Membrane-barrier Delivery of Formic Acid Vapours to Control Varroa jacobsoni Infestation in Honey Bees Colonies

by

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LIST OF ABBREVIATIONS

v/v	volume / volume
mil	milliinch
mm	millimeter
d	day
SE	standard deviation
$\bar{x}$	average
FA	formic acid
N	newton
dm	decimeter
Sep	September
UV	ultraviolet

ABSTRACT

Formic acid vapours have been shown to be an acceptable alternative to Apistan (Fluvalinate) in the control of *Varroa jacobsoni*, a parasitic mite found on honey bees. However the open delivery systems currently used in the industry put the beekeeper at unnecessary risk from direct exposure and vapour inhalation of the acid.

In this study, an alternative closed system for the delivery of formic acid vapours to beehives has been investigated. Formic acid as an aqueous solution (65%, v/v) is contained in a latex membrane through which the vapour diffuses. On reaching the outer surface it vaporizes into the hive.

Estimates of the amount of formic acid vapour that can pass through these membranes indicate that it is possible to deliver 10 g of material to the beehive on a daily basis. This would be sufficient to control Varroa mite infestation. Furthermore the method of delivery is safe, effective, and inexpensive.

1. INTRODUCTION

1.1 Disease and pests of honey bees

Like all living organisms, honey bees can be infected with diseases and pests. Some of these are more detrimental to bee colonies than others, so it is important for the beekeeper to be able to recognize conditions that might signify disease or show signs of pests and respond accordingly. Because the honey bee lives in a colony, the infection of one bee with disease will eventually lead to all bees being infected.

Bees have two distinct life forms, these being brood (young generation) and adult. Most diseases are specific to either one stage or the other. Some diseases, however, attack both broods and adults (1). The following are some of diseases that are prevalent among brood bees:

a) American Foulbrood.

b) European Foulbrood.

c) Chalkbrood.

d) Stonebrood.

e) Sacbrood.

f) Purple Brood.

g) Asian Mite *(Varroa jacobsoni)*.

Among adult bee population the following diseases are important:

a) Nosema

b) Viruses

c) Wax Moth

d) Black Bear

e) Tracheal Mite

f) Asian Mite *(Varroa jacobsoni)*

The Varroa mite (Figure 1) is one of the honey bees most serious antagonists, feeding on the haemolymph of the developing honey bee larva, pupa, and the adult bee. Unchecked, infestation will eventually lead to a hive population of malformed, non-productive bees that cannot reproduce. From an economic standpoint any infestation is intolerable because the infected bees do not produce honey and they spread their infection to other hives. Mites can spread quickly by traveling with swarms or migrating drones, and via the movement of infected equipment. Varroa mite distribution is rapidly becoming universal **(2).**

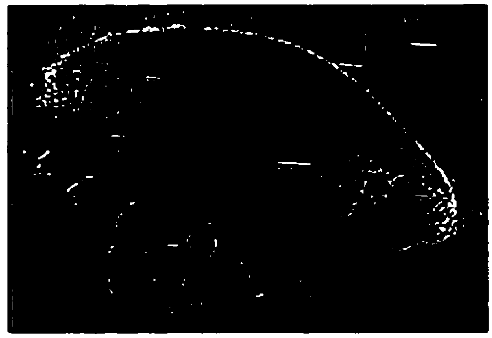


Figure. 1. Varroa jacobsoni*

1.2 History of Asian Mite (*Varroa jacobsoni*)

Varroa jacobsoni was originally found as a parasite of the eastern honey bee, *Apis cerana*, Fabricius in Asia **(3)**. However, the parasite was transferred to the European and African honey bee, *Apis mellifera*, sometime in the early 1940s, probably through the human movement of bees **(4)**. Since then, Varroa has spread throughout Europe, South and Central America, and Africa. The first reported incidence of infestation in Great Britain was in 1992 **(5)**, Costa Rica as recently as 1997**(6)** and Libya in 1979**(7)**. It was discovered in the United States in 1987 **(8)**.

In Canada, beekeepers first detected Varroa in New Brunswick in 1989 **(9)** and in Nova Scotia in 1995 **(10)**. Only a few places in the world having major apiaries can still be considered Varroa-free. Of these the more important commercial ones are in Hawaii, Australia, and New Zealand.

* Netscape, 1999, http://www.main.org/cahbs/varroa.htm.

