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MASS STORAGE OF HONEY BEE QUEENS DURING THE WINTER

by

Margriet H. Wyborn

B. Sc., Simon Fraser University, 1977

THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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Name: Margriet Hilda Wyborn

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Examining Committee:

Chairman: Dr. B.D. Roitberg, Associate Professor

Dr. M.L. Winston, Professor, Senior Supervisor,
Department of Biological Sciences, SFU

Dr. R.A. Nicholson, Associate Professor,
Department of Biological Sciences, SFU

Dr. D.R. Gillespie, Research Scientist, Public Examiner
Agriculture Canada, Agassiz Research Station

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ABSTRACT

Storage of *Apis mellifera* L. queens over the winter, within individual colony banks in screened wooden cages, is biologically and economically feasible.

Approximately 500 queens were introduced into various storage systems for three years of winter tests. Individually wintered queens in nucleus colonies were the control and these were compared to colony "banks" with queens held in (1) screened wooden cages (2) excluder cages (3) screened cages and transferred to mini-nuclei until late March, (4) excluder cages constructed in the form of a cube and held in colony banks, and (5) excluder cages held over indoor colonies. Queens held in excluder cages (2) and stored in queenright or queenless colony banks showed poor survival in all three years of testing, and is not viable for commercial use; survival for any one year, or any treatment, was never greater than 25%. Extremely high initial mortality of greater than 80 percent, in the indoor (5) and redesigned systems (4), both with excluder cages, precluded further examination.

In contrast, an average of 60% of queens survived that were stored in screened wooden cages (1) and held inside queenless colony banks. We found no differences in survival of queens that were moved between colonies and those that remained in the same colony for six months. The success of these systems was due to the preparation of colony banks that contained large numbers of adult workers produced from two-queening the colony, that were well-fed, and insulated in groups of four, to preserve heat and reduce worker clustering in the winter.

Queens from different winter storage systems were introduced to colonies, and queen attributes were examined in order to compare the effects of different storage regimes on queen function and behavior. Those queens that survived the winter were virtually identical in quality and performance in all storage systems.

Mass queen storage is economically viable for the beekeeper, because the total cost of production and storage is less than the revenue from queen sales, especially where 48 queens are stored in the colony bank. The largest proportion of total costs for wintering queens is labour. Thus, if the beekeeper does the labour himself, the labour income as well as the returns from queen sales come back to the beekeeper. We calculated that annual profit for a commercial beekeeper who does his own labour for storage and hires workers for queen production is \$16,625 when 4800 queens are successfully stored over the winter, and sold for \$10 each in the spring. If all labour costs are paid out to a hired worker, profit would be \$13,215.

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The responsibility for the study as written and all conclusions reached herein, are the author's alone. The report does not necessarily reflect the opinions of the Federal and Provincial government which funded the study.

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1. INTRODUCTION

A reliable supply of good quality queens in early April is essential for continuing the present management practices of Canadian commercial beekeeping. Queens have not been imported into Canada from the United States since 1987 due to parasitic mites, and queens cannot be produced in Canada prior to May due to climatic restrictions to natural matings. Several alternative methods for obtaining queens have been investigated over the last few years. Queens can be imported from New Zealand or Australia, or placed into storage from time of production in the summer to the following spring. However, there is no system available for overwintering queens that has been consistently successful for beekeepers other than systems for overwintering one queen per colony. Canadian self-sufficiency in queen production could be achieved if Canadian beekeepers produce summer mated queens, store them over the winter, and make them available in early spring.

Winter storage of mated queens is a technique for storing locally produced summer queens until required in April of the following year. Mated queens are required for re-queening colonies, package and nucleus production, and colony division. Sale of winter-stored queens would increase (1) the availability of locally produced queens, and increase (2) employment and (3) income. Demand for queens usually tapers off by June due to traditional colony management; with an increased demand for summer queens, the queen production season could be extended from June until August. Queen producers could double their queen production from two to four queens per year per nucleus colonies, reducing costs dramatically, since the cost of a queen is determined largely by the number of mated queens that can be obtained from each nucleus (Roberts and Stanger, 1969).

Our study on queen storage is the first that extensively tested various storage systems over the winter period, investigated which colony parameters were important

to successful queen storage, and tested queens after storage for any deterioration in reproductive potential. Beekeepers need a system that can store queens for up to six months. Such a queen storage system would increase self sufficiency for beekeepers, and increase the potential sales for spring queens. In this study we report results of three years of queen storage in various systems, and identify the "best" systems. In addition, we calculated the economic viability of the most successful systems.

2. COLONY STORAGE SYSTEMS FOR HONEY BEE QUEENS

2.1. Review of colony storage systems for honey bee queens.

Colony storage systems for honey bee queens consist of a bee colony that hosts individually caged queens. Queen storage allows the queen producer to rear mated queens and store them until required. The storage of mated queens is an important beekeeping technique, because the availability of surplus queens does not always coincide with demand. Excess queens are stored in queen banks for weeks to several months in the summer until required by the beekeeper. However, queen storage for 5-6 months under winter conditions has met with limited success.

Demand for mated queens is driven by beekeeping practices that require the majority of mated queens in early April. These queens are required for dividing colonies, requeening, and the production of packages and nucleus colonies. Locally produced mated queens are not available by early April in holarctic regions (i.e. northern temperate) such as Canada, because weather is unsuitable for rearing drones and for queen mating flights. A storage system to store locally produced queens from September to March would therefore be useful for Canadian management regimes. However, there are no queen wintering systems that have been consistently successful for different beekeepers and localities, other than those that overwinter one queen per colony.

The objective of this review is to examine systems that use the honey bee colony for winter storage of mated queens. Virgin queens are not suitable for heading a colony in early April, and therefore are not included. Queen storage in summer conditions is a commonly used technique, and is not discussed in this review, because it does not address the special problems of winter storage (Reid, 1975). Storage systems under laboratory conditions (outside of colonies), also have been excluded from this review because there is no report in the literature of such a system that will

store queens for an extended period of 5-6 months. In addition, colony storage systems are more economical than laboratory systems because standard beekeeping equipment is used and no additional buildings are required. A commercially viable storage system for overwintering queens must be economical, easy to manage, and suitable for long periods of storage, without the physiological deterioration of stored queens.

The most notable feature of studies on queen storage systems within colonies is the use of a broad range of colony parameters and management techniques (Table 1). Storage colonies have been tested with and without the colony queen, with excluder or wire screen queen cages, and in outdoor and indoor environments. However, research has not systematically focused on testing any one particular colony variable for queen survival. As a result, it is unclear what variables are important to queen survival in banks, and which variables are important to successful long term queen storage.

The most successful system of storing queens was a queenless colony that hosted queens for five months in wire screen cages (Levinsohn and Lensky, 1981). This experiment was carried out in Israel over three years, with 84% survival over the winter months. Colony maintenance included the addition of brood, bees, and syrup, and hives were painted black to absorb solar radiation and facilitate colony thermoregulation.

Queen survival (95%) also was successful in queen excluder cages where queens were held in one colony (Harp, 1969). Lower survival (72% and 21%) from a similar study indicates that this system is not reliable (Mitchell et al., 1985). Both queen excluder and wire screen cages resulted in less than 25% survival in another study after 6 months of storage; queen mortality was partially attributed to the movement of the colony cluster which resulted in the starvation and/or exposure of caged queens (Szabo, 1977b).

Table 1. Colony storage systems used for wintering a number of honey bee queens per colony.

AUTHOR	DESCRIPTION OF STORAGE SYSTEMS							
	% Queen Survival (months)	Queens ^a (# of colonies)	Bees ^c	Syrup ^d	Indoor ^e	Heat ^f	Cage ^g	Country
Walsh, 1967	100(3.5)	14(1) ^{7b}	+	+	+	+	W	N.Z.
Griffen, 1966	98(3)	96(?)	-	-	-	+	W	N.Z.
Harp, 1969	95(6)	42(1) ^b	-	+	-	-	E	U.S.A.
Harp, 1967	92(3)	18(7) ^b	+	+	-	-	E	U.S.A.
Walsh, 1967	86(3.5)	29(3)	+	+	+	+	W	N.Z.
Levinsohn & Lensky, 1981	84(5)	36(4)	+	+	-	-	W	Israel
Mitchell et al., 1985	72(6)	18(1) ^b	+	+	-	-	E	Canada

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	% Queen Survival (months)	Queens ^a (# of colonies)	Bees ^c	Syrup ^d	Indoor ^e	Heat ^f	Cage ^g	Country
Szabo, 1977	23(6)	16(15)	-	-	-	-	E	Canada
Dietz et al., 1983	20(4)	360(13)	-	+	+	+	W	U.S.A.
Szabo, 1977	20(6)	16(10)	-	-	-	-	W	Canada
Szabo, 1977	11(6)	16(8)	-	-	-	-	E	Canada
Szabo, 1977	2(6)	16(6)	-	-	-	-	E	Canada
Mitchell et al., 1985	20(6)	48(1)	+	+	-	-	E	Canada

- a. Mean number of queens stored per colony
b. The colony queen was left in the storage colony, either caged or free-running below the storage unit.
c. "+ bees" -workers or frames of capped brood added.
d. "+ syrup" -syrup or frames with honey added.
e. "+ indoor" -colony bank was kept indoors.
f. "+ heat" -colony bank was heated by an external heat source.
g. W -Wire screen cage which allows partial queen-worker contact.
E.-Excluder queen cage which allows complete queen-worker contact.

Winter survival largely depends on an adequate food supply that is accessible to the bees, and the ability of the colony to thermoregulate under winter conditions. These factors are of particular importance when a colony is used for queens storage. Thermoregulation involves the reduction of space that the bees occupy in the hive by cluster formation. This reduction of space by cluster formation is of particular importance, and potentially detrimental, to banked queens, especially if these queens are located outside the cluster. In addition, inadequate provisions for thermoregulation requirements would cause colony starvation during the winter.

Queen storage systems over shorter periods, for 3-4 months, were successful (86% survival) in some studies (Griffen, 1966; Harp, 1969; Walsh, 1967;) and unsuccessful (20% survival) in another (Dietz et al., 1983). Success was attributed to keeping colonies indoors (Walsh, 1967), providing heat (Griffen, 1966; Walsh, 1967), feeding colonies (Griffen, 1966), positioning queens in the cluster, and placing brood frames adjacent to the caged queens (Harp, 1969). Failure was attributed to the shortage of bees in storage colonies, and the inability of workers to maintain contact with the queens after the bees clustered (Dietz et al., 1983).

Several methods for provisioning and preparing colonies for winter storage are reported in the literature. Storage colonies are often provisioned with brood (Harp, 1967; Mitchell et al., 1985; Walsh, 1967), and brood or bees (Levinsohn and Lensky, 1981) during storage. The addition of syrup or frames with honey also is a common feature of colony maintenance (Dietz et al., 1983; Harp, 1967; Harp, 1969; Levinsohn and Lensky, 1981; Mitchell et al., 1985; Walsh, 1967), although colonies were not fed or manipulated in another study (Szabo, 1977b).

The presence of the colony queen, the cage type, and whether the colony is heated, are factors that also differ between systems. The colony queen was removed in the majority of systems, but the queen was caged with the banked queens in one study (Mitchell et al., 1985). In another study, the two colony queens were either

caged or one was caged, and the second remained as a free running queen below the storage unit separated by a queen excluder (Harp, 1967). The type of queen cage used was either excluder cages (Harp, 1967; Harp, 1969; Mitchell et al., 1985; Szabo, 1977b) and/or wire screen cages (Dietz et al., 1983; Griffen, 1966; Levinsohn and Lensky, 1981; Szabo, 1977b; Walsh, 1967). Colonies were kept indoors and heated (Dietz et al., 1983; Walsh, 1969) or kept outdoors and heated (Dietz et al., 1983; Griffen, 1966). Thus a variety of arrangements and management techniques can produce successful short-term storage.

Prevention of queen abandonment by workers is necessary to ensure that queens are tended throughout the winter. Queens are dependent on the workers of a colony for their survival. When the volume of space occupied by workers decreases during cluster formation, there is the potential of the cluster moving away from the queens. Worker volume is affected by the quantity of workers and is an important storage parameter for successful queen storage, yet the precise quantity of bees necessary for winter storage has not been stated explicitly (Dietz et al., 1983; Griffen, 1966; Harp, 1967; Walsh, 1967).

Ultimately, queens are dependent on the colony for their survival. Failures in storing queens for a long term may depend largely on the successful wintering of the colony itself. Successful wintering is related to colony location, size of population, honey and pollen stores, ventilation, guard against predation, use of insulation, and hardy stock (Johansson and Johansson, 1969). Worker mobility and access to stores are determined in winter storage systems by: (1) worker population, (2) colony thermoregulation, and (3) food availability. Other factors that may influence the success or failure of a storage system are: (4) type of queen cage, (5) status of colony queen, and (6) introduction techniques.

(1) Worker Population

About 5 kg, or approximately 39,000 individual bees (calculated from data in Otis, 1982) are required for wintering a colony (Farrar, 1968; Furgala, 1975; Johansson and Johansson, 1969). It follows that a colony used for queen storage over the winter also should have a minimum of 5 kg of bees (Szabo, 1977b). These bees are produced by one productive queen, or by two queens that produce brood within their independent brood nests (two-queen management, see Farrar, 1953; Farrar, 1968). Two-queen management produces a bee population that is about twice as large as in a colony headed by one queen. The larger number of workers cover a greater comb area, fill a larger volume of the hive, and create a larger volume of thermoregulated environment within the winter cluster. This warm environment permits workers to move around the hive and access pollen and honey stores to feed themselves and the stored queens.

Colony size is particularly important in queenless colony banks because no additional workers are produced by the colony. Worker population in colony banks varied from 5 kg bees (Szabo, 1977b) to 3-4 kg (Dietz et al., 1983) using standard management techniques (Farrar, 1968; Furgala, 1975). Two queen management, started in July, provided additional bees with the resultant population reaching its peak by September (Harp, 1967). Normal winter loss of workers in successful storage systems tested (>50% survival for 3-6 months) was offset by the addition of bees or frames of sealed brood every 10-14 days (Harp, 1967; Levinsohn and Lensky, 1981), or when needed (Walsh, 1967).

(2) Thermoregulation

Bees cluster together in winter to generate heat and prevent the loss of heat from the colony cluster (Farrar, 1968; Winston, 1987). Warmth within the cluster

provides sufficient heat for worker mobility, some brood rearing, food ingestion (Szabo, 1985), and queen survival. The cluster decreases in size as temperatures fall to maintain a core temperature higher than the outer areas. This results in a decrease in worker volume and an increase in queen mortality because caged queens are fixed in one location and are potentially left outside the cluster. Management techniques are therefore necessary to prevent or reduce cluster formation. Cluster formation and the resulting mortality of queens in colony banks can be prevented by assisting with thermoregulation through the placement of colonies in groups, insulating colonies, providing external heat, and/or painting the hives black.

Four colonies moved closely together into a group and insulated as one unit reduces cluster formation and bees are able to go from wall to wall and obtain stores (Peer, 1978). Packing four colonies together with insulation reduces the effect of low ambient temperatures (Szabo, 1985) when compared to single colonies (Owens, 1971). Workers do not cluster tightly in insulated colonies but remain as a larger volume within the hive, potentially covering the area of stored queens.

Cluster formation also was decreased by providing external heat to colonies in the form of light bulbs (Walsh, 1967), and a thermostatic heating unit (Dietz et al., 1983). Colonies also were placed indoors with a 500 watt heated electric tube placed below the floor of the building, (Griffen, 1966). Hives were painted black in a semi-tropical climate to assist with colony thermoregulation (Levinsohn and Lensky, 1981). Measures to protect the colony against winter temperatures were not described in some studies (Harp, 1967; Harp, 1969; Mitchell et al. 1985; Szabo, 1977b).

(3) Food availability

Sufficient stores of pollen and honey are required for the entire winter storage period of about six months. Recommended stores are 30-40 kg of honey (Furgala,

1975; Johansson and Johansson, 1969; Moeller, 1978) and 3200 cm² of pollen (Furgala, 1975). For Saskatchewan, Canada, recommended provisions are 50 kg in three hive bodies, and five combs of pollen (Szabo, 1974c). Colony stores need to be plentiful at the start of winter when storing queens, because it is difficult to feed colonies in climates where winter ambient temperatures are below freezing,. Early fall feeding allows for the proper organization of food reserves and avoids the extra costs of emergency feeding (Furgala, 1977; Johansson and Johansson, 1969). In addition, colony manipulations under winter conditions may disturb colony homeostasis, and result in increased queen mortality.

Supplemental fall feeding fulfills two requirements: it provides (1) the chemotherapeutic agents against nosema disease and foulbrood, and (2) the recommended amount of winter stores (Furgala, 1975). The timing of fall feeding is particularly important; it cannot be done too early as all cells will be full of summer honey. On the other hand, feeding should be early enough in the fall season so that bees can process and store the syrup.

Colonies mostly were fed at queen introduction in previous studies, and as late into the fall as possible for workers to be able to store the syrup into empty brood and honey cells present in the center of the colony. Provisions in the center of the hive, close to the banked queens, assist bees in obtaining provisions to feed the queens. Sugar syrup with fumagillin was fed at the time of queen introduction (Mitchell et al., 1985), and after introduction, when all brood had emerged from queenless colonies (Harp, 1967; Szabo, 1977b), or frames with stored syrup or honey were placed within the storage colony (Harp, 1969). Colonies kept indoors and heated were fed syrup throughout the winter (Dietz et al., 1983; Walsh, 1967). In Israel, syrup was fed to colonies during the winter months without providing heat. However, colonies were black to absorb sunlight and radiant heat (Levinsohn and Lensky, 1981). Thus, timing of feeding does not appear critical as long as colony

stores are available to workers at all times during storage, and food stores therefore are available to banked queens.

(4) Cage type

Worker-queen contact is necessary for survival of caged queens. The extent of this contact depends on the wire apertures of the queen cage. Two cage types have been used to store queens in colonies; wire mesh allows some worker-queen contact depending on mesh size, and queen excluder material allows full access by workers to caged queens. Small apertures, less than 2 mm, caused greater queen mortality than apertures larger than 2.5 mm, because smaller apertures restrict antennae contact, and workers will not feed queens through these smaller apertures (Free and Butler, 1958). Certain mesh sizes (1.2, 1.5 and 2.5 mm) may not result in queen mortality but do result in greater than 60% injured queens (Woyke, 1988). When the white arolium or foot pads of the queen were injured the pads were no longer white. It is suggested that workers of the colony damage queens in a colony bank. Wire screen with 2.5 mm mesh was recommended to prevent queen injury and mortality (Free and Butler, 1958; Jay, 1965). However 2.5 mm mesh may not be the optimal size; both success (Levinsohn and Lensky, 1981) and failure (Dietz et al., 1983; Szabo, 1977b; Woyke, 1988) resulted from storing queens in 3-3.5 mm mesh size cages.

Queen excluder material allows full access by workers to stored queens and also has resulted in both success and failure. Queen survival was greater than 90% in two studies (Harp, 1967; Harp, 1969), 71% in another (Mitchell et al., 1985), and less than 25% survival in two others (Mitchell et al., 1985; Szabo, 1977b). It is difficult to assess whether cage type was a factor in queen mortality because different methods were used in nearly every experiment published. Thus, although apertures of less than 2 mm cause queen mortality, it is not clear whether larger screen apertures or queen excluder material enhance queen storage.

(5) Presence of a Colony Queen.

The colony queen is central to the colony, and, together with the workers, form a cohesive working unit. Workers of a colony form a cluster during the winter which provides warmth for the queen and her brood. When a free-running colony queen is kept within the storage colony, but below a queen excluder, the colony is said to be queenright (Harp, 1967). Queenless units are storage systems without a free-running colony queen. In some studies the colony queen is caged and placed with the caged queens to be stored through the winter (Harp, 1967; Harp, 1969). Both queenright (Harp, 1967) and queenless storage units (Levinsohn and Lensky, 1981; Walsh, 1967) were successful in storing queens for periods of 3 and 5 months respectively. The advantage of a free-running colony queen in addition to the banked queens is that new workers continue to be produced by the colony queen. However, the presence of a caged or free-running colony queen may cause workers to be aggressive towards the newly banked queens (Szabo, 1977a). Other than the continuous production of brood by the colony queen, it remains uncertain whether a colony queen, in addition to banked queens, is a necessary requirement for colony banks used for winter storage.

(6) Introduction of Banked Queens

Successful queen introduction is the process of acceptance of a new queen by the workers of a colony. Colony queenlessness for a period of several days and the feeding of colonies prior to and during queen introduction are two common methods used to improve the acceptance of a new queen by workers of a colony (Laidlaw, 1981). Similar techniques are used to introduce one queen or several queens to a colony. Introducing more than one queen to a colony can be done by introducing queens simultaneously to a queenless colony (Johansson and Johansson, 1971b).

Failure to successfully introduce queens in the late summer or early fall is not caused by the lack of provisions or worker population in a storage colony, because colonies are fed ad lib. and the worker population is at its peak. It is known that failure of workers to accept a queen(s) results in queen mortality due to worker-queen aggression. Failure occurs if the newly introduced queen is different in age and / or physiology to the colony queen (Szabo, 1974b). Acceptance of a new queen by colony workers is higher if both queens are physiologically similar in egg laying capabilities or are both newly mated. Queen acceptance may depend on the amount of worker-queen contact at introduction which is determined by the size of the wire mesh of the queen cages.

Techniques used to decrease the aggressive behavior between workers and caged queens in storage banks have varied between studies. All queens were accepted 24 hours after introduction when the colony queen was caged for 20 days compared to 93% and 59% survival when the colony queen was caged for 9 days (Szabo, 1977b). Gorging the colony with honey immediately before queen introduction resulted in 95% survival (Harp, 1969). The honey gorging technique was used in two other studies, but results after introduction were not reported (Harp, 1967; Mitchell et al., 1985;). Spraying colonies with water and smearing queen cages with honey were used to introduce caged queens to a colony, but survival at introduction was not noted (Griffen, 1966). Therefore, reliability of these introduction techniques have not been demonstrated. No introduction techniques were described in other queen overwintering trials (Dietz et al., 1983; Levinsohn and Lensky, 1981; Walsh, 1967).

Summary and Conclusion

There is no one colony storage system that has been demonstrated as reliable, and that has successfully hosted queens in a northern climate for longer than 3.5

months. Five months storage and 84% survival was reported from Israel, a country with a much warmer winter climate than in northern regions. Six months storage and 95% survival was reported from the United States but good results were reported from only one colony (Harp, 1969). There are two systems that successfully stored queens for 3.5 months (86% survival; Walsh, 1967) and 3 months (92% survival; Harp, 1967) in New Zealand and the U.S. respectively. These results are more reliable as more than one colony was used in these studies. However, these two systems are not suitable for storing queens through the winter because winter conditions in holarctic regions prevail for at least 6 months. Perhaps the milder climate favours queen storage because the wintering period is less stressful to the colony.

Factors important for queen survival are those factors that influence worker-queen contact. Worker-queen contact and eventual survival of banked queens may depend on the presence of brood and young bees, the absence of worker-queen aggression, and cage type. However, conditions for maximal contact by using queen excluder material have been both successful and unsuccessful. Wire screen cages perhaps give the best trade-off between worker-queen contact and queen protection from aggression. Wire screen cages provide sufficient worker-queen contact for feeding, but restrict aggressive behavior between worker and queen.

