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INFLUENCE OF LONG TERM STORAGE ON THE NUTRITIONAL VALUE OF FROZEN POLLEN FOR BROOD REARING OF HONEY BEES

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SUMMARY

Pollen stored at different temperature conditions and for various time periods was fed to confined small colonies of newly emerged honey bees. Brood production was maintained for long periods of time by bees feeding on diets consisting of either fresh, freshly frozen or dried and subsequently frozen pollen. However, total food consumption by caged honey bees was not proportional to the number of bees reared.

Pollen collected in 1967, dried and stored frozen for 11 years was still adequate for brood rearing. Thus, pollen dried and subsequently frozen will retain a considerable amount of its nutritional value for honey bees over a long period of time.

INTRODUCTION

Pollen plays an important role in the life of honey bee colony because it provides dietary components essential for normal growth and development (DIETZ, 1975). HAYDAK (1935) showed that the nitrogen content and the weight of emerging bees is directly influenced by the pollen consumption of the nurse bees and the fluctuation in the pollen income of a colony. Growth of newly emerged bees starts as soon as they begin to feed on pollen with the resulting increase in longevity, development of brood food glands, fat bodies and other organs (MAURIZIO, 1954).

It has been demonstrated that the food value of pollen for bees diminishes with the age of the pollen (HEJTMANEK, 1933; SVOBODA, 1938; MAURIZIO, 1954; LEVIN and HAYDAK, 1957; HAYDAK, 1956, 1961, 1963; STROIKOV, 1963; DIETZ and HAYDAK, 1965; TOWNSEND and SMITH, 1969; DIETZ and STEPHENSON, 1975). Storage of pollen lowers its nutritive value for the physiological development of newly emerged bees (HAYDAK, 1961). Brood rearing on a diet including one-year-old pollen stored at 18-26 °C was considerably decreased (STROIKOV, 1963). HAGEDORN and MOELLER (1968) demonstrated reduced brood production on diets consisting of 75 per cent soya-bean flour and 25 per cent of one, two or four-year-old stored pollen. HAYDAK (1963) obtained brood rearing on diets containing two-year-old pollen, but observed that fortification of old pollen with vitamins and casein resulted in increased brood production. DIETZ and STEPHENSON (1975) found that pollen stored at room temperature for 8 years with or without sugar is nutritionally inadequate to support brood rearing. HAYDAK (1961) showed that 8 year old pollen was almost worthless for the development of young bees.

Newly emerged honey bees are not only able to subsist on a pure carbohydrate for a relatively long period of time (HAYDAK, 1937), but in some cases were able to rear brood for two weeks on a carbohydrate diet (HAYDAK, 1935). ANDERSON and DIETZ (1976) found that one group of newly emerged bees collected in the spring was not able to rear brood on a carbohydrate diet, whereas a second group collected in early summer successfully reared brood for two brood cycles. This suggests that bees may carry over nutrients in their bodies from the larval period under certain conditions.

It has been shown (DE GROOT, 1953; MAURIZIO, 1954) that for normal growth of young adult honey bees, and for the development of their hypopharyngeal glands, only a protein source is required, preferably one with adequate amounts of L-arginine and L-lysine (DIETZ and HAYDAK, 1965). However, it has also been demonstrated that vitamins of the B group are necessary for normal brood rearing of honey bees (HAYDAK, 1949; WAHL and BACK, 1955; BACK, 1956; HAYDAK and DIETZ, 1965, 1972; ANDERSON and DIETZ, 1976). Since long term storage of pollen at room temperatures affects the nutritional value of pollen for brood rearing the question arises if frozen pollen stored for long periods of time will support brood rearing of caged honey bees.

The purpose of the present study was to determine the effect of frozen and dried and subsequently frozen pollen on the brood rearing abilities of honey bees. The nutritive value of pollen stored at different temperature conditions for various periods of time was tested in terms of brood production, adult bee mortality and food consumption.

