

INDOOR WINTERING OF HONEY BEE
COLONIES IN MANITOBA

A Thesis
Submitted to the Faculty
of
Graduate Studies
The University of Manitoba
by
John Michael Gruszka

In Partial Fulfillment of the
Requirements for the Degree

of

Master of Science
Department of Entomology
May 1979

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ACKNOWLEDGMENTS

I would like to thank Dr. S.C. Jay for his encouragement and guidance and Dr. N. Holliday and Dr. R. Parker for their help and suggestions with the statistical analysis of the data. I am grateful to Mr. R. Barker, Provincial Apiarist for the Province of Manitoba, and the Manitoba Beekeeper's Association whose initiative and commitment initiated the project, and to the Manitoba Research Council for providing the funds to maintain the study. I thank Mr. L. Giguere, Mr. A. Morris, Mr. T. Morris and Mr. G. Kreutzer who provided the colonies for the trials and a great deal of time and energy to the wintering building.

I am especially grateful to Ms. J. Casey, Mr. L. Harris, and Mr. B. Fingler for their untiring and diligent assistance in collecting data; and to the following people who gave a great deal of their time to assist in the construction of the wintering building: Mr. B. Smirl, Mr. D. Couch, Mr. D. Dixon, Mr. R. Barker.

A special thank-you to my wife, Fernanda, for her patience, encouragement, and support.

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ABSTRACT

Gruszka, John Michael. M.Sc., The University of Manitoba, May, 1979.

Indoor Wintering of Honey Bee Colonies in Manitoba. Major Professor;

S. C. Jay.

An insulated indoor wintering facility was built to accommodate up to five hundred and twenty single brood chamber hives. The building contained four separate chambers, each individually heated and ventilated. Two hundred and twenty-five colonies were prepared and wintered indoors; seventy-five colonies in each of three chambers during the winter of 1976-77. A variety of treatments were used to test the effects of colony size, time of requeening, and food supplies on winter survival of honey bee colonies. Data were collected on colony weight loss and colony mortality during the winter.

Treatment did not have a significant effect on mortality. There was no significant difference in mortality among the six treatments performed.

There were significant differences in weight loss among the treatments and groups prepared. Differences were attributed to treatment and indoor conditions caused by the building construction and position of the hives within the building.

Comparisons were made between indoor wintered colonies, outdoor wintered colonies, and package bee colonies in the following spring and summer of 1977 on the basis of brood production, adult population

and honey production. Similar measurements were made to compare indoor wintered colonies requeened in the fall, in the spring, and colonies not requeened at all.

Outdoor wintered colonies had the highest brood production, largest adult populations and produced the most honey. There was no significant difference between package and indoor wintered colonies in terms of total brood production and adult populations; however, the package colonies produced more honey. The results of the requeening trial were not conclusive.

Trials were performed to test the effectiveness of orientation cues in preventing losses of adult bees from indoor wintered colonies after they were removed from winter quarters in the spring. Observations were made on the rate of adult bee losses during the first six weeks of spring. Orientation cues did not prevent adult bee loss. Substantial losses of adult bees occurred during the first week of active flight.

Samples of adult bees were taken from indoor wintered colonies, outdoor wintered colonies and package bee colonies during the spring and summer and were analyzed for Nosema disease. Indoor wintered colonies were found to have substantially higher levels of Nosema disease than outdoor or package colonies during the early spring. The level of Nosema disease decreased dramatically as the season progressed.

INTRODUCTION

Before the development of the package bee industry in the USA, beekeepers successfully wintered honey bee colonies in most parts of Canada. The use of package bees, however, quickly became the most popular method of beekeeping - packages were inexpensive, easy to operate, produced enough honey to make them profitable, and required less work than wintered colonies which needed to be tended year round.

Since the early nineteen seventies, the beekeeping industry in Canada has seen a revival of interest in wintering colonies. Beekeepers have been prompted to winter their colonies by the increased costs of package bees. Also, as the demand for package bees increased, beekeepers found it increasingly difficult to obtain their supply early in the spring. According to beekeepers, the quality of the package bees, especially queens, has deteriorated as the demand for packages has increased. Canadian beekeepers have experienced losses in production due to late arriving package bees or supersedure of queens due either to disease or poor mating. These problems, coupled with reports of the movement of Africanized bees from South America towards North America have convinced many beekeepers to attempt to become self-sufficient by wintering their colonies of honey bees.

Present-day methods of outdoor wintering are basically similar to those used in the past. Advances and adjustments have been made to accomodate current materials and management methods. There has been,

however, a radical change in indoor wintering techniques. In the past, colonies were wintered indoors in root cellars or basements. The current methods of indoor wintering utilize free standing, insulated structures and controlled environments with adjustable temperature, air flow and relative humidity. The only similarities between the old and new methods are that bees are kept undisturbed and in total darkness during the winter. Many commercial beekeepers in the three Prairie Provinces are now attempting to winter honey bee colonies both indoors and outdoors.

This study was the first part of a five year study of indoor wintering of honey bees to investigate the various aspects of the biology of a wintering colony which can be manipulated to achieve easy and successful wintering. The primary objective of this study was to determine which of several colony preparation techniques is most conducive to wintering colonies indoors. For various treatments, data were collected on colony mortality, food consumption, and colony development during the following summer. The timing of queen replacement was tested as a feasible management technique for wintered colonies. Tests were performed to determine the extent of the loss of adult bees from the colonies upon removal from wintering quarters.

The goal is to determine the optimal conditions necessary to winter bees indoors so that comparative economic and management studies can be performed with both indoor and outdoor wintering systems.

LITERATURE REVIEW

Much has been written concerning the wintering of honey bee colonies. The present literature review has been limited to research work conducted in Canada and to those geographical areas similar to that of Canada. Methods of wintering bees in milder climates were not considered to be relevant to this study.

It must be concluded that outdoor wintering is more popular than indoor (cellar) wintering since so much more information is available concerning outdoor wintering. Phillips and Demuth (1918) reported that outdoor wintering was practicable, seemed to give better results than cellar wintering, and that there was a decided change from cellar wintering to outdoor wintering by beekeepers at that time.

Indoor wintering was usually practised in areas where beekeepers believed that the winter climate was too severe, and where the winter was too long, for the colonies to survive outdoors. Indoor wintering was considered to be a more economic method since, by being kept at a more favourable temperature, honey consumption would be less than for outdoor wintered colonies. Beekeepers felt that the milder environment of cellar wintering reduced the high mortality sometimes experienced outdoors due to weather conditions (Johansson and Johansson, 1971).

Successful wintering of honey bee colonies has resulted from an understanding of the biology and needs of the colony in winter. The early beekeeper's primary concern was to reduce the effects of the cold

winter climate. Therefore, cellar wintering was popular, and at the beginning of this century, the protection of colonies outdoors with the use of insulation and some sort of packing or wrapping became common practise.

Sladen (1920) and Gooderham (1922) described wooden packing crates that were suitable for wintering under Canadian conditions. Merrill (1920, 1923) showed that packing was advantageous in the north central United States. Packing and insulation was advised for Kansas (Bayles and Parker, 1958), Indiana (Baldwin, 1919), Connecticut (Crandall, 1920, 1923), Colorado (Richmond, 1926), Iowa (Paddock, 1927), and Wisconsin (Wilson and Milum, 1927). Packing and insulation was recommended for wintering on the Canadian prairies (Braun, 1940; Le Maistre, 1942). Packing cases were used for individual colonies or groups of two or more colonies (Gooderham, 1926). Wilson and Milum (1927) tested a variety of materials (balsam-wool, wheat straw, celotex, planer shavings, clover chaff, leaves and ground cork) as to their insulating value and suitability for packing insulation.

Because of the cost and awkwardness of packing cases, other materials were tested as substitutes. Tar-paper wrapping, used with insulating material has been described by Paddock (1936), Burke and Adie (1952), Le Maistre (1942), Dyce and Morse (1960), and Edmunds (1961). A cardboard packing case was described by Boch (1964). The use of tar-paper has become more economical than the use of wooden cases so that most beekeepers are now using tar-paper wraps for wintering outdoors. High costs of materials have also eliminated the use of double-walled bee hives described by Phillips (1922) for wintering.

The formation of a cluster of bees as temperature decreases was

described by Phillips and Demuth (1915) and Milner and Demuth (1921). Knowing that the inner bees were insulated by the tightly packed outer shell of bees, some beekeepers questioned the need for insulation. However, the advantages of insulation have been demonstrated by Braun and Geiger (1955) and Haydak (1959, 1967) who showed that uninsulated hives suffered higher rates of mortality, consumed more honey during winter (Haydak, 1967) and had fewer bees in the spring than did insulated hives. Similar results were obtained by Villumstad (1959, 1960) in Norway.

The clustered bees generate heat through the metabolism of honey. Upper entrances to allow moisture, resulting from the metabolism of honey, to escape from the hive were advocated by Conner (1940), Farrar (1943, 1952), Dadant (1942), and Gooderham (1940). Baker (1942) demonstrated that colonies wintered more successfully with upper entrances than did those with no upper entrances. Provision of upper entrances has become standard practise for outdoor wintering.

Natural windbreaks are also an excellent form of protection and were recommended by Farrar (1952, 1963), Dadant (1942), Gooderham (1926), Paddock (1927) and Phillips and Demuth (1918). Johansson and Johansson (1969) suggested that windbreaks are more important than insulation for successful wintering.

The advocates of cellar wintering maintained that cellars were, in effect, sheltering the whole apiary in a protective environment, thereby achieving the same, or a better, degree of protection as packing and insulating outdoor wintered colonies. Designs for the construction of bee cellars were described by Pease (1937), Paddock (1936), Braun (1940) and Phillips and Demuth (1918).

Phillips and Demuth (1918) and Paddock (1936) elucidated the management requirements necessary for successful cellar wintering.

The colony's winter food supply is critical to successful wintering. The best food is good quality honey (Dadant, 1942; Phillips, 1922; Farrar, 1943, 1952; Johansson and Johansson, 1969). Certain honeys have been found to be unacceptable for winter stores, such as honeydew honey because it contains a high amount of particulate matter (Vorwohl, 1964; Phillips and Demuth, 1915), some late autumn-collected honeys (Phillips and Demuth, 1918; Gooderham, 1940), and honey that is prone to granulate (Richmond, 1926). Sugar syrup feeding is recommended for winter stores in areas where such honeys are a problem.

Phillips and Demuth (1918) recommended that at least forty-five pounds (20 kg) of honey be provided for winter feed. Johansson and Johansson (1966) suggested that at least seventy-five pounds (34 kg) are required under most North American conditions, while Farrar (1968) and Moeller (1977) suggested that at least ninety pounds (40 kg) are required. Braun and Geiger (1955) showed that the average weight loss of colonies during winter (thirteen year average) was between thirty-five and forty pounds (16-18 kg) per colony in Manitoba and that there was no significant difference in weight loss between outdoor wintered and cellar wintered colonies. Farrar (1952) showed that colonies, which consumed more honey during winter, also produced a higher net yield of honey during the following year.

The importance of pollen for wintering colonies has been stressed by Farrar (1934, 1952), Moeller (1977) and Johansson and Johansson (1969). All recommended the use of pollen supplements in the spring if natural pollen was inadequate.

The size of the wintered colony has been dictated by several factors. Outdoor wintered colonies, because they require substantial amounts of stored honey, have usually been wintered in two chambers. Indoor wintered colonies, because of the difficulties involved in manipulating the colonies, especially in bee cellars, were usually wintered in one brood chamber. Populous colonies (preferably with young bees) were recommended for wintering; smaller and/or weaker colonies were united with stronger colonies because weak colonies were not likely to survive the winter (Gooderham, 1940; Farrar, 1952, 1963; Moeller, 1977). However, Gooderham (1945) described a successful method of keeping small colonies (nuclei) in a bee cellar. The method was intended as a means of procuring early spring queens by wintering queens in these small colonies, but comparative tests with package colonies showed that these nuclei produced as much honey as the package colonies. A similar method employed in Nebraska was described by Barker (1975) and by Diemer and Diemer (1937).

MATERIALS AND METHODS

A. Wintering Building

(1) General Features

A free-standing structure was built in the apiary of the Entomology Department on the campus of the University of Manitoba to be used for the indoor wintering of honey bee colonies. Construction began in July and was completed on 12 December, 1976. The erection of the building was accomplished with the assistance of volunteer labour by members of the Manitoba Beekeeper's Association and the Entomology Department.

The building was of stud-wall construction, the walls and roof trusses being pre-assembled by the materials supplier. Fifteen concrete pillars, each 76 cm X 76 cm X 24.5 cm, formed the foundation on which the building stood. The external dimensions of the building were 8.5 m X 9.75 m. The interior contained four controlled environment chambers for colony storage, each approximately 3.6 m X 3.6 m X 2.4 m, a central hallway, and an equipment room at one end to house the heating and refrigeration equipment (Fig. 1). Each chamber had a double door entrance to facilitate the moving of bees into and out of the chamber.

Each chamber was insulated with fibre glass batt insulation of an R7 value in the floor, of R12 value in the walls, and R20 in the ceiling. The roof was covered with white asphalt shingles and the exterior walls painted white in an attempt to keep the effect of solar radiation to a minimum, especially during the rising temperatures of the critical

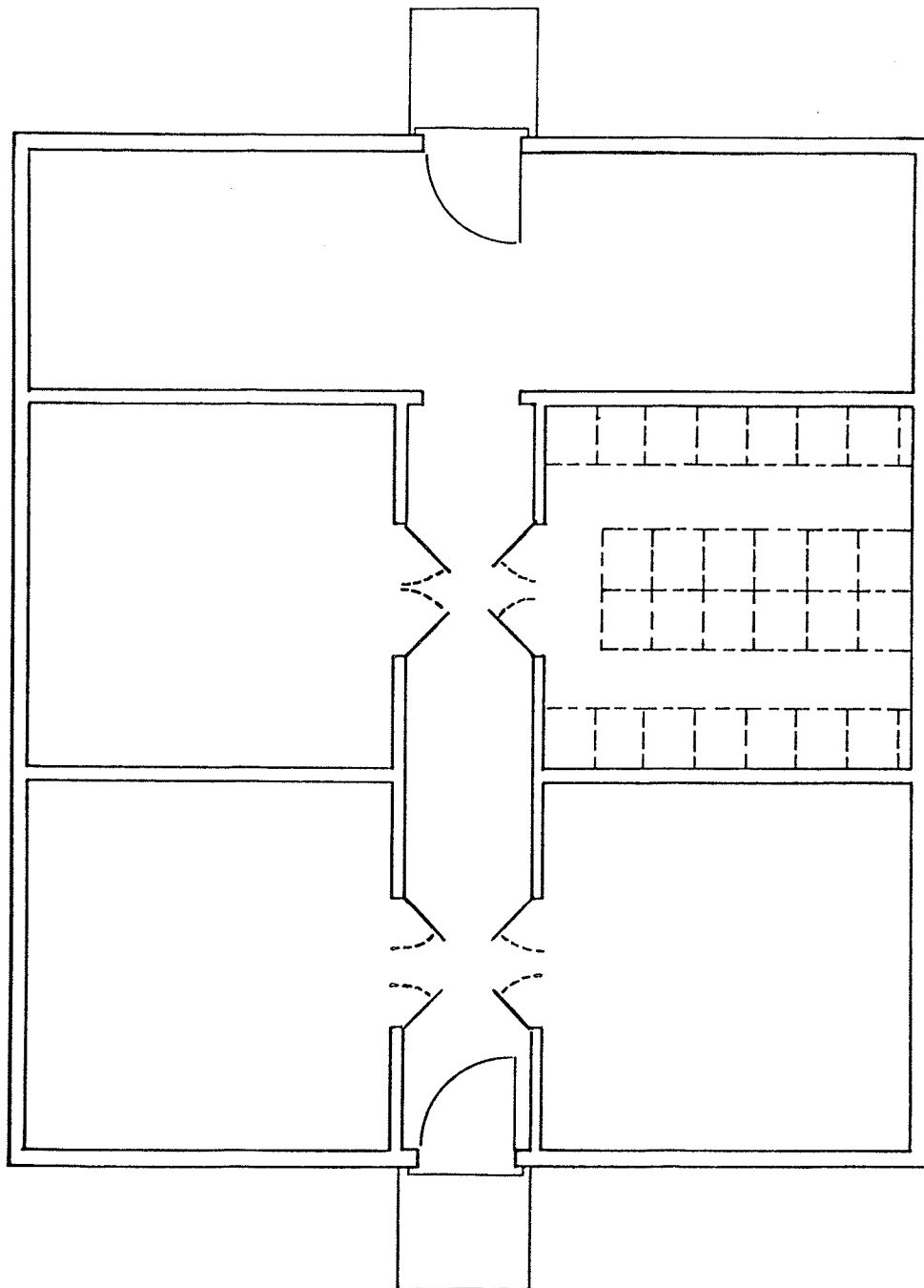


Figure 1. Diagram of wintering building to show the position of the chambers, equipment room, and position of hives.

spring period.