Factors important for successful long term storage in colonies in addition to queen survival are related to the preparation and management of the colony to survive winter conditions. Ultimately, optimal colony conditions conducive to worker-queen contact are required for queen survival because caged queens are located in a fixed position within the colony and depend on queen-worker contact for their survival.

Colony maintenance of homeostasis and eventual survival are dependent on (1) the availability of food to workers and queen(s), and (2) sufficient number of

workers to thermoregulate the colony. Therefore, colonies used for queen storage must be fed before cooler winter weather, to allow workers to store syrup. Similarly, colonies must be prepared with a worker population that can attend the banked queens. Finally, colonies are more likely to survive with an external heat source or insulation packed around the colonies to assist with the thermoregulation of the colony and reduce cluster formation.

It is clear that colonies are able to survive the winter with present management techniques, but reliable management techniques have not been developed for mass queen storage over the winter in northern regions. However, systems used both successfully and unsuccessfully point to areas that require further research before a successful system of queen storage is available. Studies reported in the literature are on the whole not comparable because different methods are used, and, to make comparisons even more difficult, details in the materials and methods are not entirely clear. We recommend that parameters noted in Table 1, plus details on insulation, be included in every report.

Further research is required in all areas of colony banking. Our research examined colony parameters because banked queens depend on the workers of a colony for their survival. We investigated (1) the different size of colony banks for the most successful and economical system; (2) queenless vs. queenright colony banks; (3) wire screen vs. excluder type cages; (4) methods of queen introduction; and (5) the importance of young workers.

2.2 Overview of Research

The aim of this study was to identify the most economical and biologically successful system to store queens en mass, through the winter. There is no overwintering system that consistently stores multiple queens for six months without considerable queen loss (Reid, 1975). Queen producers need a storage system that requires the minimum amount of equipment, can fit into their present management scheme, is economically viable, and can store more than one queen over the winter. In this study, we tested queen survival and subsequent quality in various mass storage systems and examined the economic feasibility of the more successful systems.

Winter storage allows queens that are produced during the previous summer to be available in early April. Locally produced, mated queens are not available in Canada and other northern regions at that time, because weather is unsuitable for rearing drones and queen mating flights. As a result, large numbers of mated queens have been imported every spring into Canada, either sold in packages or as individually caged queens. Queens were imported into Canada from the U.S. before the border was closed to importations in 1987 due to the threat of tracheal and varroa mites. This border closure has dictated that queens be imported from New Zealand and Australia, or produced in Canada during the summer. The annual importation of honey bee queens, however, results in a substantial revenue loss to Canada (Winston, 1983). Traditionally, queens produced during the summer are used for summer requeening, because currently these can not be stored over the winter. Requeening is usually completed during the spring because summer requeening is difficult and time consuming. If Canadian beekeepers produced summer-mated queens, stored them over the winter, and made them available in

early spring, Canadian self-sufficiency in queen production could be achieved (McCutcheon, 1983).

Laboratory storage systems in which the queen is isolated from the colony situation were excluded from this study, because there is no report in the literature of a system that will store queens for a period of 5-6 months (a typical Canadian winter) without extensive labour and specialized equipment. Colony storage systems in which the queen(s) are hosted by a colony were examined because these systems have advantages over laboratory systems. For instance, workers attending do not require replacement, whereas manual replacement of all workers is a necessary routine for laboratory systems on a bi-weekly basis (Edwards and Poole, 1971), periodically (Foti, 1957, 1958, 1962; Khidesheli, 1963), or every 1 1/2 -2 months (Avetisyan and Kotova, 1963). In addition, food for caged queens in colony banks is obtained by the workers from within the colony, whereas the feeding unit of a laboratory bank requires frequent replenishment with fresh syrup and pollen (Gary, 1966). Finally, laboratory systems dictate the additional capital expense of a building with heat and humidity controls.

Queen banks with excluder cages were tested in all three years of this study in queenless colony banks. Wire screen cages also were tested in the second and third years. Only queenless colony banks, with both cage types, were overwintered in the final year of the study. Queenright indoor colony banks were tested in the first year of the study, whereas queenless indoor colony banks were tested in the second year. In addition, an overwintering system was tested that incorporated both the colony bank and the mini-nuclei in the latter two years of the study. In all three years, survival in colony banks was compared to the survival of individual queens in 5-frame nuclei.

The majority of experiments were performed on colonies kept outdoors, because standard techniques exist for the outdoor wintering of colonies, and the

added expense of heated buildings is not required, as it is for colonies housed indoors. Colony storage banks were tested with both the minimum of winter packing for coastal British Columbia, and with winter packing as prescribed for colder interior climates. Colony survival is improved in outdoor overwintering in areas with severe winters when beekeepers insulate colonies and place them in groups of four (Szabo, 1974c; Peer, 1978).

Winter survival of colonies largely depends on colony condition prior to wintering, which includes the winter preparation with insulation (Farrar, 1968; Furgala, 1975; Johansson and Johansson, 1969). Our experimental colonies were prepared with adequate food and worker population to optimize the wintering conditions of colony banks.

Mass queen storage in colonies was compared to colonies that stored one queen with her workers, the common wintering method. The smallest wintering unit was chosen as the control treatment because hive equipment is a large cost in a beekeeping operation. Nucleus colonies can be established during the summer and wintered (Johansson and Johansson, 1970), and may consist of various sizes of hives (Gooderman, 1945; Telschow, 1962; Waller, 1977), although small units limit the success of the overwintered unit in cold climates such as in Alberta (Szabo, 1987). Five-frame nucleus colonies moved together in an eight or ten-pack were used in this study, because these have been successfully overwintered in British Columbia (Barten, personal communication; Tegart, personal communication).

Mini-nuclei were used as one of the queen banking systems because the mini-nuclei reduce queen mortality during the last two months of storage in colony banks. Queens were transferred from colony banks to mini-nuclei, in which queens were able to lay eggs and produce brood during the last two months of storage. The moderate winter climate of the study area in British Columbia permits the assembling of mini-nucleus colonies.

Colony storage systems with and without the colony queen were tested in this study because it is unclear whether the presence or absence of a colony queen promotes success in queen storage. The presence of a free-running colony queen in the storage unit is advantageous because brood is produced on a continuous basis. Furthermore, young workers replace older workers, and in this way maintain the colony population. However, the presence of a colony queen in a storage unit may interfere with the maintenance of banked queens by the colony's workers (Szabo, 1977a).

In this study, 2.5 mm mesh screen and excluder material were compared to identify the most successful queen holding cage, and to investigate whether the traditional screen mailing cages that are commercially available are as successful as excluder cages that require local manufacturing. The screen size determines the level of worker-queen accessibility. Although the minimum recommended mesh size is 2.5 mm (Free and Butler, 1958; Jay, 1965), both success and failure have been reported using larger mesh sizes. Excluder material enables workers to enter the queen cage, and feed and groom these stored queens. However, excluder material also permits aggressive worker-queen behavior that may result in queen mortality. The 2.5 mm wire screen decreases worker accessibility and thereby decreases the aggressive behavior that may lead to queen mortality.

2.3 Materials and Methods

About 500 mated honey bee queens (*Apis mellifera* L.) per year were introduced into various storage systems in mid-September of 1987, 1988, and 1989. Queens were reared from one queen mother in 1987 and 1988 by Southern Interior Apiaries, Keremeos, B.C., and from three queen mothers in 1989 by Babe's Honey, Victoria, British Columbia. These queens were removed from nucleus colonies and transported to Simon Fraser University either in queen cages with attendants (1987) or without attendants, and held in bulk bee boxes (1988 and 1989). Caged queens were temporarily stored for two days in 1987 and 1989 and eight days in 1988, prior to introduction into winter storage systems.

Both outdoor and indoor storage systems were tested during the course of this project (Table 2). Storage systems varied in size from mini-nucleus colonies to five, ten, and twenty frame standard colonies. In all cases except the mini-nuclei, frames were standard Langstroth size, (rectangle dimensions are 23 x 45 cm); the mini-nuclei frames measured 16 x 19.7 cm. Colony preparations for wintering included supplementary sugar feeding. In all cases terramycin (5ml of Terramycin Animal Formula-25 per 4.5 L) and fumagillin (100 mg /4.5 L) were added to the sugar syrup (2 parts sugar, 1 part water), to prevent American and European foulbrood, and nosema disease (see Production Data page 113). No evidence of American foulbrood, European foulbrood, nosema disease, nor *Acarapis woodi* disease was found in colonies used in this study. The number of queens hosted in one colony varied from single queens in the mini-units and the five-frame control units, to multiple queens (24-48) in ten and twenty frame hive bodies. Multiple queens were held individually in traditional wood shipping cages (wire screen cages), or in compartments covered by queen excluder material. Queens in wire screen cages were left in the same colony for six months, or else transferred monthly or bi-monthly to another colony. Colony entrances consisted of a small upper entrance and a colony-width lower entrance,

because small lower hive entrances are detrimental to the thermoregulation of overwintered colonies (Allen, 1964; Szabo, 1985). Queen survivorship was used as the indicator of a successful winter storage system. Survival of test queens was checked about every month throughout the winter period until March. In March, a sample of test queens was removed from the different storage systems and evaluated for quality and performance (Section 3).

Table 2: Outdoor and indoor colony queen systems tested in 1987-1990

STORAGE SYSTEM	1987-88	1988-89	1989-90
<u>Outdoor</u>			
5-frame Nucleus Colonies	5-frame ¹	5-frame ¹	5-frame ¹
Colony bank	Queenless 10-frame unit over a 20- frame queen-right colony ³	Queenless 20 frame unit ^{2, 3}	Queenless 20 frame unit ^{2, 3}
Mini- Nucleus colonies	-	Four hive bodies stacked condominium style ⁴	Two hive bodies stacked over a support colony ⁴
<u>Indoor</u>	Queen-right ten-frame colony ³	Queenless ten-frame colony ³	-
1. Single queen per unit 2. Wire screen cages 3. Excluder type cage 4. Queens kept in colony banks in wire screen cages until January, then transferred to single queen mini-nuclei.			

2.3.1 Outdoor storage systems

Outdoor storage systems included the large (1) colony bank, the smaller (2) 5-frame nuclei, and the (3) mini-nuclei.

1. Colony bank

The colony queen bank held outdoors consisted of a queenless colony with banked queens held in compartments placed in the center of the hive body. Two variations of the colony queen bank were used, and two variations of compartments were tested. In 1987 the colony queen bank consisted of an eight frame unit with the banked queens placed between the eight frames; this unit was located over a twenty frame, queenright colony (Fig. 1). This queen bank was separated from the colony with a double-screened, bee-proof board. In 1988 and 1989, the colony queen bank consisted of a queenless 18 frame colony with banked queens inserted into the center of the upper hive body (Fig. 2).

Queens were caged in either wire screen cages (Fig. 3; 1988, 1989), or wooden compartments attached to a frame (Fig. 4; 1987, 1988, 1990). Wire screen cages were either two (1989-90) or three-holed wooden cages (1988-89), without candy or attendants. The lacquered wire mesh screen (gaps of 2.5 mm) allowed workers to feed and attend the caged queens. Wooden compartments were constructed from laths to form a grid of 12 compartments. The framework measured 4 x 4 x 1.8 cm, and was set into comb drawn out from plastic foundation. Each compartment was covered by queen excluder material that allowed direct contact between a queen and workers; the workers could move through the excluders, but the larger queens could not.

(1). Treatments.

Treatments primarily varied with cage type, and secondarily with feeding schedule and whether young workers were introduced into the overwintering storage

unit. Colony treatments included the use of two cage types: (a) wire screen and (b) queen excluder .

(a) Wire screen cages.

Queen cages were held back to back in a wooden framework fixed within the center of a frame (Fig. 3). The remaining area of the frame, adjacent to the cages, consisted of comb filled with honey. This frame was held in the center of a 2-super queenless colony (Fig. 2a). The wire screen cage was used in four treatments:

- (i) 24 caged queens held in one colony ($n=4$) throughout the winter (1989-90).
- (ii) 48 caged queens held in one colony ($n=4$) throughout the winter (1989-90).

(iii) 24 caged queens temporarily held in one colony ($n=4$) and transferred to a newly dequeened colony every month (1988-89). The newly dequeened colony was examined one week after introducing test queens for queen cells, which were removed if found.

(iv) 24 caged queens were held in one colony ($n=4$) and transferred to a newly dequeened colony every two months (1989-90). Used storage colonies were subsequently requeened 24 hours after removal of the stored queens.

(b) Queen excluder cages

Queen excluder cages were used in five treatments. Twelve cages were located on each side of the bank frame (except in treatment 5), and were kept in one colony throughout the winter. In 1987-88 the queen bank frame was held in a queenless bank (1 super), that was placed over a 2 super queenright colony (Fig. 1).

Treatments consisted of:

- (i) The addition of one honey frame to the queen bank every two weeks ($n=5$).
- (ii) The addition of one honey frame to the queen bank every month ($n=5$).

(iii) The addition of one frame of brood and one frame of honey to the queen bank every two weeks ($n=4$).

In 1988 and 1989 the queen bank frame was held in a 2 super queenless bank (Fig.

2b). Treatments consisted of:

(i) No additional frames added to queen bank (1988-89, $n=8$; 1989-90, $n=4$).

(ii) Cube compartment (1988-89, $n=4$). Twenty-four queens were individually held in wooden queen excluder compartments constructed in a cube, and held in the center of three frames (Fig. 5). Six queens were held on each of four sides of the cube. The whole unit consisted of the cube plus part of three frames that were attached together as one unit. The cube of queens could be rotated so that all sides of the cube were observable when checking for queen survival.

(2). Colony preparation and maintenance.

Colonies were prepared in a similar manner each year. The 1989 dates are used to describe the timing of colony preparation. Preparation of colonies began at the beginning of the nectar flow on 6 July. A standard ten frame colony was placed on top of a 20-frame colony to make a two queen colony (Dietz, 1985; Farrar, 1953). The two colonies were kept separate by one hive body sandwiched between two queen excluders. Each two-queen colony was provided with additional hive bodies during the nectar flow for honey storage (placed between, and over the top of the two colonies). The total number of hive bodies of each two-queen colony was reduced to three immediately after the nectar flow and before test queens were introduced to the colony bank. In 1988-89 and 1989-90, each colony bank was further reduced to two hive bodies (lowest hive body removed) after queen introduction and before insulating the colonies in mid-October 1988 and 1989. In contrast, the two-queen colonies in 1987-88 were converted into a queenless 10-frame bank placed over the top of a 20-frame queen-right colony. First, the queen in the

upper super was removed (25 August) and a queen excluder was placed between the two units to prevent the lower colony queen from laying eggs in the upper hive body. Second, the queen excluder was replaced with a double screened bee-proof board on 21-22 September, physically isolating the upper queenless unit from the queen-right colony below. Third, three frames of brood with adhering bees, plus bees shaken from an additional 2 to 5 frames, were placed into the upper hive body to boost the worker population that covered about 6-8 frames.

Colonies were given additional provisions after the honey flow in late summer to ensure continuous brood and worker production. Pollen supplement (500 g) was given to all colonies on 24 August and 14 September, one below the lower excluder and one above the upper excluder. Each colony also was provided with an additional 10 L of syrup on 14 September, and on 4 and 27 October, 1989.

(3). Queen introduction

In 1987 the upper colony queen of the two-queen colony was removed in August, 24 hours prior to mass queen introduction, and the lower colony was left queenright. In 1988-89 and 1989-90, both queens from the two-queen colonies were removed 24 to 48 hours prior to mass queen introduction.

Immediately prior to mass queen introduction, queenless banks (1987-88) and queenless colonies (1988-89 and 1989-90), were gorged with 1.5 kg honey (twice), and were heavily smoked in 1987 and 1988, but only lightly smoked in 1989.

Frames of banked queens were inserted into the center of the queenless one hive-body queen bank (1987-88), the queenless introduction unit (1988-89), or the upper hive body of the queenless colony bank (1989-90). In 1988-89 queens initially were hosted in an introduction unit with younger workers. First, frames of brood were moved into the introduction unit (24 hours prior to queen introduction) to attract younger workers. Second, a solid board was placed below the upper super

immediately prior to queen introduction to confine the younger workers to the upper introduction unit. This solid board was replaced with newspaper 24 hours after queen introduction to enable workers of the introduction unit to unite with the remaining workers of the colony. Bee-proof wire mesh was tacked over queen excluder compartments in 1989 prior to queen introduction, and left in place for one month as a precautionary measure against worker-queen aggression.

Emergency queen cells produced during the 24 hour queenless period in the queenless colony banks were removed from the brood frames, to prevent the production of a new colony queen.

(4). Hive Insulation

Two methods of insulating colonies were used after queen introduction. In 1987, hives were wrapped individually with tar-paper, and a sheet of R12 styrofoam insulation (41.5 x 52 cm) was placed over the inner cover, underneath the lid. In 1988 and 1989, colonies were moved onto pallets in groups of four and insulated with polyethylene pillows filled with glass wool insulation (minimum R12) . One pillow (91.5cm x 1.83 m) was wrapped around the four hives, and two pillows (50 x 85 cm) were placed over the top of the four colonies on 25 October. Further, each of the four colonies was placed over a hive body filled with insulation. The insulated unit of four colonies also was covered with a sheet of plywood tied down with rubber bands (See section 4.6, page 114). Top feeders were removed (17 November) and replaced with inner covers with 6 cm top entrances. Small boards with 4 cm auger holes were placed over the insulation and nailed into position after alignment with the top entrance of each colony.

2. Five-frame nucleus colonies

Five-frame nucleus colonies were used as the control queen banking system (Fig. 6). In 1987, nucleus colonies ($n=17$) were prepared by adding 2 kg bees to five frames containing honey and pollen. In 1988-89 ($n=20$) and 1989-90 ($n=20$) nucleus colonies were started during the summer, and were prepared for the fall by equalizing brood (two frames), honey and pollen (three frames), and workers (covering four frames). In 1988-89, colonies were fed 3 kg of sugar syrup in pail feeders on 9 October, frames heavy with honey replaced empty frames on 25 January, and on 1 March frame feeders with syrup replaced one frame (1988-89). The following year (1989-90), nucleus colonies were prepared with four frames and a frame feeder. Colonies were fed on February 6 with 1.5 L of syrup and a 500 g pollen supplement patty during the winter. Test queens were caged in the nucleus colonies for two days (1987,1988) and seven days (1989) before release into each colony. Ten or 20 nucleus colonies were placed side by side and back to back to form one insulated unit in order to conserve heat. Two types of hives were used, styrofoam (7lbs/ft³) nuclei (Tegart, personal communication) in 1987 and wooden nuclei wrapped with a pillow of glass wool in 1988 and 1989. Additional insulation was placed over (fiberglass) and underneath (styrofoam) each wooden nucleus.

Each nucleus colony was fed 2 L of syrup one week prior to, and twice after, queen introduction. For 1989, feeding dates were 8 September, and 3 and 18 October. In 1990, colonies were provided with an additional 1.5 L of syrup and 500 g pollen supplement on 6 February.

3. Mini-nucleus colonies

A mini-nucleus colony (22.9 x 22.9 x 16.8 cm deep) consisted of four frames and a frame feeder (volume = 0.5 L); four mini-nuclei colonies fit into one hive body. Four hive bodies were stacked on top of each other without the support colony below in 1989, whereas two hive bodies of mini-nuclei colonies were stacked over the top of a support colony in 1990 to assist with thermoregulation (Fig. 7). In both 1988-89 and 1989-90 16 test queens were introduced into the mini-system during January after being stored from September to January in the colony bank. Each mini-nucleus was prepared in late January (1988-89) from pieces of comb filled with honey and pollen (two frames), and honey (two frames) and 0.2 kg of bees. The following year (1989-90), mini-frames of brood, honey and pollen were obtained by dismantling colonies that consisted of 40 mini-frames that were set up early in the spring.

Mini-nuclei colonies were left queenless and with a closed entrance for 24 hours before test queens were introduced (20 January, 1989; 1 February, 1990). Queens were introduced by using a wire screen cage centered in the colony, and manually released three (1989) or four (1990) days after introduction. Colonies were stored inside a heated building (20°C) for two days to improve queen acceptance, and to enable workers to store the syrup in comb prior to being moved outdoors. Colonies were placed outdoors on 23 January 1989 when each queen was released from its cage.

Each stack of mini-nuclei colonies (plus the support colony in 1990) was insulated with R15 styrofoam. An additional 0.5 L of warm sugar syrup and 0.7 kg pollen supplement was provided to each nucleus colony on 30 January 1989. No additional food was provided in 1990 as frames contained sufficient stores of pollen and honey.

Figure 1. Outdoor one-super queen bank located over a two super queenright colony, with colony entrances on opposite sides of the hive. The bank is separated from the colony by a double screened board. The modified queen holding frame is located in the center of the upper bank.

OUTDOOR QUEEN BANK

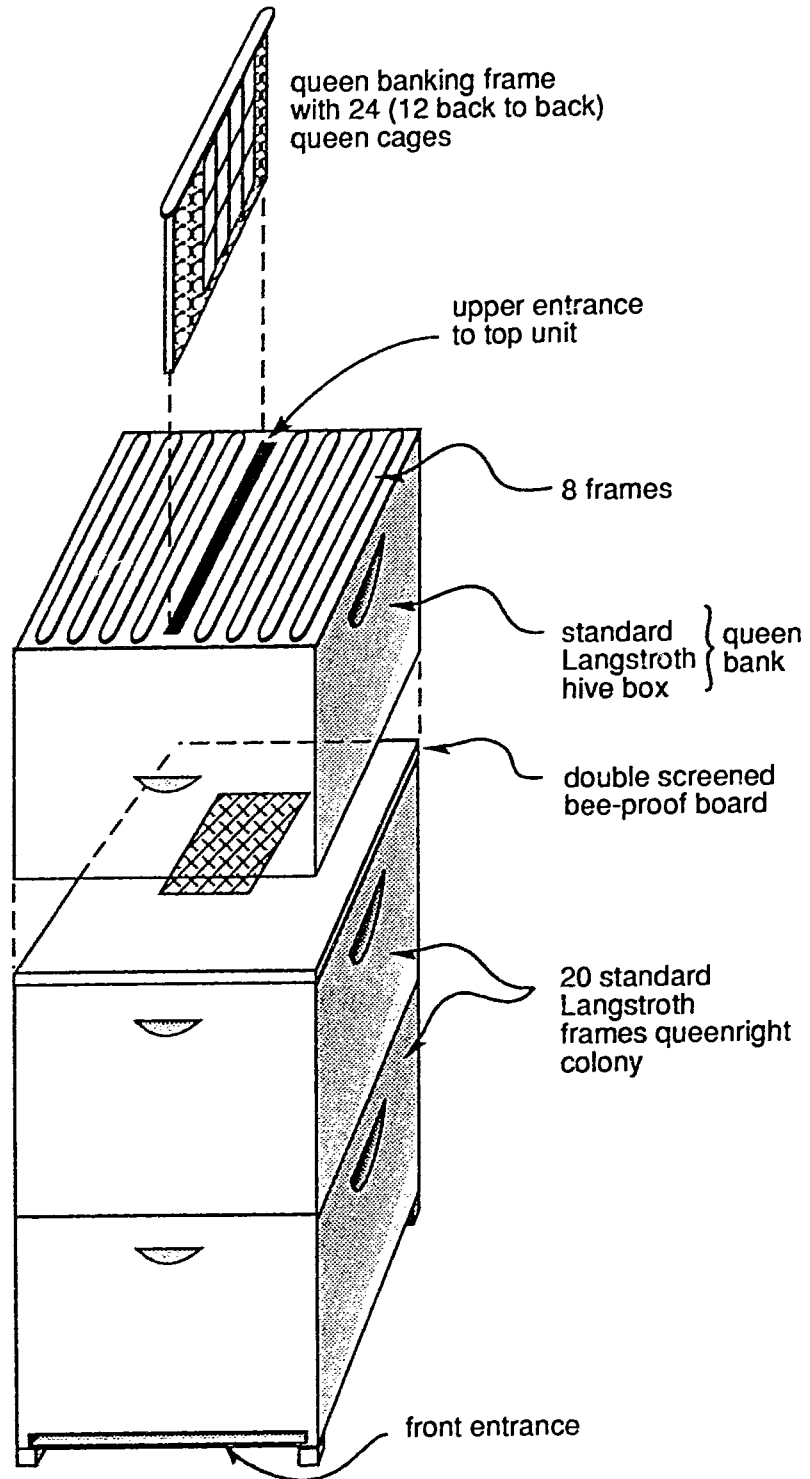


Figure 2. Outdoor two-super queen bank, with the queen banking frame placed in the center of the upper super. Each compartment contains one queen, and is covered with queen excluder material allowing workers to enter compartments. Twelve compartments are located on each side of the frame.

