Disappearing Disease – II

Effects of Certain Protein Sources on Brood Rearing and Length of Life in the Honey Bee Under Laboratory Conditions

by JOVAN M. KULINČEVIĆ, and WALTER C. ROTHENBUHLER
Department of Entomology, The Ohio State University, Columbus, Ohio 43210
and THOMAS E. RINDERER
Bee Breeding and Stock Center Laboratory, ARS, USDA, Baton Rouge, Louisiana 70808

Revised Manuscript Received for Publication Oct. 25, 1982

SUMMARY

Small colonies of bees in observation hives with flight restricted in some cases to flight cages were fed four different diets as protein sources: pollen, expeller-processed soybean flour, brewer's yeast, and a mixture of yeast and soybean flour. Observations were made on the amount of brood reared to the capped-cell stage, the longevity of adults reared, and the longevity of nurse bees after they had fed two cycles of brood. If freedom of flight of part of the colonies had little or no effect on the characteristics measured, the data suggest that the soybean flour diet (probably 3 years old) and the yeast diet (probably 2 years old), when compared with the pollen diet, led to reduced brood rearing and shortened life of both adults reared and nurse bees feeding larvae. A mixture of yeast and soybean flour approached pollen as an adequate diet, but led to shorter life of nurse bees. Colonies with 800 nurse bees, compared to 400, were superior in amount of brood reared, longevity of bees reared, and longevity of nurse bees after they had fed 2 cycles of brood. Some cases of "disappearing disease" may be explained by beekeepers feeding an inadequate pollen substitute.

INTRODUCTION

DURING THE last 50 years many materials have been tested in honey-bee colonies as pollen substitutes or pollen supplements. Generally, brood rearing by such colonies has been poor compared to that by colonies fed fresh pollen (Herbert et al. 1977). Beekeepers often do not have much choice during periods of pollen dearth and feed their bees any kind of pollen substitute in hope it will produce brood and stronger colonies.

Haydak and Tanquary (1943) found that colonies fed soybean flour or mixtures of skim milk powder with cottonseed or soybean flour reared brood, although the amount of brood reared was smaller than the amount reared by a colony fed pollen. One material used as a pollen substitute consists of a mixture of soybean flour, dried brewer's yeast, and dry skim milk (Haydak, 1967). According to Herbert et al. (1977), caged bees fed Wheast produced as much brood as those fed fresh pollen. This food material, however, is no longer commercially available.

By studying a report from a beekeeper who claimed to have "disappearing disease" (DD), we noted that colonies decreased or stagnated in population size after being fed soybean flour (Kulincevic et al., 1982). Some colonies had young worker bees, but no old ones which suggested that bees had died before reaching old age.

Testing and utilization of different materials as pollen substitutes have been mainly directed toward producing brood, and less attention has been given to the longevity of resultant worker bees. Haydak (1933) found that rye flour as a pollen substitute led to high mortality of adult bees. Maurizio (1950) reported that each of two kinds of soybean flour and dried skim milk fed to caged adult bees (as 20 per cent of their food) shortened their lives. She suggested that this was due to protein poisoning brought about by feeding too concentrated protein to bees in captivity. In 1963, deGroot reported a positive relationship between body protein content and adult longevity. Wahl (1963; as cited by Crane, 1964) found that the body weight of workers reared on certain pollen substitutes was normal, but their length of life "when fed pure sugar" was subnormal. Knox, Shimanuki, and Herbert (1971) showed that different pollens fed to caged adult bees led to different lengths of life. Eischen (1980) and Eischen et al. (1982) found a significant positive correlation between worker-larva ratio and adult longevity of larvae reared.

This report is concerned with the amount of brood reared and the longevity of workers reared by nurse bees fed natural pollen or any one of three different dietary sources of protein. The longevity of the nurse bees themselves after rearing two cycles of brood was measured and is presented.

MATERIALS AND METHODS

We tested the following diets: (1) soybean flour, (2) powdered dehydrated yeast, (3) soybean flour and yeast 1:1 by weight, and (4) pollen trapped and deep frozen a few weeks before it was fed to the bees. The soybean flour was expeller-processed by Archer Daniels and was probably three years old: Protein 50%, fat 7%, fiber 3%. The dried brewer's yeast was probably two years old: Protein 40%, fat 1%, fiber 3%. We have been informed that soybean flour deteriorates with age. The same may be true of brewer's yeast. Inasmuch as we were trying to find possible causes of disappearing bees, we fed these materials as a beekeeper might do.

