observations (survivals from dry and wet chill) from above 12 items was computed:

   $t = 4.284; \quad T_{(.01)} = 2.718$

   The wet chilled larvae had a significantly higher survival at the 1% level.

TABLE X

SURVIVAL OF EUROPEAN CORN BORER LARVAE AT -35°C., CHILLED DRY AND WET.
COLLECTED FROM FIELD ON JANUARY 19, 1968.
AVERAGE OF GLYCEROL CONTENT, 5.7%

Accumulated days at -35°C	(240 larvae) dry chilled		(240 larvae) wet chilled	
	No. healthy	% survival	No. healthy	% survival
7	17	85	15	75
21	19	95	19	95
35	17	85	16	80
49	16	80	18	90
63	20	100	20	100
77	17	85	17	85
91	14	70	11	55
105	10	50	17	85
119	17	85	18	90
133	12	60	17	85
147	11	55	16	80
161	12	60	17	85

1. 20 larvae removed at random each time for survival determination.
2. t-test for paired observations (survival from dry and wet chill) from above 12 items was computed:

   $t = 1.519; \quad T_{(.01)} = 2.718$

   The difference in survival between the dry and wet chilled larvae was insignificant at the 1% level.

TABLE XI

SURVIVAL OF EUROPEAN CORN BORER LARVAE AT -10°C., CHILLED DRY AND WET.
COLLECTED FROM FIELD ON April 4, 1968.
AVERAGE OF GLYCEROL CONTENT, 2.1%

Accumulated days at -10°C	(240 Larvae) dry Chilled		(240 Larvae) Wet Chilled	
	No. healthy	% survival	No. healthy	% survival
14	20	100	17	85
28	18	90	18	90
42	16	80	17	85
56	11	55	15	75
70	6	30	9	45
84	6	30	9	45
98	1	5	7	35
112	1	5	4	20
126	1	5	3	15
140	0	0	0	0
154	0	0	1	5
168	0	0	0	0

1. 20 larvae removed at random each time for survival determination.

2. t-test for paired observations (survivals from dry and wet chill) from above 12 items was computed:

   $t = 2.321; \quad T_{(.01)} = 2.718$

   The difference in survival between the dry and wet chilled larvae was insignificant at the 1% level.

TABLE XII

SURVIVAL OF EUROPEAN CORN BORER LARVAE AT -22°C., CHILLED DRY AND WET.
COLLECTED FROM FIELD ON APRIL 4, 1968.
AVERAGE OF GLYCEROL CONTENT, 2.1%

Accumulated days at -22°C	(240 Larvae) dry chilled No. healthy	% survival	(240 Larvae) wet chilled No. healthy	% survival
14	17	85	17	85
28	17	85	18	90
42	16	80	10	50
56	13	65	13	65
70	8	40	5	25
84	3	15	5	25
98	1	5	2	10
112	0	0	2	10
126	0	0	0	0
140	0	0	0	0
154	0	0	0	0
168	0	0	0	0

1. 20 larvae removed at random each time for survival determination.
2. t-test for paired observations (survivals from dry and wet chill) from above 12 items was computed:

   $t = 0.390; \quad T_{(.01)} = 2.718$

   The difference in survival between the dry and wet chilled larvae was insignificant at the 1% level.

TABLE XIII

SURVIVAL OF EUROPEAN CORN BORER LARVAE AT -35°C., CHILLED DRY AND WET.
COLLECTED FROM FIELD ON APRIL 4, 1968.
AVERAGE OF GLYCEROL CONTENT, 2.1%

Accumulated days at -35°C	(240 Larvae) dry chilled		(240 Larvae) wet chilled	
	No. healthy	% survival	No. healthy	% survival
14	15	75	14	70
28	15	75	7	35
42	14	70	7	35
56	6	30	8	40
70	8	40	6	30
84	6	30	12	60
98	2	10	4	20
112	5	25	4	20
126	2	10	3	15
140	2	10	3	15
154	1	5	3	15
168	2	10	2	10

1. 20 larvae removed at random each time for survival determination.
2. t-test for paired observations (survivals from dry and wet chill) from above 12 items was computed:

   $t = 0.372; \quad T_{(.01)} = 2.718$

   The difference in survival between the dry and wet chilled larvae was insignificant at the 1% level.

which varied from about -20 to -30°C.) Therefore at -22°C there were two essentially different groups of larvae; one depending on tolerance to freezing (wet larvae), the other largely on supercooling (dry larvae). The data show that the frozen state is more favorable for survival than the supercooled state at any specific subzero temperature.

Laurence (1967), in a similar survival experiment at -15 and -25°C, contributed the higher survival of the borer larvae at -25°C to the fact that the percentage of larvae in the supercooled state after various lengths of time had been lower than that at -15°C. Assuming that at lower temperatures and the associated frozen state, the degenerative biochemical processes were inhibted and therefore were advantageous to the continuation of life, Laurence concluded that the supercooled state is more injurious to the larvae than the frozen state. He testified his explanation by the recent discovery by Grant and Alburn (1966) that enzyme reactions may speed up or change their pathway in the frozen state, despite decreased kinetic energy and probable restricted diffusion.

However, that "the supercooled state is more injurious to the larvae than is the frozen state" (Laurence, 1967) without limiting the comparison to a specific temperature was not suggested by our results. In terms of T_{50D} (Table I) the supercooled fall larvae at -10°C had a longer survival than those frozen at -35°C; the supercooled spring larvae at -10°C also had a longer survival than those frozen at -22 and -35°C. That the frozen state is less injurious to the larvae than is the supercooled state cannot be concluded simply by the coincidence of longer survival of frozen larvae at one low temperature. The degree of injury on the insect caused by either the frozen state or supercooled state

might be modified by the degree of cold to which the insects were exposed. This is the reason why one specific subzero temperature was based for discussing the survival of the larvae in frozen state and those in supercooled state. No attempt was made to compare the survival among the larval groups under different subzero chilling in this study.

Survival of cold-hardy European corn borer larvae under intermittent chilling

In the study of survival of cold-hardy European corn borer larvae by Hanec & Beck (1960) intermittent rather than continuous chilling was carried out because not enough experimental insects were available to be sampled without replacement. Hanec removed all the larvae from the cold cabinets periodically and warmed them for 24 hours, checked for survival and then put them back to the cold during the course of his studies. Early winter larvae had a T_{50D} of 73 days at -20°C and 90 days at -15°C. The survival of these insects could have been different to some degree if these borers were chilled continuously. The purpose of our study was to expand this earlier work to determine the effect of cooling-warming cycles on the survival of the borer larvae during long periods of chilling and under more critical experimental conditions.

The larvae for this study were collected on November 29, 1967, the same as those for the continuous chilling experiment. A sample of 35 larvae were used for each dry and wet chilling at -10 and -22°C. The larvae were removed periodically from the cold cabinets, warmed for 48 hours at room temperature and survival was determined. The survivors were then returned to the cold for further chilling. Survival data were taken for 24 weeks.

