Apidologie 1980, **11** (2), 181-189.

A NUTRITIONAL BIOASSAY OF HONEYBEE BROOD-REARING POTENTIAL

Gerald M. LOPER and Richard L. BERDEL

U.S. Department of Agriculture, Agricultural Research, Science and Education Administration, Carl Hayden Bee Research Center, 2000 East Allen Road, Tucson, Arizona 85719

SUMMARY

A biossay which measures the relative nutritional efficiency of pollens eaten by young honeybees (*Apis mellifera* L.) rearing brood from eggs to the sealed cell stage of larval development is reported. Three hundred grams of pollen was needed to feed 1.375 bees (an average of 275 bees in each of 5 replicates) in 10-day tests. On a per bee per day basis, an average of 4.2 mg of pollen diet was consumed and 0.024 sealed cells produced. Bees fed almond *(Prunus dulcis)* pollen diet produced more sealed cells per unit of diet consumed than those fed cottonwood *(Populus deltoides)* or saguaro *(Cereus giganteus)* pollen or Yeaco-20 [R] food yeast. Fermented almond, watermelon *(Citrullus lanatus)* and creosote *(Larrea divaricata,* subsp. *tridentata)* pollen diets were as nutritionally efficient as almond. Results indicate that this biossay can be used as a screening method to test small amounts of diets for their relative nutritional value.

INTRODUCTION

Honeybees (*Apis mellifera* L.) rely on pollen and honey for food. Pollen is the main source of protein, fat, vitamins and minerals in the honeybee diet. Honey provides carbohydrates, mainly in the form of fructose and glucose.

It has been shown that pollen is necessary for full development of young bees' brood-food (hypopharyngeal) glands which secrete the food required by honeybee larvae (MAURIZIO, 1950; STANDIFER *et al.*, 1960; HAGEDORN and MOELLER, 1968; BARBIER, 1971). This indirect relationship of pollen-derived nutrients to larval development has been demonstrated by many investigators over the past 40 years (LANGER, 1931; HAYDAK, 1935; HAYDAK, 1970; DIETZ, 1975) and has more recently given rise to a number of bioassays which show some promise of elucidating the

nutritional requirements of honeybees (NATION and ROBINSON, 1968; DE GROOT, 1952; PENG and JAY, 1976; CAMPANA and MOELLER, 1977; HERBERT *et al.*, 1977). Our primary goal is to determine the nutritional requirements for brood rearing and the first step toward that goal was to develop a bioassay having greater sensitivity to nutritional stimuli than other published bioassays. The results of our bioassay development program are presented along with the relative nutritional efficiency of five bee-collected, hand-sorted pollens and one food yeast. The criterion of nutritional efficacy was the ability of young bees to rear brood from eggs to the sealed-cell stage of larval development.

MATERIALS AND METHODS

Diets were prepared from bee-collected pollen which was hand-sorted by color and then microscopically identified. The pollen samples were removed from O.A.C. type traps (SMITH, 1963) and frozen until used, but no consistent collection schedule was followed. The following pollens were bioassayed :

Almond (*Prunus dulcis* (Mill.) D. A. Webb).

Saguaro (*Cereus giganteus* Engelm.).

Cottonwood (*Populus deltoides* Bart. ex. Marsh.).

Watermelon (*Citrullus lanatus* (Thunb.) Matsum. and Nakai).

Creosote (*Larrea divaricata* Cav. subsp. *tridentata* (Sesse. and Mac. ex. D. C.) Felg. and Lowe.).

An additional diet contained Yeaco-20 (1), a commercial preparation used by beekeepers as a pollen substitute.

