

AN INVESTIGATION OF SOME ENVIRONMENTAL FACTORS INFLUENCING
THE DISTRIBUTION AND DETECTION OF THE RUSTY GRAIN BEETLE

Cryptolestes ferrugineus (STEPHENS)

(COLEOPTERA : CUCUJIDAE)

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ABSTRACT

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Laboratory and field experiments were conducted in 1970 and 1971 to study some of the physical and biological factors affecting the distribution and detection of C. ferrugineus, the most important pest of stored grain in the Prairie Provinces.

One of the laboratory experiments showed that C. ferrugineus respiration, and locomotion rates were directly correlated with temperature. This suggested that either rate could be used as an indication of the influence of temperature on the biological activity of C. ferrugineus, and that activity increased with temperature. The lower limit for movement by C. ferrugineus adults was recorded at approximately 2°C.

Tests were carried out to trap adults of C. ferrugineus in gallon jars containing wheat at moisture contents of 13.7 and 16.3 percent and at temperatures between 1.1°C and 30°C. Temperature was the major factor influencing the number of adults caught in traps. Therefore, more insects would be expected to be trapped as

the temperature increased from 1.1°C to 30.0°C. Population density, and time allowed for trapping did not significantly influence the numbers of beetles trapped.

C. ferrugineus adults were attracted through columns of grain to dishes of spoiled grain, but not to moisture in the form of water vapour. C. ferrugineus adults were also attracted to cultures of pure fungi grown on grain. Penicillium corymbiferum was most attractive, followed by Scopulariopsis brevicaulis, Fusarium sp., and spoiled grain, then Cephalosporium acremonium and Aspergillus repens. Streptomyces, wet grain and dry grain were not attractive.

Adult beetles when introduced into a bulk of wheat tended to move to the surface and periphery of the bulk. Most of the breeding took place on the south side of the bin near the wall-floor interface. Degermination in samples taken in these areas reached a maximum of 35 percent. The trapping technique was useful in detecting adult C. ferrugineus while the spear probing and vacuum probing techniques were successful in detecting more larvae than adults. The efficiency of the trap for detecting adults was comparable to that of the spear and vacuum probes for larvae. The majority of adults of C. ferrugineus tended to remain in the lower half of the bin, while most of the larvae were found in the upper half and near the wall-floor interface of the bin.

Adults tended to move from the periphery of the bin towards the centre, near the floor, as the outer portion of grain bulk cooled.

Survival in the centre of the bin was sufficient to ensure continued infestation.

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CHAPTER I

INTRODUCTION

The production of cereal grains is the most important industry on the Canadian Prairies. In some years, insects may cause damage to grain in storage, resulting in substantial financial loss to the industry. The most important stored grain insect is the rusty grain beetle, Cryptolestes ferrugineus (Stephens), and although it may feed on several cereal grains it is primarily a pest of wheat. Damage or loss may occur as a result of feeding, contamination of the grain with feces and body fragments, or by the heating of grain which is caused by dense populations of insects and micro-organisms.

Normally the Prairie climate does not provide a favourable environment for the development of serious infestations in stored grain. Occasionally, when a suitable environment does occur, insects which are normally present at low levels, congregate in the most suitable areas and increase at a rapid rate, producing heat and water, which in turn maintains an environment suitable for rapid reproduction. Under these conditions, and especially if the moisture content of the grain is high, micro-organisms associated with grain can multiply rapidly and cause grain to heat and spoil.

Economic losses may be reduced by early detection of the rusty grain beetle. To aid early detection suitable sampling techniques and procedures are necessary. The purposes of this study are to

determine the effectiveness of grain insect traps to detect the presence and distribution of the rusty grain beetle in grain, and to determine some of the environmental factors that affect the distribution of this insect in grain.

The problem

The movement and distribution of insects in grain occur in response to temperature, moisture and population density. How these and other factors effect the distribution and detection of C. ferrugineus is of prime importance in this tudy.

Since the presence of insects in stored grain is determined by sampling, it is essential to utilize adequate sampling equipment. Insect traps had not been extensively tested under field conditons therefore, it was essential to determine whether the traps would be effective in detecting low levels of infestation, and consequently be useful in determining the distribution of C. ferrugineus in bulk grain. With a knowledge of distribution, development of efficient sampling procedures are possible.

Importance of the study

A knowledge of the occurrence and distribution of insects in grain is essential before insects can be effectively controlled by chemical or physical methods. Usually, infestations of insects do not occur when grain is stored dry in adequate storage facilities. However, in recent years production and marketing of grain has been

such that farmers have been forced to store surplus grain on the ground or in temporary cribs. Thus the risk of loss due to insects and micro-organisms has increased and the need for better sampling techniques has become important.

Organization of the remainder of the thesis

The literature on the biology of C. ferrugineus and the history of the development of numerous sampling devices are reviewed in Chapter II. Chapter III describes the general experimental techniques used for rearing and handling insects, and the standard methods of analysis and environment control. Chapter IV deals with the influence of temperature on respiration and locomotion of the rusty grain beetle. The laboratory experiments in Chapter V describe the effects of temperature, moisture, time and population density on the numbers of insects trapped. Chapter VI describes the effects of the odour of the fungi and the effect of the water vapour on the movement of C. ferrugineus adults and includes a description of an apparatus used to study the effect of odour on beetle movement. Chapter VII deals with a field study of the distribution and detection of beetles in a steel granary. In this chapter some of the factors that influence distribution are examined. The results are summarized in Chapter VIII, and the conclusions are stated in Chapter IX.

CHAPTER II
REVIEW OF THE LITERATURE

The rusty grain beetle, Cryptolestes ferrugineus (Stephens), was first described and named Cucujus ferrugineus by Stephens in 1830.

The early taxonomy of this insect was confusing; circa 1848 it was placed in the genus Laemophloeus according to Rilett (1949); Ganglebauer (1899) placed it in the sub-genus Cryptolestes. Casey (1916), considered Cryptolestes as a distinct genus. Leng (1920) in his catalogue of Coleoptera of America, agreed with Ganglebauer that Cryptolestes was a sub-genus of Laemophloeus. Steele and Howe (1955) following Casey (1916), considered that Cryptolestes merited genus status. Lefkovitch (1959), in a taxonomic study of the sub-family Laemophloeinae concluded that Cryptolestes was in fact a distinct genus. Cryptolestes ferrugineus is now the official name for the rusty grain beetle.

C. ferrugineus, prior to becoming a pest of stored products, inhabited two ecologically different niches. It was a scavenger or semi-predator living primarily under the bark of trees, or a scavenger or depredator inhabiting the nests of bees and wasps (Linsley, 1942). The presence of this species has been recorded in the nests of wasps (Tuck, 1897; Richards and Herford, 1952), in haystack refuse (Fowler, 1899) under the bark of trees (Wheeler, 1921; Dolinski, unpublished data), and in an aphid gall and in soil (Lefkovitch, 1959). It has been postulated that these widespread naturally occurring resevoirs

act as foci from which new infestations may arise, and may be of some importance in determining distribution (Linsley, 1942).

C. ferrugineus has global distribution being established in most of the agricultural countries of the tropic and temperate regions (Howe and Lefkovitch, 1957). Solomon and Adamson (1955) showed that C. ferrugineus is more cold hardy than other members of the genus, thus enabling it to be extensively distributed in the cooler regions of the world.

C. ferrugineus is primarily a pest of stored cereals (Freeman, 1952; Smallman, 1944; Watters, 1955). Davies (1949) lists wheat, flour, oil seeds, cassava root, dried fruits, chilies, bean-cakes and gum damar as foodstuffs attacked by the rusty grain beetle. Howe and Lefkovitch (1957) list wheat, rice, maize, sorghum barley, ground nuts and palm kernels as products containing specimens of C. ferrugineus upon entry into Great Britain.

The first established infestations of C. ferrugineus in Canada occurred during the years 1930 - 1932 in grain stored on farms in Ontario (Stirrett and Arnott, 1932). In the Sixteenth Annual Report (1942) of the Board of Grain Commissioners for Canada (now known as the Canadian Grain Commission), the rusty grain beetle is reported as follows: "The rust-red grain beetle Laemophloeus ferrugineus (Stephens) has become a major pest, causing serious infestations in the terminal annexes and country annexes throughout the prairie provinces". The Seventeenth Annual Report (1943) reported the

rusty grain beetle as "causing more serious infestations than any other insect pest of stored grain". In 1944, it was reported as the most serious pest of stored grain in Western Canada and this reputation still stands today.

A survey carried out in Western Canada in 1958 (Liscombe and Watters, 1961) showed that 36 percent of all empty granaries examined contained low levels of infestation. A survey carried out in California by Linsley and Michelbacher (1943) showed that L. ferrugineus was found in 3 of 42 infested granaries examined in the Sacramento Valley, 9 out of 37 in the San Joaquin Valley, 7 out of 33 in the coastal region, and 5 out of 16 in the southern interior. Even though it was reported from most of the 48 states by the early 1940's, its importance was overlooked due to the seriousness of the damage by other grain pests (Rilett, 1949).

In surveys carried out in England, rusty grain beetles were commonly found in stored products (Howe, 1951) and in granaries (Coombs and Freeman, 1956).

Damage to stored grain is caused primarily by the larvae of this species. The larvae feeds mainly on the germ end of the kernel (Rilett, 1949), often degerming the kernel. This feeding reduces the germination potential if the grain is used as seed, and lowers nutritive value. The major losses due to infestations occur when population of insects produce metabolic heat and moisture leading to growth of destructive moulds and degeneration of grain

quality. Sinha (1961) found that C. ferrugineus was the most common insect associated with "hot spots". In addition to this, the presence of dead insect bodies and feces in grain may result in a loss of grade and increase the risk of losing foreign markets. In either case producers sustain economic losses.

Rilett (1949) found that when moulds were present on wheat kernels, mortality and development time of larvae decreased. Sinha (1965) found that C. ferrugineus could develop from egg to adult on 10 of 23 species of fungi tested. The shortest development period at 33⁰C was 22 days on Trichothecum roseum Link, the longest, 34 days on Fusarium moniliforme (Sheldon). Loschiavo and Sinha (1965) tested 24 fungal species and found that Nigrospora sphaerica (Mason) was, the best of all the fungi tested for feeding and oviposition by C. ferrugineus.

In wild populations, adults of the rusty grain beetle exist in a near 1:1 sex ratio (Smith, 1965). The adult life span varies with temperature and humidity. The results of Rilett (1949) and Bishop (1959) indicated a 6 - 9 month life span under controlled environmental conditions. Bishop (1959) also found that beetles lived longer at 21.1⁰C than at 32.2⁰C, and at 90 percent relative humidity than at 70 percent relative humidity.

The adults lay eggs in the grain dockage or in rough surfaces or cracks in the kernels (Rilett, 1949; Smith, 1959). Smith (1959) stated that "adults must have fine particles of food present before

their rate of oviposition on wheat grains would be very high". Later, Smith (1962) showed that at 30°C and 70 percent relative humidity, oviposition rate increased from 5.6 eggs per female day on wheat kernels to 7.5 eggs per female day on flour. Rilett, (1949) and Smith, (1965) showed that the development time decreased as the temperature increased up to the optimal temperatures of 30°C - 32.2°C. Rilett, (1949) recorded that the insects would not develop in an environment of less than 25 percent relative humidity. As the humidity increased from 50 to 90 percent at 26.7°C, developmental time decreased from 37.7 days to 35.2 days; as the relative humidity increased from 50 to 90 percent at 32.2°C the developmental time did not decrease as the relative humidity increased from 75 to 90 percent.

Smith (1963) divided the life span of an adult rusty grain beetle at 32.2°C and 70 percent relative humidity into a preoviposition period of 0.1 week, and oviposition period of 13.1 weeks, and a post-oviposition period of 2.0 weeks. An increase in temperature from 20°C to 40°C decreased the preoviposition period from 3.0 - 0.1 weeks, the oviposition period from 31.0 to 6.2 weeks and the postoviposition period from 9.7 to 1.2 weeks.

Three natural enemies of the rusty grain beetle are a gamasid mite Seuilus pomi Parst (Acarina; Mesostigmata), a wasp Cephalonomia waterstoni Gahan (Hymenoptera; Bethyliidae) and the cadelle beetle Tenebroides mauritanicus L. (Coleoptera : Ostomatidae). Of these,

Cephalonomia waterstoni is the most common in Manitoba, parasitizing larvae and feeding on eggs (Sheppard, 1936). Finlayson (1950) stated that Mattesia dispersa Naville (Protozoa, Schizogregarinaria) was another parasite attacking the larvae of the rusty grain beetle.

Because the experiments in each chapter are not inter-related, literature pertinent to each is reviewed in detail under the appropriate headings.

Detection apparatus and methods

The apparatus and methods for the detection of insects in grain kernels can frequently be highly technical and complex. The following techniques have been used with limited success, as aids to visual observations; sectioning, sanding and staining techniques (Frankenfeld, 1948; Goosens, 1948), density sectioning techniques (Apt, 1952; Katz et al, 1954), x-ray examination (Dennis, 1953; Milner et al, 1950, 1953) radiographic techniques and crack flotation tests (Harris et al, 1952; Milner et al, 1953), and aural methods (Adams et al, 1954; Bailey and McCabe, 1965).

A second group of techniques utilizes chemical or physical factors as indicators of insect infestations. These are: uric acid measurements (Rao et al, 1952), carbon dioxide production measurements (Howe and Oxley, 1944), infra red measurements (Dennis, 1958), and the ninhydrin chemical reaction to insect body fluids which contain high concentrations of free amino acids (Dennis and Decker, 1962).

To be acceptable, inspection procedures must be convenient, rapid, accurate and adaptable. In general, most of these techniques are inadequate for determining infestations because of interference from non-insect sources, low level of sensitivity, complexity of application, time required and inconsistency in detection (Decker and Dennis, 1962). These factors limit the practical and wide-spread application of these techniques and therefore, more direct methods of insect detection must be employed.

The devices used by entomologists in sampling for stored grain insects are those originally used by grain handlers and inspectors. The devices are primarily the double-tube car probe used for sampling box cars and the spear probe used for sampling deeper bulks by adding extension rods to the probe. Both types of samplers remove small quantities of grain which can be examined for the presence of insects, by sieving or by placing the grain in a Berlese funnel in which insects are driven out of a sample of grain by means of heat. Howe (1965) showed that repeated sampling with the spear probe at the same location led to vertical funnelling, which could disturb populations within the grain bulk.

Burges (1960) developed a suction spear which was considered better than the spear probe because it was more versatile.

The latest sampling device designed by Loschiavo and Atkinson (1967) and modified in 1969, (Fig. 1 and 2) consists of a cylindrical brass screen perforated with holes small enough to exclude grain

kernels yet large enough to allow the entrance of grain insects and mites. The insects that enter are collected in a compartment at the bottom of the trap. Catches of 9,000 beetles and innumerable mites have been recorded during field tests (Loschiavo and Atkinson, 1969).

CHAPTER III
GENERAL PROCEDURES

Certain general techniques were used for both laboratory and field experiments and in specific cases were modified to meet the requirements of experimental conditions.

The initial supply of C. ferrugineus was obtained in 1969, from a standard culture maintained for 8 years on a mixture of 95 percent wheat and 5 percent germ (by weight) at the Canada Department of Agriculture, Research Station, Winnipeg, Manitoba.

Insects used for experiments reported in this thesis were kept in 1 gallon jars (3600 ml) containing about 1500 ml of wheat at 14.5 ± 0.5 percent moisture content, supplemented with about 80 ml of wheat germ. The cultures were maintained in controlled cabinets at $28 \pm 0.5^{\circ}\text{C}$ and 70 percent relative humidity. The jars were sealed with No. 3 Whatman filter papers held in place with sealing wax; the papers provided adequate ventilation while preventing contamination by mites and psocids.

Insects for experiments were sifted from cultures with a No. 10 sieve, picked up with either a squirrel hair brush or an aspirator and placed into vials for conditioning or used directly. Insects were used only once for any one experiment and were of mixed age and sex unless otherwise specified.

Experiments were conducted in cabinets or modified refrigerators

where the temperature and humidity could be controlled. The moisture content of the grain used in experiments was adjusted by adding a calculated volume of water to 20 pounds of wheat, and then storing in a sealed pail at room temperature for 4 or 5 days to equilibrate the kernels with the inter-granular water vapor. Wheat moisture content was measured with a Halross dielectric moisture meter (Canadian Aviation Electronics Ltd., Winnipeg, Manitoba) or by using the oven dry method (Approved Methods Committee Amer. Assoc. of Cereal Chemists, Inc. 1969).

CHAPTER IV

THE RELATIONSHIP BETWEEN LOCOMOTOR ACTIVITY AND THE RESPIRATION RATE OF C. ferrugineus AT TEMPERATURES BETWEEN 1.1⁰C AND 30⁰C

Introduction

Environmental conditions imposed upon stored grain insects can influence their dispersal activity and distribution. The factors which may influence locomotor activity and consequently, insect distribution in grain are: temperature, moisture, food and population density. The primary influential factor in determining locomotion is temperature. Miller (1929) noted that flying insects do not fly at low temperatures, and that in crawling insects, the rate of movement becomes slower. Gunn and Hopf (1941), stated that "temperature has a great effect on the speed of biological processes". Oxley, (1949), found that thermal conductivity in grain was low, leading to a lag between air temperature and grain temperature. It is likely then, that the changes in the biological systems within a grain bulk are influenced by the interactions of its physical and biological components.

The laboratory study reported in this chapter attempts to clarify the relationship between temperature, locomotion rate and respiration rate with a view towards predicting the number of insects that can be caught by a trapping device.

Review of the literature

The apparatus and methods used in studying the locomotor activity of invertebrates are varied and numerous. The simplest method of determining locomotor activity is by measuring distance travelled in a given period of time, at a specific temperature. Mellanby (1939), followed the path of the bed-bug, Cimex lectularius L. with a pencil as it walked on a blotting paper, while time was recorded with a stop-watch. The average speed at 15°C was 59 cm/min increasing to 208 cm/min at 25°C. A maximum speed of 255 cm/min was attained at 32.2°C. Cloudsley-Thompson (1951) traced the paths of millipedes for a ten-minute period as they moved across damp filter paper. She found that speed of locomotion bore a relationship to temperature. Wellington (1960), traced the path, and calculated the rate of travel for an eighth instar larva of Halisidota argentata Pack and a third instar larva of Malacosoma disstria Hubner. In both cases the rate of movement increased with increasing temperature; also it was greater under light than under dark conditions. Perttunen and Paloheimo (1964), copied the trail and recorded the distance travelled every 3 minutes by adult Tenebrio molitor L. for a total of 15 minutes. At 100 lux illumination, they found that as temperature increased from 5°C to 20°C the total distance travelled by 20 insects increased by 904 cm to 12,450 cm in 15 minutes. At 30°C the distance travelled decreased to 10,799 cm and at 40°C increased to 29,532 cm.

Crozier (1924), measured the rate of movement of a millipede, Parajalus pennsylvanicus as a function of temperature by measuring the length of time necessary for the animal to creep a measured distance at temperatures ranging from 6⁰C to 30⁰C. He recorded that the velocity of uniform movement varied with temperature according to the Arrhenius equation. Miller (1929), measured the time necessary for blowfly larvae to travel a distance of 10 cm along a ribbon at various temperatures. The time required dropped sharply from 226.6 sec to 46.77 sec as the temperature increased from 2.8⁰C to 7.7⁰C. As the temperature gradually increased from 7.7⁰C to 40⁰C, the time to traverse the 10 cm decreased from 46.8 sec to 9.8 sec, then above 40⁰C began to increase.

Sharpley (1920, 1924), found that the speed of locomotion of ants increased from 0.44 cm/sec to 6.60 cm/sec for a 30⁰C rise in temperature. A rate of 130 cm/sec or 2.90 mph at 35⁰C was recorded for Periplaneta americana L. (McConnell and Richards, 1955).

Estimates of the proportions of insects active at a specific temperature have been used as indices for locomotor activity (Nicholson, 1934; Gunn and Hopf, 1942). Nicholson (1934), working with sheep blowflies used the measurement of the number of flies resting, moving or crawling at constant temperatures, or the numbers of flies showing these activities as the temperatures increased from 0⁰C - 45⁰C or decreased from 45⁰C - 0⁰C. Gunn and Hopf (1942), used the proportion of Ptinus tectus Boie. walking

at specific temperatures as an indicator of activity. They recorded that the frequency of locomotion depended on culture temperature, the temperature on the previous day, and the rate of temperature decrease or increase in the few minutes prior to testing.

Automatic recording devices used in behavioural studies have been adapted for use in the study of insect activity. The activity of Blatta orientalis L. was recorded on a revolving smoked drum by Gunn (1940), using an activity chamber which tilted when the insect moved. Miller (1929), attached a fly maggot to the writing lever of a kymograph. The body contractions at different temperatures were recorded on smoked paper. The number of contractions increased as temperature increased and since the contractions are responsible for locomotion indicated that locomotion speed increased with temperature.

Electrical methods have been devised to record insect activity (Brown, 1959). Photographic technology has also been utilized in monitoring insect activity over long periods of time (Edwards, 1959). These techniques are advantageous because they record with little disturbance to the animal.

Watters (1969), used emigration of C. ferrugineus from a 100 ml beaker as a criterion of locomotor activity. In testing temperature, moisture, population density, and time allowed for emigration he found that moisture was the dominant physical factor influencing locomotor activity of this species in wheat at 15^oC, 22^oC, and 28^oC.

Insect activity at any one moisture was directly related to temperature. Densities of 5 - 50 insects per 100 ml of wheat did not influence emigration.

"The increase in respiratory rate with rising temperature is perhaps the most over-confirmed fact in insect physiology" (Keister and Buck, 1964). They present numerous examples of recorded respiratory levels of several insect species of different weights, and sexes at different developmental stages and temperatures. Birch (1947), used the Warburg method for measuring the rate of oxygen consumption of adults and larvae of C. oryzae L., and R. dominica Fab. He recorded that oxygen consumption for both adults and larvae of each species increased with increasing temperature. Maximum oxygen consumption of both larvae and adults of C. oryzae reached a maximum at 32°C, while those of R. dominica reached a maximum at 38°C. With both insects these temperatures were 2°C higher than the temperatures at which the insects developed from egg to adult in the shortest time (Birch, 1945). Dehnell and Segal (1956), using a Wennesland-Scholander micro-respirometer demonstrated that cold-acclimated cockroaches consume more oxygen per gm of body weight than warm-acclimated cockroaches of equal weight.

Somme (1968), using the Kirk respirometer recorded that cold-acclimated adults of Tribolium confusum Duval respired at a lower rate than warm-acclimated adults. He suggests that cold-acclimated T. confusum which respire at a low level are poorly adapted for activity at low temperatures.

Several workers have measured the respiration rates of insects contained in bags, tubes, cages, or capsules. A search of the literature has not produced references to work on the effect of temperature on both respiration and locomotion rates in the same insect.

Materials and methods

Respiration: The oxygen consumed and distance travelled by rusty grain beetle adults 23 - 30 days old were measured in the laboratory at 1.1°C, 8.9°C, 15.6°C, 21.1°C and 30°C and 65 ± 10 percent relative humidity.

The adults were equilibrated at the experimental conditions for a period of 24 hours immediately prior to testing.