Test colonies were prepared by putting either 400 or 800 bees up to two days old, which came from a single, multiply mated queen and emerged in an incubator, into an observation hive along with a comb containing usually

300 to 400 eggs also from the same queen. A mated queen was present in each test colony but caged in a queen-excluder cage so as to prevent oviposition in comb cells, and at the same time allow worker bees access to the queen.

Two cycles of brood were reared by each test colony. Eggs were given for the first cycle (Test No. 1) when nurse bees[1] were just emerged up to two days old; for the second cycle (Test No. 2) when bees were 12 to 14 days old.

Observation hives were put into a temperature controlled shelter as described previously (Rothenbuhler et al., 1968). Colonies fed soybean flour and those fed yeast flew into flight cages, whereas those fed soybean flour and yeast mixed and those fed pollen were allowed free flight. Only a trivial amount of pollen was collected by colonies allowed free flight, and that only in the second cycle of brood rearing. Nonetheless, allowing free flight to part of the colonies causes the response of these colonies to be unsatisfactory controls on the diets. However, the data are not devoid of value. The inadequately controlled comparisons provide the basis for hypotheses. Also, several other comparisons are more rigorous since they are based on a design providing equal treatment. These comparisons include those between 400 and 800 bee units and those between the effects of soybean flour and the effects of yeast on brood rearing and length of life.

Pollen substitute[2] patties were prepared with sugar syrup. The protein concentration was kept under 30% by the addition of sugar. These substitute patties were placed on the top of observation hives in small, inverted, glass Petri dish lids. Pollen was fed from upright dishes, and sugar syrup (1 to 1 by volume) and water were supplied continuously from inverted glass jars on top of the observation hives. Both foods and water were always available to the bees.

After 12 days the sealed brood was counted and the combs were transferred to an incubator where the bees emerged. From bees reared on each comb (each number of nurse bees, cycle, and diet), three samples of 50 bees each (where available) were put into laboratory testing cages (Kulincevic, Rothenbuhler and Stairs, 1973). They were supplied with sugar syrup and given 1.5 grams of YEACO[3] pollen substitute (Rinderer and Elliott, 1977). These cages were kept in an incubator at 35°C and 50% RH. Dead bees were counted and removed daily. The number of days required for ½ of the bees to die was taken as a measure of length of life for the bees in each cage. The results from three such cages were averaged to obtain the measure of longevity for bees reared under each of the 16 conditions.

Although colonies had 400 or 800 nurse bees at the start of the experiments, a few bees were lost prior to the start of the second cycle of brood rearing. After the second cycle of brood was removed to the incubator, the nurse bees could be tested for longevity. To do so, three samples of 50 bees were taken from colonies on each of the four diets and two numbers of nurse bees. These samples were observed daily until 50% mortality had occurred in each sample.

RESULTS AND ANALYSES

The structure of the experiments and the data are presented in Tables 1 and 2.

When considering the various amounts of brood reared on the different diets, we assume that the number of eggs given, in every case, produced more larvae than the nurse bees could rear. Consequently, the actual number reared, not the percentage reared, is the figure of interest.

All diets supported brood rearing in the first cycle by the young nurse bees. Fourteen-day-old bees fed soybean flour reared almost no brood in the second cycle. Data from cycles 1 and 2 are pooled and graphed for easier visualization in Fig. 1. A glance shows that the 800 bee units always reared more brood than the 400 bee units. Analysis of variance and Duncan's New Multiple Range Test (Table 3) confirm the difference to be significant at the 5% level of probability.

The different diets also led to large differences in amount of brood reared. Soybean flour alone, and yeast alone supported brood rearing poorly. The mixture of the two, in colonies allowed free flight, supported almost as much brood rearing as pollen. The analysis of variance and Duncan's Test (Table 4) provide no evidence of a difference between pollen and the mixture of yeast and soybean flour. Pollen and the mixture (free flight) are different from either yeast or soybean flour alone (restricted flight). The latter two were also different from each other.

Fig. 2 presents the length of life of worker bees reared under different conditions. Bees reared by the 800-bee units lived about seven days longer than bees reared by the 400-bee units (Table 5). Soybean flour led to poor longevity. Bees reared by workers fed soybean flour as the only protein source lived less than five days on the average (Table 6). Bees reared by workers fed the other three diets lived more than five times as long.

