



**HAL**  
open science

# Natural products smoke and its effect on *Acarapis woodi* and honey bees

Frank Eischen, Carlos Vergara

► **To cite this version:**

Frank Eischen, Carlos Vergara. Natural products smoke and its effect on *Acarapis woodi* and honey bees. *Apidologie*, 2004, 35 (4), pp.341-349. 10.1051/apido:2004026 . hal-00891833

**HAL Id: hal-00891833**

**<https://hal.science/hal-00891833>**

Submitted on 11 May 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

## Natural products smoke and its effect on *Acarapis woodi* and honey bees<sup>†</sup>

Frank A. EISCHEN<sup>a\*</sup>, Carlos H. VERGARA<sup>b\*\*</sup>

<sup>a</sup> Honey Bee Unit, USDA-ARS-SARC, 2413 E. Hwy 83, Weslaco, TX 78596, USA

<sup>b</sup> Departamento Química Y Biología, Universidad de las Americas, Puebla, 72820 Santa Catarina Matir, Puebla, Mexico

(Received 23 September 2002; revised 26 June 2003; accepted 1st September 2003)

**Abstract** – We tested the effect natural products smoke has on the honey bee tracheal mite (*Acarapis woodi*) and honey bees. Plant materials screened for activity included coffee beans (*Coffea arabica*), corncobs (*Zea mays*), creosote bush (*Larrea tridentata*), eucalyptus (*Eucalyptus* sp.), orange peel (*Citrus sinensis*), pecan leaves (*Carya illinoensis*), dead and fresh pine needles (*Pinus cembroides*), mesquite leaves (*Prosopis glandulosa*) and tobacco (*Nicotiana tabacum*). Low but significant mite mortality was caused by the smoke of pine needles, mesquite, corncobs, and coffee beans. The smoke of *L. tridentata* killed more adult *A. woodi* than other materials (LT<sub>50</sub> = 2.4 min, single exposure). It was not effective against immatures. Mite mortality was negatively correlated with parasites/trachea, suggesting that reduced air flow while breathing may have reduced efficacy. Efficacy was modest (ca. 70%) and it caused transitory bee anesthesia. We do not recommend using this material as a control.

creosote bush / *Larrea tridentata* / smoke / *Acarapis woodi* / secondary plant products

### 1. INTRODUCTION

Mussen (2001) estimates that during the 1990s decade, US and Canadian colonies worth \$90 million dollars were lost to the parasitic honey bee mite *Acarapis woodi* (Rennie). Sporadic outbreaks of this parasite continue to cause problems, but reported losses have diminished. Menthol is the only registered treatment commercially available in the United States. Acaricidal resistance is a common and difficult agricultural problem. The recent development of resistance to popular acaricides by *Varroa destructor* Anderson & Trueman, indicates that resistance to tracheal mite controls could develop and underscores the need it for alternative management methods.

Plants limit herbivory by various defenses. Often they incorporate chemicals, i.e., secondary plant products, that are feeding deterrents, repellents, antimetabolites, or have insecticidal compounds in their tissues ((Feeny, 1970; Rosenthal and Janzen, 1979; Dobler, 2001; Lill and Marquis, 2001). The presence of active secondary plant products in many plants suggests that when applying plant smoke to their colonies beekeepers may be inadvertently fumigating them with harmful or possibly helpful secondary plant compounds. This is especially true when colonies are vigorously smoked in an effort to maintain control of the bees. Under these conditions, they may be applying significant amounts of these materials.

Our purposes were two fold: (1) determine if the heated volatiles in smoke from burning

<sup>†</sup> Mention of a proprietary product does not constitute endorsement by the Universidad de las Americas or the United States Department of Agriculture.

\* Corresponding author: feischen@WESLACO.ARS.USDA.GOV

\*\* cvergara@mail.udlap.mx

plant materials could affect the survival of the parasitic honey bee mite *Acarapis woodi* and honeybees; and (2) test a select series of plant materials, screening for those with active compounds against *A. woodi*. Some of the plants selected for screening, viz. creosote bush (*Larrea tridentata*), tobacco (*Nicotiana tabacum*), coffee (*Coffea arabica*), and orange peel (*Citrus sinensis*) are known or suspected to have insecticidal and acaricidal properties (Rodriquez and Levin, 1976; Liu, 1991; Kretschmar and Baumann, 1999). The others were selected either because they are commonly used smoker fuel (corn cobs, *Zea mays*; pine needles, *Pinus cembroides*; Johnson grass, *Sorghum halepense*) or we were asked to check their activity by beekeepers because they are highly aromatic or resinous (eucalyptus, *Eucalyptus* sp.; mesquite, *Prosopis glandulosa*; pecan, *Carya illinoensis*).

