

Nosema Control in Package Bee Production — Fumigation With Ethylene Oxide and Feeding With Fumagillin

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INTRODUCTION

MUCH has been written during the past few years about the control of Nosema disease in packages and colonies of honey bees (*Apis mellifera* L.) in North America because the disease causes economic loss to commercial beekeepers. Heavily infected colonies in the northern areas both overwintered and package type, produce substantially less honey (Farrar 1947 and Moeller 1962) and may dwindle to the point of death when winter weather is harsh. Moeller (1962) reported that at Madison, Wisconsin, naturally infected package colonies treated with fumagillin (as Fumidil B) outproduced untreated colonies by 30-40 pounds of honey; furthermore, 100% of the untreated colonies were infected within 3 weeks after establishment compared with 12.5% of those fed medicated sugar syrup. Also, L'Arrivee (1966), who studied artificially infected colonies in Manitoba, Canada, obtained an average yield of 23 more pounds of honey from a group of colonies that had not been inoculated with Nosema than from a group of heavily infected colonies. Cantwell and Shimanuki (1970) noted that in Montana artificially infected package colonies started on dry equipment and fed Fumidil B outproduced similar untreated colonies by almost 18 pounds of honey each and demonstrated (Shimanuki and Cantwell 1970) that honey will be produced by package colonies, even in a poor honey producing area

if hive equipment is fumigated with ethylene oxide gas (ETO). Furgala (1962) found that Fumidil B fed to colonies in the fall continued to have a residual effect on the number of spores of *Nosema apis* Zander 6 months after the feeding.

This test was made to determine whether Nosema-free packages of bees could be produced in a southern commercial bee yard by utilizing ETO and Fumidil B and to measure differences in honey yields when packages of bees from these colonies were shipped to the north and installed there.

METHODS AND MATERIALS

Phase I

One hundred and twenty colonies, leased from a commercial beekeeper in Louisiana, were divided by treatment into 6 groups of 20 colonies each and were moved to 6 test sites in October. The 5 treatments used were as follows:

- Group A. No treatment
- Group B. Hive equipment fumigated with ETO
- Group C. Hive equipment fumigated with ETO plus fumagillin feeding
- Group D. Bees shaken plus Fumidil B feeding
- Group E. Bees shaken
- Group F. Make up bees and hive equipment to be fumigated for Group B

The 6 sites were 300 feet to 1 mile apart, and the hives were arranged about 12 feet apart. All sites had the same ecological terrain, and each site had ample orientation material such as trees and fences to reduce drifting. Nectar and pollen plants and water were nearby and equally available to all colonies.

Brood restriction

All queens used in the colonies were young and produced by the same breeder. Each queen was confined in a $\frac{3}{4} \times 2\frac{1}{2} \times 2\frac{1}{2}$ in. wire cage (with queen excluder material on one side) that was impressed into the face of a comb to eliminate brood rearing prior to ETO fumigation of equipment for Groups B and C. These cages permitted the queens to lay and the workers to enter and groom the queens. Also, the colonies were checked after a week so we could cut out any queen cells and recage any queens that had been released. Three weeks were required for all the brood to emerge and the combs to be ready for ETO fumigation. The elimination of brood rearing at this time of year reduced the populations of adult bees, especially of young adults for overwintering. Thus, on November 21, bees from Group F were blown out of their hives into screen cages with a bee blower, and the hive equipment was fumigated with ETO prior to being used for Group B. Bees from Group F were used to bolster nuclei at another site.

Fumigation

A fumigation package was prepared by placing two 4 x 8 ft. plywood panels side by side on flat ground and centering a 10 x 15 ft. black sheet of 6-mil polyethylene sheeting on the plywood. Then five 2 x 4's were arranged on edge on the sheet so hive bodies could be spaced equally on them in four rows of five bodies per row (stacked two high). Also, boards were placed on top of the stack in a random fashion to permit gas circulation, and a hygrothermograph was placed inside one of the hive bodies to record the temperature and humidity

FOOTNOTES

¹ In cooperation with the Louisiana Agricultural Experiment Station.

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during the fumigation. Another sheet of polyethylene was then placed over the equipment, and the edges were weighed down against the bottom tarp with sand to make a gas-tight seal. In addition, a heating chamber was arranged around the package to maintain the minimum temperature during fumigation at 70° F. This chamber was produced by using a framework of pipe to hold another sheet of polyethylene over the package (the edges along the ground were covered with sand) and locating one catalytic heater (5,000-8,000 BTU) inside the sheeting at each end of the package. Also, a small opening was made at either end of the polyethylene to insure an adequate supply of air for the heaters.