The entrances at both ends of the building had small platforms at the top of the stairs at approximately truck-bed level to facilitate the moving of hives into the building.

The floors of the chambers, hallway and equipment room were painted with a grey enamel paint. Hives were placed along each wall and down the center of each room, leaving aisles 76 cm wide. The center pallet supported two rows of hives, back to back. These pallets could accommodate twenty-six hives, so that each chamber had a maximum capacity of one hundred and thirty hives (five high) if only single storey hives were used, less if two storey hives were utilized.

(2) Heating System

Each chamber had its own heat supply, an electric forced-air furnace of a type common to domestic use, i.e., a model EL-10 (10 K.W., 630 c.f.m.) manufactured by Inter-City of Winnipeg, Manitoba. These were positioned in the equipment room (Fig. 2). The two furnaces servicing the chambers on one side of the hallway had a common air intake duct bringing in outside air through an external wall of the equipment room.

Ducts (45 cm X 30.5 cm) from each furnace carried the heated air into their respective chambers and opened into the center of the room at the ceiling. The opening was equipped with a baffle so that the incoming air was evenly dispersed throughout the room. The inflow air duct had a baffle control to adjust manually the rate of air changes per hour entering each chamber. A return air duct, opening half-way down one wall, provided recirculation of the chamber air; the amount recirculated could be adjusted manually by baffles inserted within the duct.

Thermostat controls were located within each chamber. Initially,

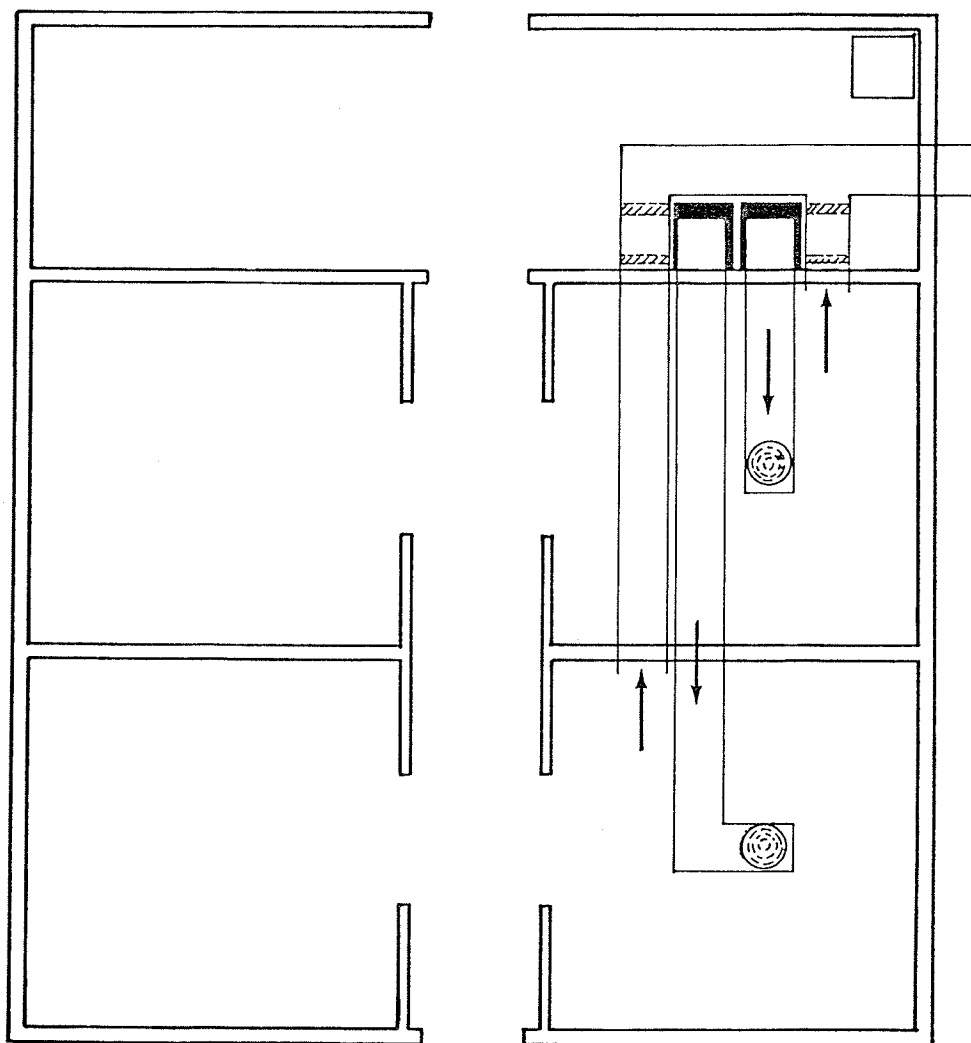


Figure 2. Diagram to show position of ventilation system and air flow into the chambers.

these controls had a range of $\pm 3^{\circ}\text{F}$. This was found to be inadequate so that controls with a 1°F range were installed. To have such a narrow range, the thermostat was connected to both heat and refrigeration systems simultaneously. This meant that the refrigeration equipment was turned off during most of the winter.

(3) Refrigeration System

Two refrigeration systems were installed, one for each pair of rooms. Each system consisted of a compressor unit and two cooling coils, one mounted on top of each furnace within the ducts leading into the chambers. The compressor units were mounted on the floor in the equipment room rather than exposed outdoors. This prevented cold damage to the compressors. Each compressor was three horsepower with a cooling capacity of 32,000 B.T.U. per hour. The systems were equipped with a hot gas by-pass.

The coolant temperature was set slightly above 0°C to avoid ice build-up on the cooling coils from the humid re-cycled air. This resulted in a slight reduction of cooling capacity.

(4) Ventilation

Those ducts used for the heating/cooling system also provided the ventilation for the building. The furnace fans operated continuously, thereby forcing air constantly into the chambers. By manually adjusting dampers within the inflow ducts, the fresh air:recirculated air ratio could be adjusted. Dampers could also be adjusted to alter the amount of air entering each chamber. These were set for the greater part of the winter so that a volume of air equal to four air changes per hour entered each chamber.

The volume of air entering each chamber, i.e. air changes per hour,

was calibrated and the appropriate settings of the baffles were marked so that the volume could be reset without recalibration. This volume was increased in the spring to help cool the chambers without the use of the cooling system and decreased again to obtain maximum efficiency of the cooling system once outdoor temperatures increased to a point where the cooling system was required.

Due to the constant inflow of air into each chamber, a positive pressure was created in the chambers. This forced air out of the rooms via the exhaust channels beneath the pallets on the floor. The exhaust ducts rose vertically 0.6 m from the pallets along the wall, through the wall and then down along the outside of the building (Fig. 3). This system was designed to prevent carbon dioxide build-up on the floor of the chambers. The doors to the chambers were fitted with weather stripping, to ensure that the rooms would be fairly airtight. The forced air system ensured that a certain amount of carbon dioxide-laden air was constantly exhausted. Return air ducts for recirculated air were 1.2 m from the floor to decrease the possibility of recirculating carbon dioxide.

At a capacity of seventy-five hives per chamber, the volume:hive ratio was $0.44 \text{ m}^3/\text{hive}$ (15.56 cu. ft./hive); at the maximum hive capacity of one hundred and thirty hives, this would be reduced to $0.25 \text{ m}^3/\text{hive}$ (8.86 cu. ft./hive).

The equipment room contained a fan, thermostatically controlled, to function when the temperature in this room increased beyond 16 to 18°C. This fan removed the heat created in the room by the refrigeration systems. In the late spring, the removal of the heat was assisted by leaving the outside door open.

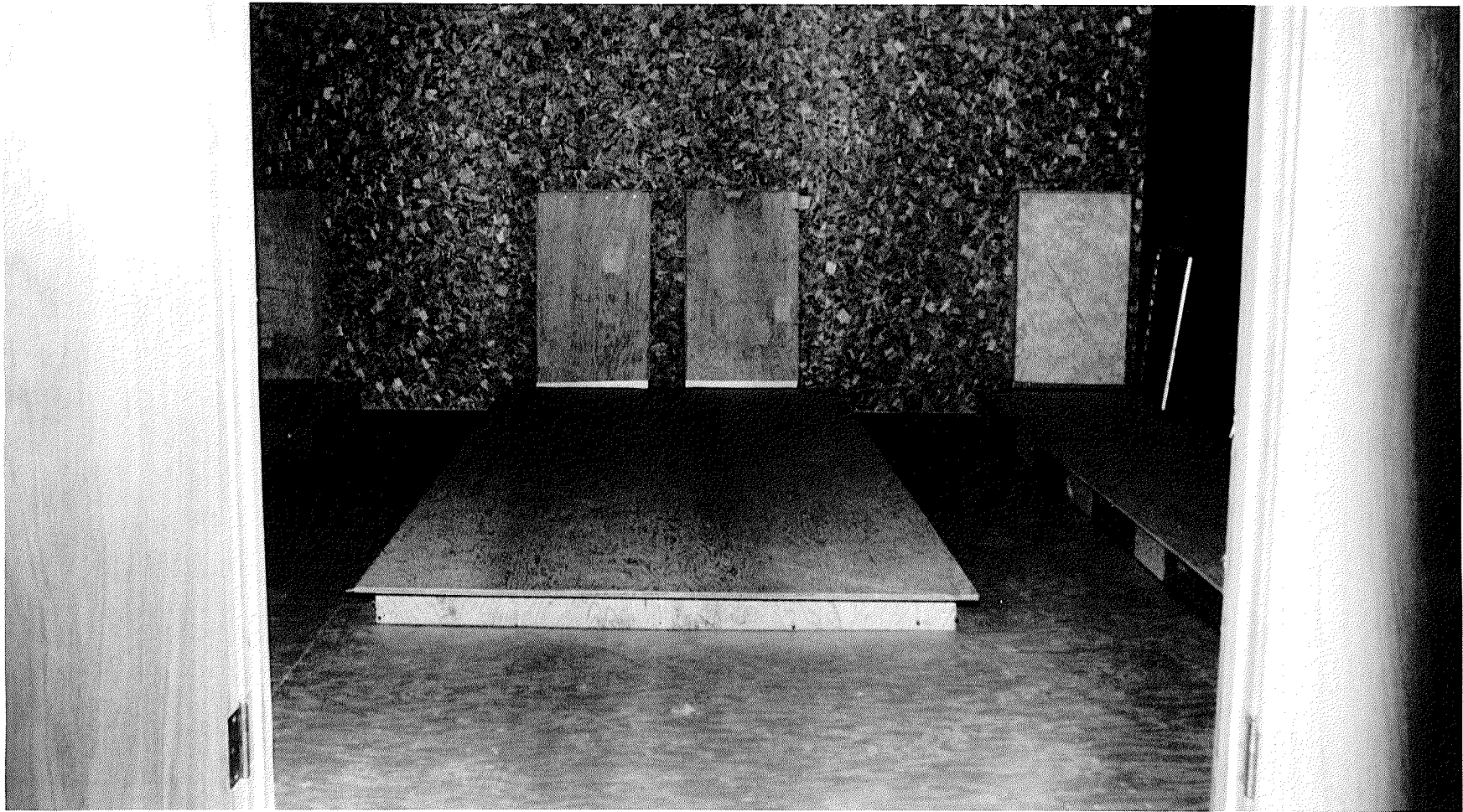


Figure 3. Exhaust ducts at floor level under pallets, leading outside through the wall.

(5) Lighting

All doorways were made to be light-proof so that work and movement in the hallway was possible without causing undue excitement of the bees in the chambers. Each chamber had two light sockets which contained red-coloured, fifty watt incandescent light bulbs to provide light in the chambers when it was necessary to enter. The hallway and equipment room were both well lit.

(6) Alarm System

An alarm system, thermostatically controlled in each chamber, was set to function whenever the temperature reached 11°C , i.e. the temperature at which bees break cluster. When this temperature was reached, a breaker system was activated which would signal the operators of the alarm system who would immediately telephone the author.

The alarm system would de-activate and re-set itself automatically once the temperature of the chamber returned below the critical emergency temperature.

(7) Operation

It was not possible to test the equipment before the hives were moved into the chambers on 14 December, 1976. All chambers were kept at $5^{\circ}\text{C} \pm 1^{\circ}\text{C}$ and the ventilation system was adjusted to deliver four air changes per hour to each chamber. During the winter, four air changes per hour was just adequate to maintain a temperature of 5°C with little supplemental heat.

The aisles were swept four or five times a week to remove dead bees from the floor. The hygrothermograph charts were replaced at the same time. Care was taken to disturb the hives as little as possible during these operations.

The refrigeration equipment was able to maintain a stable temperature in the chambers during the spring. There were, however, difficulties on two occasions. On the first occasion, the motor to the exhaust fan in the equipment room had shorted out, causing the temperature in the equipment room to reach 38 to 40°C so that the cooling system could not function.

The second occurrence, in the late spring, was caused by the moisture in the air freezing and forming a block of ice on the cooling coils, thereby incapacitating the refrigeration system. This occurred because the coolant temperature in the system was set to operate below 0°C.

On both occasions the temperature of the chambers increased to near 22°C for periods of approximately twelve hours before the situation was rectified.

The daily temperature within a chamber was $5^{\circ}\text{C} \pm 1^{\circ}\text{C}$ during the winter. The critical period, however, was during the spring period. Air circulation alone did not maintain a temperature of 5°C. The daily fluctuation increased to 3.3 to 5.5°C even when the number of air changes per hour was increased. In the early spring, this fluctuation was eliminated by activating the cooling system. However, by late spring, when the outdoor temperature reached 16°C and higher, the daily temperature fluctuation again increased to 3 to 4°C so that the temperature in the chambers approached 10°C.

The colonies were removed from the wintering quarters between 4 - 14 April, 1977.

B. General Methods

(1) Introduction

The large number of hives necessary for the project were loaned to the Department by three commercial beekeepers. Each beekeeper agreed to make available seventy-five colonies as well as all sugar and honey feed necessary to winter the colonies, all necessary hive equipment, as well as transportation of the hives to and from the wintering quarters. In addition, two of the beekeepers were asked to supply another twenty-five colonies which were prepared and wintered outdoors.

Colonies were prepared for winter following honey harvest. Preparation of the first group of colonies commenced 8 September and was completed 14 September, the second group was prepared between 22 and 30 September, and the final group between 16 and 20 October. This extended preparation period was unavoidable due to a shortage of manpower.

(2) Preparation of Indoor Wintered Colonies

Colonies to be wintered were selected on the basis of large population, presence of a healthy laying queen, and absence of disease. Most colonies were housed in two boxes but some were in three. Each hive was reduced to a single chamber in which were placed nine frames: two or three central combs of pollen and brood and six or seven combs of honey. The adult bee population was not altered, all adults being allowed to remain in the hive. The colonies were left for a day and then weighed to determine the original colony weight.

Once prepared, one of the following treatments was performed:

- A) - given a second box of honey
 - wintered as a double storey hive
 - 15 colonies

- B) - given 8 lbs. of sugar syrup (2 parts sugar, 1 part water)
 - given a second box of honey
 - wintered as a double
 - it was intended that this group would have its honey reserves replenished with honey as it was utilized by the bees
 - 10 colonies
- C) - similar to B except that the colony was to have its food reserves replenished with sugar syrup as necessary
 - 10 colonies
- D) - wintered as a single storey hive
 - 15 colonies
- E) - wintered as a single storey hive as in D except that a new queen was introduced when the colony was prepared
 - 10 colonies
- F) - this group remained as single storey hives until they were brought into the building at which time they received a box of honey
 - 15 colonies

The colonies were moved into the wintering building during 13 to 15 December, 1976. Each colony was weighed as it entered the building to ascertain weight loss for the period of time it remained outdoors. The colonies were also weighed when removed from the building to determine weight loss during their winter confinement.

Two of the three groups of single storey colonies were given supplemental feeding when it was noticed that their food supplies were running low. The colonies were removed from the chamber singly, weighed, empty combs were replaced by combs of honey, and the colonies were re-

weighed before being replaced in the chamber. This was done on 28 January and 14 to 15 February, 1977. It was not necessary to feed the third group which was the last to be prepared in the fall.

All of the colonies of one beekeeper were kept in one chamber. The three chambers were kept at the same temperature with similar rates of air flow. Colonies were removed from the building between 4 to 14 April, 1977.

Each colony was given a 0.5 kg patty of pollen supplement shortly after it was removed from the wintering building. The patty consisted of Brewer's yeast (95%) and pollen (5%) and sugar syrup (2:1, sugar: water) mixed to a paste .

All colonies were reduced to a single storey shortly after they were placed in the apiary. The management of the hives once in the apiary (i.e. drug feeding, queen checks, addition of supers, etc.) was the responsibility of the beekeeper.

Further treatments of these groups are detailed in separate chapters.

(3) Preparation of Outdoor Wintered Colonies

Colonies to be wintered outdoors were prepared at the same time as those to be wintered indoors. Only colonies that had large populations, a laying queen, and were disease-free were prepared. The colonies were fed sugar syrup in an attempt to attain an initial gross weight of at least one hundred and thirty pounds (60 kg).