MATERIALS AND METHODS

Fourteen three-frame nucleus hives (15 × 10 × 25 cm) constructed from wood and nylon screen were placed inside flight cages. Approximately 300 grams of newly emerged worker honey bees, less than

twelve hours old, were placed in the nuclei, and fertile queens were introduced. An initial feeding of the diets in a paste form was supplied in the combs. One week after installing the first bees in each nucleus, the nuclei were opened to allow the bees access to the larger flight area (35 × 107 × 132 cm). The mortality during this 7 day period ranged from roughly 3.6 to 5.4 % and was determined for each colony by counting the dead bees inside the flight cage. For every eight dead bees, one gram of newly emerged bees was added to each colony to bring the total back up to approximately 300 grams. After this intial replacement, no more bees were added and mortality counts were made at regular intervals.

The pollen used in this study was removed on a daily basis from the hives provisioned with pollen traps. After removal of any refuse, the fresh pollen was either placed in airtight containers and immediately frozen or placed on paper towels in cardboard boxes to be lamp dried. A 100 watt light bulb, positioned 10 to 12 inches above the pollen, served as the heat source. The drying period lasted from 24 to 48 hours depending on the amount of pollen available. The lamp dried pollen was also put in airtight containers and placed in the freezer (lamp dried and frozen pollen) or stored in the dark at room temperature (dried pollen) in the laboratory.

The bees in the 14 colonies were fed the following seven diets : 1, honey, powdered sugar and fresh lamp dried pollen; 2, honey, powdered sugar, and 1978 lamp dried and frozen pollen; 3, honey, powdered sugar and 1967 lamp dried and frozen pollen; 4, honey, powdered sugar and 1978 fresh frozen pollen; 5, honey, powdered sugar and 1967 fresh frozen pollen; 6, honey, powdered sugar and 1967 pollen stored at room temperature; 7, honey and powdered sugar. The pollens used in diets 3, 5 and 6 were mixed pollens collected at College Park, Maryland, and after drying were either stored in the dark at room temperature or in a freezer. The fresh pollen, collected in Athens, Georgia, and used in diets 1, 2 and 4 was also of mixed composition. Each diet consisted of 20-25 percent pollen and had a consistency moist enough for the bees to consume it readily.

The diets were offered in petri dishes placed upside down on the frames. Additional food was supplied according to consumption. The food given to each colony and the residues removed were weighed and recorded. Supplementary feedings of sugar syrup were provided at the onset and at one point midway in the experiment to stimulate flight activity. Fresh water was available *ad libitum*. The colonies were examined at regular intervals to determine the presence of brood. In the colonies that reached the sealed brood stage, brood counts were made every 10 days for several brood cycles to determine the number of open and sealed cells of brood.

RESULTS AND DISCUSSION

The results from both colonies on each diet are shown in Table 1. Differences between the seven diets were seen in their effects on food consumption, mortality and brood rearing. All colonies consumed the diets readily. There was considerable variation in the total amount of food consumed not only between colonies on the same diet, but also between different diets. As expected, bees on diet 6 consumed the smallest amount of food. The largest amount was consumed by bees on diet 5.

Bees feeding on diet 6 (lamp dried pollen, 1967) had eggs for 2 brood cycle periods, but we did not observe any larvae. Only in one of the two colonies of bees on diet 7 (control) did we observe some eggs during the first brood cycle period. Brood of all stages were present continuously in colonies which received diets 1, 2, 3, 4 and 5 for at least 4 brood cycles.

The amount of brood produced by bees fed on fresh pollen (diets 1 and 2) was not substantially different. In both instances the pollen was dried after collection and either frozen after drying or fed to bees without freezing. Pollen treated in this manner was nutritionally the most adequate for brood rearing of honey bees. Fresh frozen

TABL. 1. — *Food consumption and brood production of caged honey bees on various diets* (S = number of sealed cells, L = number of unsealed cells).