Oxygen consumption was measured in a Gilson Differential Respirometer. Each test was replicated twice at each temperature, using 100 adults per test. Two respirometer flasks containing all materials except the insects were used as controls. The CO₂ absorbing chambers were filled with 10 percent KOH, and supplied with filter paper wicks 1/2 inch long to increase the CO₂ absorbing surface. The insects were placed into tygon tube cages 1.5 cm long and 0.9 cm in diameter. The ends of the tubular cages were covered with 100 mesh nylon bolting cloth to permit air movement. The insects were placed into the cages via a funnel inserted into a slit through the wall. A loaded cage was placed horizontally on the floor of each flask. The flasks were

lowered into the water bath and equilibrated for 15 min., then the apparatus was closed to the external environment and allowed to equilibrate for another 15 min. The manometer fluid was brought to zero and the reading on each manometer recorded. Readings were taken every hour for 4 hours after which the insects were removed and weighed. The oxygen consumption at each temperature was recorded as ml of O_2 consumed/gm wt body wt/hr.

Locomotion: The locomotory behaviour and distances travelled by adults of the rusty grain beetle were measured in controlled environment rooms maintained at temperatures ranging from $1.1^{\circ}C$ to $30^{\circ}C$. Since the rusty grain beetle is negatively phototactic, observations were made under dim red light to minimize the possibility that light may affect their behaviour.

The paths of individual adult insects walking on Kraft paper overlying a 26 x 20 inch horizontal glass plate was traced with a pencil at a distance of about 1/2 inch behind the insect for 2 minutes or for a shorter period if the insect left the tracing surface sooner. The materials were equilibrated under the conditions of the experiment for one hour prior to testing. There were two replicates each with 10 to 15 insects traced at each temperature. The length of the lines, representing the distance travelled by each insect was measured with a map meter. The data recorded in terms of distance per unit of time at each temperature.

Results

Effect of temperature on the respiration and locomotion of C. ferrugineus. The role of temperature in regulating respiration rate and locomotor activity was clearly demonstrated (Table I, Fig. 1). The rate of respiration increased gradually from 1.1⁰C and 15.6⁰C then rose sharply from 15.6⁰C to 30⁰C. The rate of locomotion increased at a constant rate in relation to temperature (Table I, Figure 1). The rates of increase for respiration and locomotion within the range 15.6⁰C and 30⁰C are parallel, indicating that both increased at the same rate. The regression analysis and coefficients of determination for respiration (97 percent) and locomotion (93 percent) indicate that both are directly related to temperature.(Figure 2). Consequently, respiration rate can be correlated with locomotion and either one used as an indicator of the effect of temperature on the biological activity of this insect. Extension of the plotted graph for locomotion at the lower end in Figure 1 indicate that locomotion ceases at approximately 2⁰C. Prediction of the temperature at which respiration ceases does not appear possible from the graphs in Figure 1 and 2. The straight line relationship at the lower end of the respiration lines does not hold. The insects would be killed at a higher temperature than the extrapolated lines would indicate.

The reduction in distance travelled, in response to temperature change by C. ferrugineus adults is visually apparent upon examination of some actual traced paths manoeuvred by adult insects at the 4

TABLE I
 THE INFLUENCE OF TEMPERATURE ON THE RATES OF RESPIRATION AND LOCOMOTION OF C. ferrugineus

Temperature °C	No. of groups tested	<u>Respiration</u>		<u>Locomotion</u>	
		Mean rate of respiration ul/gm/hr	Number of insects tested	Mean rate of locomotion; mm/sec **	±S.E.
1.1	11	338.53 ± 12.82	25	1.75 ± 0.07	
8.9	11	692.20 ± 19.10	23	3.53 ± 0.13	
15.6	10	807.92 ± 15.00	11	6.46 ± 0.32	
21.1	12	1596.35 ± 35.15	30	10.48 ± 0.40	
30.0	9	3462.68 ± 55.00			

* 100 insects per group

** Respiration was measured at hourly intervals for 4 hours.

S.E. - Standard Error

FIGURE 1
INFLUENCE OF TEMPERATURE ON THE RESPIRATION
AND LOCOMOTION RATES OF
C. ferrugineus

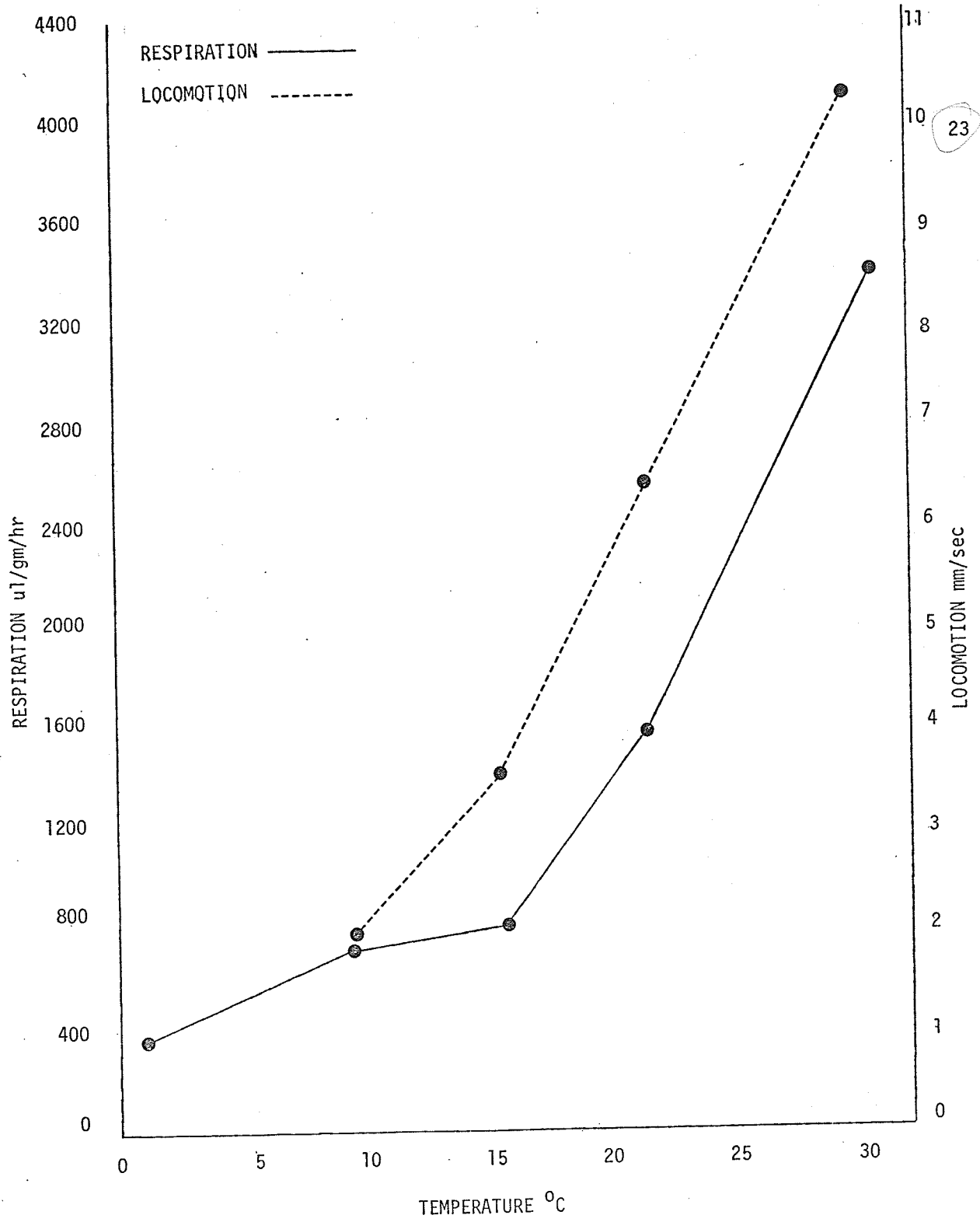


FIGURE 2
RELATIONSHIP BETWEEN RESPIRATION,
LOCOMOTION AND TEMPERATURE FOR
C. ferrugineus

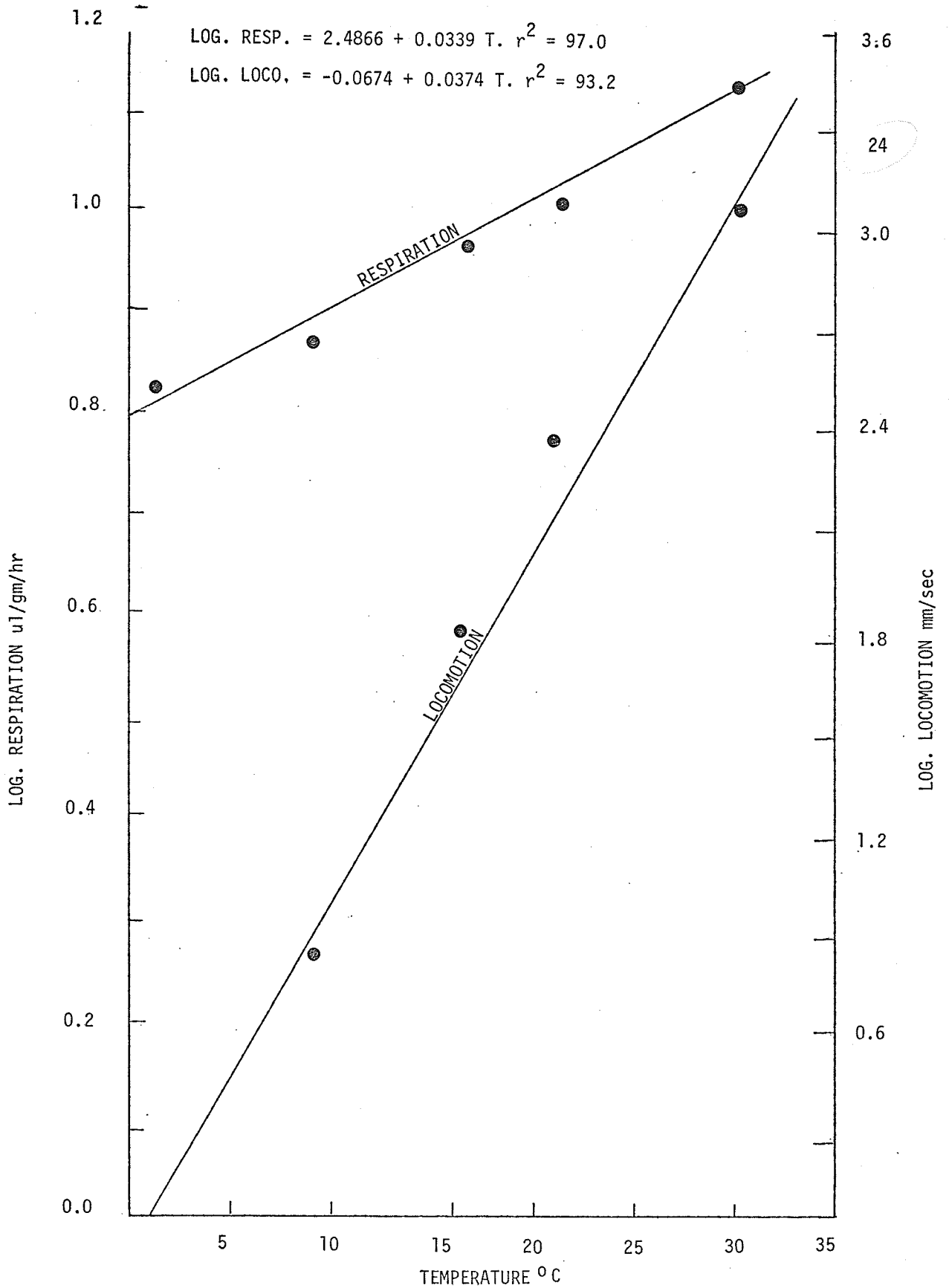
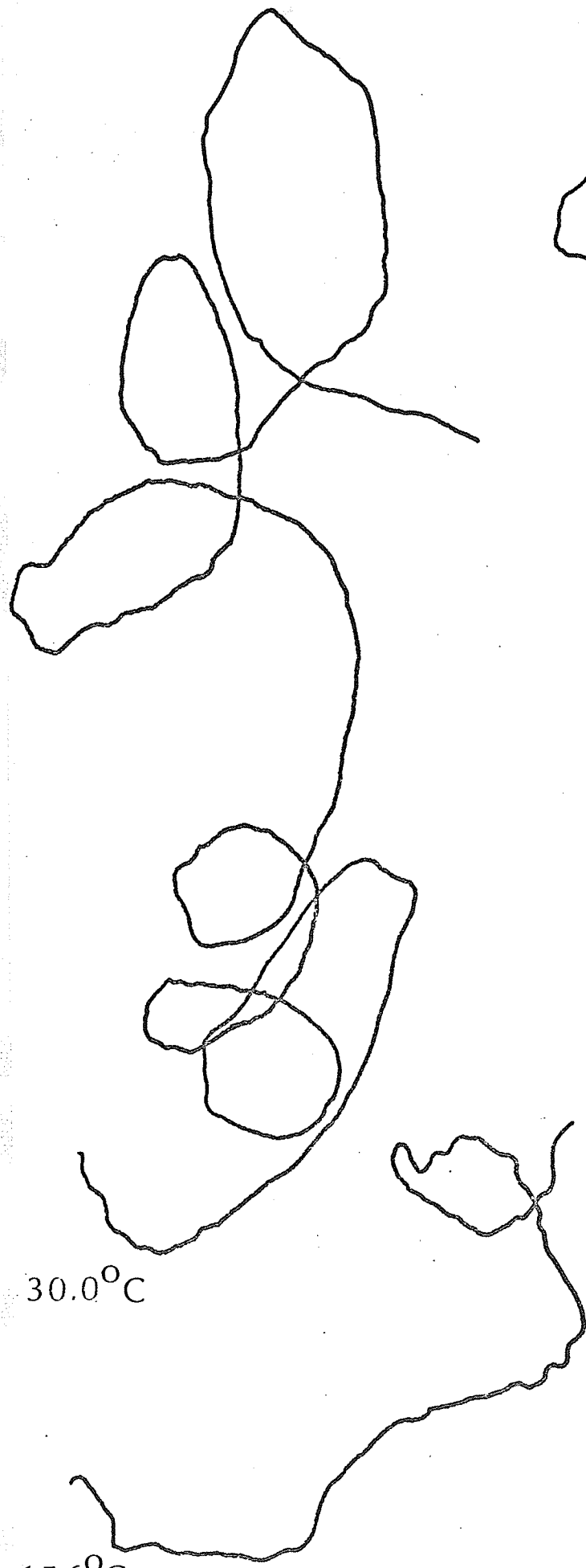
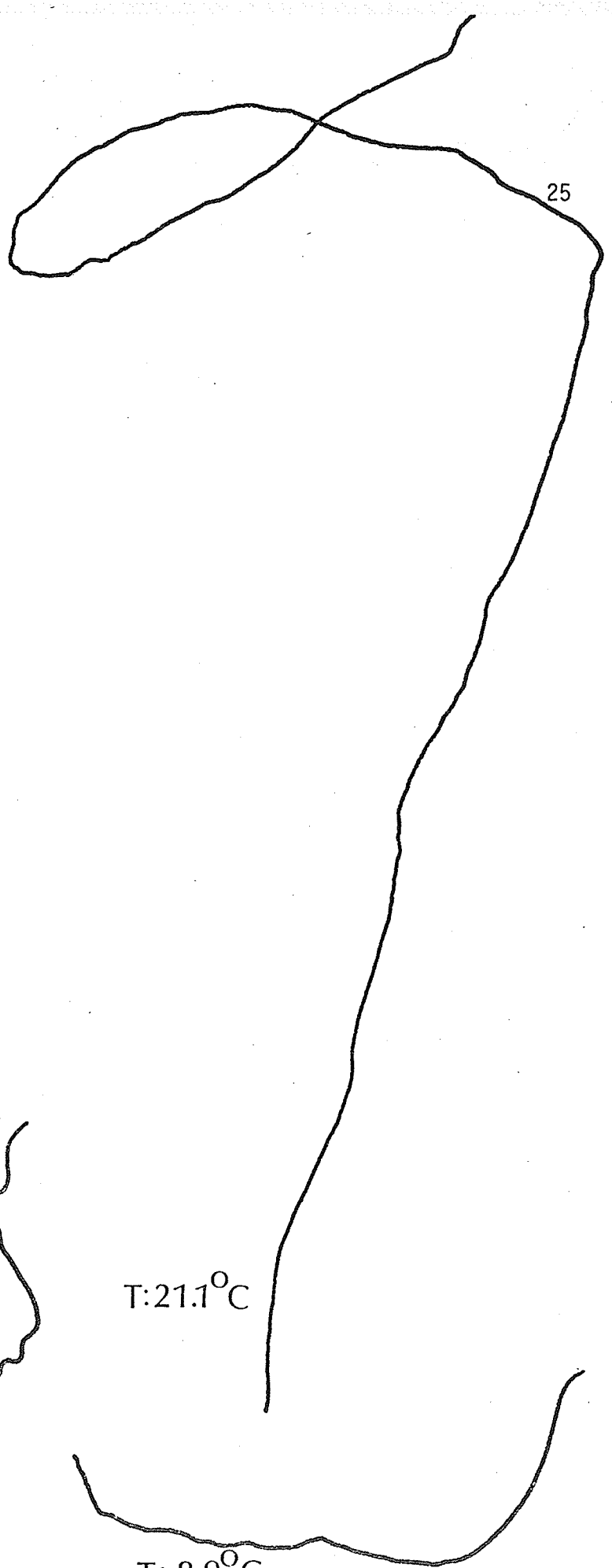


FIGURE 3
PATHS TRAVELLED BY C. ferrugineus IN A ONE MINUTE
PERIOD AT VARIOUS TEMPERATURES



TIME: 1MINUTE



experimental temperatures (Figure 3).

Discussion

The locomotion-temperature response appears to be almost a straight line relationship. These results agree with the type of results reported by Mellanby (1939), for the bed bug, Cimex lectularius L., he found that above the chill-coma temperature healthy bugs moved at approximately the same speed, and within limits the higher the temperature the higher the speed. According to Perttunen and Paloheimo (1964), this simple correlation between temperature and the velocity of an insect's movement is not common. Work by Henson (1964), studying Conophthorus coneperdus, and Perttunen and Paloheimo (1964) studying Tenebrio molitor L. showed that there was a middle temperature range within which the speed of movement did not change when the temperature was raised. In C. coneperdus the middle range was between 20°C and 25°C, while in T. molitor it was between 20°C and 30°C. As temperature increased to the lower limit of the middle temperature range locomotion increased and above the upper limit of the middle temperature range locomotion increased as temperature increased, but between these temperatures locomotion did not increase.

The respiration-temperature response of C. ferrugineus where respiration increased gradually from 1.1°C to 15.6°C and then rose sharply from 15.6°C to 30°C resembles that found by many workers for other insects. For example Kiester and Buck (1964), list a number of

insect species which show the typical respiration-temperature curve. They found that the absolute respiratory rate was low at low temperatures, increased rapidly through the middle range temperatures, then dropped sharply as lethal temperatures were reached. The curve in Figure 1 follows this description closely; however, it does not approach the lethal temperature.

Birch (1947), recorded the respiratory rate of Rhizopertha dominica Fab. over a wide range of temperature. The adults were confined in a bag that contained wheat as food. The insects were free to move, but the interaction between the grain and insect may have affected the respiratory rate.

In the present experiment the insects were caged but free to move. Interaction among the insects may have affected respiration to some degree.

The humidity during the experiment was not controlled but since the experimental apparatus was kept continuously in the same room relative humidity probably remained constant from test to test. Birch (1947), recorded that the effect of humidity on the respiration rate of Calandra oryzae L. and R. dominica was negligible. Gunn and Cosway (1938), recorded similar results for the cockroach Blatta orientalis L.

The results of respiration experiments were presented as ul/gm/live weight of 100 insects/hr at any given temperature. Kiester and Buck (1964), advocate wet body weight over dry body weight as an

expression of metabolic rate. Investigations of many insects has shown a directly proportional relationship between respiration and body weight. Consequently, the rate of respiration has been expressed as μl per weight, rather than μl per insect since the groups of 100 insects used in the tests were of differing weights.

Prior to this experiment, the biological relationship of respiration and locomotion in C. ferrugineus had not been compared to determine the relationship between the two biological processes. The relationship presented may or may not be consistent in beetle populations. However, in individual experiments with respiration or locomotion, there is a definite tendency for the rates of both these activities to increase with rising temperature.

Experiments concerning locomotion have been conducted primarily by physiologists and behaviourists to determine the theoretical relationships between insect activity and environmental stimuli. Few economically orientated studies concerning the relationship between temperature and locomotion have been recorded.

This experiment indicates that locomotor activity increases with rising temperatures. Since the capture of beetles in traps depends on insect locomotion, more C. ferrugineus should be trapped at 30°C than at lower temperatures.

The experiment reported in this chapter suggests that if traps were present at temperatures near 2°C , insects would not move, therefore they would not be trapped. The experiment was not conducted in

the presence of actual grain kernels, therefore, the theory must be tested in a three dimensional grain system to determine whether this simple temperature-locomotion relationship can be substantiated. Chapter V documents laboratory experiments performed to test the effect of temperature on the number of insects trapped in grain.

CHAPTER V
LABORATORY STUDIES OF SOME FACTORS AFFECTING THE
NUMBERS OF C. ferrugineus ADULTS CAUGHT BY TRAPS PLACED IN ONE-
GALLON JARS OF WHEAT

Introduction

Insect activity is controlled by environmental stimuli. Either individually or in combination, temperature, moisture, insect density and food regulate insect distribution. The part played by each of these factors in regulating insect distribution is complex and not clearly understood.

The data presented in Chapter IV (Table I) showed that the rate of locomotion of C. ferrugineus on a flat surface is controlled by temperature. However, the environment of a grain bulk is not as discrete or controllable a system as the laboratory and the effects of other environmental factors could possibly influence insect activity, off-setting the simple temperature-locomotion relationship.

Field bulk grain systems are continuously changing due to variations in the external and internal environments of the bulk. Insects respond to the changes either through acclimation or relocation. The changes in locomotor activity in response to environmental stimuli would be of importance in determining proper usage of insect traps.

The purpose of the laboratory study described in this chapter is to determine by trapping, the movement of C. ferrugineus in wheat over

1, 2, and 3 day intervals. Temperature and moisture of the wheat, plus the population densities were varied to determine the importance of each factor, and the importance of the interaction of these factors in controlling movement.

Review of the literature

Few trapping devices have been used to study the response of insects to temperature, moisture and insect density over a given time period. Watters and Cox (1957), designed a pit-fall trap for the detection of stored product insects moving on the surface of the grain. They placed empty and water-filled jars into the grain so that the lips of the jars were level with the grain surface. Results indicated that the traps captured a few specimens of the common insects present in the infested grain. Watters (1964), used this same technique for studying the locomotor activity of the hairy spider beetle, Ptinus tectus Boie.

Graham (1962), designed an efficient trap for removing Tribolium castaneum (Hbst.) from grain samples and also for sampling large stocks of bagged maize. The trap consisted of a vertical glass vial contained in a cover of wire gauze that provided a small pointed platform over its open top. Insects were trapped when they passed through the wire gauze into the vial.

Watters (1964), determined by tube traps, the spatial distribution of C. ferrugineus in boxes of wheat at different moisture contents

after measured time intervals. The tube traps consisted of pyrex tubes in different locations extending upwards from the bottom of boxes to various heights. The openings of the tubes were covered with wire mesh which allowed the passage of insects but not grain into the tube and down into the collecting area at the bottom of the box. He recorded that locomotor activity in wheat at all moisture contents decreased with time.

Watters (1969), studied the interaction between temperature, grain moisture, insect density and time on the emigration of C. ferrugineus from 100 ml beakers of wheat. He showed that insect emigration was directly related to temperature and that more insects emigrated from dry than from damp wheat.