Once feeding was complete, two hives were placed together, wrapped with a layer of fibre-glass insulation (R10) and then with a layer of black tar-paper. The tops of the hives were insulated as well. The hives were provided with a reduced lower entrance and an upper entrance

of approximately 1 cm X 7.5 cm in the inner cover. Mouse bait was used to prevent rodents entering the hives. The colonies were left in well sheltered apiaries for the winter.

Pollen supplements and sugar syrup were fed beginning 20 March, 1977. The colonies were removed from their wrapping during the third week in April and moved to the experimental apiaries.

The colonies of one outdoor wintered group were divided into two equal portions. The adult bees, brood combs and honey combs were separated into two equal parts to form two new colonies. One colony remained with the original queen, the other colony was given a newly-mated queen. The colony with the original queen was monitored throughout the summer; the one given a new queen was not included in the tests. The second group of outdoor wintered colonies was not divided.

(4) Spring Treatments

In the spring, two apiaries were established containing indoor wintered colonies, outdoor wintered colonies and package colonies. Each apiary contained only the hives of one beekeeper.

The following treatments were represented in each apiary, each treatment initially containing seven colonies:

1. - wintered as a double indoors (A)
2. - wintered as a double indoors (B)
new queen introduced in the spring
3. - wintered as a single indoors (D)
4. - wintered as a single indoors (E)
requeened prior to wintering
5. - prepared as a single, wintered as a double indoors (F)
6. - outdoor wintered colony (OW)

7. - package colony (P)

One apiary was located five miles north of Portage la Prairie, Manitoba; the other was located near Winnipeg, one mile north of the Perimeter Highway and two miles east of Highway # 59.

(5) Measurements

Scale colonies were maintained in each apiary and weights were taken every twelve days.

The apiaries were visited every twelve days at which time the amount of capped brood was recorded from each colony. This was done by placing a grid, made of a wooden frame and strung at one inch intervals (2.5 cm), over each brood frame containing capped brood and estimating the area of capped brood (Fig. 4).

During each visit, a sample of fifty or more adult bees was taken from a honey comb adjacent to the brood area from each hive. Bees were collected from the top bar into a plastic bag and brought to the laboratory within one hour and deep frozen. These bees were later checked for Nosema disease.

For analysis, twenty-five bees were mascerated whole in 10 ml of distilled water. A drop of the resultant fluid was placed on a slide and examined under high power (X440) for the presence of spores. The number of spores per field of vision (three replicates) for each sample was recorded (Anon., 1966).

Some of these mascerated samples were diluted by adding a further 15 ml to attain a dilution of one bee per ml. Samples were thoroughly mixed and the number of spores per ml (thereby, number of spores/bee) determined using a haemocytometer (Cantwell, 1970).

At each apiary, two hives from each treatment were selected and

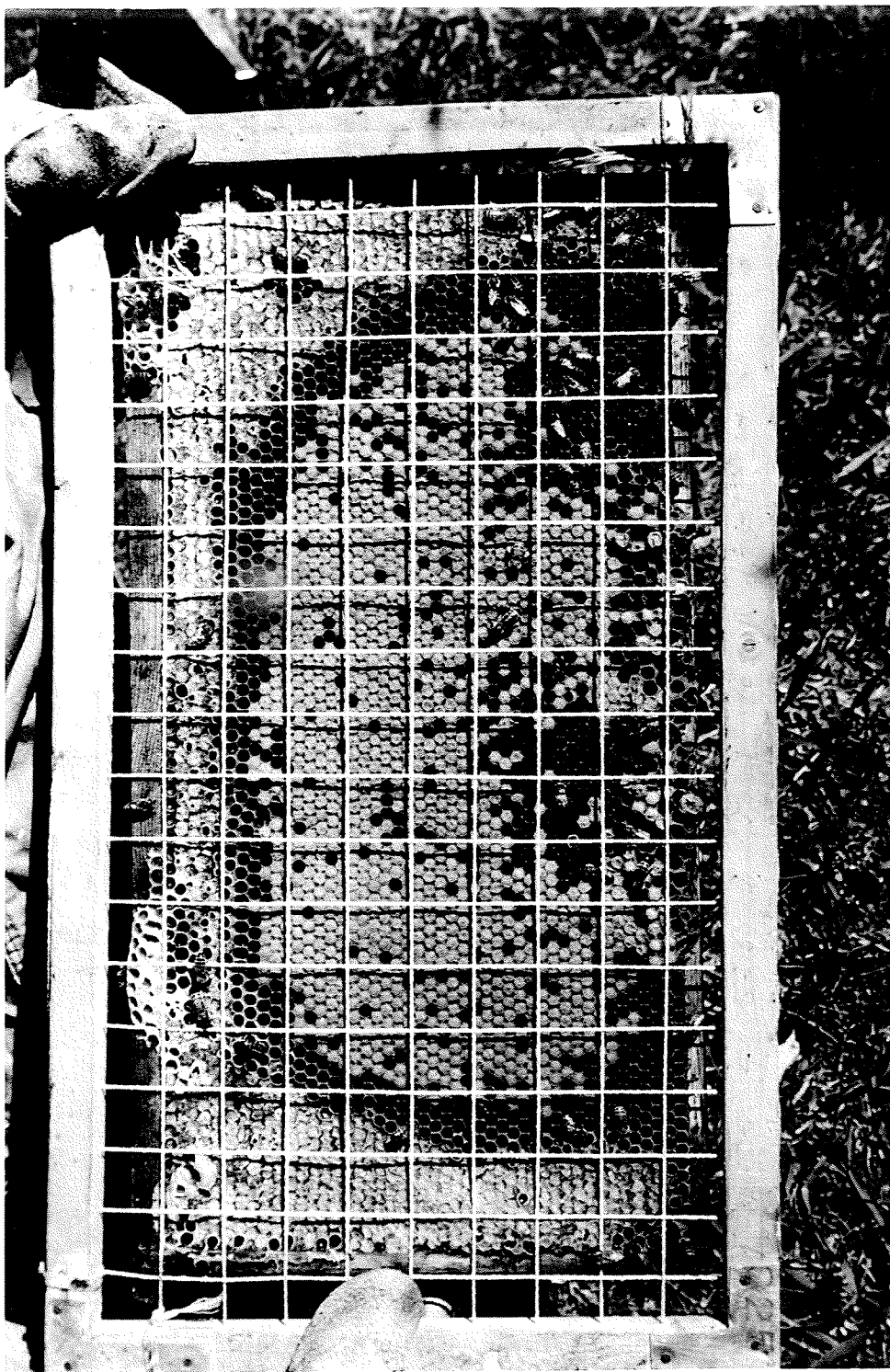


Figure 4. Grid used to measure sealed brood area.

sampled for pupae each twelve days. A total of fifty pupae were taken each time and sealed in a plastic bag. An attempt was made to take pupae of a uniform age; the eyes were dark but the bodies had not yet begun to colour. Samples were taken immediately prior to leaving the apiary and were weighed at once upon arrival at the laboratory.

Adult population estimates were recorded every twenty-four days (readings were made early in the morning before foraging had commenced). This was done by estimating the area of the frame covered by bees. The population was then estimated as follows: number of frames of bees \times surface area of both sides of a frame \times 7 (bees/sq. in.) as derived from photographs taken of bees on frames (Jeffree, 1951).

Honey production data were recorded for each treatment. The total weight of all the honey boxes for each treatment was recorded before and after the honey was extracted to obtain a gross honey weight for each treatment. This was divided by the number of hives in the treatment to obtain the honey production per colony.

MORTALITY AND WEIGHT LOSS OF OUTDOOR AND INDOOR WINTERED COLONIES

(1) Introduction

It is a common belief that the main cause of mortality in outdoor wintered colonies is starvation (Farrar, 1952; Johansson and Johansson, 1971) coupled with the fact that long periods of extreme cold, such as occur in Manitoba, prevent the colony from breaking cluster and repositioning itself on combs of honey. If outdoor wintering is to be successful on the prairies or northwest U.S.A. special preparation methods must be used (Farrar, 1963; Haydak, 1967; Johansson and Johansson, 1969; L'Arrivee, 1961).

The advantage of indoor wintering is that it eliminates the effect of the extreme winter environment which is likely to cause mortality. Haydak (1967) summarized work done by a number of researchers who determined conclusively that under extremely cold winter conditions, mortality and honey consumption was lower in colonies that were wrapped (insulated) and wintered outdoors than when they were not so protected. It follows that indoor wintering conditions, providing even greater insulation from the environment, should reduce mortality and honey consumption even further. However, indoor wintering introduces complications associated with the long periods of confinement (up to six months) in total darkness.

In the past, indoor wintering in Canada was conducted in root cellars (Braun, 1934; Geiger and L'Arrivee, 1965; Gooderham, 1939). More

recently indoor wintering has been performed in controlled environment chambers. By controlling the environment in which the bees are maintained it is possible to determine the ideal conditions of temperature, humidity and air exchange most suitable to the successful indoor storage of honey bee colonies. It is the aim of beekeepers, using this system, to reduce winter mortality to a low level. All preparation conditions being optimal, outdoor wintered colonies are still subjected to a changeable environment, and are therefore subject to a higher risk of mortality (Johansson and Johansson, 1971).

Indoor wintering allows for the possibility of decreasing honey consumption during winter. Recommended procedures for outdoor wintered colonies are to leave seventy to ninety pounds (32-41 kg) of honey for winter and early spring consumption (Farrar, 1957; Haydak, 1959). This is necessary because outdoor wintered colonies consume large amounts of honey to maintain cluster temperature. The colder the external temperature, the more honey is consumed. Average consumption during winter has been reported to be near forty pounds (18 kg) in Manitoba (Geiger, 1967; Geiger and L'Arrivee, 1965) and in northern Alberta it has been reported to be as high as ninety pounds (41 kg) (Pankiw, 1968). To ensure that the colonies do not starve, beekeepers need to leave more honey than these amounts reported in the hive for winter.

(2) Methods and Materials

A total of two hundred and twenty-five colonies were prepared for indoor wintering and fifty colonies were prepared for outdoor wintering. The methods of preparation and the six treatments were described earlier (see Chapter 3).

Final preparation and initial weighing of the three groups took

place during the following periods: Group 1, 8-14 September, 1976; Group 2, 22-30 September, 1976; Group 3, 16-20 October, 1976. The colonies remained outdoors until 13-15 December, 1976 when they were brought into the building. Hives were weighed when moved into, and out of, the winter quarters. Any additional honey that was fed to colonies during storage was recorded.

Loss in colony weight was calculated for each hive for the period before winter storage and for the period during storage. Colonies that had died were not used in the calculation of weight loss since it could not be reliably ascertained when they had died.

(3) Results and Discussion

(A) Mortality. Percent survival for the different treatments within each group is shown in Table 1. These data were transformed using an angular transformation and then analysed using analysis of variance. Analysis of variance revealed that there was no significant difference in mortality among the three groups of hives wintered, nor was there any significant difference in mortality among the six treatments.

Although losses were uniform, they were higher than anticipated. Only nine of the two hundred and twenty-five colonies prepared (i.e. 4%) were dead before 15 December, 1976 when the colonies were brought into winter quarters. Although this was low, the prolonged outdoor exposure may have been responsible for a large part of the subsequent mortality indoors. Twenty-nine percent (64 of 216) of the colonies died during storage.

It was intended to bring the colonies into storage in early November. This date was postponed due to delays experienced in completing the installation of the heating and cooling systems in the wintering

TABLE 1. Percent survival of indoor wintered colonies for the period
September, 1976 to April, 1977.

<u>TREATMENT</u>	<u>GROUP 1</u>	<u>GROUP 2</u>	<u>GROUP 3</u>
Double (A)	80.0	80.0	73.3
Double, honey (B)	60.0	80.0	90.0
Double, syrup (C)	50.0	60.0	60.0
Single, old queen (D)	66.6	60.0	80.0
Single, new queen (E)	20.0	80.0	50.0
Single, double (F)	20.0	86.6	93.3

facility. The colonies had to remain outdoors where they experienced a month of temperatures below 0°C . Most hives were almost completely covered with snow by 15 December. Figure 5 shows ice build-up within the hives resulting from condensation of moisture-laden air due to a lack of ventilation. These colonies were not prepared to endure sub-zero temperatures since they were not insulated and did not have upper entrances to allow for some ventilation.

Figure 6 shows a histogram depicting the total mortality for all treatments. The total combined indoor treatment mortality was 32.8 percent. This was very similar to the 33.9 percent mortality which occurred in the outdoor wintered treatment. It was hoped that the rate of mortality of indoor wintered hives would be at least as low as mortalities reported in successful outdoor wintering trials. Outdoor winter mortalities of ten to fifteen percent have been reported as normal by Haydak (1959,1967) and Johansson and Johansson (1969) and others.

Group 1 sustained the highest mortality. Part of this was due to the action of skunks which was not noticed until December. Six of the nine colonies found dead before storage were from this group. The unrestrained actions of the skunks seriously depleted the colony populations. Skunks are known to sit at the front of a hive and scratch at the entrance. The disturbance alarms the bees which come out to investigate, only to be eaten by the skunk as they emerge from the hive. The loss of adult bees due to skunks was likely responsible for the subsequent high mortality in that group during storage.

Most of the mortality was due to inadvertent starvation. It was not realized until too late, that although the hives contained sufficient



Figure 5. Ice build-up on inner covers before colonies were moved indoors.

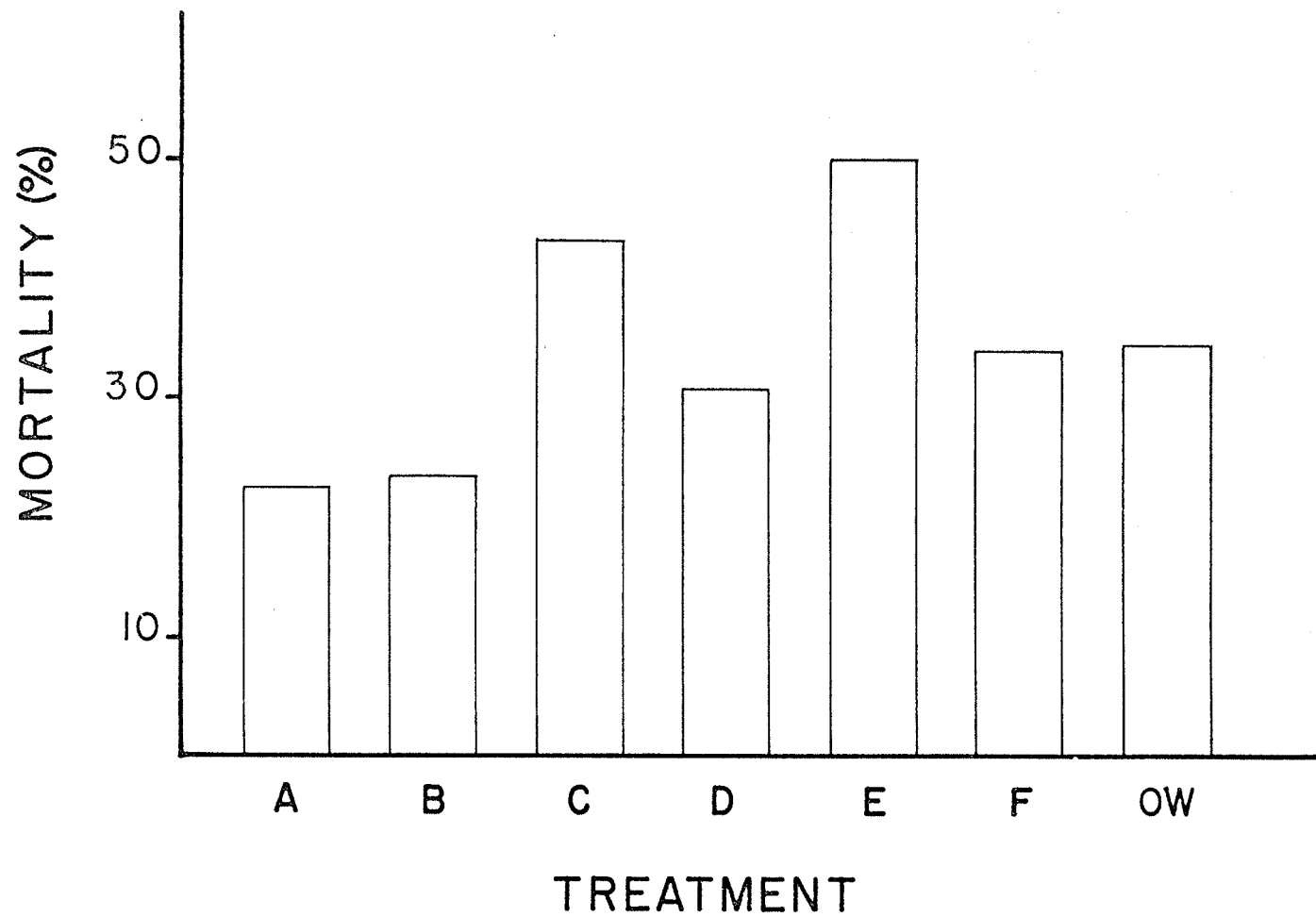


Figure 6. Histogram of combined mortality for all treatments during indoor storage.

honey, some of it had crystallized and hence was not available to the colony for consumption. Had this problem been recognized earlier, water could have been provided to the colonies which would have enabled them to liquify and utilize the honey.