Colony No.	Colony size (g)	Diet #	Diet Consumption (g)	Brood counts I S	I L	II S	II L	III S	III L	IV S	IV L	V S	V L	VI S	VI L
1	300	1 Dried Pollen (1978)	823 ab[z]	569	119	283	219	454	294	347	146	216	149	105	10 a[z]
2	310		364	422	239	178	x	x	356	95	66	2	5	...	...
3	313	2 Dried and Frozen Pollen (1978)	887 ab	753	152	264	271	y	y	299	38	194	154	144	95 ab
4	316		548	6	22	x	11	25	68	124	40	81	23	...	...
5	318	3 Dried and Frozen Pollen (1967)	511 ab	367	3	36	82	52	73	80	22	7	2	6	... b
6	306		303	255	199	105	72	92	81	110	47	9	13	...	...
7	300	4 Fresh Frozen Pollen (1978)	515 ab	159	117	64	126	96	43	17	15	...	...	...	... b
8	339		522	60	0	7	3	...	...	...	...	...	...	...	...
9	315	5 Fresh Frozen Pollen (1967)	692 a	55	91	38	5	...	...	...	...	...	...	...	... b
10	312		824	291	96	137	114	117	51	35	14	...	...	...	...
11	245	6 Dried Pollen (1967)	278 b	Eggs		Eggs		...	...	...	...	...	...	...	...
12	255		266	Eggs		Eggs		...	...	...	...	...	...	...	...
13	311	7 No Pollen (sugar solution)		Eggs		...	...	...	...	...	...	...	...	...	...
14	309			...		...	...	...	...	...	...	...	...	...	...

x : requeened.

y : missing data.

z : any 2 means followed by the same letter are not significantly different according to Duncan's multiple range test.

pollen, regardless of age, appeared to be nutritionally less adequate for long term brood production of caged honey bees. As a matter of fact there was little difference in total number bees reared on these 2 types of pollen (diets 4 and 5), even though one of them had been stored for eleven years. The reason why bees on these pollens failed to rear brood beyond the fourth brood cycle is not clear, especially since there is statistically no difference ($P < 0.05$) in brood production of bees on diets 2, 3, 4 and 5.

Figure 1 shows the mortality trend of the bees during several brood cycles. Bees on diet 7 (control) which were deprived entirely of pollen dwindled the fastest. Diet 6 (1967 lamp dried pollen) was only slightly better. The difference between these two