Materials and methods

Three 128-oz jars each containing approximately 5.75 lbs No. 2 Northern wheat of 16.3 percent moisture content and 3 containing wheat of 13.7 percent moisture content were set up in each of 4 cabinets maintained at constant temperatures of 8.9^oC, 15.6^oC, 21.1^oC and 30^oC, respectively, at 3 levels of infestation of 100, 250 and 400 respectively, and for 3 time periods of 1, 2, and 3 days respectively.

Loschiavo and Atkinson (1967), designed a device for the detection of stored grain insects (Figure 4 and 6). The device was a cylindrical brass tube 7.6 inches long, tapered to a point at one

FIGURE 4
INSECT TRAP DESIGNED BY LOSCHIAVO AND ATKINSON, 1967.

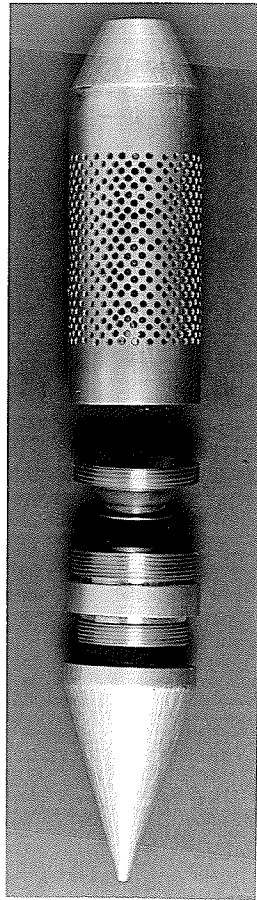


FIGURE 5

INSECT DETECTING TRAP DESIGNED BY LOSCHIAVO AND ATKINSON, 1969.

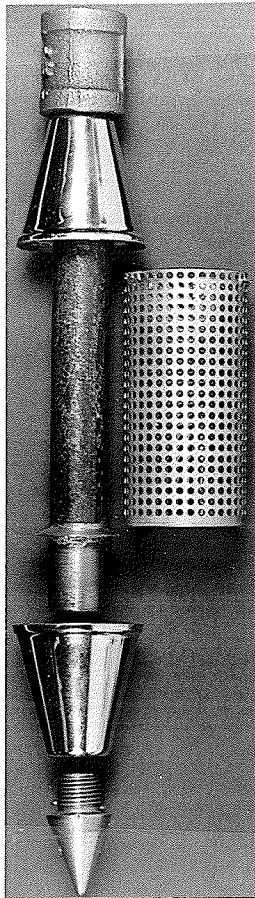
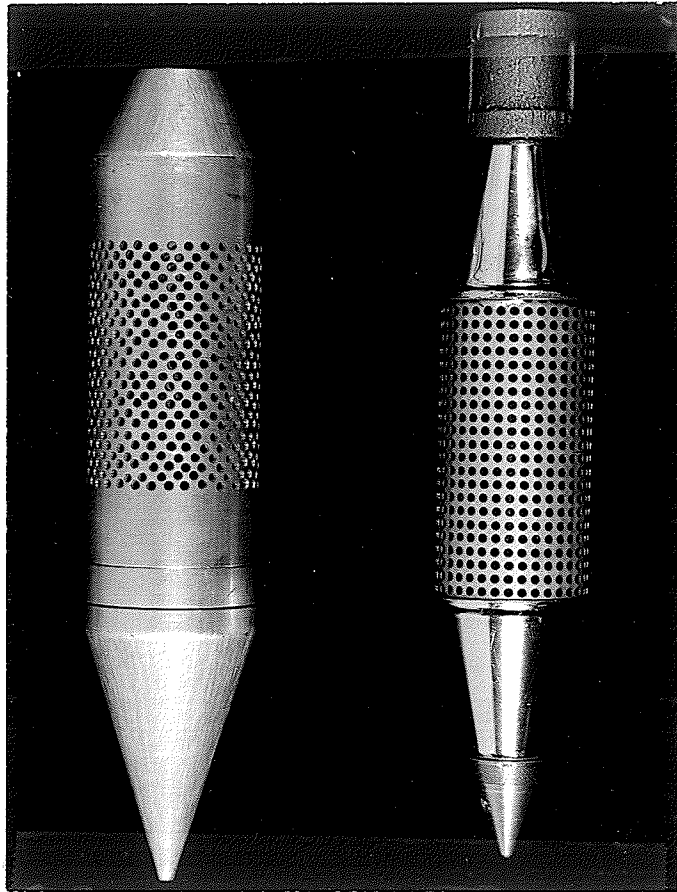


FIGURE 6
COMPARISON OF THE INSECT-DETECTING TRAPS.



end, and threaded at the opposite end so that extension rods could be attached to the trap for probing in deep bins. The central section of the trap was perforated with holes small enough to exclude grain kernels, yet large enough to allow the entry of insects. Insects entering the trap through the perforations in the wall pass down through the trap to a holding chamber near the bottom. The insects are prevented from escaping by a flange around the entrance to the holding chamber. In 1969, a simplified model of the device operating on the same principle as the original device was described (Figure 5 and 6). A central brass screen was terminated at both ends by a 1.5 inch long cone. The cones and screen were assembled around a central steel shaft. Insects entering the trap were collected in the lower cone; escape was prevented by a flange located at the upper end of the cone, and on the central shaft. Insects were removed from the trap by unscrewing the lower cone. Throughout the remainder of this thesis, this device will be referred to as the trap.

The bulletin introducing the revised trap claimed that escape from the trap is prevented by the 2 flanges located in the trap.

Tests in the laboratory showed that some C. ferrugineus adults could manoeuver around the flange on the centre post. Tests also showed that the flange positioned at the top of the nose cone was not completely escape-proof. Actually, it was the smoothness of the walls of the nose cones that prevented the insects from climbing up to the top of the cone and escaping. Casual observations revealed

that after extensive use in the field, the smooth finish on the nose cone tarnished and consequently some insects were able to climb the walls and escape. It was observed that escape from the trap was prevented by the application of polytetrafluoroethylene (Fluon) (Imperial Chemical Industries, Welwyn Garden City, Herts, England) to the inner surface of the cone, and on the lower section of the central steel shaft. Therefore, Fluon-coated traps were used in all laboratory experiments.

A trap was inserted vertically into each jar 24 hours prior to experimentation, to allow temperature equilibration. Adults of C. ferrugineus of mixed age were taken from a stock culture, counted into lots of 100, 250 and 400, and conditioned in the respective cabinets for at least 1 hour. Then they were sprinkled into each of the jars at the different moisture contents and temperatures. The jars were covered loosely with steel lids to keep moisture loss to a minimum. To maintain constant light conditions, each jar was placed in a double-walled Kraft paper bag. The experiment was replicated 3 times.

The traps were left in the grain for 1, 2, or 3 days respectively. The number of insects in each trap was counted and recorded at the end of each time period. The insects remaining in the grain were removed by sifting over a 10-mesh sieve. The moisture content of the grain was measured at the end of each experiment to determine the amount of water loss. The grain was reconditioned if and when moisture

content varied more than 0.5 percent. The grain in the jars was completely changed after 25 - 26 days at 30°C and, at appropriate times at the other temperatures to prevent the occurrence of second generation adults (see Smith, 1959).

The number of insects found in each trap was expressed as a percentage of the total in each jar. Each percentage was subjected to a square root transformation (Steele and Torrie, 1960) and factorial analysis of variance. Due to heterogeneity of error variance, the error sum of squares was sub-divided into sources corresponding to each factorial effect. Each factorial effect was tested against its corresponding error mean square.

Results

The effect of temperature, moisture and insects on the number of insects trapped after 1, 2 and 3 days. Temperature was the primary factor influencing the locomotor activity of C. ferrugineus adults in grain as indicated by the numbers caught in the trap (Table II, Figures 7 - 9). With the exception of the 1 day trapping period in grain at 16.3 percent moisture content and containing a population of 100 beetles, more beetles were caught in traps at 30°C than at any of the lower temperatures in jars containing grain at moisture contents of 16.3 and 13.7 percent (Table II, Figure 7).

Analysis of the transformed (\sqrt{m}) data supported the results showing that temperature significantly influenced the number of C. ferrugineus trapped for the 1 and 2 day periods but was not a

TABLE II

THE NUMBER OF *C. ferrugineus* ADULTS CAUGHT IN TRAPS AT INTERVALS OF ONE, TWO AND THREE DAYS AT POPULATION DENSITIES OF 100, 250, 400 ADULTS PER JAR AT TEMPERATURES OF 8.9°C, 15.6°C, 21.1°C AND 30°C AT GRAIN MOISTURE CONTENTS OF 13.7 AND 16.3 PERCENT

Population Density	Trapping Time (Days)	Temperature °C								
		8.9	15.6	21.1	30.0					
		13.7	16.3	13.7	16.3	13.7	16.3	13.7	16.3	
	1	1	6	6	11	16	12	9	11	
		3	15	25	12	6	2	26	3	
		3	2	5	15	7	3	37	8	
		\bar{x} -S.E.*	2.3 [±] 1.2	7.7 [±] 3.8	12.0 [±] 6.5	12.7 [±] 1.2	9.7 [±] 3.2	5.7 [±] 3.2	24.0 [±] 8.1	7.3 [±] 2.3
100	2	1	0	6	20	17	13	27	31	
		0	0	4	2	7	3	47	16	
		2	0	9	5	19	13	36	34	
		\bar{x} -S.E.	1.0 [±] 0	0.0 [±] 0.0	6.3 [±] 1.5	9.0 [±] 5.6	14.3 [±] 3.7	9.7 [±] 3.3	37.0 [±] 5.2	27.0 [±] 5.6
	3	4	7	5	2	23	12	42	22	
		8	4	24	17	19	17	15	14	
		5	20	10	15	24	6	19	12	
		\bar{x} -S.E.	5.7 [±] 1.2	10.3 [±] 4.9	13.0 [±] 5.7	11.3 [±] 4.7	22.0 [±] 1.5	11.7 [±] 3.2	25.3 [±] 8.4	16.0 [±] 3.0

TABLE II CONTINUED

THE NUMBER OF *C. ferrugineus* ADULTS CAUGHT IN TRAPS AT INTERVALS OF ONE, TWO AND THREE DAYS AT POPULATION DENSITIES OF 100, 250, 400 ADULTS PER JAR AT TEMPERATURES OF 8.9°C, 15.6°C, 21.1°C AND 30°C AT GRAIN MOISTURE CONTENTS OF 13.7 AND 16.3 PERCENT

Population Density	Trapping Time (Days)	Temperature °C								
		8.9	15.6	21.1	30.0					
		Moisture content of grain in jars								
		13.7	16.3	13.7	16.3	13.7	16.3	13.7	16.3	
	1	11 13 5	11 4 3	38 51 11	18 39 41	39 50 22	16 27 13	54 49 75	26 20 24	
		\bar{x}^{\pm} S.E.	9.7 [±] -2.4	6.0 [±] -2.5	33.3 [±] -11.8	32.7 [±] -7.4	37.0 [±] -8.1	18.7 [±] -4.3	59.3 [±] -8.0	23.3 [±] -1.8
250	2	11 8 4	3 0 3	32 30 11	18 3 12	36 10 29	18 12 20	33 96 63	13 11 32	
		\bar{x}^{\pm} S.E.	7.7 [±] -2.0	2.0 [±] -1.0	24.3 [±] -6.7	11.0 [±] -4.4	25.0 [±] -7.8	16.7 [±] -2.4	64.0 [±] -18.2	18.7 [±] -6.7
	3	13 13 32	24 10 16	31 31 33	15 50 27	43 35 39	19 30 55	87 50 11	49 74 28	
		\bar{x}^{\pm} S.E.	19.3 [±] -6.3	16.7 [±] -4.3	31.7 [±] -0.7	30.7 [±] -10.3	30.0 [±] -2.3	34.7 [±] -10.7	49.3 [±] -21.0	50.0 [±] -13.0

TABLE II CONTINUED

THE NUMBER OF C. ferrugineus ADULTS CAUGHT IN TRAPS AT INTERVALS OF ONE, TWO AND THREE DAYS AT POPULATION DENSITIES OF 100, 250, 400 ADULTS PER JAR AT TEMPERATURES OF 8.9°C, 15.6°C, 21.1°C AND 30°C AT GRAIN MOISTURE CONTENTS OF 13.7 AND 16.3 PERCENT

Population Density	Trapping Time (Days)	Temperature °C								
		8.9	15.6	21.1	30.0					
		Moisture content of grain in jars								
		13.7	16.3	13.7	16.3	13.7	16.3	13.7	16.3	
400	1	12	9	17	66	37	47	82	48	
		7	9	33	35	37	47	99	22	
		2	7	32	50	50	51	113	38	
		\bar{x}^{\pm} S.E.	7.0 ⁺ -2.9	8.3 ⁺ -0.6	27.3 ⁺ -5.2	50.0 ⁺ -9.0	41.0 ⁺ -4.3	48.0 ⁺ -1.3	98.0 ⁺ -9.0	36.0 ⁺ -7.6
400	2	19	13	12	71	31	18	113	54	
		5	13	10	17	37	21	185	38	
		3	7	14	32	92	35	198	84	
		\bar{x}^{\pm} S.E.	9.0 ⁺ -5.0	11.0 ⁺ -2.0	12.0 ⁺ -1.2	40.0 ⁺ -16.1	53.3 ⁺ -19.4	24.7 ⁺ -5.2	165.3 ⁺ -26.4	58.7 ⁺ -13.5
400	3	17	4	3	28	76	37	151	83	
		20	12	27	51	61	49	178	89	
		40	63	94	51	73	76	109	58	
		\bar{x}^{\pm} S.E.	25.7 ⁺ -7.2	26.3 ⁺ -18.5	44.7 ⁺ -25.0	43.3 ⁺ -7.7	70.0 ⁺ -4.6	54.0 ⁺ -11.5	146.0 ⁺ -20.1	80.0 ⁺ -11.0

* Mean ± standard error

ANALYSIS OF VARIANCE OF TRANSFORMED DATA FOR NUMBER OF *C. ferrugineus* TRAPPED AT INTERVALS OF

1, 2 OR 3 DAYS, AT TEMPERATURES OF 30°C, 21.1°C, 15.6°C, AND 8.9°C AT POPULATION DENSITIES OF 100, 250 AND 400 ADULTS, AT MOISTURE CONTENTS OF 13.7 AND 16.3.

Source	d.f.	1 Day		2 Days		3 Days	
		M.S.	F.	M.S.	F.	M.S.	F.
Total	71						
Treatments	23						
Numbers (N)	2	0.00269123	0.88	0.01492185	2.39	0.00089386	0.12
Temperatures (T)	3	0.15287139	13.40**	0.43238048	27.03**	0.14112610	3.89
Moisture (M)	1	0.03632410	4.05	0.15827814	14.80	0.02483106	3.39
N x T	6	0.00626423	1.23	0.02112639	3.52*	0.00626540	0.95
N x M	2	0.00704627	1.43	0.00417702	1.38	0.003478 6	2.30
T x M	3	0.05404583	7.29*	0.02124980	2.49	0.00843591	2.12
N x T x M	6	0.00436737	0.89	0.00908314	1.47	0.00617237	0.82
Replicates (R)	2						
Error	46						
N x R	4	0.00305842		0.00623750		0.00704105	
T x R	6	0.01140549		0.01599630		0.03626360	
M x R	2	0.00897807		0.01069134		0.00733208	
N x T x R	12	0.00509075		0.00598766		0.00658713	
N x M x R	4	0.00490946		0.00301779		0.00151146	
T x M x R	6	0.00740926		0.00853003		0.00396858	
N x T x M x R	12	0.00491768		0.00614105		0.00751133	

* Significant at 0.05% level

** Significant at 0.01% level

FIGURE 7

THE INFLUENCE OF TEMPERATURE ON THE MEAN CUMULATIVE
PERCENTAGE OF C. ferrugineus ADULTS COLLECTED IN
TRAPS AFTER A ONE DAY TRAPPING PERIOD.
(RESULTS AT DIFFERENT DENSITIES COMBINED).

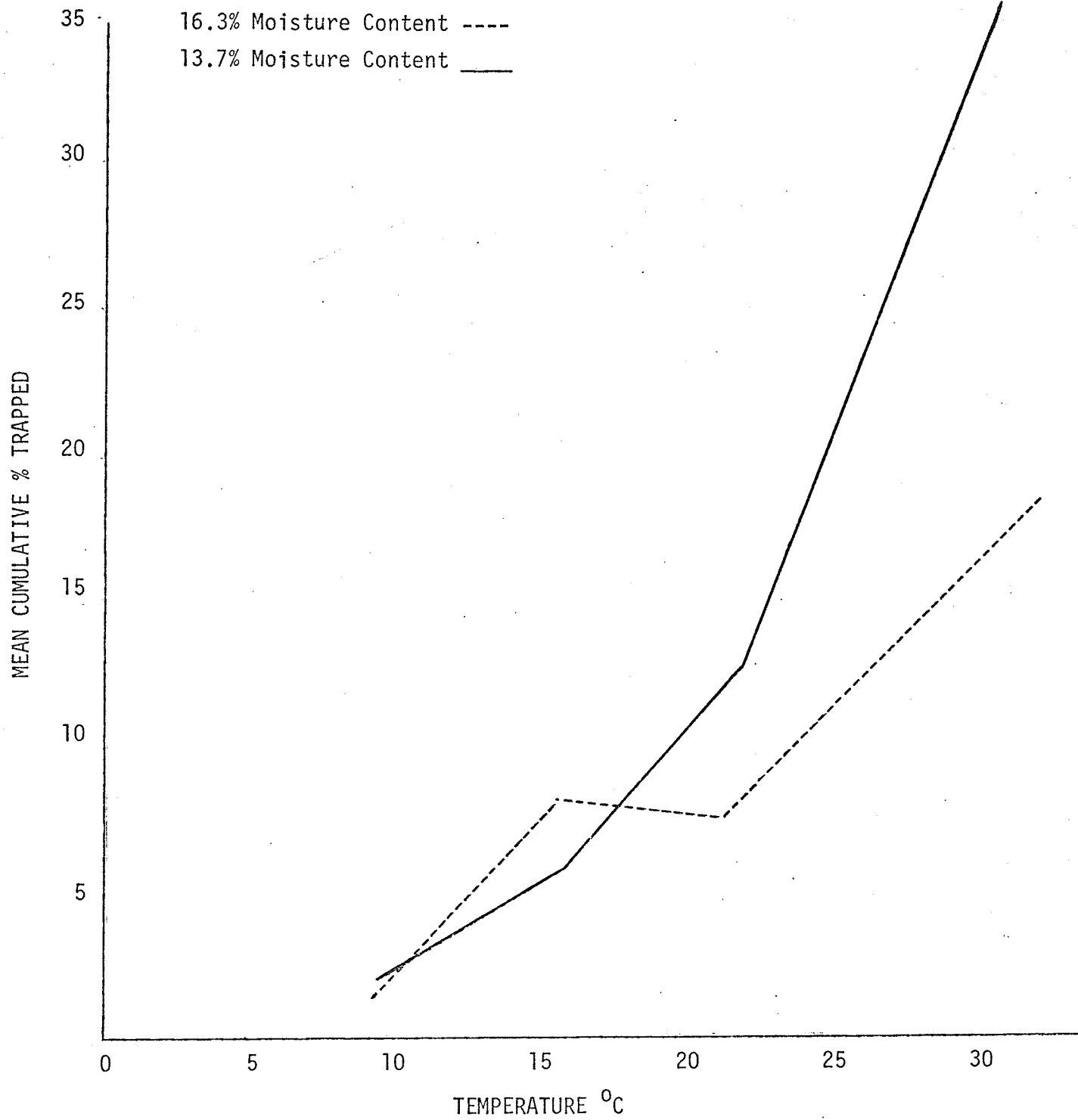


FIGURE 8

THE INFLUENCE OF TEMPERATURE ON THE MEAN CUMULATIVE
PERCENTAGE OF C. ferrugineus ADULTS COLLECTED IN
TRAPS AFTER A TWO DAY TRAPPING PERIOD.
(RESULTS AT DIFFERENT DENSITIES COMBINED).

