

# Method of Application of Tylosin, an Antibiotic for American Foulbrood Control, with Effects on Small Hive Beetle (Coleoptera: Nitidulidae) Populations

P. J. ELZEN, D. WESTERVELT,<sup>1</sup> D. CAUSEY,<sup>1</sup> J. ELLIS,<sup>2</sup> H. R. HEPBURN,<sup>2</sup> AND P. NEUMANN<sup>3</sup>

USDA-ARS, Kika de la Garza Subtropical Agricultural Research Center, 2413 E. Highway 83, Weslaco, TX 78596

J. Econ. Entomol. 95(6): 1119–1122 (2002)

**ABSTRACT** The method of application of the antibiotic tylosin (Tylan) for control of oxytetracycline-resistant American foulbrood (*Paenibacillus larvae* White) was tested in honeybee (*Apis mellifera* L.) colonies. A powdered sugar mixture with tylosin, applied as a dust, was efficacious in eliminating American foulbrood symptoms at a rate of 200-mg Tylan per 20 g of powdered sugar, applied at weekly intervals for 3 weeks. A second method of treatment consisting of Tylan mixed with granulated sugar and vegetable shortening and applied once as a patty, at an equivalent total dose as the dust method, to diseased colonies also effectively eliminated symptoms of disease. In all colonies treated with patties, however, small hive beetle (*Aethina tumida* Murray) populations significantly increased, compared with the powder sugar method or untreated controls. Bee populations in patty-treated colonies also were significantly reduced, most likely the result of the invasion and proliferation of adult and larval small hive beetles. Such reduction in colony strength was not seen in dust-treated colonies. Because of the obvious damaging populations of small hive beetles, concerns about development of disease resistance, unknown risks of residues, and lack of support by regulatory agencies for the use of the patty method, the use of the dust method of tylosin is greatly favored over the patty method.

**KEY WORDS** honey bee, American foulbrood, tylosin, small hive beetle

THE BACTERIAL DISEASE American foulbrood (AFB) (*Paenibacillus larvae* White) is the most serious disease affecting commercial and hobbyist beekeepers in the U.S. (Shimanuki et al. 1992). Diseased brood acts as a source of infectious spores that can spread throughout an entire apiary and killing it if left unchecked. For the past ca. 40 yr, the antibiotic oxytetracycline has been used to prevent and cure AFB symptoms (Shimanuki et al. 1992). Oxytetracycline remains the only legally approved antibiotic registered for AFB control in the U.S. (Shimanuki 1997). In recent years, however, AFB resistance to oxytetracycline has been detected in the U.S. (Miyagi et al. 2000), resulting in the expensive practice of burning severely contaminated hive equipment as the most common means to control resistant disease outbreaks. Oxytetracycline resistance by AFB has also been detected in South America (Alippi 2000). New antibiotics have been investigated for AFB control (Hitchcock 1970;

Peng et al. 1996), however, with the compound tylosin being in the forefront. These authors tested tylosin on oxytetracycline-susceptible AFB, not resistant strains. Tylosin has been shown to control oxytetracycline-resistant AFB in the laboratory (Kochansky et al. 2001) and is currently undergoing consideration by the U.S. Food and Drug Administration (FDA) for approval of use in U.S. honey bee hives.

During the same period that resistance to oxytetracycline by AFB was recorded in the U.S., a newly introduced coleopteran pest species of honey bees was discovered in the southeastern U.S. (Sanford 1998). This species, the small hive beetle (*Aethina tumida* Murray), damages hives as adults and larvae by invading bee hives and consuming honey, pollen, and most preferentially bee brood (Elzen et al. 1999). Honey stored in frames for eventual extraction is also subject to attack, which results in the fouling of the honey that renders it unusable (Lundie 1940). Chemical controls, both within the hive and ground treatment (for pupating larvae) have been approved, but concerns by beekeepers of hive product pesticide contamination have arisen correspondingly. As of this writing, the small hive beetle has been confirmed in virtually all states east of the Mississippi River (J. Pettis, personal communication) with very probable spread to the rest of the U.S. in the near future.

