

Development of a Gel Formulation of Formic Acid for Control of Parasitic Mites of Honey Bees

Jan Kochansky* and Hachiro Shimanuki

USDA ARS, Bee Research Laboratory, Building 476, Beltsville Agricultural Research Center - East, Beltsville, Maryland 20705-2350

Formic acid has been used in various countries for the control of parasitic mites of honey bees (*Apis mellifera*), particularly the *Varroa* mite (*Varroa jacobsoni*) and the tracheal mite (*Acarapis woodi*). Its corrosivity and consequent fear of liability have precluded commercial interest in the United States, and its rapid vaporization requires frequent reapplication. We have developed a gel formulation of formic acid which provides controlled release over 2–3 weeks and improves the convenience and safety of handling of formic acid. The strong acidity of formic acid restricts the choice of gelling agents; vegetable gellants such as agar are destroyed, and bentonite clay derivatives do not gel, even with high-shear mixing. Polyacrylamides lead to viscous liquids lacking thixotropic properties. High-molecular-weight poly(acrylic acids) and fumed silicas provided gels with suitable physical characteristics. The poly(acrylic acid) gels were difficult to mix and gave slower and nonlinear release behavior, while the fumed silica gels were easy to prepare and linear in formic acid vaporization.

Keywords: Honey bees; *Apis mellifera*; formic acid; *Varroa jacobsoni*; tracheal mite; *Acarapis woodi*; acaricide; gel; poly(acrylic acid); fumed silica

Two species of mites parasitic on honey bees (*Apis mellifera*) have become major problems in the United States in the last 15 years, in addition to being serious problems in most beekeeping countries of the world. The tracheal mite (*Acarapis woodi* (Rennie)) is an endoparasite, living in the adult bee's tracheae. It was introduced into the U.S. after 1983 (Shimanuki and Knox, 1989). The *Varroa* mite (*Varroa jacobsoni* (Oudemans)) is an ectoparasite of both brood and adult bees, and entered the U.S. within a few years prior to 1987 (Anonymous, 1987). Both mites now inhabit honey bee colonies in most of the country. Infestation of a honey bee colony with either mite can lead to loss of the colony, but colony mortality caused by the *Varroa* mite can reach essentially 100% in temperate regions (De Jong, 1990). Not counting fumigants for unoccupied equipment, only one compound is currently registered for control of each mite in the U. S., menthol for the tracheal mite and fluvalinate (Apistan, Wellmark International, Dallas, TX) for the *Varroa* mite. Section 18 emergency registrations for a coumaphos strip were granted recently for use against Apistan-resistant *Varroa* in some states, but residues may become a problem. It is now well-established that *Varroa* has become resistant to fluvalinate in Northern Italy and surrounding regions (Lodesani et al., 1995; Milani, 1995), and resistance has now been reported in the United States (Eischen, 1998).

Alternate acaricides registered or under consideration elsewhere include amitraz, bromopropylate, chloropropylate, and coumaphos (Dietz and Hermann, 1988), but for various reasons none of these is likely to be registered in the United States. Essential oils or their isolated components have also been tried (Calderone et al., 1997 and references therein) but frequently have a

very small margin of safety for the bees (Ellis and Baxendale, 1997).

The first major report on the use of formic acid as a miticide was published nearly twenty years ago (Ritter and Ruttner, 1980). Wachendörfer et al. (1985) developed the "Illertisser Milbenplatte", consisting of a square of absorbent cardboard (bearing a remarkable resemblance to an overgrown beer coaster) soaked in formic acid before placing in the hive. This method or a modification has also been tested in other countries (Hoppe et al., 1989; Fries, 1989; Bracey and Fischer, 1989). Other methods of dispensing liquid formic acid include formic acid-soaked cheesecloth (Liu and Nasr, 1992; Liu et al., 1993), wood shavings ("Varroa Treatment Device", Popodi Development, Naramata, BC, Canada), and liquid reservoirs with wicks (Ritter and Ruttner, 1980; Sharma et al., 1983; Lupo and Gerling, 1990; one such is available commercially as the "Nassenheider Evaporator" from J. Weiland Werkzeugbau, Berlin).