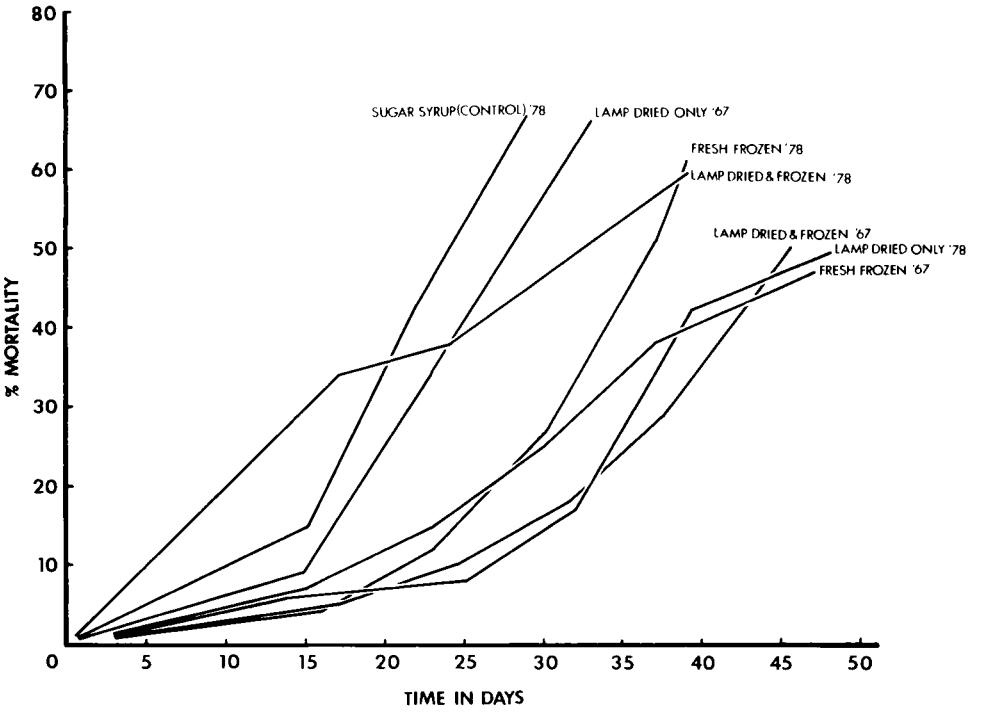

FIG. 1. — *Per cent mortality of caged honey bees on various diets.*

diets, however, is not statistically significant. Bees on diets 1, 3, 4 and 5 showed a fairly consistent rate of decline and did not differ substantially from each other after 4 brood cycles. The reason for the elevated mortality observed in bees on diet 2 during the first two brood cycles is unknown. Nevertheless, there was no statistical difference in the rate of mortality observed in bees on diets 1, 2, 3, 4 and 5 throughout the test period. HAGEDORN and BURGER (1968) reported that the amount of ascorbic acid decreased upon storage of dried and frozen pollen and suggested that this vitamin may

be an essential nutrient for brood rearing of honey bees. However, experimental evidence for this suggestion is still lacking (DIETZ, 1975).

In the early stages of the experiment, bees on diet 7 (no pollen) were fairly active, while the bees in the other colonies displayed only slight to moderate flight activity levels. Similar to our earlier observations (DIETZ and STEPHENSON, 1975) we again noticed marked differences in flight activity between the morning or afternoon and the evening observations. The caged bees were much more active in the late afternoon than any other period of the day, which confirms our observation of a diurnal rhythm in caged bees especially since all cages were left in darkness except when being examined.

The fact that bees on diet 5 ate more food than any of the bees tested indicates food consumption by caged honey bees may not be proportional to the number of bees reared (Tabl. 1). This point is also evident in bees feeding on dried and frozen pollen (diet 3). In this instance, test colony No. 6 consumed about 40 % less food than colony No. 5 but produced essentially the same number of sealed cells. In general, brood production was sustained for a considerable length of time by bees on diets consisting of either recently dried, fresh pollen (diet 1) or dried and frozen (diets 2 and 3). However, dried and subsequently frozen pollen collected in 1967 and stored frozen in airtight containers for 11 years was still adequate for brood production. Consequently, pollen dried and frozen will retain a considerable amount of its nutritional value for bees over a long period of time.

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RÉSUMÉ

INFLUENCE DU STOCKAGE A LONG TERME DU POLLEN CONGELÉ SUR SA VALEUR NUTRITIONNELLE POUR L'ÉLEVAGE DU COUVAIN CHEZ LES ABEILLES

On a donné à 14 colonies installées dans des ruchettes (15 × 20 × 25 cm) en chambre de vol du pollen récolté par les abeilles et stocké à différentes températures et pendant des durées variées. Chaque nucleus comprenait 300 g d'abeilles fraîchement écloses, une reine féconde et 3 rayons de cellules de mâles sans pollen ni miel. Les régimes ont été offerts initialement sous forme de pâte dans les rayons; par la suite ils ont tous été donnés dans des boîtes de Pétri placées la tête en bas sur le dessus des rayons. Du sirop de sucre a été fourni une fois au début et une autre fois au milieu de l'expérience. Après l'apparition des premières cellules de couvain operculé on a compté tous les 10 jours pendant plusieurs cycles de couvain le nombre de cellules operculées et non operculées.

Le pollen utilisé était récolté journellement dans des ruches équipées d'une trappe à pollen. Le pollen frais était placé soit dans des récipients hermétiques et immédiatement congelé, soit sur des serviettes en papier dans des boîtes en carton pour être séché pendant 24-48 heures par la chaleur dégagée par une ampoule de 100 watts. Le pollen séché à la lampe était mis également dans des récipients hermétiques placés dans un congélateur ou stockés à l'obscurité à la température ambiante. On a donné aux abeilles des colonies testées les 7 régimes suivants : 1) miel, sucre en poudre et pollen frais séché à la lampe; 2) miel, sucre en poudre et pollen de 1978 séché à la lampe et congelé; 3) miel, sucre en poudre et pollen de 1967 séché à la lampe et congelé; 4) miel, sucre en poudre, pollen de 1978 frais et congelé; 5) miel, sucre en pou-