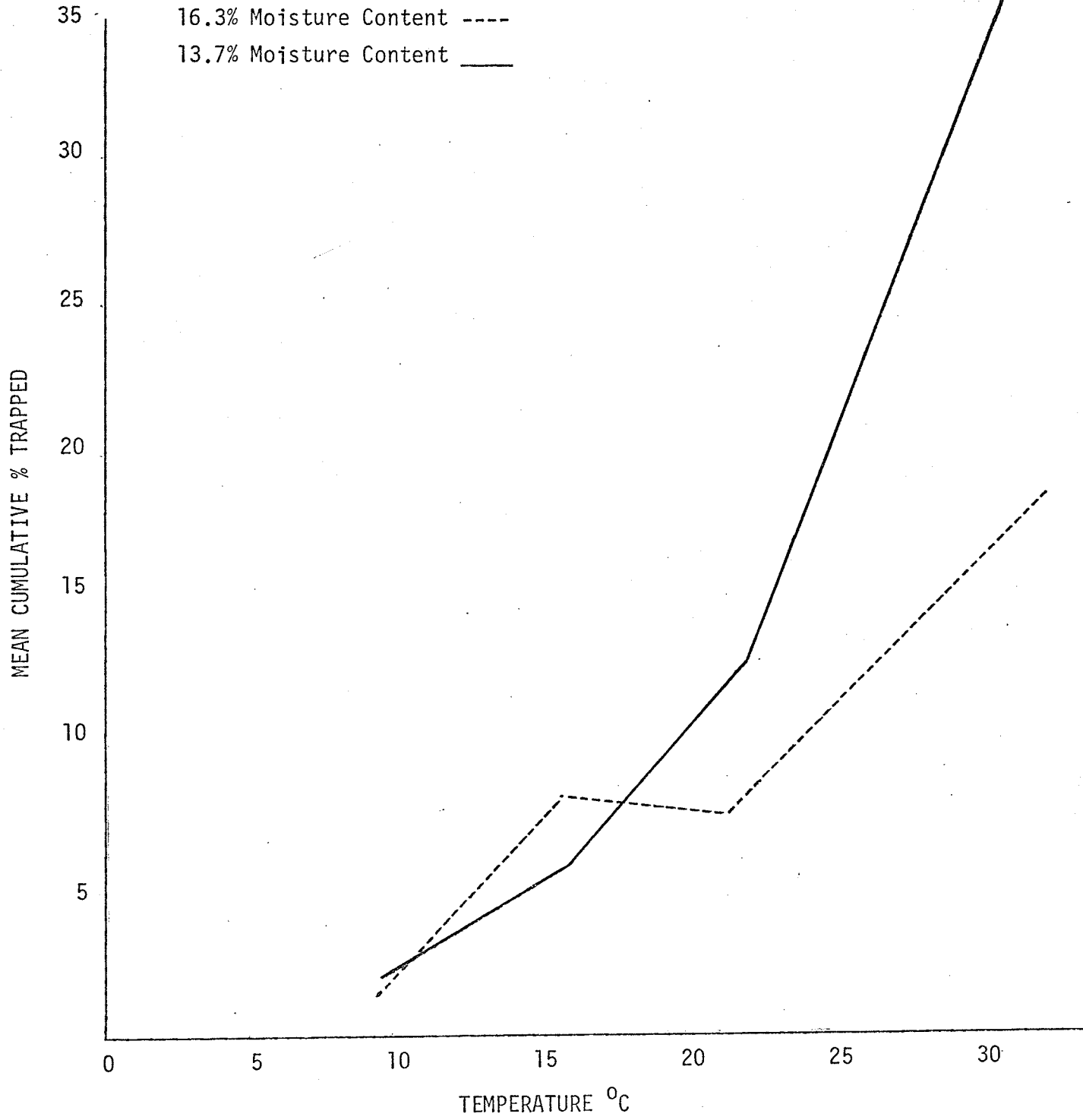
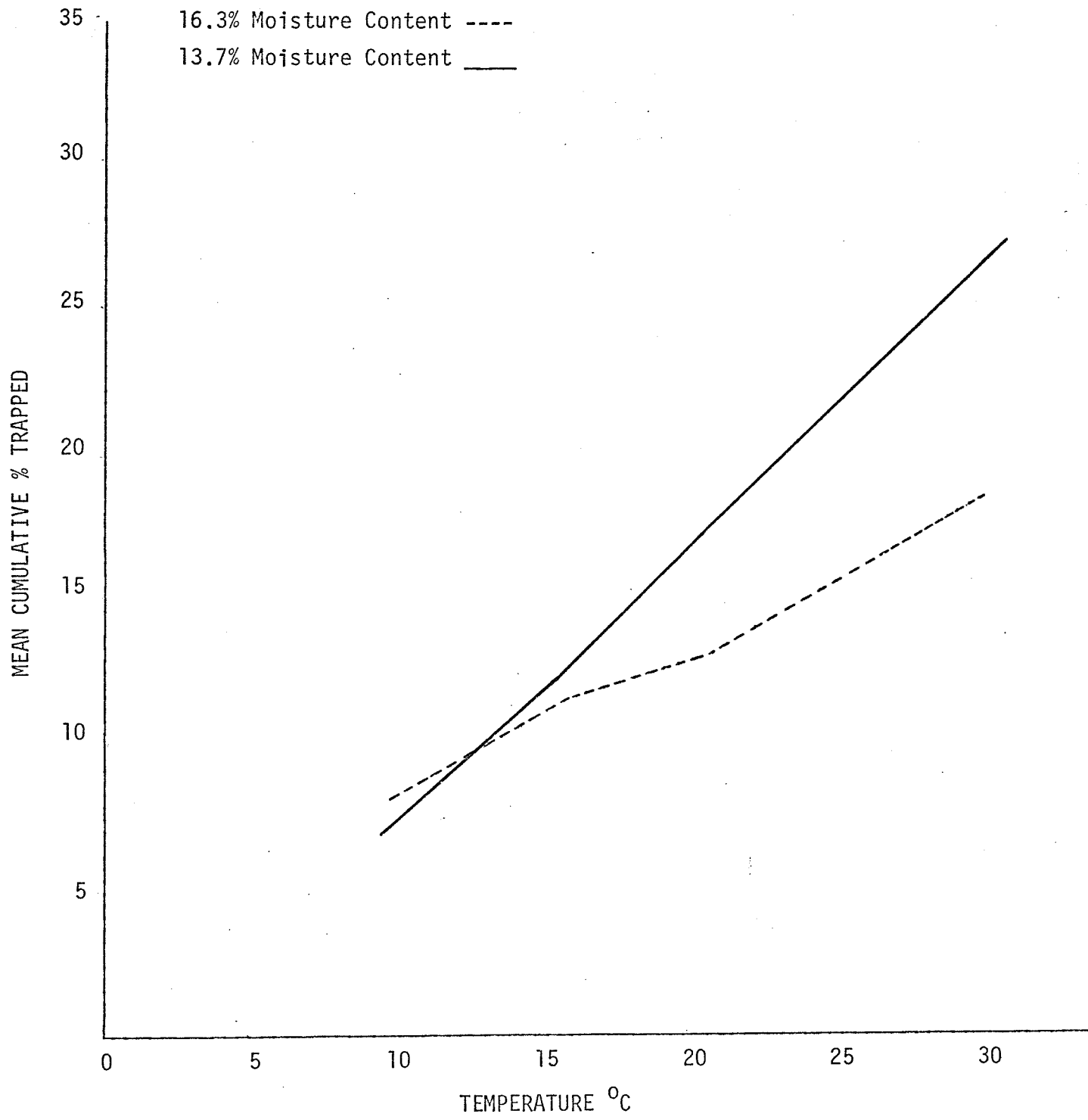


FIGURE 9

THE INFLUENCE OF TEMPERATURE ON THE MEAN CUMULATIVE
PERCENTAGE OF C. ferrugineus ADULTS COLLECTED IN
TRAPS AFTER A THREE DAY TRAPPING PERIOD.
(RESULTS AT DIFFERENT DENSITIES COMBINED)



significant factor influencing the number trapped for the 3 day trapping period (Table III). The lack of significance for temperature at the 3 day trapping period may be due to a high temperature replicate interaction, due to the low percentage trapped at 30⁰C and 16.3 percent moisture content for the third replicate.

Statistically moisture content did not influence the number of insects trapped in any test, but at higher temperatures more insects were caught in grain at 13.7 than 16.3 percent moisture content (Table III, Figures 7 - 9). The effect of moisture does appear to be a factor which is more important at high temperatures (30⁰C) than at low temperatures (8.9⁰C).

It was thought that mutual stimulation of insects crowded together at the highest density would result in a higher percentage of insects being trapped than at the lowest density. The data (Table II) and analysis of the data (Table III) show that population density did not influence the number of insects caught in traps. Although more insects were caught as density increased, the percentage trapped (number of insects caught/number of insects present) did not differ significantly when the jars were at the same temperature, contained grain of the same moisture content and were trapped for the same length of time.

There was no significant difference between the percentages of insects trapped after 1, 2, or 3 day trapping periods (Figures 7 - 9).

Analysis of variance (Table III) showed that there was a sig-

nificant interaction ($P=0.05$) between temperature and moisture for the 1 day trapping period. The 2 day trapping data indicated a significant interaction ($P=0.05$) between temperature and insect density. Since trends are of primary concern in this experiment, no explanation is given for these interactions.

Discussion

The results show that adults of C. ferrugineus are more active at 30°C than at lower temperatures. In general, the numbers of insects trapped increased as temperature increased (Tables II and III). This pattern of increasing numbers of insects being trapped as temperature increased was more stable in grain at 13.7 percent moisture content than in grain at 16.3 percent moisture content. These results basically confirm the conclusion reached in Chapter IV that locomotor activity of adults of C. ferrugineus is regulated primarily by temperature.

Watters (1969), recorded a similar temperature locomotor relationship for C. ferrugineus. His results, obtained by recording the number of insects emigrating from the surface of wheat in 100 ml beakers at 28°C , 22°C , and 15°C , showed that the number emigrating declined directly with the reduction of temperature.

Surtees (1964 c), traced the paths manoeuvred by C. ferrugineus in a vertical sandwich of grain 2 kernels thick, held between 2 glass plates. He found that the distance travelled and the linear dis-

placement (straight line distance from start of movement to completion of movement) were higher at 25°C and 20°C than at 30°C. Surtees attributed the reduction in linear displacement at 30°C to reduce rate of movement (orthokinesis) coupled with an increase in turning intensity (klinokinesis). The results of experiments conducted in this chapter, contradict those of Surtees since more insects were trapped at 30°C than at lower temperatures, indicating an increase in locomotor activity at the higher temperatures.

Although an effect of wheat moisture content on the locomotor activity of C. ferrugineus was not clearly demonstrated, moisture did appear to affect locomotor activity. For example, at high wheat moisture content (16.3 percent), fewer insects were caught than at low moisture content (13.7 percent). However, since the analysis of variance did not show any significant relationship, moisture was not considered as a factor which affected locomotor activity appreciably.

Watters (1969), in his studies on emigration, recorded that emigration of insects from the surface of wheat in 100-ml beakers was higher at a wheat moisture content of 9.8 percent than of 13.3, 14.9, and 17.8 percent at temperatures of 15°C, 22°C, and 28°C. These results agree with those of Surtees (1963 c) which show that between 15°C and 35°C more C. ferrugineus appeared at the surface of wheat at 9 percent moisture content than at 12 or 13 percent moisture content. Watters (1957), in a field experiment found that

more C. ferrugineus and Sitophilus granarius were captured in water traps in wheat at 12 or 15 percent moisture content than at 18 percent moisture content. He attributed this to increased locomotor activity of insects in dry wheat. Both workers measured the number of insects appearing at the surface as an indication of locomotor activity; their results indicate that more C. ferrugineus come to the surface in dry grain than in moist grain. In the experiments described in this chapter, locomotor activity was measured in terms of horizontal movement, and not vertical movement. It can therefore, be postulated that in grain, moisture affects vertical movement, but not horizontal movement.

There was no indication that high insect density itself influenced the percentage of insects trapped. Results for individual gallons varied, but the analysis of variance did not indicate that density was a factor either stimulating or depressing locomotor activity. Similarly, Watters (1969), showed that high insect density did not affect locomotor activity. He found that the emigration of C. ferrugineus from the surface of wheat in 100-ml beakers was neither increased or decreased as a result of differences in population densities at temperatures of 28⁰C, 22⁰C, or 15⁰C.

The time allowed for trapping did not significantly affect the number of C. ferrugineus trapped ($P=0.05$). Only slightly more were trapped after 3 days than after 1 or 2 days of trapping. Therefore, we can assume that a 2 day trapping period would be sufficient for

detecting the presence of stored grain insects under field conditions. Watters (1969), recorded the number of insects emigrating from the surface of beakers of wheat in 100-ml beakers and found that over an 8 day period, emigration increased with time, irrespective of temperature, moisture or insect density. The time allowed for trapping in this experiment presented in this chapter may have been too short to indicate its influence on the number of insects trapped; but at least some guideline for the use of the trap has been established.

The results of these laboratory experiments show that of the 4 factors considered, temperature, moisture, insect density and time, temperature is the factor primarily responsible in determining the number of insects caught in a trap. These results are of little significance in determining proper usage of the trap under field conditions, but it is apparent that the chances of trapping C. ferrugineus would be greater in the warmer than in the cooler areas of the grain bulk.

CHAPTER VI
THE INFLUENCE OF FUNGI ON THE DOWNWARD MOVEMENT OF
C. ferrugineus IN A COLUMN OF WHEAT

Introduction

Over 100 species of micro-organisms have been isolated from stored grain in Canada (Machacek et al, 1951). The relationship between stored grain insects, mites and micro-organisms present in stored grain has been studied by numerous workers. (Hinton, 1945; Agrawal et al, 1957; Van Wyk et al, 1959; Griffiths et al, 1959; Misra et al, 1961; Sinha et al, 1962; Wallace and Sinha, 1962; Sinha, 1964; Sikorowski, 1964; Abdel-Rahman et al, 1969; Sinha et al, 1969; Van Bronswijk and Sinha, 1971; and Thomas and Dicke, 1971). Most of these workers were concerned with some aspect of the feeding of stored grain insects on fungi, the changes in the rate of growth, development and reproduction of stored grain insect as influenced by fungi, the influence of stored grain insects on the growth of storage fungi, the dynamic equilibrium between micro-organisms and insects or the attraction of insects to grain kernels or discs covered with fungi.

Storage moulds do not grow in grain at a moisture content of 12 percent or less (Agrawal et al, 1957). Pockets of moist grain frequently occur in dry bulks of grain as a result of roof leakage, seepage of moisture through the wall of storage facilities or trans-

location of moisture within the grain bulk. These pockets of moist grain promote the growth of fungi present in stored grain and are normally the areas where stored grain insects cause severe damage.

Experiments in this chapter were designed to determine if grain severely infected by fungi, attract C. ferrugineus. In the same experiment, the attraction of C. ferrugineus to water vapour was also tested. In addition, experiments were conducted to determine whether pure fungi grown on autoclaved grain attract adults of C. ferrugineus and to determine whether moisture alone, in terms of relative humidity in autoclaved grain could act as an attractant.

Review of the literature

The role of the odour of micro-organisms, especially fungi, influencing insect behaviour is obscure. Weldman (1933), used several types of mould and yeast growths on dried waste fruits as attractants to trap the dried fruit beetle, Carpophilus hemipterus (L.). He found that inoculated cull fruits even in the presence of natural fruits were capable of attracting the beetle in fairly large numbers. Weldman (1933), speculated that there may be a possibility of using microbiologically active substances capable of attracting the dried fruit beetle as a means of control.

Thomas and Dicke (1971), devised a method to study the response of the grain mite, Acarus siro L., to fungi associated with stored food commodities. The mites introduced into the centre of an arena could choose between a disc of fungus and an untreated control disc.

The investigation revealed that certain fungi served as sources of attraction for the grain mite. The mites responded to the attractive fungi at the 1 hour period in large numbers, but maximum response occurred after 3 hours of exposure. Once this peak was reached, the response was reduced, and as the response to the fungi declined, a slight increase in mite response to the controls was demonstrated.

Materials and methods

Nine containers, to support columns of wheat were made from plexiglass cylinders 9.5 x 50 cm. A brass screen 26 guage in thickness with 0.085 inch diameter holes, 1/8 inch apart was fitted in a horizontal plane to the bottom of each cylinder. This was then joined with tape to a 2.5 cm section of plexiglass cylinder of the same dimensions (Figure 10). The cylinders were filled with wheat and placed over petri dishes 9.4 cm in outside diameter. The test petri dishes contained grain covered with fungal flora; the control petri dishes were empty, filled with water, or autoclaved grain, depending on the experiment. The experiment was conducted in a controlled chamber set at $26.5 \pm 1^{\circ}\text{C}$ and 70 ± 5 percent relative humidity (Figure 11).

The test and control petri dishes were placed in the test chamber for 1 hour to allow the grain to come into equilibrium with the experimental temperature and humidity. One hundred adults of mixed age, which had been equilibrated for 45 minutes in the test

FIGURE 10
COLUMN USED FOR TESTING THE EFFECT OF FUNGI AND MOISTURE
ON THE DOWNWARD MOVEMENT OF ADULT C. ferrugineus.

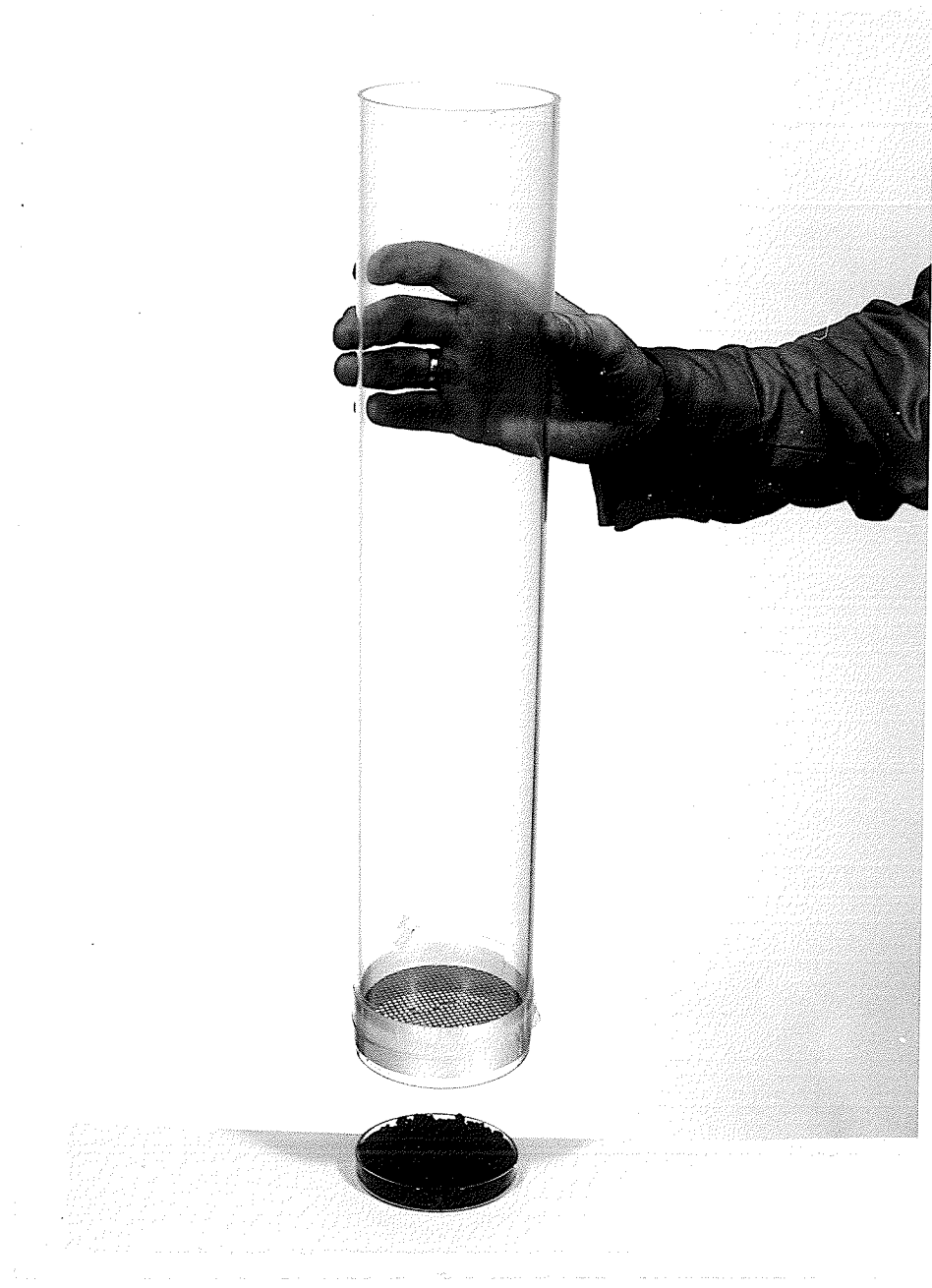


FIGURE 11
CHAMBER USED TO CONTROL ENVIRONMENTAL CONDITIONS DURING
THE TEST, WITH THE TEST APPARATUS IN POSITION.



chamber were sprinkled on the surface of the grain in each column. Forty-eight hours later, the number of insects which had passed through the brass screen into each petri dish was recorded. To prevent insects from moving back up into the column, a coating of vaseline was applied to the inside surface of the column below the screen.

Spoiled grain. In initial tests, the number of insects recovered from petri dishes containing spoiled grain obtained from the field and covered with a mixture of fungi, was compared with the number recovered from empty dishes or those containing sterile distilled water. The number of insects recovered from empty dishes was used as an indication of the natural downward movement of the insect; the number recovered from the water was used to indicate whether moisture had any effect on the movement of the insects under these circumstances. The wheat in the columns above the screen was No. 4 Northern, conditioned at 13.7 percent moisture content.

Each test consisted of 3 columns for each treatment and was repeated 3 times, providing 9 replicates for each treatment.

Pure single species of fungi on wheat. Results of initial tests where beetles were attracted to spoiled grain indicated that odours from fungi or from the fungi-grain mixture might be acting as attractants. Therefore, experiments were conducted to ascertain whether fungi, isolated in pure culture and cultured on autoclaved grain mould could act as attractants.

Six species of fungi were isolated from the rotting grain used in initial tests, according to the method of Sinha and Wallace (1964), and grown in pure culture on potato-sugar-agar (PSA) slants at room temperature. Seven 500 ml flasks each containing 375 ml of distilled water, and 57 1000 ml flasks each containing 300 ml of No. 2 Northern wheat stoppered with cotton were steam-autoclaved. Each species of fungi was placed into a separate flask of water. The flasks were shaken for 1 minute to disperse the fungal spores and mycelia. Thirty, 40 and 50 ml of a suspension of each fungus were then added with an autoclaved glass syringe to each of 3 flasks of wheat, respectively. Each treatment was replicated 3 times for each fungus. This procedure increased the probability of obtaining uncontaminated grain-fungus cultures. Also, since different fungi have different moisture requirements, the provision of 3 different moisture contents increased the probability of obtaining optimal growth for each fungal species. The flasks were shaken twice daily for 2 days after inoculation, then placed in a cabinet at $29 \pm 1^{\circ}\text{C}$ and 70 ± 5 percent relative humidity for 30 days. Fungal growth for all 6 fungi was best in flasks inoculated with 50 ml of suspension. After 30 days of incubation, the contents of these flasks were emptied into quart jars, 1 fungal species per jar and kept at minus 6.5°C to retard further fungal growth.

The wheat in the columns above the screen was No. 2 Northern, at 14.3 percent moisture content. The conditions of the grain in

TABLE IV
 MEAN NUMBERS OF C. ferrugineus ADULTS RECOVERED FROM COLUMNS OF
 WHEAT AFTER 48 HOURS AT $26.5 \pm 1^{\circ}\text{C}$

Treatment	Test *			Mean
	1	2	3	
Spoiled grain	90.5	75.7	84.7	83.6
Water	47.0	53.0	45.6	48.5
Empty dish	42.7	47.7	52.3	47.6

L.S.D. at 1 percent level = 25.6

* Three replicates per test

TABLE V
RELATIVE DISTRIBUTION OF SIX SPECIES
OF FUNGI ON THE SPOILED WHEAT *

Fungi	Percentage kernels infected
<u>Streptomyces</u> sp.	72.7
<u>Cephalosporium acremonium</u> Corba	36.4
<u>Penicillium corymbiferum</u> Westling	27.3
<u>Fusarium</u> sp.	18.2
<u>Aspergillus repens</u> De Bary	4.5
<u>Scopulariopsis brevicaulis</u> (Sacc.) Bain	Present

* Total seeds examined = 22

TABLE VI
 MEAN * NUMBER OF C. ferrugineus ADULTS MOVING OUT OF
 COLUMNS OF WHEAT AFTER 48 HOURS AT 26.5 \pm 1°C

Treatment	Mean ** \pm S.E.
<u>Penicillium corymbiferum</u> Westling	80.0 \pm 3.67 a
<u>Scopulariopsis brevicaulis</u> (Sacc.) Bain	62.7 \pm 5.91 b
<u>Fusarium</u> sp.	59.4 \pm 4.30 b
Spoiled grain	56.9 \pm 4.18 b
<u>Cephalosporium acremonium</u> Corda	42.9 \pm 4.53 c
<u>Aspergillus repens</u> De Bary	40.4 \pm 6.49 c
<u>Streptomyces</u> sp.	26.4 \pm 4.01 d
Wet grain	23.2 \pm 3.30 d
Dry grain	17.8 \pm 3.07 d

* Mean of nine replicates

** Means followed by the same letters are not significantly different from each other at the 5 percent level (Duncan's multiple range test).

the columns were different in the test of mixed fungi, and pure fungi for 2 reasons: (1) the supply of grain at the Research Station had changed; (2) the experiments were not carried out simultaneously and comparisons between the 2 experiments were not considered possible. Each test fungal-wheat mixture was placed under each of 6 columns respectively, while dry autoclaved grain (14.3 percent), moist grain (22 percent), and spoiled grain (from the same source as that used in the initial experiment) were placed under 3 other columns respectively. These 9 columns were placed in the cabinet for 48 hours, after which counts were made. This test was repeated 9 times. The data obtained was subjected to a Duncan's multiple range test (Duncan, 1955).

Results

Movement of C. ferrugineus in columns of wheat over spoiled fungus infected wheat, water or empty dishes. Significantly more insects ($P= 0.01$) moved from the grain into dishes with spoiled grain than into dishes with water or those that were empty (Table IV). There was no significant difference between the number of insects in the empty and water-filled dishes.

Of the 6 species of fungi isolated from the spoiled wheat, Streptomyces sp. was the most common, infecting 72.7 percent of the kernels tested (Table V). Cephalosporium acremonium Corda, Penicillium corymbiferum Westling and Fusarium sp. were relatively common, whereas Aspergillus repens De Bary was rare. Scopulariospsis

brevicaulis (Sacc.) Bain. was present, but the percentage of seeds infected with this fungus could not be determined with certainty. The seeds used for fungal analysis showed no germination and were dark brown to black, indicating severe damage by micro-organisms.