Mention of a proprietary product does not constitute endorsement by the USDA.

<sup>1</sup> Florida Department of Agriculture, 1911 S.W. 34th Street, Gainesville, FL 32614 (e-mail: pelzen@weslaco.ars.usda.gov).

<sup>2</sup> Department. Zool. & Entomol., Rhodes University, Grahamstown, South Africa 6140.

<sup>3</sup> Martin-Luther Universität, Krollwitzer Str. 44, Halle/Saale, Germany 06099.

Beekeepers in Florida, one of the states most heavily affected by the small hive beetle, have observed that the presence of hive supplemental patties are associated with higher beetle numbers and damage. Such patties include pollen substitute patties for feeding of adult bees within the hive, grease patties for tracheal mite control, and patties incorporating oxytetracycline for AFB control. The future potential method of application of tylosin is now being questioned by beekeepers, with their interest in options of applying the antibiotic in a confectioner sugar dust method or by incorporating into grease patties, as described for application methods for oxytetracycline (Shimanuki et al. 1992). Therefore, we initiated the current study to investigate the efficacy of tylosin applied in dust or patty form and subsequent comparative quantification of small hive beetle infestations and hive strength over an extended period.

### Materials and Methods

The trial of method of application of tylosin and resulting effect on small hive beetle populations was initiated at Altoona, FL, on 26 Jun 2001. Only one apiary was used in the study, because of concerns by local beekeepers in maintaining diseased hives in an area of high density of commercial operations, as well as concerns by the state apiary regulatory agency that only a limited location be used to maintain diseased hives. Standard Langstroth queenright hives free of AFB symptoms, two-deep with 10 frames per box, with established honey bee (*Apis mellifera* Ligustica) populations were used. Ample stores of honey and pollen were present in each hive. All hives were inoculated two weeks before initiation of the trial. The inoculation process consisted of removing 150 cells of actively diseased brood from a local commercial apiary (before burning) and agitating them in 56.8 liters of high fructose corn syrup. All hives were then fed, via top jar feeders, two liters of this syrup/slurry mixture until all was consumed. Before inoculation, a sample of this diseased material was sent to the USDA-ARS Beltsville Honey Bee Laboratory and verified as moderately resistant to oxytetracycline, using the disk-diffusion method (Shimanuki and Knox 2000). Hives contained strong populations of bees at test initiation and initial disease progression.

At initiation, AFB disease evaluation was determined by removing brood frames from each individual hive and categorizing (Hitchcock et al. 1970) disease symptoms by the presence of diseased brood and scale. All hives were determined to be uniformly heavily diseased at onset, with >100 diseased cells apparent in each hive. Numbers of frames of adult bees per hive were counted by considering both sides of a frame as one frame of bees, either fully covered or rounded to the nearest third or half. Initial small hive beetle populations were assessed by counting beetles on hive bottoms, frames, and under the top covers per hive.

Three treatments were then randomly assigned to hives. Treatment one consisted of a confectioner's

sugar dust, which was made by combining 200 mg of Tylan (100 g base tylosin tartrate, obtained from Elanco, Indianapolis, IN) with 20 g confectioner's sugar (a dose found efficacious in a previous study (P. Elzen, D. Westervelt, and M. Feldlaufer, unpublished data). The full 20 g of this dust were applied by sprinkling over ends of top bars for three weeks at one week intervals, for a total dose of 600-mg Tylan over 3 wk. Treatment two consisted of 15-g Tylan combined with 1814 g granulated sugar, then mixed with 907 g vegetable shortening. Each hive received a 227-g patty of this mixture. Grease patties were previously found to remain in hives 5 to 6 wk, for a Tylan dose of  $\approx$ 200 mg/wk, roughly equivalent to the dust dosage. Treatment three consisted of an untreated control. Eight hives were randomly assigned to each treatment group.