The results in Tables XIV and XV show that dry chilled larvae survived longer than those chilled wet at both -10 and -22°C and that survival was higher at -10°C than it was at -22°C. At -22°C, T_{50D} for dry larvae was 80 days and only 75 days for the wet larvae. At -10°C the T_{50D} for dry larvae was not reached even after 167 days of intermittent chilling, 83% were still alive. The wet larvae reached T_{50D} after about 105 days of chilling. The survival difference between wet and dry larvae at -22°C is probably not significant.

To compare the survivals obtained by both the continuous and intermittent chilling, Table XVI is listed. It is apparent that the continuously chilled larvae had a higher survival than did the intermittent chilled ones at -22°C but not at -10°C. A possible explanation to this is that at -22°C or colder, the intermittent chilled larvae suffered from the harmful frequent freezing-thawing cycles and therefore had a lower survival. But the larvae seem to tolerate the frequent cooling-warming cycles at a mild -10°C. No explanation is offered for the large difference in survival of the dry chilled larvae and wet chilled larvae under intermittent and continuous chilling at -10°C.

TABLE XIV

SURVIVAL OF EUROPEAN CORN BORER LARVAE AT -10°C., CHILLED INTERMITTENTLY COLLECTED FROM FIELD NOVEMBER 29, 1967

Accumulated days at -10°C	(35 Larvae) dry chilled		(35 Larvae) wet chilled	
	No. healthy	% survival	No. healthy	% survival
14	35	100	34	97
28	34	97	34	97
42	34	97	31	89
56	34	97	29	83
70	33	94	29	83
84	33	94	28	80
97	33	94	24	69
111	32	91	16	46
125	32	91	10	29
139	31	89	3	9
153	29	83	3	9
167	29	83	0	0

TABLE XV

SURVIVAL OF EUROPEAN CORN BORER LARVAE AT -22°C., CHILLED INTERMITTENTLY COLLECTED FROM FIELD NOVEMBER 29, 1967

Accumulated days at -22°C	(35 Larvae) dry chilled		(35 Larvae) wet chilled	
	No. healthy	% survival	No. healthy	% survival
14	32	89	30	86
28	30	86	27	77
42	24	69	26	74
56	19	54	23	66
70	19	54	20	58
84	17	49	13	37
97	11	31	6	18
111	3	9	1	3
125	3	9	0	0
139	1	3		
153	1	3		
167	0	0		

TABLE XVI

A COMPARISON OF THE INTERMITTENT AND CONTINUOUS CHILLING ON THE SURVIVAL OF THE COLD-HARDY EUROPEAN CORN BORER LARVAE AT -10 AND -22°C COLLECTED FROM FIELD NOVEMBER 29, 1967

T_{50D}*	-10°C		-22°C	
	Intermittent	Continuous	Intermittent	Continuous
Dry Chilled	> 167+	115	80	104
Wet Chilled	105	120	75	134

* T_{50D} is the exposure time in days required for 50% mortality.

+ Percent survival higher than 50% all through the incubation period of 167 days.

PART II

ECOLOGICAL FIELD STUDIES AND OTHER PRELIMINARY OBSERVATIONS ON THE EUROPEAN CORN BORER

CHAPTER V

FIELD STUDIES

Vertical distribution of European corn borer larvae in corn plants and their survival in the field

The vertical distribution of overwintering larvae in corn stalks could be a factor in their winter survival. Snow levels in fields of standing stalks rarely exceeds 18 inches and within this protective cover the larvae could be protected from extreme cold, temperature fluctuations and predators such as birds and mice.

On November 13, 1967 several hundred corn plants were dissected to determine the distribution of borer larvae along the length of the stem. The stem was divided into six inch sections beginning from the roots to a height of 36 inches. Thirty-six inches was chosen as the arbitrary height because above that, most of the stalks were broken off at various heights.

Table XVII shows the vertical distribution of the diapausing borer larvae in corn plants; about 72% of the population overwintered in the sections of the plant below the average snow level of 18 inches. The same distribution study in the field was repeated in the spring and the survival was compared between the larvae from the upper 18 inches of stalk and those from the lower 18 inch portion. The results are shown in Table XVIII. The survival of larvae below the snow level was as high as 85% while only 70% of the larvae survived the winter above the snow level.

It is apparent that the location under the snow cover has a more

TABLE XVII

VERTICAL DISTRIBUTION OF EUROPEAN CORN BORER LARVAE IN CORN PLANTS
SURVEYED ON NOVEMBER 13, 1967 IN MORDEN MANITOBA
36 PLANTS WERE SAMPLED

	Soil level (0")	0-6"	6"-12"	12"-18"	18"-24"	24"-30"	30"-36"	Total larvae
No. of larvae	57	59	34	22	24	24	11	231
% of total in plant	24.7	25.6	14.7	9.5	10.4	10.4	4.7	

TABLE XVIII

SURVIVAL SURVEY ON EUROPEAN CORN BORER LARVAE IN 261 CORN PLANTS BELOW AND ABOVE SNOW LEVEL COLLECTED ON MAY 10, 1968

	No. of larvae	% of total in plant	Number dead	Number paralyzed	Number healthy	% survival
Above 18"	53	27	10	6	37	69.8
Below 18"	145	73	18	3	124	85.5

unvarying temperature and is warmer than any exposed part of the corn stalk. This can be well illustrated by Laurence's (1967) temperature data (Table XIX). The borers overwintered in the stem located close to the ground level would experience very little variation in temperature and, therefore, would not suffer from the lethal freezing-thawing cycles. The larvae close to the ground level were also favored by the relatively higher temperature below the snow level. Table XIX shows that the temperature at ground level was no lower than -5°C even when it was as low as -35°C above the snow level. That the freezing-thawing cycles are lethal has already been illustrated in Chapter IV. Temperatures in the range from -3.0 to -4.5°C would not be expected to be lethal to the borer even over long durations of exposure. Preliminary tests in the laboratory showed that at -2.0°C 90 per cent of cold-hardy larvae could survive 184 days of exposure. While at -22°C and intermittent chilling T_{50D} was reached in 75 to 80 days under laboratory conditions (Tables XIV and XV).

Comparison of winter survivals of larvae in standing and snow-buried corn plants

In order to determine whether the larvae in the snow-buried corn stalks survive longer than those in standing plants in the field during the winter, about 400 corn plants were laid on the ground and covered with snow about one foot thick in the early winter. Another sample of stalks was left standing and the vicinity around the plants was kept clear of snow. Survival was checked in the late spring of 1968.