dre et pollen de 1967 frais et congelé; 6) miel, sucre en poudre et pollen de 1967 conservé à température ambiante; 7) miel et sucre en poudre. Le pollen récolté en 1967 à College Park (Maryland) provenait de plantes non déterminées; celui de 1978, récolté à Athènes (Géorgie), provenait également de plantes non déterminées. Chaque régime comprenait 20-25 % de pollen et possédait une humidité suffisante pour être aisément consommé par les abeilles.

On a étudié la valeur nutritionnelle du pollen stocké à différentes températures et pendant des durées variées sur le plan de la production de couvain, de la mortalité des adultes et de la consommation de nourriture. Les résultats des groupes de 2 colonies testant chaque régime sont représentés dans le Tableau 1 et la Figure 1.

La quantité de couvain produite par les abeilles nourries avec le pollen frais (régime 1) ou le pollen congelé après séchage (régime 2) indique que ces régimes sont les plus appropriés du point de vue nutritionnel pour l'élevage du couvain par les abeilles. Le pollen frais congelé, quel que soit son âge, apparaît moins approprié du point de vue nutritionnel pour la production à long terme de couvain par les abeilles encagées. La raison pour laquelle les abeilles nourries avec du pollen frais congelé n'ont pas réussi à élever de couvain au-delà du 4^{e} cycle de couvain n'est pas claire, puisqu'il n'y a en particulier aucune différence statistique ($P < 0,05$) dans la production de couvain par les abeilles nourries avec les régimes 2, 3, 4 et 5.

La Figure 1 montre la tendance de la mortalité durant plusieurs cycles de couvain. Il n'y a pas de différence statistique dans le taux de mortalité des abeilles nourries avec les régimes 1, 2, 3, 4 et 5 tout au long de la période de test. Il n'y a également aucune différence statistique entre le taux de mortalité des abeilles élevées sur régime 6 et celui des abeilles élevées sur régime 7. Ces 2 régimes sont pourtant significativement différents des 5 autres.

Il existe une très grande variation dans la quantité totale de nourriture ingérée, non seulement entre colonies nourries avec le même régime, mais aussi entre colonies élevées sur des régimes différents. En général la production de couvain est maintenue pendant une très longue durée par les abeilles nourries avec les régimes comprenant soit du pollen frais récemment séché (régime 1), soit du pollen séché et congelé (régimes 2 et 3). Pourtant le pollen séché puis congelé récolté en 1967 et stocké tel quel dans des récipients hermétiques durant 11 ans est encore approprié à la production de couvain. Le pollen séché et congelé conserve donc une très forte proportion de sa valeur nutritionnelle pour les abeilles sur une très longue période de temps.

ZUSAMMENFASSUNG

DER EINFLUSS VON LANGZEITLAGERUNG AUF DEN NÄHRWERT VON TIEFGEFRORENEM POLLEN FÜR DIE AUFZUCHT VON HONIGBIENEN

Der von Bienen gesammelte Pollen wurde unter verschiedenen Temperaturbedingungen und für unterschiedliche Dauer aufbewahrt und an 14 Testvölker in kleinen Kästen (15 × 20 × 25 cm) in einem Flugraum verfüttert. Jede Kolonie bestand aus 300 g frisch geschlüpften Bienen, einer befruchteten Königin und drei ausgebauten Waben ohne jeglichen Pollen oder Honig. In der ersten Fütterung wurde die Nahrung zu einer Paste gemischt in den Waben angeboten, später wurde sie jedoch leicht angefeuchtet in Petri-Schalen gereicht, die mit der Oberseite nach unten auf den Rahmen plaziert wurden. Jeweils zu Anfang und zur Mitte des Experiments wurde Zuckersirup gefüttert. Nachdem die ersten geschlossenen Brutzellen vorhanden waren, wurden alle 10 Tage für mehrere Brutzyklen die offenen und geschlossenen Zellen gezählt.