For the fumigation, two 80-lb. charges of sterilizing gas, 12% ethylene oxide and 88% Freon-12^R (measured on a platform scale) were released into the fumigation package at 5-hour intervals. A vent hole was cut at the top of the fumigation package to permit the displacement of air by the introduced gas and then sealed after the prescribed level of gas was reached. The heating chamber was removed, and gas samples were taken at the top and bottom of the fumigation package with a Kitagawa Unico 400 Precision gas detector; these calculations for gas concentration indicated that a minimum of 3.5% ETO remained inside the package, though we believe the concentration was actually greater. The day of the first fumigation, the temperature and relative humidity inside the package during the following 24 hour period ranged from 60 to 88° F. and from 40 to 79, respectively.

For the second fumigation of hive equipment from Group B to be used for Group C, the equipment was preheated for 12 hours to achieve a higher temperature. Thus, temperature and relative humidity during the 24-hour period after the second charge ranged from 78° to 115° F. and from 35 to 78%, respectively. Once the polyethylene cover was removed, the fumigated equipment was aerated for 1 week.

On December 12, the 60 colonies of Groups A, B, and E received 1 gallon each of untreated Drivert sugar syrup while those of C and D were fed with 1 gallon of Drivert syrup treated with Fumidil B. Little of the medicated syrup, given to Groups C and D on December 12, had been consumed by the bees by late January due to cold temperatures. The remainder of the uneaten Fumidil B treated Drivert syrup in each feeder of Groups C and D was measured and discarded in January. The 4 subsequent feedings, Feb-

ruary 18, March 12, March 18-19, and March 28, of Fumidil B treated syrup to Groups C and D were adjusted so that each colony received a total of approximately 2 gallons of treated Drivert syrup (100 mg. fumagillin activity/gallon). By March 19, 21 weak colonies in Groups B, C, D, and E were dead or had been combined with others. On April 4th, all feeders in colonies of Groups C and D with less than 1 quart of medicated sugar syrup were emptied by pouring the syrup into the cells of empty combs. The empty feeders were then filled with plain sugar (sucrose) syrup to increase colony stores. By April 4th, all the colonies in Groups A, B, C, D, and E had received a total of approximately 3.75 gallons of syrup (sucrose sugar or Fumidil B + Drivert). No additional feeding was necessary after the onset of the nectar flow after the first week in April.

Honey bee and hive manipulations

On December 2, bees of Group B were blown from their untreated equipment into the fumigated equipment of Group F with a bee blower, after the queens had been removed and recaged on 1 of the fumigated frames. All queens were clipped and marked during the blowing operation. On December 4, bees of Groups D and E were blown out of their untreated hives into cages and then immediately shaken back into their untreated equipment. On December 10, bees of Group C were blown out of their untreated hives into the fumigated equipment of Group B.

During the project, all hive equipment was scraped free of burr comb and propolis as much as possible to enable easier frame and bee manipulation. As the populations in the colonies began to increase during January because of the early red maple nectar and pollen flow, the two brood chambers in each hive were reversed. This reversal placed the brood cluster close to the hive entrances and permitted the queens to expand their brood nests upward in the hives. After the colonies had built to swarm populations, queen cells were removed from each hive every 10 days to prevent swarming and loss of bees and queens.

Nosema sample collection

On December 6, the first samples of adult bees (30-45 per colony) were taken from the entrances of each of the 100 hives during the early morning hours every 2 weeks except when temperatures were low or rain fell.

Seven sets of bee samples were collected between December 6 and March 19 at the hive entrance (during December when temperatures were low and rain fell, bees were taken from the outside frame of the cluster). The abdomens of 20 bees per colony were detached from the thorax and homogenized for 2 minutes with an equal volume of water in a blender at 2000 rpm and for an additional minute at 3000 rpm. A small amount of the suspension was drawn out by means of a pipette, and a platinum milk sample loop was used to place 0.01 ml of the homogenate on a haemocytometer for spore counting.

Package bee study

The second phase of this study consisted of preparing and sending 100 2-lb. packages of bees for a honey production study under northern conditions.