## 2. MATERIALS AND METHODS

### 2.1. Experiments with eight plant materials (Experiment 1)

Plant materials were collected in Nuevo Leon or Tamaulipas, Mexico, i.e., coffee beans (*C. arabica* purchased in Allende, NL Mexico), white corncobs (*Z. mays*), eucalyptus leaves (*Eucalyptus* sp.), orange peel (*C. sinensis* L.), pecan leaves (*C. illinoensis*), dead pine needles (*P. cembroides*), mesquite leaves (*P. glandulosa*) and tobacco (*N. tabacum*; crushed cigars, Ejecutivos Santa Clara SA, purchased in Monterrey, NL Mexico). Living plant material was gathered and air dried under ambient conditions (excepting coffee and tobacco, which were purchased in Mexico). Corncobs were obtained from a garden by hand-shelling dry ears of corn and breaking them into small pieces (ca. 1.0 cm<sup>3</sup>) by hammering. Dried grass (tentatively identified as Johnson Grass, *S. halepense*), another common smoker fuel was used for comparison in later experiments. Care was taken to collect materials away from roadways and agriculturally active areas (coffee and tobacco, were commercial products) to avoid contamination. Smoke was generated with a smoker (Dadant®; 25.4 cm Hamilton, Illinois) by preparing a bed of hot coals using paper and the test material and then filling the smoker with the test material. The smoker was filled only half full in the case of coffee beans and tobacco. About 10 puffs on the smoker were made to insure that the test material was burning. Then the smoker was set aside for a few minutes to let the temperature of the burning materials mod-

erate. In all tests we avoided blowing hot smoke (> 37 °C) on bees, and regularly measured the temperature of test smokes with a thermometer inside the test container.

#### 2.1.1. Trial 1

Twelve bees were randomly selected from the inner cover of a honeybee colony considered to be *Apis mellifera ligustica* heavily infested with tracheal mites (over several months of sampling, 100% of bees were infested with 30.4 mites of all stages per trachea found during the trial). Bees were placed in standard Benton queen mailing cages with queen candy (Root, 1983). Efficacy was determined by placing the caged bees at the bottom of a 3.8-liter jar. A circular hole was cut in the lid fitted with a cork stopper and thermometer. Two puffs of cool smoke (36 ± 2 °C) were directed into the jar and its lid closed. The bees were held in the smoke for six minutes, and then removed. Bees were allowed a 20-minute recovery period, offered a drop of water, placed in a dark incubator (35 °C; ca. 50% RH), and examined daily for mortality (18 day period). Four days after treatment, five bees from each cage were removed and one of their first thoracic tracheal trunks examined for the presence of living and dead mites using the technique described by Eischen et al. (1987) using a M5A Wild Heerbrugg dissecting microscope. All stages of mites were counted and the criteria for death applied except to the eggs. Their status was indeterminable. The survival of the remaining seven bees was monitored. Before testing a second material, the smoker was carefully cleaned and residues burnt off.

#### 2.1.2. Trial 2

This trial was conducted on a shortened list of plant materials: white corncobs (*Z. mays*), green pine needles (*P. cembroides*) and *Eucalyptus* sp. leaves using 10 bees per Benton cage. Final mortality was assessed for *A. woodi* and honey bees after five days. This test was conducted under the same conditions as described above and done to verify our finding in Trial 1.

### 2.2. Experiments with creosote bush (*L. tridentata*)

#### 2.2.1. Experiment 2

Above ground portions of *L. tridentata* were collected about 30 km south of Saltillo, Coahuila, air dried, and cut into small pieces (2–3 cm). Smoke from the whole plant was applied to *A. woodi* infested bees for varying lengths of time (2–8 min).

After treatment, bees were allowed to recover in ambient conditions for 20 minutes and then each Benton cage was given a drop of water and placed in the incubator (35 °C; ca. 50% RH). Mite mortality was checked after four days.

### 2.2.2. Experiment 3

A test based on hive volume with one application of smoke from creosote bush prepared as in Experiment 2 was conducted. Infested honey bee workers were placed in Benton mailing cages, screen side down, on a queen excluder. The excluder was then placed between two empty standard deep Langstroth hive bodies (241 mm). Hive bodies rested on a standard bottom board and were covered by a telescoping cover. Smoke was applied through a standard-sized bottom entrance. Cool smoke was slowly blown into the hive for 30 seconds, then cages were removed after varying lengths of time (2–8 min). Dried grass (tentatively identified as Johnson Grass, *S. halepense*), was included in this test for comparison. Twenty minutes after treatment, each Benton cage was given a drop of water and placed in the incubator (35 °C; ca. 50% RH). *A. woodi* infestations were examined four days after treatment.

### 2.2.3. Experiment 4

Hive volume was simulated and two treatments of *L. tridentata* smoke applied on days one and four. Mite mortality was assessed 5 days after the second application. Other conditions were the same as in Experiment 3.

### 2.2.4. Experiment 5

Smoke from new growth and old growth parts of creosote bush collected from the same plant. All conditions were the same as in Experiment 2. As a separate issue, we examined the effect of heat on mite mortality in this experiment. Our test apparatus (3.8 liter jar) was placed in a water bath and its inside temperature adjusted to 48 °C by mixing hot and cold water in the bath. Infested honey bee workers were held at that temperature for six minutes by placing them, in their cages, on the bottom of the jar.

## 2.3. Statistical analyses

A one-way ANOVA was conducted on plant materials screened for activity in Experiments 1 and 5. Means were evaluated using the Least Significant Difference method (LSD). A correlation coefficient was calculated between the number of mites per tra-

chea and mite mortality. Statistical analyses of dose responses (mite mortality) to stepwise increases to smoke exposures were performed using a probit analysis (logit transformation; SAS Institute, 1990) and the time required to cause 50% mortality ( $LT_{50}$ ) calculated in Experiments 2, 3, and 4.