The survival of the larvae from the snow-buried plants was higher as compared with those from the standing stalks. Larvae from standing

TABLE XIX

TEMPERATURES AT VARIOUS POSITIONS INSIDE AND OUTSIDE A CORN STALK SET UP OUTSIDE AT THE UNIVERSITY OF MANITOBA, WINNIPEG, DURING THE WINTER OF 1965-66
DATA SUMMARIZED FROM LAURENCE (1967)

		Temperature (°C)							
		Ground level		Snow level (2 ft.)		4 ft.		6 ft.	
Date	Time	Inside stalk	Outside stalk	Inside stalk	Outside stalk	Inside stalk	Outside stalk	Inside stalk	Outside stalk
19 Jan. 1966	9:30 a.m.	-4.0	-3.0	-16.5	-17.0	-17.5	-18.0	-17.0	-17.0
21 Jan. 1966	9:00 a.m.	-3.5	-3.0	-33.5	-33.5	-35.0	-35.5	-34.0	-33.5
27 Jan. 1966	3:30 p.m.	-4.5	-4.5	-29.0	-29.0	-31.0	-31.0	-30.0	-31.0
3 Feb. 1966	2:30 p.m.	-3.0	-3.0	-16.5	-17.5	-19.0	-20.0	-18.5	-18.5

plants in early spring had a 62 per cent survival while those from the snow-buried stalks had 72 per cent survival (Table XX).

The protective role of snow during the severe winter on the survival of the European corn borer is further apparent.

Winter survival, glycerol concentration and undercooling temperature of mature European corn borer larvae in Winnipeg and Morden

Larval winter survival from 1965 through 1969 (Table XXI) was recorded. Infested corn stalks were collected at Morden, bundled and stored in an open field near the university. No attempts were made to influence the normal snow cover. Survival was checked regularly during the winter. For this survey larvae were cut out of the corn stalks, removed to room temperature and the number of healthy larvae determined. As the winter progressed, many living larvae were incapable of locomotion and eventually died even when placed under warm conditions. The larvae were termed "paralyzed" and were considered as dead in the calculation of survival percentages.

Table XXI shows that the survival during the 1965-66 winter was considerably higher than that of the larvae in the three winters which followed. The survival was essentially similar during 1966-69 winters.

Data from 1967-1969 indicate that the greatest mortality increase occurs during March, past the season of extremely low temperatures. The relationship between winter survival and temperature has not yet been analyzed.

The readings of glycerol concentration and undercooling temperature of the borer larvae collected in the field at Morden during 1967-68 winter are presented in Table XXII.

TABLE XX

COMPARISON OF EUROPEAN CORN BORER WINTER SURVIVAL IN STANDING AND SNOW-BURIED CORN STALKS

Date of survey	Condition of corn stalks	No. of plants	No. of larvae	No. dead	No. paralyzed	No. healthy	% survival
Mar. 7, 1968	Standing in the field	147	135	54	18	163	69.4
Mar. 27, 1968		660	2,907	754	355	1,798	61.9
Apr. 26, 1968	Buried in the snow	300	802	163	65	574	71.6

TABLE XXI

WINTER SURVIVAL OF DIAPAUSE LARVAE KEPT OUTSIDE IN STOCKED CORN STALKS IN OPEN FIELD

Year	Date	No. larvae collected	No. dead	No. paralyzed	No. healthy	% survival
1966	April 4	209	20	-	189	90.4
	April 26	184	23	-	161	87.0
	May 1	107	13	-	94	87.9
	May 22	61	8	-	53	87.0
1967	Jan. 5	102	10	-	92	82.3
	Feb. 3	97	15	-	82	84.5
	Mar. 2	90	34	-	56	62.2
	Mar. 21	100	44	-	56	56.0
	Apr. 9	146	58	-	88	60.3
	May 10	605	358	-	247	57.5
1967	Nov. 7	1,182*	7	-	1,175	99.4
1968	Jan. 6	169	6	-	163	96.4
	Jan. 17	1,122*	45	51	1,026	91.4
	Jan. 23	643*	34	45	564	87.7
	Mar. 9	235	54	18	163	69.4
	Apr. 2	2,907*	754	355	1,798	61.9
1968	Oct. 24	42	2	-	40	95.2
1969	Jan. 2	98	5	-	93	94.8
	Jan. 29	81	8	3	70	86.4
	Mar. 1	102	9	12	81	79.4
	Apr. 25	76	17	6	53	69.7

* Large number collected because larvae used for other experiments.

TABLE XXII

GLYCEROL CONCENTRATION AND UNDERCOOLING TEMPERATURE OF THE EUROPEAN CORN BORER LARVAE AT MORDEN, MANITOBA DURING 1967-68 WINTER

Date	Mean glycerol conc. (% live wt.)	Undercooling temperature (°C)
Nov. 20, 1967	5.0±0.35	-21.4±3.8°C
Dec. 11, 1967	5.5±0.26	-
Jan. 6, 1968	6.2±0.64	-27.3±4.3
Jan. 19, 1968	5.7±0.68	-26.5±6.3
Feb. 8, 1968	6.9±0.70	-22.9±3.3
Feb. 20, 1968	5.3±0.66	-19.7±3.8
Mar. 9, 1968	4.3±0.98	-11.1±2.7
April 3, 1968	2.1±0.10	-13.5±1.8
April 23, 1968	0.9±0.17	-7.5±3.7
May 22, 1968	0.4±0.20	-9.8±3.0

Pupation

During 1965 and 1966 corn stalks were brought in from Morden to Winnipeg. The stalks were overwintered outside and examined periodically for pupae during the spring and summer of the same year. From 1967 through 1969, however, this study was done in corn fields at Morden. Figure 4 shows the pupation records of the borer from 1965 to 1969. Since it was not possible to make surveys frequently enough to find out the exact date of 50% pupation, the best possible deduction of the dates (Table XXIII)--which would not be more than one or two days from the exact dates--was made by fitting the curves from Figure 4. Both Figure 4 and Table XXIII show that the initiation of pupation and date of 50% pupation varied greatly from 1965 to 1969. The earliest date recorded for the beginning of pupation was June 4 in 1968, the latest, June 30 in 1965. The time required for the larvae to reach 50% pupation was approximately two weeks after pupation began. There was no conclusive evidence to suggest a correlation between mean air temperature during April, May and June and the pupation pattern of the borer. Possibly soil and stalk temperatures would be a more valid criterion.

Adult flight activity

This study was carried out in the Morden area. Daily counts were made of adults caught in four modified New Jersey mosquito light traps which were located on the edge of the corn field and were operated between the hours of 9:00 p.m. and 5:00 a.m. It is assumed that maximum moth captures coincide with peaks of flight activity.

The seasonal limits of flight are shown in Table XXIV. Adult

FIGURE 4

Pupation records of European corn borer in southern Manitoba, 1965-1969.