Most of these techniques involve handling of liquid formic acid by the beekeeper, and many involve removal of one or more frames from the hive, leaving space that the bees fill with unwanted (by the beekeeper) honeycomb. Because of the mobility and highly corrosive nature of liquid formic acid, none of the potential manufacturers of beekeeping supplies in the United States has been willing to risk the liability associated with it.

Formic acid is also active against the tracheal mite, and Nelson et al. (1994) reported on a formic acid "gel-strip" in tests against tracheal mite. While it did lower mite levels in colonies, it was much less effective than liquid formic acid or menthol formulations. We reproduced this gel formulation, based on polyacrylamide, and found it exceedingly difficult to mix, with working times measured in seconds, and having poor rheological

* Corresponding author. Telephone: (301) 504-8668. Fax: (301) 504-8736. E-mail: jkochans@asrr.arsusda.gov.

properties. Rather than a gel, the material was an exceedingly viscous liquid, which eventually flowed out of dispensers unless contained.

Because of the potential value of formic acid against both species of parasitic mites and the immediate need for an alternate to fluvalinate for use against *Varroa*, we have developed a gel formulation with improved characteristics. We wanted a thixotropic material, capable of flowing under load, but which would revert to a nonslumping gel under the weight of amounts up to several hundred grams. It should be easy to mix, stable in storage, and relatively inexpensive. This paper describes the successful accomplishment of these goals.

MATERIALS AND METHODS

Formic acid was obtained from Fisher Scientific (Pittsburgh, PA, 90%) or Aldrich (Milwaukee, WI, 95%) and diluted to the desired concentration by volume. Agar was bacteriological grade from Difco Laboratories (Detroit, MI). Other vegetable gellants (acacia, xanthan gum, and guar gum) were obtained from Aldrich.

Synthetic gelling agents were commercial samples: Cab-O-Sil M-5, EH-5, and TS-720 (Cabot Corp., Tuscola, IL), Cyanamer P-21, N-300 LMW, and N-300 (Cytec Industries, West Patterson, NJ), Alcomer 120, Percol 156, 351, and 753 (Allied Colloids, Suffolk VA), Bentone RV and MA, Rheolate 255, 300, and EA-2670 (Rheox, Inc., Hightstown, NJ), and SGP 104 water absorbent polymer (Henkel Corp., Minneapolis, MN). They were chosen to be representative of types available, but are by no means a complete set.

High-shear mixing was performed with a Polytron instrument (Brinkmann, Westbury, NY). Other mixing was done with either a magnetic stirrer or by hand. Polymers were sifted slowly onto the surface of magnetically stirred formic acid solutions. When the viscosity became too high for the magnetic stirrer, stirring was continued with a stainless steel spatula. The Alcomer 120 high-concentration solution was prepared as described by Nelson et al. (1994).

Silicate gels were prepared by diluting sodium silicate solution (Aldrich) with the required amount of water to give the desired concentration after addition of formic acid. The acid was added, and the solution was swirled until homogeneous and allowed to stand for gelation.

Viscosity was measured on a Brookfield DV-II+ instrument with a No. 7 spindle (Brookfield Engineering Laboratories, Stoughton, MA).

Plastic films for packaging experiments were obtained locally (polyethylene, polypropylene) or from Mr. Steve Epstein, Mid-Continent Packaging, Enid, OK. Evaporation rates were determined in a laboratory hood at ambient laboratory temperature. Formic acid concentrations in air were determined with monitoring tubes (National Dräger, Pittsburgh, PA).

Data reduction and plotting were carried out using Prism ver. 3.0 (GraphPad Software, Inc., San Diego, CA).