## 3. RESULTS

### 3.1. Experiment 1

#### 3.1.1. Bee mortality

In Trial 1 (Tab. I) 71.4% of bees treated with crushed corncob smoke died within 18 days of fumigation. Bees treated with pecan and mesquite leaf smoke were comatose after fumigation but recovered.

In Trial 2, 100% of workers treated with the smoke of crushed corncobs died within five days of fumigation. A few bees were comatose immediately after treatment, but appeared normal within a few minutes.

#### 3.1.2. Mite mortality

No material caused high levels of mite mortality (Tab. I), but the smoke of pine needles, corncobs, tobacco, mesquite, pecan and coffee caused significant adult mite mortality ranging from 25.8–51.9%. A negative correlation ( $r = -0.85$ ,  $n = 8$ ,  $P < 0.01$ ) between the mite population and % mite mortality was found (orange peel was deleted from this correlation because it did not burn well and we do not think it has been fairly tested).

### 3.2. Experiment 2

#### 3.2.1. Bee mortality

A few bees were comatose when removed from the smoke. All recovered within 10 minutes. After seven days, 18.0% of the bees in the two-minute exposure to *L. tridentata* had died, whereas all other bees survived (Tab. II). Hence bee mortality was not dose related, and it is not known if *L. tridentata* smoke has long term detrimental effects on honey bees.

**Table I.** Experiment I. *Acarapis woodi* fate in one first thoracic tracheal trunk of infested bees<sup>1</sup> treated for six minutes with smoke from 8 plant materials. Mortality assessment 4 days after treatment.

treatment	trial	bee <sup>2</sup> death	<i>A. woodi</i>							
		(%)	adults	larvae	eggs <sup>3</sup>	alive (x ± SD)	dead (x ± SD)	% dead	alive (x ± SD)	dead (x ± SD)
Untreated control	1	0	19.6 ± 8.4	3.4 ± 0.9	14.8 a <sup>4</sup>	10.6 ± 3.0	0	10.8 ± 8.7		
Orange peel	1	14	13.4 ± 5.4	1.0 ± 1.0	6.9 a	4.4 ± 3.1	0	7.6 ± 5.3		
Eucalyptus leaves	1	0	16.0 ± 6.4	5.0 ± 0.7	23.8 abc	12.2 ± 1.3	0.2	5.8 ± 2.6		
Tobacco (cigars)	1	0	18.0 ± 10.6	6.4 ± 3.9	26.2 abc	7.2 ± 6.4	0	5.0 ± 1.6		
Pine needles (dead)	1	0	14.4 ± 9.0	5.0 ± 2.3	25.8 bc	5.6 ± 4.0	0	6.8 ± 3.7		
Crushed corncobs	1	71	10.2 ± 4.1	5.6 ± 1.1	35.4 bc	7.2 ± 4.4	0.2	5.6 ± 2.9		
Pecan leaves <sup>5</sup>	1	0	10.2 ± 8.6	3.6 ± 1.1	26.1 bc	6.4 ± 5.1	0	5.0 ± 4.0		
Mesquite leaves <sup>5</sup>	1	14	9.0 ± 7.1	6.0 ± 4.1	40.0 c	5.2 ± 4.5	0.4	1.2 ± 1.3		
Coffee beans	1	0	5.2 ± 2.8	5.6 ± 2.5	51.9 c	3.8 ± 2.9	0	4.4 ± 1.8		
Untreated control	2	0	20.0 ± 8.2	10.0 ± 4.3	33.3 a	7.4 ± 4.7	0	5.2 ± 3.8		
Crushed corncobs	2	100	22.7 ± 10.2	19.5 ± 2.6	46.2 a	19.5 ± 9.7	0.5	4.0 ± 1.4		
Pine needles(fresh)	2	10	16.6 ± 7.6	11.2 ± 1.9	40.3 a	14.6 ± 5.9	0.2	3.8 ± 0.8		
Eucalyptus leaves	2	10	14.4 ± 7.5	9.8 ± 2.8	40.5 a	11.4 ± 5.1	0.2	4.8 ± 1.5		

<sup>1</sup> Mite mortality are averages from five bees.

<sup>2</sup> Bee mortality at 18 and 5 days, for Trial 1 (n = 7) and 2 (n = 10), respectively.

<sup>3</sup> Unable to determine egg viability.

<sup>4</sup> Means in a column followed by the same letter are not significantly different (LSD;  $P > 0.05$ ).

<sup>5</sup> Pecan and mesquite leaf smoke caused regurgitation and anesthesia.

### 3.2.2. Mite mortality

In general, longer exposures to *L. tridentata* smoke resulted in higher *A. woodi* mortality. The 70.1% mortality observed by the longest exposure is approaching economic levels (LT<sub>50</sub> = 2.4 min; 95%CL = 0.2–3.7, n = 5, intercept = -0.3, slope = 0.9 SE = 0.7, chi-square = 8.8). Low levels of larval mite mortality were observed as in Experiment 1.

### 3.3. Experiment 3

*L. tridentata*: hive simulation with one application of smoke. Increasing the exposure time from two to six minutes of *L. tridentata* smoke caused increasing mite mortality. Exposure times of 8 or 10 minutes did not cause additional mite mortality (Tab. II; LT<sub>50</sub> = 3.8 min; 95%CL = 2.7–4.7, n = 6,

intercept = -0.7, slope = 1.2, SE = 0.4, chi-square = 9.2). In a follow up experiment, we found no significant increases in mite mortality when mites were examined eight days after fumigation compared with four days. This suggests that most mite mortality occurs soon after fumigation with this plant. Grass smoke did not appear to affect mite mortality.