All pollens were collected in 1977 except saguaro which was collected in 1976. These « pure » pollens were analyzed for protein content (Kjeldahl N × 6.25). Previous tests showed increased consumption of diet when powdered sugar was added to pollen; therefore, all pollen diets used in the work reported here had powdered sugar (9.29 ± 0.24 % dry wgt.) added to them. Powdered sugar (13 % dry wgt.) was also added to the Yeaco-20 diet to stimulate consumption. Distilled water was mixed into all diets to achieve the consistency required to form pellets. The average water content of the diets was 33 ± 4 %. After formulation, all diets, except the fermented almond, were frozen until the start of the test. The fermented almond pollen diet contained the same amount of sugar and water as the standard almond pollen diet except that after mixing the diet, the diet was kept at 34 °C in a water bath for 3 days. Fermentation produced a watery suspension which was dried with warm air while mixing until desired consistency was reached.

Diet pellets were made by pressing 20 gm of diet into a circle 5.5 cm diameter × 1-cm thick. This was done to reduce variation in density and surface area which might affect consumption. The pellet was placed in a screen envelope (21 × 10 × 1 cm) made from No. 5 mesh galvanized wire screening.

Almond pollen diet was used as a standard against which all other diets were tested. We chose almond because it was available early in the year and was recommended as a highly nutritious pollen (Keith DOULL, personal communication).

Tests were conducted in July through October, 1977. The test procedure was repeated every 4 weeks and began by collecting 25-30 frames of emerging brood from a similar number of hives with genetically diverse queens. Frames were placed in an incubation room (31.7 ± 1.7 °C, 52 ± 9 % RH) and emerging bees were brushed off at 2-hour intervals to minimize exposure to colony-stored pollen and honey

(1) Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

(DIETZ, 1969). Frames were brushed in a rotating manner that assured equal distribution of bees from different colonies to each treatment. Bees were brushed into standard (0.505 × 0.415 × 0.165 M) Dadant hive bodies for 24 ± 5 hours until about 2000 were in each. A separate hive body was used for each dietary treatment; the hive bodies contained 100 gm of diet, an amount previously found to be more than the bees would consume. Inverted 100-ml bottles with 3 holes in each cap provided continuous feeding of 60 % (total solids) covered holes in the top board. Bees matured for 4 days under these conditions; water and sugar syrup were added when necessary, using red light for illumination.

On the fifth day after brushing the newly emerged bees, tests were started. The incubation room was chilled to 22.3 ± 1.5 °C to force clustering of bees and 30 to 40 gm of bees were put into small (0.260 × 0.130 × 0.205 M) wooden boxes (nucs). For future tests, we recommend counting an exact number of bees (360) into each test nuc. Each nuc was ventilated with four 1.3 cm screen-covered holes, two at the bottom of a side and two on the top. Also on the top were two 3.2-cm screen-covered holes upon which sugar syrup and water bottles were inverted. The nuc floor was made of No. 7 mesh screening elevated 1 cm so bees could not reach feces which fell through the screen. A small 21 × 16 cm frame of honeycomb with not less than 200 eggs in it was placed in the nuc (2). All visible pollen and honey were removed by aspiration from the comb prior to its introduction. Eggs were acquired by placing genetically diverse apiary queens and 2 empty frames of comb in a cage made of queen excluder screening into the brood nest of apiary colonies 2 days before starting the test. Also placed in each nuc were two 20-gm diet pellets (in the No. 5 mesh screen envelopes) a quantity about twice the amount bees consumed in previous tests. One diet pellet was put on each side of the comb of eggs close enough that bees could crawl from diet to comb (TABER, 1973). A virgin queen was put in each nuc to inhibit queen cell production (BUTLER, 1954); the top was put on and the nuc was placed according to completely randomized design on a table. Fives nucs were prepared for each diet tested. Water and 60 % sucrose syrup in 100 ml bottles were inverted on each nuc and test start time was recorded. Diet, sugar syrup and water were available to bees at all times.

Tests were conducted in a room (6 × 3 × 2.4 M) with controlled temperature (28.2 ± 3.6 °C) and relative humidity (56 ± 10 RH). Two 60 W red lights were used for illumination.