Der eingebrachte Pollen wurde täglich von den mit Pollenfallen ausgestatteten Völkern gesammelt. Dieser frische Pollen wurde dann entweder sofort in luftdichten Behältern gefroren oder für 24-28 Stunden auf Papiertüchern in Kartons unter einer 100 Watt Glühlampe getrocknet und dann in luftdichten Behältern entweder gefroren oder bei Zimmertemperatur im Dunklen aufbewahrt.

Die an die Testvölker verfütterte Nahrung setzte sich wie folgt zusammen : 1) Honig, Puderzucker und frischer (1978) lampengetrockneter Pollen, 2) Honig, Puderzucker und in 1978 lampengetrockneter und gefrorener Pollen, 3) Honig, Puderzucker und in 1967 lampengetrockneter und gefrorener Pollen, 4) Honig, Puderzucker und in 1978 frisch gefrorener Pollen, 5) Honig, Puderzucker und in 1967 frisch

gefrorener Pollen, 6) Honig, Puderzucker und seit 1967 bei Zimmertemperatur gelagerter Pollen, 7) Honig und Puderzucker. Der in 1967 gesammelte Pollen stammt von unbestimmten Pflanzen in College Park, Maryland. Der frische Pollen stammt ebenfalls von unbestimmten Pflanzen und wurde 1978 in Athens, Georgia gesammelt. Jede Nahrungsmischung bestand aus 20-25 % Pollen und war feucht genug für eine einfache Aufnahme durch die Bienen.

Der Nährwert des verschieden gelagerten Pollens wurde untersucht an Hand von Brutproduktion, Arbeitsbienenmortalität und Nahrungskonsum. Die Ergebnisse von jeweils beiden Völkern für jede Nahrungsmischung sind in Tabelle 1 und Figur 1 zusammengefasst.

Der Nährwert von frischem Pollen (Diät 1) oder getrocknet und gefrorenem Pollen (Diät 2) war ausreichend hinsichtlich der Anzahl von aufgezogener Brut. Frisch gefrorener Pollen, unabhängig vom Alter erscheint weniger geeignet für Langzeit-Brutproduktion von gekäfigten Honigbienen. Mit frisch gefrorenem Pollen ernährte Bienen beendeten die Brutaufzucht nach dem vierten Brutzyklus. Der Grund für dieses Verhalten ist nicht klar, besonders da statistisch kein Unterschied ($P < 0.05$) in der Brutproduktion zu sehen ist zwischen Bienen, die mit den Diäten 2, 3, 4 und 5 ernährt wurden.

Die Sterblichkeitsrate der Bienen während mehrerer Brutzyklen ist in Figur 1 aufgezeigt. Während der gesamten Testperiode konnten keine statistischen Unterschiede in der Sterblichkeitsrate festgestellt werden für Bienen mit den Diäten 1, 2, 3, 4 und 5. Ebenso war kein statistischer Unterschied in der Sterblichkeitsrate für Bienen mit Diäten 6 und 7 beobachtet worden. Jedoch war zwischen diesen beiden Gruppen von Diäten eine signifikante Abweichung zu erkennen.

Eine erhebliche Variation bestand im gesamten Nahrungskonsum nicht nur zwischen Völkern mit der gleichen Mischung, sondern auch zwischen Kolonien, die mit verschiedenen Mischungen gefüttert wurden. Im allgemeinen wurde die Brutproduktion für eine erhebliche Zeit aufrecht erhalten durch Bienen, deren Nahrung entweder kurzzeitig gelagerten, getrockneten Pollen (Diät 1) oder getrockneten und gefrorenen Pollen (Diäten 2 und 3) enthielt. Der in 1967 gesammelte Pollen, der getrocknet und dann gefroren gelagert wurde, war nach 11 Jahren immer noch von ausreichendem Nährwert für die Brutaufzucht. Die Ergebnisse zeigen, dass getrockneter und anschliessend gefrorener Pollen über einen langen Zeitraum hinweg einen ausreichenden Nährwert für Bienen behält.

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