Movement of C. ferrugineus in columns of wheat over pure single species of fungal cultures, moist grain, and dry grain. The possibility that fungi acting as attractants were responsible for the downward movement of C. ferrugineus in the initial experiment prompted further studies. The number of adults of C. ferrugineus moving out of the columns of wheat in response to various treatments fell into 4 distinct groups (Table VI). Significantly more insects were recovered from dishes that contained Penicillium corymbiferum, Scopulariopsis brevicaulis, Fusarium sp., spoiled grain, Cephalosporium acremonium and Aspergillus repens than from those with Streptomyces sp., wet grain and dry grain. This indicated that all the fungi tested except Streptomyces attract C. ferrugineus, some more so than others (Table VI).

Discussion

This experiments has shown that some fungi serve as attractants to C. ferrugineus, and that they vary in attractivness. The nature of the attractant is not understood. Thomas and Dicke (1971), found that the grain mite Acarus siro showed a strong, positive response to dry fungal extracts, eliminating the possibility that mites are attracted by relative humidity CO₂ concentrations produced by fungi.

The results (Table VI) of these experiments confirm the results of Thomas and Dicke, that high relative humidity per se does not act as an attractant.

The source of the stimuli that elicit a directional response in C. ferrugineus are probably chemical components in the living fungi. Since the design of the experiment was such that physical contact between the insect and the fungi was prevented, the odours of the fungi, or the chemicals produced by the fungi-grain complex must have been responsible for attracting insects to areas infected with micro-organisms.

The results obtained for water and empty dishes indicate a mean natural downward movement of 47.6 - 48.5 percent of the insects (Table IV) which is attributed to positive geotaxis (Watters, 1969). Nevertheless, despite this natural downward movement, significantly ($P = 0.01$) more C. ferrugineus were found in dishes that contained fungi than in those without. An interesting phenomenon was observed in the second experiment using pure fungi as attractants. The mean number of insects out of 100 found in the dishes of spoiled grain was 83.6 in the first experiment (Table IV) and 56.9 in the second experiment (Table VI). Since temperature, relative humidity in the cabinet, and methods of assessment were the same in both experiments, the most feasible explanation is the difference in the grains used in the columns above the test dishes in the two experiments, namely, No. 4 wheat at 13.7 percent moisture content in the first experiment

and No. 2 wheat at 14.3 percent moisture content in the second. The reasons for these differences were not examined but by making a few assumptions, a theoretical explanation may be presented. If the grain in the column used in the second experiment was infected with more mould spores than the grain used in the first experiment, this more heavily infected grain might attract beetles and consequently cause a reduction of locomotor activity. This reduced activity coupled with a possible increase in feeding could lead to a reduction in the downward movement of the insect.

The numbers of insects moving downwards in response to relative humidity did not differ significantly from the numbers moving down in response to natural tactic responses.

The attractive effects of fungi can be grouped according to the mean number of insects found in each fungus-wheat mixture. The statistical analysis divided the fungi and control materials into 4 distinct groups, with no overlap between the groups (Table V). It was noted that P. corymbiferum greatly affected the consistency of the kernels, turning the kernels punky, and allowing the insects to enter the kernel easily. A similar consistency was noted in the spoiled grain obtained from the field. The effect of the other fungi on the kernels of wheat upon which they were grown was not as obvious. P. corymbiferum, A. repens, and S. brevicaulis produced great quantities of spores, while the only obvious presence of the other fungi was the growth of mycelium on the germ ends of the kernels.

The significance of fungi in the biology of C. ferrugineus has been reported by many workers. Rilett (1949), found that when taka-diastase, a substance commonly occurring in many moulds and fungi, was added to wheat endosperm, mortality of C. ferrugineus was reduced from 90 to 10 percent, and the developmental time from egg hatch to adult was reduced from 42 to 40.6 days. On endosperm supporting mould growth, the developmental time was reduced to 37.8 days. He concluded that the growth of mould on stored wheat greatly increased the total amount of food for the developing larvae by making the starchy endosperm more readily available as larval food.

Sinha (1965), reported that C. ferrugineus completed development on 10 species of fungi. Loschiavo and Sinha (1966), showed that C. ferrugineus would aggregate, feed, grow and develop on some species of pure fungi. Since Sinha (1965), and Loschiavo and Sinha (1966), grew their fungi on artificial medias and not grain, their results may have been different had the fungi been grown on grain.

Sinha (1961), found that C. ferrugineus was the commonest stored grain insect associated with hot spots. Hot spots support intense mould growths, and provide basic physical requirements necessary for development. Wallace and Sinha (1962), and Sinha (1964), found that some unidentified species of Penicillium sp. were the commonest fungi found in hot spots. Grain infected with a mixture of fungi is not always adequate for experimentation, since 1 fungus will tend to dominate and thus mask the response that might otherwise

be elicited by the test insects. For this reason, experiments were conducted with mixed fungi as well as with pure fungi to determine whether 1 fungus or a combination of fungi could produce a response in an insect.

The influence of mould on the biology of other stored product insects have been documented by many workers. Van Wyk et al (1959), observed that the confused flour beetle, Tribolium confusum (Duval) was attracted to, and developed better in flour infected with storage fungi than in flour free of fungi. Woodroffe (1962), reported that the foreign grain beetle Ahasverus advena (Waltl) thrived in mouldy foodstuffs. Chang and Loschiavo (1971), reported that the fungal concentrations in flour and rate of larval development of Cryptolestes turcicus (Grouvelle) were directly correlated.

The importance of the odour of fungi in determining the distribution of stored grain insects such as C. ferrugineus has been grossly overlooked. These tests have conclusively shown that insects can detect, and orient themselves to a source of fungal odour. Through locomotor activity, the insect can reach the source of attractive odours, or move away from repellent odours. When grain is placed into a bin it is usually of uniform moisture content and temperature. Damage in stored grain frequently occurs when pockets of high moisture are produced in the grain. High moisture and moderate temperature supply an adequate environment for the production of fungi. Under these optimal conditions, C. ferrugineus can grow and develop

rapidly. Insects detecting the odour of these fungi could orient themselves to the source and through locomotor activity reach a favourable environment. The accumulation of insects in moist areas can be explained not only in terms of a purely klino-kinetic response to humidity but also as this experiment has shown, in terms of an olfactory response to chemical stimuli from components in the natural environment.

Detection and sampling of moist areas within a bulk could be more effective if we had some idea of insect distribution. These experiments have shown that C. ferrugineus may accumulate in moist, damp pockets of grain in response to olfactory stimuli from micro-organisms or near the bottom of a grain bulk in response to geotactic stimuli. The probability of detecting infestations is thus greatly increased by placing traps in these suspected areas. Frequently, areas of moist, often rotting, grain can be located by visual inspection of the storage facility.

The effect of temperature, moisture and spoiled grain on the distribution of C. ferrugineus in a bulk grain system are discussed in Chapter VII.

CHAPTER VII

FIELD STUDIES ON THE DETECTION AND DISTRIBUTION OF ADULTS OF C. ferrugineus IN A BULK OF WHEAT.

Introduction

Insect pests are very seldom distributed evenly in a bulk of stored grain and infestations have been reported from all depths and locations. The physical and biological gradients in a grain bulk vary and since stored grain insects respond to these gradients, uniform distribution of insects is rare. Temperature and humidity gradients are the primary factors influencing insect distribution.

An understanding of the distribution of insects in stored grain is essential for rapid, accurate sampling and the application of proper control measures. The abundance and location of the insects determines the type of control required. In many cases, lack of early detection leads to severe damage and economic loss.

Areas of severe infestation are determined by the physical and biological properties of the grain and the source of infestation. Liscombe and Watters (1962), found that mites and insects lived in cracks of walls and floors of empty granaries; when these granaries are filled, grain near the walls and at the floor tends to become infested. Subsequent entry of insects may also occur at the periphery of the grain bulk namely at the surface, through the walls or from the soil bin surface. Temperatures at the surface and walls of bins

are normally high due to absorption of radiant heat from the sun. If the moisture content of grain is high these zones can provide excellent breeding sites. The movement of insects from these breeding sites depends on changes within the grain bulk.

The purpose of the studies described in this chapter was to follow by trapping, the movement, distribution, and build-up of C. ferrugineus in a bulk of wheat subject to normal environmental changes. The effectiveness of various sampling methods in detecting insects was also tested.

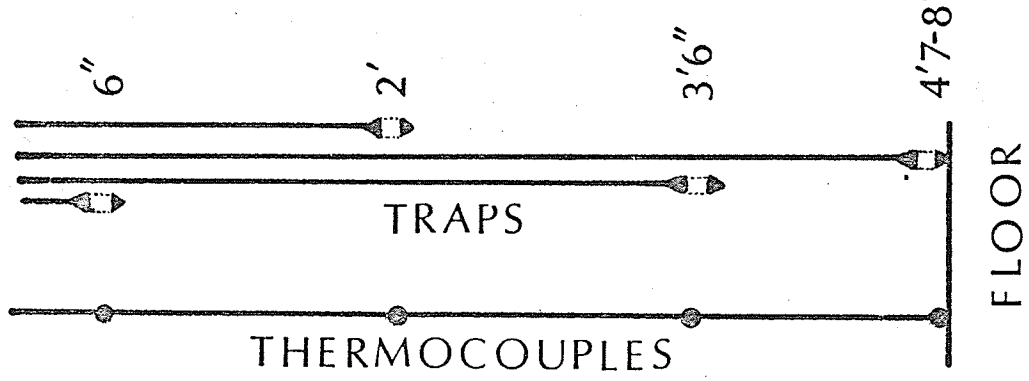
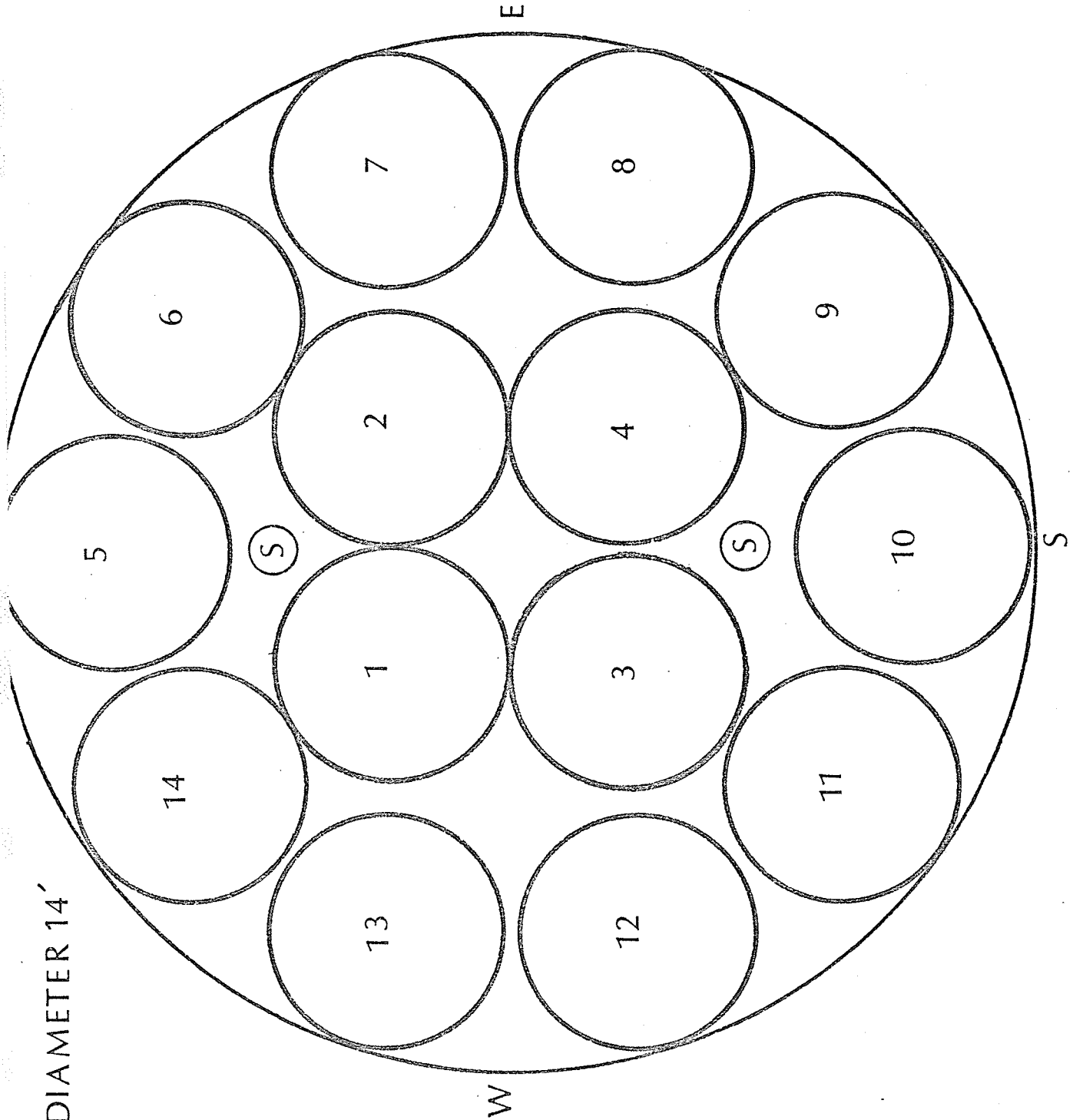
Review of the literature

The main problem in studying the distribution of stored grain insects in large bulks of grain is to devise suitable sampling methods. Most methods are time-consuming and tend to be inaccurate because insects tend to congregate in pockets of grain where environmental conditions are most favourable.

Several laboratory studies have been designed to study the distribution of stored product insects within stored cereals. Cox and Smith (1957), studied the distribution of Tribolium confusum (Jacquelin du Val) in a box of flour by dividing the box into 4 sections and sieving each section. Surtees (1965), studied the distribution of C. ferrugineus in an acrylic plastic box containing 25 kg of wheat in 64 3-inch cubes. The insects were released in the centre of the upper surface, the bulk was broken down a week later,

FIGURE 12
DIAGRAM OF THE SURFACE, AND VERTICAL LOCATIONS OF THERMOCOUPLES
AND TRAPS IN THE BIN AT GLENLEA, MANITOBA. (A SET OF TRAPS
AND THERMOCOUPLES WERE POSITIONED IN THE CENTRE OF
EACH CIRCLE)

DIAMETER 14'



and the number of insects in each 3-inch cube recorded. Watters (1964), using a box, 50 by 30.6 cm equipped with the tube traps rising from the floor of the box, studied spatial distribution of C. ferrugineus in wheat. The box was filled with grain, and insects were introduced on the surface of the grain near the centre of the box. The number of insects trapped in each tube trap was used as an indication of insect distribution.

Reports on the distribution of stored grain insects in large bulks of grain are lacking. Walker (1960), removed samples with a standard bin tier, from the top 100 inches of grain in bins during the middle of each month from November through April. The numbers of insects in each sample were used to determine distribution over the trapping period.

Materials and methods

Grain to a depth of 4'8" was placed in a concrete-floored steel granary, 14' in diameter, at Glenlea, Manitoba. The grain was primarily red spring wheat, but there was also a small admixture of barley, durum wheat, buckwheat and dust.

Sampling sites were selected at 3'4" intervals on concentric circles 1'8" and 5' from the wall, respectively. There were thus 10 sites on the outer circle and 4 on the inner circle (Figure 12). At each site sampling was carried out at 6", 2', 3'6", and 4'6" depths for a total of 56 samples. For convenience of reference,

FIGURE 13

EXPERIMENTAL BIN AND RECORDING HUT AT GLENLEA, MANITOBA, 1970.

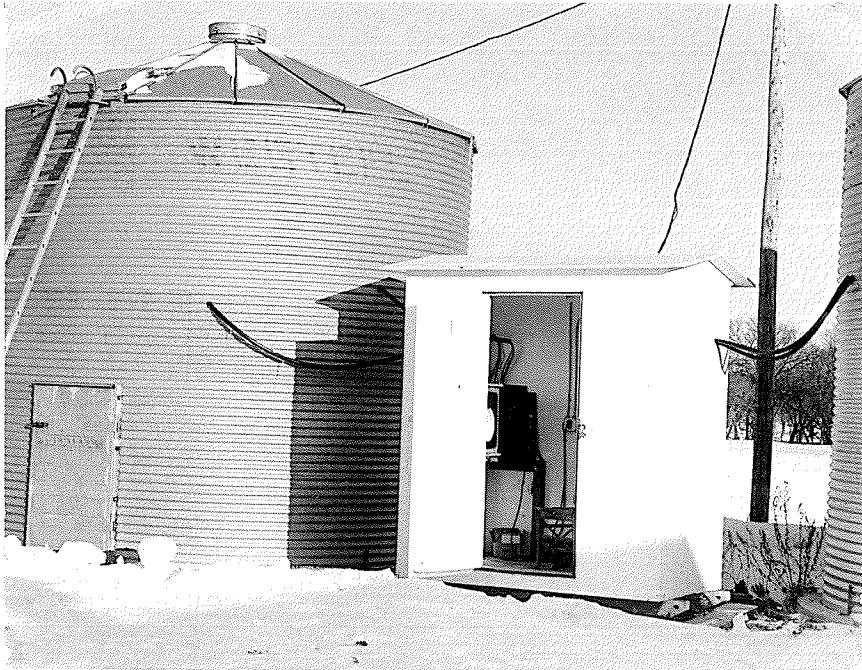


FIGURE 14
VIEW OF THE INNER AND OUTER TRAPPING LOCATIONS, AND THE
TRAP POSITIONS AT EACH LOCATION IN THE BIN.

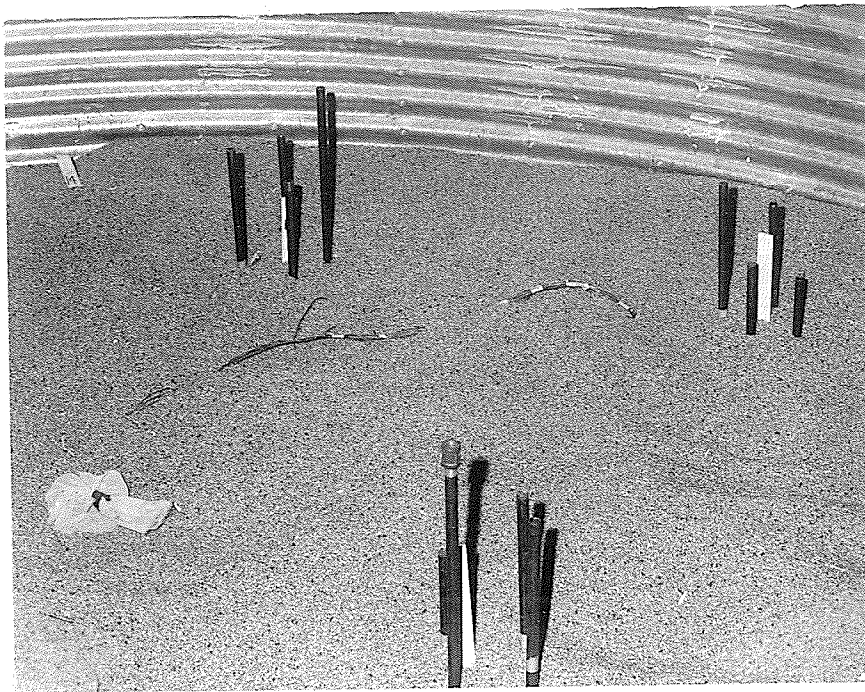


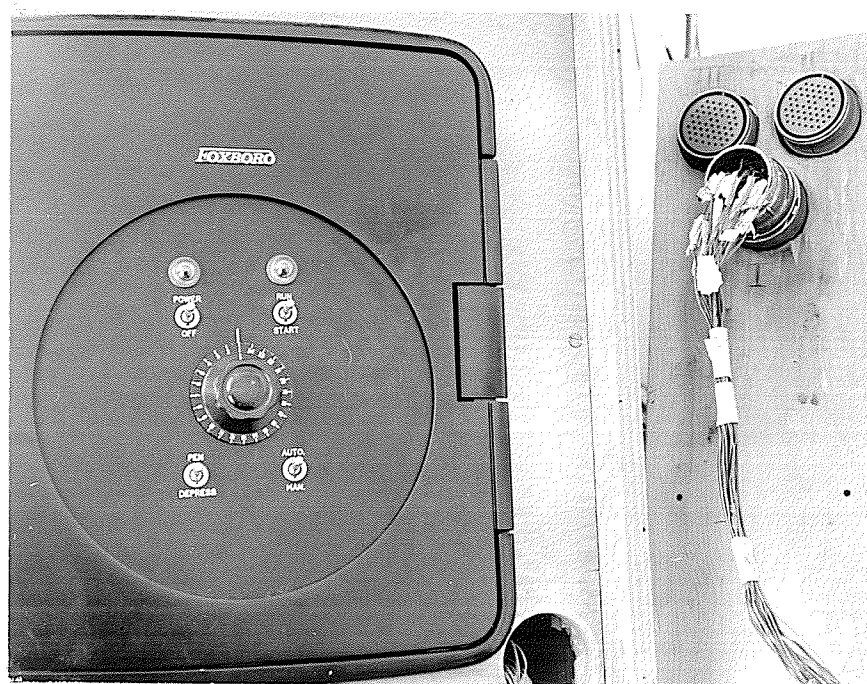
FIGURE 15
POSITIONS OF THE TRAPS AND THERMOCOUPLES AT EACH LOCATION IN
THE BIN.



FIGURE 16
RECORDING EQUIPMENT IN THE HUT NEXT TO THE BIN.



FIGURE 17
CANNON-COUPERS USED TO CONNECT EACH SET OF THERMOCOUPLES TO
THE RECORDER IN THE HUT.



the sites were numbered from 1 - 14, with 1 - 4 in the inner circle and 5 - 14 in the outer circle as shown in Figure 12. Thermocouples were placed at each of the 4 levels at each sampling site, with 24, 24, and 8 connections respectively to each of 3 cannon-couplers connected to a 24 Point Foxboro Recorder. Temperatures were recorded continuously at sites 1 to 6 inclusive and manually at bi-weekly intervals at the remaining sites until September 16th and weekly thereafter until November 25th, 1970.

On July 20th, 1970, a sample of 8 oz. of grain was taken with a vacuum probe at each of the sampling locations. The samples were sealed as they were taken and returned to the laboratory. The moisture content of the grain at each location was determined by a two-stage moisture test on a portion of each sample. After the moisture test, a portion of the grain from each sample was placed in a plastic vial (3 inches by 3/4 inch) with a snap-on cap, previously labelled according to the sampling location.

On July 26th, approximately 1,500 rusty grain beetles, as determined by weight, (0.3287)gm) were placed into each vial. The following day, the contents of the vials were placed in the grain in the bin, each at its appropriate sampling location. The insects were actually released 6 inches to the right of the thermocouples at each location.

The release apparatus was a 5 foot length of 3/4 inch copper pipe and a similar length of 1/4 inch steel rod, on the end of which was

FIGURE 18
EMPTY CONTROL CHAMBER EXTENDING FROM THE SURFACE TO THE
FLOOR OF THE BIN.



soldered a 3/4 inch washer which served as a plug for the pipe as it was being pushed into the grain. Once in position, the rod and washer were removed leaving an empty open-ended pipe. At each site, the pipe was pushed to the deepest sampling location (4'6") and the contents of the appropriate vial poured down the pipe. The pipe was then raised to the next sampling location and again the contents of the appropriate vial was poured down the pipe. The insects were released in this manner at each sampling location. In tests at the laboratory, there was no evidence that insects were injured in the drop down the pipe.