OUTDOOR TWO SUPER QUEEN BANK

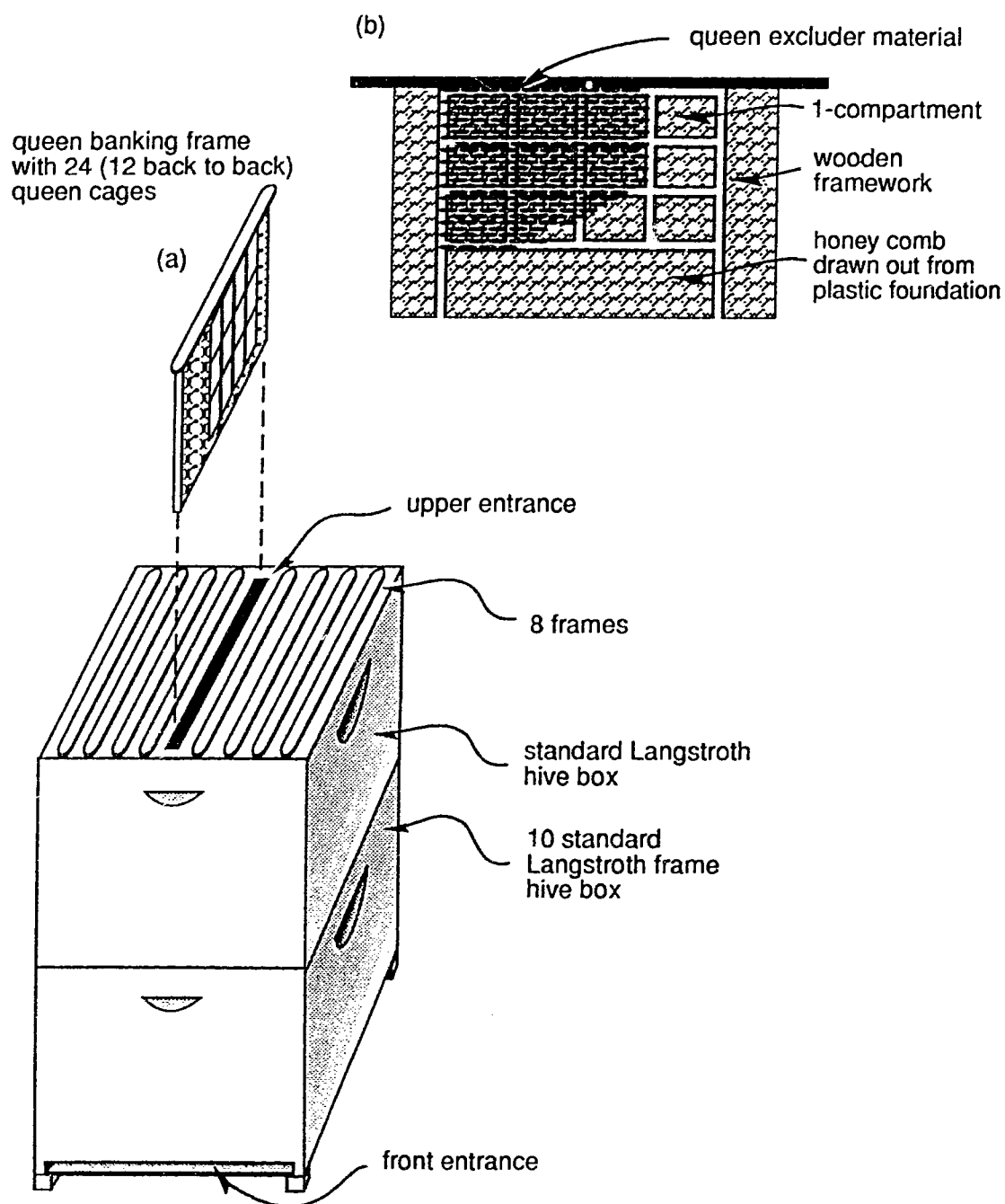


Figure 3. Modified frame for holding 24 wooden cages (12 on each side) with screen on one side. Four cages are held back to back in each of three trays. Each cage contains one queen with bee-proof screen allowing partial queen-worker contact.

FRAME FOR HOLDING 24 QUEEN CAGES

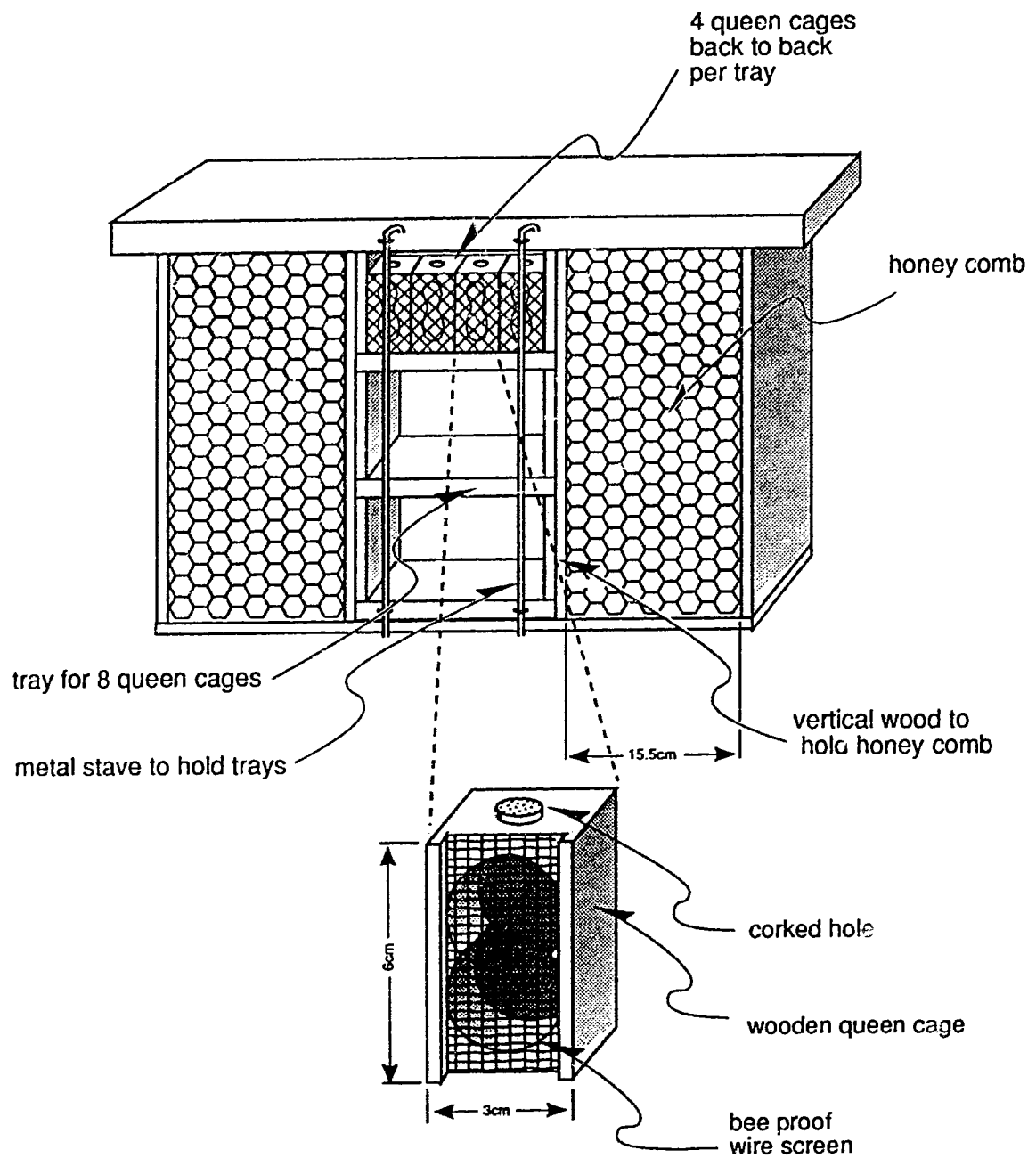


Figure 4. Modified frame with twelve partitions covered with excluder material, that are inserted into the honey comb drawn out of plastic foundation. The frame holds twelve compartments on both sides of the frame.

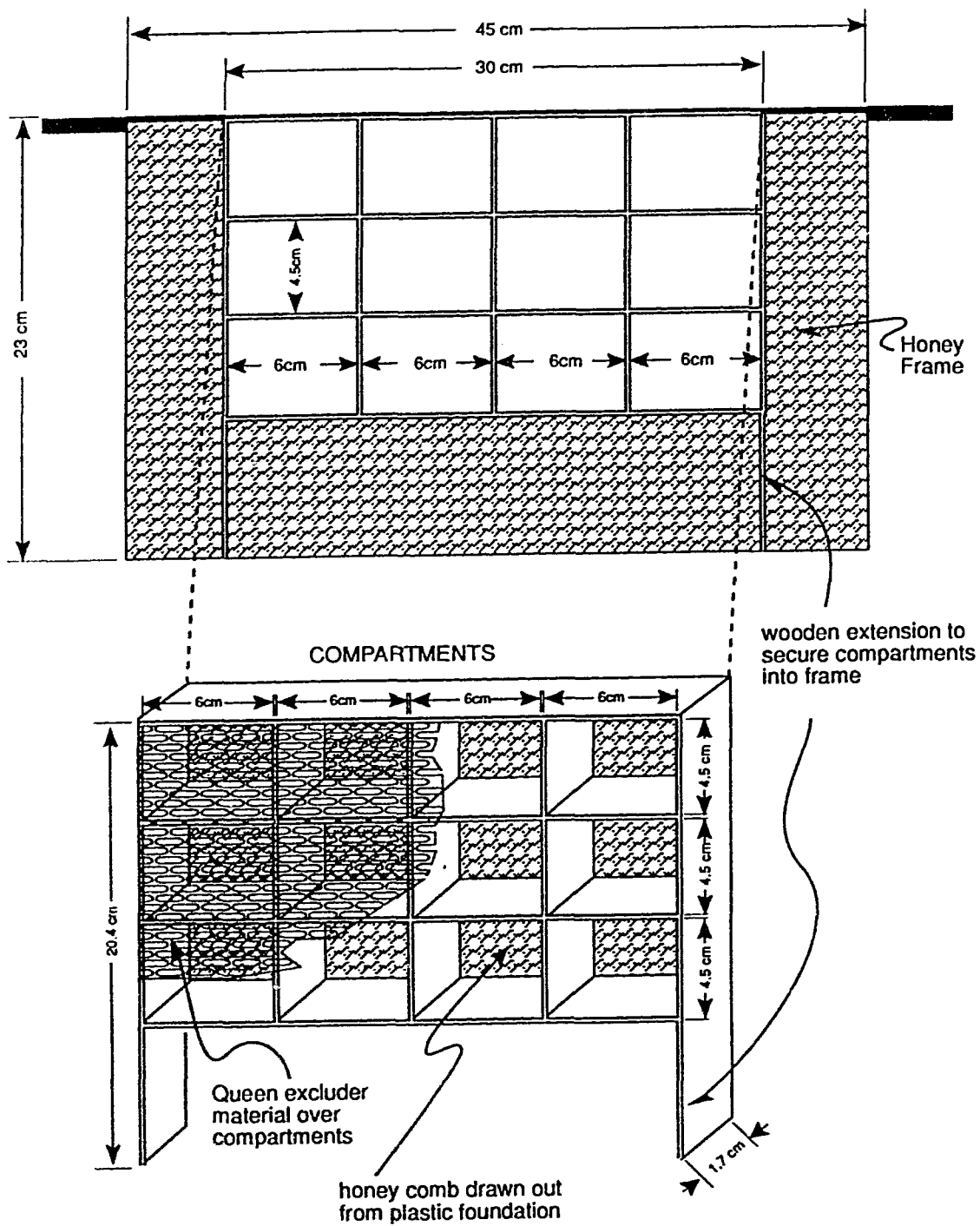


Figure 5. Cube compartment with six excluder cages on each side of the cube. The cube is held in the center of three frames. Each compartment contains one queen and is covered with queen excluder.

CUBE COMPARTMENT

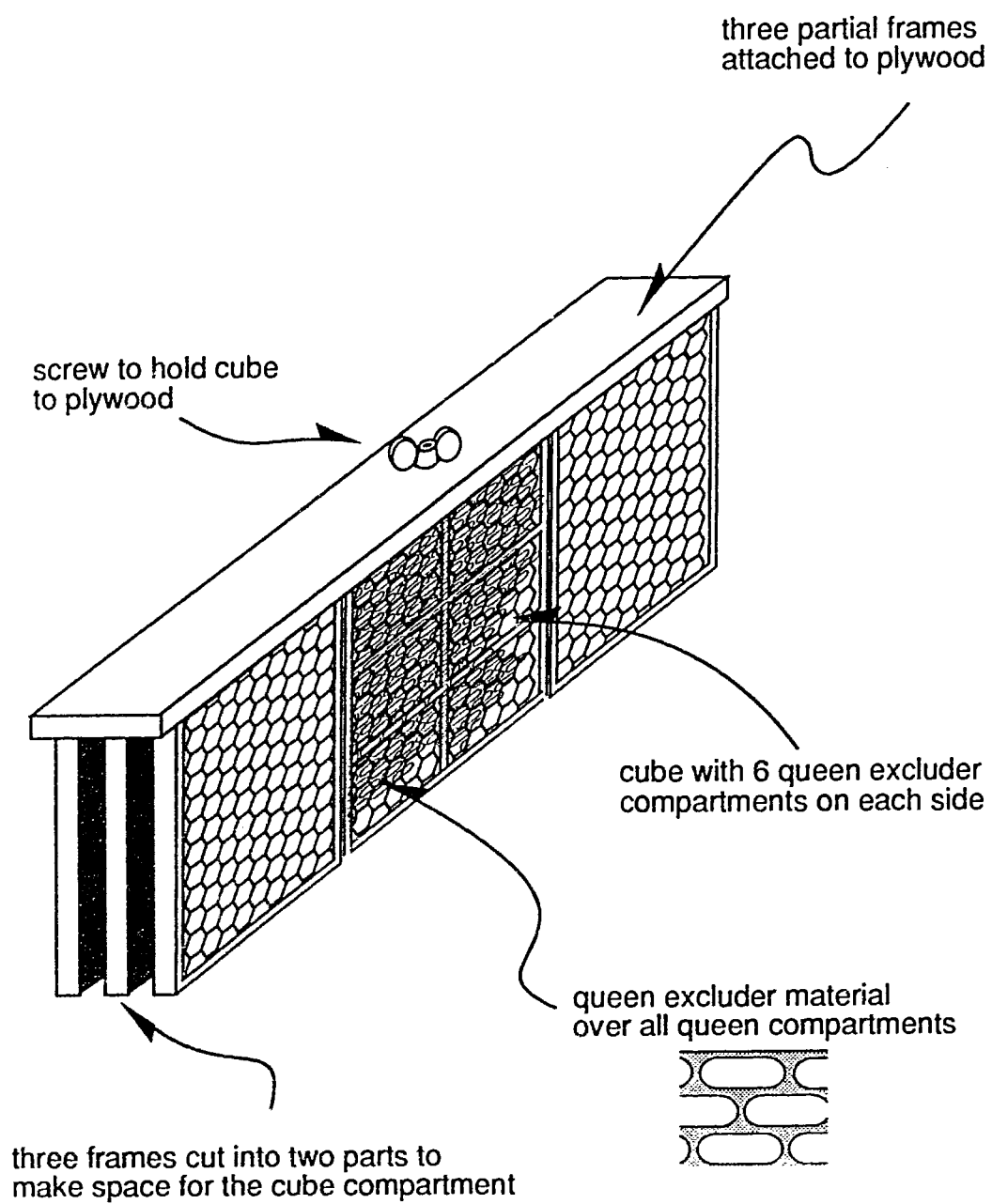


Figure 6. Five-Frame nucleus colony shown here with four frames and one frame-feeder. The hive lid is not drawn, but covers the whole unit.

5 - FRAME NUCLEUS COLONY

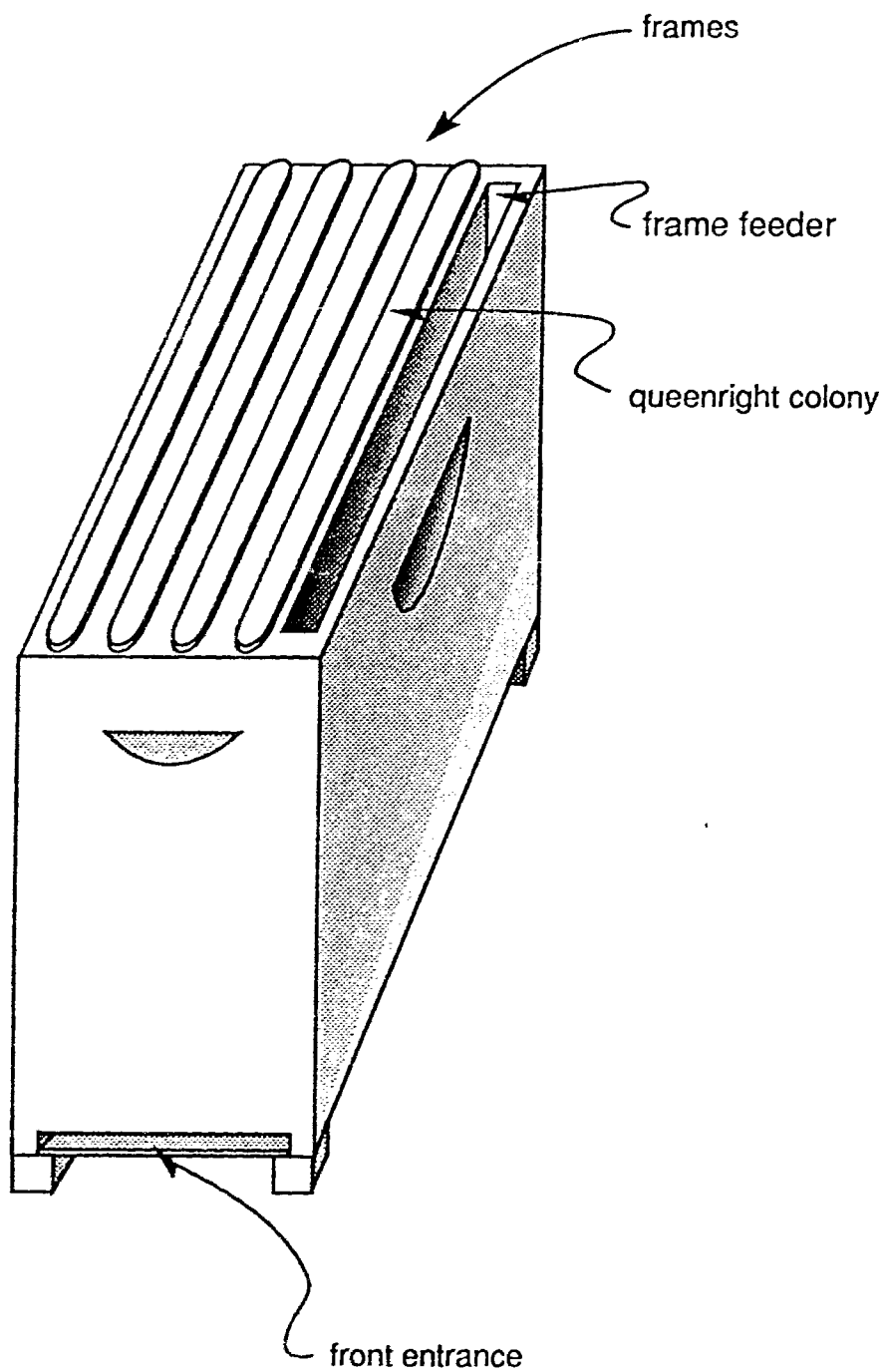
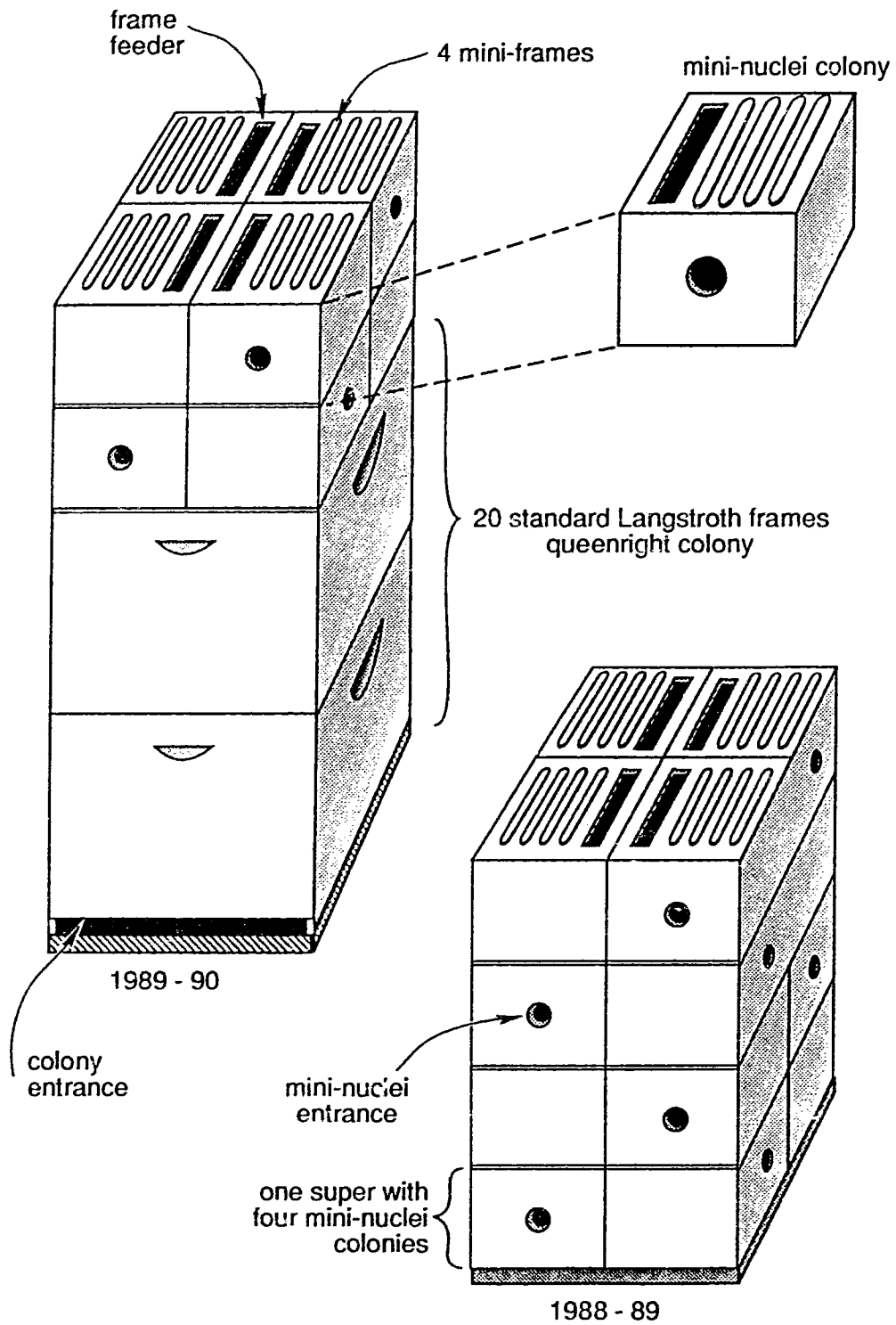


Figure 7. The mini-nuclei system consisted of supers stacked condominium style. Two supers were stacked over a support colony in 1989-90, whereas four supers were stacked without a support colony in 1988-89. One super consisted of four nuclei, each with four frames and a frame feeder.