On May 1, forty 2-lb. packages of bees were shaken from Groups A and B (a young, caged queen hung inside each package) and shipped by air freight from the test colonies at Baton Rouge, Louisiana, to the Bee Management Laboratory at Madison, Wisconsin. Samples of 30-45 honey bees were collected from each package for determination of Nosema disease as the packages were being prepared for shipment. Similarly, sixty 2-lb. packages from Groups C, D, and E were prepared and shipped on May 12 (more than 1 package had to be made up from some colonies because of the loss of the 21 colonies). Twenty-five additional queens from the same breeder source were shipped separately on May 12 to replace any losses in shipment and introduction.

All the packages were made up by using a commercial blower and exact weights read on a scales as the packages were being filled. Each package was numbered and color coded by treatment and group. The 100 package colonies were divided between Zerbruchen and Groth bee yards of the Madison laboratory.

Subsequently, at the Madison laboratory samples of bees were collected at the entrances of the hives every 2 weeks as long as weather permitted flight. The final samples were obtained from the top of the brood cluster with salve cans on October 27. All samples of honey bees were packed in dry ice and airmailed to the Bee Breeding Investigations Laboratory at Baton Rouge so examination procedures could be standardized by making all spore counts at one laboratory.

Table 1. Average number of spores of NOSEMA APIS/bee ($\times 10^6$) determined from 20 bees/sample per colony, 20 colonies in each of five groups. Baton Rouge, Louisiana.

Dates	Group A (untreated)	Group B (ETO-fumigated)	Group C ETO-Fumigated + (Fumidil B syrup)	Group D Shaken + (Fumidil B syrup)	Group E (shaken)
December 6	0.01	0.01	1.38	0.01	0.00
December 24	0.01	2.00	0.38	0.75	0.00
January 7	0.00	0.00	0.38	0.06	0.00
January 24	0.00	0.01	0.29	0.25	0.55
February 10	0.63	0.10	1.63	0.00	0.00
February 27	1.25	0.88	0.09	0.00	0.00
March 19	0.78	0.00	0.00	0.00	0.00
April 3	0.00	0.00	0.00	0.02	0.00
April 18	0.14	1.00	1.25	0.03	0.51
May 1*	0.90	0.00	--	--	--
May 12*	--	--	0.01	0.02	0.01
Total	3.72	4.00	5.41	1.08	1.07
Average	0.37	0.40	0.54	0.11	0.11

* Samples were taken prior to shipment of package bees to Madison, Wisconsin.

Table 2. Average number of spores of NOSEMA APIS/bee ($\times 10^6$) determined from 20 bees/sample per colony, 20 colonies in each of five groups. Madison, Wisconsin.

Dates	Group A (untreated)	Group B (ETO-fumigated)	Group C ETO-Fumigated + (Fumidil B syrup)	Group D Shaken + (Fumidil B syrup)	Group E (shaken only)
May 5*	0.17	1.00	--	--	--
May 14*	--	--	0.03	0.01	0.01
May 27	5.03	8.84	0.51	1.78	1.33
June 10	2.80	2.32	1.42	2.53	1.60
June 24	1.06	1.84	3.04	1.45	1.60
July 7	0.88	1.24	1.37	1.01	1.04
July 22	1.20	1.01	0.58	1.46	1.01
August 14	0.37	0.68	0.18	0.24	0.36
August 18	0.18	0.20	0.60	0.06	0.83
September 2	0.00	0.05	0.12	0.01	0.78
September 15	0.20	0.28	0.45	0.38	0.21
September 29	1.78	3.96	6.31	3.01	3.48
October 14	0.46	1.50	2.40	1.94	1.29
October 27	2.63	0.00	0.00	6.25	0.62
Total	16.76	22.92	17.01	20.13	14.16
Average	1.28	1.76	1.30	1.54	1.08

* Nosema spore samples taken after receipt of packages from Baton Rouge, Louisiana.

RESULTS AND DISCUSSION

The infection of colonies by Nosema disease from samples taken from December 6 through May 12 is shown in Table 1. Infection levels were erratic throughout the months of December to May. Table 1 shows that of sampling dates from December 6 to May 1, 70% showed Nosema infections for Group A, 60% for Group B, 80% for Group C, 60% for Group D, and 30% for Group E. No pronounced spring Nosema infection peaks were observed in any of the 5 groups, as has been reported in literature (Oertel 1964).