1.3. The life cycle of Varroa mites

Varroa is a parasite specific to honey bees. It cannot survive on bumble bees, wasps, or any other insects. Like many other parasites, Varroa depends upon hormones obtained from its host to regulate its own metabolism, including egg production.

The Varroa mite will feed on both adult bees and brood bees to enable it to reproduce. In the case of adult bees it will feed on them for up to thirteen days before it enters brood cells. There, larvae that are preparing to pupate are particularly susceptible to infestation. The cell is normally infected just before the cell is sealed.

Mite eggs are produced every thirty hours. The first offspring is always a male while subsequent newborns are predominantly female. Immature mites are white and soft-bodied, but they mature rapidly. Varroa females mature in six days, become reddish-brown, and are hard-bodied. They must complete their development before the adult bee emerges from the cell, or they are not viable and die.

Varroa mite numbers increase only slowly in cells containing worker bees because their brood cycle is relatively short and the mite will not mature before the worker bees emerge. In the case of drones, however, the brood cycle is longer and this allows the Varroa mite to develop to full adulthood. This results in a rapid population increase in Varroa mites from incubation in drone brood cells (11).

Figure 2. Varroa mites on worker bee*

Figure 2 shows a newly emerged worker bee that has been badly damaged as an immature bee by these "Varroa" mites. She has no wings, hardly any abdomen, and her rear legs are paralyzed. She cannot work, and will not live long.

Figure 3. Several mites on a drone larva*

Figure 3 shows several mites on a drone larva. Inspecting a few drone larvae is a quick way to check for the presence of these mites. Mites are reddish-brown and stand out against the white baby drone. Finding one mite while checking ten or twenty drones is

* Netscape, 1999, http://www.main.org/cahbs/varroa.htm.

cause for treatment. Finding several mites on each drone probably indicates a hive beyond redemption.

1.4. Economic importance of honey bees

The economic importance of honey bees is tied to agriculture. Honey bees are credited with approximately 85 % of the pollinating activity necessary to supply about one-third of the American food supply **(12)**.

According to Statistics Canada **(13)** in 1997, about 11,000 beekeepers kept 520,000 honey bee colonies in Canada. During the same year United States beekeepers cared for over 2,579,000 colonies **(14)**. Numbers of both bees and beekeepers have been declining steadily, however, since the Varroa mite was introduced. The only way the honey bee industry in North America can be stabilized is by introducing a strategy of mite management treatment on an annual basis.

To further complicate the situation, a second mite species Acarapis woodi is also spreading throughout North America. Although these mites are currently present in most Canadian provinces, they have yet to be found in Nova Scotia.

1.5. Chemical treatments against Varroa mite

Varroa mite infestations have been treated successfully by application of several organic acids and other organic compounds such as ethereal oils, methanol, acetic acid, oxalic acid, Apistan (Fluvalinate), and formic acid **(15)**

In Canada three chemicals have been approved for the control of parasitic mites on honey bees these being: menthol and formic acid for tracheal mites; and Apistan (Fluvalinate) and formic acid for Varroa. Apistan (Figure 4) is the treatment of choice for Varroa, but concern is already being expressed about its suitability, since Apistan-resistant mites have been reported in Italy, Florida and recently in France. Not only is this resistance a potential disaster for beekeepers, but the use and misuse of Apistan can contaminate honey and wax **(16)**.

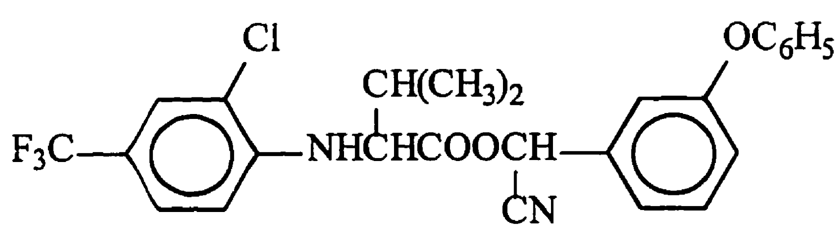


Figure. 4. Molecular formula of Apistan or Fluvalinate

Formic acid gives results that are as good if not better in treating bees than other organic acids. It is inexpensive, and occurs naturally at low levels in honey **(17)**. It is not surprising, therefore, that the use of formic acid for mite control is becoming more widespread in Canada **(18)**.

