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# The Influence of Pollen on the Susceptibility of Honey-Bee Larvae to *Bacillus larvae*[1,2]

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Pollen, water, or nothing was added to the food of worker honey-bee, *Apis mellifera,* larvae aged 6–18 hr. Six hr later larvae from each of these three groups received, in their food, either *Bacillus larvae* spores in water, or water only. Pooled data from seven replicates of this procedure show that spore feeding preceded by pollen feeding resulted in a 71.17% mortality; spore feeding preceded by water feeding resulted in 94.84% mortality; and spore feeding preceded by nonexperimental feeding resulted in 92.75% mortality. Mortality in groups not receiving spores was below 11.00% in all cases. Chi-square analyses showed a highly significant reduction in mortality associated with pollen feeding.

## INTRODUCTION

Honey-bee larvae, *Apis mellifera,* display variations in resistance to *Bacillus larvae,* the causative agent of American foulbrood. Woodrow (1942) demonstrated that younger larvae are highly susceptible, whereas larvae of about 2 days of age are highly resistant. Larvae of different genetic lines vary in susceptibility (Rothenbuhler and Thompson, 1956; Bamrick, 1960; Lewis and Rothenbuhler, 1961; Bamrick and Rothenbuhler, 1961). Larvae of the three honey-bee castes also vary in resistance to this pathogen (Rinderer and Rothenbuhler, 1969).

Rinderer and Rothenbuhler (1969) concluded that caste resistance stems from environmental differences. The most obvious environmental difference between castes concerns the food supplied by adult bees to larvae. The nutritional regimes of queens, workers, and drones are quite dissimilar; e.g., see Haydak (1957); Townsend and Shuel (1962). One element of this dissimilarity is the amount of pollen present in the larval diets of the three castes. Drone larvae receive the most pollen and are the most resistant; queen larvae receive the least pollen and are the least resistant; and worker larvae receive intermediate amounts of pollen and are intermediate in resistance to *B. larvae.* This investigation was designed to test the effect of pollen in the diet of worker larvae on their resistance to *B. larvae.*

## MATERIALS AND METHODS

Three hybrid queens confined to individual combs for 12 hr provided fertilized eggs of a known age range. After oviposition, the eggs, contained in experimental combs, were isolated from the queen in one of two nurse colonies for subsequent development.

On each comb the resulting larvae, aged

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$12 \pm 6$ hr, were separated into six experimental groups by the sequential application of two sets of treatments. The first set of treatments consisted of placing in the food of each larva on the comb either a microdroplet, 0.574 $mm^3$, of a 1:1 suspension by volume of sterile distilled water and pollen of a variety of floral sources collected fresh from pollen cells of a single active colony; a microdroplet, 0.574 $mm^3$, of sterile distilled water; or nothing. Rows of larvae were selected for inoculation procedures in a way that eliminated bias. Following this first set of treatments, the larvae were returned to their nurse colony for 6 hr until the second set of treatments was administered.

The second set of treatments consisted of placing either a microdroplet, 0.287 $mm^3$, containing 400 *B. larvae* spores (determined by the use of a bacteria-counting chamber) suspended in sterile distilled water, or a microdroplet, 0.287 $mm^3$, of sterile distilled water in the food of larvae. Rothenbuhler and Thompson (1956) discussed the method of food inoculation in greater detail. Half of each group of larvae resulting from the first set of treatments received spores, and half received water. Thus, six experimental groups of larvae were produced (see tabulation).

| Group | First feeding | Second feeding |
|---|---|---|
| 1 | Pollen in water | Spores in water |
| 2 | Pollen in water | Water only |
| 3 | Water only | Spores in water |
| 4 | Water only | Water only |
| 5 | Nothing | Spores in water |
| 6 | Nothing | Water only |

After receiving the second set of treatments the larvae were returned to the nurse colony. After 24 hr, larvae in each experimental group were counted. These counts served as the basis for subsequent calculations.

When all cells containing larvae were capped by nurse-colony bees, the combs were removed from the nurse colony and placed in an incubator. The day before the expected emergence of adult bees, the caps were removed, and survivors and nonsurvivors were counted from seven replications of this procedure.