MINI NUCLEUS COLONIES



2.3.2 Indoor storage system

The indoor queen storage system consisted of a ten frame colony held indoors, with free flight through an outdoor exit hole (Fig. 8). Three free-flying queenright (1987-88), and four free-flying queenless units with young workers (1988-89), were used for queen storage. Each colony consisted of four frames with pollen and honey, six with brood, and eight frames covered with adult workers. Sugar syrup was fed to colonies ad lib. using an inverted bottle, prior to and after queen introduction. Indoor conditions were kept at a constant temperature of 15°C, and with ambient day-light regime.

Colonies were left queenless for one day (1988-89) prior to queen introduction (16 September). Colonies were then lightly smoked and 1 kg of honey was poured over the top of the frames (1987-88, 1988-89). Honey was provided to each colony at least twice to ensure bees were gorged with honey. Queens were introduced one at a time, and, if worker-queen aggression was observed, honey was placed on the queen to reduce any further aggression.

Twenty-four (1987-88) and twelve (1988-89) queens were stored over the top of each colony. Queens were held above the colony in wooden compartments with queen excluder material at the base of all compartments. This allowed workers to mingle with each banked queen, and within the queenright colonies in 1987-88, and queenless colonies in 1988-89. Compartments were constructed from a wooden grid (1 x 1 cm wood; inside measurements of each compartment of 5 x 6 x 5 cm). Plexiglass over the top of each compartment provided good visibility of queens and workers. Drawn comb with honey was placed on one inside wall of each compartment. Queen compartments and feeding jars were supported over the colony by a 3 mm plywood board. Areas of the plywood board were cut away for the placement of queen compartments, feeding jars, and two areas of screen (25 x 5 cm)

for colony ventilation. Additional ventilation was provided by a hole covered with bee proof screen located on one side of each compartment.

2.3.3 Introduction of excluder caged queens

Twenty-four colonies in Abbotsford, B.C. were used to compare four colony treatments for introducing 12 queens simultaneously into a queenless colony bank. Each colony was equalized for brood and bees. Each colony consisted of two supers with a minimum of seven frames of brood, and a minimum of seventeen frames of bees with sufficient honey and pollen stores. All queens were reared from one queen mother by Southern Interior Apiaries, Keremeos, B.C.. Queens were transported to Burnaby in queen cages that were held in a mailing cage containing sufficient workers and queen candy. Queens were left in cages and placed in queen banks until used in the experiment. Queens were individually held in queen excluder compartments, six compartments to each side of the frame (see Fig. 5).

Six colonies were used in each of four treatments. Two colonies in each treatment were set up on each of three consecutive days (20, 21, and 22 June, 1988). The four treatments were as follows:

(1) Smoke. The colony was left queenless for 24 hours, and smoke was lightly blown over the top bars of the colony at introduction.

(2) Smoke and honey. The colony bank was kept queenless for 24 hours and about 2 kg of honey was poured over the top bars. Immediately prior to and after honey consumption the colony was heavily smoked and the queens introduced to the colony.

(3) Smoke, honey, and young workers. Younger workers were isolated in the upper super by placing all brood in the upper super 24 hours prior to queen introduction. Four hours prior to introduction, a solid board was placed under the upper super to isolate it from the remaining part of the colony. In addition, 2 kg of

honey was poured over the top bars, and the upper unit was heavily smoked immediately prior to queen introduction. Twenty-four hours after introduction, the solid board was replaced with newspaper, to slow down the combination of the two supers.

(4) Smoke, honey, young workers, and queen mandibular pheromones. This treatment was similar to (3), except for the addition of 10 queen equivalents of a five-component synthetic blend of queen mandibular pheromone (Slessor et al., 1988). This blend consists of three acids: 9-keto-2 (E) - decenoic acid, and R-(-)- and S-(+)-9-hydroxy-2 (E) - decenoic acid, and two aromatics: methyl *p*-hydroxybenzoate and 4-hydroxy-3-methoxyphenylethanol. Two queen equivalents of the pheromone blend were placed on the center of each of five glass slides. The slides were attached to top of the frame bars of each colony. Queen survival was checked one and two weeks after queen introduction.

2. Queen cage screen type.

Eight colonies were used to compare queen survival in queen cages with a single screen, and queen cages with double screens, that is with screen over the excluder material. The colony banks had either four or seven queens and was replicated four times. Colonies were equalized with a minimum of eight frames of brood, and stores. One day prior to queen introduction, the colony queen was removed and each colony was fed syrup with fumagillin. At introduction the colonies were lightly smoked and the frame with the queens inserted into the centre of each colony. Queen survival was checked weekly over four weeks.

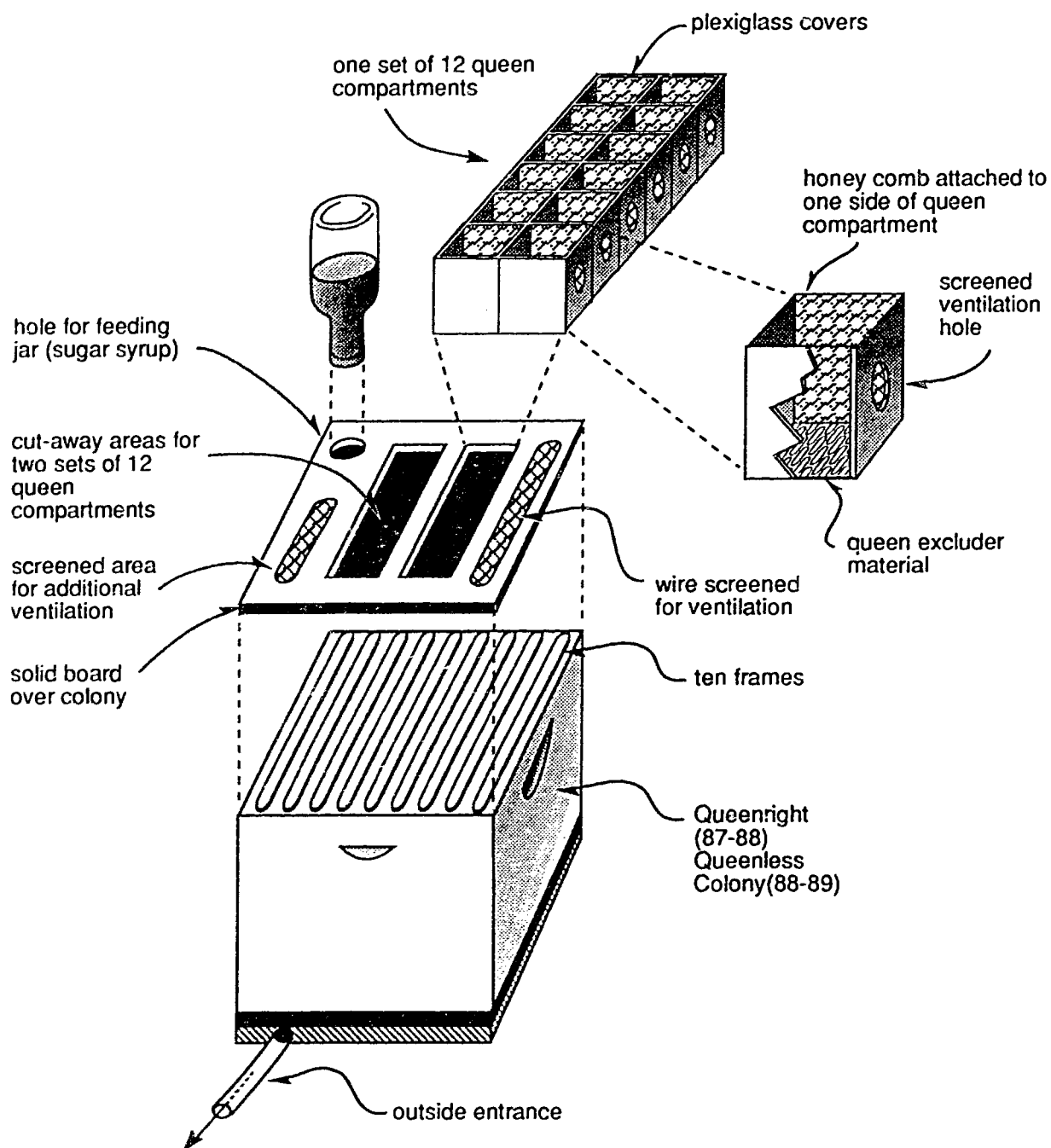
2.3.4. Statistical analyses

The "Lifetest" statistical test was used to determine if there were differences in the rate of queen mortality between storage systems. The Wilcoxon test indicated

early differences and the Log Rank test indicated differences later in the experiment (SAS Institute Inc., 1984). The CATMOD PROCEDURE (SAS Institute Inc., 1984) was used to analyze for differences in locational mortality of banked queens, between rows and between columns.

Figure 8. Indoor colony bank (queenright in 1987 and queenless in 1988) with free flight through an outdoor exit hole. Twenty-four (1987-88) and twelve (1988-89) queens were stored over the top of each colony separated from the queenright hive below, with excluder screen. Each compartment contained a piece of drawn comb and was covered by a piece of plexiglass. Sugar syrup was fed to colonies ad lib. using an inverted bottle. Ventilation was provided above the colony and into each compartment via screened areas.

INDOOR SYSTEM



2.4 RESULTS

2.4.1. Outdoor Storage Systems

There were several trends in queen survival that were apparent in all of the three study years. Single queens in the 5-frame nuclei (control) consistently showed low mortality; this treatment was significantly better ("Lifetest" $P < .05$) than any other treatment except for the mini treatment ($P > .05$) in 1989-90. Second, significantly fewer queens survived ($P < .05$) in all excluder-based treatments than in any other treatments. Third, a greater number of queens survived at the inner and upper areas of the queen holding frame held in colony banks. Finally, queen survival was consistently between 50% and 65% in all screen-based treatments after six months of winter storage. In this study we found no differences in the variance of queen survival between years.

In 1987-88, queen survival was significantly different ($P < .05$) between individual queens held in 5-frame nuclei, and queens held in excluder cages that were hosted in queenless banks located above a queenright colony (Fig. 9). Colony banks with excluder cages that were treated with brood and honey had similar queen survival ($P > .05$); neither the feeding schedule nor the addition of brood affected queen survival. The excluder cage treatment in colony banks did not result in acceptable levels of queen survival; only 6.5% of queens survived after six months of storage.

In 1988-89, survival also was significantly different ($P < .05$) between 5-frame nuclei and all colony bank treatments with excluder and screen cages (Fig. 10). However, the survival of queens held in screen cages and in the mini-system were identical ($P > .05$). Similar to 1987-88, excluder cages in colony banks were not successful in storing queens through the winter. In addition, survival in excluder cages was lower after one week in the cube formation than in the frame formation;

queen survival was 10% in the cube (1988-89), and 35% (1988-89) and 96% (1989-90) in the frame after one week of storage. However, survival in the frame formation for both years after six months storage was only 20%.

A distinct positional pattern of living queens was found in queen holding frames after six months of storage. The distance between the outer queen cages and the edge of the holding frame influenced whether queens survived winter storage. When cages were at least 15.5 cm from the frame edge in insulated colonies there was no difference in queen mortality ($P > .05$; 1989, Table 3). Greater queen mortality occurred when queens were 13 cm and 10.2 cm from the edge of the frame ($P < .05$; 1988, 1989). Minimum insulation around colony banks in 1987 also increased mortality of queens located in the outer excluder cages (7.5 cm from frame edge; $P < .05$; 1987). On the other hand, there was no difference ($P > .05$) in mortality between queens in excluder cages of insulated colonies. There was a slight tendency for more queens to die at the lower rows of the minimum insulated colony (1987) and where 48 queens were stored in the one colony (1989). However, the difference was not significant ($P > .05$).

In 1989-90, queen survival in the mini-system was not statistically different from the 5-frame nuclei; it was the only treatment in the three-year study period, that had similar queen survival to the 5-frame nucleus control (Fig. 11). The mini-system also was similar ($P > .05$) to the 24-screen cage system even though the 24-cage system was statistically different from the 5-frame nuclei ($P < .05$). The 5-frame nuclei also were significantly different ($P < .05$) from the remaining treatments: 48-screen-cage, 24 screen cage-switch, and excluder cage. Survival in the 24 and 48-screen-cage was similar (60% and 61%, $P < .05$). In addition, there were no significant differences between screen cage treatments with 24 queens, 24 queens switched to new colonies every two months, and 48 queens per colony ($P > .05$). Therefore, there was no advantage in moving queens bi-monthly to newly dequeened

colonies, and there was no detrimental effect on queen survival if either 24 or 48 queens were housed in one colony. Finally, excluder cages did not store queens successfully through the winter; survival was less than 25%, similar to the low survival in the previous two years.

2.4.2. Indoor Storage Systems

In both queenright (1987-88) and queenless (1988-89) indoor colonies, more than 95% of the queens died after one month of storage. The majority of queens died within the first week of introduction for both years. Queen survival was 33% after three days in three queenright colonies (1987-88), and 19% after six days in four queenless colonies (1988-89).

2.4.3 Introduction of excluder caged queens

Although there was no significant difference ($P > .05$; Table 4) between treatments introducing 12 caged queens simultaneously to a queenless bank, there was a significant difference between days ($P < .05$). Marginal significance ($P = .096$; Student Newman-Keuls test) suggests that, with more colony replicates, a significant difference would have emerged between treatments. The treatment with excessive smoke, honey, and younger workers had the greatest number of surviving queens; this technique resulted in 80.6% queen survival. Queen survival varied between treatments: (1) light smoke (50%), (2) excess smoke and honey (33%), and (4) excess smoke, honey, young workers, and queen mandibular pheromone (50%).

Fig. 9. Survivorship curves (1987-88) of queens held over the winter in (1) 5-frame nuclei, and (2) excluder cages placed in queenless units, which were treated with (i) honey every two weeks, (ii) honey every month, and (iii) brood and honey every two weeks. Different letters indicate significant differences ($P < .05$, "Lifetest").

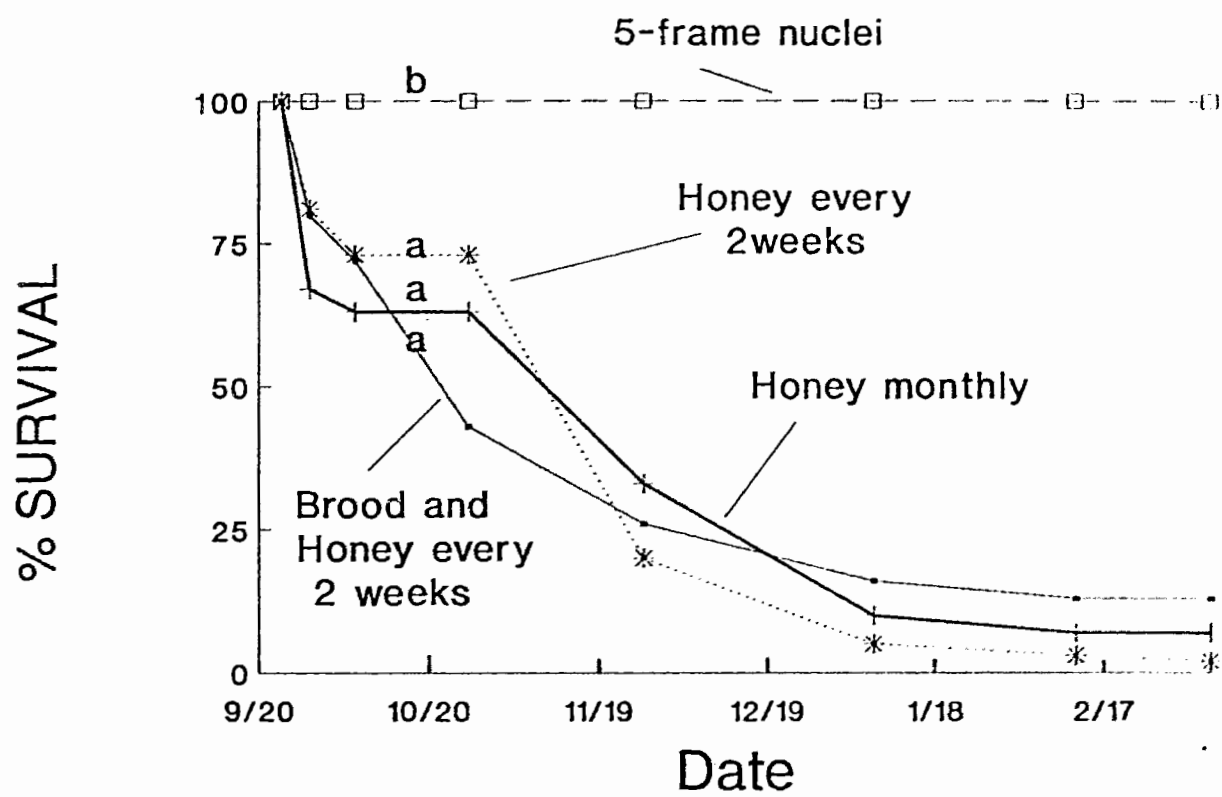


Fig. 10. Survivorship curves (1988-89) of queens held over the winter in (1) 5-frame nuclei, (2) excluder cages (cube and frame formation), (3) screen cages that were moved to new colony banks every month, and (4) mini nuclei. Different letters indicate significant differences ($P < .05$, "Lifetest").

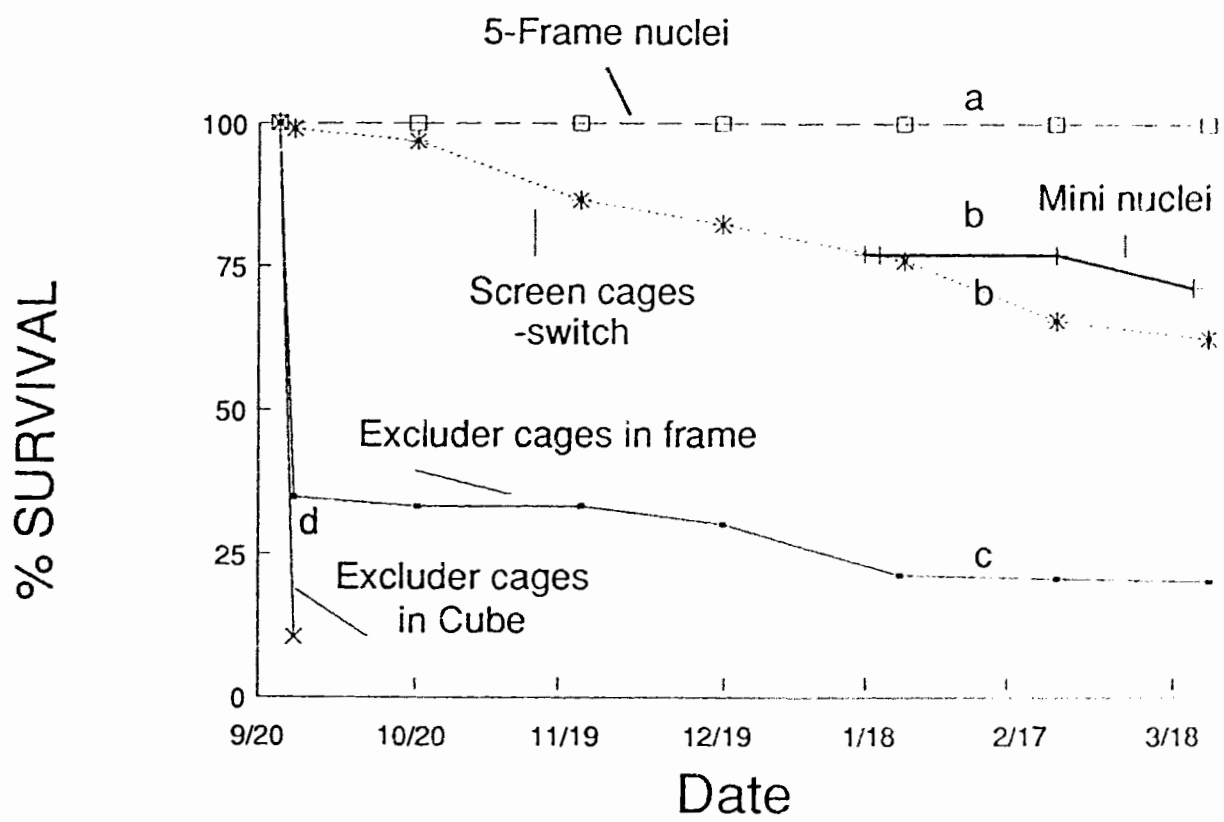


Figure 11. Survivorship curves (1989-90) of queens held over the winter in (1) 5-frame nuclei, (2) excluder cages (frame formation), (3) screen cages with (i) 24 queen, (ii) 24 queens-switch, that were moved to new colony banks every two months, and (iii) 48 queens, and in (4) mini nuclei. Different letters indicate significant differences ($P < .05$, "Lifetest")

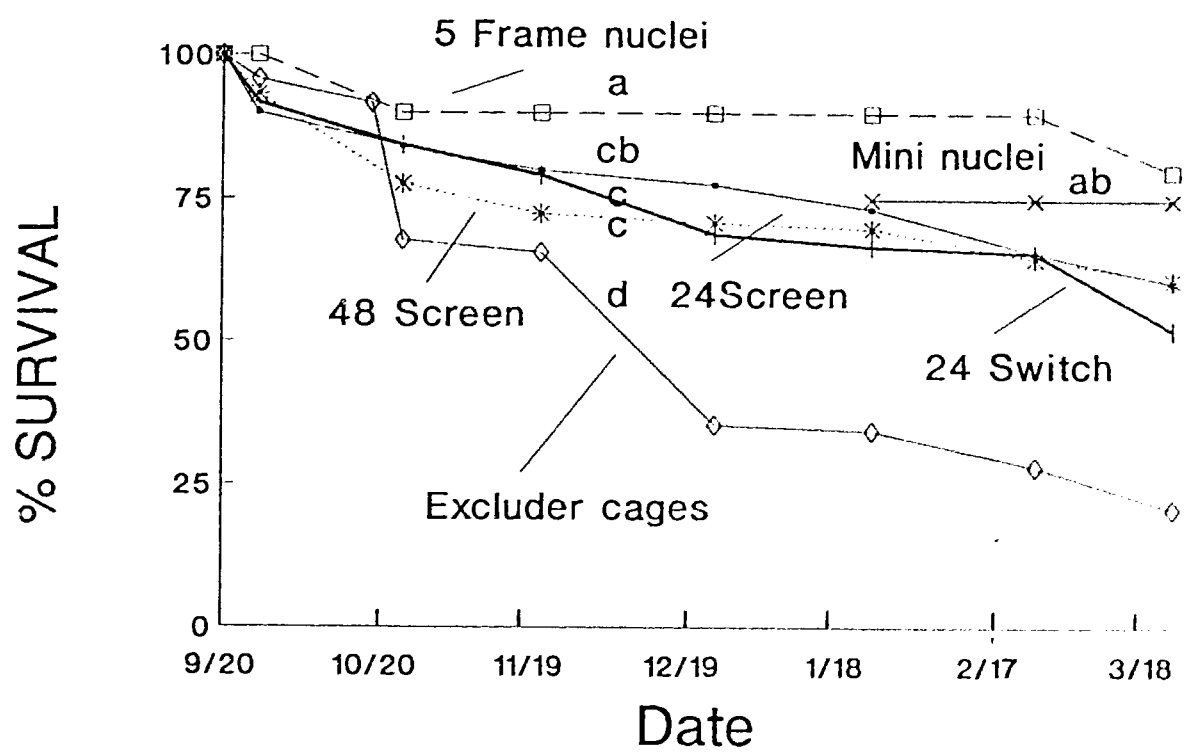


Table 3: A comparison of locational mortality in queen cage holding frames in colony banks with minimum and maximum insulation. Mortality between the center and outside columns (COL), and between rows of caged queens are statistically significantly different (values followed by *) or not at the .05 level.