1.6 Formic acid (methanoic acid)

1.6.1 Preparation of formic acid

Ants secrete significant quantities of formic acid as part of their defence mechanism against predators. In fact the name "formic" is derived from the Latin word for ant, "formica". It is hardly surprising, therefore, that formic acid was first isolated as a natural product from these insects. Nowadays, however, formic acid is prepared more cheaply by synthetic means. Three of these are indicated below: **(19)**

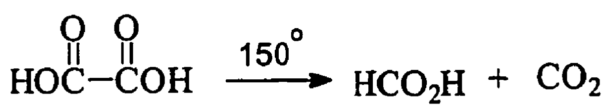
1. Ester hydrolysis

Esters can be hydrolyzed to carboxylic acids in an aqueous solution containing strong mineral acid. In most cases the reaction is slow.

$$H\text{—}\overset{\overset{\displaystyle O}{\|}}{C}\text{—}OR \xrightarrow[H^+]{H_2O} H\text{—}\overset{\overset{\displaystyle O}{\|}}{C}\text{—}OH + ROH$$

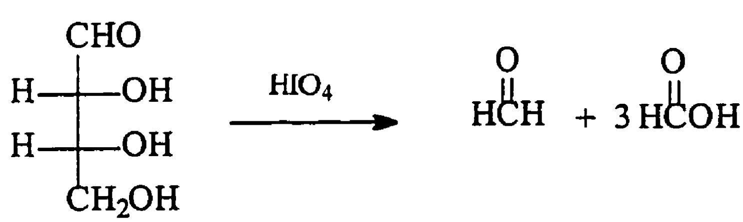
2. Decarboxylation of oxalic acid

A few α-carbonyl acids such as oxalic acid that contain carbonyl groups on adjacent carbon atoms can undergo decarboxylation on heating. In the case of oxalic acid one of the products is formic acid.



3. Oxidation of erythrose

Erythrose is oxidized to formaldehyde and formic acid in the molar ratio 1:3 by periodic acid at room temperature.



1.6.2 Physical properties of formic acid

Some of the main physical characteristics of formic acid are provided in Table I.

Table I. Physical properties of formic acid (20)

Molecular weight	46.03 g. mol^{-1}
Boiling point	100.7 °C
Melting point	8.4 °C
Density	1.22 g. mL^{-1} at 20 °C
Solubility	Completely soluble in water, alcohol, ether, acetone and benzene
Enthalpy of vaporization of formic acid	20.10 kJ. mol^{-1} at 25 °C

1.6.3 Toxicology of formic acid

Formic acid is a significant skin and eye irritant. In sufficient quantities it can burn. Apiculturists must be careful, therefore, not to allow contact of formic acid with sensitive parts of their bodies, nor to inhale the vapours. Ambient vapour concentrations of formic acid can exceed 100 ppm. Table II summarizes some of the toxicological data available for formic acid.

Table II. Summary of vapour inhalation toxicology data (21)

Measure	Value
Threshold limit value (TLV)	5 ppm
Acute dose (immediate danger)	100 ppm
LC50 (rat)	15 000 ppm / 15 min
LC50 (mouse)	6200 ppm / 15 min

1.7 Application of formic acid to beehives

The delivery of formic acid to beehives is usually undertaken in one of three ways. Each is an "open" method in which the beekeeper is continually exposed to formic acid vapours, and is therefore at risk.

1.7.1 Nassenheider device **(13)**

The Nassenheider device consists of a fibrous wick, which dips into a reservoir of an aqueous solution of formic acid held in a plastic container. As the formic acid rises in the wick it evaporates. The amount of formic acid vapour delivered to beehives is controlled by the size of the wick. Free air circulation around the wick is maintained, while keeping it away from possible body contact, by enclosing it in a cage-like structure.

1.7.2 Krämer plate treatment (22)

Soft fiber plates impregnated with formic acid are sealed in a plastic bag of 0.15 mm thickness. The formic acid vapour is transported to the beehive by passing through holes cut in the plastic bag. These holes are usually about 1.5 cm in diameter. The number of these holes in a given situation varies according to temperature and general climatic conditions. Each unit has to be tested before it is placed in a given hive.

1.7.3 Gel formulation (23)

A gel formulation of 65% v/v formic acid has also been used. In this instance the gel is placed in polyethylene bags which are heat-sealed to form a packet with dimensions 15.25 cm ×15.25 cm ×1 cm. These can then be placed over the top bars of the upper brood chamber and do not, then interfere with hive closure. Formic acid vapour enters the hive through holes in the bag.