## Results and Analyses

The data are presented in Table 1, which shows numbers of survivors and nonsurvivors for all seven tests, pooled data, and percentages of survival and nonsurvival calculated from pooled data. Inspection of the data shows that in all cases spore-fed groups of larvae displayed much higher mortality than groups not fed spores. Mortality in groups not fed spores was below 11.00% in all cases. Spore feeding preceded by unaltered feeding by nurse bees resulted in 92.75% mortality; spore feeding preceded by the addition of water to brood food resulted in 94.84% mortality; and spore feeding preceded by the addition of pollen to brood food resulted in 71.17% mortality.

Chi-square analyses are presented in Table 2. Interaction analyses comparing groups of water-fed (second feeding) larvae are all nonsignificant; thus, homogeneity among the data is concluded. Of the comparisons among these control groups of larvae, only one individual chi square (replicate 6, pollen vs water) is significant. Since no other $\chi^2$ (individual, pooled, or summed) provided evidence to the contrary, it is concluded that no differences in mortality existed among the three control groups.

Analyses comparing groups of spore-fed larvae all have nonsignificant interaction chi squares; thus, these data are concluded to be homogeneous. Individual, summed, and pooled $\chi^2$'s testing the effect of adding water to brood food prior to spore feeding as compared to adding nothing to brood food prior to spore feeding (spore-fed, water vs nothing) are all nonsignificant. Hence, it is concluded that the addition of a small volume of water to brood food does not have an effect on larval mortality by substantially diluting a spore dosage given to larvae 6 hr later. Comparisons of larvae that

TABLE 1
CLASSIFICATION OF LARVAE

| Test replicate number | Treatments | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Nothing | | | | Water | | | | Pollen | | | |
| | Spores | | Water | | Spores | | Water | | Spores | | Water | |
| | D[a] | A[b] | D | A | D | A | D | A | D | A | D | A |
| 1 | 60 | 2 | 15 | 71 | 52 | 1 | 13 | 50 | 13 | 6 | 2 | 16 |
| 2 | 96 | 8 | 10 | 80 | 77 | 2 | 11 | 88 | 63 | 8 | 9 | 78 |
| 3 | 65 | 7 | 3 | 92 | 63 | 11 | 6 | 79 | 44 | 15 | 5 | 45 |
| 4 | 62 | 2 | 7[c] | 66 | 51 | 0 | 9[d] | 64 | 37 | 10 | 2 | 35 |
| 5 | 78 | 13 | 12 | 88 | 82 | 9 | 5 | 80 | 38 | 32 | 10 | 74 |
| 6 | 101 | 6 | 2 | 93 | 105 | 5 | 1 | 105 | 43 | 16 | 7 | 85 |
| 7 | 75 | 4 | 9 | 74 | 85 | 0 | 12 | 69 | 36 | 24 | 13 | 58 |
| Pooled data | 537 | 42 | 58 | 564 | 515 | 28 | 57 | 535 | 274 | 111 | 48 | 391 |
| Treatment % | 92.75 | 7.25 | 9.32 | 90.68 | 94.84 | 5.16 | 9.63 | 90.37 | 71.17 | 28.83 | 10.93 | 89.07 |

[a] The D headings include larvae and pupae either dead of American foulbrood or missing.
[b] Alive.
[c] One of these individuals was a larva dead of American foulbrood. All other dead control larvae, save those indicated by footnote *d*, were removed by adult bees between the base count and the final count.
[d] Three of these individuals were pupae apparently eaten by a greater wax-moth larva in the test comb.

TABLE 2
$\chi^2$ COMPARISONS OF MORTALITY FOR TEST GROUPS

| Test replicate number | df | Between groups of water[a]-fed larvae (second feeding) | | | Between groups of spore-fed larvae (second feeding) | | |
|---|---|---|---|---|---|---|---|
| | | Pollen vs water[b] | Pollen vs nothing[c] | Water[b] vs nothing[c] | Pollen vs water[b] | Pollen vs nothing[c] | Water[b] vs nothing[c] |
| 1 | 1 | 0.84 | 0.44 | 0.24 | 14.04**[d] | 13.62** | 0.16 |
| 2 | 1 | 0.00 | 0.00 | 0.00 | 4.58 | 0.65 | 2.31 |
| 3 | 1 | 0.36 | 2.94 | 1.44 | 2.32 | 5.72* | 0.89 |
| 4 | 1 | 1.31 | 0.62 | 0.28 | 12.08** | 9.26** | 1.27 |
| 5 | 1 | 1.89 | 0.00 | 2.06 | 26.75** | 19.40** | 0.83 |
| 6 | 1 | 5.64*[d] | 3.09 | 0.45 | 13.02** | 15.31** | 0.13 |
| 7 | 1 | 0.34 | 1.74 | 0.58 | 26.42** | 26.87** | 4.33 |
| Sum of chi-squares | 7 | 10.38 | 8.83 | 5.05 | 99.21** | 90.83** | 9.92 |
| Pooled chi-squares | 1 | 0.45 | 0.74 | 0.03 | 99.15** | 81.06** | 2.37 |
| Interaction chi-squares | 6 | 9.93 | 8.09 | 5.02 | 0.06 | 9.77 | 7.55 |