The presence of disease in all treatment groups was reassessed on 10 Jul and finally on 30 Aug. Hives were categorized as diseased or not diseased by the detection of diseased brood in frames examined per hive. Adult and larval small hive beetle numbers were reassessed on 3 Jul, 10 Jul, and finally on 30 Aug. Hive strength was assessed after initial evaluation on 26 Jun by counting numbers of frames of adult bees on 30 Aug at the termination of the trial.

**Statistical Analyses.** Efficacy of tylosin on post-treatment evaluation dates was determined by comparing the proportion of diseased hives for each treatment on 10 Jul and 30 Aug versus disease proportion of hives on 26 Jun (initial) by chi-square analysis (Zar 1974). By 10 Jul, two patty-treated hives and one control hive absconded, and by 30 Aug an additional patty treated and control hive absconded, as well as one dust treated hive. Absconding has been observed associated with SHB infestations in central Florida (D. W., unpublished data). These hives were removed from statistical analysis for tylosin efficacy. Mean numbers of adult or larval small hive beetles between application type for each evaluation date were compared by analysis of variance (ANOVA) and means were separated by least significance tests (SYSTAT, Inc. 1990). Mean number of frames of bees at test initiation and at final assessment were compared as to method of application by *t*-tests (Zar 1974).

### Results and Discussion

Tylosin applied in a confectioner's sugar dust was effective in reducing and eliminating symptoms of oxytetracycline-resistant American foulbrood disease in the apiary of the current study. Dust treated hives initially began with 100% diseased hives, which was significantly reduced to 0% diseased hives 65 d after initial treatment (Table 1). An equivalent weekly dosage of tylosin in grease patties was also found to be effective in treating symptoms of AFB in diseased hives in the current study, with ready consumption of patties by the bees and control of even heavy infection. The proportion of diseased hives was initially 100%, which was reduced to 0% disease symptoms detected 65 d after initial treatment (Table 1). Disease

**Table 1. Efficacy of two application methods of tylosin for control of oxytetracycline-resistant American foulbrood**

| Treatment    | Percentage of Hives With Detectable AFB Disease |        |        |
|--------------|-------------------------------------------------|--------|--------|
|              | 26 Jun                                          | 10 Jul | 30 Aug |
| Control      | 100                                             | 87.5   | 83.3   |
| Dust         | 100                                             | 37.5   | 0.0*   |
| Grease patty | 100                                             | 66.7   | 0.0*   |

Proportions within a row followed by an asterisk are significantly different from initial 26 Jun value (chi-square for both treatments = 8,  $df = 1$ ,  $P < 0.05$ ; analyses performed on actual numbers of hives diseased/undiseased, not on proportional values).

symptoms were still readily detectable 14 d after initial treatment (dust: chi-square = 3.13,  $df = 1$ ,  $P > 0.05$ ; patty: chi-square = 2.0,  $df = 1$ ,  $P > 0.05$ ), indicating a longer evaluation period is necessary to expect bees to remove diseased brood. It should be noted that while the dust and patty treatments provided equivalent weekly dosages, the patty treatment was present twice as long and so provided an extended period of treatment.

Method of tylosin application played a significant role in the development of small hive beetles in treated hives. As early as one week after initial treatment, hives treated with grease patties exhibited a highly significantly greater number of adult small hive beetles, compared with the untreated control and dust-treated hives ( $F = 35.64$ ,  $df = 2$ , 21,  $P < 0.0001$ ; Table 2). Such a rapid increase of adults from initially low numbers would suggest heavy immigration from surrounding areas into patty-treated hives; this region of Florida contains a large number of beekeeping operations that continue to report moderate-heavy small hive beetle infestations. As time progressed, small hive beetle numbers began to equalize between treatments and controls ( $F = 0.040$ ,  $df = 2$ , 15,  $P < 0.961$ ; Table 2).