RESULTS

Initial experiments with agar indicated that the strong acidity of the formic acid degraded the gellant. Preformed 1.5% agar/water gels dissolved in 65% formic acid. A warm solution of 1.5 g agar in 25 mL of water did not gel when mixed with 65 mL of 90% formic acid. Dry agar powder, on exposure to 65% formic acid gradually dissolved, but the solution showed no thickening. A starch-graft absorbent polymer (SGP 104) apparently did not absorb formic acid, as the powder did not expand and instead gradually dissolved. This acid sensitivity also applied to other vegetable gellants. Acacia did not give any useful increase in viscosity to 65% formic acid. Gels with xanthan gum and guar gum

Table 1. Gellation of Formic Acid Solutions with Cab-O-Sil M-5

formic acid concentration, %	Cab-O-Sil required, %	gel description
0 (water)	ca. 15	initial acceptable gel, but gel degraded over a few weeks to a mobile slurry
25	10	acceptable gel
45	8.5	acceptable gel
65	7	standard gel
90	<7 (7% was too thick, could have used less)	acceptable gel

Table 2. Gellation of Formic Acid Solutions with High-Molecular-Weight Poly(acrylic acids)

formic acid concentration, %	5% Carbopol 934	5% Carbopol 941
25	acceptable gel	acceptable gel
45	acceptable gel	acceptable gel
65	acceptable gel	acceptable gel
75	acceptable gel	acceptable gel
85	white mobile slurry	viscous lumpy liquid
90	colorless liquid with white sediment, no apparent thickening	colorless liquid with white sediment, no apparent thickening

(1 g/30 mL 65% formic acid) were initially satisfactory, but thixotropic properties were lost after a few days, and the solution viscosity decreased visibly over 1 week at room temperature, with the viscosity of the guar gum solution decreasing more rapidly than that of a xanthan gum solution.

Silicic acid gels formed from sodium silicate and 65% formic acid were prepared with various concentrations of sodium silicate. Silicate at 5, 10, and 20% formed a firm brittle gel after 15 min (20%) to 24 h (5%), but liquid separated from all after a few days.

An old sample of fumed silica, Cab-O-Sil M-5, showed considerable promise, giving easily mixed gels with the desirable nonslumping properties, despite the high polarity and hydrogen-bonding properties of formic acid. The higher the concentration of formic acid, the less gellant was required to produce a satisfactory gel (Table 1). New samples of Cab-O-Sil M-5 and Cab-O-Sil EH-5 required slightly less material, probably because of moisture pickup during storage of the old samples. Viscosities of two batches, after standing overnight, were 30 000 and 50 000 cP at 25 °C. As expected, the viscosity was strongly dependent on spindle rotation rate. A new batch of gel, after standing for 2 h at 22.5 °C, showed viscosities of 690 000 cP at 0.5 rpm, 450 000 at 1 rpm, 180 000 at 2 rpm, 82 000 at 4 rpm, 35 000 at 10 rpm, 18 500 at 20 rpm, and 8150 at 50 rpm. Similar fumed silica products are manufactured by other companies and would also presumably be suitable but were not tried. A silane-treated version, Cab-O-Sil TS-720, was not wet by the formic acid and was totally unsuitable.

High-molecular-weight poly(acrylic acids) (Carbopol 934 and 941) gave acceptable gels (Table 2) but took longer to mix. Preliminary experiments suggested that evaporation of formic acid from poly(acrylic acid) gels was slower and also nonlinear (see below).

All of the polyacrylamides tested gave viscous, non-thixotropic solutions (Table 3).