INSULATION ¹	DISTANCE ² (cm)	SYSTEM	N ³	YEAR	COL	ROW
MAX	15.5	24-Screen ⁴⁵	8	1989 ⁵	.88	.07
MAX	13	24-Screen	4	1988 ⁵	.02*	.10.
MAX	10.2	48-Screen	4	1989 ⁶	.05*	.09
MIN	7.5	24-Excluder ⁶	14	1987 ⁵	.0005*	.27.
MAX	7.5	24-Excluder	8	1988 ⁵	.50	.89
MAX	7.5	24-Excluder	4	1989 ⁵	.67	.36.

1. Type of insulation.

2. Distance between edge of frame and queen cage.

3. The number of colony queen banks.

4. Queen cages covered with bee-proof screen.

5. 24 or 48 caged queens per colony.

6. Queen compartments/cages covered with queen excluder.

Values followed by * were significantly different (CATMOD Procedure, SAS)

Table 4: The number of queens alive 14 days after introducing queens in excluder cages to four groups of six colonies in June, 1988. Colonies were prepared and treated with (1) smoke, (2) smoke and honey, (3) smoke, honey and young workers, and (4) smoke, honey, young workers and pheromone.

TREATMENTS	NUMBER OF LIVE QUEENS PER COLONY (out of 12)			TOTAL ALIVE	% ALIVE
	DAY OF EXPERIMENT				
	1	2	3		
1.	6	4	7	36	50% ^c
	1	10	8		
2	0	1	3	24	33% ^c
	2	11	7		
3	10	2	12	58	80% ^c
	10	12	12		
4	1	12	10	36	50% ^c
	0	4	9		
TOTAL ALIVE	30	56	68	154	
PERCENT ALIVE	31.3% ^a	58.3% ^{ab}	70.8% ^b		

Values followed by different letters were significantly different (Student Newman-Keuls test; $P < 0.05$)

2.5 Discussion

Multiple storage of queens in screen cages is a viable overwintering method for a period of about six months in queenless colonies. Queen storage with 24 queens per colony bank was successful for two successive winters, with average survival of 59% (1988-89 and 1989-90). Colony banks with up to 48 queens also survived six months of winter storage in queenless colony banks, with average survival of 61% (1989-90). Survival in individual colonies was variable, between 30% and 83%. Successful storage (84-100% survival) with screen cages has been reported previously (Griffin, 1966; Levinsohn and Lensky, 1981; Walsh, 1967), although two studies reported a much lower and less successful queen survival (20%; Dietz et al. 1983; Szabo, 1977b).

In contrast, queens held in excluder cages and stored in queenright or queenless colony banks repeatedly failed to survive testing over three winters. Mean survival for any one year, or any treatment, was never greater than 25%. Similar results were reported in two studies where survival for 6 months was less than 20% (Mitchell et al., 1985; Szabo, 1977b). However, excluder cages were successful for six months in two studies (95% queen survival (n=1); Harp, 1969; 72% queen survival (n=1); Mitchell et al., 1985), and successful for 3 months in another study (92% queen survival (n=7); Harp, 1967). We conclude that queen survival in storage colonies with excluder cages is too variable for commercial use.

The specialized system of excluder cages in a "cube" formation resulted in high mortality (90%) in the first month. This mortality may have resulted from the workers failing to keep the whole cube with the queens warm, because the center of the cube was closed and not accessible to the workers. Also, the configuration of the cube in the center of the colony divided the colony in half, decreasing effective thermoregulation.

Individual queens were stored successfully in mini-nuclei from January to March, after four months of storage in colony banks with screen cages. The success of transferring queens depends on environmental conditions because handling queens under inclement weather conditions could harm the queen. Thus the transfer of queens would be suitable only in mild climates such as coastal British Columbia, or more southerly climates. Nevertheless, the low mortality of mini-nuclei compared favorably with mortality in the larger 5-frame nuclei. This makes the mini-nuclei a biologically viable alternative to the 5-frame nuclei. However, our economic analyses indicated that the mini-system are not economically viable because of the specialized equipment required and the time needed to set up the system in January (Section 4, Table 9).

Fundamental management techniques for wintering and storing queens included insulating colony banks, and placing colonies in 4-packs. Insulation allows colonies to thermoregulate without the workers clustering together, and workers are spread from one side of the hive to the other. This mobility within the hive enables workers to move honey to where it is required. Thus, colonies that are well insulated and provisioned prior to winter do not require additional honey during queen storage (screen cage treatments in 1988-89 and 1989-90).

In contrast, workers in partially insulated colony banks did cluster, and depleted honey stores from frames in the center of the colony cluster (1987-88). Clustered workers are restricted to obtaining food from within the area of the cluster, which includes the frames adjacent to the caged queens. Thus, the replacement of empty frames with honey-filled frames would be essential for the management of uninsulated colony banks. Queen survival was similar when frames were replaced every two weeks and every month (1987-88).

Cluster size is determined by ambient temperatures and by the worker population of a colony. In 1987-88, the worker population obtained from a single

colony was an additional factor that contributed to queen mortality in uninsulated banks. A single colony cluster is about half the volume of a cluster from a two-queen colony. The cluster size obtained from a single colony was small, and resulted in isolating some of the queens outside the cluster. The larger worker population in 1988-89 and 1989-90, plus the use of colony insulation, ensured queens would not be isolated from worker bees. The larger worker populations was produced by two-queening colonies at the start of the honey flow in early July. Thus, colony insulation and high worker population reduce the chance of cluster formation and appear to improve queen survival, both in colony banks and 5-frame nuclei.

Mortality of queens at the outer edges of the queen holding frame was greater than mortality of more central queens when the distance of the outer cage to the hive wall was 13 cm or less, and in particular when the colony bank was not well insulated (1987). Queens close to the hive wall or to the edge of the frame are more susceptible to being abandoned by the workers of the colony than queens near the centre of the frame, especially in uninsulated hives when the cluster contracts in size. When storing large numbers (48) of queens in a colony, a greater number of queens are closer to the edge of the frame than in a colony with 24 queens. An external source of heat applied to the colony might decrease the movement of workers away from the banked queens. Queen location on the frame contributes towards the isolation and eventual starvation of queens in colonies that cluster.

The same technique for introducing multiple queens under fall conditions resulted in higher queen mortality (65% in 1988-89 excluder cage treatment, see Fig 10) than under summer conditions (20% in introduction of excluder caged queens; Table 4, treatment 3). Under summer and winter conditions, the age structure and the brood-adult ratio differ depending on seasonal factors such as the timing and number of brood cycles, and worker longevity. (Winston, 1987). Perhaps the age

structure of the colony and/or the brood-worker ratio influences worker behavior towards newly introduced queens.

Mortality at introduction of caged queens was more severe in excluder cages with full worker-queen contact than in screen cages with partial worker-queen contact (1989-90). When wire screen was fixed over excluder cages at introduction, mortality initially was low (4%). However, when the wire screen was removed one month later, mortality increased to 32%. High mortality in excluder cages at introduction and during storage indicates that full worker-queen contact with excluder cages is detrimental to queen survival.

The interplay of queen attractiveness and worker aggression at the colony and at the individual level is likely to result in different levels of queen care, and influence the variation in queen survival between colonies, and within treatments. Workers respond to levels of queen attractiveness by either aggressive or queen care behavior. It is not known whether different levels of attractiveness determine worker aggression or worker care towards the queen. There is significant variation of mandibular pheromone produced by queens of similar age and development (Slessor *et al.*, 1990), and some queens are more attractive than others to the workers of a colony (Szabo, 1974a). Thus, certain queens in a queen bank might be less attractive to workers than others, and perhaps experience higher mortality. There also is a genetic component in the response of workers to queen mandibular pheromone at the colony level (Pankiw, personal communication), and workers in some colonies may be more attracted to particular queens. Whether certain strains of honey bees could be bred for queen-care and not queen-aggression requires investigation.

Queen storage in queenless colony banks was successful without the addition of brood (younger workers), or the transfer of queens to colonies with younger workers. These results were not anticipated, since young workers with active glands generally tend queens (Winston, 1987). The age-range of summer workers known to

tend queens (from three studies) is 1-50 days, with a mean age of just less than 20 days (Winston, 1987). Perhaps active glands are retained by workers when queen contact is maintained (Avetisyan and Vasiliadi, 1967). The queens' pheromone(s) may act as an stimulant that ensures that worker gland(s) remain active. In our study, many workers were more than six months old (180 days) towards the end of winter storage in several successful treatments; these colonies nevertheless tended queens successfully (60% survival) until the end of the storage season.

Indoor queen storage with excluder cages in queenless and queenright colony banks was not successful. Considering the importance of cluster formation and temperature to queen abandonment, the indoor system should be the best. The reason for failure is probably due to the excluder cages. Lack of time did not allow us to test screen cages in the indoor colony banks.

Queenright storage colonies kept indoors or outdoors were not successful in storing queens, unlike a similar study in which queenright colonies were kept outdoors with 92% queen survival for three months (Harp, 1967). The advantage of queenright storage colonies is that brood is continually produced, providing a continuous supply of adult workers. Conversely, the presence of a colony queen in the queen bank may confound the problem of queen storage by attracting workers from the stored queens held above the colony to the colony queen.

Our economic analyses (Section 4) indicate that mass queen storage in colony banks is economically viable, because the total cost is less than the total revenue when queens are sold for \$10 each. The level of queen survival, the number of queens per colony bank, and whether the beekeeper hires outside labour all influence the expected profits. The "best" system economically was 48 queens caged in wooden screened cages, earning the beekeeper \$13,215 for every 100 colonies used as storage banks (Table 10). If the beekeeper does the labour himself, an additional

23% of the total cost, the beekeepers income is increased to \$16,625. These are convincing figures that colony banks are economically as well as biologically viable.

Our study has demonstrated that queenless colony banks can be used to store mated queens for six months over the winter when the colony bank is supplied with sufficient workers, food, and insulation. The presence of a laying queen to provide additional workers, and/or the addition of brood, was not necessary for successful queen banking. Our economic analyses indicate that, at 60% queen survival, with 48 screen cages per colony, queen storage is both economically viable and biologically feasible.

3. QUEEN QUALITY AFTER WINTER STORAGE

3.1 Introduction

An important part of our study on queen storage was the assessment of queen quality after storage, to determine any treatment effects. Queen attributes have been examined previously to determine whether a simple, measurable queen characteristic would be indicative of queen quality, but the evidence is weak that any one characteristic defines a "good" queen (Avetisyan, Rakhmatov and Ziedov; Boch and Jamieson, 1960; Nelson and Gary, 1983; Eckert, 1934,1937;). We examined queen quality by measuring both queen attributes and colony performance. The ability to cull poor-quality queens has important economic benefits for the commercial beekeeping industry. Time, labour, and equipment costs would all be saved if inferior queens were identified prior to installation into honey-producing colonies.

The reproductive capacity of honey bee queens is an important component of queen quality, because the colony depends on the queen's reproductive capacity to grow to an economical size for honey production. Ideally, the reproductive capacity of banked queens should not be different from queens overwintered individually in colonies. However, there is no simple method of determining reproductive capacity and overall queen quality. Colony performance in terms of population (Burgett et al., 1985) and honey production are the most commonly-used methods of assessing queens, but these are both indirect and time-consuming methods. Usually, colony size measurements are only used as a research tool. Other parameters such as internal and external queen measurements also have been used in research as indicators of queen quality, although the evidence is weak that these measurements are correlated with colony performance. Both colony size measurements and queen measurements have been used to assess queen quality in some studies (Scott-Dupree et al., 1988; Van Eaton, 1986).

Colony performance has been assessed by noting queen survival during the trial (Szabo, 1977b), or her egg-laying pattern (Foti, 1958; Jordan, 1959; Levinsohn and Lensky, 1981; Nelson and Roberts, 1967; Shehata, 1982). More detailed assessments have included brood measurements alone (Poole et al., 1973), brood, wax and honey (Avetisyan and Vasiliadi, 1967), colony weight (Szabo, 1975), honey weight (Avetisyan and Vasiliadi, 1966), and sealed brood, pollen, honey and colony weight (Mitchell et al., 1985). In some queen storage studies, queens were not tested for colony performance, presumably because it is expensive and time-consuming to do so (Edward and Poole, 1971; Dietz et al., 1983; Harp, 1967, 1969; Walsh, 1967).

There is conflicting evidence concerning whether internal and external queen characteristics are directly related to colony performance such as brood and honey production. Correlations were weak between queen weight and brood (Boch and Jamieson, 1960; Makarov, 1969; Nelson and Gary, 1983) and honey (Nelson and Gary, 1983) production. Further, the number of ovarioles was weakly correlated with brood production in one case (Avetisyan, 1961) and no relationship in another (Eckert, 1937). The number of ovarioles are strongly correlated to queen weight (Avetisyan et al., 1967; Wen-Cheng and Chong-Yuan, 1985; Woyke, 1987) and queen pupae weight (Hoopingarner and Farrar, 1959), as would be expected because the ovaries make up a large proportion of the body mass. However, the number of ovarioles were weakly correlated with queen weight (Avetisyan, 1961; Weaver, 1957). Other measurements such as wing, tibia, and head size were not correlated with the number of ovarioles (Eckert, 1934), but the number of bristles per wing area and the number of ovarioles were highly correlated (Woyke, 1987). Overall, there does not appear to be one good indicator for queen quality. Honey production is strongly correlated to brood production (Szabo, 1982a), but this fact does not link colony performance to queen characteristics such as ovariole number or queen weight. In this study, we compared colony attributes including areas of brood, adult worker

bers, honey, pollen and drawn-out comb, and weight of honey between storage treatments. Also, we compared measures of queen quality, specifically queen weight, ovariole number and ovary weight between storage treatments, and tested correlations between these variables and colony performance.

3.2 Materials and Methods

Six to eight queens were randomly selected from colony storage systems and tested for colony performance and queen attributes after about six months of storage (1989, 1990). High mortality during the 1987-88 storage trial preempted further queen analyses.

Queen performance was gauged by comparing the growth of test colonies. Each queen was introduced into a 5-frame nucleus in early April with the same amount of stores (two frames of honey, one frame of pollen) and bees (1-1, 2-2 frames covered by bees) in each nucleus. Total areas of brood, pollen, honey and adult worker bees were measured in early June and August. Areas of newly drawn comb and weight of extracted honey also were measured in August.

Queen attributes were examined for eight queens from each treatment in 1989 and again in 1990, and included: (1) queen weight, (2) ovariole number, and (3) dry ovary weight. Queens were weighed alive (Mettler H20T balance), immediately after winter storage in late March, in an open tube (8 x 30 mm) with a 1 mm opening at the smaller end, and a cap at the larger end of the tube. Both ovaries were dissected from dead queens and stored in 70% alcohol until examined. The number of ovarioles in both ovaries was examined with a dissecting microscope (20X), after softening the tissue for four days in 10% glycerol under refrigerated conditions, and counted after cutting both ends of each ovary off, and pulling the ovary apart into smaller bundles of ovarioles (10-15 ovarioles) with two insect pins (El-Helaly *et al.*, 1980). Ovaries were dried at 42°C for three days, and then weighed.

Queen attributes and queen colony-performance were compared between treatments using the analyses of variance technique (GLM procedure in SAS), and correlations were made between the variables (CORRELATION procedure in SAS).

3.3 Results

3.3.1 Queen attributes

There were some statistical differences for queen attributes between treatments, but these were not consistent over the two years (1988-89 or 1989-90) (Fig. 12). In 1988-89 queen weight was different ($P < .05$) between the 5-frame nucleus and the 24-standard-switch treatments, and not different ($P > .05$) to the mini-nuclei and the 24-excluder treatments. In contrast, there was no difference for queen weight ($P > .05$) in the following year (1989-90) for all treatments (5 frame nuclei, switch, 24 and 48 standard, 24-excluder, and mini-nuclei). Similarly, differences occurred between treatments for ovary weight but again were not consistent between years. In 1989-90 there was no difference between treatments for ovary weight ($P > .05$), whereas ovary weight was greater ($P < .05$) in queens stored in mini-nuclei than in any other treatment in 1989-90. In contrast, the number of ovarioles were not different ($P > .05$) between treatments for both 1988-89 and 1989-90.

3.3.2 Colony performance

There was no difference ($P > .05$) in colony performance by queens stored in colony banks and queens overwintered in 5-frame control colonies over two years of testing (1988-89 and 1989-90). Measurements were taken in June 1989 (Fig. 13), August 1989 (Fig. 14), June 1990 (Fig. 15), and August 1990 (Fig. 16). The weight of honey in the 48-standard was low compared to the other treatments in August 1990 (Fig. 16), whereas the honey area for the 48-standard system was similar to the other treatments. Less honey was stored in the honey super (which was extracted and weighed), and more honey was stored in the brood chamber, which was measured by area, in that treatment.

3.3.3 Correlations

There were no significant correlations ($P > .05$) between queen weight (measured in April), and colony performance (measured in August), as indicated by areas of brood, bees, honey, pollen and comb, and honey weight (1989 and 1990). However, inconsistent relationships were found between queen attributes. In 1989, ovary weight was correlated with queen weight ($r = 0.51$, $P < .01$) and ovariole number ($r = 0.39$, $P < .01$), but queen weight was not correlated with ovariole number ($P > .05$). In contrast, there were no significant correlations found in 1990 between queen weight, ovariole number and ovary weight ($P > .05$).

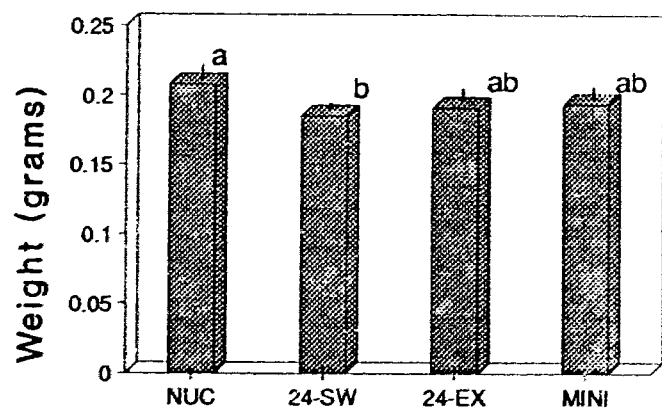
3.3.4 Queen survival in colony performance tests.

On average, 11% of winter-stored queens placed in spring colonies died or became drone layers during the spring and summer performance trials. One out of eight queens died by 1 May in each of the 1989 treatments (5-frame nuclei, 24-standard, 24-switch, 24-excluder, and mini-nuclei). Fewer queens died in 1990 than in 1989. No queens died from the 24-switch, 5-frame nuclei, and 24-excluder systems, and two out of eight queens died from both mini and 24-standard system. Two out of six 48-standard queens became drone layers by mid-June.

Figure 12. A comparison between treatments of queen weight, ovary weight, and ovariole number after winter storage (1988-89 and 1989-90). Queens were either wintered singly in (1) 5-frame nuclei (NUC) and (2) mini-nuclei (MINI), or in groups. Colony banks held either of (3) 24 or (4) 48 queens in screened cages. Queens (24) were also (5) switched (24-SW) to a new colony banks (monthly in 1989 and bi-monthly in 1990). Queens (24) were also caged in (6) excluder cages (24-EX). Different letters indicate significant differences ($P < .05$).