The development of larval small hive beetle numbers in patty-treated hives was significant and also dramatic. One week after initial treatment of hives, significant larval populations developed in patty-treated hives, but not in control or dust treated hives ( $F = 21.39$ ,  $df = 2$ , 21,  $P < 0.0001$ ; Table 3). Larval numbers continued to increase by the second week after treatment in patty treated hives, but again not in control or dust-treated hives ( $F = 3.793$ ,  $df = 2$ , 20,  $P < 0.04$ ; Table 3). Larvae were observed crawling through

**Table 2. Mean ( $\pm$ SE) of adult small hive beetles in hives undergoing various treatment methods of tylosin for American foulbrood control**

| Treatment    | Adult Beetles |                  |                  |                  |
|--------------|---------------|------------------|------------------|------------------|
|              | 26 Jun        | 3 Jul            | 10 Jul           | 30 Aug           |
| Control      | <5            | 11.1 $\pm$ 4.1a  | 46.3 $\pm$ 24.6a | 91.7 $\pm$ 35.2a |
| Grease patty | <5            | 88.1 $\pm$ 11.9b | 93.6 $\pm$ 30.8a | 70.0 $\pm$ 34.8a |
| Dust         | <5            | 5.0 $\pm$ 3.0a   | 10.6 $\pm$ 6.2b  | 82.1 $\pm$ 31.7a |

Values within a column followed by different letters are significantly different; ANOVA and LSD means separation for each date ( $P < 0.05$ ).

**Table 3. Mean  $\pm$  SE number of larval small hive beetles in hives undergoing various treatments methods of tylosin for American foulbrood control**

| Treatment    | Larvae |                  |                    |        |
|--------------|--------|------------------|--------------------|--------|
|              | 26 Jun | 3 Jul            | 10 Jul             | 30 Aug |
| Control      | 0a     | 0a               | 0a                 | 0a     |
| Grease patty | 0a     | 67.9 $\pm$ 11.8b | 213.6 $\pm$ 133.1b | 0a     |
| Dust         | 0a     | 0a               | 0a                 | 0a     |

Values within a column followed by different letters are significantly different; ANOVA and LSD means separation for each date ( $P < 0.05$ ).

the patties, appearing to feed on them. By the completion of the trial, all hives were larvae-free, even in the patty-treated hives, suggesting in these hives that beetle generations followed a discrete cycle rather than continuous egg laying by adult female beetles. It is unclear why larvae developed only in the patty treated hives during the treatment period.

Hive strength was also significantly impacted by method of application of tylosin. By the end of the evaluation period, control hives exhibited significantly reduced numbers of frames of bees ( $t = 2.07$ ,  $df = 14$ ,  $P < 0.05$ ; Table 4) compared with initial numbers. Hives treated with grease patties also exhibited decline in bee numbers over time ( $t = 3.374$ ,  $df = 14$ ,  $P < 0.05$ ; Table 4). No significant overall reduction of bee populations was seen in hives treated with the dust method of application.

Therefore, we demonstrated that tylosin is an effective compound in controlling oxytetracycline-resistant AFB in the apiary tested, using both dust and grease patty methods of application. The grease patty method was associated with significant outbreaks of the small hive beetle and reduced colony strength, however. Given the wide distribution of the small hive beetle in the eastern U.S. and likely spread to the entire continental U.S., application of tylosin via patty method would seem a poor management practice based on the current study. There also exists the possibility of increased residues of tylosin in hive products when applied as a grease patty versus dust application: the patty remains longer in the hive than a dust application, increasing chances of residues accumulating in wax and honey. Such a chance of increased residues is one reason the FDA looks unfavorably on the approval of tylosin grease patties for AFB control. Also, such increased exposure time of antibiotic with patho-

**Table 4. Mean  $\pm$  SE number of frames of bees per hives undergoing various methods of application of tylosin for American foulbrood control**

| Treatment    | Frames Adult Bees Per Hive |                |
|--------------|----------------------------|----------------|
|              | 26 Jun                     | 30 Aug         |
| Control      | 9.5 $\pm$ 0.5a             | 6.3 $\pm$ 0.8b |
| Grease patty | 9.3 $\pm$ 0.5a             | 4.0 $\pm$ 1.5b |
| Dust         | 10.0 $\pm$ 0.0a            | 8.3 $\pm$ 1.3a |

Values within a row followed by different letters are significantly different ( $t$ -test,  $P < 0.05$ ).

gen also increases the chances for AFB to more rapidly become resistant to tylosin, compared with the less persistent dust method of application.