Other products (Rheolate 255, Rheolate 300, and Rheolate EA-2670 (Rheox, Inc.)) were proprietary emulsions of unspecified composition and gave little or no thickening of 65% formic acid at recommended concen-

Table 3. Effect on Formic Acid Solution of Various Poly(acrylamides)^a

product	concentration, %	description
Alcomer 120	5	viscous liquid
Alcomer 120	15	rubbery material, flows slowly. Became even harder as formic acid evaporated. Difficult to handle, becomes too thick to stir after only ca. 15 s. This was the material and concentration used by Nelson et al. (1994).
Percol 753	5	viscous liquid
Percol 753	3	viscous liquid, flows slowly
Percol 753	2	viscous liquid, not as thick as honey
Percol 351	5	almost acceptable gel but concentration sensitive (see lower concentration)
Percol 351	3	viscous liquid, about like honey
Percol 156	5	viscous liquid
Cyanamer N-300	5	viscous liquid
Cyanamer N-300 LMW	5	less viscous than Cyanamer N-300
Cyanamer P-21	5	less viscous than Cyanamer N-300

^a All in 65% formic acid.

trations of 9–15%. Bentone MA and Bentone RV (also from Rheox) were treated bentonite clay products. Neither gave useful thickening of 65% acid after either normal or manufacturer-recommended high-shear mixing. Untreated bentonite gave similar results.

The desirable form for dispensing the formic acid would be some sort of prefilled packet, which would only need to be opened by a suitable mechanism before placing it in the bee hive. As a prototype, we used ~15 cm square plastic bags that were heat-sealed after the addition of the gel. A packet this size, packed with 200 g of gel, was about 8–9 mm thick, and would fit beneath the inner cover of a bee hive without requiring any modifications to the hive. Initial experiments with polyethylene and polypropylene indicated that the plastics were too permeable to formic acid, although they were used in our initial field experiment (Feldlaufer et al., 1997). The odor of formic acid could be perceived through the plastic. Enough formic acid vapor diffused through the plastic and into a formic acid monitoring tube taped to the outside of a sealed packet to exceed the scale after standing overnight. A polyethylene/polyester laminate showed lower permeation by a factor of approximately 10 by use of a formic acid detector tube, but the laminate separated after a few days. A sample of Barex film, suggested on the basis of its low water vapor permeability, was totally unsuitable, essentially dissolving in formic acid. The best laminate found to date, although it could still be improved considerably, is a valeron/surlyn combination. Permeation was low enough to meet the DOT specifications for internal packagings (49CFR, part 173, appendix B) when tested at room temperature. Delamination eventually occurred with this material also but required about 6 months to become apparent. Work is continuing on suitable packaging materials.

Evaporation tests of the best gels (7% Cab-O-Sil M-5, 5% Carbopol 934, and 5% Carbopol 941 in 65% formic acid) were carried out from packets. Gel samples (200 g) were sealed in Valeron/Surlyn packets (~140 × 135 mm after sealing) and four evenly distributed slits were cut the width of the packet. The opened packets were placed in a hood and weighed periodically (the tare weight of the packet plus gelling agent is 18–20 g). Results are shown in Figure 1a. The rate of evaporation from the fumed silica packets seemed essentially linear over all but the end of the range. Linear regression on the first 12 days of the run gave r^2 values of 0.9973 and 0.9915 for the two replicates considered separately, or 0.9859 for the two runs taken together (Figure 1b). Rate