### 3.4. Experiment 4

Table II shows that a second fumigation with *L. tridentata* smoke in a simulated hive condition did not cause a higher adult mite mortality compared with mites given only one exposure to smoke. Similar to Experiment 3, adult mite mortality reached a plateau with a six-minute exposure (LT<sub>50</sub> = 4.3; 95%CL = 3.6–5.0, n = 6, intercept = -0.96, slope = 1.5, SE = 0.5, chi-square = 20.5).

**Table II.** *A. woodi* fate in infested bees<sup>1</sup> treated for varying lengths of time with smoke of above ground portions of *Larrea tridentata*. Mortality assessment 4 days after treatment<sup>2</sup>.

treatment	exp.	<i>A. woodi</i>					
		adults			larvae		eggs <sup>3</sup>
		alive (x ± SD)	dead (x ± SD)	% dead	alive (x ± SD)	% dead (x ± SD)	(x ± SD)
Untreated control	2	14.8 ± 6.7	0.8 ± 0.8	5.1	11.2 ± 4.1	0	5.8 ± 2.6
2 min. <i>L. tridentata</i>	2	3.8 ± 4.3	5.2 ± 0.8	57.8	6.2 ± 4.3	1.0 ± 1.7	3.6 ± 5.0
4 min. <i>L. tridentata</i>	2	9.4 ± 5.4	7.6 ± 3.4	44.7	6.6 ± 5.6	0.8 ± 0.8	3.6 ± 2.5
6 min. <i>L. tridentata</i>	2	6.0 ± 4.5	12.4 ± 3.4	67.4	6.2 ± 5.3	1.0 ± 1.7	5.0 ± 4.3
8 min. <i>L. tridentata</i>	2	5.2 ± 5.8	12.2 ± 6.5	70.1	7.2 ± 7.6	1.4 ± 1.9	1.8 ± 2.5
Untreated control	3	12.4 ± 6.4	5.0 ± 2.7	28.4	3.8 ± 2.2	0	2.2 ± 0.4
2 min. <i>S. halepense</i>	3	15.8 ± 7.3	3.6 ± 2.5	18.6	14.0 ± 8.3	0.2 ± 0.4	6.2 ± 3.8
2 min. <i>L. tridentata</i>	3	12.2 ± 10.2	7.0 ± 2.0	36.5	14.8 ± 10.2	0	3.6 ± 2.9
4 min. <i>L. tridentata</i>	3	9.8 ± 5.5	7.8 ± 3.9	44.3	9.2 ± 2.8	0.6 ± 1.5	2.4 ± 1.5
6 min. <i>L. tridentata</i>	3	6.8 ± 7.0	16.4 ± 2.9	70.7	7.0 ± 7.8	0	2.4 ± 2.1
8 min. <i>L. tridentata</i>	3	8.4 ± 6.5	14.2 ± 6.1	62.8	11.8 ± 3.5	0	4.6 ± 2.8
10 min. <i>L. tridentata</i>	3	8.0 ± 6.7	14.8 ± 6.3	64.9	7.4 ± 2.9	1.0 ± 1.4	3.8 ± 2.2
Untreated control	4	26.0 ± 6.8	3.0 ± 1.4	10.3	10.8 ± 5.9	0	14.6 ± 5.5
2 min. <i>S. halepense</i>	4	19.8 ± 5.1	5.0 ± 2.0	20.2	6.8 ± 3.0	0	11.0 ± 2.2
2 min. <i>L. tridentata</i>	4	19.4 ± 4.5	7.2 ± 4.2	27.1	9.6 ± 8.4	0	9.4 ± 6.9
4 min. <i>L. tridentata</i>	4	21.8 ± 2.3	20.4 ± 2.2	48.3	8.2 ± 0.8	0.8 ± 1.3	7.2 ± 2.4
6 min. <i>L. tridentata</i>	4	8.4 ± 1.8	22.4 ± 6.4	71.8	10.4 ± 4.4	0	4.6 ± 1.7
8 min. <i>L. tridentata</i>	4	10.8 ± 9.0	12.8 ± 3.7	52.6	9.6 ± 5.5	2.6 ± 1.7	3.0 ± 1.6
10 min. <i>L. tridentata</i>	4	12.6 ± 16.5	29.8 ± 13.9	70.3	9.4 ± 7.8	4.8 ± 2.8	6.2 ± 11.1

<sup>1</sup> Five bees per treatment, one first thoracic tracheal trunk examined/bee.

<sup>2</sup> Experiment 2 conducted in a 3.8 liter jar and a probit analysis performed on numbers of dead adult mites (LT<sub>50</sub> = 2.4 min). Experiment 3 simulated hive smoke; 1 application (LT<sub>50</sub> = 3.8 min). Experiment 4 simulated hive smoke; 2 applications (LT<sub>50</sub> = 4.3 min), the 2nd given four days after the first.

<sup>3</sup> Unable to determine egg viability.

### 3.5. Experiment 5

Table III shows that smoke from *L. tridentata* young leaves killed significantly fewer adult mites than the smoke of old leaves. Smoke from whole plants caused intermediate adult mite mortality. In bees exposed to 48 °C *A. woodi* mortality was not different than in the control. Heated bees became agitated. Bee mortality was not monitored in Experiments 3, 4, and 5.