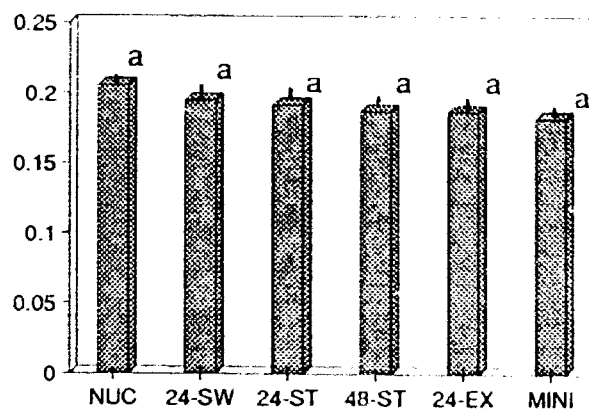
1988-89

QUEEN

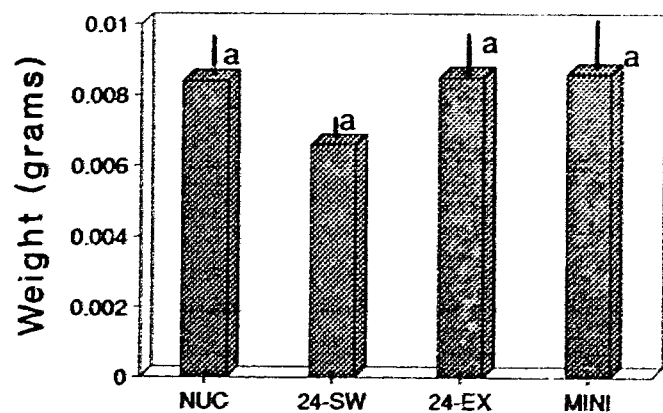


1989-90

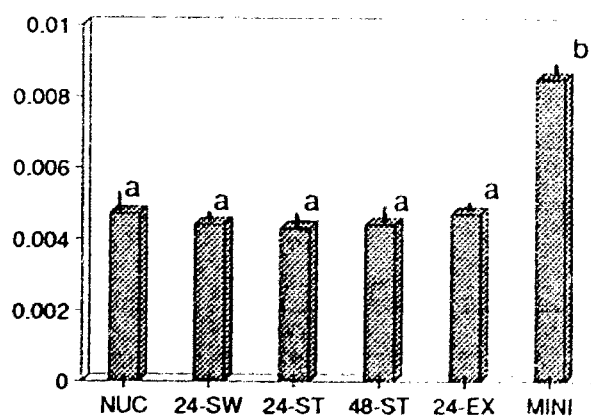
QUEEN



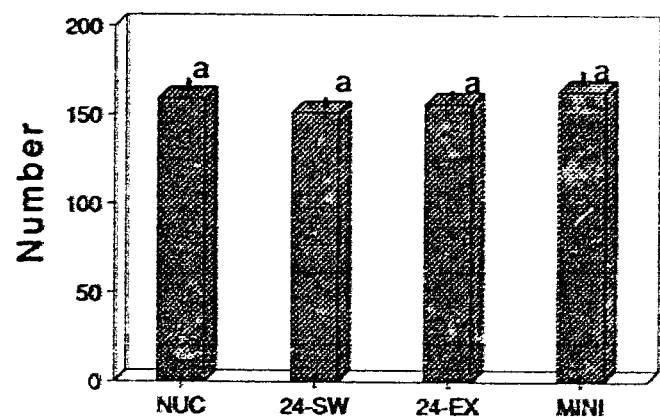
OVARY



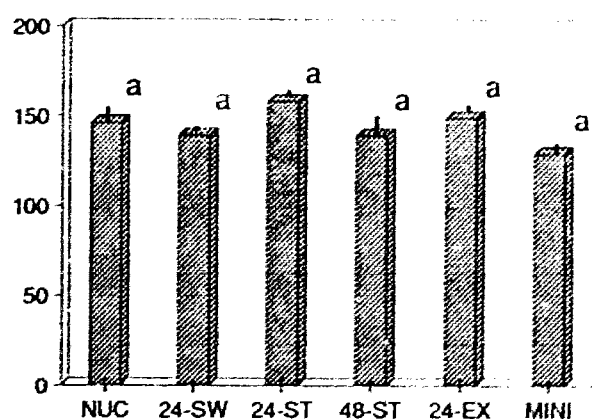
OVARY



OVARIOLES



OVARIOLES



TREATMENTS

Figure 13. A comparison of colony parameters between queens that were overwintered in five treatments. Areas of brood (cm²), adult worker bees, pollen, and honey were measured with a plastic grid in June, 1989. Queens were either wintered singly in (1) 5-frame nuclei (NUC) and (2) mini-nuclei (MINI), or in groups held in colony banks. Colony banks held queens either in screen cages or excluder cages. 24 queens were caged in screened cages and (3) switched (24-SW) to new colony banks (monthly in 1989) or (4) left in the same colony bank (24-ST); (5) 24 queens were also caged in excluder cages (24-EX). Different letters indicate significant differences ($P \leq .05$).

COLONY PERFORMANCE, June 1989

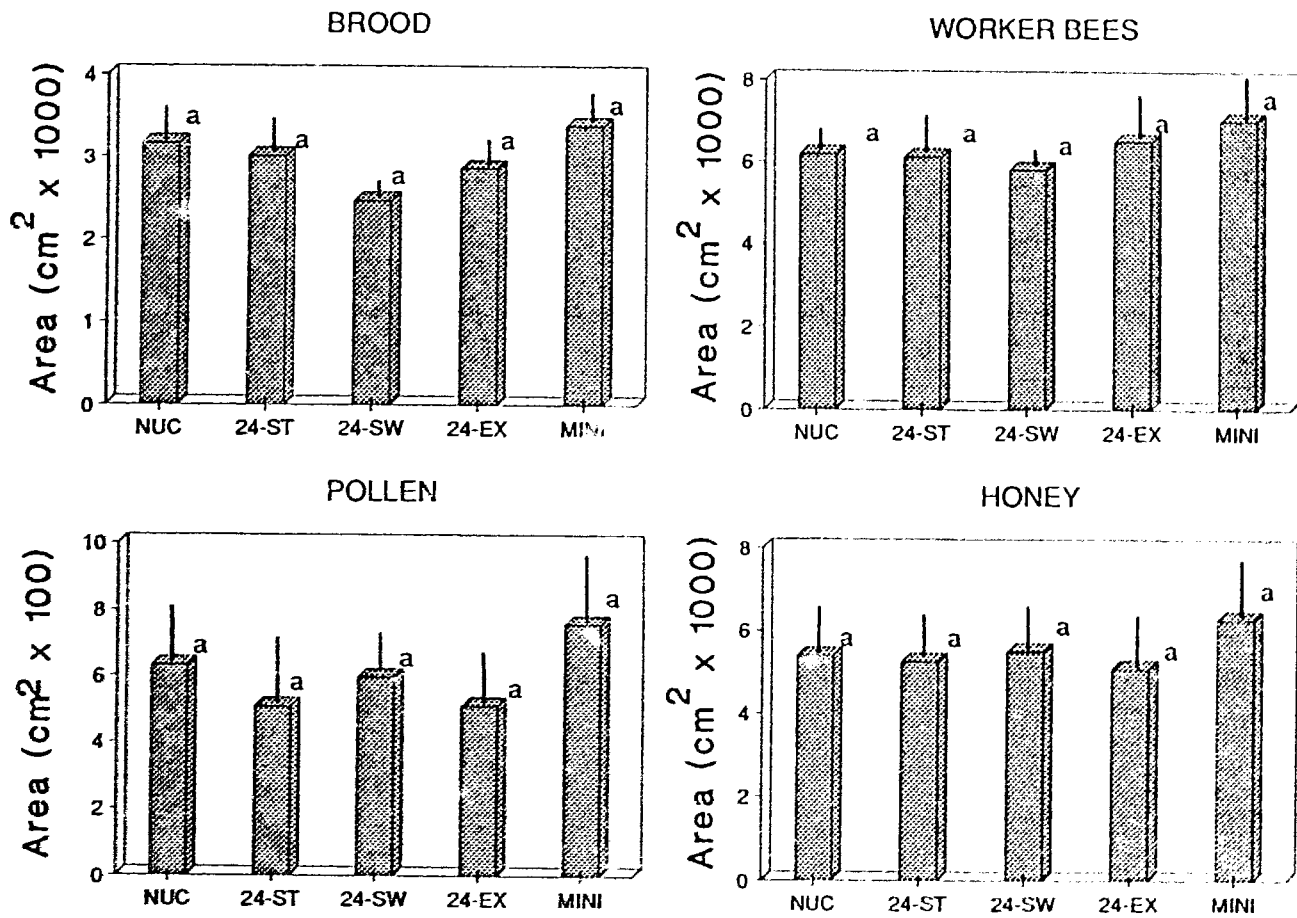
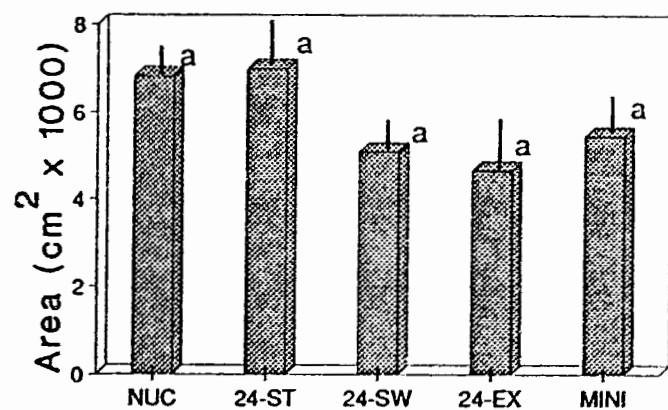


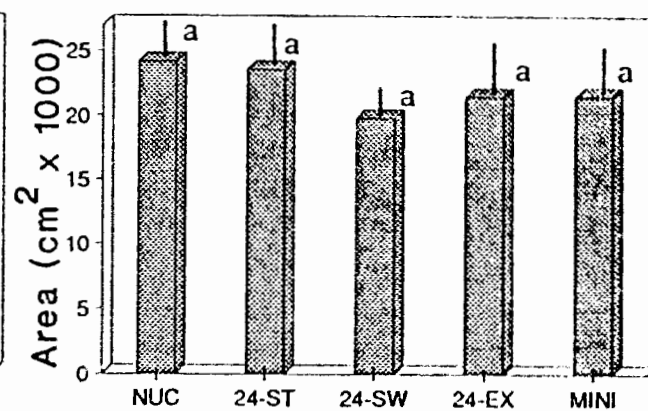
Figure 14. A comparison of colony parameters between queens that were overwintered in five treatments. A plastic grid was used to measure the areas (cm²) of brood, adult worker bees, pollen, honey, and comb, and honey was weighed on 9 August, 1989. Queens were either wintered singly in (1) 5-frame nuclei (NUC) and (2) mini-nuclei (MINI), or in groups held in colony banks. Colony banks held (3) 24 queens in screened cages (24-ST) or (4) switched (24-SW) to new colony banks (monthly). 24 queens were also (5) caged in excluder cages (24-EX). Different letters indicate significant differences ($P < .05$).

COLONY PERFORMANCE, August 1989

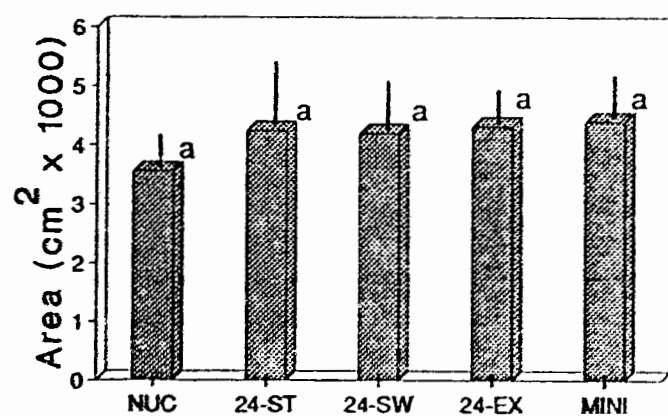
BROOD



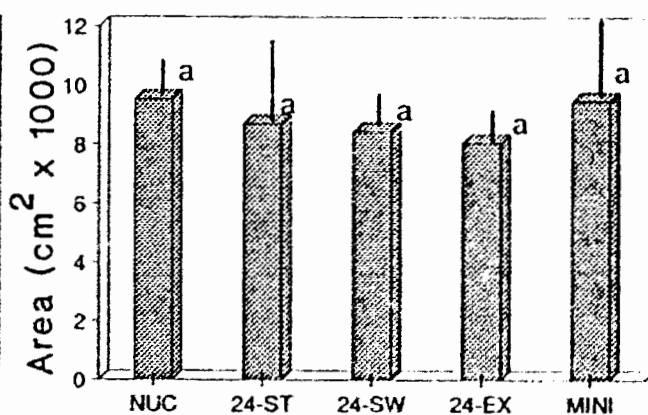
WORKER BEES



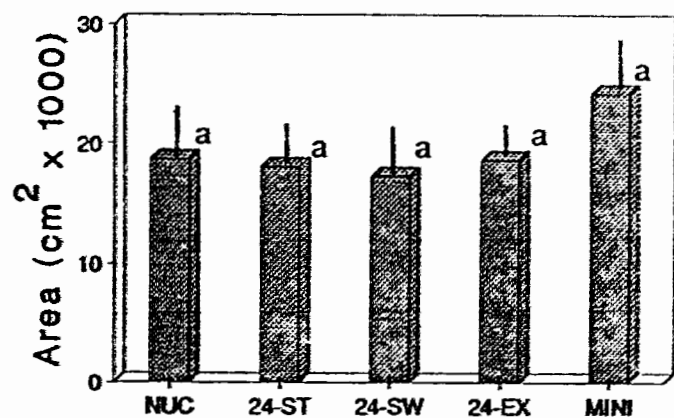
POLLEN



HONEY



COMB



HONEY

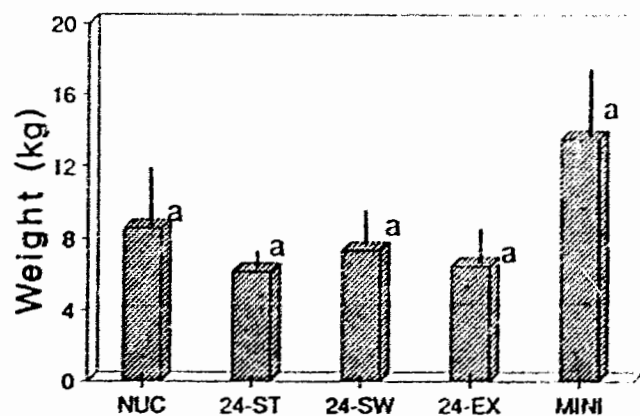
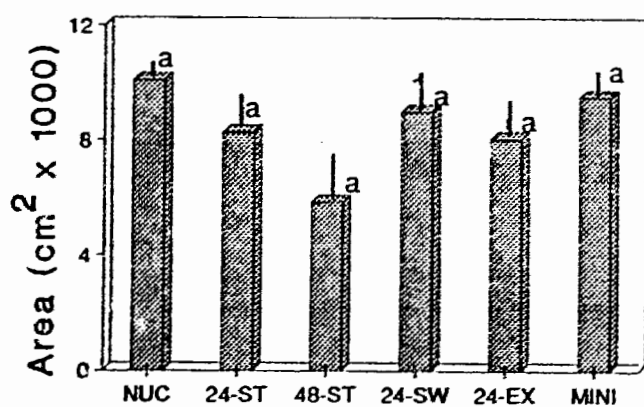


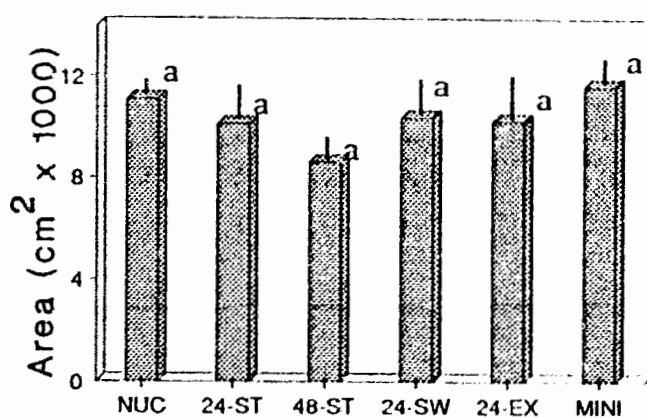
Figure 15. A comparison of colony parameters between queens that were overwintered in five treatments. A plastic grid was used to measure the area (cm^2) of brood, bees, pollen, and honey on 5 June, 1990. Colony banks held queens either in screen cages or excluder cages. Queens were caged in screened cages and (3) switched (24-SW) to new colony banks (bimonthly in 1990) or (4) left in the same colony bank (24-standard - 24ST; and 48-standard- 48ST). Queens were also caged in (5) excluder cages (24-EX). Different letters indicate significant differences ($P < .05$).

COLONY PERFORMANCE, June 1990

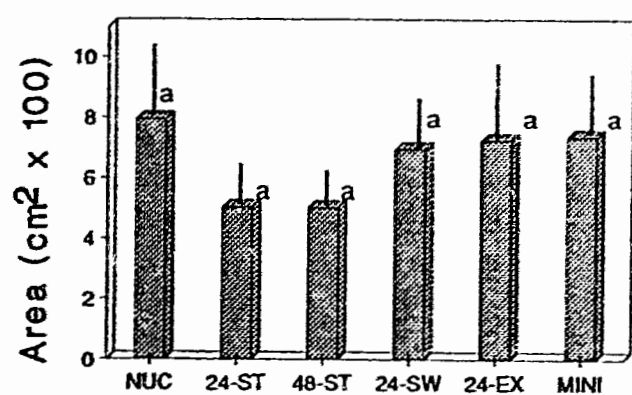
BROOD



WORKER BEES



POLLEN



HONEY

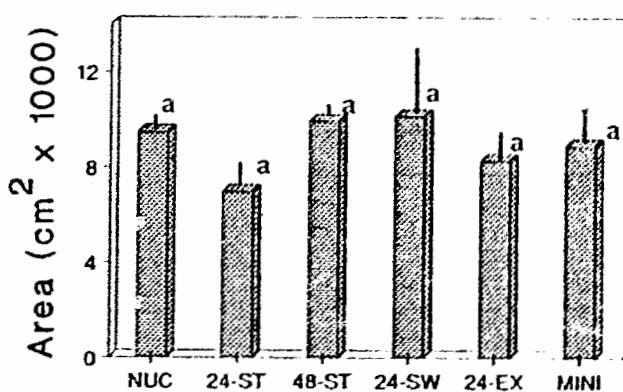
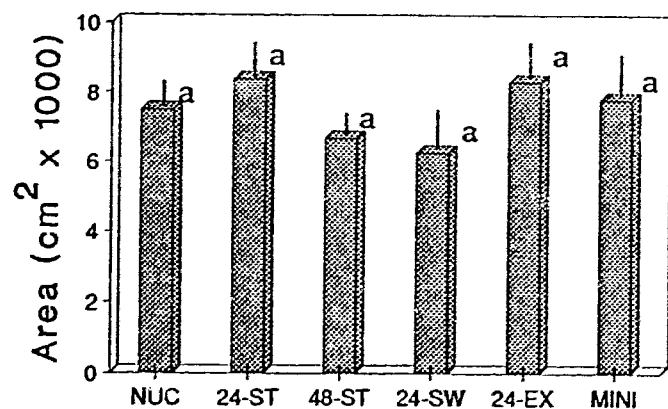


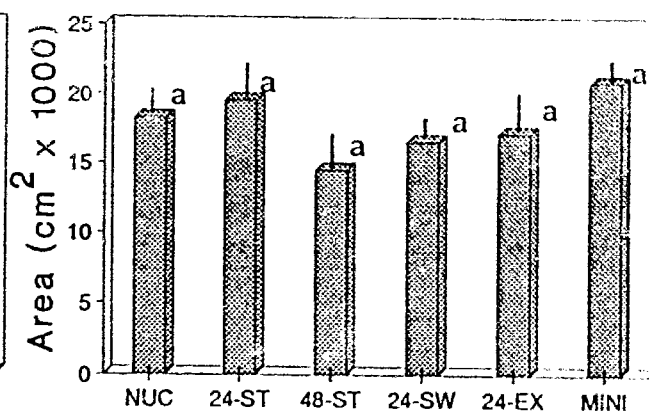
Figure 16. A comparison of colony parameters between queens that were overwintered in five treatments. A plastic grid was used to measure areas of brood (cm^2), adult worker bees, pollen, honey, and comb on 7/8 August, 1990. Excess honey was weighed on the same date. Queens were either wintered singly in (1) 5-frame nuclei (NUC) and (2) mini-nuclei (MINI), or in groups held in colony banks. Colony banks held queens in screened cages and (3) switched (24-SW) to new colony banks (bimonthly in 1990) or (4) left in the same colony bank (24-standard (24ST) and (5) 48-standard (48ST). Queens were also caged in (6) excluder cages (24-excluder (24EX). Different letters indicate significant differences ($P < .05$).

COLONY PERFORMANCE, August 1990

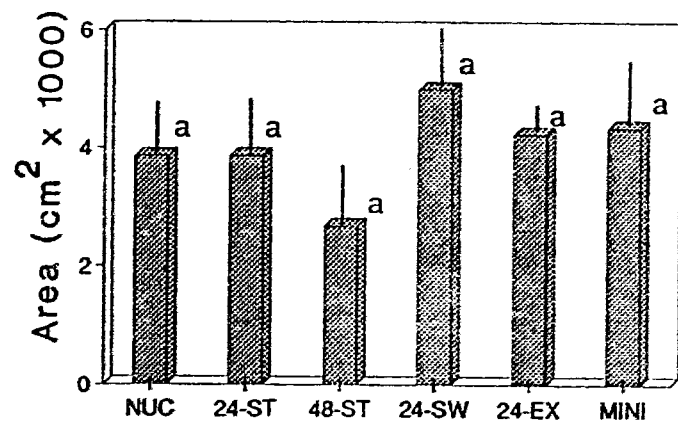
BROOD



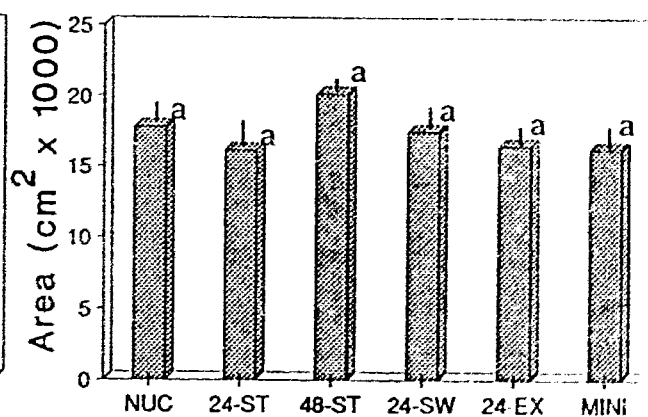
WORKER BEES



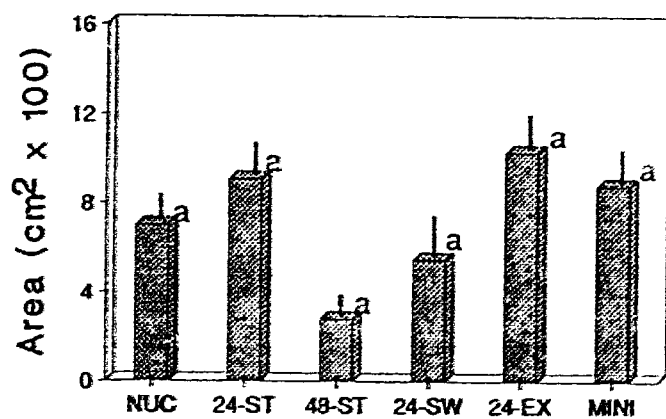
POLLEN



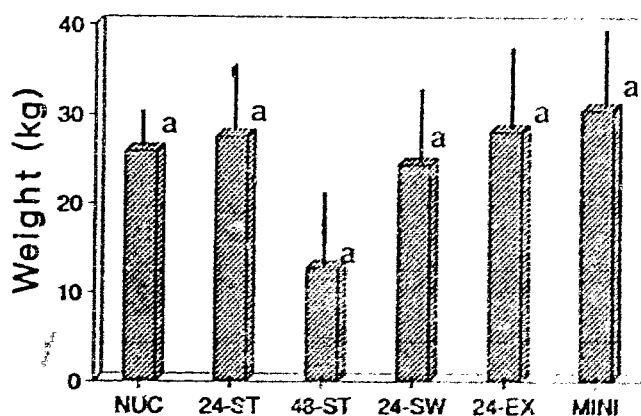
HONEY



COMB



HONEY



3.4 Discussion

There were no significant differences between the control and most treatments, except for the mini-system, in queen weight, ovary weight and ovariole number after 6 months of storage in two years of testing. This is an important result, because it indicates that the potential fecundity of queens was not affected by long-term storage. The lack of ovary degeneration in stored queens was particularly noteworthy.