Tests were terminated after 10 days. Dead bees on screen bottoms were counted and the nucs were placed in a carbon dioxide chamber to anesthetize live bees which were then stored in plastic bags and frozen. Water and sugar syrup were measured and dietary remains were weighed and recorded for each nuc, including the control which contained water, sugar syrup and diet but no bees. Losses from evaporation of controls were subtracted from consumption data. Sealed cells were counted and the number of frozen bees was recorded.

Ten bees from each test unit were crushed in 10 ml H_2O and microscopically examined for *Nosema apis* spores with the use of a hemacytometer as described by SHIMANUKI and CANTWELL (1978).

Consumption data from each nuc were divided by the number of bees (live at the end of the test) in that nuc and then divided by the days elapsed from test start time to arrive at the amount of food consumed or sealed cells produced per bee per day. The data were then expressed as numbers of sealed cells per unit of diet and protein consumed.

RESULTS AND DISCUSSION

Table 1 gives the « raw » data from the 4 bioassays conducted in 1977 while Table 2 gives the calculated data from the same tests. All the pollens in these tests had relatively high inherent protein levels, which was diluted in the diet by the addition of water and sugar, thus, dietary protein levels ranged from 12 to 19

(2) The idea to provide a frame of eggs excess in number to what the small number of nurse bees could rear was a contribution from Stephen TABER III and Keith DOULL which we gratefully acknowledge.

TABL. 1. — *Protein content of pollens and diets — 1977.*

Test	Treatment	Pollen Percent Protein (dry wgt. basis)	Diet (1) Percent Protein (wet wgt. basis)	Diet (2) Consumed (gm)	Sealed (2) Cells	Bees (3)
1	1977 Almond	30.1	17.6	9.7 ± 1.2	90 ± 23	316 ± 26
1	1976 Saguaro	22.8	14.0	13.5 ± .4	71 ± 11	278 ± 16
2	1977 Almond	30.1	18.4	7.8 ± .8	76 ± 10	272 ± 17
2	1977 Cottonwood	20.9	12.0	12.2 ± 1.8	52 ± 8	260 ± 40
3	1977 Almond	30.1	15.1	10.2 ± .8	47 ± 21	243 ± 11
3	1977 Fermented Almond	30.1	16.4	8.4 ± .7	44 ± 13	257 ± 8
3	Yeaco-20	44.9	19.0	4.4 ± .9	.2 ± .4	306 ± 22
4	1977 Almond	30.1	18.5	9.9 ± 2.4	60 ± 26	260 ± 60
4	1977 Watermelon	29.1	17.9	11.2 ± 2.0	62 ± 21	260 ± 50
4	1977 Creosote	30.1	18.5	15.5 ± .7	84 ± 17	302 ± 14

(1) Calculated values based on percent protein.
(2) Mean of five replications in pollens and amount of sugar and water added.
(3) Mean of five replications at start of test.

percent. Although water and sugar consumption were measured, we did not discern any consistent or nutritionally meaningful correlations with brood rearing. Almond pollen diet consumption (Tabl. 1) was rather constant across all tests and generally lower than the consumption of the other pollens indicating perhaps a lower content of phagostimulants. The variation in number of sealed cells on the almond diet prohibits making comparisons across individual tests. Comparisons within tests are more easily made on a per bee per day basis as shown in Table 2.

In test No. 1 (July), the bees consumed significantly more ($P < 0.01$) saguaro pollen diet than almond pollen diet and thus more ($P < 0.05$) protein but produced fewer ($P < 0.01$) sealed cells/kg diet consumed than when fed almond diet. It should be remembered that the saguaro pollen was a year older than the almond pollen.

In test No. 2 (August), almond pollen diet was tested against cottonwood pollen diet. Bees consumed significantly more ($P < 0.01$) cottonwood diet than almond diet and consequently ate more ($P < 0.01$) protein from cottonwood than from almond, but the bees produced significantly more ($P < 0.01$) brood on almond than on cottonwood.