If one eliminates from consideration those colonies allowed free flight (pollen; soybean flour + yeast) and

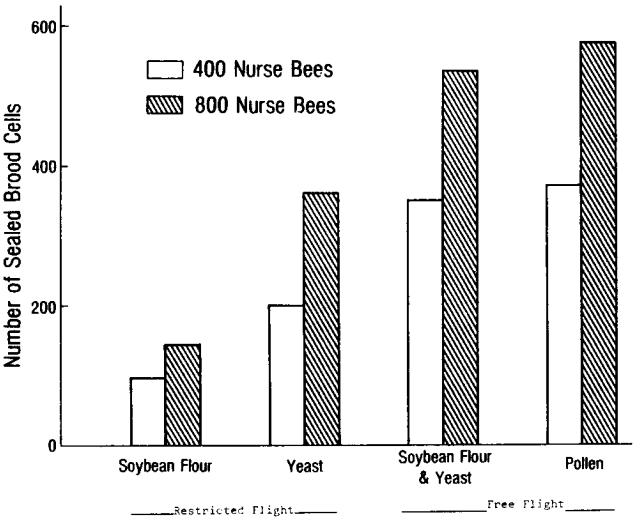

Fig. 1. Cells of sealed brood reared by nucleus colonies of 400 and 800 nurse bees fed the indicated diets and allowed the indicated flights.

FOOTNOTES

[1] The adult bees in the colonies are called nurse bees for the sake of simplicity and with no implication that all of them are involved in nursing brood.

[2] Neither substitute nor supplement seems correct as commonly defined. We have arbitrarily chosen to use substitute for diets containing no pollen.

[3] Mention of a trademark, proprietary product or vendor 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 or vendors that may also be suitable.

TABLE 1. Amount of sealed brood production by the 800-bee units on different diets, and longevity of resultant emerged worker bees. Test No. 2 is a second test using the same nurse bees as in Test No. 1. Age of nurse bees in fourth column is age at start of each test. Last column represents the longevity of nurse bees after nursing 2 cycles of brood. The bees in observation hives A and B flew only in flight cages (restricted flight) whereas those in C and D were allowed free flight. Comparisons of diets across the broken line are confounded by the additional flight variable.

Observ. Hive	Test No. or Cycle	Kind of diet	Age of nurse bees in days	No. of nurse bees	No. of eggs given	No. of sealed brood cells	Days to 50% mortality	Days to 50% mort. after nursing brood
A	1	Soybean	0-2	800	361	138	3.4	-
	2	Soybean	12-14	800	322	6	-	24.0
B	1	Yeast	0-2	800	400	220	30.3	-
	2	Yeast	12-14	800	422	140	30.7	23.7
C	1	Soybean & Yeast	0-2	800	340	260	34.0	-
	2	Soybean & Yeast	12-14	800	389	276	34.7	17.3
D	1	Pollen	0-2	800	341	260	33.7	-
	2	Pollen	12-14	800	432	315	33.3	37.0

TABLE 2. Amount of sealed brood production by the 400-bee units on different diets, and longevity of resultant emerged worker bees. Test No. 2 is a second test using the same nurse bees tested in Test No. 1. Age of nurse bees in fourth column is age at start of each test. Last column represents the longevity of nurse bees after nursing 2 cycles of brood. The bees in observation hives A and B flew only in flight cages whereas those in C and D were allowed free flight. Comparisons across the broken line are confounded by the additional flight variable.

Observ. Hive	Test No. or Cycle	Kind of diet	Age of nurse bees in days	No. of nurse bees	No. of eggs given	No. of sealed brood cells	Days to 50% mortality	Days to 50% mort. after nursing brood
A	1	Soybean	0-2	400	158	92	5.3	-
	2	Soybean	12-14	400	370	6	-	19.0
B	1	Yeast	0-2	400	343	120	23.3	-
	2	Yeast	12-14	400	382	80	20.0	11.7
C	1	Soybean & Yeast	0-2	400	359	208	20.3	-
	2	Soybean & Yeast	12-14	400	366	141	25.7	17.3
D	1	Pollen	0-2	400	341	191	23.7	-
	2	Pollen	12-14	400	369	181	27.3	24.6

TABLE 3. Mean amounts ± SE of sealed brood reared by the 2 nurse bee populations, pooling the results from the 4 diets and the 2 cycles of brood. Means followed by different letters are significantly different at the 5% level of probability by Duncan's New Multiple Range Test.

No. of nurse bees	N	Mean number of sealed brood cells
800	8	201.87 ± 35.77 A
400	8	127.37 ± 23.87 B

TABLE 4. Mean amounts ± SE of sealed brood reared on the 4 diets, pooling results from the 2 numbers of nurse bees and the 2 cycles of brood.