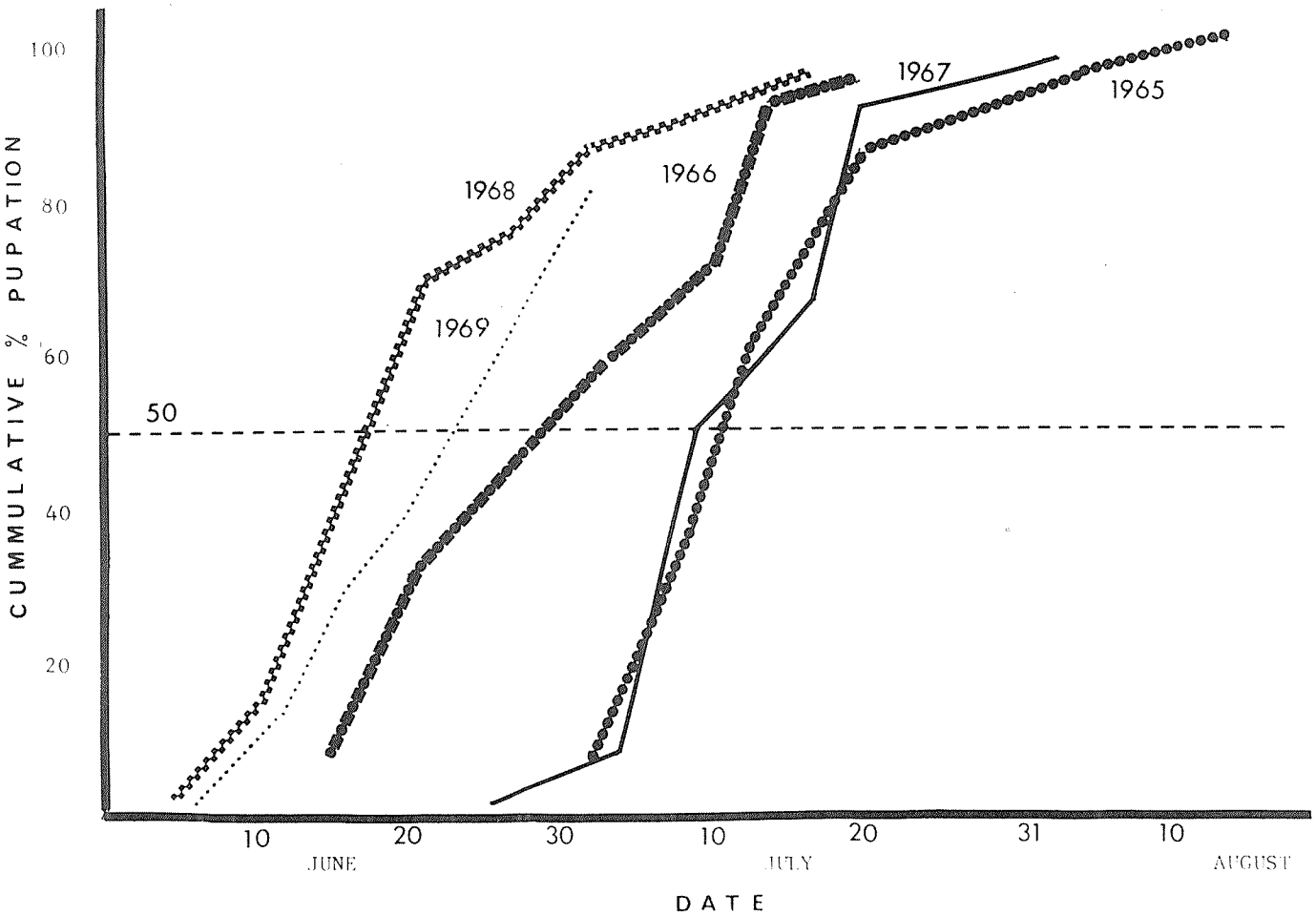

CUMMULATIVE % PUPATION
100
80
60
40
20
50
1968
1969
1966
1967
1965
10
20
30
10
20
31
10
JUNE
JULY
AUGUST
DATE

TABLE XXIII

DATES OF 50% PUPATION OF OVERWINTERED CORN BORER LARVAE IN SOUTHERN MANITOBA 1965-1969

Year	Location	Date of 50% pupation
1965	Winnipeg	July 11
1966	Winnipeg	July 1
1967	Morden	July 9
1968	Morden	June 17
1969	Morden	June 24

TABLE XXIV

THE SEASONAL LIMITS OF MOTH FLIGHT OF THE EUROPEAN CORN BORER FROM 1965 TO 1969, MORDEN, MANITOBA

Year	Commencement of flight	Peak of flight	Termination of flight	Duration of flight
1965	July 16	July 23	August 14	30 days
1966	July 1	July 13	August 1	32 days
1967	July 7	July 16	August 20	45 days*
1968	July 8	July 11	August 7	31 days
1969	July 16	August 5	September 2	49 days*

* May be partial second generation.

emergence, insofar as it is reflected by flight to the light traps ranged from 30 days to 49 days. Figure 5 shows that the central 50% of the moth catches occurred within one to two weeks, mostly in July but sometimes overlapping into August.