TABL. 2. — *Honeybee diet consumption, sealed cell production and diet efficiency data (1) — 1977.*

Test	Treatment	Days	Bees (2)	Diet (mg)	Protein (μg)	Cells sealed ($\times 10^{-2}$)	Cells sealed per kg protein consumed	Cells sealed per kg diet consumed
1	1977 Almond	9.9	304 ± 24	3.2 ± .4 b	590.0 ± .7 b	3.0 ± .7 a	16.5 ± 2.8 a	3.0 ± .5 a**
1	1976 Saguaro		274 ± 15	5.00 ± .17 a** (3)	697 ± 23 a**	2.6 ± .5 a	14.0 ± 2.4 a	1.9 ± 4 b
2	1977 Almond	9.9	266 ± 15	3.0 ± .23 b	560 ± 4 b	2.9 ± .4 a**	21. ± 4 a**	3.8 ± .7 a**
2	1977 Cottonwood		252 ± 4	5.0 ± .20 a**	644 ± 28 a**	2.1 ± .2 b	13.6 ± 2.3 b	1.8 ± .3 b
3	1977 Almond	9.8	236 ± 10	4.4 ± .4 a**	700 ± 70 a**	2.0 ± .9 a**	12. ± 5 a**	2.0 ± .9 a**
3	1977 Fermented Almond		254 ± 7	3.3 ± .21 a	700 ± 40 a	1.7 ± .5 a	9.7 ± 2.2 a	2.1 ± .5 a
3	Yeaco-20		299 ± 28	1.5 ± .20 b	310 ± 40 b	.01 ± .02 b	.1 ± .2 b	.02 ± .04 b
4	1977 Almond	9.9	260 ± 50	3.9 ± .25 c	720 ± 50 b	2.3 ± .7 a	12.7 ± 3.2 a	2.4 ± .6 a
4	1977 Watermelon		260 ± 50	4.4 ± .09 b	780 ± 16 b	2.4 ± .7 a	12. ± 4 a	2.2 ± .8 a
4	1977 Creosote		300 ± 15	5.2 ± .25 a**	990 ± 50 a**	2.8 ± .5 a	9.6 ± 2.0 a	1.8 ± .4 a

(1) Consumption and sealed cell production data are reported on a per bee per day basis and are means of five replications.

(2) Mean of five test nucs at end of test.

(3) * = $P < .05$; ** = $P < .01$ — treatments having different letters within tests vary significantly according to Duncan's multiple range test.

The third test (September) compared the standard almond diet with the fermented almond diet and a diet based on a food yeast, Yeaco-20 (1). Fermented almond was fed to test the hypothesis that fermentation would produce a product similar to bee bread, resulting in a material which would be consumed faster and produce more sealed cells than the standard almond diet. A bee diet reported by JOHANSSON and JOHANSSON (977) contained Yeaco-20, corn syrup (Isosweet-100 (1)) and water (6 : 6 : 3 by wgt.) and provided 18 % protein and 28 % sugar. Our Yeaco-20 dict contained about the same percentage of protein but only 13 % sugar. Lower dietary sugar content could have been the reason that so little of the Yeaco-20 diet was eaten. More ($P < 0.01$) diet and therefore more protein was eaten by bees fed almond and fermented almond than those fed the diet containing Yeaco-20 (Tabl. 2). Cells sealed per unit of diet consumed varied significantly ($P < 0.01$), demonstrating that almond and fermented almond were used more efficiently than the Yeaco-20 diet. Our hypothesis that fermented almond pollen was more efficient nutritionally than standard almond pollen was not proven. However, it should be noted that the coefficient of variation for the numbers of sealed cells per unit of diet consumed for fermented almond (24 %) was only about half that of almond (45 %). This might indicate that fermentation slightly increased bee assimilation of nutrients or reduced concentration of a toxic factor(s). Fermentation of pollen for the 3 days was probably insufficient time for a stable microbial population to evolve. Pain and Maugenet (1966) studied modifications in stored pollen caused by microorganisms and concluded that pollen required 12 days of storage for complete fermentation.