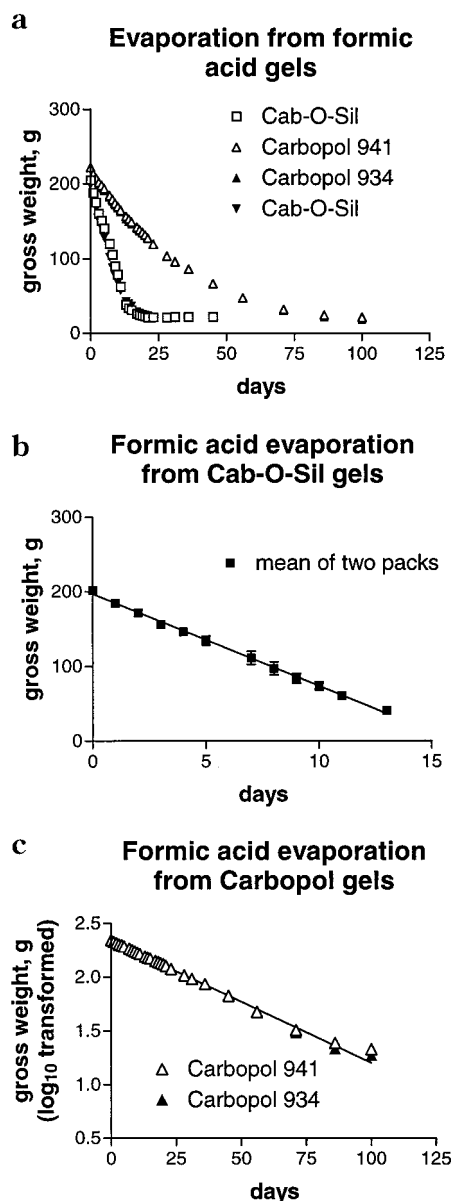


Figure 1. (a) Evaporation of 65% formic acid from fumed silica (7% Cab-O-Sil M-5) and poly(acrylic acid) (5% Carbopol 934 and 941) gel packets, linear scale. (b) Cab-O-Sil data, first 12 days. (c) Poly(acrylic acid) data, log-transformed.

of evaporation from the poly(acrylic acid) gels slowed considerably (seemingly exponentially) when plotted on a linear scale; when log-transformed and replotted, the

data gave a straight line ($r^2 = 0.9935$ and 0.9958 for the two gels) (Figure 1c). Ninety percent of the formic acid had evaporated from the fumed silica packets in 12–13 days, while the poly(acrylic acid) packets took approximately 63 days to reach this stage.

CONCLUSIONS

Of all these products, the fumed silicas and the high-molecular-weight poly(acrylic acids) were the only ones that gave gels with satisfactory physical properties. The desired gel was soft but thixotropic and nonslumping, with a consistency resembling that of chocolate pudding. The fumed silicas were much easier to use, requiring only brief agitation to achieve satisfactory properties. The poly(acrylic acid) products took several hours to disperse, since they had to be added slowly to well-agitated formic acid to avoid the formation of lumps which persisted for a long time once formed. On an industrial scale, this might be overcome by the use of proper equipment, although manufacturer's literature indicates that use of high-shear mixing would lead to degradation of the polymer and reduction of viscosity. Evaporation from the fumed silica gels was optimum for application to bee hives, requiring 2–3 weeks for evaporation of all the formic acid from a packet. This is about the time required in our preliminary study (Feldlaufer et al., 1997) during which the formic acid level in the hive remained in the range of 10–50 ppm, with most readings in the range of 10–20 ppm.

An additional advantage of the fumed silicas is that they do not dissolve in the formic acid but form instead a well-dispersed solid phase, so that the rate of evaporation of the formic acid does not change greatly with time. The poly(acrylic acids) and polyacrylamides dissolve in the formic acid, and the viscosity increases rapidly with evaporation of the acid, reducing the rate of evaporation as the quantity of acid remaining decreased.