Examination of the dead colonies revealed that some were queenless, which may have been the cause of death. The queens may have been lost at the time of preparation or may have died due to skunks or disease (i.e. Nosema). One queen was found wandering on the floor of a chamber when the hives were being brought into the building.

None of the dead colonies showed any symptoms of American foulbrood disease. No disease "scales" were found in the dead colonies; however, since very little brood was raised while in storage, it was not conclusively established that foulbrood diseases were not present.

Much of the mortality occurred in the spring and was associated with the loss of adult bees when the colonies were removed from winter quarters. Once outdoors, after spending the winter in complete darkness, the bees would fly from the hives to defecate at the first available opportunity. Many of these bees became disoriented, became chilled, or for some other reason were not able to find their way back to the hive and so were lost. This "dwindling" of colony population was so great in some cases that recruitment to the population by brood rearing could not keep pace with adult losses and the colony eventually died within a period of three weeks.

Four colonies had American foulbrood disease in the spring. Although these colonies did not die during winter and were not included in mortality calculations, they were so weakened by the disease as to be economically unviable.

(B) Weight Losses. Colony weight loss was due both to loss of bees as they died and to honey consumption. Unfortunately, it was not possible to determine what proportion of the total weight loss was due to loss of bees or honey consumption. However, based on an average fall colony population of 40,000 bees weighing approximately ten pounds (4.5 kg) then the total weight loss due to loss of bees would be six to eight pounds (2.7 to 3.6 kg). If this loss is relatively constant, then the weight loss due to loss of bees would approach three to four pounds (1.4 to 1.8 kg) for each period (i.e. before and during storage).

(I) Before Storage. Weight loss for all groups for the period before storage is shown in Figure 7. Since the three groups were prepared at different times (September to mid-October), the amount of time spent outdoors by each group was different. Consequently, the weight losses for the period before storage were converted to a weekly weight loss to eliminate the differences in weight loss caused by the differing lengths of time from preparation to storage in the indoor quarters. Table 2 shows the adjusted weekly weight losses for the treatments.

The weight loss data for the various treatments and for the three groups were tested for significant differences using analysis of variance. The Student-Newman-Keuls (SNK) test was used to determine which treatments were significantly different from the others. The results of these analyses follow:

(1) Groups. There proved to be a significant difference in weight loss between the three groups tested. This was not expected since all three groups were treated similarly. However, the cause may be due to the attempted adjustment of weight loss to a weekly weight loss. There were three possible sources of error. First, there was a difference in

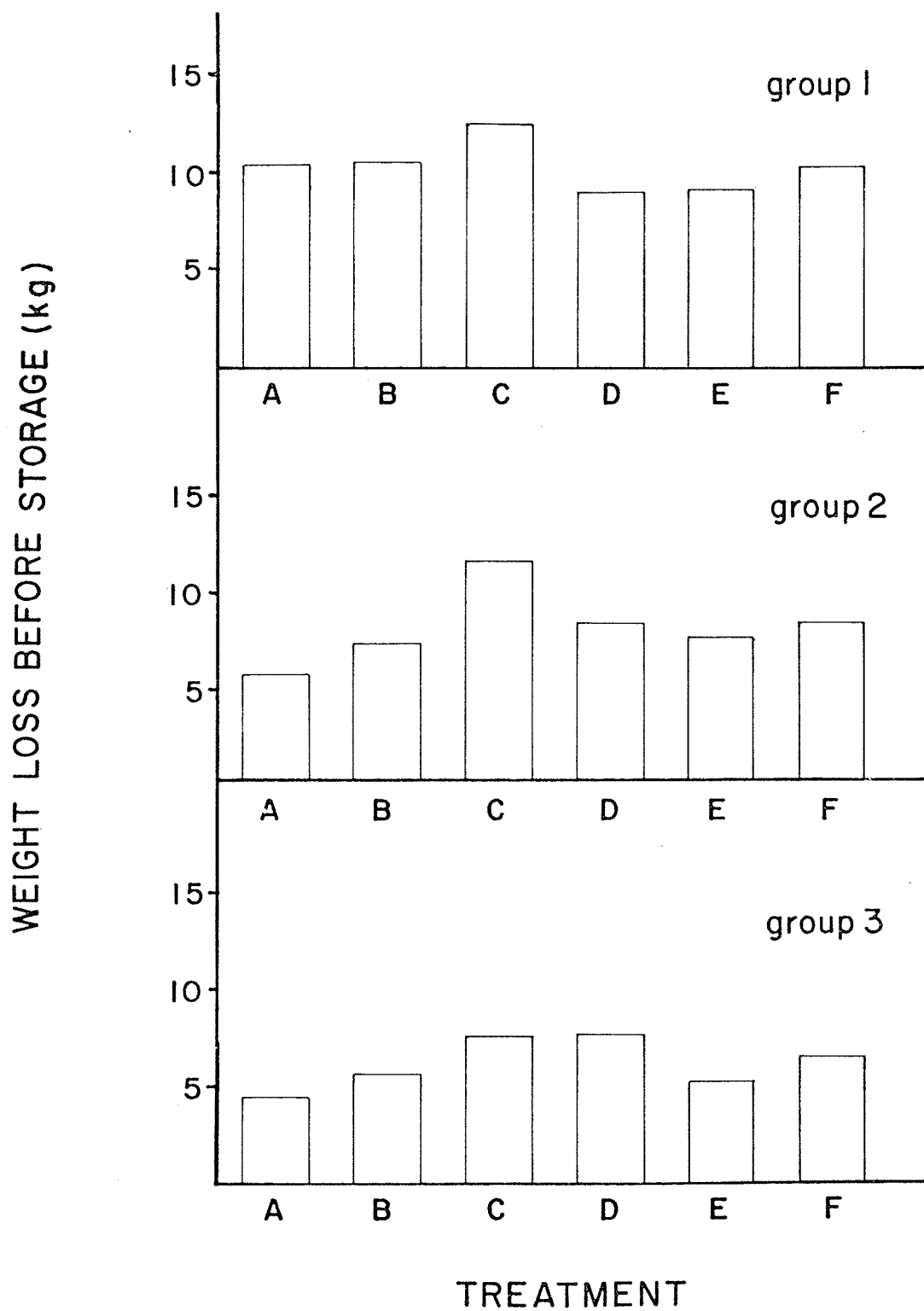


Figure 7. Weight loss, for all groups, for the period before indoor storage.

TABLE 2. Mean weekly weight loss (kg) during September to December, 1976.

<u>TREATMENT</u>	<u>GROUP 1</u>	<u>GROUP 2</u>	<u>GROUP 3</u>	<u>TREATMENT AVERAGE</u>
Double (A)	0.83 ± 0.05	0.47 ± 0.04	0.53 ± 0.04	0.61 ± 0.034
Double, honey (B)	0.84 ± 0.08	0.59 ± 0.05	0.67 ± 0.07	0.70 ± 0.043
Double, syrup (C)	0.99 ± 0.06	0.97 ± 0.04	0.91 ± 0.04	0.96 ± 0.028
Single, old queen (D)	0.72 ± 0.04	0.69 ± 0.04	0.91 ± 0.07	0.77 ± 0.034
Single, new queen (E)	0.74 ± 0.04	0.61 ± 0.07	0.64 ± 0.05	0.66 ± 0.033
Single, double (F)	0.81 ± 0.03	0.69 ± 0.04	0.78 ± 0.07	0.76 ± 0.031
Group average	0.82 ± 0.023	0.66 ± 0.025	0.74 ± 0.03	

duration of the outdoor period of thirty-one days from the group prepared first to that prepared last. Secondly, the seasonal difference during this elapsed time may have caused different rates of consumption for September and October which were not possible to detect. A third possibility was that the difference in weight loss may have been due to possible genetic differences in the bees themselves.

(2) Treatments. When the treatments were analyzed, it was found that there was a significant difference in weight loss only between the treatment with the lowest weekly weight loss (treatment A, double) and that with the highest weekly weight loss (treatment C, double replenished with sugar syrup). This was not expected since both were treatments using double storey hives: however, the highest weight loss treatment was one which was fed sugar syrup during preparation. Not being able to determine how much of the eight pounds (3.6 kg) of sugar syrup fed would be stored in the hive or how much utilized by the bees, the weight of the sugar syrup was added to the total hive weight. The difference may be, then, only a reflection of the error in estimating the weight gain due to the sugar syrup feeding. Alternately, the higher weight loss may have been caused by a stimulating effect created by the feeding of sugar syrup which may have induced the colony to consume more of its honey stores.

(II) During Storage. The weight loss during storage for each colony was calculated directly from the weight of each colony as it entered and was removed from the building. Mean weight losses for all treatments are shown in Figure 8. An analysis of variance revealed that there was a significant difference ($p < .001$) among treatments and among groups. The results of these analyses follow:

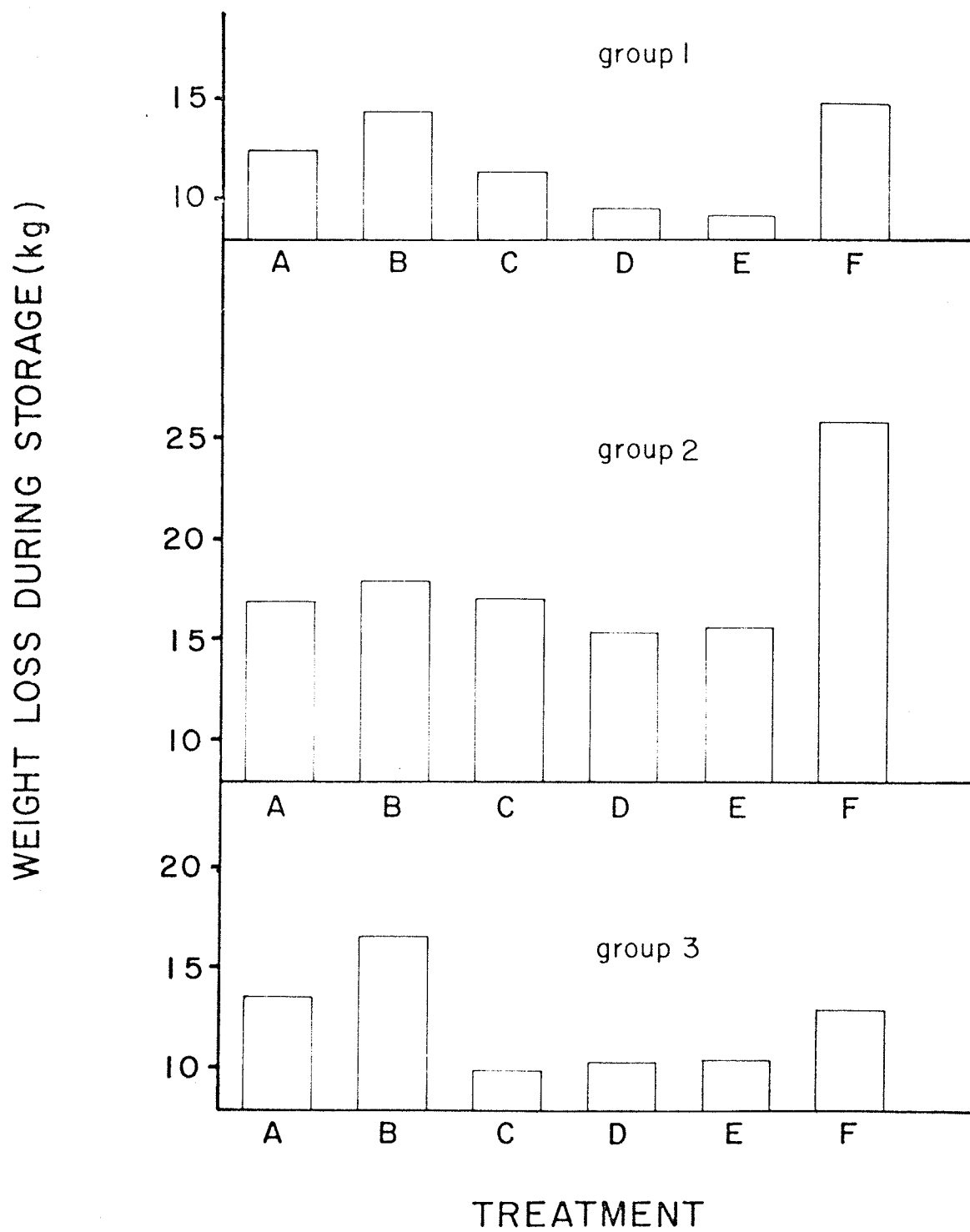


Figure 8. Weight loss, for all groups, during indoor storage.

(1) Groups. Using the SNK test, it was shown that Group 2 was significantly different ($p < .05$) from the others. This group had a significantly higher weight loss than the other two (Table 3). This is thought to be a result of differences in storage conditions (i.e. machinery vibrations).

Group 2 was stored in the room adjacent to the equipment storage room, (Fig. 1). The vibrations from the equipment operation were transmitted via the wooden floor and appeared to disturb the bees. The vibrations were constant due to the running of the fans in the furnaces to provide ventilation in the chambers; they were particularly severe in the spring when the compressors for the refrigeration equipment were operating. It is thought that this constant stress may have caused the bees in this chamber to consume more honey.

The other two groups were contained in the chambers furthest away from the equipment room. The effects of the vibrations were likely not as great in these chambers, hence weight loss was lower. However, some effect on the bees may have taken place because the weight losses for these two groups, although significantly lower than the first group, were still considerably higher than anticipated.

It is unlikely that some other factor may have caused the higher weight loss since this group with the highest weight loss during storage had the lowest mean weekly weight loss for the period before storage, suggesting that the storage conditions were the determining factor.

(2) Treatments. Analysis revealed that there was a significant difference ($p < .05$) in weight loss during storage between the two treatments with the lowest weight loss (both singles treatments, D and E) and the treatment with the highest weight loss (single + super, F).

TABLE 3. Mean weight loss (kg) for the period of indoor storage (December, 1976 to April, 1977).

<u>TREATMENT</u>	<u>GROUP 1</u>	<u>GROUP 2</u>	<u>GROUP 3</u>	<u>TREATMENT AVERAGE</u>
Double (A)	12.6 \pm 0.7	16.8 \pm 1.1	13.4 \pm 1.5	14.3 \pm 0.69
Double, honey (B)	14.6 \pm 1.5	17.7 \pm 1.3	16.6 \pm 1.9	16.5 \pm 0.96
Double, syrup (E)	11.3 \pm 0.8	17.1 \pm 1.0	19.9 \pm 1.9	12.9 \pm 1.08
Single, old queen (D)	9.8 \pm 0.8	15.3 \pm 0.6	11.0 \pm 0.5	11.9 \pm 0.59
Single, new queen (E)	9.2 \pm 1.0	15.4 \pm 1.2	9.1 \pm 0.9	12.7 \pm 1.24
Single, double (F)	15.3 \pm 0.6	25.9 \pm 1.9	12.9 \pm 2.0	20.5 \pm 1.82
Group average	12.1 \pm 0.5	18.6 \pm 0.78	12.7 \pm 0.72	

Weight loss in the doubles treatments (A,B,C) was intermediate but not significantly different from either the single treatments (D,E) or the single + super treatment (F).

It is difficult to conclude if the difference in weight loss is a treatment effect or if the vibrations are again having an effect on the weight loss encountered by each treatment. The singles treatments were located, in all cases, at the top of a stack of five boxes within the chambers. It was felt that if any of the treatments needed to be fed during the winter storage period, it would likely be the singles treatments since they had the least amount of stored honey. They were placed on top, therefore, for easy access. By the same reasoning, the least likely to need extra feed were the single + super treatments (F) since they received a box of honey on entering the quarters and hence were placed on the floor, at the bottom of the stacks.

This difference in weight loss may then reflect the difference in position in the stack. Those colonies at the bottom would likely be disturbed by the vibrations from the floor more severely than colonies at the top of the stack.

The effects of other factors such as carbon dioxide accumulation, air circulation and heat distribution, which may also have been related to position in the stack, may have had a part in accounting for weight loss. Carbon dioxide accumulation was not measured but seems unlikely, since the rate of air exchange in each chamber was adequate (four air changes per hour).

There was no significant difference between the treatments with the lowest weight loss. These two were both single treatments, one with the original queen (D), the other with a new queen introduced in the

fall (E). This suggests that the replacement of the old queen by a new queen in the fall before storage has no significant effect on weight loss during storage.