In test No. 4 (October), almond pollen diet was compared with diets based on watermelon and creosote pollens (Tabl. 2). Bees consumed significantly ($P < 0.01$) more watermelon pollen diet than almond pollen diet. The creosote pollen diet, in turn, was consumed in significantly ($P < 0.01$) greater amounts than was the watermelon pollen diet.

Parallelling dietary consumption, significantly ($P < 0.01$) more creosote pollen protein was consumed than almond and watermelon pollen proteins. There were no significant differences in the number of sealed cells or sealed cells per unit of diet consumed in this test; watermelon and creosote were as efficient as almond in supporting brood-rearing.

Observed coefficients of variation for numbers of cells sealed among the treatments were high. Nosema infection could be a source of such variation (MAURIZIO, 1950; RINDERER and ELLIOTT, 1977). However, bees from all nucs were examined for Nosema spores and none were found.

Using the number of cells sealed per unit of diet consumed as the criterion of nutritional efficacy, almond pollen was found to support brood-rearing more efficiently than cottonwood or saguaro pollens. The almond pollen diet was not more efficient than diets containing fermented almond, watermelon, or creosote pollens. The lower

sugar content and consumption of the Yeaco-20 diet is the probable explanation for its failure to support brood-rearing.

So far, we have only used this bioassay procedure to test pollens as they can be trapped without any attempt to make up diets of equal protein content. However, we feel that the test can be used to test experimental diets, artificial or semi-artificial, since almost all dietary sources can be controlled. We feel this test, which uses a limited supply of eggs, eliminates possible dietary input provided by excess eggs from actively laying queens as is the case with most other honeybee bioassay procedures. We hope that this bioassay will stimulate further honeybee nutritional research designed to elucidate the chemistry and biochemistry of honeybee nutrition.

Received for publication in February 1980.

ACKNOWLEDGMENTS

We wish to thank Joseph WAGNER and LYNUS RICHARDS for Kjeldahl analysis of pollens, Robert SCHMALZEL for microscopic identification of pollens, Stephen TABER, III for helpful criticism of our procedures, A. J. NACHBAUER, Jr. and Gordon D. WALLER for providing bee-collected pollens and Bruce CAMPBELL and Steve MCILVAIN for technical support.

RÉSUMÉ

UN TEST BIOLOGIQUE NUTRITIONNEL DU POTENTIEL D'ÉLEVAGE DU COUVAIN CHEZ L'ABEILLE DOMESTIQUE

Cet article décrit une technique de test biologique, et quelques uns de ses résultats, qui utilise un nouveau procédé dans lequel on ne donne aux abeilles nourrices (*Apis mellifera* L.) que quelques œufs en plus de ceux qu'elles peuvent élever. On a placé les nourrices récemment écloses (environ 275 abeilles/unité de test) dans une petite ruchette en bois (0,26 × 0,13 × 0,20 m) avec plus de 200 œufs dans un rayon propre. De petites boulettes des régimes testés sont placées dans la ruchette; de l'eau et du sirop de sucre à 60 % sont également disponibles. Les régimes testés comprenaient du pollen récolté par les abeilles sur l'amandier *(Prunus dulcis),* la cactée *Cereus giganteus,* le peuplier *(Populus deltoides),* le melon d'eau *(Citrullus lanatus)* et le « creosote bush » *(Larrea divaricata subsp. tridentata).* 300 g seulement de pollen sont nécessaires pour chaque test. Nous avons également testé un régime utilisant un sous-produit de levure comme source de protéines et de vitamines. Tous les régimes étaient mélangés à l'eau et au saccharose pour en accroître la consommation et il y a eu 5 répétitions de chaque régime pour chaque test. Les tests se sont terminés au bout de 10 jours lorsque les cellules du couvain ont été operculées. A la fin de chaque test on a déterminé le nombre d'abeilles vivantes et d'abeilles mortes, compté le nombre de cellules operculées et calculé le poids de régime et de protéines consommé.