The relationship between the number of moths caught and the temperature at night

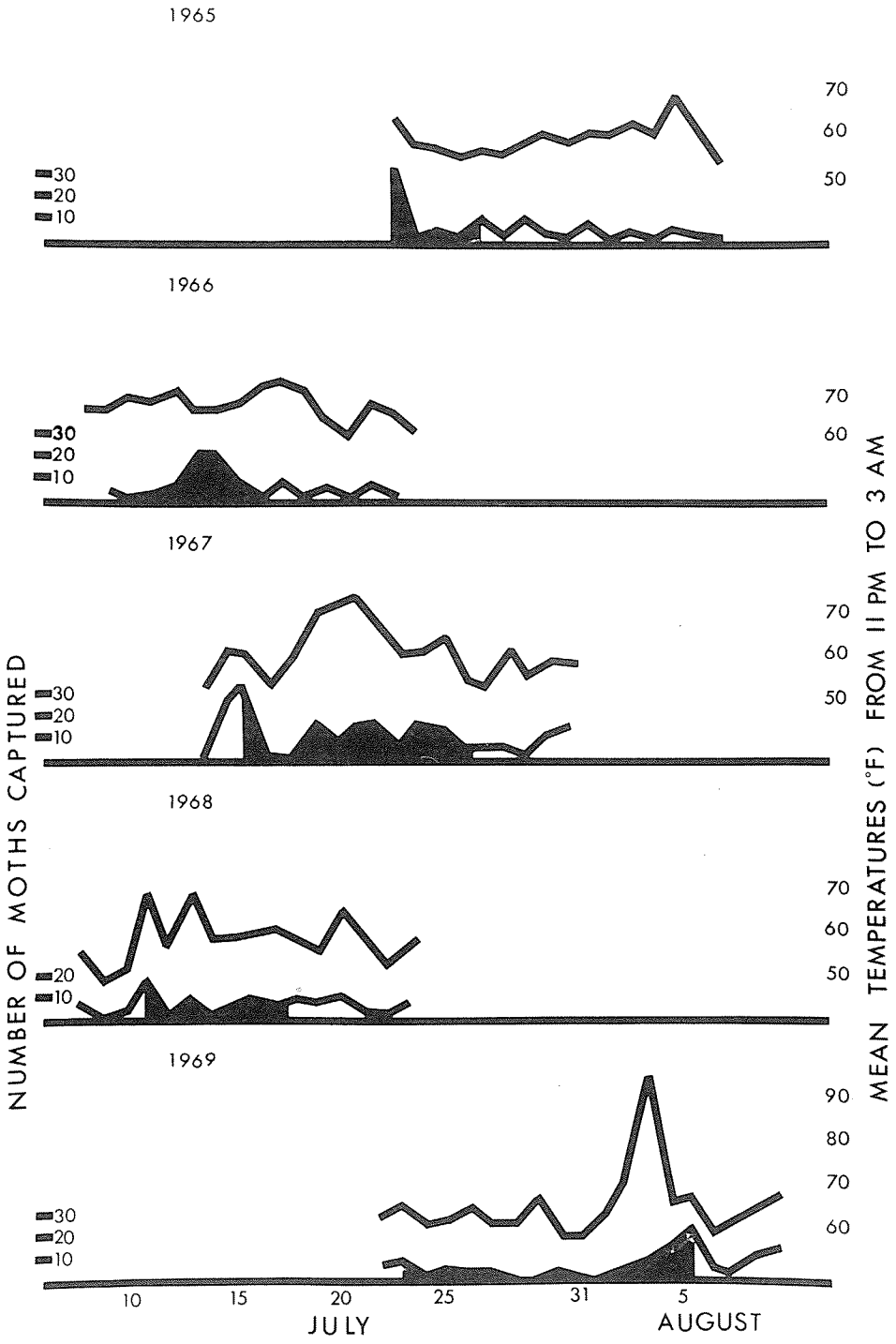
In Figure 5, the catches of the central 50% borer moths during their period of flight are compared with the mean of the hourly temperatures from 11:00 p.m. to 3:00 a.m. of the corresponding night. The remaining "tail parts" of the moth catches both before and after the central part were not shown because the numbers of daily moth catches were too small to reliably indicate any trends. Even so, no obvious trends were indicated between the percentage of moths caught during the night and the corresponding mean hourly temperatures. Similar results were also shown by Laurence (1967). Laurence found no apparent relationship between the percentage of moths caught during the night and the minimum temperature of the corresponding night, and the maximum temperature of the preceding day. The mean of the hourly temperatures from 11:00 p.m. to 3:00 a.m. was used because up to 70% of the total catches were collected by the traps during this four hour period. Windless nights during which the temperature was above 55°F were the most favorable for moth activity. Rain and wind disrupted flight even when night temperatures were favorable.

Predicting adult emergence by the use of heat units

The use of heat unit accumulation as an index of corn borer

FIGURE 5

Daily catches of the corn borer moths and corresponding mean temperatures from 11:00 p.m. to 3:00 a.m., Morden, Manitoba, 1965-69. Dark area indicates central 50 per cent moth catches.


1965
1966
1967
1968
1969
NUMBER OF MOTHS CAPTURED
MEAN TEMPERATURES (°F) FROM 11 PM TO 3 AM
30
20
10
70
60
50
90
80
10
15
20
25
31
5
JULY
AUGUST

development was introduced by Apple (1952) who used a summation of the mean of the daily minimum and maximum temperatures above 50°F to predict the first appearance of the different stages of the corn borer in Illinois and Wisconsin. With the same technique, Jarvis and Brindley (1965) developed a method for predicting the cumulative percentages of moth flight in Boone County, Iowa. Both Apple and Jarvis and Brindley used 50°F (10°C) as the base in calculating the heat unit accumulations. However, this technique for predicting moth flight of the corn borer was not satisfactory for the Morden area. The base temperature of 50°F for the conditions in Morden was reported too low and instead 55°F was suggested as the best base temperature (Laurence, 1967).

The correct base temperature, according to Arnold (1959) is the one giving the smallest coefficient of variation for summations in respect to different years based on it. Laurence (1967) used several base temperatures, i.e., from 50 to 57°F, to compute the coefficient and found that the smallest value could be obtained when corresponded to a base of 55°F.

When the minimum temperature is below and the maximum above the base temperature, some deviation may be introduced. Arnold (1960) proposed a method to correct this type of deviation. In computing day degrees his method was applied to the data collected in Morden.

The dates into which the 1%, 25%, 50% 75% and 95% of the cumulative percentage of moth flight fell were noted and cumulative day-degrees on these dates were calculated using 55°F as a base. Expected numbers of day degrees in Fahrenheit necessary to reach 1, 25, 50, 70 and 95% moth flight were obtained by taking the average of the day degrees of

corresponding levels of moth flight in five years. By using the above expected number of day degrees, the date of any of the five levels of accumulative percentage of moth flight can be predicted. Table XXV shows the predicted and actual dates and the expected number of day degrees necessary to reach 1, 25, 50, 75 and 95% moth flight. In general, the actual and predicted dates agreed closely. The closest estimates occurred in 1966 and 1967. The poorest estimates occurred in 1969, an abnormally cooler year than normally expected. In all the years except 1969 temperatures were near or above normal, the predicted dates coincided with the actual dates.

Oviposition and development of the larvae

From 1965 through 1969 egg-mass counts were made at Morden in the fields where moth flight was surveyed. Table XXVI shows periods of main flight activity and date of greatest egg-mass density. In all five years studied, the former always coincided with the latter. However, in 1967 and 1969 the dates of greatest egg-mass density fell into the second greatest flight activity period instead of the greatest flight activity period; i.e., the main flight period was on July 15 and 16, but the greatest egg-mass density occurred on July 30 and 31, in 1967.

The greatest egg-mass density and infestation of grain corn by the larvae in various years are also listed in Table XXVI. The former is shown correlated to the latter. The greater egg-mass density is always followed by the heavier infestation.

Field collections of the larvae during their development period were made periodically during the 1965-69 period. Figure 6 shows that

TABLE XXV

ACTUAL AND PREDICTED DATES OF THE MOTH FLIGHT MORDEN, MANITOBA 1965-69

Cumulative percent	1965 Date		1966 Date		1967 Date		1968 Date		1969 Date		Expected number of day degrees
	A*	P**	A	P	A	P	A	P	A	P	
1%	7-16	7-9	7-1	7-3	7-7	7-9	7-8	7-9	7-16	7-13	469
25%	7-23	7-18	7-11	7-10	7-16	7-18	7-11	7-16	7-23	7-19	574
50%	7-25	7-24	7-14	7-15	7-21	7-22	7-16	7-23	8-4	7-26	666
75%	7-29	7-30	7-17	7-18	7-30	7-27	7-20	7-30	8-8	7-30	732
95%	8-5	8-7	7-25	7-27	8-8	8-4	8-3	8-10	8-11	8-5	841