(III) Total Weight Loss. The total weight loss for the indoor wintered treatments was higher than anticipated. Table 4 shows the group mean total weight losses for the indoor wintered treatments of each group. Figure 9 is a histogram of the mean total weight loss and includes the outdoor wintered treatment (OW) for Group 2.

The total weight loss for each colony was compared using analysis of variance. There proved to be significant differences between groups as well as between treatments.

(1) Groups. The SNK test revealed that there was a significant difference in weight loss among all three groups. This difference was mainly due to the weight loss during the period of storage. The weight losses were greatest during storage and so a similar trend is shown for the total weight loss as for that incurred during storage. Again, the group with the highest weight loss (Group 2) was the group nearest the equipment room, while those groups in the chambers furthest from the equipment room had lower weight losses.

(2) Treatments. Analysis of total weight loss for the treatments revealed a trend similar to that shown for weight loss during storage. There were three distinct groupings. The treatments with the highest (F, single + super) and lowest (E, single, new queen) weight loss were significantly different from the rest of the treatments.

The two treatments with the highest weight loss (F, single + super; and B, double, honey) were significantly different from the other treatments.

TABLE 4. Mean total weight loss (kg) for the period September, 1976 to April, 1977.

<u>TREATMENT</u>	<u>GROUP 1</u>	<u>GROUP 2</u>	<u>GROUP 3</u>	<u>TREATMENT AVERAGE</u>
Double (A)	23.5 \pm 1.1	22.0 \pm 1.2	18.1 \pm 1.7	21.3 \pm 0.83
Double, honey (B)	24.9 \pm 2.3	24.9 \pm 1.3	22.4 \pm 2.4	23.9 \pm 1.23
Double, syrup (C)	24.1 \pm 0.7	28.0 \pm 1.1	17.4 \pm 1.9	23.1 \pm 1.38
Single, old queen (D)	19.0 \pm 0.9	22.4 \pm 1.1	17.8 \pm 0.9	19.5 \pm 0.64
Single, new queen (E)	17.9 \pm 0.9	22.8 \pm 1.9	14.2 \pm 1.7	19.6 \pm 1.57
Single, double (F)	24.3 \pm 0.8	33.9 \pm 2.4	19.4 \pm 2.2	26.2 \pm 1.91
Group average	22.4 \pm 0.68	25.9 \pm 0.96	18.8 \pm 0.81	

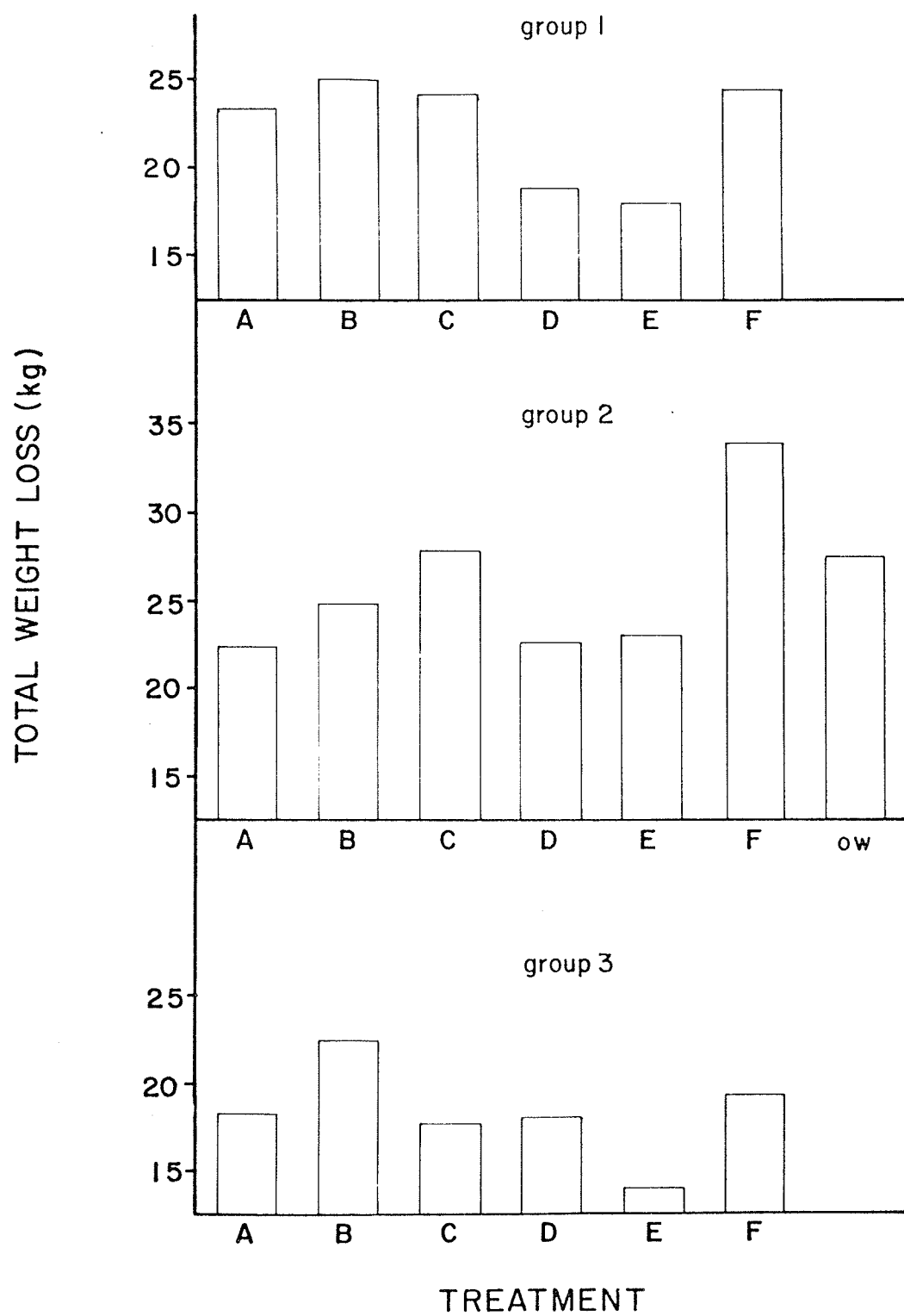


Figure 9. Histogram of total weight loss for all treatments.



The two treatments with the lowest weight loss (D, single, old queen; and E, single, new queen) were significantly different from the remainder of the treatments.

It seemed that those colonies with the smallest amount of stored feed and housed in one storey hives (treatments D and E) consumed the least amount of feed. The colonies with the largest amount of stored feed and in the largest hives (two storeys, treatments A, B, C, and F) consumed significantly more food.

Food consumption may have been related to the colony winter adult population. The double storey hives may have maintained a larger population during the fall preparation period and may have gone into winter quarters with more adult bees than did the single storey colonies. Colony weight loss may have been a reflection of colony population; the larger the population, the greater the consumption.

The significant differences found in total weight loss during winter among the treatments was likely influenced by the pattern of weight loss observed during the period of indoor storage. The double storey colonies were likely influenced more by the refrigeration machinery vibrations, since they were at the bottom of the stack, than were the single storey colonies which were at the top of the stacks.

It was not possible in this study to determine the relative importance of each of these aspects in the observed differences in weight loss during the winter.

COMPARISON OF BROOD REARING BY INDOOR WINTERED COLONIES, OUTDOOR
WINTERED COLONIES, AND COLONIES STARTED FROM TWO POUND PACKAGE BEES

(1) Introduction

A method was devised for comparing the various indoor treatments against outdoor wintered treatments and colonies established from package bees (two pounds, 0.9 kg). Brood production and the increase in colony population during the spring (up to the time of honey flow) was used to measure the relative performance of the various indoor wintered treatments. Various authors have reported methods of wintering bees, either indoors or outdoors, and several comparisons of techniques have been made (Braun, 1941; Braun and Geiger, 1955; Gooderham, 1926; and Le Maistre, 1942). These authors used the size of the spring colony as a measure of the value of a wintering technique; however, none reported a method of measure accurate enough to enable comparisons to be made between experiments. Measurements of adult populations, in terms of number of frames of bees in strong, medium and weak colonies may determine relative spring size (adult population) within a particular group of wintered colonies but do not allow others to compare results to those reported.

Baker (1942) used brood measurements as a method of comparing wintering treatments. Accurate brood counts were made of all colonies in the test over a period of time shortly after the bees were removed from winter packing. The mean brood counts, for different treatments,

were compared at each period of measurement using analysis of variance to determine if there was a significant difference in amount of brood reared by the different treatments. This was taken to show a measure of "success" of treatment.

During these investigations, an attempt was made to compare wintering treatments by measuring the amount of brood reared and the population increase up to the time of honey flow. Total brood production, adult population, and honey production were the criteria used to determine relative performance of each wintered group.

Three hive sizes were tested: single storey (D,E), double storey (A,B,C), and single storey plus super (F, a single storey hive converted to a double upon entry into winter quarters by the addition of a super of honey). The first comparison was to determine if any of these three sizes was more conducive to indoor wintering than the others.

Secondly, the effect of replacement of queens at different times was tested to determine if any one treatment would be superior to the others. And lastly, by including outdoor wintered colonies and colonies started from package bees in the same apiary as indoor treatments, a comparison could be made of the three types of management techniques.

(2) Materials and Methods

The surviving colonies of Group 1 and Group 2 were taken from the wintering building, placed into separate apiaries, and monitored during the spring and early summer. The colonies from Group 3 were not included, but rather, were used to monitor the loss of adult bees (chapter 6).

Every twenty-four days, an estimation of adult population was done early in the morning, before foraging activity had begun, to ensure that

the total population was present. Adult estimates were completed for all hives in an apiary before foraging had commenced. To ensure this, the operation usually began at day break.

Due to the large number of colonies in each apiary, it was decided that a visual estimate would be the best method of ensuring that all colonies could be observed on any one day. Even so, near the time of honey flow, when colonies were three storeys high (twenty-seven frames) the method was time consuming.

Each frame was examined separately, and an estimate of the area on both sides of the frame which was covered by bees was recorded (Nelson, 1971). Each frame estimate was judged to the nearest tenth of a frame (i.e. a frame completely covered with bees on both sides would be rated as 1.0, a frame that had 0.7 of the surface of one side and 0.3 of the surface area of the other side covered with bees would be recorded as having 0.5 of a frame of bees).

The number of bees in a hive was recorded as the number of frames covered by bees. This was converted to number of bees using 322 square inches (2078 cm^2) as the surface area of a standard Langstroth frame (both sides) and a density of 7.2 bees per square inch. This density was calculated from photographs and is similar to that used by Jeffree (1951).

Brood measurements were performed every twelve days. On alternate inspections, brood measurements were taken after the adult estimates had been completed. Bees were shaken from the brood combs and the area of sealed brood was measured using a grid (Fig. 4) of one inch squares ($2.5 \text{ cm} \times 2.5 \text{ cm}$).

The brood data for each treatment were analyzed using regression

analysis. The regression lines for each treatment were then compared by analysis of covariance to determine if there were any significant differences among treatments with respect to amount of brood or rate of increase of brood. These parameters were used as indicators of strength of colony and, therefore, success of treatment.

(3) Results and Discussion

(A) Brood Production. A total of eight brood estimations were performed between 8 April and 28 June, 1977. The results are shown in Table 5 (Group 1) and Table 6 (Group 2) as mean sealed brood area and total sealed brood area for each treatment. The sealed brood measurements for outdoor wintered colonies and package colonies are included in these tables.

From the total brood recorded per treatment, it would appear that the outdoor wintered treatments performed better in both groups. The package colonies in Group 1 produced more sealed brood than the average of all indoor treatments. However, in Group 2, the package colonies produced slightly less brood than did the indoor treatments up to the time of final observation.

This difference in brood production by package colonies probably occurred because the package colonies in Group 1 were installed ten days ahead of those in Group 2, thus allowing for an extra, earlier brood reading in the Group 1 package colonies.

The total brood production figures are misleading and suggest that the indoor colonies and package colonies were of equal strength and honey producing potential. In fact, this was not the case. Although the total brood counts were similar, the impact of those emerging adults joining the adult foraging population was not similar for the two types

TABLE 5. Mean sealed brood area for treatments to Group 1 hives.

<u>TREATMENT</u>	<u>AREA OF SEALED BROOD (cm²)</u>								Total
	Apr.8	Apr.18	Apr.30	May12	May23	June5	June17	June29	
Double (A)	4	144	447	339	1060	728	1571	2749	7042
Spring queen (B)	0	150	368	308	219	512	1107	1876	4540
Old queen (D)	50	498	1936	1723	2599	2030	3271	4116	16214
Fall queen (E)	0	32	1213	723	1871	1123	3155	4271	12388
Single, double (F)	52	323	784	803	1516	1603	1784	1174	8039
Combined indoor	17	216	852	700	1247	1085	1999	2677	8793
Outdoor		807	1826	2365	3211	2441	3588	3779	18017
Package				890	1536	1830	3297	3345	10898

TABLE 6. Mean sealed brood area for treatments to Group 2 hives.

<u>TREATMENT</u>	<u>AREA OF SEALED BROOD (cm²)</u>								Total
	Apr.8	Apr.17	Apr.29	May11	May24	June4	June16	June28	
Double (A)	25	159	510	463	1014	1420	2151	3078	8820
Spring queen (B)	16	223	961	1587	1543	2721	3020	3203	13274
Old queen (D)	1	62	314	454	1205	1549	2237	2587	8409
Fall queen (E)	4	127	710	707	1172	1225	1558	2102	7605
Single, double (F)	13	88	626	424	824	1045	2932	3207	9159
Combined indoor	12	147	681	870	1217	1780	2487	2896	10090
Outdoor		1341	1946	*1433	2423	3106	3677	4584	18510
Package						2319	3123	3569	9011

* approximately half of the brood combs removed 30 April, 1977 to start new colonies

of treatments. The brood counts of the indoor treatments were done over a period of time which was twice as long as that of the package colonies. As well, all of the early brood reared by indoor wintered bees during the first half of the observation period might have been "exhausted" raising brood and not become part of the foraging population at the time of honey flow. However, it is likely that almost all of the brood measured in the package colonies would have been part of the foraging population at the beginning of the honey flow.

Regression analysis (with analysis of variance) was used to analyse the rates of brood production during the time of observations for each treatment in both groups. All treatments were found to be significant ($p < 0.01$). The regression lines for brood production versus time for similar treatments in each apiary were then compared. It was found that a significant difference in brood production ($p < 0.05$) existed between the two groups for all treatments except one - treatment A, wintered as doubles (Table 7).

The differences in brood production of similar treatments in the two groups may have been due to the fact that the apiary locations were fifty miles (80 km) apart and the foraging conditions may not have been similar. Secondly, these differences may have been a manifestation of the differences experienced by these groups during indoor storage. There were significant differences in honey consumption during storage for the two groups. It was suggested that this may have been caused by stress due to vibrations in the building. As a result of the environmental differences experienced by the two groups both during indoor storage and in the apiaries, a treatment in one apiary was not necessarily similar to the same treatment in the second

TABLE 7. Comparison of regression lines for brood production against time (similar treatments between apiaries) with analysis of covariance.

	Double (A)	New queen (B)	Single old queen (D)	Single fall queen (E)	Single double (F)	Outdoor	Package
F-slope	$p > 0.2$	$p < 0.001$	$p > 0.1$	$p < 0.05$	$p < 0.05$	$p > 0.2$	$p < 0.01$
F-intercept	$p > 0.2$	$p < 0.001$	$p < 0.001$	$p < 0.05$	$p < 0.05$	$p < 0.01$	$p > 0.2$
F-variance ratio	$p > 0.05$	$p > 0.05$	$p < 0.05$	$p < 0.05$	$p > 0.05$	$p > 0.05$	$p < 0.05$
N(1)	8	8	8	8	8	7	3
N(2)	8	8	8	8	8	7	5

apiary. No attempt was made to alter the initial spring population since this was seen to be an indicator of success of the treatment during indoor storage.

The treatments, in the two groups, were not combined for statistical analysis but were analyzed separately. The regression lines for each treatment in a group were compared with those of each other treatment using analysis of covariance. The results are shown in the form of a probability matrix in Table 8 (F-slope) and Table 9 (F-intercept). There were no significant differences in F-variance ratio.

(I) Group 1

(1) Hive Size. There was no significant difference in the slope of the regression lines for either single (D), double (A) or single plus super (F) treatments. This suggests there was no significant difference in the rate of increase of brood rearing and that brood rearing was not influenced by the size of the hive during indoor storage. However, there was a significant difference in the F-intercept values, that of the singles treatment (D) being higher than the other two. The singles treatment (D) had a significantly larger amount of capped brood resulting in higher populations and ultimately in higher honey production (Table 10).

(2) Requeening. It was anticipated that those treatments which received new queens either in the previous fall (E) or in the spring (B) would have produced more brood than treatments with old queens. Comparing regression lines, it was evident that the treatments with the most rapid rate of increase were the fall requeening treatments (E) and treatments which were not requeened (D). These were not significantly different from each other or from one other non-requeened treatment (A) in rate of increase, but were significantly different from the spring requeened

TABLE 8. Probability matrix (F-slope) obtained when comparing regression lines for brood production plotted against time for indoor wintered treatments for Group 1 and Group 2 apiaries.