Immediately after release of the insects, 2 cylinders, made of 100-mesh bolting cloth, were placed vertically in the grain to provide an enclosed area where movement and development of the beetles could be checked. The cylinders were 6 inches in diameter and extended from the floor of the bin to the surface of the grain. They were located 3 1/2 feet from the most northerly and southerly points, respectively, of the bin. The cylinders were placed in position with the aid of a 10-inch galvanized steel pipe which was forced into the grain and then evacuated with a vacuum cleaner. The cloth cylinder was lowered into the pipe, filled with some of the evacuated grain and then the pipe was removed. Forty rusty grain beetles were released within each cylinder at each of the 4 previously noted levels. The cylinder on the North was checked 4 times each at 12 day intervals. On each occasion, the grain was removed in layers, 0 - 15". 15 - 30",

30 - 45", and 45 to 56" and the numbers of adults and larvae, alive and dead, in each layer were recorded. After completion of each count, the grain and insects were returned to their proper zone. The cylinder on the South was left undisturbed for the 48-day period. The results from the cylinder experiments were used as a basis of comparison for the movement and development of the beetles in other areas of the grain.

To follow the distribution of the beetles in the grain bulk, several methods were used at various time intervals after release of the beetles. In one method, traps were placed at each sampling location 24 hours after the beetles were released. After 2 days, the traps were removed and the number of beetles in each was recorded. When there were more than 15 beetles in a trap, they were replaced at the location of capture the next day, allowed to redistribute for 1 day, then the traps were reinserted into the grain. This procedure was repeated 13 times using the same time intervals, then extended to weekly intervals with continuous trapping and no return of insects when it was noted that new generation adults were being taken. A spear probe and a vacuum probe were other devices used for sampling. On September 16th, 1970, 51 days after the original release of the insects, spear probe samples were taken 6" to the right of the traps, but only at the 3 upper locations since the spear probe was not suitable for sampling near the floor. On the same date, an additional set of samples were taken with a vacuum probe 6" to the left of the trapping locations at each of the 4 levels. The samples were placed in jars

which were sealed and taken to the laboratory where they were sifted and the numbers of adult beetles in each recorded. The moisture content of the grain at each location was also determined. After the moisture content was determined, the individual samples were placed in Berlese funnels, and the numbers of larvae in the samples determined. The same procedure was repeated 41 days later on October 28th, 1970. The results obtained by the different sampling methods were compared to determine the efficiency of the various methods, but were used primarily to determine the distribution pattern of the insect.

Further samples were taken at all sampling locations with a spear probe and a vacuum probe on February 15th and 16th, 1971. As before, the samples were sealed in jars and taken to the laboratory where the numbers of insects were recorded. Percentage degermination in different areas of the bin was determined by an examination of grain from the vacuum probe samples. To limit the amount of work involved, samples were combined according to the sampling pattern. At sampling sites 1 to 4 and similarly at sites 5 to 14 (Figure 12), the samples were combined by level, ie. samples at the 6" level were combined, as were the samples from each of the other levels. The combined samples were thus representative of grain at the 4 levels at 2 distances from the bin wall. Each of the combined samples was thoroughly mixed and a subsample of 100 kernels, taken at random with a teaspoon, was examined to determine the percentage degermination. Degermination was determined by removing the pericarps from the grain end of the kernel with a scalpel.

From the results of the samples taken in February, 1971, it was considered that valuable information on the distribution and development of the rusty grain beetle could be obtained from more selective sampling in the grain bin. Consequently, samples were taken twice in March and once in April. On March 1st, samples were taken with a vacuum probe at 3 levels - floor, 1 foot above the floor and 2 feet above the floor, at each of the 44 locations shown in Figure 24. The numbers of insects and moisture content of the grain was determined for each sample. On March 17th, vacuum probe samples were taken from grain in the area at the juncture of the wall and floor every 15" around the periphery of the bin. As before, the number of insects and moisture content were determined. In addition, percentage degermination was determined on the basis of 100 kernels removed at random from each sample. A final set of samples was taken on April 7th, 1971 in 2 areas of the bin (Figure 25) to examine in more detail the pattern of insect distribution and kernel degermination.

Results

Distribution of *C. ferrugineus* in relation to temperature and moisture changes in the grain bulk: For convenience of presentation, the data have been grouped arbitrarily to represent 4 zones in the bin. Zone I refers to the 6" level, Zone II to the 2' level, Zone III to the 3'6" level and Zone IV to the 4'8" level. Each zone was also divided vertically; the inner 4 sampling sites being termed the inner

set, and the outer 10 the outer set (Figure 12). The temperature records are the average temperatures of the grain in the inner set at sampling site 2 and in the outer set at site 11 (Figure 12).

Zone I: The highest temperatures recorded were in Zone I early in August (Figure 19). However, the temperature dropped most rapidly in this zone with the onset of cool weather and reached 0°C by the middle of November. The temperature did not vary greatly between the inner and the outer sets.

Very few insects were trapped in this zone although the temperature was the most suitable for biological activity. However, the low moisture content of the grain in this area may have made the zone unsuitable for insect activity. The record of occurrence of beetles in this zone confirms the observations of Watters and Cox (1957) that C. ferrugineus comes to the surface of wheat bulks. Adults of C. ferrugineus have also been observed in flight in a steel granary, thus providing further confirmation that the beetles do move to some extent into the surface zone of a grain bulk (Dolinski, unpublished data).

Zone II: The temperature in this zone was near the 20°C range until September 16th (Figure 20) when a gradual but steady decline began. The temperature in the outer set was higher than in the inner set from July 30th to September 16th, but thereafter the situation was reversed. This was brought about by a rapid loss of heat from the grain near the walls of the bin as compared to a slow loss of heat from

FIGURE 19
RECORD OF TEMPERATURE AND NUMBERS OF INSECTS TRAPPED IN THE
INNER AND OUTER SETS OF LOCATIONS IN ZONE I (6" LEVEL)
FROM JULY 27TH TO NOVEMBER 25TH, 1970.

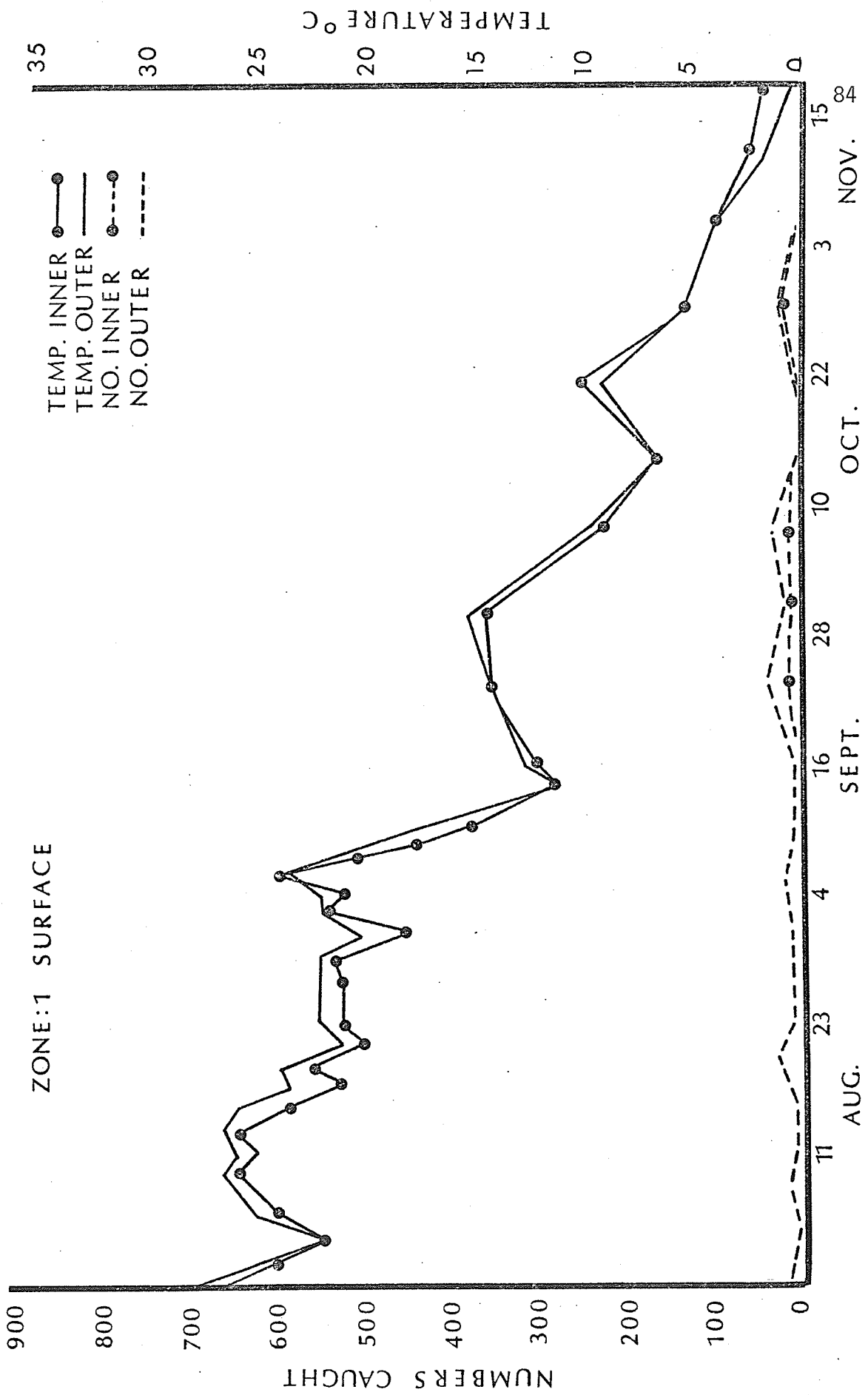


FIGURE 20
RECORD OF TEMPERATURE AND NUMBER OF INSECTS TRAPPED IN THE
INNER AND OUTER SETS OF LOCATIONS IN ZONE II (2' LEVEL)
FROM JULY 27TH TO NOVEMBER 25TH, 1970.

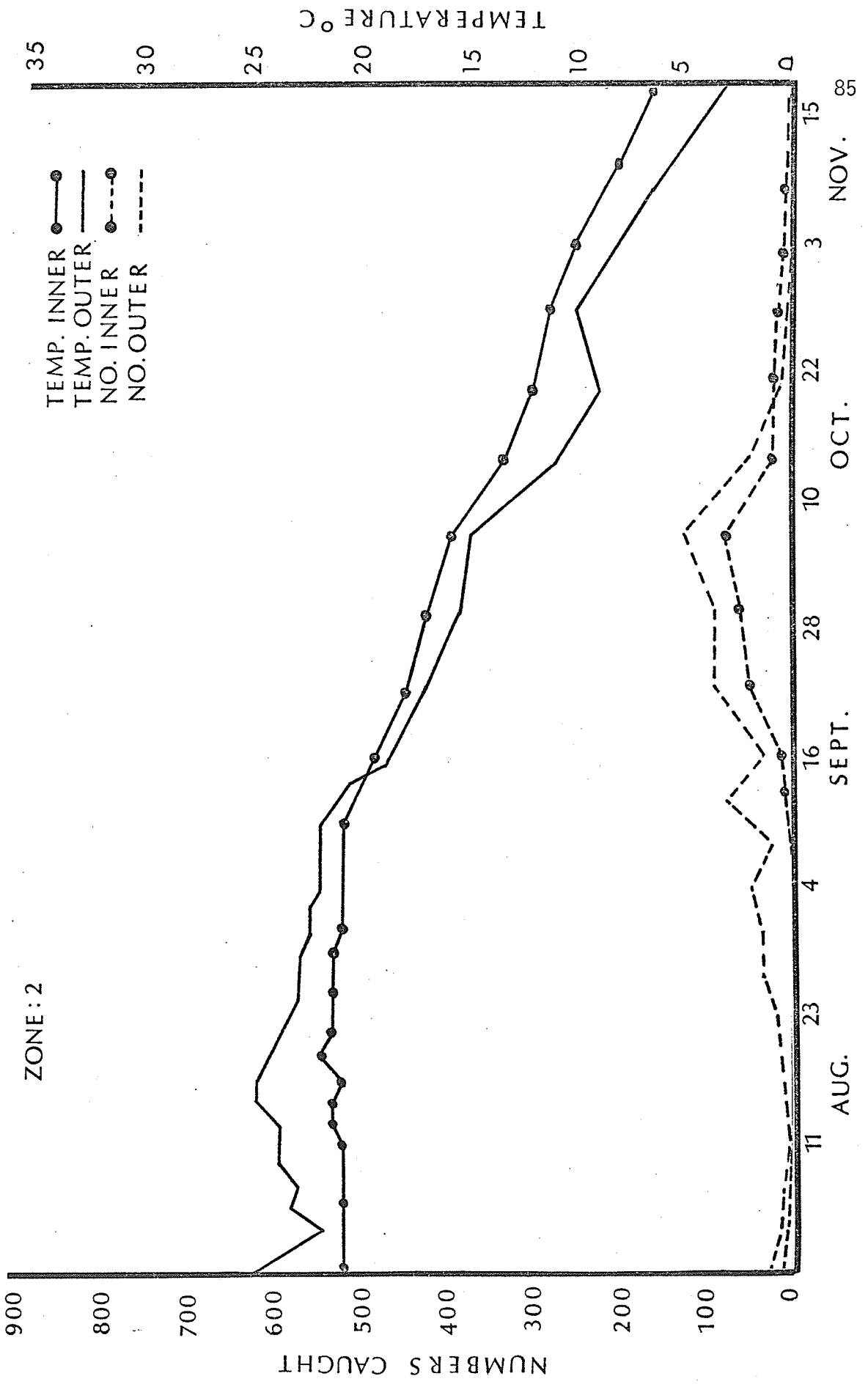


FIGURE 21
RECORD OF TEMPERATURE AND NUMBER OF INSECTS TRAPPED IN THE
INNER AND OUTER SETS OF LOCATIONS IN ZONE III (3'6" LEVEL)
FROM JULY 27TH TO NOVEMBER 25TH, 1970.

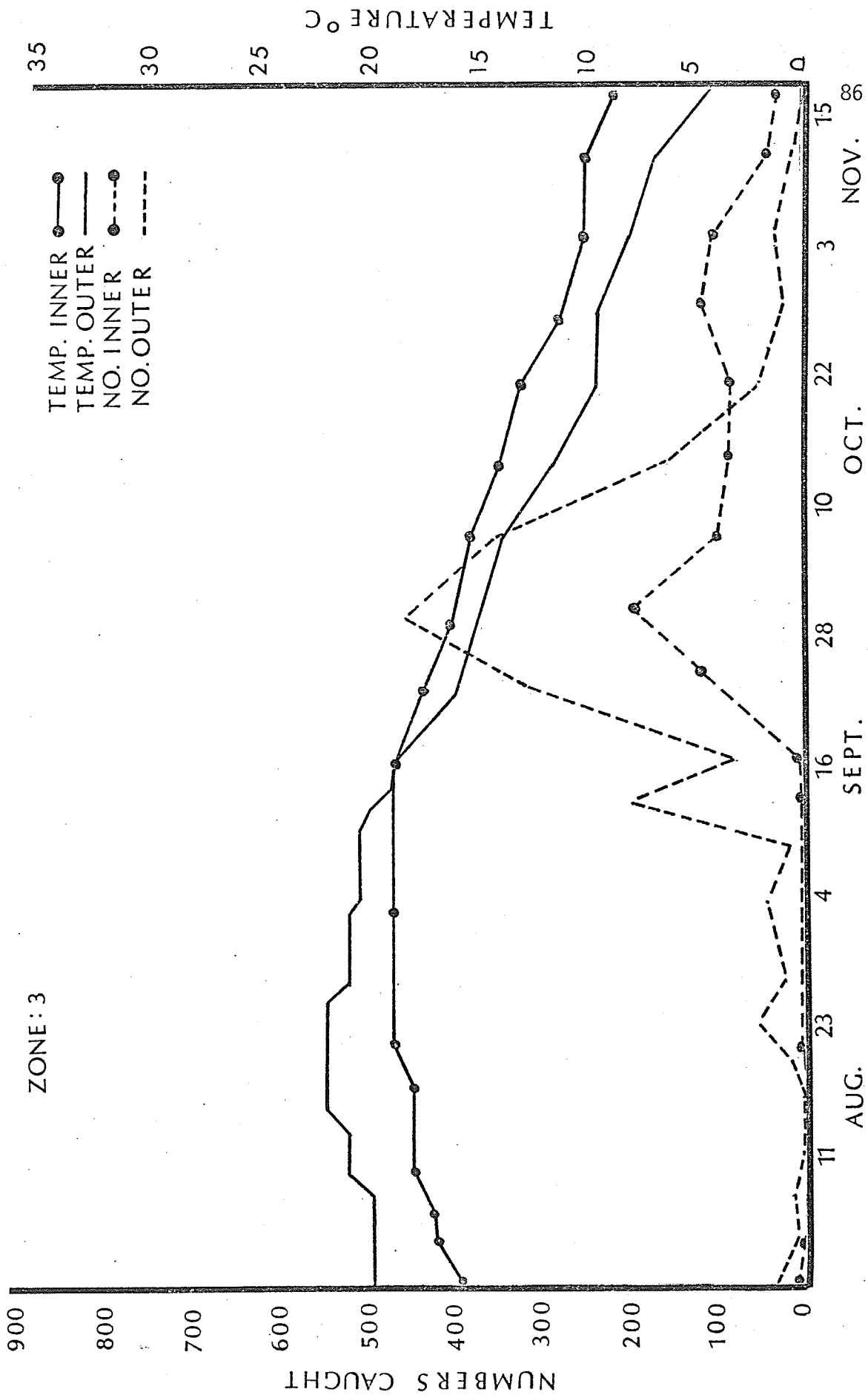


FIGURE 22
RECORD OF TEMPERATURE AND NUMBER OF INSECTS TRAPPED IN THE
INNER AND OUTER SETS OF LOCATIONS IN ZONE IV (4'8" LEVEL)
FROM JULY 27TH TO NOVEMBER 25TH, 1970.

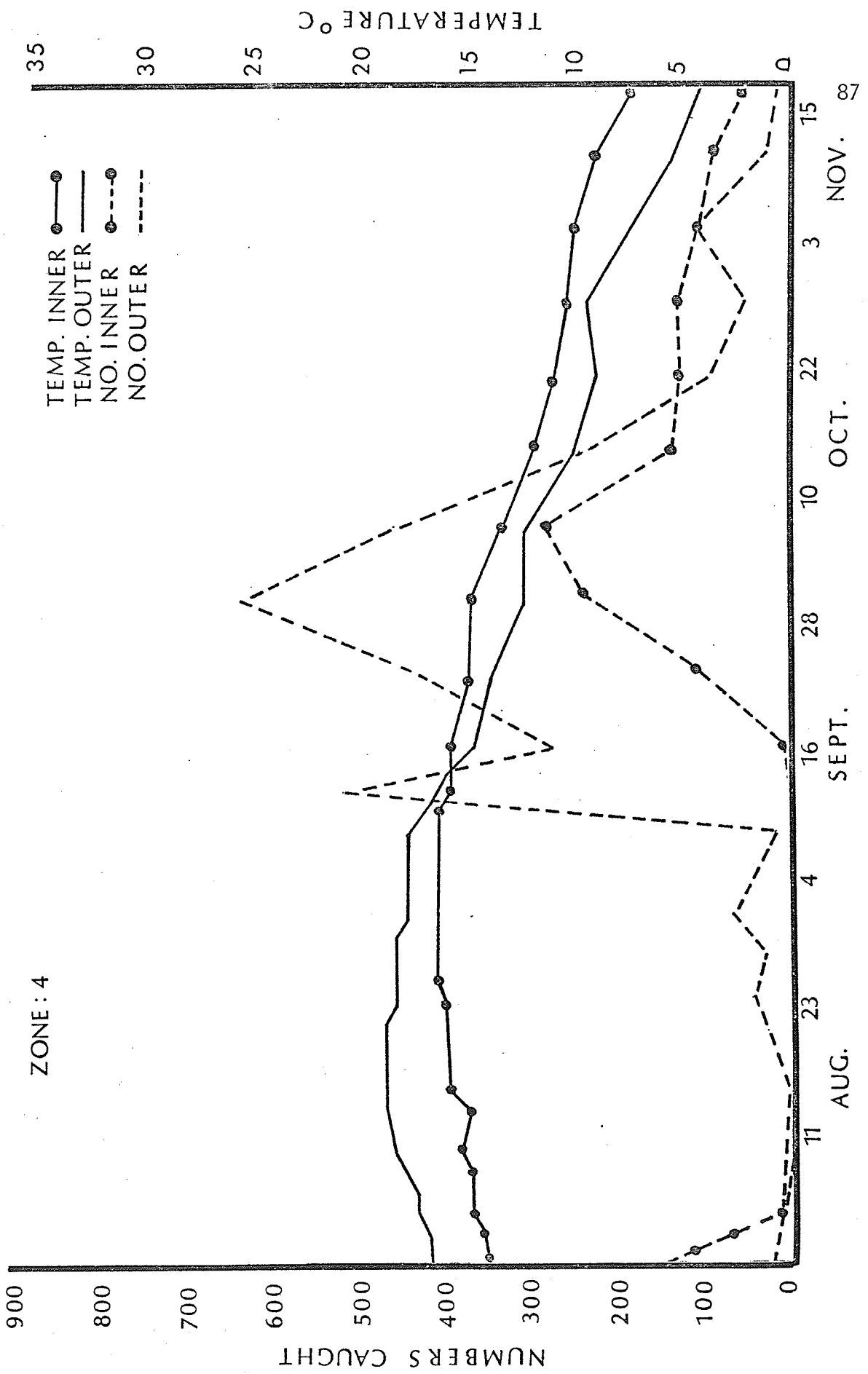


TABLE VII
 MOISTURE CONTENTS OF THE GRAIN IN THE EXPERIMENTAL BIN AT GLENLEA, ON JULY 20TH, AND
 SEPTEMBER 16TH, 1970

Location * in bin	Depth (in inches)					
	6	24	42	58		
	July 20	Sept. 16	July 20	Sept. 16	July 20	Sept. 16
1	14.1	14.1	14.9	14.9	15.3	15.5
2	14.8	14.1	14.1	14.1	15.2	14.9
3	14.0	14.1	14.9	15.1	15.2	15.5
4	14.9	14.1	14.2	14.0	15.0	15.3
5	14.5	14.1	14.0	14.0	15.1	14.9
6	14.2	14.2	14.6	14.6	14.5	14.6
7	14.4	14.2	14.6	14.2	13.8	13.8
8	14.3	14.1	14.7	14.5	14.4	14.3
9	14.0	14.2	14.2	14.2	14.4	14.4
10	14.6	14.5	14.2	14.4	14.9	15.1
11	13.9	14.6	15.1	15.2	15.2	15.2
12	14.8	14.2	15.8	15.1	15.1	13.8
13	14.3	14.2	15.4	15.1	15.6	15.6
14	14.2	14.3	14.7	14.6	15.2	15.4

* Refer to Figure 12

the central region of the grain bulk.

More insects were trapped in this zone than in Zone I and, as in Zone I, the greatest number were taken in late September and early October (Figure 20). The number of C. ferrugineus trapped in the outer set exceeded those trapped in the inner set up to October 22nd; thereafter, the number trapped in each set were equal. The moisture content of the grain in this zone averaged near 14.6 percent, a moisture content adequate for insect activity and reproduction.

Zone III: Except for a short period in August, the temperature in this zone did not exceed 20⁰C (Figure 21). As in Zone II, the temperature in the outer set was higher than in the inner set until about September 16th, after which the reverse situation prevailed. The moisture content of the grain in this zone averaged 15 percent, somewhat higher and more suitable for the beetles than that in Zone II.