Ovary weight was significantly greater in queens from the mini-system in 1990, but there were no differences between the mini-system and other treatments in 1989. There were no differences between treatments in both years for number of ovarioles. We expected that heavier ovaries would have larger number of ovarioles. On the contrary, mini queens had the lowest number of ovarioles (only in 1989-90) of any treatments. Perhaps, egg-laying queens from the mini-nuclei had fewer ovarioles that contained larger, more developed eggs that were ready for laying. We expected that laying queens from the 5-frame nuclei also would have heavier ovaries than banked queens. This was not the case, as the ovary weight of the 5-frame nuclei was not different from the banked queens.

It also was an important result that there were no differences between the performance of queens that were stored in either mass storage colonies or singly in 5-frame nucleus colonies. Similarly, there were no differences in brood (Pool et al., 1973) and colony development, or wax and honey production, (Avetisyan and Vasiliadi, 1967), in other queen storage studies in which stored queens were tested for differences in colony performance. This confirms the hypothesis that long-term winter storage does not decrease a queen's reproductive potential, at least for the following spring and summer season. Therefore, queens that survive for 6 months in

storage banks are potentially equal in reproductive capacity to those queens overwintered singly in a colony.

Significantly less brood was produced by queens from excluder cages in one study, in which six queens from one storage colony were tested for performance (Mitchell *et al.* 1985). We found no differences in performance between queens from excluder cages in colony banks and the control over two years. Our results were obtained by testing fourteen queens randomly selected from ten colony banks over two years. Our larger sample suggests that storage of queens in excluder cages does not necessarily effect their performance.

We found no correlations between queen weight and colony variables when these were measured in April and August respectively (1989 and 1990). Other studies have shown weak correlations between queen weight and colony brood production when queen and colony measurements were taken in the same time period (Boch and Jamieson, 1960; Nelson and Gary, 1983). This indicates that the weight of a queen may be an indicator of colony performance at the time of measurement, but is not necessarily associated with colony performance at a later date.

We found queen weight weakly correlated with ovary weight in 1989, and no correlation was found between these variables in 1990. Results in other studies vary from weak correlations (Avetisyan, 1961; Weaver, 1957) to strong correlations (Avetisyan *et al.*, 1967; Wen-Cheng, 1985; Woyke, 1987). It is unclear why the relationships between these variables differed between studies.

In this study, supersedure occurred in 8% (1989) and 11% (1990) of queens from all treatments (including the controls) during the spring and summer performance trials, similar to 8% supersedure reported for non-stored queens (Poole *et al.* 1973). In other studies, the supersedure rate was greater than 10% in the control or non-banked queens (20% in Mitchell *et al.*, 1985; 33% in Szabo, 1977b). Supersedure rates also were high for queens stored in excluder cages (42%, Szabo,

1977b: 83%, Mitchell et al., 1985) and screen cages (62%, Szabo, 1977b) in colony banks. Queens stored under laboratory conditions superseded at 63% (Poole et al., 1973) and 50% (Szabo, 1975b), and 29% became drone layers in another study (Nelson and Roberts, 1967). It is significant that the supersedure rate of queens from our control and our banked queens was less than 12 percent, which is significantly less than the supersedure rate of stored queens and most of the non-banked queens in other reported studies. The consistently low level of supersedure of both stored and non-stored queens in our study suggests that properly stored queens perform as well as colony queens, and that both queen types were of good quality using our methodology .

Our results demonstrate that queens can be stored for six months without any detrimental effect from the storage procedure when proper storage techniques are used. Canadian queen producers could produce queens during the summer, store them over the winter, and sell these queens early the following spring. Thus, Canadian producers could compete successfully with the more costly queens currently imported from Australia and New Zealand each spring. In addition, if queens from certain regions of Canada remain free of parasitic mites, in particular *Varroa jacobsoni* and *Acarapis woodi*, then the demand from U.S. beekeepers could increase, because the two mites have recently become endemic in the United States. The additional threat of Africanized bees moving into the queen rearing areas of the southern States can only increase the demand for queens from Canada, Australia and New Zealand. The results presented here, indicating good performance from overwintered banked queens, suggest that Canadian queen producers can now compete for the lucrative Canadian and export markets for spring queens.

4. ECONOMIC ASSESSMENT OF STORAGE SYSTEMS

4.1 Introduction

This economic analysis was prepared to evaluate the cost of overwintering mated queens in a colony bank, compared to the cost of overwintering one queen in a 5-frame nucleus colony. The methods of mass queen storage we have developed are biologically viable, but they also must be economically practical for the beekeeper. We evaluated the cost of storing queens in colony banks with (1) 24 (24Q) and (2) 48 (48Q) queens, and in the (3) mini-nucleus system. We only included the cost for syrup, pollen supplement, and queen cages as the material costs for queen production and winter storage. All other beekeeping supplies were assumed to be available within the beekeeping operation.

The time period covered in this study was from July in one year to April of the following year, and included the production of queens, preparation of colony banks, colony preparation for wintering, removal of banked queens, and the introduction of colony queens into the queenless colony banks in the spring. The cost of queen production and storage was obtained by estimating the hours of labour and materials required.

4.2 Methods

The total cost of storing queens was calculated by adding the cost of producing queens to the cost of winter storage. Methods and materials used in our queen storage study (1987-90) were used to estimate the hours required for each manipulation or procedure. Assumptions (Section 4.5) were made in relation to beekeeping techniques and costs that are usually part of the beekeeping operation. For example, we assumed that all beekeeping activities were carried out at the home

yard, and that travelling time and mileage was minimal and therefore not included in the costs. In these analyses we used 20 colony banks that had either 24 or 48 caged queens per colony bank. We used this number of colony banks because we assumed that the minimum number of queens required by a queen producer was about 240 (50% survival from 480 queens). Calculations for the cost of pollen supplement and sugar syrup are included in Section 4.6 on Production Data.

The cost of producing queens for storage in the fall was calculated by estimating the number of hours required to produce queens in the summer prior to winter storage (Table 5 and 6). Besides labour and feeding costs, the cost of two-hole queen-cages (1989 prices) were included in the cost estimate for queen production. We assumed that mating nuclei would require two feedings from June to mid-September. Labour was charged out at \$10 per hour. We estimated that 700 (1400) mating nuclei were required to produce sufficient queens to fill 20 colonies with either 24 (480) or 48 (960) queens per colony. The number of mating nuclei (700) is greater than the number of mated queens required in the fall (480), because mating success is never 100%. We estimated mating success to be 75%. Similarly, a greater number of queen cells are needed for 700 mating nuclei because cell acceptance is about 75%. We estimate that 960 (1920) queen cells are required for grafting. This would result in 720 (1440) cells for placement into 700 (1400) mating nuclei. The number of queens available for storage is 525 (1080) for a storage system that would hold 480 (960) queens. Queen production data were obtained from Phil LaFlamme who produced the test queens for Simon Fraser University in 1987-88 and 1988-89.

The total labour costs in hours for banking queens were calculated by subtracting the number of hours required to winter a colony with one queen, from the number of hours required to winter 24 queens in the 24Q storage system (Table 7). The estimate of labour for preparing and managing colony banks over the winter

was divided into colony management, queen introduction, colony feeding, and colony insulation. Colonies are managed with two-queens until banking begins, to provide sufficient workers for winter storage over 6 months. Insulated wrap was not included in the cost of wintering colony banks, although the wrap could be classed as extra equipment for wintering colony banks in areas such as coastal B.C.. Nevertheless, insulated wrap is necessary for colony banking to reduce cluster formation and prevent queen isolation from the workers of the colony during the winter.

Total labour and material costs for producing and storing queens (Table 8) were calculated by adding fall production (Table 5) to labour (Table 6) and material costs by using 20 colony banks with either 24 or 48 queens per colony.

The total cost of labour and material for setting up and managing sixteen mini-nuclei from late January to late March (Table 9) was calculated by estimating labour required for preparing nuclei, transferring queens from colony banks to the nuclei, and feeding colonies. Syrup and pollen supplement were the only material costs included in the cost of using mini-nuclei.

Cost estimates for queen storage were calculated from labour and material costs needed for preparing and managing twenty colony banks (Table 10). Colony banks had either 24 or 48 queens per bank. From these cost estimates further projections were made for larger production and storage trials.

4.3 Results

The cost to produce 480 queens (24 queens in each of 20 colonies) was \$1.95 per queen (Table 6). The cost was slightly less for one queen when 960 queens were produced (\$1.89 per queen). This small difference in costs between the two scales of production occurs because labour hours are the same per queen except in the preparation of cell starters, finishers and breeders which was the same for both

production levels. Labour constituted about 20% of the production cost for both levels of production.

The number of hours (74.7) required to prepare and manage 20 colony banks was about twelve times the number of hours required for overwintering one queen in twentyt 5-frame nucleus colony (6.5 hours). Thus, the number of extra hours needed for storing queens in colony banks was 68.2 hours over the entire winter period (Table 7).

Labour hours constitute a significant portion of the total costs required for producing and storing queens, 43% and 35% of the total labour and material costs for the two levels of production and storage (calculated from data in Tables 5 and 7). The total cost per queen for fall production and wintering is \$6.09 and \$8.47 for 48Q and 24Q per colony bank respectively, assuming 50% survival (Table 8).

The total cost of labour and material for setting up and managing sixteen mini-nuclei from late January to late March is \$107.64 (Table 9). The cost for each queen is \$6.72. A large proportion of this cost (88%) is for labour; the remainder is the cost for syrup and pollen supplement.

4.4 Discussion

Mass queen storage is economically viable for the beekeeper because the total cost of production and storage is less than the total revenue based on a sale price of \$10 per queen. If the beekeeper uses his own labour and does not hire outside labour, an additional 40% of the production costs or labour income goes to the beekeeper. Profits can be increased further when a greater number of queens are stored in a colony bank, and if larger numbers of queens survive storage.

We estimated an average profit value for a beekeeper using our "best" system; that is, one that we have demonstrated is biologically feasible and produces consistent results. We used the 48Q system as "best" since that system yielded the

best survival, but we also calculated profits from the 24Q system for comparison. A survival value of 66% was used in the calculations, since that was the average survival in all screen cage treatments in our study. We examined profits by extrapolating from 20 to 100 colony banks (Table 8), but it was unrealistic to expect the beekeeper to do the labour required for the production of 4800 queens. The number of mating nuclei one beekeeper can manage is about 1000 nuclei without additional labour. Thus, in both calculations, labour cost for queen production are included in the total costs; in other words, profits are income above the cost of queen production. This is an important aspect of the analyses, because the most significant proportion of queen production and wintering cost is labour, which in most commercial beekeeping operations would stay within the beekeepers operation. The remaining cost is largely food.

We calculated profits for queen storage by examining a commercial beekeeping operation that banked 4800 queens in 100 colony banks, that (1) hires labour for production and storage phases and (2) hires labour only for production of queens and has the beekeeper perform colony bank management. We assumed that queens are sold for \$10 each in April. When the beekeeper hires help for all the work, queen production and storage, the profits were \$13,215 (Table 10). When the beekeeper hires labour for queen production only, but does the labour for colony bank preparation and management, profit is increased to \$16,625 (labour for preparing and managing colony banks is 23% of total cost). These calculations indicate that queen storage is economically viable.

Both the level of queen survival and the number of queens per colony bank alter the expected profit (Table 10). When larger number of queens survive storage, the increase in profits are substantial (Fig. 17). These data indicate that, at a level of 50% survival, with labour costs included, returns are minimal (\$835) when only 24 queens are placed into a colony bank. On the other hand, the profits for queens

surviving in the 45Q system with a 50% survival are \$8.415. In the 48Q system, a further 64% increase in profit results from an increase in queen survival to 60%, and even greater profits of \$18.015 can be expected if survival is 70% or greater in the 48Q system. These potential returns would increase if even greater numbers of queens are banked. However, the 70% level of survival was not reached in our storage study. The highest survival rate in any one system was 63% (in this study 1988-89). Clearly, even minor improvements in survival would dramatically improve the economic returns from surviving queens.

Viable beekeeping operations are usually broad-based, and income usually comes from other sources than solely from the sale of spring queens. Additional income would result from honey production using the 100 colonies prepared for colony banks.

Colony banks would be useful for both small and large beekeeping operations. The smaller operator with 100-200 honey producing colonies could produce queens for re-queening these colonies, and save the cost of buying spring queens. The use of colony banks in operations larger than what we have indicated would also be possible; there would be higher labour costs, but also greater profits.

The mini-nuclei system costs a further \$6.72 to bank each queen, increasing the total cost of producing and wintering queens to \$15.19 per queen (\$8.47 + \$6.72). Thus, mini-nuclei are not an economical method for storing queens. However, the cost of setting up and managing mini-nuclei is largely labour (88%), which is a greater proportion of labour costs than the labour required for colony banks (42%). If the beekeeper performs his own labour, returns can be increased by labour costs. Therefore, the mini-nuclei could be more economical if the beekeeper performs his own labour. In our cost analyses we did not include the cost of the mini-nuclei and have assumed that these are available in the beekeepers operation. However, the mini-nuclei could be classed as special equipment and may not be found in the

beekeeper's operation, unless these nuclei (4-way nuclei) are used as mating nuclei in the spring and summer. The main advantage of the mini-nuclei is that mortality for the period from late January to late March, is lower (6%) than if the queens are kept in colony banks (16%). Overall, the utility of the mini-nuclei is limited for economic reasons. Further, the mini-nuclei can only be used in locations with moderate winter climate such as coastal British Columbia or warmer climates south of Canada.

These results demonstrate that mass queen storage with 48 queens per colony bank, a biologically viable system, is the most economical system for the beekeeper. This system uses beekeeping equipment that is already used in beekeeping operations, and therefore requires no additional equipment. The economic viability of queen storage and the significant returns that are possible from queen storage would allow the beekeeper to expand his business, and specialize in producing and supplying queens, in addition to being involved in a variety of beekeeping activities such as honey, pollen, and bee production, and pollination. The availability of competitively priced queens from Canadian producers throughout the spring and summer could boost demand throughout this period, providing a more stable income for the queen producer. Canadian queen producers can now compete for the lucrative Canadian and export markets for spring queens. Future research into colony banks will undoubtedly improve queen survival and further increase returns for the beekeeper.

Figure 17: A comparison of profits when all labour costs for queen production and storage are included in the total costs, and when labour for queen production only is included in the total costs of queen production and storage. These data are for 100 colony banks with 24 or 48 queens per bank, when survival is 50%, 60%, or 70% after winter storage.

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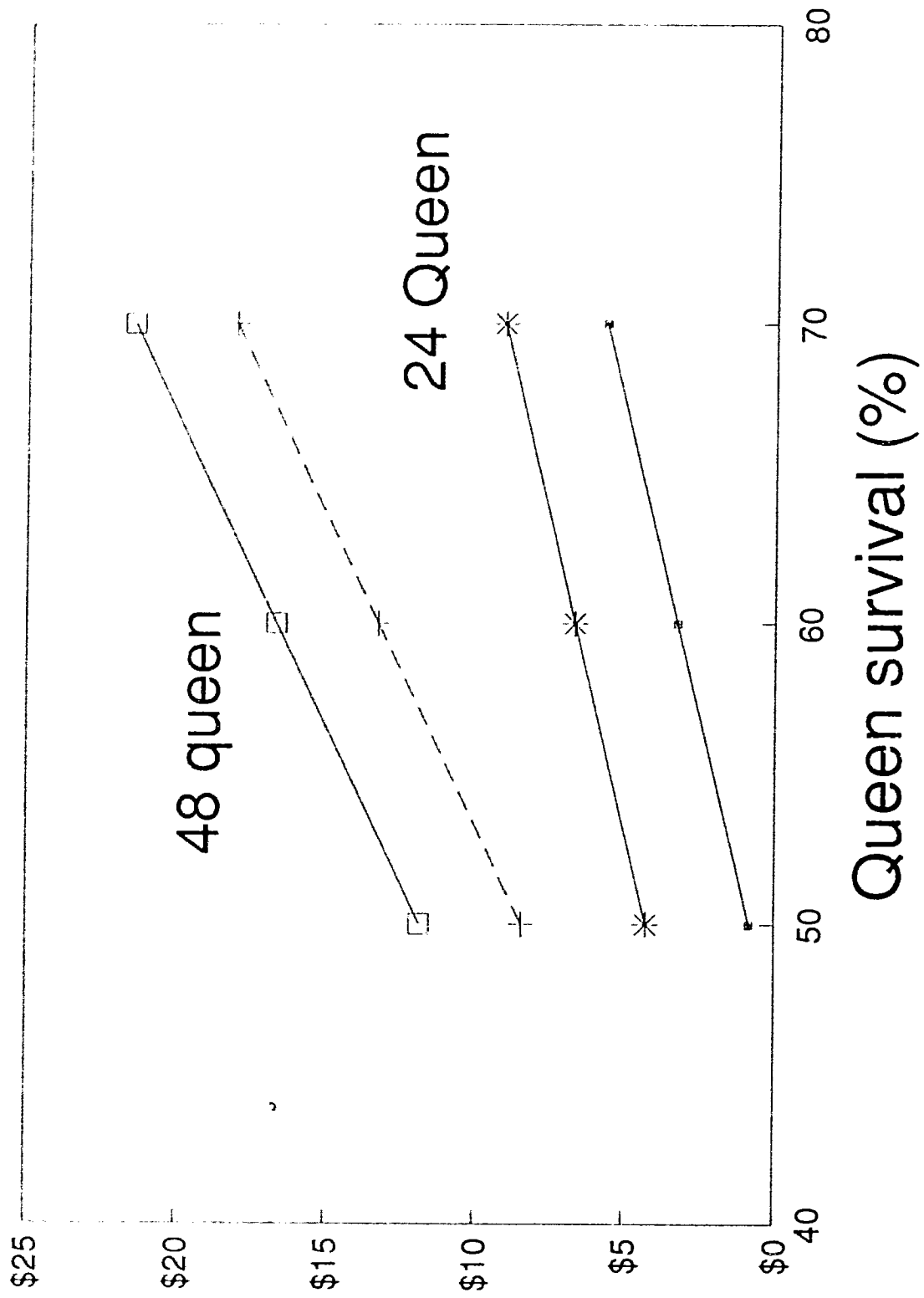


Table 5: Labour costs for preparing nuclei and producing sufficient queens to stock 700 (1400) mating nuclei in mid-June. Labour costs noted in hours.

DETAILS	<u>HOURS</u>	
	<u>Number of Nuclei</u>	
	700	1400
1. Feeding syrup to 700 nuclei to 1400 nuclei	3	6
2. Dipping cups (approx 300 per hour) 960 cups 1920 cups	3.2	6.4
3. Preparing 8 cell starters, 16 cell finishers, and 3 breeders	5	5
4. Collecting grafting frames, grafting, placing cups in cell starters. Grafting 480/day: (700)- for 2 days (1400)-for 4 days	3.2	6.4
5. Moving cells from starters to finishers. (700) (1400)	.5	1.0
6. Cutting cells from bars, culling, and placing cells in carrier (700) (1400)	1.0	2.0
7. Cells to nuclei (700) (1400)	3.0	6.0
Total (hours)	<u>18.9</u>	<u>32.8</u>

Table 6: Production costs for preparing nuclei and producing sufficient queens to stock 700 (1400) mating nuclei in mid-June.

DETAILS	<u>COSTS</u>	
	<u>Number of Nuclei</u>	
	700	1400
Labour from Table 5 18.9 and 32.8 hours @ \$10/hour	\$189	\$328
MATERIALS		
1. Feeding 0.5 L of syrup to mating nuclei twice @ \$.90/L -700 X 2 X 0.5l X \$.90 = \$630 -1400 X 2 X 0.5l X \$.90 = \$1260	\$630	\$1260
2. Cost of 2-hole cages in 1989: 1000 cages for \$170 + shipping \$34= U.S.\$204= Can\$ 240 unit price=0.24c. for 480 for 960	\$115.20	\$230.40
<u>TOTAL FALL PRODUCTION COSTS</u>	<u>\$934.20</u>	<u>\$1818.40</u>
Cost per queen (for 480) (for 960)	\$1.95	\$1.89

Table 7. Labour costs for preparing and managing (1) twenty colony banks with 24 or 48 queens (24Q or 48Q), prepared from two-queen colonies¹, and (2) twenty 5-frame nucleus colonies with one queen (1Q). Labour cost are noted in hours.

TIMING OF COLONY MANAGEMENT	LABOUR (Hours)	
	24Q/48	1Q
COLONY PREPARATION		
July, early		
1. Preparation and moving 1-super colonies	1	-
2. Unloading	1	-
3. Prep. of bottom colony	1	-
- add excluder and newspaper over brood chamber, and below honey super.		
July, 2nd and third week.		
1. Check for queen in upper colony	1	-
2. Super with honey supers (twice)	2	2
August, early		
1. Remove honey supers		
2. Reduce to 4 supers	2	2
August, 24 (3 weeks prior to Q intro)		
1. Prep. of 84 pollen patties	1	-
2. Feeding pollen patties (2 per colony)	1	-
3. Modify frames for holding queen cages (one time cost only)	10	-
Sept, 14		
1. Feeding one pollen patty /colony	1	-
2. Preparation of sugar syrup		
10 L/ colony	1.5	1.5
3. Feeding sugar syrup	1	1
Sept, 21		
1. De-queen and assess colony size at 20 min./ colony (20 mins X 28 colonies ¹)	9.2	-
Sub total	32.7	6.5

1. We assume that 28 two-queen colonies are required to obtain 20 colony banks with adequate worker population in the fall.

continued on next page

Continued from previous page.

TIMING OF COLONY MANAGEMENT	LABOUR (Hours)	
	24Q/48Q	1Q
Sub-total from previous page	32.7	6.5
QUEEN INTRODUCTION		
Sept, 22		
1. Transfer queens from mating -nuclei to queen cages. For 480 queens at 20 -nuclei per hour.	24	-
2. Transfer queen cages into queen banks	2	-
Sept, 27		
1. Check for queen cells on marked brood frames (12 min. per colony).	4	-
COLONY FEEDING AND INSULATION		
October, 4 and 27		
1. Prepare sugar syrup	2	-
Two feedings of 10 Litres/ colony	1	-
Feeding sugar syrup	1	-
October, 25		
1. Preparation of insulated wrapping ¹	-	-
2. Wrapping 20 colonies in 4-packs ¹	-	-
March, 31		
1. Removal of queens from storage colonies at 15 mins / colony assuming 50% survival in a 24 queen colony bank ²	5	-
2. Requeening of storage colonies at 10 mins / colony	3	-
<u>TOTAL HOURS</u>	<u>74.7</u>	<u>6.5</u>

1. Insulated wrapping was prepared as outlined in the Agriculture Canada publication (1984). Four colonies are wrapped back to back in a four pack. The wrapping lasts for about five years.

2. Queens from colony banks are used by the beekeeper for requeening, making packages or nucleus colonies. Therefore this does not include labour for adding attendants to queen cages.

Table 8: Total labour and material costs for fall production and queen winter storage using twenty colony banks with 24 and 48 queens per bank.