* A - Actual

** P - Predicted

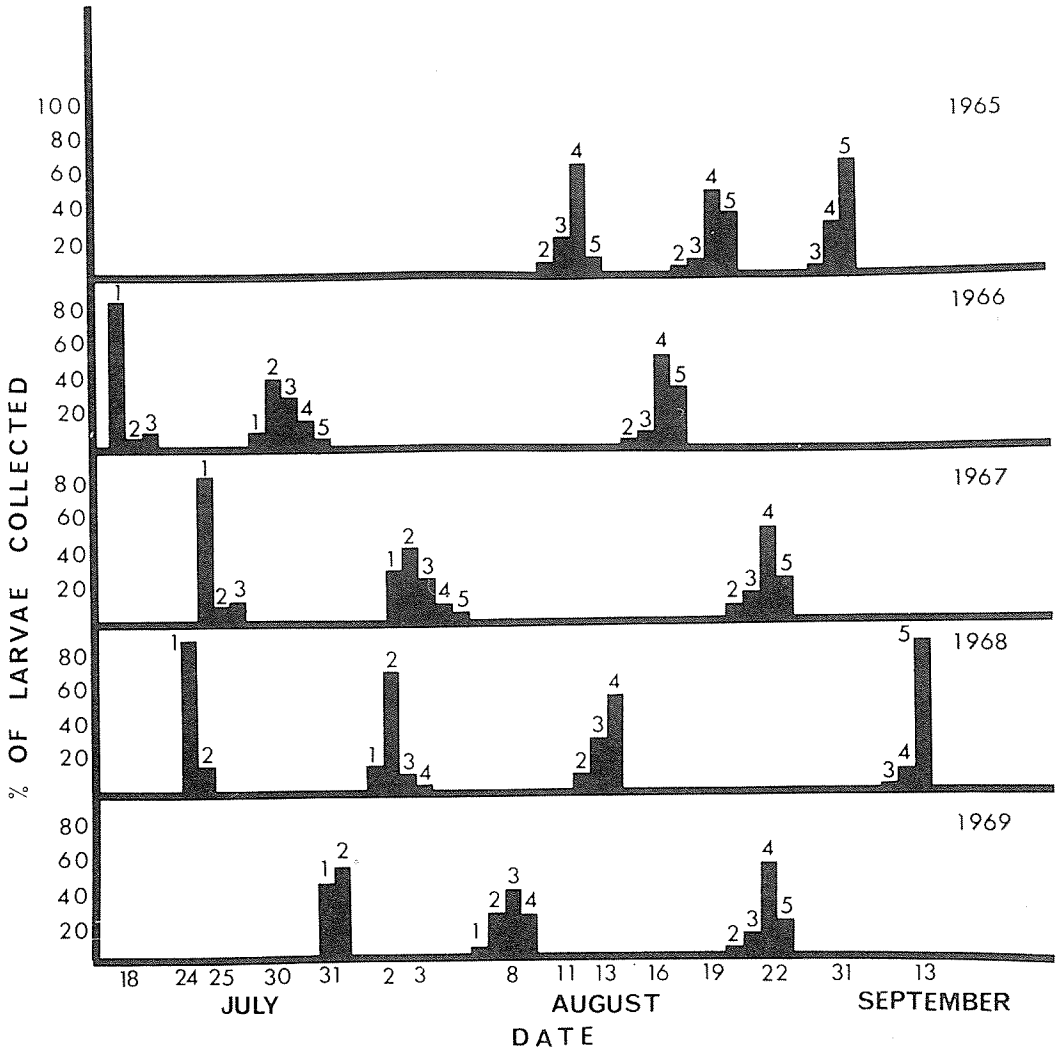
TABLE XXVI

DATES OF MAIN FLIGHT ACTIVITY OF CORN BORER MOTHS, GREATEST EGG-MASS DENSITY AND LARVAL INFESTATION ON GRAIN CORN AT MORDEN, MANITOBA 1965-69

Year	Periods of main flight activity	Greatest egg-mass density (per 100 plants)	Infestation on corn (larvae found in 100 plants)
1965	7-23	70	650
1966	7-13 & 14	120	710
1967	7-15 & 16 7-30 & 31	40	510
1968	7-11	15	42
1969	8-5 7-22 & 23	34	344

FIGURE 6

Seasonal history of the larval stages of the European corn borer in one corn field in Morden, Manitoba, 1965-1969.


% OF LARVAE COLLECTED
1965
1966
1967
1968
1969
100
80
60
40
20
18 24 25 30 31 2 3 8 11 13 16 19 22 31 13
JULY
AUGUST
SEPTEMBER
DATE

the percentage of larvae in each instar in the five-year period.

During 1965 no systematic collection was made until August 11. In 1966 the first larvae appeared on July 18; 1967, on July 20; 1968, around July 16 and 1969 on July 22. In general, the first larvae showed up during the third week of July, five to seven days after the first appearance of egg-masses. By the end of the second week of September more than 90% of the larvae molted into the fifth instar. In 1965 and 1966 all the larvae were in fifth instar by September 15; in 1967, 95% of the larvae were in fifth instar on September 12; in 1968, 88% were in fifth instar on September 13 and in 1969, 98% were in the fifth instar on September 16 (not shown in figure).

CHAPTER VI

PRELIMINARY STUDIES ON GAMMA IRRADIATION OF EUROPEAN CORN BORER LARVAE

Walker and Brindley (1963) tested the possibility of using the "sterile male technique" as a means of controlling the European corn borer and concluded that the cost of treating large numbers of moths by x-rays would be economically unsound. The authors suggested that a cobalt^{60} source would be desired for any large scale tests.

Raun, Lewis, Picken and Hotchkiss (1967) made a series of tests to investigate the effects of irradiation with gamma rays from the Co^{60} source on the larvae of the borer. They concluded that somatic damage caused by irradiation of non-diapausing larvae was too extensive to make this a practical method of corn borer control. However, diapausing larvae showed little somatic damage and pupation, moth emergence and mating were nearly normal at levels of irradiation as high as 5,000 rads.

Materials and methods

In this study cold-hardy and non-cold-hardy diapausing larvae were irradiated to determine whether irradiation would have different effects on the two groups of larvae. The cold-hardy larvae were collected in December, 1968 and March, 1969 and undercooled to about -22°C. The non-cold-hardy larvae were collected during April, 1969 and had an undercooling temperature of -13.5°C.

The larvae were dissected from the plants, placed in samples of 50 specimens each and each sample was irradiated with Co^{60} at 1,000, 5,000, 10,000, 20,000 and 50,000 rads. After irradiation the larvae were placed

in individual vials at 26.7°C and pupation and adult emergence was observed.

Results and discussion

The results (Tables XXVII-XXIX) of irradiating cold-hardy and non-cold-hardy larvae are inconclusive. In all tests irradiation over 10,000 rads resulted both in reduction in pupation and also a large decrease in adult emergence. Irradiations of 50,000 rads caused mortality in all larvae.