1.8. Permeation technology

Barrier technology has been known for many years **(24)**. For instance, permeation tubes have been used in gas chromatography for the determination of trace organics in aqueous samples. In this particular instance water containing the analyte of interest is pumped past a silicone rubber tube membrane through which flows the carrier gas. The organic compounds in the water are preferentially absorbed into the membrane, pass through it into the carrier gas, and are thence transported to the detector **(25).**

The amount of vapour produced via permeation (closed system) is primarily a function of five variables, these being (1) the chemical structure of the evaporating substance, (2) the type of material through which permeation takes place, (3) the thickness of this material, (4) the temperature, and (5) the surface area of the permeable material.

In dynamic systems, the air velocity across the surface of the permeable material is another significant factor to be considered. The major driving force behind permeation characteristics of any permeable material, through, is the concentration gradient across the membrane.

1.8.1 Permeation theory

The rate of permeation of a liquid through a membrane, (p) is directly proportional to the vapour pressure of the liquid (P),

$$p = K\,\mathrm{P} \qquad (1)$$

Where k is proportionality constant.

The Clausius-Clapeyron equation (equation 2) describes the relationship between the vapour pressure of a liquid, its enthalpy of vaporization (ΔH_v) and the absolute temperature, T. This equation may be written in the form

$$P = e^{-\frac{\Delta H_v}{RT}+C} \qquad (2)$$

Where C is a constant.

Since the rate of permeation of a liquid through a membrane is directly proportional to its vapour pressure, the rate of permeation at temperature, T_1, is related to the rate of permeation at temperature, T_2, by:

$$p_2 = p_1 e^{\frac{\Delta H_v}{R}\left(\frac{1}{T_1}-\frac{1}{T_2}\right)} \qquad (3)$$

1.9. Beehives temperature and formic acid

In the hive, worker bees have their own system of temperature control, so that the hive temperature never falls below 18 °C (winter) and never climbs above 35 °C (summer) (**26**). In winter, energy derived from stored honey generates sufficient body heat to keep the hive at an adequate temperature for adult survival. If the temperature appears to be falling dramatically outside the hive, the worker bees cluster together for warmth. In summer, worker bees ventilate the hive by fanning their wings. This produces cooling air currents and helps move the hotter interior air outside.

The two temperature extremes inside the hive, 18 °C in winter and 35 °C in summer, are well within the temperature range for which formic acid is a liquid (Table I). It is not surprising, therefore, to note the extensive use of this compound in Varroa mite control.

1.10 Thesis proposal

The idea that formic acid could be delivered to a beehive through a permeation membrane is appealing because the system would be intrinsically safer than "open" methods. This research therefore, is directed toward the development of a static method for the safe, effective, and inexpensive delivery of formic acid (65%, v/v) vapour through a closed membrane system into beehives to control Varroa jacobsoni in honey bee colonies.

2. EXPERIMENTAL

Table III lists the different membranes (latex membranes) used in this study. All membranes were washed in a dilute soap solution and hung overnight to dry prior to use. Membranes were charged with approximately 100 to 120 g of (65%, v/v) formic acid prepared from concentrated acid (90 % BDH Chemicals, AnalR B10114) as shown in Appendix 1 page B.

Table III. Latex membranes types

Latex Membrane Number.	Description
1	Johnson & Jonhson (lubricated, Ortho Shields Dura Thin)
2	Carter-Wallace N.S. Inc. (lubricated, Trojan-Enz Large)
3	Durex (lubricated, Ramses Ribbed & Thin)
4	Durex (nonlubricated, Sheik)
5	Ramses (nonlubricated)

2.1 Latex permeation and open diffusion of 65% (v/v) formic acid – ambient temperature

A mass of 27.6 g of formic acid (65%, v/v) was placed in a 150 mL beaker. Another 35.3 g of formic acid (65%, v/v) was also placed in 150 ml beaker and sealed with a non-lubricated latex membrane (number 2, Table III) stretched over the top. Both beakers were placed inside a fumehood with the fan off. The mass of formic acid in each beaker was recorded three times per day (morning, mid day, and evening) during seventeen day period. The average temperature during this time was 21.4 °C.