### 4. DISCUSSION

Smoke has been reported as an effective method of applying acaricidal materials for controlling parasitic honey bee mites (Dag et al., 1997; Herbert et al., 1989; Van Laere and de Wael, 1987; Watanabe, 1986). The commercial products Folbex VA (bromopropylate) and Varamit (amitraz), both marketed outside the U.S., are strips designed to be burned to release the active ingredients and

**Table III.** Experiment 5: *A. woodi* infesting bees<sup>1</sup> after 6-min fumigation with *L. tridentata* smoke or exposure to elevated temperature (48 °C).

treatment	<i>A. woodi</i>					
	adults			larvae		eggs <sup>2</sup>
	alive (x ± SD)	dead (x ± SD)	% dead	alive (x ± SD)	dead (x ± SD)	(x ± SD)
Untreated control	18.4 ± 3.6	4.8 ± 1.5	20.7 a <sup>3</sup>	9.6 ± 3.4	0	3.6 ± 2.2
Heated control (48 °C)	14.0 ± 6.1	3.4 ± 1.9	19.5 a	8.0 ± 4.8	0	3.6 ± 1.5
New leaves <i>L. tridentata</i>	22.0 ± 13.4	7.0 ± 4.5	24.1 ab	5.2 ± 1.6	0.4 ± 0.5	1.2 ± 1.1
Whole plant <i>L. tridentata</i>	16.0 ± 8.0	11.8 ± 8.7	40.0 bc	10.2 ± 2.8	0	5.4 ± 2.1
Old leaves <i>L. tridentata</i>	15.0 ± 3.8	15.0 ± 4.9	50.0 c	7.0 ± 3.5	0.2 ± 0.4	3.8 ± 0.8

<sup>1</sup> Five bees were used in each treatment; one first thoracic tracheal trunk examined/bee.

<sup>2</sup> Unable to determine egg viability.

<sup>3</sup> Means in a column followed by the same letter are not significantly different (LSD;  $P > 0.05$ ).

have been reported to be effective against *A. woodi* and *V. destructor*. Our tests probably involved more than combustion products. We suspect that plant material above the burning embers in the smoker got hot and volatilized. These unburned gases may have had an effect on bees and tracheal mites. Eischen (1997) and Elzen et al. (2001) report that some natural products smoke have a negative impact on *V. destructor*.

There were variable responses by both *A. woodi* and adult bees to different plant smokes. Corncob smoke was toxic to adult bees, whereas it caused only moderate adult mite mortality. We are not aware of what compound(s) in corncobs caused bee mortality. It is possible that residues contaminated the corncobs. We can not vouch that the corn, tobacco, or coffee were free of artificial contaminants as they were either collected in a garden or purchased. The other materials used in our tests were collected in remote areas and it is very unlikely that these contained residues. Smoke given off by burning coffee beans also gave moderately high *A. woodi* mortality, but was harmless to bees. Further testing is warranted with this material.

Tobacco smoke was ineffective in our tests, which was contrary to a number of reports. Liu (1991) found that it caused a relatively high tracheal mite mortality, but that excessive nicotine would kill bees. De Ruijter (1982) reported that it is effective against *V. destructor*. Cook and Griffiths (1985) noted its value in

detecting *V. destructor* infestations. It is possible we did not apply sufficient smoke or that the cigar tobacco used had a low nicotine content. Beekeepers inclined to try this material as a smoker fuel should exercise caution as anesthesia can cause colony suffocation, especially during hot weather.

Our tests with *L. tridentata* smoke showed that it contains compounds capable of killing adult *A. woodi* infesting honey bees. Increasing exposures were generally associated with higher rates of mite mortality. A second exposure to *L. tridentata* smoke four days after the first did not increase mite mortality. Lengthening the incubation time after treatment did not detect mite mortality at a later time suggesting that most *A. woodi* mortality occurs acutely after exposure to the smoke. Generally, longer exposures killed more adult mites, but a plateau was reached around 70% mortality after a 6 min. exposure. A possible cause for this may have been the extreme density of mites in the tracheal tubes. We observed a significant negative correlation between control and parasite numbers in the trachea. It is likely that when tightly packed, mites are shielded to a degree by reduced airflow (Bailey, 1954). They may also receive reduced doses simply because the proximity of bodies shielded a portion of their surface to exposure. *L. tridentata*, as other plant materials, killed few mite larvae. This has been noted previously with other acaricides (Eischen et al., 1988), but is poorly understood.

A number of compounds derived from *L. tridentata* have been shown to be feeding deterrents and antimetabolites against herbivores (Rhoades, 1977; Greenfield et al., 1987; Greenfield et al., 1989; Chapman et al., 1988; Meyer and Karasov, 1989; Hyder et al., 2002). It is a source of medicinal herbal products used in Mexico (D. Cardoso-Tamez, personal communication). Nordihydroguaiaretic acid (NDGA), which can comprise 10% of its dry leaf matter, has been shown to have antiviral and antimicrobial as well as antifeedant properties (Chapman et al., 1988; Craigo et al., 2000; Verastegui et al., 1996). Worker bees treated with *L. tridentata* smoke uniformly displayed anesthesia and vomiting similar to that caused by tobacco smoke. However, longevity tests revealed no reduction in lifespan with any duration of exposure to *L. tridentata* smoke.