[a] Fed to larvae as a control to spore-fed larvae.
[b] Fed to larvae as a control to pollen-fed larvae.
[c] A group of larvae fed nothing at the time of pollen or water feeding as a control on spore dilution effects.
[d]** Significant at the 1% level of probability. * Significant at the 5% level of probability.

had pollen and then spores added to their brood food with larvae that received water and then spores (spore-fed, pollen vs water) show 5 highly significant $\chi^2$'s in 7 replicates. Furthermore, the sums of $\chi^2$'s and the pooled $\chi^2$ are both highly significant. Comparisons of spore and pollen-fed larvae with those which received spores

only (spore-fed, pollen vs nothing) also show many significant $\chi^2$'s in the replicates. Likewise, the sum of $\chi^2$'s and the pooled $\chi^2$ are highly significant. Thus the null hypotheses that there are no significant differences between groups of larvae are rejected. It is concluded that a highly significant reduction in mortality is associated with the addition of pollen to the larval brood food.

## Discussion

This investigation provides two important pieces of information. First, since the differences presented in this paper could not have arisen from genetic differences because of the structure of the experiment, it provides added evidence for the conclusion of Rinderer and Rothenbuhler (1969) that environmental differences can result in variation in larval resistance to *B. larvae*. Second, the results show that some attribute of bee-collected pollen brings about a decrease in larval mortality caused by *B. larvae*.

The specific material in bee-collected pollen which is responsible for reducing susceptibility remains to be found; however, a number of possibilities exist. The increased amount of protein may result in increased resistance via a protein-requiring resistance mechanism present in honey-bee larvae. The pollen itself could contain a bacteriostatic, or bacteriocidal, component, which becomes immediately available in brood food or is released by the partial digestion of pollen. Adult bees may add some substance to the pollen which is bacteriocidal or bacteriostatic. Further investigations of pollen and its mode of antibacterial action are being pursued.

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## References

Bamrick, J. F. 1960. "Larval Resistance and Histopathogenesis in Two Genetically Different Lines of Honey Bees (*Apis mellifera L.*) Fed Spores of *Bacillus larvae* White." Ph.D. Thesis, Iowa State University of Science and Technology, Ames, Iowa.

Bamrick, J. F., and Rothenbuhler, W. C. 1961. Resistance to American foulbrood in honey bees. IV. The relationship between larval age at inoculation and mortality in a resistant and a susceptible line. *J. Insect Pathol.*, **3**, 381–390.

Haydak, M. H. 1957. The food of the drone larvae. *Ann. Entomol. Soc. Amer.*, **50**, 73–75.

Lewis, L. F., and Rothenbuhler, W. C. 1961. Resistance to American foulbrood in honey bees. III. Differential survival of the two kinds of larvae from two-drone matings. *J. Insect Pathol.*, **3**, 197–215.

Rinderer, T. E., and Rothenbuhler, W. C. 1969. Resistance to American foulbrood in honey bees. X. Comparative mortality of queen, worker, and drone larvae. *J. Invertebr. Pathol.*, **13**, 81–86.

Rothenbuhler, W. C., and Thompson, V. C. 1956. Resistance to American foulbrood in honey bees. I. Differential survival of larvae of different genetic lines. *J. Econ. Entomol.*, **49**, 470–475.

Townsend, G. F., and Shuel, R. W. 1962. Some recent advances in apicultural research. *Annu. Rev. Entomol.*, **7**, 481–500.

Woodrow, A. W. 1942. Susceptibility of honeybee larvae to individual inoculations with spores of *Bacillus larvae*. *J. Econ. Entomol.*, **35**, 892–895.