2.2 Indoor permeation of 65% (v/v) formic acid through different membranes– ambient temperature

One of each of five different latex membranes (1, 2, 3, 4, and 5 in Table III) was filled with formic acid (65%, v/v) at an ambient indoor temperature of 20 °C. Each membrane was tied shut and placed in a 150 mL beaker which was then positioned in a fumehood so that there was equal airflow around each beaker. The mass of each filled membrane was recorded three times per day (morning, mid-day and evening) over an eighteen day period.

2.3 Membrane structural integrity study after and before exposure to formic acid (65%, v/v).

Exposure of membranes to formic acid (65%, v/v) over an eighteen day period samples used in this study were taken from different membranes in experimental 2.2. Each of these membranes, along with one of each membrane type that had not been in contact with formic acid, was cut with scissors to provide two pieces of latex measuring approximately 2.5 cm x 7.0 cm. These pieces of latex were clamped in a universal testing machine (Istron model 4302) along the short length, and the strain break point in newtons was measured, by applying a load speed of 20 cm. min^{-1}.

2.4 Membrane thickness as a variable affecting permeation.

Latex membranes of different thichnesses (Apex Medical Technologies, San Diego, CA) were used in this study. Six membranes, two each of approximately the same thickness were studied. These varied in thickness from 0.071-0.65 mm. Each was charged with approximately 110g of formic acid (65% v/v), tied off, and placed in a trough inside a fumehood with the fan off. The mass of each membrane was measured three times daily for fifteen days.

2.5 Outdoor studies on the permeation of formic acid (65%, v/v) through latex membranes no honey bees present.

One of each of five different types of membranes (numbers 1-5 in Table III) were charged with approximately 100g of formic acid solution (65%, v/v) and the open ends tied off. These membranes were then placed in beakers, which were positioned between the sixth and eighth frames of a standard Langstroth type hive.

Three other membranes (numbers 1-3 in Table III) were also charged in similar manner. These were hung by strings attached to spatulas lying on the top bars of the beehive frames.

The mass of each of the above eight membranes was recorded three times daily, morning, mid-day, and evening, during a twelve day period starting on May 30, 1997. The temperature inside the hive at the midpoint of frame seven was recorded with a mercury thermometer, each time membranes were removed for weighing.

2.6 Outdoor studies on the permeation of formic acid (65%, v/v) through latex membranes in hives with honey bees present.

Three outdoor hives, each composed of two-super standard Langstroth bodies, were used in this study which took place between September 12-26, 1997. Experiments were conducted in the upper part of each hive. All hives contained a healthy queen and workers.

For each hive, six filled membranes (number one in Table III) were filled with formic acid (65%, v/v), after which the open ends were tied off. The middle frame (frame five) was removed from each hive so that the filled membranes could be inserted. These were then hung from spatulas lying across the top bars of the beehive frame.

The mass of each filled membrane (± 0.1 g), the temperature inside each hive adjacent to the membranes, and the outdoor temperature adjacent to the hive were recorded daily between 13:00h and 14:00 h. Temperature was recorded at 72 s intervals using Optic StowAway® temperature data loggers (Onset Computer Corp.).

2.7 Permeation and diffusion studies of water and formic acid

Five different solutions containing varying amount of formic acid were prepared 0%; water only, 20%, 35%, 50% and 65% formic acid (v/v). In the permeation study (closed system), five latex membranes (number 1 in Table III) were each filled with approximately 100 g of each of the above solutions, and placed inside a trough in a fume hood with the fan off. The mass of each membrane and the temperature inside the fume hood were recorded at approximately noon daily for fourteen days consecutively.

In the diffusion study (open system), approximately 60 g of each of the above formic acid- water solutions was placed in separate beakers. Each beaker was placed in a fume hood with the fan off. The mass of the liquid remaining in the beaker was measured each day at noon for fourteen days.

2.8 Radiation effect on latex membranes

A total of ten latex membranes (number one, Table III) were each washed with dilute soap solution and hung to dry as noted earlier. All membranes were then charged with approximately 100 g of formic acid solution (65%, v/v). A group of five membranes was placed in separate troughs, after which each trough was placed in a fumehood, in different rooms.

Five of the filled membranes were studied in a dark environment, the light in both room and fumehood being extinguished while a blind covered the windows. The mass of formic acid in each membrane was recorded once a day (at mid day) for fourteen days.

The other five filled membranes were studied in a light environment, the fluorescent light in both room and fume fumehood being turned on. The mass of formic acid in each membrane was recorded once a day (mid day) for fourteen days

The lamp used in the second study was a Cool White lamp (Phillips T12/Cw/ 30 watts, Code) which is the fluorescent lamp most frequently used in normal room illumination today (27). The lamp output was approximately 0.8 candelas per cm^2. The following figure 5 shows the spectral distribution of Cool White lamps.