The sharp decrease in both pupation and adult emergence of the April larvae at 1,000 rads may not necessarily be due to the irradiation. The population as a whole appeared to be weak because only 60 per cent of the controls pupated vs. 70 and 78 per cent of the December and March larvae respectively. Adult emergence in the controls was also abnormally low compared to the other two populations. These experiments should be repeated before any conclusions can be made as to the effects of irradiation on cold-hardy and non-cold-hardy borer larvae.

TABLE XXVII

RESULTS OBTAINED FROM GAMMA IRRADIATION (AT A DOSE RATE OF 19,320 RAD/MIN FROM A CO^{60} SOURCE) OF DIAPAUSING EUROPEAN CORN BORER LARVAE COLLECTED MARCH 14, 1968 (50 larvae per treatment)

Treatment (rad)	Pupation		Normal moths emerging	
	No. pupae	%	No. moths	%
None	39	78	34	87
1,000	43	86	36	83.7
5,000	42	84	32	76.2
10,000	34	68	26	76.5
20,000	33	66	7	21.2
50,000	0	0	0	0

TABLE XXVIII

RESULTS OBTAINED FROM GAMMA IRRADIATION (AT A DOSE RATE OF 19,320 RAD/MIN FROM A CO^{60} SOURCE) OF DIAPAUSING EUROPEAN CORN BORER LARVAE COLLECTED ON APRIL 4, 1968 (50 larvae per treatment)

Treatment (rad)	Pupation		Normal moths emerging	
	No. pupae	%	No. moths	%
None	30	60	21	70
1,000	24	48	13	54.2
5,000	32	64	18	56.3
10,000	32	64	15	46.9
20,000	25	50	4	16.0
50,000	0	0	0	0

TABLE XXIX

RESULTS OBTAINED FROM GAMMA IRRADIATION (AT A DOSE RATE OF 18,570 RAD/MIN FROM A CO^{60} SOURCE) OF DIAPAUSING EUROPEAN CORN BORER LARVAE COLLECTED ON DECEMBER 12, 1968 (50 larvae per treatment)

Treatment (rad)	Pupation		No. moths emerging			% Normal moths emerging
	No. pupae	%	Normal male	Normal female	Mal-formed	
None	35	70	13	15	1	80.0
1,000	45	90	18	17	3	77.8
5,800*	44	88	14	14	6	63.6
10,000	41	82	14	15	6	70.8
20,000	38	76	9	5	11	36.8
50,000	0	0	0	0	0	0

Larvae chilled at 4.4°C for four weeks before irradiation.

* 5,800 rad instead of 5,000 rad due to technical error.

CHAPTER VII

PRELIMINARY ANALYSIS OF SOME ORGANIC ACIDS IN EUROPEAN CORN BORER BY GAS-LIQUID CHROMATOGRAPHY

There have been very few analyses of organic acids in the haemolymph of any insect. One of the few comprehensive works is that of Levenbook (1961) on organic acids common in insect haemolymph which contained the following, succinnate, malate, fumarate, citrate, lactate and α-ketoglutarate. No information on the organic acids of the European corn borer in particular is available. The present study of organic acid analysis is part of the cold-hardiness studies. Since glycerol content cannot fully explain the cold-hardiness of the overwintering larvae, some other factors must be involved and organic acids could be one of the solutes.

Materials and methods

Insect material

Both field-collected and chilled larvae were used. Field larvae were collected from January through May and the chilled larvae (collected in November) were kept for 72 days at -10, -22 and -35°C with and without contact moisture. The borer larvae weighed about 100 mg. and were ground in a mortar with sand in hot 80% (V/V) ethanol. The ground material was subsequently extracted with hot solution of 80% ethanol, 40% ethanol, water and again with 80% ethanol. The extracts were centrifuged, combined and evaporated to dryness in vacuum. The dry residue was successively extracted with 5 ml. of ether and the same volume of water to separate lipids and water-soluble materials. The latter were fractionated

with ion-exchange columns and the organic acid fraction recovered. The methods of preparing standards, methyl esters, columns and chromatography are the same as Canvin's (1965) in his analysis of plant materials for organic acids.

Standards

Trimethyl citrate (m.p. 78°C), dimethyl malonate (b.p. 180°C) and dimethyl succinate (b.p. 191°C) were prepared from the corresponding acids by refluxing 1 g of the acids in 24 ml. methanol with 1 ml. of 30% fuming H_2SO_4 for one hour. After the addition of water the esters were recovered in chloroform and the chloroform evaporated to dryness. The esters of the first one were further purified by crystallization from hot ether while the other two were further purified by distillation. Dimethyl malate was prepared by methylation of purified malic acid with diazomethane. The acid was purified by dissolving 2.2 g malic acid in 8 ml. acetone, filtering the solution, and then diluting it with 32 ml. benzene. After the solution was allowed to stand at room temperature for two hours, the malic acid crystals were recovered by filtration, washed with benzene, and dried overnight at 80°C. Dimethyl tartaric was prepared from the acid with diazomethane and used without purification. Trans-aconitic acid was recrystallized twice from hot water before esterification with diazomethane to yield trimethyl trans-aconitate.

Preparation of methyl esters

The above-mentioned water-soluble organic acid fraction was evaporated to dryness in vacuum. The dry residue was then dissolved in ether-methanol (9:1 V/V). All methyl esters of the insect material were prepared by adding an ethereal solution of freshly distilled

diazomethane to the acids dissolved in ether-methanol. Diazomethane was generated from an ethereal solution of N-methyl-N-nitroso-p-toluenesulfonamide by adding an alcoholic base and heating the mixture to 70°C. Excess diazomethane and solvents were removed by evaporation and the methyl esters dissolved in a known volume of methanol. A portion of this solution was injected into the gas chromatograph.

Preparation of columns

Chromosorb W (60-80 mesh) was coated with 10% Reoplex 400 by dissolving the latter in chloroform and then adding the chromosorb W. The mixture was stirred until excess chloroform had evaporated and was further dried overnight at 100°C. The coated material was sized and the 60-80 mesh fraction was packed in copper tubes (15 inches by 1/4 inch). The column was equilibrated overnight in an Aerograph A-90-P_2 gas chromatograph at 150°C and 10 ml/min helium flow rate.

Chromatography

After the Reoplex 400 column was equilibrated, the oven temperature was adjusted to 55°C, the injector temperature to 180°C, the detector temperature to 212°C, and the carrier gas (helium) flow rate to 100 ml/min. Samples were injected with 10-μl or 50-μl Hamilton syringes. After injection of the sample the column temperature was programmed at 4°C per minute until a temperature of 175°C was reached. This temperature was maintained for five minutes to make sure that no more methyl ester emerged. The signals from the thermal conductivity detector were led to a Brown recorder with a chart speed of 0.5 inch/minute.