DETAILS OF COSTS	DOLLARS	
	24Q	48Q
FALL PRODUCTION COSTS from Table 6.		
1. Fall production and feeding costs for 480 queens=	<u>\$934.20</u>	
costs for 960 queens=		<u>\$1818.40</u>
WINTER STORAGE COSTS		
1. Labour costs for 20 colony banks minus total hours for overwintering 20 colonies with 1Q (Table 8). 74.7hrs - 6.5hrs = 68.2 X \$10=	\$682	\$682
2. Pollen patties 24 August: Two pollen patties per colony @ \$.68/patty \$.68 X 2 X 28 colonies=	\$38.08	\$38.08
14 September: One pollen patty per colony @ \$.68/patty X 28 colonies=	\$19.04	\$19.04
3. Sugar syrup. 14 September: no cost ¹ 1. 27 October: Two feedings of 10 Litres/ colony for 20 colonies @ \$.90/Litre 2 X 10 X 20 X \$.90=	\$360.00	\$360.00
Cost of wintering 480 with 240 queens surviving ²	<u>\$1099.12</u>	
Cost of wintering 960 with 480 queens surviving ²		<u>\$1099.12</u>
TOTAL WINTERING AND FALL PRODUCTION COST	\$2033.32	\$2917.52
COST / SURVIVING QUEEN	\$8.47	\$6.08

1. Cost of sugar syrup for 14 September is not included in the winter costs because both treatments are fed sugar syrup at this time.

2. We assume 50% survival of 480/960 queens.

Table 9. Total cost of labour and material for setting up and management of 16 mini-nuclei from late-January to late-March.

DETAILS	HOURS	COSTS
LABOUR		
29 January		
1. Preparation of nuclei by dismantling consolidated -nuclei		
2. Insulating colonies	4	
3. Preparation of insulation for stacked nuclei (one time cost)	3	
30 January		
1. Pulling queens from storage colonies at 5 mins per colony, and introduction of queens into mini-nucs	1.5	
7 February		
1. Manual release of queens @2 mins/queen	.5	
2. Queen check 1 week later	.5	
TOTAL HOURS	<u>9.5hrs</u>	
TOTAL COSTS		
1. Labour:		
9.5 hours X \$10 per hour	=	\$95
2. Syrup .5 Litre/colony for 16 colonies @ \$.90/Litre		
=.5 X 16 X \$.90	=	\$7.20
3. Pollen supplement @ half a patty per colony, for 16 colonies @ \$.68/patty		
=.5 X 16 X \$.68	=	<u>\$5.44</u>
TOTAL COSTS FOR 16 MINI-NUCLEI	=	<u>\$107.64</u>
MINI-NUCLEI WINTERING COST / QUEEN	=	<u>\$6.72</u>

Table 10: A comparison of profit for queen production and storage in 100 colony banks when costs include (1) all labour (column 5/6) and (2) labour for queen production only (column 7/8). 24 or 48 queens are banked per colony when survival is 50%, 60%, or 70% after winter storage.

1	2	3	4	5	6	7	8
Queens/ colony bank	Queens into storage	Queen winter Survival (%)	Queens alive after storage	Total cost ¹ (labour & mater- ials)	Profit ² (4x\$10)-5	Total ³ cost (part- labour & materials)	Profit (4x\$10)-7
24	2400	50%	1100 ⁴	\$10,165	\$835	\$6,755	\$4,249
24	2400	60%	1340	\$10,165	\$3,235	\$6,755	\$6,645
24	2400	70%	1580	\$10,165	\$5,635	\$6,755	\$9,045
48	4800	50%	2300	\$14,585	\$8,415	\$11,175	\$11,825
48	4800	60%	2780	\$14,585	\$13,215	\$11,175	\$16,625
48	4800	70%	3260	\$14,585	\$18,015	\$11,175	\$21,425

1. Total labour and material costs for queen production and queen winter storage for 100 colony banks (total wintering and fall production cost x 5 from Table 8). It is assumed that labour does not increase with increasing survival of queens.

2. We assumed that queens are sold for \$10 each in April.

3. These costs include part of the labour and all materials. Labour for queen production is included in the costs whereas the labour costs for queen storage (\$682 x 5 from table 8) is not included.

4. We assumed that 20 queens are removed from 240 queens (50% of 480) to re-queen the 20 colony banks.

4.5 Assumptions

(1) Colony banks

1. Time estimates used in preparing cost of labour data were based on the actions of experienced beekeepers. Estimates would be low for inexperienced beekeepers.
2. Only the cost of materials and supplies of winter queen storage, over and above the cost of overwintering a colony, were included in this study. Other costs such as repairs, maintenance, taxes, insurance, and travelling costs, as well as allowances for depreciation and interest, were not included. These costs are already included by the operation whether or not queens are stored over the winter.
3. Colony banks were prepared by two-queening colonies at the beginning of the honey flow to produce populous colonies by September as described in Section 2.3.1.1.
4. All the necessary equipment and expertise is available because the beekeeper is already in the queen production business.
5. Honey production and the management of colonies is the same for both one-queen and two-queen colonies.
6. Colony banks are used only for honey production after removal of banked queens. These colonies are not used for pollination, queen rearing, or package production.
7. All equipment is standard beekeeping equipment and no extra equipment is required for overwintering queens. It is assumed that overwintered colonies are wrapped with insulation for winter, and therefore there is no extra cost for wrapping colony banks. Colony banks were insulated as described in Section 2.3.1.1.
8. All queen rearing activities take place at the home location.
9. Production costs for summer queens include only labour costs and feeding costs.

(2) Mini-nuclei

1. Mini -nuclei are prepared from dismantled, consolidated nucleus colonies.
2. Consolidated nucleus colonies are prepared as described in Section 2.3.1.3.
3. Sixteen queens are obtained from a 24-queen storage bank in January,

assuming a mortality of 20%.

4.6 Production Data

1. Pollen supplement mixture is approximately 10% pollen by weight. A typical batch for 75 - 500gm patties consists of:

8.6 kg sugar @ \$32.00/40kg	=	\$ 6.88
8.6 kg water		
12.7 kg brewers yeast @ \$1.10/kg	=	\$ 13.97
3.2 kg pollen @ \$8.81/kg	=	\$ 28.20
Wax paper @ \$3.00/30m	=	<u>\$ 2.25</u>
TOTAL	=	\$ 51.30

Therefore the cost of materials/patty is \$0.684

2. Sugar syrup consists of a two-part sugar to one-part water, by volume.

Terramycin and Fumagillin B for the control of bee diseases are added at a rate of one teaspoon for each 4 L of syrup. Recommendations for Terramycin are based on Terramycin Animal Formula-25 which is a water soluble powder containing 25 g active ingredient in 400 g carrier. The recommended dosage for Fumagillin is 100 mg /4.5 L of syrup. The following materials are used for each 45 L of syrup:

40 kg sugar	=	\$32
10 tsp terramycin @ \$.36/tsp	=	\$ 3.60
10 tsp Fumagillin B @ \$.50/tsp	=	<u>\$ 5.00</u>
TOTAL	=	\$40.60

Therefore cost of materials per L is \$0.90

3. Winter Insulation Wrap. Complete details are available in the Alberta Agriculture publication Agdex 616-21, 1984. For a discussion on this method see Canadian Beekeeping, Vol 7, No.3. Materials required for wrapping four colonies placed back to back are as follows:

Black polyethylene sheet, 6 mil., 4.42m (14.5') long by 1.83m (6') wide

Pink fibreglass insulation, 3.5 batts, R12, 4.27m X 58cm (14' X 23").

Plywood used as a common lid, 1cm X 1.23m X 1.83m (3/8" X 4' X 6').

Plywood with holes to correspond to the upper entrance of the colonies,

7.6cm X 12.7cm X 1cm (3/8" X 3" X 5")

5. RECOMMENDED STORAGE METHOD AND CONCLUSIONS FOR THE BEEKEEPER

5.1 Description

The colony bank for mated queens consists of a queenless colony with banked queens held in cages placed in the center of the upper hive body. The hive consists of a minimum of 18 frames or two supers. Queens are caged in wooden cages screened with bee-proof screen on one side, that allows adult workers to feed and attend the caged queens. Queen cages were held back to back in a wooden framework fixed within the center frame. We successfully stored up to 48 queens in a colony bank. The frame of caged queens remains in the same colony bank throughout the storage period.

Three major areas of colony management were identified as critical to the success of queen survival through the winter:

1. Large adult worker population.
2. Adequate honey stores.
3. Prevention of winter cluster formation.

A large worker population is essential for queen storage over the winter, due to the lack of a colony queen to replenish the worker population. In addition, a large worker population is essential to ensure that workers do not cluster and withdraw from some of the stored queens. We produced colonies with surplus workers by creating a two-queen colony from a one super and a two super colony in July, at the start of the honey flow. Supering was done as required and by September, the number of supers were again three. The colony queens were removed at introduction of the banked queens. Colonies were placed in a 4-pack configuration and insulated on all sides including the bottom of the colony, to prevent colony workers from forming a winter cluster and withdrawing from the banked queens.

The minimum requirement for a bank is a 3-Langstroth deep-super colony with adequate reserves of pollen and honey that are properly organized within the brood nest, plus a large worker population (minimum of 20 frames of workers). Twenty-four hours prior to installing the banked queens both colony queens are removed and the bank remains as a queenless unit (no free-running colony queen).

5.2 Preparation of Colony Banks.

This method describes the preparation of colonies as queen banks, and is suitable to our management system on the coast of British Columbia. For cost effectiveness, the preparation of colonies must be adapted to the individuals' management system.

(1) Colony preparation

1. Prepare a pallet platform for colonies in groups of four. Place each colony on an empty super filled with insulation (on top of the pallet).
2. The first colony manipulation involves making a two queen colony prior to the summer honey flow. In early July, combine a standard 20 frame colony with a 10 frame colony. Keep the two colonies separate with a honey super sandwiched between two queen excluders.
3. Add extra supers to colonies as required during the honey flow.
4. Ensure that adequate stores are present in the colony by allowing workers to take honey stores from the extra supers into the brood chambers.
5. Place two pollen patties (one below and one above the excluder) in each of the two queen colony during August to stimulate brood production.
6. Reduce the number of supers of each two-queen colony to three by mid-September.

7. Feed syrup with Fumagillin and Terramycin by using a top feeder in September, to ensure that empty brood cells are filled with syrup, and that honey frames are well filled with syrup.

(2) Colony preparation 24 hours before queen introduction.

(mid to late September):

1. Remove the two colony queens.
2. Check for adequate amounts of brood in both colonies (20 frames with brood).
3. Choose colonies for banks that contain a minimum of 20 frames with brood, honey and pollen, and the remaining frames (five) with honey.
4. Create space for the queen bank frame by removing two frames in the upper third super. Remove frames with the least pollen and honey stores.
5. Use thumb tacks to identify brood frames for queen cell removal after one week.

(3) Queen introduction

1. Place queens in queen cages (2.5 mm mesh) without attendants.
2. Place up to 48 cages in a banking frame.
3. Lightly smoke colony at the upper and lower entrances. We recommend that honey or syrup spray not be used on the queen cages because it can encourage robbing.
4. Insert bank frame slowly between the colony frames.
5. One week after introducing banked queens, cut all queen cells from brood frames.

(4) Colony Maintenance

1. Reduce the number of supers to two by early October.
2. Feed colonies with syrup plus Fumagillin using a top feeder in early and late October. In most areas of Canada it is too cool by October to feed syrup.

Therefore colonies must be fed as much as possible just before and after queen bank introduction in September.

3. Remove top feeders, and replace with inner cover with an entrance.
4. Insulate (minimum R12) and wrap the four colonies as a unit including the top of the colonies.
5. Cover the insulated unit with a sheet of plywood to protect the insulation, and tie down with large rubber bands (inner tubes). See the Alberta Agriculture publication (Agdex 616-21) "A polyethylene insulated wrap for overwintering honey bee colonies out of doors in Alberta". This publication can be obtained from the Media Branch, Alberta Agriculture, 7000 -113 Street, Edmonton T6H 5T6.

5.3 Conclusions

1. Queen banking is both biologically and economically feasible. The level of queen survival determines the expected profits.
2. The performance of banked queens is not altered by the banking process.
3. Queen storage would be useful to both the small and large beekeeping operations, to bank queens until required for up to 6 months.
4. Canadian queen producers could produce queens during the summer, store them over the winter, and sell these queens early the following spring. Thus, Canadian producers could compete successfully with the more costly queens currently imported from Australia and New Zealand each spring. In addition, if queens from certain regions of Canada remain free of parasitic mites, in particular *Varroa jacobsoni* and *Acarapis woodi*, then the demand from U.S. beekeepers could increase, because the two mites recently have become endemic in the United States.

6. GLOSSARY OF APICULTURE TERMS

American foulbrood - A brood disease of bees caused by the bacteria *Bacillus larvae*.

Apiarist - Beekeeper.

Apiary - The location and sum total of colonies, hives and other equipment assembled on one site for beekeeping operations.

Apiculture - The science and art of raising honey bees for economic benefits or personal enjoyment.

Attendants - Adult worker bees that attend the queen.

Apis mellifera - The common honey bee found throughout the western world though originally from the near-east. Humans have carried *Apis mellifera* from Europe to all continents.

Balling a queen - An attack on a queen by a number of worker bees intent on killing her by pulling at her legs and wings, stinging and suffocation. In this process the bees form a tight ball of bees around the queen.

Bank - A laboratory or colony type storage system for honey bee queens.

Bee - See *Apis mellifera*

Beehive - Domicile for colony of honey bees.

Beekeeper, commercial - A beekeeper managing 200 or more hives, as a main or sole source of livelihood. He or she generally sells his or her crop in bulk.

Bee space - The natural space found between combs built by bees. This space is big enough to permit free passage of bees. It measures 8mm (5/16"). Spaces larger than bee space will be filled by bees with burr and some comb or full honey comb. Spaces less than a bee space will be sealed by bees with propolis. Langstroth applied this observation to North American hive equipment construction.

Breeder - A colony selected for young larvae production.

Brood - Immature or developing stages of bees; includes eggs, larvae and pupae.

Brood chamber - The part of a hive where the brood is reared and food is stored. May include one or more hive bodies and the combs within.

Brood comb - Wax comb from the brood chamber of a hive that contains brood.

Brood food - Secretion of highly nutritious food, used to feed young larvae and queens, produced in the hypopharyngeal glands in the heads of adult bees.

Brood nest - Area of hive where bees are densely clustered and brood is reared.

Battery boxes - Partially screened cardboard boxes that hold queens in queen cages. These queens are fed by free running workers that feed from candy located in the bottom of the box.

Candy - See queen cage candy.

Capped brood - Brood whose cells have been sealed by the bees with a porous cover to isolate the immature bees within, during their non-feeding prepupal and pupa periods.

Capped honey - Honey stored in sealed cells.

Castes - The three forms of bees; workers, drones and a queen comprising the adult population of a honey bee colony.

Cell - Single unit of space in comb in which honey is stored or a bee can be raised; there are about 4 worker cells per square cm of comb.

Cell starter - A queenless colony used in the initial stage of queen cell production.

Cell finishers - A queenright colony used to finish cells begun in cell starter colony.

Chilled brood - Immature bees that have died from being too cold.

Cluster - The form or arrangement of bees within a hive when ambient temperature drops below 14°C.

Colony - An aggregate of several thousand worker bees, drones and a queen bee living together in a hive or in any other dwelling as one social unit.

Colony storage system - A colony that hosts individually caged queens.

Comb - A back-to-back arrangement of two series of hexagonal wax cells which hold eggs, brood, pollen or honey.

Comb foundation - Manufactured sheets of beeswax with the foundation pattern of worker cells embossed into the wax.

Cups - Wax cups that hold the young larvae after grafting. Cups when fully developed become queen cells.

Deep super - A super used to hold standard, full-depth frames; the usual depth is 241 mm or 244 mm (Canada).

Dividing colonies - A beekeeping technique of splitting one colony to produce two or more colonies.

Drawn combs - Combs having the cells built out by honey bees from a sheet of bees wax or plastic foundation.

Drifting - The tendency of bees to transfer to colonies other than their own because of a lack of visual cues to orient them to their "home" hive.

Drone - Male bee.

Drone layers - Queens that only produce unfertilized eggs or drones.

Emerging brood - Young adult worker bees that emerge from their cells.

Emergency queen cells - Queen cells that are produced when colony is queenless.

Entrance blocks - Pieces of wood used in regulating the size of hive entrances.

European foulbrood - A brood disease of bees caused by the bacteria
Streptococcus pluton.

Excluder - See queen excluder.

Excluder queen cage - A cage made from wooden strips fixed into the honey comb, and covered with excluder material.

Feeder / top feeder - A wooden container that holds syrup for feeding a colony of bees. It fits on top of a super. Also called a Miller feeder.

Fertile queen - A queen which has been inseminated artificially or naturally with drone spermatozoa and is capable of laying fertilized eggs.

Field bees - Worker bees which are usually 21 or more days old and work in the field to collect nectar, pollen, water and propolis.

Foundation - See comb foundation.

Frame - Four pieces of wood designed to hold honey comb. It consists of one top bar, one bottom bar and two end bars.

Frame feeder - A wooden or plastic trough which is hung in a hive in place of a frame and contains a solution of syrup to feed bees.

Free running queen - A queen that is not confined to a cage and has the freedom to move in the hive.

Free-flying - A colony with an exit to the outside.

Fumagillin - The common name for Fumidil-B, the antibiotic for controlling nosema disease.

Grafting - A technique for transferring larvae from brood comb to wax cups to produce queens.

Grafting frames - Modified frames that hold two or three bars with queen cups, or queen cells.

Hive - A constructed home for bees.

Hive body - A wooden box which encloses the frames.

Honey bee - The common name for *Apis mellifera*. Order Hymenoptera, Family Apidae.

Honey flow - See nectar flow.

Hormone - Substance produce in small quantity in/on part of body (usually in gland of internal secretion) and transported to other parts, where it exerts its action.

Hymenoptera - An order of insects to which all bees belong, as well as ants, wasps and sawflies.

Inner cover - A lightweight cover used under a standard telescoping cover on a bee hive.

Introduction unit.- Part of a colony that is prepared to accept queens that will increase the likelihood of queen acceptance.

Introduction Techniques - Techniques to improve the probability of success in introducing a queen (s) into a colony.

Laboratory queen banks - A system of storing queens without a colony. Queens are either kept in isolation or held with some adult workers.

Langstroth frame - A frame measuring 45 cm long by 23 cm deep, exclusive of the lugs or shoulders that support the frame.

Langstroth hive - Hive with movable frames. Dimensions such that the beespace is allowed between surfaces.

Larva - The second stage in the complete development of an insect, such as the honey bee, having complete metamorphosis or four stages - egg, larva, pupa, adult.

Mandibles - The jaws of an insect. In the honey bee and most insects the mandibles move in a horizontal plane.

Manipulation of colonies - Using hive equipment to the best advantage in aiding colony development.

Mating nuclei - Nuclei specifically used for mating virgin queens.

Mailing cage - A wooden cage with bee-proof screen for holding a queen (and her attendants).

Mini-nucleus - A small Langstroth colony consisting of four frames (16 x 19.7 cm) and a feeder.

Movable frame - A frame or comb constructed on the principle of the "bee space". When placed in a hive it remains unattached to its surrounding by pieces of burr comb or heavy deposits of propolis, and is therefore, easily removed for inspection.

Nectar flow - The production of nectar by various plant species sufficient that bees gather and store the nectar and convert it to honey.

Nosema disease - Disease of adult bees caused by the protozoan, spore-forming, mid-gut parasite *Nosema apis* Zander.

Nucleus/nuclei - A small colony of bees often used in queen rearing or mating.

Nurse bees - Young worker bees that feed larvae.

Overwintering Queens - A method of storing queens other than singly in a colony.

Package bees - Two or three pounds of bees which are shaken from combs of beehives into wire mesh cages. A caged, mated queen is placed in each package. Packages are used to establish honey bee colonies.

Pheromone - A chemical secretion released externally by one animal which stimulates a response in a second animal of the same species.

Pollen - The male sex cells of plants gathered by worker bees from the anthers of flowers. Pollen provides protein, minerals, fats and vitamins which are consumed by the young nurse bees, that in turn produce brood food or "royal jelly".

Pollen substitute - A food material which is used to substitute wholly for pollen to supply the needed protein, vitamins and fats to a bee's diet. It includes water, sugar and other materials such as brewer's yeast.

Pollen supplement - A mixture of pollen and other protein sources along with sugar and water, fed to colonies to supplement their protein needs.

Propolis - Resins and gums gathered from trees and used by bees in sealing cracks, repairing cracks, and covering objectionable material within the hive.

Pupa - Third stage in the development of an insect having complete metamorphosis. In this stage the organs of the larva are replaced by those which will be used as an adult.

Queen - Sexually developed female bee, larger and longer than a worker bee.

Queen banks - Colonies in which caged queens are placed for storage until use.

Queen banking frame - A modified frame that holds queen cages or consists of compartments inserted into the wax foundation.

Queen cage - A small cage in which a queen and five or six worker bees may be confined for shipping and / or introduction into a colony.

Queen cage candy - Candy made by kneading powdered sugar with invert sugar syrup until it forms a stiff dough used in queen cages.

Queen cell - A cell that contains an immature virgin queen.

Queen excluder - A screen device made of wire, zinc, or plastic, which has openings of about 5 mm that permit workers to pass through the screen, but exclude queens and drones. It is used to confine the queen to a specific part of the hive or compartment/cage.

Queen introduction - See Introduction Techniques

Queenless - Without a queen.

Queen mandibular pheromone - A blend of compounds from the queen mandibular gland that elicits retinue behavior, attracts swarms, and suppresses queen rearing.

Queen mother colony - A colony chosen to provide larvae for grafting into queen cells.

Queen producer - A beekeeper who specializes in producing queens.

Queenright - With a queen.

Queen storage - A method of storing queens in colony queen banks or laboratory banks.

Requeening - Adding a queen to a queenless colony.

Robbing bees - Bees which enter colonies other than their own in order to remove honey and carry it to their own hive.

Royal jelly - A highly nutritious glandular secretion produced by young adult worker bees; it is used to feed the queen and young brood.

Screen cages - A queen cage that consists entirely of bee-proof wire screening.

Sealed brood - Brood in late larval and pupal stages with cells sealed.

Shaking - The process of placing bees from parent colonies into packages.

Smoke - A method of calming adult worker bees into a less aggressive behavior prior to colony manipulation such as queen introduction.

Storage systems - See Colony Storage Systems.

Stores - Provisions of honey and pollen that are found in honey comb of a hive.

Supers - A hive box or hive bodies with or without frames.

Supersedure - Replacement of the existing queen by a young queen produced by the bees within the colony from their own larvae or eggs.

Swarm - The aggregate of worker bees, drones and a queen that leaves the mother colony to establish a new colony. Swarming results in the division and dispersal of honey bee colonies.

Syrup - A sugar solution that is fed to colonies and provides a carbohydrate source for the colony.

Terramycin - A trade name of oxytetracycline, a drug commonly used to prevent European and American foulbrood.

Thermoregulation of a colony - The regulation of heat by adult worker bees by clustering together in winter. Heat is generated and loss of heat from the cluster is prevented by clustering.

Thorax - The middle region of the bee's body that carries the wings and legs.

Two queen management - A method of managing a colony of bees to increase the number of adult workers to about twice that of a single queen colony.

Unsealed brood - Brood in egg and larval stages only.

Virgin queen - Unmated queen.

Winter packing - Enclosing the hive with materials that decreases heat loss from cold temperatures and windy conditions, e.g. tar paper, insulated wrap.

Wire screen cages - see Queen cage.

Wooden queen cage - Wooden cage covered on one side with bee proof screen.

Worker bee - A female bee whose organs of reproduction are only partially developed and that is responsible for carrying on all the routine work of the colony.

Worker comb - Honeycomb with about 4 cells / cm²

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