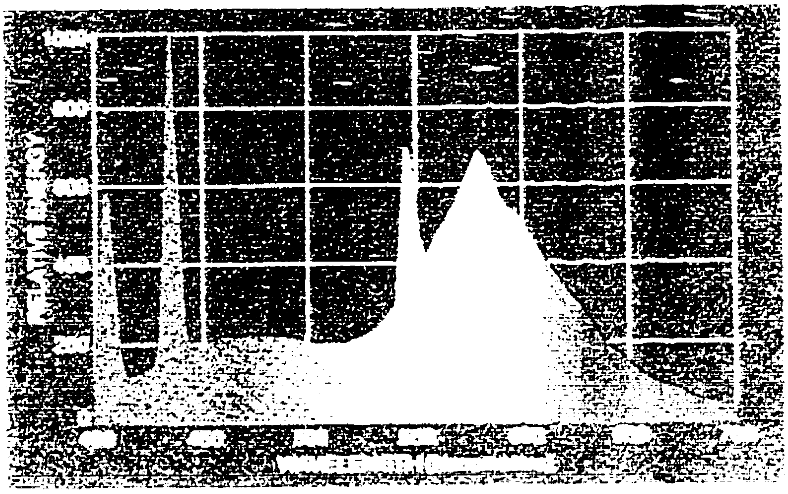


Figure 5. Shows the spectral distribution of Cool White lamps

A universal testing machine (Istron model 4302) was used to test the structural integrity of each of the membranes used in this study. The experimental procedure used was that given in Section 1.3.

3. RESULTS AND DISCUSSION

3.1 Comparison of diffusion and permeation rate for formic acid (65%, v/v)

This experiment was performed to find out whether latex membranes allow permeation by formic acid solution and whether there is a difference in the rate of evaporation of formic acid (65%, v/v) from an open beaker (direct diffusion) or from one covered with a latex membrane (permeation). Results showed that the formic acid solution in the uncovered beaker evaporated at a faster rate than that in the covered one (Figure 6). Furthermore, the actual rate at which formic acid permeated the latex membrane and evaporated was relatively constant, and comparable to the behaviour of formic acid in the uncovered beaker (Figure 7).

In the diffusion study, exceptionally scattered data were observed in the initial stages of the experiment. These are thought to be due to experimental design and were eliminated from further consideration.

In contrast, data obtained from the covered beaker shown no such variability (Figure 7). It is assumed in this case that the rate of transfer of formic acid solution through the latex membrane and its rate of evaporation from the membrane surface are essentially constant. It suggests that if formic acid vapour is to be delivered to a beehive from inside a latex membrane or from a closed system, the delivery can be achieved at a relatively constant rate.

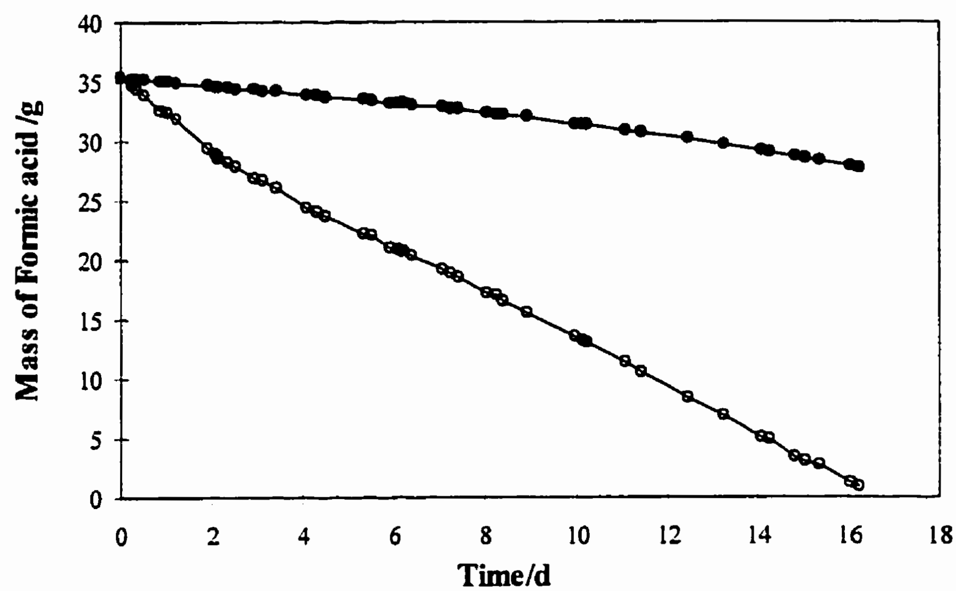


Figure 6. Difference between permeation and diffusion of 65% (v/v) formic acid where (•) is permeation through latex membrane and (o) is diffusion into atmosphere from open beaker.