Diet	N	Mean number of sealed brood cells
Pollen (free flight)	4	236.75 ± 31.44 A
Soybean & Yeast (free flight)	4	221.25 ± 30.43 A
Yeast (restricted flight)	4	140.00 ± 29.44 B
Soybean (restricted flight)	4	60.50 ± 32.84 C

TABLE 5. Mean longevities ± SE of bees reared by the 2 different numbers of nurse bees, pooling the results from the 4 diets and the 2 cycles of brood reared.

No. of nurse bees	N	Mean days to 50% Mortality
800	8	25.44 ± 4.84 A
400	8	18.86 ± 3.08 B

compares those confined to flight cages (soybean flour; yeast), one sees that the yeast diet exceeds the soybean flour diet in brood reared and in length of life of bees reared (Tables 4 and 6). This out-dated soybean flour was incredibly poor bee food, and would diminish rather than increase colony population.

Do these diets or the number of nurse bees have any effect on the longevity of nurse bees? By counting the days to 50% mortality of nurse bees after they had reared two cycles of brood and were 24 to 26 days of age, one can learn something about the strain of brood rearing and the adequacy of the diets for nurse bees (Fig. 3). Nurse bees from the 800-bee units lived longer than those from the 400-bee units (Table 7). Nurse bees that had been fed pollen lived about nine days longer than nurse bees on the next best diet (Table 8). Soybean flour, yeast, and their mixture did not differ in effect on nurse bee longevity. All reduced longevity of nurse bees below that of pollen.

DISCUSSION

When bees had only yeast or soybean flour as a protein source, amount of brood reared was decreased. Longevity of bees reared and longevity of nurse bees was decreased below that found when pollen was fed. Feeding a mixture of soybean flour and yeast was comparable to pollen feeding with respect to amount of brood reared and its longevity, but resulted in a shortening of nurse bee life as did the feeding of either material alone.

What is the significance of these results for the beekeeper? Certainly it is not profitable for a beekeeper to feed old expeller-processed soybean flour (no longer avail-

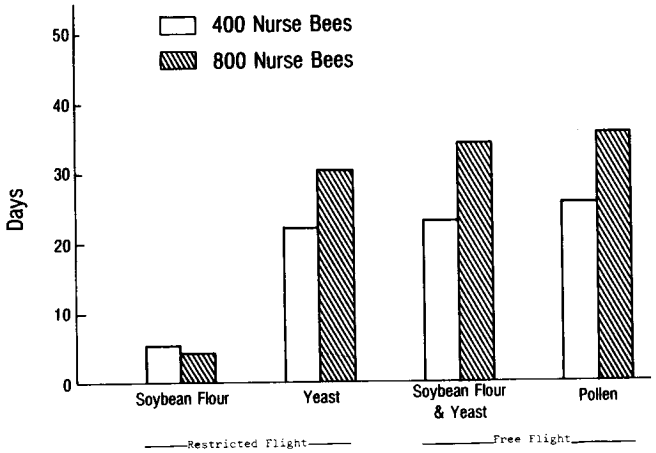

Fig. 2. Days to 50% mortality of bees reared by the indicated number of nurse bees and fed the indicated diets and allowed the indicated flights.

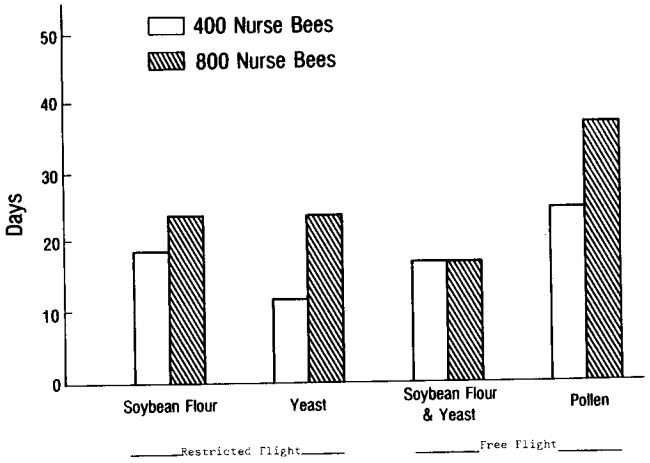

Fig. 3. Days to 50% mortality of nurse bees which had survived to 24-26 days of age, nursed 2 cycles of brood, been fed the indicated diets, and allowed the indicated flights.