<u>TREATMENT</u>	<u>GROUP 1</u>				<u>GROUP 2</u>			
	(B)	(D)	(E)	(F)	(B)	(D)	(E)	(F)
(A)	$p>0.1$	$p>0.05$	$p>0.1$	$p>0.1$	$p>0.1$	$p>0.75$	$p\approx 0.05$	$p>0.5$
(B)		$p<0.01$	$p<0.025$	$p>0.75$		$p>0.05$	$p<0.001$	$p>0.5$
(D)			$p>0.75$	$p<0.01$			$p<0.025$	$p>0.5$
(E)				$p<0.025$				$p<0.05$

(A) Double

(B) Spring queen

(D) Old queen

(E) Fall queen

(F) Single, double

TABLE 9. Probability matrix (F-intercept) obtained when comparing regression lines for brood production plotted against time for indoor wintered treatments for Group 1 and Group 2 apiaries.

<u>TREATMENT</u>	<u>GROUP 1</u>				<u>GROUP 2</u>			
	(B)	(D)	(E)	(F)	(B)	(D)	(E)	(F)
(A)	p>0.1	p<0.001	p>0.05	p>0.5	p<0.005	p>0.5	p>0.5	p>0.75
(B)		p<0.001	p<0.025	p<0.025		p<0.001	p<0.001	p<0.05
(D)			p>0.1	p<0.005			p>0.25	p>0.5
(E)				p>0.1				p>0.25

(A) Double

(B) Spring queen

(D) Old queen

(E) Fall queen

(F) Single, double

TABLE 10. Honey production (kg) for Group 1 and Group 2 apiaries, 1977.

<u>TREATMENT</u>	<u>GROUP 1</u>	<u>GROUP 2</u>
Double (A)	8.6	17.2
Double (B) new queen	16.8	31.3
Single (D) old queen	34.0	31.3
Single (E) fall queen	45.8	14.1
Single (F) double	7.3	10.4
Outdoor	53.1	90.7
Package	66.7	37.2

treatment (B) and the other non-requeened treatment (F).

This more rapid rate of increase of brood resulted in larger total brood production (Table 5) and thus higher honey production (Table 10) for treatments (D) and (E). Probably the poor showing of the spring requeened treatment (B) resulted from the small initial adult population of this treatment in the spring. The queens were not able to lay to their full potential but were restricted by the amount of brood that could be tended by the small number of adult bees.

(3) Indoor Wintering, Outdoor Wintering and Packages. Comparison of the slopes of all indoor treatment regression lines (Table 11) with outdoor and package colony regression lines revealed that the outdoor wintered treatments were significantly different only from the two weakest indoor treatments (B and F). However, comparing F-intercept values, the outdoor wintered treatment was significantly different (higher) than all other indoor wintered treatments (except D) and the package bee treatment.

Similarly, the package bee treatment had a significantly different rate of increase from indoor treatments (A), (B) and (F) but was not significantly different from the rate of increase of the two better indoor treatments (D and E) or the outdoor wintered treatment (Table 11). Comparison of the F-intercept values revealed that the package colonies were significantly different from all others except the indoor treatment (E).

The outdoor wintered treatment and package treatment produced more total brood than the combined indoor average (Table 5). Treatments (D) and (E), however, had values similar to the outdoor wintered and package treatments. However, for the indoor treatments, due to mortality, not all of this brood would be part of the adult foraging

TABLE 11. Probability matrices for F-slope and F-intercept (F-variance ratio not significant) obtained when comparing regression lines for brood production of indoor wintered treatments versus outdoor wintered and package treatments for Group 1 and Group 2 apiaries.

<u>GROUP 1</u>	<u>F-SLOPE</u>		<u>F-INTERCEPT</u>	
TREATMENT	OUTDOOR	PACKAGE	OUTDOOR	PACKAGE
(A)	$p > 0.25$	$p < 0.05$	$p < 0.001$	$p < 0.05$
(B)	$p < 0.05$	$p < 0.01$	$p < 0.001$	$p < 0.005$
(D)	$p > 0.5$	$p > 0.25$	$p > 0.1$	$p < 0.025$
(E)	$p > 0.25$	$p > 0.5$	$p \approx 0.025$	$p > 0.25$
(F)	$p < 0.05$	$p < 0.005$	$p < 0.001$	$p < 0.05$
PACKAGE	$p > 0.1$		$p < 0.01$	

<u>GROUP 2</u>	<u>F-SLOPE</u>		<u>F-INTERCEPT</u>	
TREATMENT	OUTDOOR	PACKAGE	OUTDOOR	PACKAGE
(A)	$p > 0.1$	$p > 0.25$	$p < 0.001$	$p < 0.025$
(B)	$p > 0.5$	$p > 0.25$	$p < 0.001$	$p > 0.5$
(D)	$p < 0.001$	$p > 0.1$	$p < 0.05$	$p < 0.005$
(E)	$p < 0.01$	$p < 0.025$	$p < 0.001$	$p < 0.001$
(F)	$p > 0.5$	$p > 0.5$	$p < 0.001$	$p > 0.05$
PACKAGE	$p > 0.5$		$p < 0.05$	

- (A) Double
 (B) Spring queen
 (D) Old queen
 (E) Fall queen
 (F) Single-double

population at the time of honey flow; whereas, most of the total recorded for the package colonies would be part of the foraging population. This would explain the differences in honey production (Table 10).

(II) Group 2

(1) Hive Size. Whether the colony was wintered as a single, double or was a single and became a double when moved indoors did not seem to have any effect on colony growth. There was no significant difference between the doubles colonies (A) and singles (D) or the single plus super (F) treatments with respect to both rate of increase in amount of brood or initial amount of brood (Table 5).

(2) Requeening. When the slopes of the treatment regression lines were compared (Table 8) it was found that only that of the fall requeened treatment (E) was significantly different from all of the others. There was no significant difference in slope between any other treatments. Treatment (E) had a significantly lower rate of brood production.

The replacement queens used in treatment (E) were produced and mated at the campus of the University of Manitoba. They were mated in four frame nuclei colonies and selected after they began to lay. Although all replacement queens were tested for egg production, it is possible that some of the queens may have been poorly mated, which may account for their lower brood production. Secondly, the queens were replaced in the colonies in September. The ideal time would have been during, or shortly before, the end of honey flow. This may have produced better results.

A comparison of F-intercept values (Table 9) revealed that there was a significant difference between the spring requeened treatment (B) and all other treatments. This group (B) had a higher initial amount of

brood due to a higher population. Although the introduction of a new queen in the spring did not affect the rate of brood increase, the larger initial colony size allowed for a substantially larger amount of brood to be reared than in the other treatments and, therefore, resulted in a higher amount of honey production.

(3) Indoor Wintering, Outdoor Wintering and Packages. Table 11 shows the F-values for outdoor wintered and package colony regression lines as compared to the indoor treatment regression lines for Group 2. The trend is similar to that displayed in the indoor treatment comparisons in that treatment (E) is significantly different from both outdoor and package regression lines (F-slope). There was also a significant difference in F-slope values between the indoor single treatment (D) and the outdoor wintered treatment. The rate of brood increase for the other indoor treatments did not differ from the outdoor and package treatments.

However, when the intercepts of the regression lines were compared, it was evident that the outdoor treatment was significantly different from all other treatments. The outdoor treatments had a significantly higher initial amount of brood which translated into a larger foraging population and thus a higher amount of surplus honey (Table 10).

The package treatment was significantly different (F-intercept) from all other treatments except (D) and (F) and, consequently, recorded a higher honey production.

(III) Summary

(1) Hive Size. The results were similar for both groups. The size of the wintered colony, whether a single storey (D), double storey (A), or a single converted to a double upon storage (F), did not seem to influence the rate of brood production in the following year. However, the singles

treatment (D) in Group 1 had a significantly larger spring population enabling it to rear a larger amount of brood (Fig. 10).

(2) Requeening. The results of the requeening test are shown in Figure 11. The combined data suggest that there was little difference among the treatments. However, the results were not conclusive because of the wide variation exhibited. The amount of brood raised by each treatment was biased by the original spring size of the various treatments. Queen performance was then a function of adult population and not necessarily a function of the queen's age. A second element of bias was introduced because requeening took place between six and twenty-two days earlier in Group 1 than in Group 2.

In hindsight, it is suspected that the fall requeening treatment was also biased in that the queens were replaced too late in the season. Fall requeening was intended to perform two functions: first, to improve the colony's wintering capability by having a new queen rear a substantial amount of brood late into the fall thereby increasing the proportion of young bees in the hive. Secondly, a young queen should have a greater potential egg laying capacity in the spring than a queen entering its second season.

Due to the late timing, the first condition was not realized. The colonies did not have a significantly larger spring population from other treatments and so the second premise of increased egg production was limited by the colony size.

(3) Indoor Wintering, Outdoor Wintering and Packages. Although the comparison of these three management methods has been done, the results should not be taken to be indicative of the superiority of one method over another. While the superior results of outdoor wintering in terms

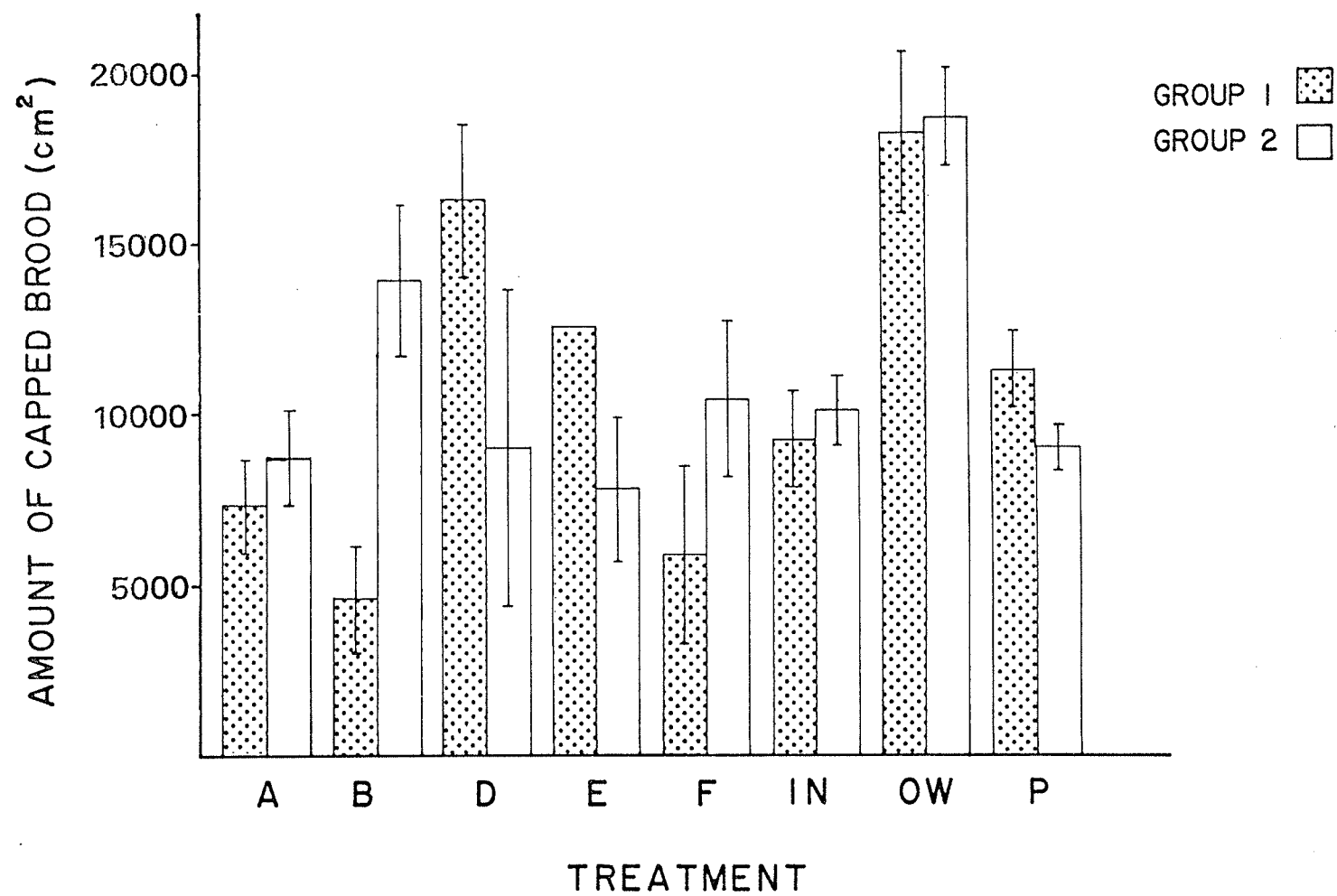


Figure 10. Histogram to illustrate total amount of sealed brood produced by each treatment (both apiaries).

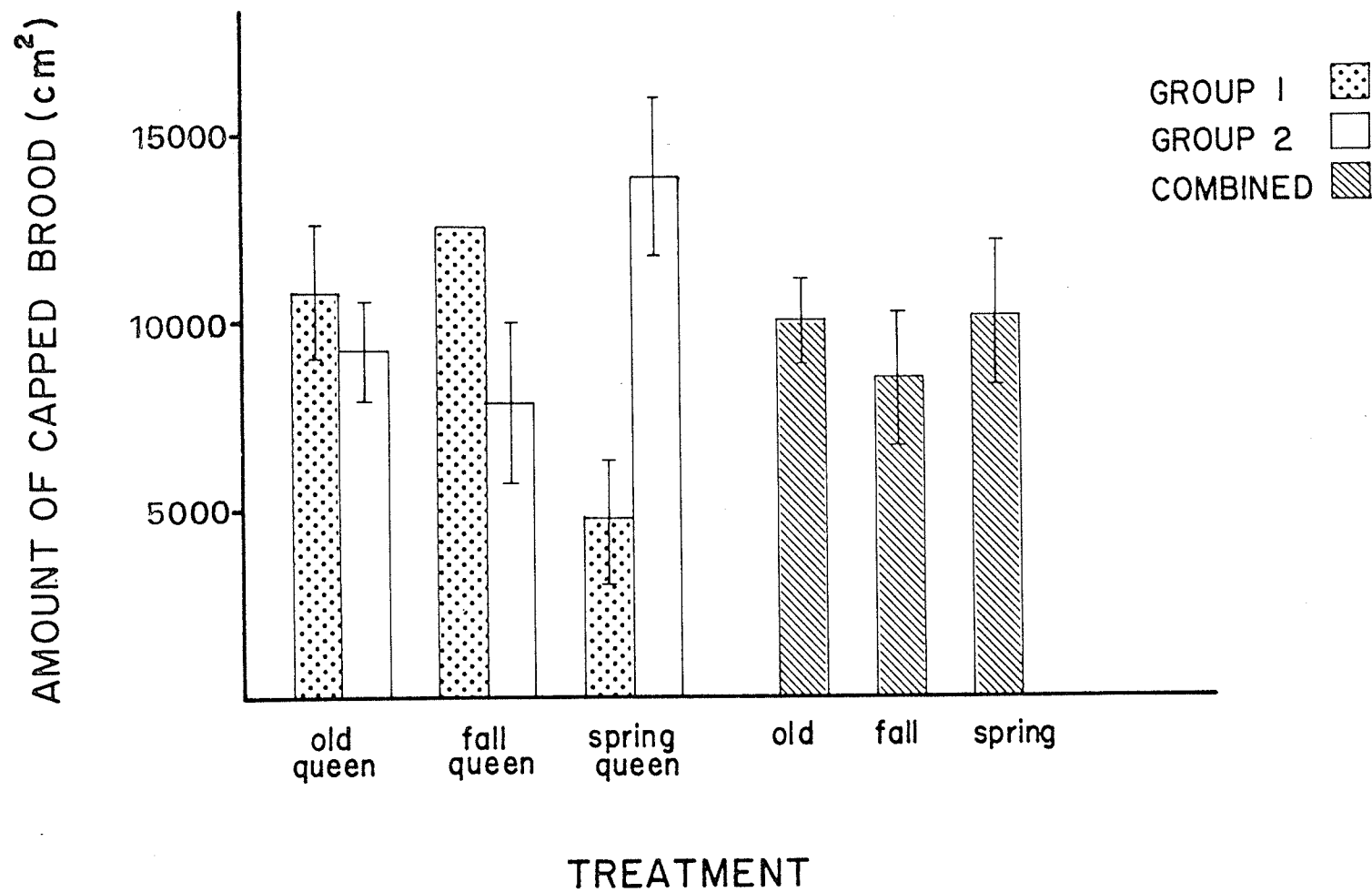


Figure 11. Comparison of amount of sealed brood produced in colonies with old queens (treatment A), fall-replaced queens (treatment E), and spring-replaced queens (treatment B).

of brood rearing (Fig. 12) and honey production over package treatments were expected, the poor results of the indoor wintered treatments were not, and were most likely due to the small initial size of the colonies due to conditions experienced during the winter.