Secondary plant product levels in *L. tridentata* vary by tissue type (Hyder et al., 2002), by variants within a locality (Greenfield et al., 1987; Greenfield et al., 1989; Ernest, 1994), by soil fertility (Lightfoot and Whitford, 1989) and over its range (Downum et al., 1988). Some of the variation observed in our tests may be due to plant variability, though all samples were taken from a small area. Further, our test of new and old leaves came from the same plants. The higher mortality of adult *A. woodi* treated with the smoke of old leaves indicates a higher level of active compounds in older leaves.

Efficacy varied in this study due to differences in the host, parasite and plant. Most of these await exploration. Though mindful of the importance of variation, our primary goal was to screen a selected group of plant materials for active compounds. Worst-case infestations were used to insure that results with lesser infestations would likely be as good or better. Unfortunately, large mite loads increase the time required to score them and limited the number bees examined. This probably did not detract from our screening objective, but did reduce sensitivity to measuring variation.

The smoke of creosote bush was the most effective tested, but other plants showed a modest activity. We conclude from these experiments that tracheal mite mortality was caused by plant volatiles. However, because there are possible, but unknown side effects on

honey bees, we do not advocate attempting to control *A. woodi* with any of them. These data do indicate that unknown compounds may be therapeutic and identification of them could lead to novel approaches for managing *A. woodi*.

## ACKNOWLEDGEMENTS

This paper is dedicated to the memory of Sr. David Cardoso Tamez, ApiCar, Allende, Nuevo Leon, Mexico who urged us to begin this line of inquiry, provided honey bee colonies, apiary locations, living facilities and made us welcome in Mexico. David's two sons, David Jr. and Guillermo were extraordinarily helpful. We could not have done this work without them. Dr. Lambert Kanga and Mr. R. Henry Graham helped with the statistical analysis. Drs. Robert Danka (USDA-ARS) and Eric Mussen (Univ. Calif., Davis) reviewed the manuscript and made valuable comments. This research was supported in part by the California State Beekeepers Association.

**Résumé – Effets de la fumée de divers produits naturels sur *Acarapis woodi* et l'Abeille domestique.** Les plantes se protègent des herbivores de diverses façons. Elles élaborent souvent des composés chimiques, i.e. des substances végétales secondaires tels que les dissuasifs, les répulsifs, les antimétabolites ou possèdent des insecticides dans leurs tissus. Les insectes développent en retour des stratégies de défense contre ces substances et un processus de coévolution s'ensuit. Les apiculteurs utilisent une grande variété de matériaux d'origine végétale comme combustible pour l'enfumeur afin de calmer les colonies. Il se peut donc qu'ils enfument par inadvertance les colonies avec des substances végétales secondaires qui sont nuisibles (ou bénéfiques) pour les abeilles et les acariens parasites.

Cette recherche évalue l'efficacité de la fumée issue de produits naturels pour tuer l'Acararien des trachées, *Acarapis woodi* Rennie. L'efficacité a été déterminée en plaçant des abeilles dans un récipient en verre de 3,8 L et en y envoyant deux bouffées de fumée. Certains produits testés ont eu des effets sur la santé des abeilles et cela a été noté. L'activité des plantes suivantes a été testée : grains de café (*Coffea arabica*), épis de maïs (*Zea mays*), un buisson des régions sèches d'Amérique du Sud *Larrea tridentata*, eucalyptus (*Eucalyptus* sp.), écorce d'orange (*Citrus sinensis*), feuilles de pacanier (*Carya illinoensis*), aiguilles de pin (*Pinus cembroides*), feuilles de l'arbre *Prosopis glandulosa* (« mesquite ») et tabac (*Nicotiana tabacum*). La fumée d'aiguilles de pin, de *Prosopis*, d'épis de maïs et de grains de café a provoqué une mortalité relativement modeste (environ 25–50 %) mais significative des acariens adultes (Tab. I).



La fumée de *L. tridentata* a tué les *A. woodi* adultes ( $TL_{50} = 2,4$  min, une seule exposition) quatre jours après le traitement. L'efficacité n'a pas été améliorée par une seconde fumigation ni en repoussant à huit jours après le traitement l'examen de la mortalité. La fumée de *L. tridentata* a été inefficace contre les stades immatures. Nous ne recommandons pas ce matériau pour lutter contre l'Acarien des trachées. L'efficacité est modeste (environ 70 %) et provoque une anesthésie passagère. Les données indiquent néanmoins que cette plante, lorsqu'elle est brûlée ou chauffée, dégage des substances qui peuvent tuer *A. woodi* (Tab. II). La fumée provenant des vieilles parties (tiges et feuilles) a tué significativement plus d'acariens que la fumée des jeunes pousses (Tab. III). Dans tous nos tests, la fumée avait une température de 37 °C environ. Maintenir les abeilles infestées à 48 °C n'a pas amélioré l'efficacité de la fumée.

La plupart des matériaux végétaux testés n'a pas semblé réduire la durée de vie des abeilles adultes ; pourtant la fumée des épis de maïs (d'origine mexicaine) s'est montrée toxique pour les abeilles. Celles-ci ont survécu apparemment en bonne santé à la fumigation d'épis de maïs écrasés mais se sont mises à mourir 4 à 5 jours plus tard. L'action sur les abeilles de certaines plantes (par exemple le pacanier et *Prosopis glandulosa*) a été très nette pendant le traitement mais apparemment sans effet durable (Tab. I).