Near the end of September, almost 500 adult beetles were trapped in the outer set and 200 in the inner set in 1 48-hour trapping period. This peak corresponds in general, to the period of greatest activity recorded in Zones I and II. An interesting change is recorded in the number trapped from approximately October 18th to the end of the fall sampling. During this period more insects were trapped in the inner set of traps than in the outer set. This may have been due to higher temperatures in the inner set and therefore, greater locomotor activity or possibly to a movement of insects from the periphery of the bin into the central regions or both. Although the moisture content was suitable,

reproduction in this zone must have been limited because the temperature did not exceed 20 degrees.

Zone IV: The temperature pattern in this zone was basically the same as that in Zone III (Figure 22). The moisture content of the grain averaged 15.4 percent, an optimum for rapid insect growth and reproduction.

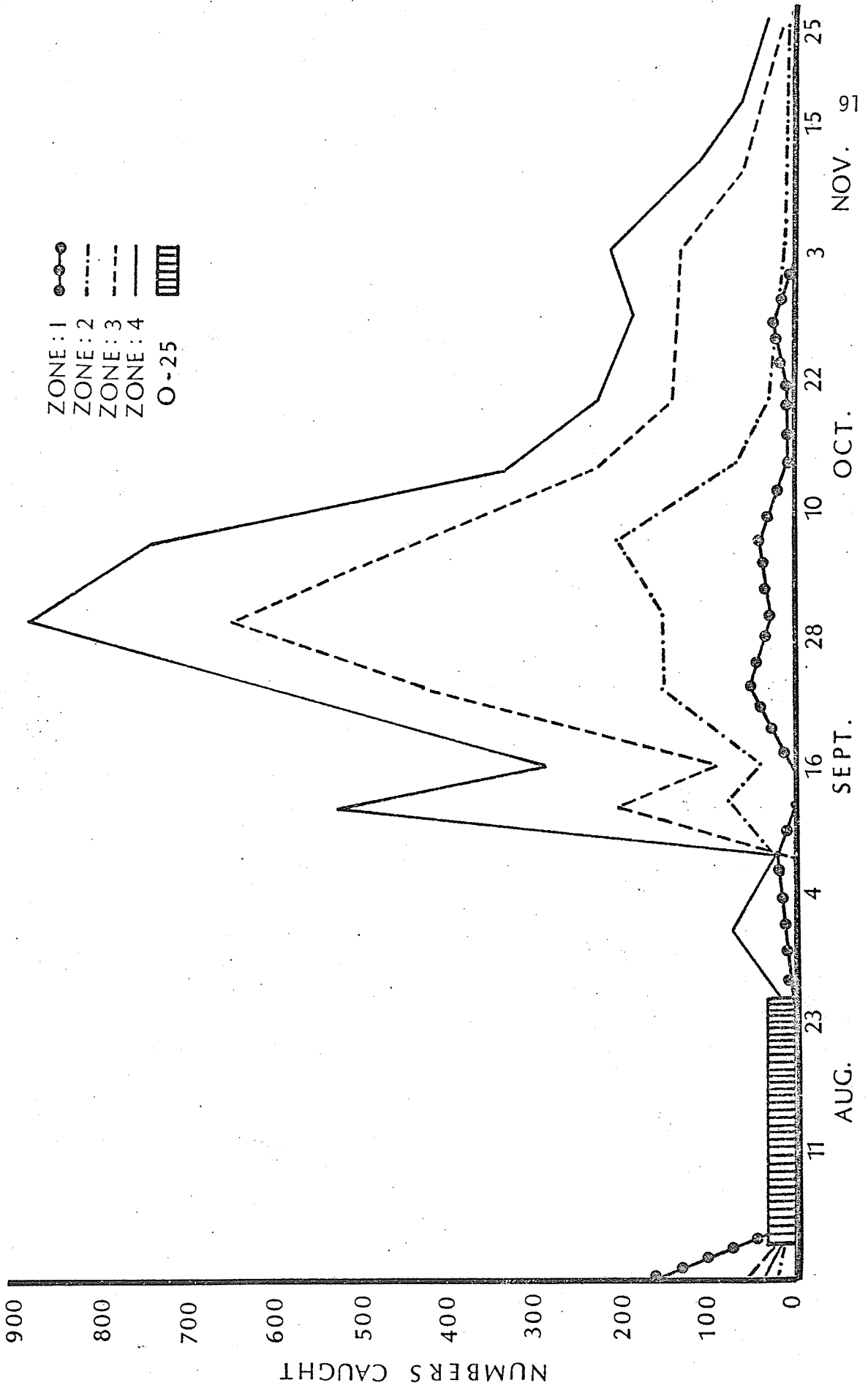
The number of insects trapped in the outer set of traps increased sharply during the second week of September, dropped somewhat near mid-month and then increased to a maximum of nearly 700 adults on September 28th, (Figure 22). After September 28th, the numbers trapped dropped sharply again. The peak capture in the inner set of traps occurred early in October, slightly behind the peak in the outer set. However, the average number of insects per trap was roughly the same in the inner and outer sets. After mid October, more insects were trapped in the inner set than in the outer set. Whether or not this increase is due to higher locomotor activity in the central region or movement toward the centre of the bin will be discussed later.

Although the moisture content of the grain, in this zone, was optimal for rapid insect development and reproduction, the low temperature was a limiting factor.

Throughout August, very few insects were trapped in any of the zones (Figure 23). The peak catches occurred around September 28th, nearly 2 months after introduction of the insects into the grain bulk. This probably corresponds to the development of the first

FIGURE 23

TOTAL NUMBERS OF ADULT C. ferrugineus CAUGHT IN TRAPS IN EACH
ZONE OF THE BIN DURING THE PERIOD JULY 30TH TO NOVEMBER 25TH, 1970.



generation of beetles. Thereafter, the temperature and the number of insects trapped in the grain bulk began to decrease. The reduction in numbers of insects trapped was probably due to reduced locomotor activity associated with the reduced temperature.

Perhaps the most significant trend noted in this study is that the number of insects trapped increased with increasing depth in the grain, the fewest being trapped at the surface and the greatest number near the floor of the bins. This suggests that infestations could be detected more successfully by trapping near the bottom of a bin than at any other depth. The accumulation of insects near the floor may be a response to a high moisture content of the grain at this level in the bin. Since temperatures near the floor were too low for rapid reproduction, it is suggested that the insects must have bred elsewhere and then moved to the area near the floor in response to a moisture gradient.

Distribution of insects in the control cylinders: Examination of the control cylinder on the North side of the bin 11 days after introduction of the insects showed that 72 percent were located in the top 15 inches of grain in the cylinder (Table VIII). There were very few insects in the lower 30 - 56" of the grain. By August 19th, 23 days after introduction, approximately 57 percent of the insects recovered in the previous examination were in the 15 - 30" depth, while 35 percent of the insects still remained in the upper 15 inches of the grain (Table VIII). Examination on August 31st and September 14th,

indicated that the adult beetles tended to concentrate in the 15 - 45" depth whereas the larvae were concentrated in the upper 30 inches and lower 11 inches of the cylinder (Table VIII).

In the South cylinder, examined on September 15th, 47 percent of the adults recovered were located in a layer of grain 15 - 30" below the surface of the grain bulk. Thirty-three percent of the remaining adults were recovered from the bottom 11 inches. The distribution of larvae in this cylinder clearly indicates that reproduction took place within the top 30 inches of grain. No larvae were found at a depth below 30 inches of the surface. The recovery of 142 live adults, out of the 160 introduced, indicated low mortality.

A comparison of the use of trapping, spear probing, and vacuum probing methods for determining the presence, abundance and distribution of C. ferrugineus in bulk grain. The data (Tables IX, X, XI and XII) shows that in all cases, more larvae than adults were recovered from each sampling site using the vacuum and spear probes, while more adults than larvae were caught using the trap. The efficiency, (No. of samples with insects/No of samples x 100) of the spear and vacuum probes was higher (55 - 65 percent) when larvae were used as an indication of infestation, than when adults were used to determine the efficiency of the 2 devices (efficiency for adults 27 - 34 percent) (compare Tables IX and XI and XI to X and XII respectively).

The trap recovered more adults from the grain than did either the vacuum, or spear probe devices. Only 2 larvae were caught in the

TABLE VIII
 NUMBERS OF INSECTS RECOVERED FROM CONTROL SOCKS
 LOCATED IN THE BIN AT GLENLEA, MAN., 1970

		<u>Number of insects recovered in each zone</u>									
Sock 1 (North)	Date	0 - 15"		15 - 30"		30 - 45"		45 - 56"		Total	
		A.*	L.**	A.	L.	A.	L.	A.	L.	A.	L.
	Aug. 7	98		29		5		5			137
	Aug. 19	47		76		6		4			134
	Aug. 31	7		57		33		6			103
	Sept 14	3	13	28	13	36	4	18	21	85	32
Sock 2 (South)	Sept 15	2	16	69	11	23		48		142	27

* A = adults

** L = larvae

TABLE IX
 TOTAL NUMBERS OF C. ferrugineus LARVAE OBTAINED FROM SAMPLES
 TAKEN AT 6", 2', 3'6" AND 4'7 - 8" DEPTHS AT THE 14 LOCATIONS
 DESIGNATED IN FIGURE 12, ON SEPTEMBER 16, 1970 WITH A VACUUM
 PROBE, SPEAR PROBE AND 56 TRAPS

Depth	VACUUM PROBE		TRAP		SPEAR PROBE	
	No. samples with larvae	No. larvae	No. samples with larvae	No. larvae	No. samples with larvae	No. larvae
6"	14	129	0	0	12	87
2'	11	168	0	0	8	233
3'6"	7	46	1	2	10	74
4'7 - 8"	4	5	0	0	No sample possible	
Total	36	348	1	2	30	394
Efficiency	64.3		1.8		71.5	

TABLE X

TOTAL NUMBERS OF C. ferrugineus ADULTS OBTAINED FROM SAMPLES TAKEN AT 6", 2', 3'6" AND 4'7 - 8" DEPTHS AT THE 14 LOCATIONS DESIGNATED IN FIGURE 12, ON SEPTEMBER 16, 1970 WITH A VACUUM PROBE, SPEAR PROBE AND 56 TRAPS

Depth	VACUUM PROBE		TRAP		SPEAR PROBE	
	No. samples with adults	No. adults	No. samples with adults	No. adults	No. samples with adults	No. adults
6"	1	1	5	6	0	0
2'	4	17	10	40	5	73
3'6"	4	16	9	86	4	8
4'7 - 8"	7	63	11	281	No sample possible	
Total	16	97	35	413	9	81
Efficiency*	28.6		62.5		21.4	

* Efficiency = Number of samples with insects/Number of samples x 100

TABLE XI

TOTAL NUMBERS OF C. ferrugineus LARVAE OBTAINED FROM SAMPLES TAKEN AT 6", 2', 3'6" AND 4'7 - 8" DEPTHS AT THE 14 LOCATIONS DESIGNATED IN FIGURE 12, ON OCTOBER 28, 1970 WITH A VACUUM PROBE, SPEAR PROBE AND 56 TRAPS

Depth	VACUUM PROBE		TRAP		SPEAR PROBE	
	No. samples with larvae	No. larvae	No. samples with larvae	No. larvae	No. samples with larvae	No. larvae
6"	10	8	0	0	14	98
2'	9	209	0	0	9	337
3'6"	8	64	0	0	7	22
4'7 - 8"	4	14	0	0	No sample possible	
Total	31	368	0	0	30	457
Efficiency	55.4		0		71.5	

TABLE XII
 TOTAL NUMBERS OF C. ferrugineus ADULTS OBTAINED FROM SAMPLES
 TAKEN AT 6", 2', 3'6" AND 4'7 - 8" DEPTHS AT THE 14 LOCATIONS
 DESIGNATED IN FIGURE 12, ON OCTOBER 28, 1970 WITH A VACUUM
 PROBE, SPEAR PROBE AND 56 TRAPS

Depth	VACUUM PROBE		TRAP		SPEAR PROBE	
	No. samples with adults	No. adults	No. samples with adults	No. adults	No. samples with adults	No. adults
6"	2	2	10	25	3	3
2'	4	6	8	26	4	10
3'6"	7	53	9	137	5	6
4'7 - 8"	6	62	10	186	No sample possible	
Total	19	123	37	374	12	19
Efficiency	33.9		66.1		28.6	

TABLE XIII

TOTAL NUMBER OF C. ferrugineus ADULTS OBTAINED FROM SAMPLES TAKEN AT 6", 2', 3'6" AND 4'7 - 8" DEPTHS IN THE INNER FOUR AND OUTER TEN SETS OF TRAPPING LOCATIONS USING A VACUUM PROBE AND TRAPS, ON SEPTEMBER 16, AND OCTOBER 28, 1970.

(COMPILED FROM DATA COLLECTED FOR TABLES VII - X)

Date:	Depth	VACUUM PROBE		TRAP	
		No. adults inner set	No. adults outer set	No. adults inner set	No. adults outer set
Sept. 16/70	6"	0	1	1	5
	2'	0	17	10	30
	3'6"	0	16	5	75
	3'7 - 8"	3	60	7	274
Oct. 28/70	6"	1	1	16	10
	2'	3	3	15	11
	3'6"	39	14	114	23
	4'7 - 8"	15	47	133	53

traps (Table IX). Thirty-five of 56 traps contained adults, while only 16 of 56 (28.6 percent) samples taken with the vacuum probe, and 9 of 42 (21.4 percent) taken with the spear probe recovered adults from samples taken on September 16th (Table X). Similar results were obtained upon examination of the samples taken on October 28th (Table XII). The efficiency of the trap in detecting adults is approximately double that of either of the other sampling methods used for detection of adults. It was found that the efficiency of the spear probe and vacuum probe devices for detecting larvae, was equal to the efficiency of the trap for detecting adults (Table IX - XII).

When comparing the number of adults, and larvae recovered from the samples taken with the vacuum probe on September 16th, only 16 of 56 (28.6 percent) samples contained adult insects while 36 of 56 (64.3 percent) of the same samples contained larvae. Similar results were obtained using the spear probe. Larvae appear to be a better indication of infestation than adults when using the vacuum or spear probes.

The data presented in Tables IX and XI confirmed the results obtained in the control cylinders, that the larvae tend to concentrate in the top 2 feet of the grain bulk. Adult distribution is similar to the pattern presented in Figure 8, adults tended to concentrate in the lower 1/2 of the grain bulk (Tables X, XII). The data collected, using the vacuum probe and trap was subdivided into the outer set of 10 locations, and the inner set of 4 locations (Figure 12) to determine the changes in the numbers of insects present in each set on September

16th and on October 28th (Table XIII). On September 16th, using a vacuum probe, a total of 3 adult beetles was recovered from the inner set of sites and 94 from the outer set of sampling sites. Six weeks later, on October 28th, 58 adults were recovered from the inner set, and 65 from the outer set.

On September 16th, using the traps, 384 adults were removed from the outer set and 23 from the inner set of sampling sites. On October 28th, only 97 adults were recovered from the outer set of sampling sites while 278 were recovered from those in the inner set. These data (Table XIII) indicates that the number of insects in the central set increased from September 16th to October 28th, 1970 and that the adults of C. ferrugineus tend to concentrate near the floor of the bin as the fall season progresses.

Winter distribution of C. ferrugineus in a grain bulk. The temperature at which C. ferrugineus adults stop moving has been set at approximately 2⁰C (Figure 2, Chapter 4). By March 1971, the temperature of the entire experimental bulk of grain was below 2⁰C. At various intervals from March 17th, 1971, samples were taken from various locations in the bin with a vacuum probe to determine in what area of a granary C. ferrugineus adults spend the winter. Examination of the samples taken from various locations (Figure 24) revealed that the bulk of the live insects in the grain were concentrated in a small triangular region near the floor, extending from approximately 1 foot east to 4 feet east of the centre of the bin (Figure 24). The actual numbers of live and

FIGURE 24

SITES IN THE BIN AT GLENLEA FROM WHICH SAMPLES WERE REMOVED ON
MARCH 1st, 1971, TO DETERMINE WINTER DISTRIBUTION OF C. ferrugineus
(NUMBERS INDICATE DISTANCE IN FEET FROM CENTRE)

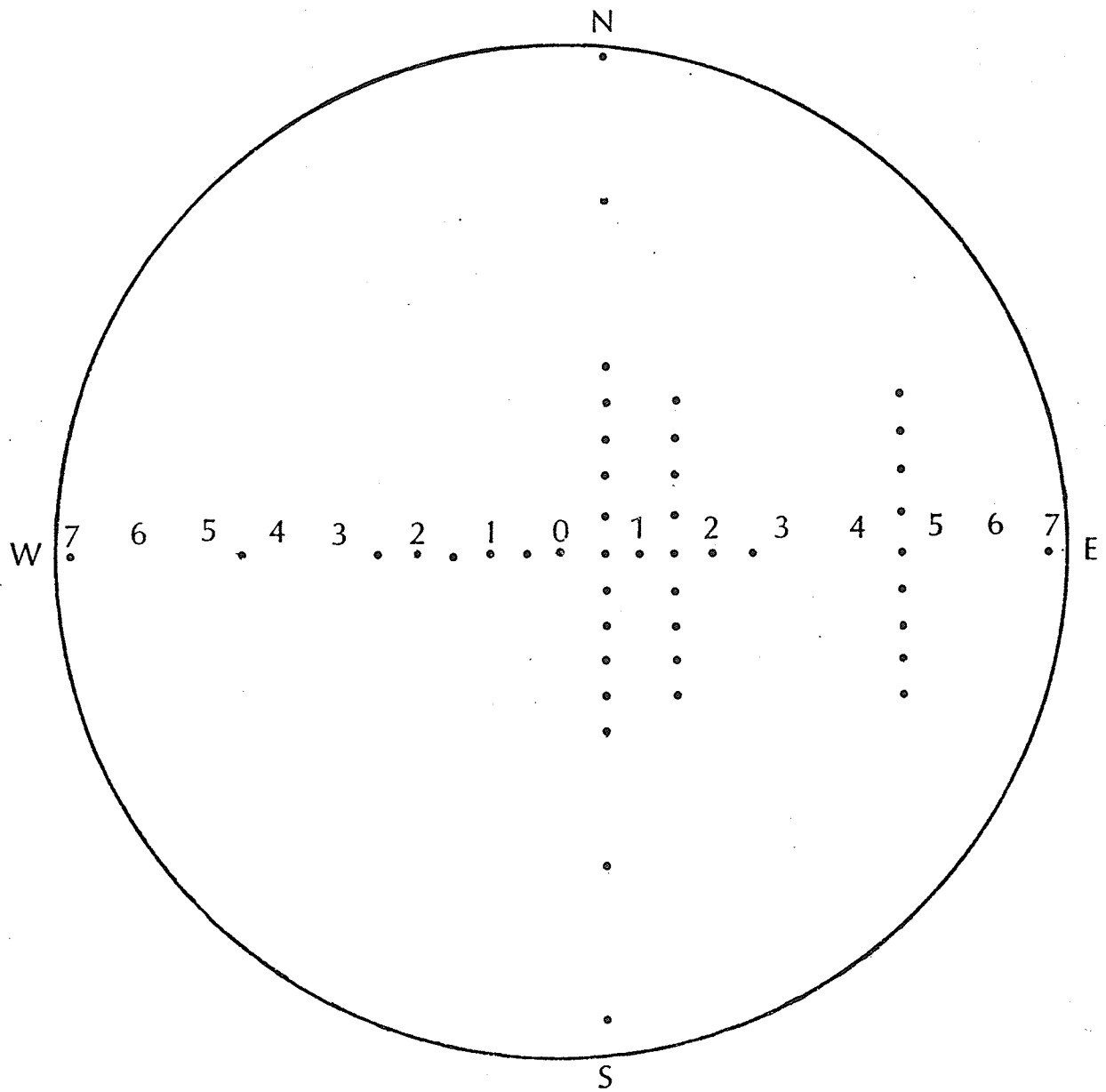


TABLE XIV

LOCATIONS FROM WHICH GRAIN SAMPLES WERE TAKEN WITH A VACUUM PROBE,
ON MARCH 1ST, 1972 AND THE NUMBERS OF INSECTS IN EACH SAMPLE
(REFER TO FIGURE 24)

103

Location of sampling points from centre of bin	Distance from floor		
	0 - 1"	1'	2'
E6"	5,39*	20,13	0,6
E6"N6"	2,34	0,6	0,1
E6"N1'	0,3	0,1	0,0
E6"N1'6"	1,3	0,0	0,0
E6"N2'	0,0	0,0	0,0
E6"N2'6"	0,0	0,0	0,0
E6"N4'6"	0,0	0,0	0,0
E6"N6'10"	0,0	0,0	0,0
E6"S6"	8,36	27,28	2,16
E6"S1'	7,18	2,0	0,0
E6"S1'6"	0,4	0,0	0,1
E6"S2'	0,2	0,0	0,0
E6"S2'6"	0,1	0,0	0,0
E6"S4'6"	0,1	0,0	0,0
E6"S6'6"	6,9	0,0	0,0
E1'	75,439	131,350	12,29
E1'6"	133,139	311,600	15,49
E1'6"N6"	0,4	0,0	0,1
E1'6"N1'	0,1	0,1	0,1
E1'6"N1'6"	1,1	0,0	0,0
E1'6"N2'	0,0	0,0	0,0
E1'6"S6"	48,183	33,176	10,86
E1'6"S1'	7,64	5,8	0,1
E1'6"S1'6"	3,14	0,1	0,0
E1'6"S2'	25,78	4,4	0,0
E2'	69,38	23,20	15,31
E2'6"	56,32	27,40	5,17
E4'6"	24,2	0,3	0,0
E4'6"N6"	3,9	0,1	0,0
E4'6"N1'	0,1	0,0	0,0
E4'6"N1'6"	1,0	0,0	0,0
E4'6"N2'	0,0	0,0	0,0
E4'6"S6"	14,7	1,0	1,0
E4'6"S1'	12,0	0,0	0,0
E4'6"S1'6"	16,5	0,1	0,0
E4'6"S2'	12,12	0,0	0,0
E6'10"	11,92	0,7	0,0
W6"	0,5	2,7	0,0
W1'	21,16	0,1	0,1
W1'6"	1,6	0,0	0,0
W2'	3,3	0,0	13,13
W2'6"	0,4	0,0	0,2
W4'6"	1,0	0,0	0,0
W6'10"	1,0	0,0	0,0

* Number live, Number dead

dead insects found at each point, are recorded in Table XIV. Examination of kernels removed from the heavily infested area showed no degermination of kernels by C. ferrugineus larvae. Consequently, it is apparent that the adults recovered from this region, developed in some other section of the grain bin, then moved to this central location.

Percentage degermination by C. ferrugineus in an 8 month period.

The percentage degermination of the samples obtained within a few inches of the floor and wall of the grain bin at Glenlea was highest from the East to South to West region of the bin, approximately from point 35 through 0 to 15 (Table XV, Figure 25). There were great variations from location to location, but the average degermination in this zone was 20 percent. There was another area of high degermination, extending from location 21 to 24 (Table XV). The area from the West to North to East was relatively free of seriously degermed kernels near the wall-floor interface. The number of insects found in each of the samples was directly correlated with the number of kernels degermed in each sample. Only 9 adult C. ferrugineus were found alive in the 35 samples taken near the wall. The moisture content of the grain in the 35 samples ranged from 15.1 to 17.7 percent and was sufficient for C. ferrugineus development. There was no obvious relationship between moisture, and percentage degermination. No pupae or adults were found alive in the samples examined.