The potential approval of tylosin would be one tool in the management of AFB, along with the breeding of hygienic bee strains that remove AFB-diseased larvae from the hive (Spivak and Reuter 1998). Long-term management of AFB requires the combination of these multiple approaches, to best ward off the development of resistance to tylosin and lessen usage of chemical compounds within the hive.

#### References Cited

- Alippi, A. 2000. Is Terramycin R losing its effectiveness against AFB? *Bee Biz* 11: 27–29.
- Elzen, P. J., J. R. Baxter, D. Westervelt, C. Randall, K. S. Delaplane, L. Cutts, and W. T. Wilson. 1999. Field control and biology studies of a new pest species, *Aethina tumida* Murray (Coleoptera: Nitidulidae), attacking European honey bees in the Western Hemisphere. *Apidologie* 30: 361–366.
- Hitchcock, J. D., J. O. Moffet, J. J. Lockett, and J. R. Elliott. 1970. Tylosin for control of American foulbrood disease in honey bees. *J. Econ. Entomol.* 63: 204–207.
- Kochansky, J. P., D. A. Knox, M. F. Feldlaufer, and J. S. Pettis. 2001. Screening alternative antibiotics against oxytetracycline-susceptible and resistant American foulbrood. *Apidologie* 32: 547–554.
- Lundie, A. E. 1940. The small hive beetle, *Aethina tumida*. *Sci. Bull* 220: Union of South Africa, Dep. Agric. Forestry, Entomological Series 3, 30 pp.
- Miyagi, Y., C.Y.S. Peng, R. Chuang, E. C. Mussen, M. S. Spivak, and R. H. Doi. 2000. Verification of oxytetracycline-resistant American foulbrood pathogen *Paenibacillus larvae* in the United States. *J. Invert. Pathol.* 75: 95–96.
- Peng, C.Y.S., E. Mussen, A. Fong, P. Cheng, G. Wong, and M. A. Montague. 1996. Laboratory and field studies on the effects of the antibiotic tylosin on honey bee *Apis mellifera* L. (Hymenoptera: Apidae) development and prevention of American foulbrood disease. *J. Invert. Pathol.* 67: 65–71.
- Sanford, M. T. 1998. *Aethina tumida*: a new beehive pest in the Western Hemisphere. *APIS* 16: 1–5.
- Shimanuki, H., D. A. Knox, B. Furgala, D. M. Caron, and J. L. Williams. 1992. Diseases and pests of honey bees, p. 1083–1151. *In* J. Graham [ed.], *The Hive and the Honey Bee*. Dadant and Sons, Hamilton, IL.
- Shimanuki, H. 1997. Bacteria, pp. 33–54. *In* R.A. Morse and K. Flottum [eds.], *Honey bee pests, predators, and diseases*. A.I. Root Co., Medina, OH.
- Shimanuki, H. And D. A. Knox. 2000. Diagnosis of honey bee diseases. U.S. Dep. Agric. Handbook No. AH-690.
- Spivak, M. And G. S. Reuter. 1998. Honey bee hygienic behavior. *Am. Bee J.* 138: 283–286.
- SYSTAT, Inc. 1990. Intelligent software. Evanston, IL.
- Zar, J. H. 1974. *Biostatistical Analysis*. Prentice-Hall, Englewood Cliffs, NJ.

Received for publication 20 May 2002; accepted 16 August 2002.