#### *Acarapis woodi* / fumée / toxicité / *Larrea tridentata* / substance végétale secondaire

**Zusammenfassung – Rauch aus natürlichen Produkten und seine Wirkung auf *Acarapis woodi* und Honigbienen.** Pflanzen schützen sich gegen Pflanzenfresser in unterschiedlicher Weise. Oft bauen sie chemische Substanzen (z.B. sekundäre Pflanzenstoffe) zur Fraßabschreckung, Abstoßung oder als Antimetaboliten ein oder sie haben Insektizide in ihrem Gewebe. Insekten wiederum entwickeln Abwehrstrategien gegen diese Stoffe und es entsteht ein sich gegenseitig beeinflussender Evolutionsprozess. Imker nutzen viele unterschiedliche pflanzliche Materialien für ihren Raucher zur Beruhigung der Völker. Dabei könnten sie bei der Bearbeitung der Völker unbemerkt Pflanzenmaterial zum Rauchen einsetzen, das zwar gegen die parasitischen Milben hilft jedoch für die Bienen selbst schädlich ist.

Die folgende Untersuchung bewertet die Wirkung von Rauch aus natürlichen Produkten zur Tötung der Tracheenmilbe *Acarapis woodi*. Zur Bestimmung der Wirkung wurden gekäfigte Bienen in einen 3,8-Liter Glasbehälter gestellt und 2 Rauchstöße dazugegeben. Einige der getesteten Produkte beeinflussten die Gesundheit der Bienen, was ebenfalls notiert wurde. Das Pflanzenmaterial, dessen Aktivität getestet (screening) wurde, bestand aus Kaffeebohnen (*Coffea arabica*), Maiskolben (*Zea*

*mays*), einem Busch aus den Trockengebieten Südamerikas (*Larrea tridentata*), Eucalyptus Arten (*Eucalyptus* sp.), Orangerinde (*Citrus sinensis*), Blättern der Pekan Nussbäume (*Carya illinoensis*), Kiefernnadeln (*Pinus cembroides*), Blättern der Mesquite Bäume (*Prosopis glandulosa*) und der Tabakpflanzen (*Nicotiana tabacum*). Ein Absterben der adulten Milben durch den Rauch von Kiefernnadeln, Mesquiteblättern, Maiskolben und Kaffeebohnen war gering aber signifikant (ca. 25–50 %) (Tab. I).

Der Rauch mit flüchtigen Stoffen von *L. tridentata* tötete adulte *A. woodi* ( $LT_{50} = 2,4$  min bei einer Anwendung). Die Messung erfolgte 4 Tage nach der Behandlung. Die Wirkung wurde durch eine 2. Rauchbehandlung nicht erhöht. *L. tridentata* Rauch blieb bei Larven unwirksam. Wir empfehlen diese Pflanze nicht für eine Behandlung gegen die Tracheenmilbe. Der Behandlungserfolg war gering (ca. 70 %) und hatte eine vorübergehende Narkosewirkung. Die Daten zeigen aber, dass diese Pflanze beim Verbrennen eine Substanz(en) freigibt, die *A. woodi* töten kann (Tab. II). Der Rauch von alten Pflanzenteilen (Stamm und Blätter) tötete signifikant mehr Milben als Rauch von frisch gesprossenen Teilen (Tab. III). In unseren gesamten Testreihen hatte der Rauch eine Temperatur von etwa 37 °C. Die Haltung der infizierten Bienen im Glas bei 48 °C erhöhte die Wirkung nicht.

Das meiste im Rauch getestete Pflanzenmaterial schien keine Wirkung auf die Lebensdauer der adulten Honigbienen zu haben. Allerdings erwies sich der Rauch von Maiskolben (mexikanischer Herkunft) als bienengiftig. Bienen überlebten zunächst den Rauch von zerkleinerten Maiskolben, begannen aber nach 4–5 Tagen zu sterben. Die Reaktion der Bienen auf manch anderen Pflanzenrauch, wie z.B. Pekan oder Mesquite, war im Moment der Behandlung sehr deutlich aber offensichtlich ohne Dauerschäden. (Tab. I).

#### *Larrea tridentata* / Rauch / *Acarapis woodi* / sekundäre Pflanzenstoffe

## REFERENCES

- Bailey L. (1954) The respiratory currents in the tracheal system of the adult honey-bee, J. Exp. Biol. 31, 589–593.
- Cook V.A., Griffiths D.A. (1985) Varroasis of bees: tobacco smoke detection, Ministry of Agriculture, Fisheries and Food, Great Britain, Pam. 936, 2 p.
- Craig J., Callahan M., Huang R.C.C., DeLucia A.L. (2000) Inhibition of human papillomavirus type 16 gene expression by nordihydroguaiaretic acid plant lignan derivatives, Antiviral Res. 47, 19–28.
- Chapman R.F., Bernays E.A., Wyatt T. (1988) Chemical aspects of host-plant specificity in three *Larrea* feeding grasshoppers, J. Chem. Ecol. 14, 561–580.