The bees comprising the 2-lb. packages were mildly infected when they were shipped May 1 and 12. Also, the samples taken from the packages upon arrival at Madison May 5 and 14 still showed only mild infection (Table 2). However, by May 27, the levels of infection had increased considerably in all except Group C. This rise coincided with the lack of brood emergence

Table 3. Average pounds gained by five groups of 20 package colonies each shipped from Baton Rouge, Louisiana, and managed at Madison, Wisconsin, for honey production.

Groups	Apiary Sites		Av. of Yards
	Groth Yard	Zerbruchen Yard	
A — Check	65.8	92.3	79.1
B — ETO-fumigated	77.9	79.3	78.6
C — ETO-fumigated + Fumidil syrup	94.8	124.2	109.5
D — Shaken + Fumidil syrup	98.2	146.2	122.2
E — Shaken	82.9	112.3	97.6

during the first 3 weeks after package installation. The highest levels of infections May 27 were in Group A (untreated) and B (fumigated), but a month later Group C (fumigated and Fumidil B fed) had the highest level (3.04×10^6 spores/bee).

The peak of infection in Group C may have occurred because of an increase in protein consumption since pollen is abundant at Madison during May and June. Gontarski and Mebs (1964) showed that more Nosema spores developed in broodless bees fed

pollen than in those fed only a honey-sugar candy and speculated that some vitamins in pollen probably expedited the development of the spores. Also, Beutler and Opfinger (1950) noted that bees held in cages and caged colonies had increased levels of Nosema disease when they were fed pollen. Furthermore, the effects of Fumidil B feeding and ETO-fumigation of equipment on the development of Nosema may have been reduced by increased consumption of protein.

After the two June readings the levels of disease tapered off until September 29 which coincided with the rearing of young bees. The rise in infection levels in all of the groups on September 29 coincided with the normal reduction or cessation in egg laying and brood rearing in the north central area of the United States. Thus, the reduction or cessation of brood rearing in September in Wisconsin reduces the number of new bees that can replace older infected bees. Then since the life expectancy of bees increases when foraging decreases in the fall and since older bees probably have a higher level of *Nosema* infection, a rise in the disease might be expected when brood rearing stops in northern regions.

Honey production data, Table 3, show a location variation in the total

weight colonies gained. Group C and D which received ETO-fumigated equipment plus Fumidil B and were shaken plus received Fumidil B, respectively, had the largest weight gains. This result is in agreement with the results obtained previously by Shimanuki and Cantwell (1970). However, in view of the cost of Fumidil B, Drivert, and sucrose sugar and the labor, time, and feeders used in treating the colonies, the weight gain may not have been great enough to show a commercial profit. The value of honey was computed at 15c per lb. ●

LITERATURE CITATION

Bentler, E., and E. Opfinger. 1950. Pollenernahrung und *Nosema*-befall der Honigbiene (*Apis mellifica*). (Pollen nutrition and *Nosema* infection of the honey bee (*Apis mellifera*.) Zt. Vgl. Physiol. 32(5): 383-421.

Cantwell, G., and H. Shimanuki. 1970.

The use of heat to control *Nosema* and increase production for the commercial beekeeper. Amer. Bee J. 98(7): 263.

Farrar, C. L. 1947. *Nosema* losses in package bees as related to queen supersedeure and honey yields. J. Econ. Entomol. 40(3): 333-338.

Furgala, B. 1962. Residual fumagillin activity in sugar syrup stored by wintering honey bee colonies. J. Apic. Res. 1: 35-37.

Gontarski, H., and M. Dietrich. 1964. Eiweissfütterung und *Nosema*-entwicklung (The feeding of proteins to honey bees and the development of *Nosema apis* Zander.) Zt. Bienenforsch. 7(3): 53-62.

L'Arrivee, J. C. M. 1966. Effects de la noseuse sur le rendement en miel (Effects of *nosema* disease on honey yield). Can. Agri. 11(3): 24-25.

Moeller, F. E. 1962. *Nosema* disease control in package bees. Amer. Bee J. 90(10): 390-392.

Oertel, E. 1964. *Nosema* disease in the Baton Rouge area. Glean. Bee Cult. 92(7): 427-437.

Shimanuki, H., and G. Cantwell. 1970. The role of heat and ethylene oxide in *Nosema* disease prevention. Bull. Apicole 12(1): 35-41.

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