Les données ont été calculées pour fournir 2 mesures de l' « efficacité » nutritionnelle des régimes : 1) cellules operculées par kg de protéines, par abeille et par jour et 2) cellules operculées par kg de régime, par abeille et par jour. A l'aide de ces mesures on a pu mettre en évidence des différences nutritionnelles significatives entre les pollens, particulièrement en utilisant les données de consommation des régimes. Les abeilles nourries avec le régime au pollen d'amandier ont produit plus de cellules operculées par régime consommé que celles nourries avec les régimes au pollen de cactée et à la levure. Les abeilles nourries avec le régime au pollen de melon d'eau et au pollen de « creosote bush » ont fait exactement aussi bien que cel-

les nourries avec le régime au pollen d'amandier. La méthode de test biologique fournit une analyse rapide des différences dans la valeur nutritionnelle relative des régimes puisqu'ils influent sur une réaction biologique significative des abeilles.

ZUSAMMENFASSUNG

EIN ERNÄHRUNGS-BIOTEST FÜR DIE LEISTUNGSFÄHIGKEIT VON HONIGBIENEN ZUR BRUTAUFZUCHT.

Die Arbeit beschreibt die Technik einer biologischen Prüfmethode und einige Resultate mit einem neuen Verfahren, bei dem Pflegebienen *(Apis mellifera)* nur wenig mehr Eier angeboten werden, als sie aufziehen können. Frisch geschlüpfte Pflegebienen (etwa 275 Bienen je Testeinheit) wurden in kleine hölzerne Käfige gebracht (0,26 × 0,13 × 0,20 m) zusammen mit über 200 Eiern in einer sauberen Wabe. Die Käfige wurden mit kleinen Mengen der Test-Futtermischung versehen, ebenso mit Wasser und 60 % Zuckerlösung.

Das Testfutter enthielt von Bienen gesammelten Pollen der Mandel *(Prunus dulcis)*, Saguaro *(Cereus giganteus)*, Pappel *(Populus deltoides)*, Wassermelone *(Citrullus lanatus)* und Kreosotstrauch (Larrea *divaricata subsp. tridentata*). Für jeden Versuch wurden nur 300 g Pollen benötigt. Wir prüften auch eine Diät aus einem Hefe-Nebenprodukt als Eiweiss- und Vitaminquelle. Alle Futterproben wurden als Mischung mit Wasser und Zucker angeboten, um die Aufnahme zu erhöhen. Für jeden Versuch wurden fünf Wiederholungen angesetzt. Am 10. Tag wurden die Versuche beendet, sowie die Brutzellen verdeckelt waren.

Am Ende jedes Versuches wurde die Zahl der lebenden und der toten Bienen bestimmt, die Zahl verdeckelter Brutzellen gezählt und das Gewicht der Menge an Futtermischung und Eiweiss berechnet.

Die Daten wurden mit dem Ziel bearbeitet, zwei Messzahlen für die « Wertigkeit » (Effizienz) einer Futtermischung für die Ernährung der Bienen zu gewinnen :

1) Zahl der verdeckelten Zellen pro kg Eiweiss^{-1} Bienen^{-1} Tage^{-1}.

2) Zahl der verdeckelten Zellen pro kg Futter^{-1} Bienen^{-1} Tage^{-1}.

Bei Verwendung dieser Messzahlen konnten signifikante Unterschiede im Nährwert zwischen den verschiedenen Pollensorten nachgewiesen werden, besonders wenn man die Daten des Futterverbrauchs verwendet. Bienen, die ein Futter mit Mandelpollen erhielten, erzeugten mehr verdeckelte Zellen in Bezug auf die Menge des verbrauchten Futters als die mit Saguaro- und Hefefutter. Die Bienen, die Wassermelonen- und Kreosotpollen erhielten, leisteten ebenso viel wie die, welche mit Mandelpollen gefüttert wurden.