With experience and modifications to the indoor wintering building, indoor wintered colonies will undoubtedly winter more successfully and should prove to be better honey producers than was shown during 1977.

(B) Adult Population Estimates

The results of the adult estimations are shown in Table 12 for the treatments in both groups. The combined average of all indoor treatment estimates has been plotted in Figure 13 with population estimate averages for the outdoor wintered and package treatments.

The projected adult estimates (June 28) were derived from the total sealed brood estimates for 23-24 May until 16-18 June, inclusive. Brood that had emerged on or after 23-24 May, 1977 was assumed to be part of the foraging population on 28 June, 1977. Bodenheimer (1937) assumed, in his population models, that workers died forty-two days after emergence. Brood measured on 16 June, 1977 would have emerged by 28 June.

(1) Estimate of Error. Forecasting the population size on 28 June was necessary since the honey flow had begun and collection of sealed brood and adult data was terminated. Adults, that had emerged prior to 24 May were not included in the projected estimate. Mortality for those adults emerging after 24 May was not determined, but was assumed to be zero.

Less emphasis was placed on the adult estimations as a criterion in determining success of a wintering treatment. Since the data collection for adult estimates was more subjective than that used for sealed brood data, the possibility of error was greater for adult

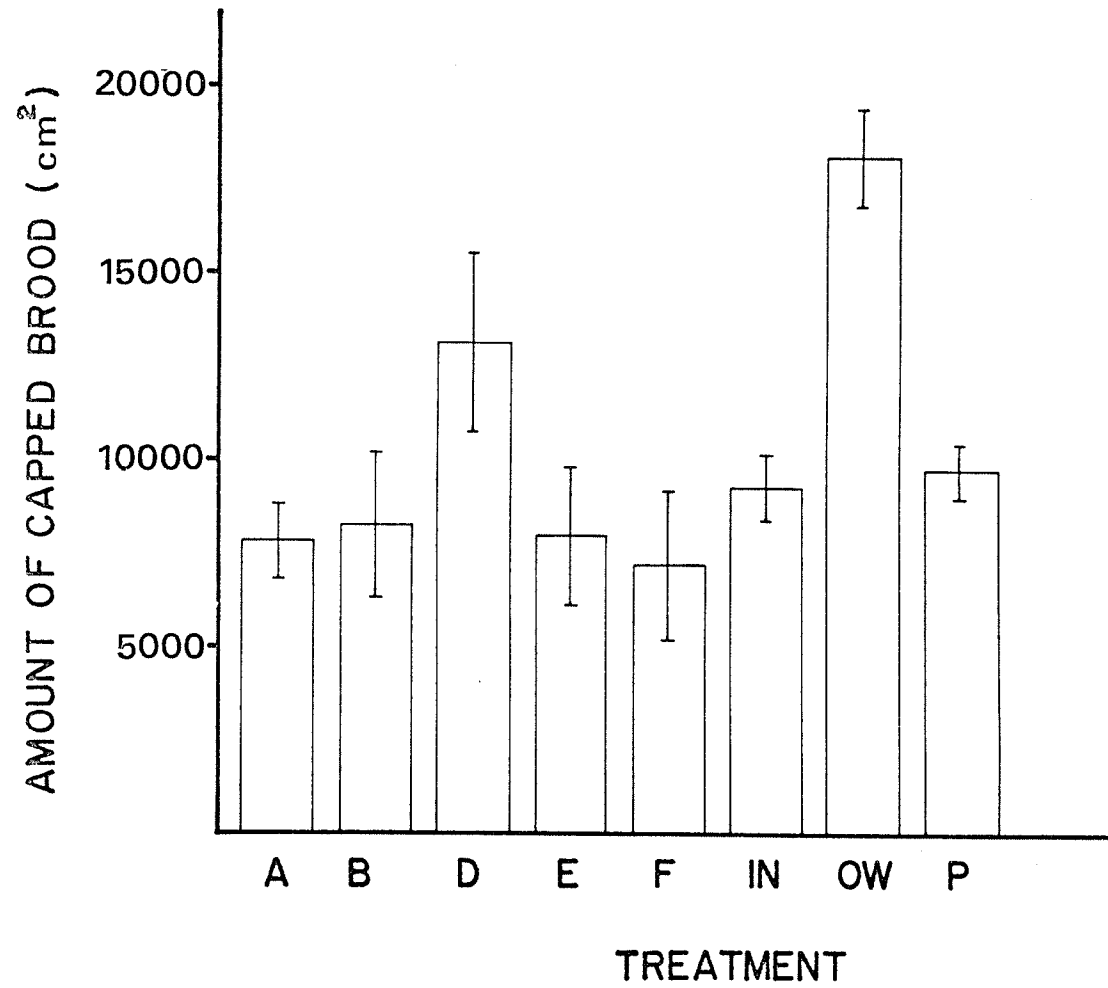


Figure 12. Mean total brood production for all treatments.

TABLE 12. Results of adult estimations (number of adult bees) recorded as treatment means for both apiaries.

TREATMENT	ADULT ESTIMATE				
	Apr. 8-9	Apr. 30-May 2	May 23-24	June 16-18	June 28 (projected)
(A) G1	3426	3105	3587	8032	13012
G2	4436	3120	3732	10709	17768
Ave.	3931	3112	3659	9370	15390
(B) G1	4583	2484	2998	5097	7935
G2	8139	5507	11658	18390	27754
Ave.	6361	3995	7328	11743	17844
(D) G1	8139	6896	11823	21248	30610
G2	2713	1570	3997	11637	19333
Ave.	5426	4233	7910	16442	24971
(E) G1	3427	4284	7282	14137	23775
G2	4069	2355	4776	12173	15325
Ave.	3748	3319	6029	13155	19550
(F) G1	5247	3534	6211	10281	13341
G2	3105	2891	5033	12637	21562
Ave.	4176	3212	5622	11459	17452
Combined indoor ave.	4728	3575	6110	12434	19042
Outdoor G1	10189	9608	16279	27305	35807
G2	9424		9883	22551	35672
Ave.	9806		13081	24928	35739
Package G1		4122	6184	16064	25818
G2			5660	16982	22232
Ave.			5922	16523	24025

(A) Double
 (B) Spring queen
 (D) Old queen
 (E) Fall queen
 (F) Single-double
 G1 Group 1
 G2 Group 2

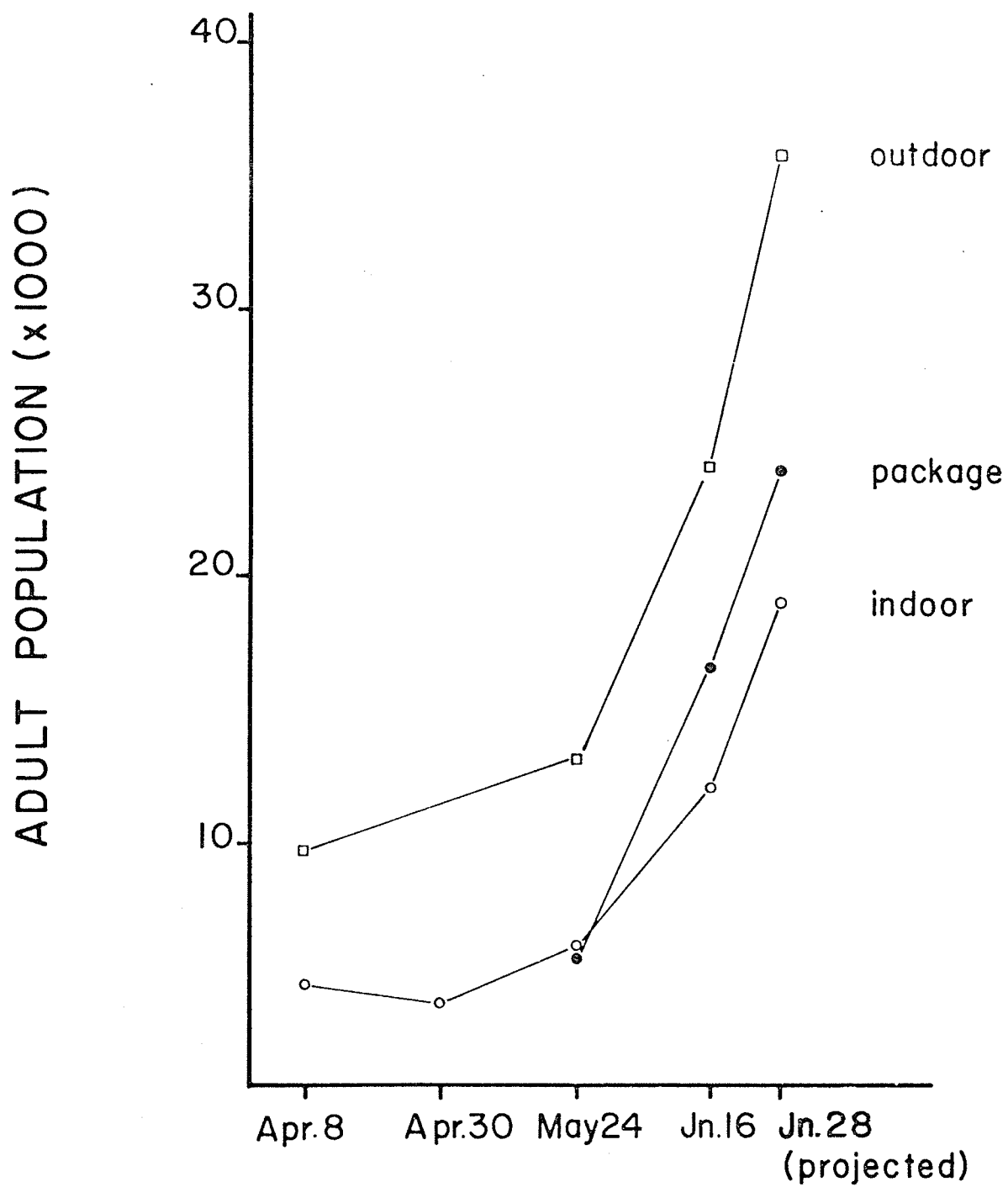


Figure 13. Estimated adult population (treatment mean) for indoor wintered, outdoor wintered, and package colonies.

estimates.

During each observation period, data were recorded by four observers. Attempts were made frequently to check the degree of similarity and accuracy among observers.

The degree of accuracy for the early readings, when colonies were relatively small, was similar to the five to ten percent error reported by Nelson (1971) using a system of photographs.

The adult estimates were used solely to depict the relative colony size of the treatments for a comparison of the three management techniques and to suggest an additional reason for the difference in honey production.

The adult estimates reflect the trend demonstrated by the sealed brood estimates. The outdoor wintered colonies had the largest amount of sealed brood and consequently, the largest populations. The package colonies, although not differing greatly from the indoor treatments in total sealed brood produced, displayed larger populations. The larger populations were responsible for the larger amounts of surplus honey production recorded by the outdoor and package treatments.

(2) "Dwindling" in the Indoor Wintered Treatments. Dwindling describes the decrease in colony size during the spring period. It is caused by the loss of old bees from the colony once they commence flying in the spring.

The indoor wintered treatments were severely affected by dwindling. The adult estimates (Table 12) revealed that most colonies had suffered substantial losses during the first month after removal from winter quarters. This loss of adult bees retarded colony development by decreasing the amount of replacement brood the colony was capable of producing. As a result, the colony populations did not return to their

original size until the latter part of May and did not begin to increase in size until early June.

LOSSES OF ADULT BEES FROM INDOOR WINTERED
COLONIES WHEN MOVED OUTDOORS IN SPRING

(1) Introduction

The death of adult bees within a colony during winter is a natural phenomenon. In wintering buildings, where the temperature is usually held between 4° and 8°C . (38° to 46°F .), this low mortality is evidenced by the daily accumulation of dead bees on the floor. Outdoor wintered colonies, due to their colder environment, appear to lose older bees only when the temperature rises sufficiently to allow flight, at which time, large numbers of dead bees will often be found in the snow around a colony. Many authors report that comparative studies of colonies wintered indoors and outdoors show that outdoor wintered colonies tend to have larger populations in the spring than indoor wintered colonies (eg. Phillips and Demuth, 1918). This may be caused by higher mortality of adult bees from the indoor wintered colonies after they are moved outdoors. The mortality of adult bees and consequent reduction in colony size during the spring has been referred to as "dwindling". For indoor wintered colonies, dwindling is thought to be caused by the influence of light on bees that have been kept in darkness for four months, and by stresses associated with accumulated rectal contents. This heavy mortality, immediately after removal from wintering quarters, can severely hamper a colony's development. So many adults are lost at once that the amount of brood that the colony is able to rear decreases

substantially.

Colonies wintered outdoors do not seem to suffer dwindling losses to the same extent as do indoor wintered colonies. Perhaps the constant exposure to sunlight causes a low level of mortality of those adults needing to fly due to stress (eg. accumulated rectal contents) throughout the late winter and early spring. Thus, outdoor wintered colonies seldom experience the large adult bee mortalities that indoor wintered colonies experience when moved outdoors. Outdoor colonies usually have a large number of young bees in the spring due to winter brood rearing. These replacement adults maintain the colony population so that the death of the older wintered adults does not appear to affect seriously the spring adult population.

In this experiment, the colonies were confined indoors from 15 December, 1976 until 8 April, 1977 in complete darkness. A number of tests were performed to determine the extent of losses of adult bees in the spring when colonies were moved outdoors and to determine if orientation cues could be effectively used to reduce these losses to a minimum.

(2) Materials and Methods

All of the colonies used in these orientation tests were from one source (Group 3). They were prepared for wintering the previous fall and had wintered in one of the chambers of the wintering building. A total of thirty colonies were used in the trials in two apiary sites which were approximately five kilometers apart.

(i) Marking Bees. Thirty colonies were removed from the winter quarters during the evening of 6 April, 1977. Two hundred bees, randomly chosen from each colony, were marked on the thorax with fast-drying enamel

paint. The paint was applied using a syringe (Fig. 14). Bees were marked directly as they moved about on the combs or on the top cover. The bees in any one hive all received the same colour of paint; five different colours were used, one for each of the treatments in an apiary.

(ii) Moving Hives to Field Positions. Hives were moved to their field positions during the night, immediately after the marking was complete.

Both apiaries were similar in respect to the following conditions:

hives were sheltered by a windbreak to the north, had a southern exposure, and they and the surrounding areas were clear of snow cover and orientation cues (eg. trees, rocks, etc.).

Five treatments, four test treatments and a control (three replicates each), were placed in each apiary. Hives were spaced five metres apart to reduce the chances of drifting between colonies. Hives were positioned sequentially along the windbreak so that each replicate was separated by four other treatments. Each treatment contained different coloured marked bees so that drifting could be easily recognized.

(iii) Orientation Cues. The control hives had no orientation cues, had reduced entrances (2.5 cm X 1.0 cm), and faced south. The following treatments were tested as to their affect in reducing the loss of adult bees:

1. East - hives facing east
2. West - hives facing west
3. Large entrance - hives with large entrances (15 cm X 1.5 cm)
4. Coloured obstacle - hives with a coloured obstruction in front of the entrance (Fig. 15)
5. Blue - hives with blue painted fronts
6. Yellow - hives with yellow painted fronts



Figure 14. Applying paint to thorax of honey bee using a syringe.



Figure 15. View of coloured obstruction in front of hive entrance.

7. Bars - hives with four blue bars on a white background
(known to be recognizable by bees)
8. Circle - hives with a blue circle on a white background

Treatments 1 to 4 were included in one apiary and treatments 5 to 8 were in the other. All treatment hives were similar in size and appearance to the control hives except for the tested variable.

(iv) Field Observations. The first data were collected on 8 April, 1977 after the colonies had had one day of good flying weather during which the bees were able to leave the hive. Subsequently, the hives were examined every seventh day for six weeks.

During the first observations, any dead marked bees in the hive or at the front of the hive were noted. Observations were made in the morning before the bees had commenced foraging. The number of coloured bees remaining in each hive was recorded. Any drifting bees (differently coloured marked bees) were not recorded as part of the marked bees of the colony since they were effectively lost to the parent colony but were recorded to provide data on the frequency of occurrence of drifting. Observations were terminated on May 20, 1977.

(v) Analyses of Data. The data recorded were transformed to show percent loss of marked bees over time. Arcsin transformation was performed on the percentage data and subsequently regression analyses were done for each treatment. The regression lines for each treatment were compared with all other treatments using analysis of covariance.

(3) Results and Discussion

Three colonies (one each of west, control and blue) became queenless during the course of the experiment and were excluded from the analysis.

The percentage of marked bees remaining in a hive was calculated from the initial number of marked bees after accounting for any dead marked bees found in or near the hive during the first observations. The percentage loss (replicate average) of marked bees for each group is shown in Table 13 and graphically in Figure 16.