TABLE 6. Mean longevities ± SE of bees reared on the 4 diets with results pooled from the 2 numbers of nurse bees and the 2 cycles of brood.

Diet	N	Mean days to 50% Mortality
Pollen (free flight)	4	29.50 ± 2.42 A
Soybean & Yeast (free flight)	4	28.67 ± 3.46 A
Yeast (restricted flight)	4	26.75 ± 2.64 B
Soybean (restricted flight)	4	4.35 ± 0.55 C

TABLE 7. Mean longevities ± SE of nurse bees following the nursing of 2 cycles of brood. The 4 diets are pooled.

No. of nurse bees	N	Mean days to 50% Mortality
800	4	25.50 ± 4.13 A
400	4	18.17 ± 2.66 B

TABLE 8. Mean longevities ± SE of bees that had nursed 2 cycles of brood and lived to 24-26 days of age. Results from the 2 numbers of nurse bees are pooled.

Diet	N	Mean days to 50% Mortality
Pollen (free flight)	2	30.83 ± 6.22 A
Soybean (restricted flight)	2	21.50 ± 2.51 B
Yeast (restricted flight)	2	17.67 ± 6.02 B
Soybean & Yeast (free flight)	2	17.33 ± 0.00 B

able) or brewer's yeast alone as a pollen substitute. No material can be fed to bees with confidence until its effect on the amount of brood reared and the longevity of both nurse bees and the bees they rear have been tested. A poor protein source can lead to a reduced colony population. Much excellent work is being done now, in several laboratories, in an effort to define an adequate pollen substitute.

ACKNOWLEDGEMENTS

This investigation was supported by a cooperative agreement between The Ohio State University and the Agricultural Research Service of the United States Department of Agriculture, and by the Ohio Agricultural Research and Development Center. This research was done in cooperation with the Louisiana Agricultural Experiment Station.

REFERENCES

Crane, E. E. 1964. Abstract of (Wahl, O. 1963. Vergleichende Untersuchungen uber de Nahrwert von Pollen, Hefe, Sojamehl and Trockenmilch fur die Honigbiene (**Apis mellifera**)). Z. Bienenforsch. 6(8), 209-280.) Apicultural Abstracts 15: 160 No. 873/64.

Eischen, Frank A. 1980. The worker/larva ratio and its effect on the longevity of worker honey bees, **Apis mellifera** L. Ph.D. Dissertation. The Ohio State University, Columbus, Ohio. pp. 91.

Eischen, Frank A., Walter C. Rothenbuhler, and Jovan M. Kulincevic. 1982. Length of life and dry weight of worker honeybees reared in colonies with different worker-larva ratios. J. Apic. Res. 21: 19-25.

deGroot, A. P. 1953. Protein and amino acid requirements of the honeybee (**Apis mellifica** L.). Physiologia Comparata et Oecologia 3:197-285.

Haydak, M. H. 1933. Der Nahrwert von Pollenersatzstoffen bei Bienen. Arch. Bienenknd. 14: 185-219.

Haydak, M. H. 1967. Bee nutrition and pollen substitutes. Apiacta 1:3-8.

Haydak, M. H. and M. C. Tanquary. 1943. Pollen and pollen substitutes in the nutrition of the honey bee. Tech. Bull. Minn. Agr. Exp. Sta. No. 160.

Herbert, E. W., H. Shimanuki and D. Caron. 1977. Caged honey bees (Hymenoptera, Apidae): comparative value of some proteins for initiating and maintaining brood rearing. Apidologie, 8(3): 229-235.

Knox, D. A., H. Shimanuki, and E. W. Herbert. 1971. Diet and longevity of adult honey bees. J. Econ. Entomol. 64: 1415-1416.

Kulincevic, J. M., W. C. Rothenbuhler, and G. R. Stairs. 1973 The effect of presence of a queen upon outbreak of a hairless-black syndrome in the honey bee. J. Invertebr. Pathol. 21: 241-247.

Kulincevic, J. M., W. C. Rothenbuhler, and T. E. Rinderer. 1982. Disappearing Disease. I. Effects of certain protein sources given to honey bee colonies in Florida. Amer. Bee J. 122: 189-191.

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.

Rinderer, T. E. and K. D. Elliott. 1977. The effect of comb on the longevity of caged adult honey bees. Ann. Entomol. Soc. Amer. 70: 365-366.

Rothenbuhler, W. C., V. C. Thompson and J. J. McDermot. 1968. Control of the environment of honey bee observation colonies by the use of hive shelters and flight cages. J. Apic. Res. 7(3): 151-155.