Samples were taken inward towards the centre of the bin, from positions 7 and 13, (Figure 25). The percentage degermination was

TABLE XV

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MOISTURE CONTENT, PERCENT DEGERMINATION, AND NUMBER OF
C. ferrugineus FOUND IN SAMPLES TAKEN AT 15 INCH INTERVALS
 AROUND THE PERIPHERY OF THE GRAIN BULK AT GLENLEA (MARCH 17, 1971)

Location* in bin	Moisture content	No. <u>C. ferrugineus</u> adults in each sample		No. of kernels/100 containing dead larvae, pupae or adults	Percent degermination
		Live	Dead		
0	15.8	0	2	13	17
1	16.0	2	14	7	8
2	16.4	0	32	1	2
3	16.6	1	15	4	31
4	16.9	2	93	7	29
5	16.5	5	365	8	31
6	16.0	2	12	9	22
7	16.6	0	3	18	33
8	16.6	0	8	10	21
9	16.4	0	1	1	2
10	17.0	0	19	8	12
11	17.0	0	15	4	6
12	16.4	0	53	12	16
13	17.2	0	300	20	30
14	16.8	0	17	15	22
15	16.1	0	1	9	30
16	15.1	0	21	3	9
17	16.2	0	0	0	2
18	16.2	0	0	1	1
19	16.5	0	2	4	4
20	16.6	0	5	12	18
21	17.3	0	7	12	20
22	17.6	0	17	12	19
23	15.9	0	48	10	11
24	16.4	0	2	0	0
25	17.7	0	1	1	2
26	17.3	0	0	1	1
27	17.4	0	1	2	2
28	17.2	0	4	0	1
29	16.2	0	1	0	1
30	17.0	0	0	0	0
31	16.6	0	36	3	3
32	16.5	0	2	2	2
33	16.7	0	8	5	6
34	16.3	0	6	0	2
35	16.4	0	34	10	20

* To determine location refer to Figure 20

FIGURE 25

SITES NEAR THE PERIPHERY OF THE BIN AT GLENLEA FROM WHICH SAMPLES WERE REMOVED ON MARCH 1ST, 1971, TO DETERMINE DEGERMINATION AND DISTRIBUTION, AND SITES 7 AND 13 FROM WHICH SAMPLES WERE REMOVED TOWARDS THE CENTRE OF THE BIN ON APRIL 7TH, 1971.

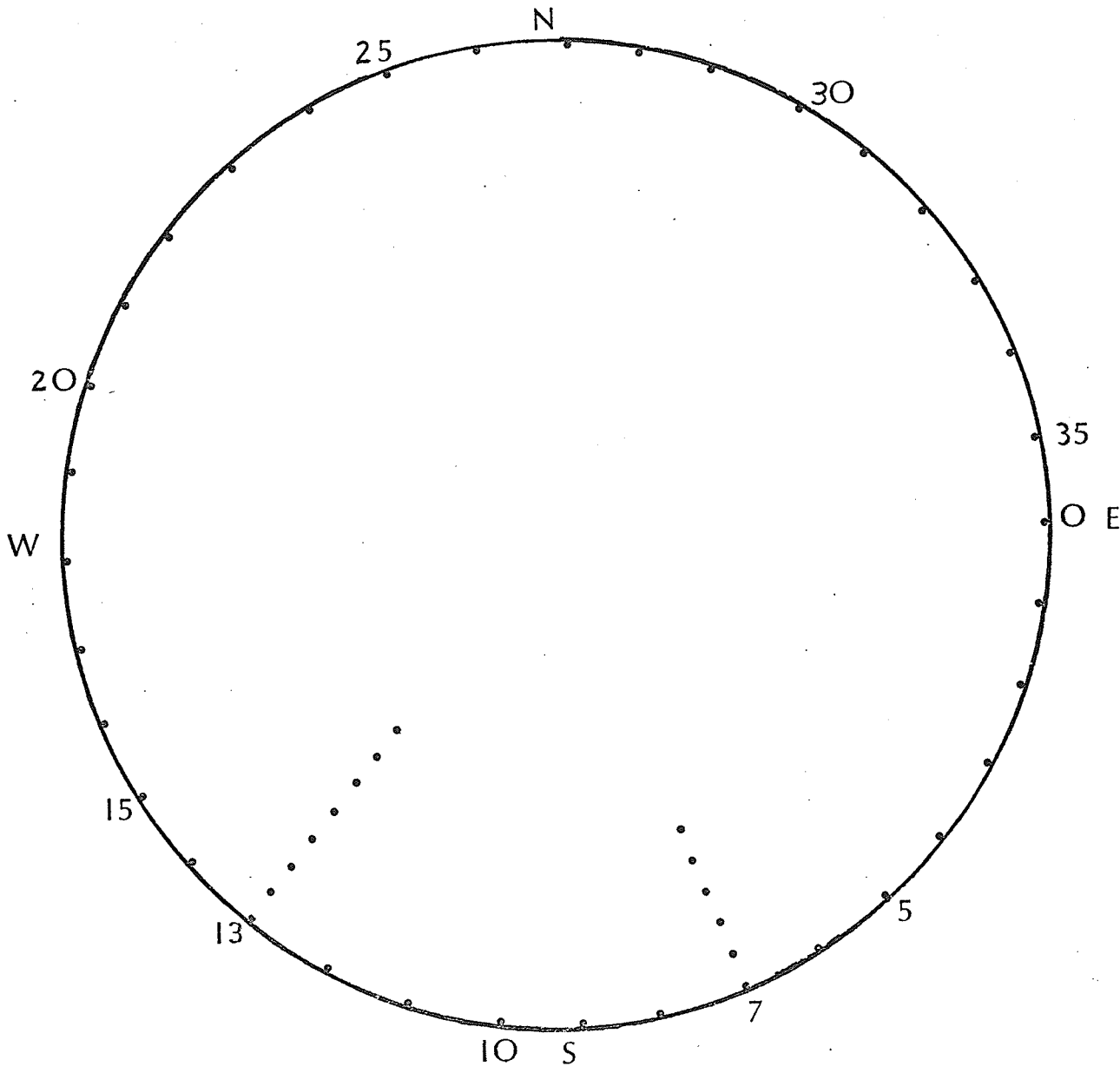


TABLE XVI

MOISTURE CONTENT, AND PERCENT DEGERMINATION OF KERNELS REMOVED AT 6 INCH INTERVALS VERTICALLY AND HORIZONTALLY FROM LOCATIONS 7 AND 13 IN THE BIN AT GLENLEA (MARCH 17, 1971)

Location* in bin	Distance from wall (in inches)	Distance from floor (in inches)											
		0-1	12	24	36	48	M.C.	%	M.C.	%	M.C.	%	
7	1-2	16.2	17	13.7	3	13.9	3	13.9	3	13.9	1	14.0	5
7	6	15.2	0	14.5	0	14.1	0	14.3	2	14.1	2	14.1	0
7	12	15.6	1	14.3	0	13.8	0	14.1	4	14.2	4	14.2	2
7	18	15.8	0	14.9	0	13.8	0	14.0	1	14.6	1	14.6	0
7	24	15.6	0	-----	-	-----	-	-----	-	-----	-	-----	-
13	1-2	17.5	29	15.0	6	14.5	2	13.9	0	13.7	0	13.7	0
13	6	16.2	1	14.7	0	14.2	0	13.9	0	13.6	0	13.6	0
13	12	15.7	0	-----	-	-----	-	-----	-	-----	-	-----	-
13	18	15.8	0	-----	-	-----	-	-----	-	-----	-	-----	-
13	24	15.8	0	-----	-	-----	-	-----	-	-----	-	-----	-
13	30	15.9	0	-----	-	-----	-	-----	-	-----	-	-----	-
13	36	15.9	0	-----	-	-----	-	-----	-	-----	-	-----	-

* To determine location refer to Figure 20

----- No sample taken

highest at the floor as near the wall as possible at both sampling positions (Table XVI). The percentage degermination of kernels decreased from that in samples taken at positions 7 and 13 at the inner wall-floor interface of the bin as distance from these positions increased vertically and horizontally.

Sampling at all other portions of the bin revealed that in general, the percentage degermination of the wheat kernels ranged from 0 - 1 percent. These data are not presented, but in all the samples taken at the 56 sampling points shown diagrammatically in Figure 12, percentage degermination was either 0 or 1 percent.

The species and numbers of insects caught using the trapping method are recorded in Table XVII. This table includes data from other experiments that are not part of this thesis but are presented to indicate the variety of insects and mites that are detected by the trapping technique.

Discussion

The distribution of C. ferrugineus in a large bulk of wheat appears to be influenced primarily by temperature with the site of maximum reproduction occurring where temperature and moisture are optimal.

The movement of the 84,000 adult C. ferrugineus initially introduced into the grain bulk on July 27th, 1970 could not be traced by the trap method until late fall. The initial population may have been

TABLE XVII

SPECIES AND NUMBERS OF INSECTS CAUGHT IN TRAPS AT THREE DIFFERENT
SAMPLING LOCATIONS DURING THE PERIOD SEPTEMBER, 1969 TO DECEMBER 1971

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Species	Numbers* trapped	Trapping site
Coleoptera		
Anthicidae		
<u>Anthicus floralis</u> (Linne.)	6	Selkirk
Cryptophagidae		
<u>Cryptophagus varus</u> (Woodroffe & Coombs)	43	Glenlea, La Salle
Cucujidae		
<u>Cryptolestes ferrugineus</u>	15,000+	Glenlea, La Salle, Manitowish
<u>C. ferrugineus</u> (Steph.) Larvae	979	Glenlea, La Salle
<u>Oryzaephilus surinamensis</u> (Linne.)	2	Glenlea
Lathridiidae		
<u>Coninomus constrictus</u> (Gyll.)	37	Glenlea, La Salle
<u>Lathridius minutus</u> (Linne.)	66	Glenlea, La Salle
<u>Microgramme arga</u> (Reit.)	3	La Salle
Monitomidae		
<u>Monitoma picipes</u>	2	La Salle
Silvanidae		
<u>Ahasverus advena</u> (Waltl.)	136	Glenlea
Tenebrionidae		
<u>Tenebrio molitor</u> (Linne.)	2	La Salle
<u>Tribolium castaneum</u> (Herbst.)	2	Glenlea
Hemiptera		
Anthocoridae		
<u>Xylocoris galactinus</u> (Fieb.)	10	Selkirk
Hymenoptera		
Bethylinidae		
<u>Cephalonomia waterstonii</u> (Gahan)	4	Glenlea
Psocoptera		
Atropidae		
<u>Lepinotus reticulatus</u> (Endl.)	100,000+	Glenlea, La Salle
Acarina		
Acaridae		
<u>Acarus siro</u> (Linne.)	50,000+	Glenlea
Cheyletidae		
<u>Cheyletus eruditus</u> (Schr.)	500,000+	Glenlea
Cunuxidae (sp. undetermined)	124	Glenlea
Glycophagidae		
<u>Glycophagus destructor</u> (Schr.)	500,000	Glenlea
Laelaptidae		
<u>Haemolaelaps casalis</u> (Berlese)	1,000	Glenlea, La Salle
Tydeidae		
<u>Tydeus interruptus</u> (Thor.)	20,000+	Glenlea

* Numbers followed by + indicate an estimate

too small to indicate movement, but results obtained from the control cylinders indicated that the adults may have moved to areas not sampled by the trap. In the controls, the insects moved to the top 2 feet of grain within 11 days, or possibly sooner after introduction into the bin. This movement which was most likely in response to the vertical temperature gradient could also have been followed horizontally, leading many insects to the periphery of the bin and out of the sampling area (Table VIII). Either the beetles moved to the surface or the periphery of the bin and out of the sampling area or too few insects were present to indicate actual distribution. Only after approximately a month and a half were substantially large numbers of beetles trapped. The larger number trapped was due to increased population as a result of reproduction. By this time however, the population size was unknown, and exact distribution undeterminable.

The results from the controls also indicated that in the confined area of the cylinder, reproduction as indicated by the larvae sifted out of the grain took place in the top 30 inches of grain. Later sampling with the vacuum and spear probes also showed that more larvae were in the upper half than the lower half of most of the bin. Counts of the numbers of kernels degermed in each zone indicated that more larvae were present near the surface than the floor. However, the differences between counts were not significant, and the number of larvae was a better indicator of distribution in those zones. The greater number of larvae in the upper half of the bin than the lower

half was attributed to emigration from the cooler lower half, and to inhibition of oviposition due to cooler temperatures in the lower half.

Sampling near the end of the experiment revealed that the number of larvae present in the upper portion of the bin were low compared to the numbers near the wall-floor interface (Table XV and XVI) (Fig. 25). The temperature in this latter area of the bin was high, ranging from approximately 45°C at the wall to 25°C 4 to 5 inches from the wall. This temperature range was suitable for insect development. This area also contained sufficient moisture from seepage along the concrete floor-wall intersection. This seepage is typical in many steel bins, and often provides the conditions conducive to the development of moulds or fungi which may have been responsible for attracting insects to the area. As shown in Chapter VI, micro-organisms can attract adult C. ferrugineus.

Records of insects trapped indicated that more adult C. ferrugineus were trapped near the floor of the bin than the surface (Figure 23). This was due to the greater re-production of insects near the wall-floor interface than in the upper portion of the bin and possibly to a geotactic response. The insects moved from the wall into the bin as the grain temperature increased. From July 27th to October 20th, the number of insects trapped in the outer set of traps was higher than that trapped in the inner set. This indicated that the number of insects present or locomotor activity was greater in the central portion of the bin after October 20th. Data obtained with the traps and vacuum probes on

September 16th and October 28th show increases in the number of insects recovered in the inner set of sampling sites and decreases in the outer set. (The data for the spear probe were not included because samples could not be taken near the floor). The data obtained with the vacuum probe clearly showed that the number of insects present in the area of the inner set of traps had increased; the number recovered did not depend on insect movement (Table XIII). There were 2 1/2 times the number of traps in the outer set of sampling locations than in the inner set, but the number of insects in the inner portion of the bin, especially near the floor increased substantially from September 16th to October 28th, 1970.

The increase in the number of insects in the inner section of the bin was related to the decrease in temperature in the peripheral portion of the bin, while the inner portion remained at a relatively higher temperature. Evidently the insects moved from the periphery of the bin into the central region in response to temperature. This was confirmed by sampling in March which showed that most of the adults of C. ferrugineus were near the centre of the bin (Table IV). Since degermination in that area was zero, the adults must have migrated to the centre rather than developed there. Whether these adults will move back to the periphery of the bin as the grain warms will be the subject of future experiments.

The number of live adults in the centre of the bin during the winter was sufficient to ensure continued infestation the following year. Many dead insects were noted in the central area. These were probably

the less hardy insects which could not survive the low temperature, in winter. A few insects survived the winter within a few inches of the wall, indicating that some individuals of this species can survive low temperatures (Table XIV and XV). These few insects became mobile as soon as the sun began to warm the walls of the bin in spring and commence reproduction in this small micro-zone by approximately May 1st of any given year. No larvae or pupae were found alive in the bin in March.

Sampling methods used in these studies varied in effectiveness in detecting insects in stored grain. The traps collected adults exclusively, while the spear and vacuum probes collected both larvae and adults. In one instance during the entire sampling period, a total of 189 larvae were recovered from a single trap, indicating that larvae do at times leave the kernels and move freely in the grain bulk. The efficiency of the traps was significantly affected by the depth at which they were placed, more insects being trapped as depth increased.

The vacuum and spear probes were more efficient for detecting larvae than adults (Table IX and XI). Most of the larvae were at the 6 inch level on September 16th; by October 28th, 1970 the bulk of the larvae were at the 2 foot level. This was brought about by movement deeper into the bin as the surface cooled. Adults were collected by both probing methods, but these methods were more efficient for larvae than for adults. On the other hand only adults

are collected with the traps. Therefore, when using probes, the possibility of determining whether a bin is infested is greater if larvae rather than adults are used as an indicator of infestation.

The rusty grain beetle was not the only insect caught in the traps. Table XVII shows that many species of insects and mites were collected. Both Tenebrio molitor and Pyralis farinalis adults were found in the traps but these were collected when these insects climbed down the extension pipe from the surface into the trap. The traps would be a good device for studying mite distribution, since millions of mites were collected. The population of the minor species of insects collected was low, and their distribution could not be determined with accuracy.

In summary, the insects which were introduced into the bin moved to the surface and sides of the bin. Reproduction took place in both areas, but was highest on the South side, near the wall-floor interface. As the grain cooled from the outside, the bulk of the adults moved towards the warmer centre of the bin. The larvae and pupae which were near the periphery and surface of the bin died as the grain cooled. Many adults in the central region of the bin and a few near the peripheries survived to perpetuate the infestation.

From these experiments the following sampling procedures are recommended for different seasons: (1) to sample a bin with a trap in spring and summer place the trap as near the floor-wall interface as possible at the South side. (2) to sample with the trap in late fall,

place the traps approximately 5 feet from the wall near the floor. (3) to sample with the probes in summer, take samples from the top 2 feet of grain in the bin, and use larval populations as well as adult populations as an indication of infestation. (4) if the vacuum probe is used during the summer, sample near the floor-wall interface. (5) if the vacuum probe is used during the winter, sample near the centre of the bin at the floor and check the samples for adults, or remove samples from the area of the floor-wall interface, and examine for wheat kernel degermination. If these practices are followed when sampling a steel bin, an accurate assessment of infestation will be possible.

CHAPTER VIII

SUMMARY

The present investigation of the influence of environmental factors on the distribution and detection of C. ferrugineus in wheat, were initiated to expand our knowledge of stored grain insect activity. Proper control procedures in stored grain depend on the use of proper detection methods, and an understanding of insect distribution. Laboratory experiments were designed to determine: (1) locomotor and respiratory activity of insects at different temperatures (2) the effect of temperature, moisture, insect density and time, on the number of insects caught in traps, and (3) the effect of fungi, and water vapour on the downward movement of C. ferrugineus through columns of wheat. A field study was conducted to determine the distribution of C. ferrugineus in a large bulk of wheat, and examine the efficiency of various sampling devices. The results of these studies are briefly summarized in this chapter.

As temperature increased from 1.1^oC to 30^oC, the speed of locomotion on a flat surface increased from 1.75 mm/sec to 10.48 mm/sec., while oxygen consumption in the absence of wheat increased from 338.53 ul/mg/hr to 3462.68 ul/mg/hr. The coefficient of determination (R^2) for locomotion was 93.2 percent while the coefficient of determination was 97.0 percent for respiration. Both speed of locomotion and rate of respiration are directly determined by temperature, and

change in direct relation to temperature change. C. ferrugineus adults became motionless at 2°C, thereby suggesting that the trap would be ineffective at lower temperatures.

Experiments conducted with adult C. ferrugineus in 1-gallon jars indicated that of environmental factors temperature, moisture, insect density and time allowed for trapping, temperature was the primary factor determining the number of insects trapped. As the temperature increased from 8.9°C to 30°C, the number of insects found in traps located in grain at moisture contents of 13.7 percent and 16.3 percent increased.

High temperature (30°C) and low moisture (13.7 percent) as compared to high temperature and high moisture (16.3 percent) appeared to facilitate an increase in numbers trapped, but the difference was not statistically significant.

The results of experiments up to this point indicate that as temperature increased, insect activity and numbers of insects caught in traps, increased.

One hundred insects placed at the surface of columns of wheat placed over dishes of either single fungi, mixed fungi, dry or moist grain or water, were not affected equally. Grain covered with fungi caused more insects to move down in to the dishes of wheat, than any of the other treatments. The results also suggest that different fungi vary in ability to attract adult C. ferrugineus. P. corymbiferum attracted 80 of 100 adults while Streptomyces sp. only attracted 26.4

of 100 adults. Five other grain-fungi treatments fell between this range (Table VI). Wet grain, and dry grain attracted the fewest insects, and were not significantly different from each other.

The fact that fungi play a role in insect biology has been confirmed by many workers. These results definitely indicate that fungi play a part in determining insect movement in grain, leading to changes in distribution, and therefore regulating sampling procedures.

Insects when artificially distributed in a bulk of wheat tended to move to the warmer areas of the bin, namely near the surface and walls. The populations of adult beetles tended to increase in the bottom half of the bin, with the maximum number trapped nearest the floor. Sampling with spear and vacuum probes, and results from controls indicated that many larvae were present in the top 2 feet of grain. Results of sampling near the wall however; showed that the bulk of the reproduction in the bin took place at the floor-wall interface along the entire South side of the bin. The areas of reproduction were determined by counting the numbers of degermed kernels. Degermination on the South side near the all reached a maximum of 33 percent.

The number of insects trapped in each zone of the bin reached a maximum near the end of October, then decreased as environmental temperature decreased with the onset of the fall, and winter seasons. On approximately October 18th, the number of insects trapped near the centre of the bin increased over the number trapped in the outer set. This increase in numbers trapped in the inner set was due to the

movement of C. ferrugineus adults from the periphery to the centre of the bin. Sampling during the winter confirmed this movement, since the bulk of the live adults were congregated near the centre of the bin close to the floor. A few adult C. ferrugineus were able to survive the winter near the wall of the bin.

Sampling in the bin was carried out with 3 sampling devices. The trap was efficient in sampling for adult C. ferrugineus. The spear and vacuum probe devices were more efficient in collecting larvae than adults. The efficiency for collecting larvae with the spear and vacuum probes was approximately equal to the efficiency of the trap for detecting adults.

Many species of insects and mites were collected with the trap. Work dealing with the detection of various insects using this device could be facilitated successfully, and provide valuable information.

A summary of sampling methodology is provided in Chapter VII, and is therefore not summarized in this chapter.

CHAPTER IX

CONCLUSIONS

1. Rates of locomotion on flat surfaces and respiration in the absence of wheat are determined by temperature, and change in response to temperature.
2. Locomotion of adult C. ferrugineus ceases at approximately $2\pm 1^{\circ}\text{C}$.
3. Of environmental factors temperature, moisture, numbers, time and temperature is the primary factor responsible for determining the number of insects caught by the traps.
4. As temperature increased from 8.9°C to 30°C the number of insects trapped increased.
5. A 2 day trapping period is sufficient for detecting adults of C. ferrugineus.
6. A mixture of fungi growing in spoiled grain attracted more adults of C. ferrugineus to the bottom of columns, than did water.
7. Penicillium corymbiferum attracted more insects to the bottom of the columns of wheat than the group of Scopulariopsis brevicaulis, Fusarium sp., spoiled grain, than the group of Cephalosporium acremonium, Aspergillus repens, than the group of Streptomyces sp., wet grain and dry grain, respectively.
8. Fungi may play a vital role in influencing the movement, thus distribution of adult C. ferrugineus.

9. Insects when distributed evenly in grain move to the surface and possibly the periphery of a grain bin.
10. Reproduction of C. ferrugineus takes place near the surface of the bin, and near the floor-wall interface on the South side of the bin, where moisture and temperature conditions are optimal.
11. Adult C. ferrugineus concentrate near the floor of a steel bin.
12. As the environmental temperature decreases, the adults of C. ferrugineus tend to move towards the warmer central area of the bin, near the floor.
13. Adults of C. ferrugineus can survive winter conditions within 3" of the wall of the bin.
14. The spear and vacuum probes are more efficient in detecting larvae than adults of C. ferrugineus while the trap is efficient in detecting only adults of C. ferrugineus.
15. The trap is efficient in detecting many species of insects and mites, and may be useful in studying summer distribution of these animals.
16. To determine whether a steel bin is infested during summer, sample with a spear or vacuum probe near the surface of the bin, and check for larvae, or sample with the trap near the floor-wall interface with the trap, or in moist areas which provide an environment conducive to the survival of C. ferrugineus. In winter, only a vacuum probe should be used, and samples taken near the floor-wall interface should be checked for degermination.

In addition, samples should be taken in the central region of the bin near the floor, and checked for adult C. ferrugineus. The presence of feces in vacuum probe samples can also indicate an infestation.

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