It will be noticed in Table 13 that the percent loss decreased for the April 22 reading for one of the control groups. A similar decrease occurred for the April 15 and April 22 counts for the "circle" test group. These were the result of human errors in counting marked bees during the preceding observation. Due to the cold weather during the early morning observations, the bees were tightly packed in the brood nest and, as a result, the marked bees were difficult to find. The observed fluctuations were likely the result of missed bees, which were counted during the subsequent observation, causing a decreased percent loss.

As seen in Table 13, the percent loss of marked bees was high after only one day of flight activity. The losses ranged from a low of 35.5 percent to a high of 72.9 percent. The average loss of marked bees of all treatments was 51.2 percent after one day, increased to 76.6 percent on 29 April, 1977 by which time brood had begun to emerge, and was at 95.7 percent six weeks after the hives were removed from winter quarters, at which time the observations were terminated.

The colonies were placed into what would be considered ideal spring locations. They were sheltered from cold spring winds, predominantly from the north, and had a southern exposure to assist in warming of the colony during the day for brood rearing. There was no snow cover in the immediate area to cause disorientation and result in

TABLE 13. Percent loss (treatment average) of marked bees from hives moved outdoors after one day and at weekly intervals thereafter.

<u>TREATMENT</u>	<u>PERCENT LOSS</u>						
	Apr.8	Apr.15	Apr.22	Apr.29	May6	May13	May20
Control	35.9	63.5	59.9	73.9	81.8	91.6	96.6
East	71.1	80.0	82.4	85.6	87.6	92.8	94.6
Large entrance	35.5	65.5	68.7	84.6	89.3	96.4	97.0
Obstruction	49.2	60.0	65.2	70.5	81.8	89.7	93.4
West	44.0	61.8	65.2	71.3	78.8	85.8	91.9
Control	49.8	66.0	71.3	78.5	87.1	93.1	96.6
Bars	42.9	56.8	60.9	74.2	84.2	92.0	96.8
Blue	54.4	68.4	72.0	78.2	87.3	92.2	94.8
Circle	72.9	70.2	71.8	78.4	85.4	94.7	97.6
Yellow	56.1	62.0	66.8	70.8	80.8	89.7	96.9
Average	51.2	65.4	68.4	76.6	84.4	91.8	95.7

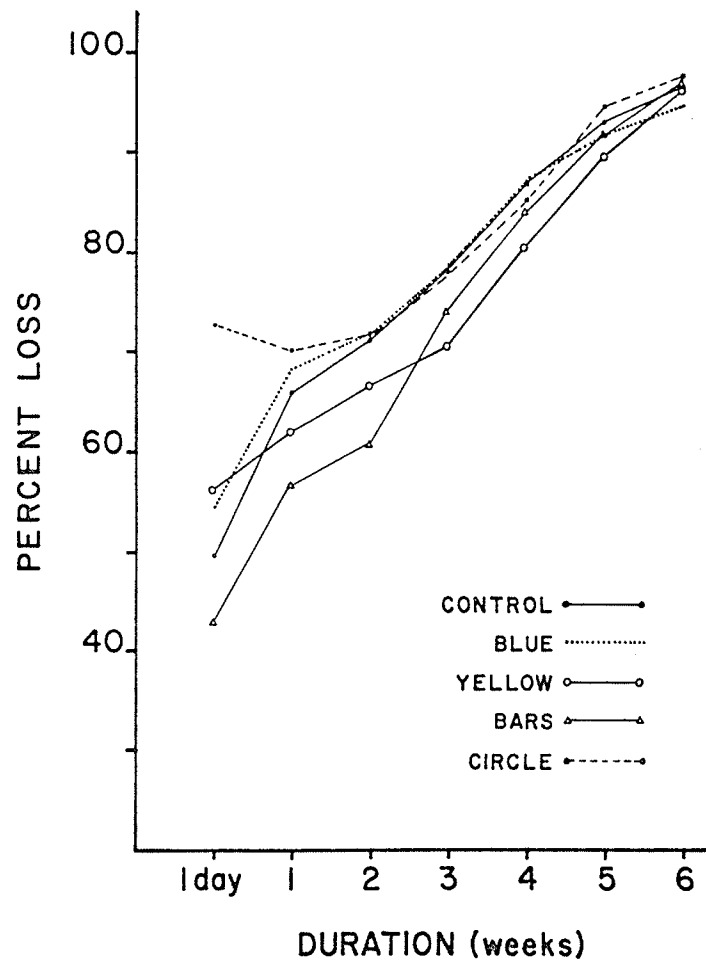
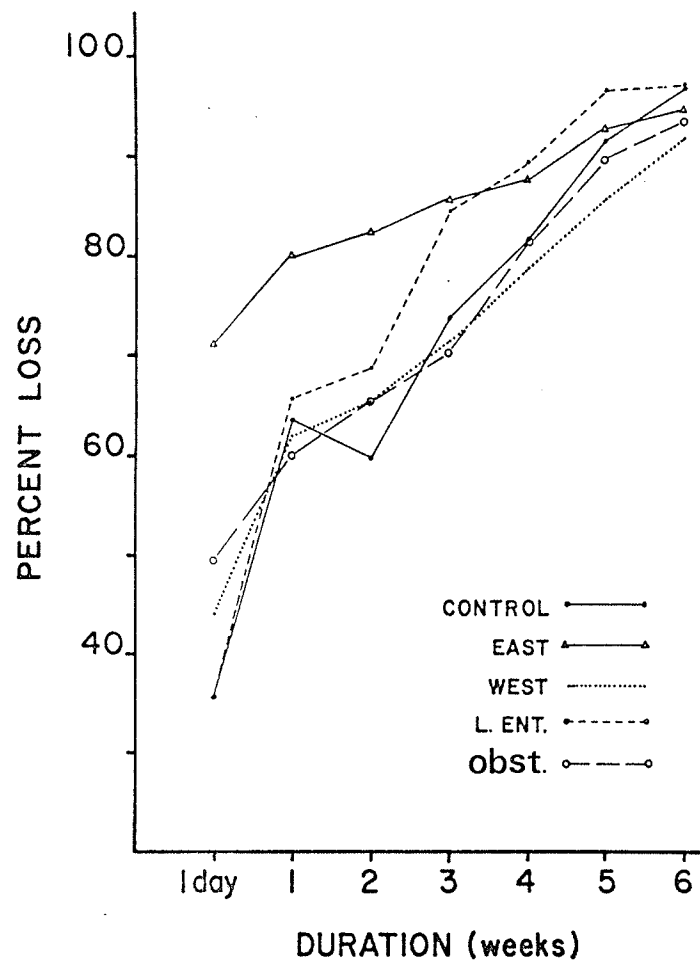


Figure 16. Percent loss of marked adult bees during first six weeks after removal from indoor wintering quarters.

increased losses of adult bees. Even so, the losses of marked bees were high.

It was assumed, for the purposes of this experiment, that the marked bees would be representative of the colony as a whole since the bees were randomly marked. Thus, the percent loss of marked bees would be indicative of the percent loss of the total population. Substantial losses of marked bees occurred during the first day of active colony flight. Adult bees left the colony and were not able to return and, presumably died. The percent loss of marked bees was similar to the apparent decrease in colony population, i.e. if fifty percent of the marked bees were missing, the colony also appeared to have decreased in adult population by about half.

Undoubtedly, "stress" on the bees was responsible for some of these losses. The bees, stressed by the need to defecate, which may have been complicated in some cases by the occurrence of Nosema disease, were stimulated by the light and the warmth of the sun to fly immediately. Once airborne, they may have become chilled and/or disoriented and could not return to the colony. Secondly, the bees could have been leaving the hives to forage. Whatever factors were responsible, the losses were enormous, eg., after one day, 51.2 percent were lost and a further 14.2 percent loss occurred during the first week.

During the first week outdoors, brood rearing was increased and the losses of marked bees during this period may have been representative of adult mortality associated with foraging and aging due to brood rearing. The rate of loss of marked bees during the second and third weeks was 3.0 percent and 8.2 percent, respectively.

The high losses were likely the result of death of the older

adult bees in the population that survived through the winter from the previous fall. Older bees would be more stressed by accumulated rectal contents than would the adult bees raised during the winter. Also, the older bees would be exhausted from brood rearing during the winter. Perhaps the percent loss of adult bees from the population would have been lower if there was a higher proportion of young bees (ie. raised during the winter) in the population. The proportion of young bees in the population when the colonies were removed outdoors would be dependant on the amount of brood rearing that occurred while the colonies were stored indoors.

Orientation cues did not appear to decrease substantially the loss of marked bees from the hives. The results of comparing regression lines for each treatment are shown in the form of probability matrices in Table 14 (F-intercept) comparing initial loss, and Table 15 (F-slope) comparing rate of loss. The F variance ratio values were not significant. No one treatment was significantly different from all other treatments with respect to initial loss of bees (F-intercept) or rate of loss of marked bees (F-slope). However, the treatment East (which had its hive entrance facing east) had a significantly higher initial loss of marked bees when compared to most of the other treatments. The rate of loss of marked bees for the East treatment was also significantly higher than most of the other treatments.

Because the hives faced east, it was suspected that the early morning sun striking the hive fronts directly may have influenced the bees to fly earlier in the day. If so, once outside the hives, the bees may have become chilled and not been able to return to the hives, with the result that these colonies suffered higher losses than the other

TABLE 14. Probability matrix (F-intercept) obtained when comparing treatment regression lines.

[illegible]

TABLE 15. Probability matrix (F-slope) obtained when comparing treatment regression lines.

[illegible]

treatments. No other treatment was significantly different from the control treatments with respect to initial loss of marked bees. Therefore, none of the orientation cues reduced the initial loss of marked bees from the colonies. It appeared that certain factors mentioned earlier (eg., light, defecation, Nosema disease, stress) may have prevailed and that orientation cues were, therefore, not effective in reducing losses.

Very few bees were found to have drifted to other hives. For the duration of the experiment, fewer than ten bees were found to have drifted among hives. The orientation cues and the distance between colonies were likely responsible for the small amount of drifting experienced. Losses experienced by the colonies in the experiment resulted from mortality of the adult bees after they flew from the hives and not due to drifting to other hives.

NOSEMA DISEASE ANALYSIS

(1) INTRODUCTION

Nosema disease in honey bees is caused by a protozoan parasite, Nosema apis Zander. Until the discovery of drugs to counter the effects of the disease (Farrar, 1954; Jamieson, 1955), Nosema disease was considered a serious economic concern, causing infected colonies to grow at a slower rate than non-infected colonies. Burnside and Revell (1948) showed that caged bees infected with Nosema died sooner than non-infected bees and speculated this was the reason for slower development of infected colonies. Farrar (1947) showed that Nosema disease in package bees was responsible for early queen supersedure and reduced honey yeilds. Jeffree and Allen (1956) found that colonies infected with Nosema disease had a higher rate of loss of adult bees during winter than did disease-free colonies.

(2) MATERIALS AND METHODS

A sample of approximately thirty bees was obtained from each hive every twelve days from the time the colonies were placed in the apiary to the time of honey flow (June 28-29). Adult bees were collected from the tops of the frames above the brood cluster, placed in plastic bags and kept frozen until analyzed.

For analysis, twenty-five bees were mascerated in ten millilitres of water. A drop of this fluid was placed on a slide and the number of spores per field of vision were recorded (magnifivation X440). Three

counts per sample were made to obtain an average count per sample.

Selected samples had another fifteen millilitres of water added to obtain a dilution of one bee per millilitre. A Haemocytometer was used to estimate the number of spores per bee (L'Arrivee, 1963) at various levels of infection as determined by the number of spores per field of vision.

(3) RESULTS AND DISCUSSION

L'Arrivee (1963) determined that foraging bees, collected at the colony entrance, exhibited higher rates of infection than did bees collected in the interior of the hive. Moeller (1956) found that *Nosema* infected bees in a winter cluster tended to select the warmest part of the cluster and recommended sampling from the top of the center of the cluster.

Since this investigation was begun early in the spring and the samples had to be taken, of necessity, when the bees were clustered and little or no foraging was occurring, adult bees were taken from the bees situated at the top of the cluster. For uniformity, this practise was continued throughout the experiment. According to L'Arrivee (1963), these bees would show lower rates of infection than would foraging bees.

A level of infection for each colony was determined based on the number of spores per field of vision. This method was used to facilitate the diagnosis of the more than five hundred samples. A composite sample of twenty-five adult bees (as described by L'Arrivee, 1963) was used to determine the infection levels for selected samples in terms of number of spores per bee. The results of the *Nosema* analysis are shown in Table 16. Unfortunately, some of the samples for Group 2 (May 13) were lost.

TABLE 16. Treatment average spore count (spores per field of vision) for each treatment during the sampling period.

GROUP 1

TREATMENT	Apr. 18	May 4	May 12	May 23	June 5	June 18	June 29
A	97.6	254.6	1.2	5.7	5.6	2.5	2.6
B	134.1	145.1	17.2	29.7	9.1	3.9	16.2
D	138.5	179.8	10.8	15.6	8.7	20.4	9.8
E	94.0	219.5	1.0	2.0	19.3	5.3	0
F	148.9	210.0	6.5	64.7	15.9	3.2	1.5
OW		10.9	32.3	4.6	10.4	2.5	7.3
P		17.7	32.3	29.4	9.8	2.1	3.0

GROUP 2

TREATMENT	Apr. 20	May 1	May 13	May 24	June 4	June 16	June 28
A	171.5	81.9	22.8	11.5	0.5	3.2	1.0
B	111.8	81.4	*	15.5	8.2	3.3	9.5
D	125.4	118.9	*	10.0	1.6	5.9	0.6
E	78.6	54.7	*	26.9	5.8	5.4	4.6
F	148.7	165.5	*	7.8	7.0	1.5	5.3
OW	1.4	*	*	3.7	3.7	6.9	5.0
P			*	3.5	5.1	2.4	3.8

* samples lost

It can be seen from Table 16 that all of the indoor wintered colonies in both groups had very high Nosema spore counts for the 18 April and 4 May, 1977 samples. These counts correspond to infection rates well above a million spores per bee and would be classified as "heavily infected" (L'Arrivee, 1963). The level of infection decreased dramatically in the 12 May, 1977 sample for the Group 1 apiary and remained at a low level for the remainder of the sample period. It is likely that Group 2 had a similar decline in the May 13 sample period and henceforth showed low infection rates.

This seasonal variation has been noted by many authors (eg. Burnside and Revell, 1948; Cantwell and Shimanuki, 1970). The apparent decreased rate of infection may have been caused by a natural decrease in Nosema infection. Burnside and Revell (1948), in their studies of the effects of temperature on the growth of Nosema disease, determined that brood nest temperatures of 34° to 35°C ($93-95^{\circ}\text{F}$) are above the optimum for Nosema disease and consequently inhibit the spread of Nosema. The increased brood rearing, and warmer weather, resulting in hive temperatures above the optimum for Nosema disease, may have been responsible for the observed decrease in May.

Part of the observed decrease may have been due to the sampling techniques. L'Arrivee (1963) found that foraging bees were the most heavily infected and that house bees had the lowest rates of infection. Because samples were taken from the top of the cluster, the samples in May likely contained fewer foraging bees than did the earlier samples, thus showing a decreased infection rate.

It is not possible to assess the effect of the high levels of Nosema disease on the indoor wintered colonies. However, the high

incidence of disease during the critical first three weeks is believed to have retarded the colonies development during the first month outdoors in the spring through the loss of adult bees and reduced brood rearing. None of the indoor wintered colonies were fed prophylactic drugs for the prevention of Nosema disease. Those colonies used in the losses experiment (Chapter 6) may have experienced losses due, in part, to Nosema disease. It is likely that the indoor wintered colonies in the Group 1 and Group 2 apiaries were under similar stresses and could have experienced similar losses. The losses observed were certainly detrimental and served to reduce the rate of growth of the colonies during the spring.

The outdoor, and package, colonies were not observed to have as high levels of Nosema disease as did the indoor wintered colonies. The low rates of Nosema infection in the outdoor wintered colonies may have been due to the loss of infected bees during the winter or to the fact that these colonies were large and had already begun to raise substantial amounts of brood by the time the first samples were taken. Unfortunately, the Group 2 samples were incomplete for the outdoor wintered colonies and the package colonies were installed late so that the first Nosema sample from package colonies was not taken until 24 May, 1977 at which time, only a low level of infection existed. It is unlikely that colony development was hampered by Nosema disease in these treatments since the levels of infection were likely comparable to those for Group 1 outdoor and package colonies.

The indoor wintered colonies produced smaller amounts of brood during the build-up period prior to honey-flow than did the outdoor wintered and package bee colonies. This resulted in smaller adult

populations for the indoor wintered colonies and consequently, they produced less honey. Nosema disease may have been an important factor by reducing the lifespan of the adult bees and thus, decreasing the amount of brood raised during the early spring. The indoor wintered colonies had much higher infections than did the outdoor wintered and package colonies.

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