Dieses biologische Testverfahren ergibt eine rasche Analyse der Unterschiede bei dem relativen Nährwert von Nahrungsmitteln, da sie eine signifikante biologische Leistung der Bienen beinflussen.

REFERENCES

BARBIER M., 1971. – Chemistry and biochemistry of pollens. *Prog. Phytochem.*, **2,** 1-28.

BUTLER C. G., 1954. – The method and importance of the recognition by a colony of honeybees *(A. mellifera)* of the presence of its queen. *Trans. R. Ent. Soc. Lond.*, **105**. Pt. 2. 11-29.

CAMPANA B. J., MOELLER, F. E., 1977. – Honeybees : preference for and nutritive value of pollen from five plant sources. *J. Econ. Entomol.*, **70,** 39-41.

DIETZ A., 1969. – Initiation of pollen consumption and pollen movement through the alimentary canal of newly emerged honeybees. *Ann. Entomol. Soc. Amer.*, **62,** 43-46.

DIETZ A., 1975. – Nutrition of the adult honeybee. Chap. V *in : The Hive and The Honey Bee.* Dadant and Sons, Inc.

GROOT A. P. DE., 1952. – Amino acid requirements for growth of the honeybee (*Apis mellifera* L.). *Experientia,* **8,** 192-193.

HAGEDORN H. H., MOELLER F. E., 1968. – Effect of age of pollen used in pollen supplements on their nutritive value for the honeybee. I. Effect of thoracic weight, development of hypopharyngeal glands, and brood-rearing. *J. Apic. Res.,* **7,** 89-95.

HAYDAK M. H., 1935. – Brood-rearing by honeybees confined to a pure carbohydrate diet. *J. Econ. Entomol.,* **28,** 657-660.

HAYDAK M. H., 1970. – Honeybee nutrition. *Ann. Rev. Ent.,* **15,** 143-156.

HERBERT E. W., Jr., SHIMANUKI H., CARON D., 1977. – Optimum protein levels required by honeybees (Hymenoptera : Apidae) to initiative and maintain brood-rearing. *Apidologie,* **8,** 141-146.

JOHANSSON T. S. K., JOHANSSON M. P., 1977. – Feeding honeybees pollen and pollen substitutes. *Bee World,* **58,** 161-164.

LANGER J., 1931. – New experiments with pollen and bee bread investigations. *Märkische Bienen-Zeitung,* **21,** 317-320.

MAURIZIO A., 1950. – The influence of pollen feeding and brood-rearing on the length of life and physiological condition of the honeybee. *Bee World,* **31,** 9-12.

NATION J. L., ROBINSON F. A., 1968. – Brood-rearing by caged honeybees in response to inositol and certain pollen fractions in their diet. *Ann. Ent. Soc. Am.,* **61,** 514-517.

PAIN J., MAUGENET J., 1966. – Biochemical and physiological research on pollen stored by bees. *Ann. Abeille,* **9,** 209-236.

PENG Y., JAY S. C., 1976. – The effect of diet on queen rearing by caged worker honey bees. *Can. J. Zool.,* **54,** 1156-1160.

RINDERER T. E., ELLIOTT K. D., 1977. – Worker honeybee response to infection with *Nosema apis :* inflkuence of diet. *J. Econ. Entomol.,* **70,** 431-433.

SHIMANUKI H., CANTWELL G. E., 1978. – *Diagnosis of Honey Bee Diseases, Parasites, and Pests.* ARSNE-87, USDA, ARS Publ. p. 6-8.

SMITH M. V., 1963. – The O.A.C. pollen trap. *Can. Bee J.,* **74,** 4-5, 8.

STANDIFER L. N., MCCAUGHEY W. F., TODD F. E., KEMMERER A. R., 1960. – Relative availability of various proteins to the honeybee. *Ann. Ent. Soc. Am.,* **53,** 618-625.

TABER S., III., 1973. – Influence of pollen location in the hive on its utilization by the honeybee colony. *J. Apic. Res.,* **12,** 17-20.