Results and discussion

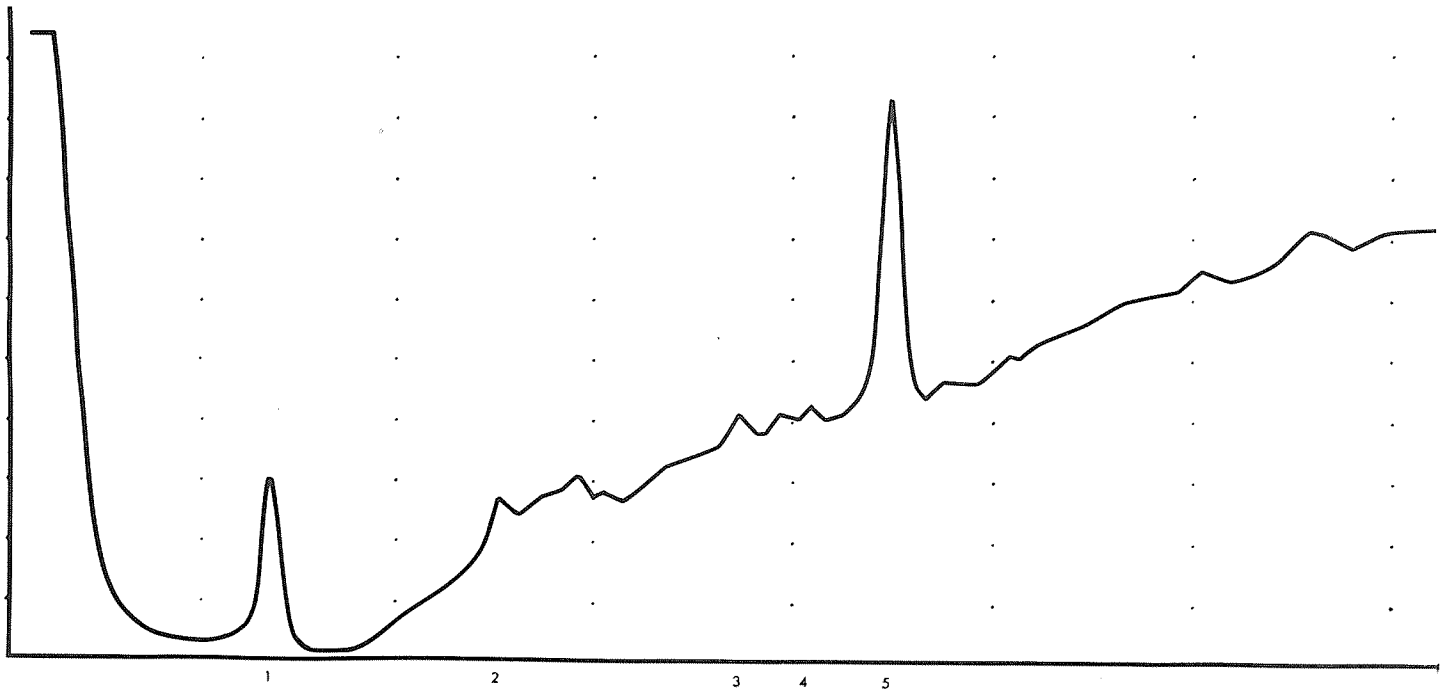
The separation of the methyl esters of several organic acids in a programmed run with Reoplex 400 as the stationary phase is shown in Figure 7. The analysis of the organic acids from the insect tissue was complicated by the appearance of several unknown peaks in the chromatographic trace. Two of these (retention times 17.7 and 30.6) could be impurities derived from the ion-exchange column. The results of some analyses of the organic acids from the corn borer tissues of different groups and treatments all showed similar patterns as shown in Figure 7. The integration units of the area below each peak and above the baseline were modified as suggested by Canvin (1965). Relative detector response of a thermal conductivity detector to various organic acid methyl esters, their relative amount of acids in percentage were obtained as follows, citric acid, 45-50%, succinic acid, 25-30%, aconitic acid, 6%, malic and tartaric acid less than 5% each. Some unknown acids in small quantity were also found. One of these (retention time 36.3) could be isocitric acid, another (retention time 39.7) could be fumaric acid. For the identification of these unknown, further tests are required.

The qualitative and quantitative similarity of the organic acid samples, samples from both cold-hardy January larvae and non-cold-hardy May larvae, from dry chilled or wet chilled larvae, from larvae in the frozen state or in supercooled state, indicate that organic acids were not directly involved in cold-hardening of the European corn borer.

FIGURE 7

Gas chromatogram of the methyl esters of some organic acids on Reoplex 400. Dotted lines show intervals of six minutes. Peaks (retention time in minutes):

1. dimethyl succinate (8.1)
2. dimethyl malate (15.2)
3. trimethyl aconitate (22.4)
4. dimethyl tartarate (24.6)
5. trimethyl citrate (27.0)


1
2
3
4
5

CHAPTER VIII

SUMMARY

Cold-hardiness of the European corn borer was studied in terms of number of days required for the larvae to reach 50% mortality (T_{50D}) at three temperatures, -10, -22 and -35°C. The degree of cold-hardening of the field collected diapausing corn borer larvae was synchronized with their glycerol quantity induced under the natural environment. The winter larvae (containing 5.7% glycerol) were cold-hardy at all three subzero temerpature levels while the fall larvae (containing 5.0% glycerol) were cold-hardy only at -10 and -22°C. The spring larvae (containing 2.1% glycerol) were not cold-hardy.

The frozen state was found to be less injurious to the cold-hardy larvae than the supercooled state when larvae were exposed to continuous subzero chilling at a specific temperature. This was most apparent at -22°C. The wet chilling caused rapid initial freezing of all larvae. Dry chilling caused random freezing at a rate dependent on the temperature. The survival data show that the wet and dry chilled larvae were essentially equally susceptible to cold at -10°C and at -35°C respectively.

A comparison was made between intermittent and continuous chilling on survival. The larvae chilled intermittently had a lower survival than those under continuous chilling at -22°C. The frequent freezing-thawing cycles which were experienced by the intermittently chilled larvae were more injurious than continuous freezing.

Five year data on the ecological studies of the corn borer near Morden were updated. The protective function of snow on the winter

survival of the borer larvae was established. By insulating the outside cold, the thick layer of snow not only gives considerable warmer surrounding environment around the corn stem close to the ground level, but also lessens the temperature fluctuation. The larvae under the snow level were therefore benefited by not being exposed to frequent harmful freezing-thawing cycles.

Prediction of moth flight activity in Morden at 1%, 25%, 50%, 75% and 95% levels were made possible by the use of the past five year moth flight activity data and heat units.

Irradiations by Co^{60} from 1,000 to 50,000 rads produced inconclusive evidence that non-cold-hardy larvae were more susceptible than cold-hardy larvae.

Five organic acids in the mature corn borer larvae were identified by gas-liquid chromatography, these were, citric, succinic, aconitic, malic and tartaric acid. The preliminary study shows that these acids are not directly involved in the cold-hardening of the borer larvae.

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