- Dag A., Slabezki Y., Efrat H., Kamer Y., Yakobson B.A., Mozes Koch R., Gerson U. (1997) Control of honey bee tracheal mite infestations with amitraz fumigation in Israel, *Am. Bee J.* 137, 599–602.
- De Ruijter A. (1982) Tobacco smoke can kill *Varroa* mites, *Bee World* 63, 138.
- Dobler S. (2001) Evolutionary aspects of defense by recycled plant compounds in herbivorous insects, *Basic Appl. Ecol.* 2, 15–26.
- Downum K.D., Dole J., Rodriguez E. (1988) Nordihydroguaiaretic acid interpopulational and intrapopulational variation in the Sonoran desert USA Mexico creosote bush *Larrea tridentata* Zygophyllaceae, *Biochem. Syst. Ecol.* 16, 551–556.
- Eischen F.A. (1997) Natural products smoke and *Varroa*, *Am. Bee J.* 137, 107.
- Eischen F.A., Pettis J.S., Dietz A. (1987) A rapid method of evaluating compounds for the control of *Acarapis woodi* (Rennie), *Am. Bee J.* 127, 99–101.
- Eischen F.A., Vargara C., Dietz A., Cardoso-Tamez D. (1988) Cymiazole, a systemic acaricide for the control of *Acarapis woodi* (Rennie) infesting honey bees. I. Laboratory Tests, *Apidologie* 19, 367–376.
- Elzen P.J., Stipanovic R.D., Rivera R. (2001) Activity of two preparations of natural smoke products on the behavior of *Varroa jacobsoni* Oud., *Am. Bee J.* 141, 289–291.
- Ernest K.A. (1994) Resistance of creosote bush to mammalian herbivory: Temporal consistency and browsing-induced changes, *Ecology* 75, 1684–1692.
- Feeny P. (1970) Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars, *Ecology* 51, 565–581.
- Greenfield M.D., Shelly T.E., Gonzalez-Coloma A. (1989) Territory selection in a desert grasshopper, the maximization of conversion efficiency on a chemically defended shrub, *J. Anim. Ecol.* 58, 761–772.
- Greenfield M.D., Shelly T.E., Downum K.R. (1987) Variation in host-plant quality, implications for territoriality in a desert grasshopper, *Ecology* 68, 828–838.
- Herbert E.W. Jr., Witherell P.C., Bruce W.A., Shimanuki H. (1989) Evaluation of six methods of detecting *Varroa* mites in beehives, including the experimental use of acaricidal smoke containing fluralinate or amitraz, *Am. Bee J.* 129, 605–608.
- Hyder P.W., Fedrickson E.L., Estell R.E., Tellez M., Gibbens R.P. (2002) Distribution and concentration of total phenolics, condensed tannins, and nordihydroguaiaretic acid (NDGA) in creosote bush (*Larrea tridentata*), *Biochem. Syst. Ecol.* 30, 905–912.
- Kretschmar J.A., Baumann T.W. (1999) Caffeine in *Citrus* flowers, *Phytochemistry* 52, 19–23.
- Lightfoot D.C., Whitford W.G. (1989) Interplant variation in creosote bush foliage characteristics and canopy arthropods, *Oecologia* 81, 166–175.
- Lill J.T., Marquis R.J. (2001) The effects of leaf quality on herbivore performance and attack from natural enemies, *Oecologia* 126, 418–428.
- Liu T.P. (1991) Tobacco smoke and tracheal mites, *Am. Bee J.* 131, 435.
- Meyer M.W., Karasov W.H. (1989) Antiherbivore chemistry of *Larrea tridentata* effects of woodrat *Neotoma lepida* feeding and nutrition, *Ecology* 70, 953–961.
- Mussen E.C. (2001) Introduction, spread, and economic impact of tracheal mites in North America, in: Webster T.C., Delaplane K.S. (Eds.), *Mites of the Honey Bee*, Dadant & Sons, Hamilton Illinois, pp. 43–56.
- Rhoades D.F. (1977) The antiherbivore chemistry of *Larrea*, in: Mabry T.J., Hunziker J.H., Difeo D.R. Jr. (Eds.), *Creosotebush; Biology and Chemistry of Larrea in New World deserts*, Dowden, Hutchinson & Ross, Stroudsburg, Pennsylvania, pp. 135–175.
- Rodriguez E., Levin D.A. (1976) Biochemical parallelisms of repellents and attractants in higher plants and arthropods, *Recent Adv. Phytochem.* 10, 214–270.
- Root A.I. (1983) *The ABC and XYZ of Bee Culture*, The AI Root Company, Medina, Ohio, 712 p.
- Rosenthal G.A., Janzen D.H. (1979) *Herbivores. Their interaction with secondary plant metabolites*, Academic Press, New York.
- SAS Institute (1990) *SAS/STAT User's Guide*, 6th ed., SAS Institute, Inc. Cary, NC.
- Van Laere O., de Wael L. (1987) Techniques for therapeutic smoke treatment of bee colonies, *Apiacta* 22, 74–80.
- Verastegui M.A., Sanchez C.A., Heredia N.L., Garcia-Alvarado J.S. (1996) Antimicrobial activity of extracts of three major plants from the Chihuahuan desert, *J. Ethnopharmacol.* 52, 175–177.
- Watanabe K. (1986) Screening tests of acaricides to control *Varroa* by smoking, *Honeybee Sci.* 7, 161–164 (In Japanese).