LITERATURE CITED

- Anonymous. Varroa mites found in the United States. *Am. Bee J.* **1987**, *127*, 745–746.
- Bracey, S.; Fischer, F. Initial results of the field treatment of honey bee colonies infested with *Varroa jacobsoni* using formic acid in hot climates. *Am. Bee J.* **1989**, *129*, 735–737.
- Calderone, N. W.; Wilson, W. T.; Spivak, M. Plant extracts used for control of the parasitic mites *Varroa jacobsoni* (Acari: Varroidae) and *Acarapis woodi* (Acari: Tarsonemidae) in colonies of *Apis mellifera* (Hymenoptera: Apidae). *J. Econ. Entomol.* **1997**, *90*, 1080–1086.
- De Jong, D. Mites: Varroa and other parasites of brood. In *Honey bee pests, predators, and diseases*, 2nd ed.; Morse, R. A., Nowogrodzki, R., Eds.; Cornell University Press: Ithaca, NY, 1990.
- Dietz, A.; Hermann, H. R. Control methods. In *Biology, detection and control of Varroa jacobsoni: a parasitic mite on honey bees*; Lei-Act Publishers: Commerce, GA, 1988; Chapter VIII, pp 54–65.
- Eischen, F. A. Varroa control problems: more answers from Florida. *Am. Bee J.* **1998**, *138*, 267–268.
- Ellis, M. D.; Baxendale, F. P. Toxicity of seven monoterpenoids to tracheal mites (Acari: Tarsonemidae) and their honey bee (Hymenoptera: Apidae) hosts when applied as fumigants. *J. Econ. Entomol.* **1997**, *90*, 1087–1091.
- Feldlaufer, M. F.; Pettis, J. S.; Kochansky, J. P.; Shimanuki, H. A gel formulation of formic acid for the control of parasitic mites of honey bees. *Am. Bee J.* **1997**, *137*, 661–663.
- Fries, I. Short-interval treatments with formic acid for control of *Varroa jacobsoni* in honey bee (*Apis mellifera*) colonies in cold climates. *Swedish J. Agric. Res.* **1989**, *19*, 213–216.
- Hoppe, H.; Ritter, W.; and Stephen, E. W. C. The control of parasitic bee mites: *Varroa jacobsoni*, *Acarapis woodi* and *Tropilaelaps clareae* with formic acid. *Am. Bee J.* **1989**, *129*, 739–742.
- Liu, T. P.; Nasr, M. Effects of formic acid treatment on the infestation of tracheal mites, *Acarapis woodi* (Rennie) in the honey bee, *Apis mellifera* L. *Am. Bee J.* **1992**, *132*, 666–668.
- Liu, T. P.; Chu, L. T. Y.; Sporns, P. Formic acid residues in honey in relation to application rate and timing of formic acid for control of tracheal mites, *Acarapis woodi* (Rennie). *Am. Bee J.* **1993**, *133*, 719–721.
- Lodesani, M.; Colombo, M.; Spreafico, M. Ineffectiveness of Apistan treatment against the mite *Varroa jacobsoni* Oud in several districts of Lombardy (Italy). *Apidologie* **1995**, *26*, 67–72.
- Lupo, A.; Gerling, D. A comparison between the efficiency of summer treatments using formic acid and Taktic against *Varroa jacobsoni* in beehives. *Apidologie* **1990**, *21*, 261–267.
- Milani, N. The resistance of *Varroa jacobsoni* Oud to pyrethroids: a laboratory assay. *Apidologie* **1995**, *26*, 415–429.
- Nelson, D.; Mills, P.; Sporns, P.; Ooraikul, S.; Mole, D. Formic acid application methods for the control of honey bee tracheal mites. *Bee Science* **1994**, *3*, 128–134.
- Ritter, W.; Ruttner, F. Neue Wege in der Behandlung der Varroatose (New methods in the treatment of varroatosis). *Allg. Dtsch. Imkerztg.* **1980**, *14*, 151–155.
- Sharma, O. P.; Garg, R.; Dogra, G. S. Efficacy of formic acid against *Acarapis woodi* (Rennie). *Indian Bee J.* **1983**, *45*, 1–2.
- Shimanuki, H.; Knox, D. A. Tracheal mite surveys. *Am. Bee J.* **1989**, *129*, 671–672.
- Wachendörfer, G.; Fijalkowski, J.; Kaiser, E.; Seinsche, D.; Siebentritt, J. Laboratory and field tests with Illertisser Milbenplatte (= mite plate), a new way of application of formic acid to control varroatosis. *Apidologie* **1985**, *16*, 291–305.

Received for review February 9, 1999. Revised manuscript received June 29, 1999. Accepted July 6, 1999. Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

JF9901439