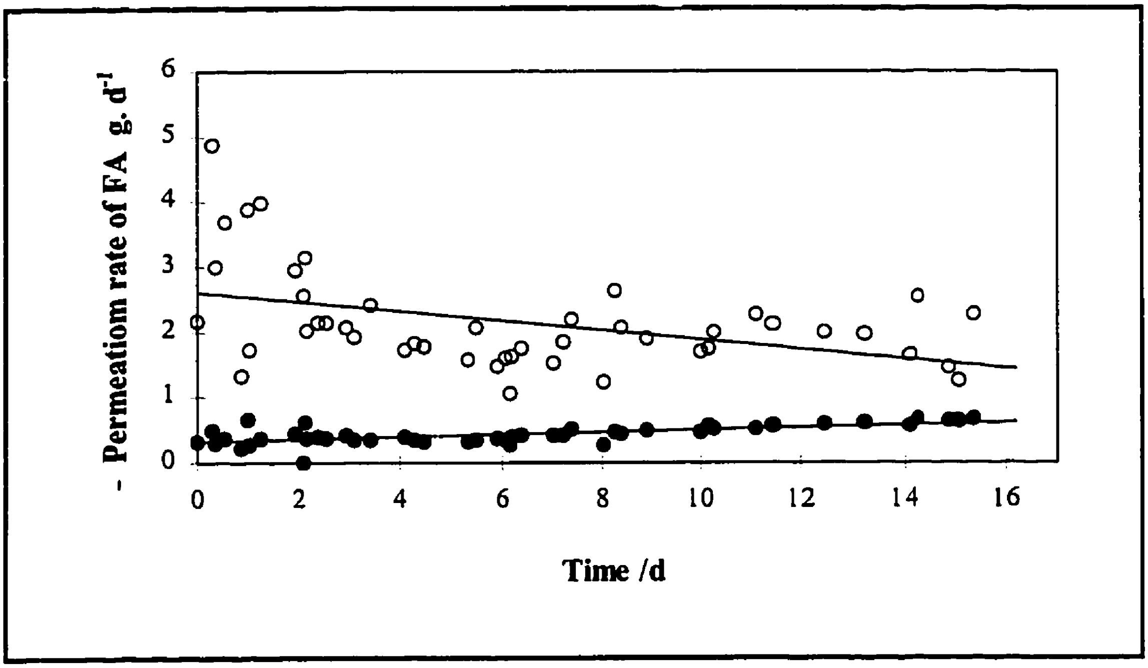


Figure 7. Differences between (•) permeation (65%, v/v) of formic acid through latex membrane and (o) its direct diffusion into atmosphere from open beaker (change in mass/ change in time)

3.2 Indoor permeation study

This experiment was conducted to determine which of the commonly available latex membranes showed the best permeation characteristics for formic acid. Membranes from five different suppliers were used (Table III). Using the experimental method described in Section 2.2, it was determined that one of these five membranes (number one Table III) showed a greater rate of permeation than any of the others. This is significant because it would allow the most rapid delivery of formic acid to a beehive from a closed system.

All membranes except one (number five Table III) appeared to be structurally unaffected by the formic acid solution after fifteen days. This particular membrane composition would not be suitable for containing formic acid solution in a beehive.

In another experiment, the five membranes were filled with formic acid, as described in Section 2.2. Their mass loss through permeation was recorded over a period of eighteen days. The results of this study show that the rate of mass loss actually decreases somewhat over time. In the case of membrane number one the decrease in rate was approximately 0.029 ± 0.003 g. d^{-2} as determined by a linear regression of the data points. This behaviour contrasts with the covered beaker experiment where the rate of mass loss was constant. The difference can be attributed to the fact that where the formic acid solution is in direct contact with the latex membrane, it tends to shrink it, thus decreasing the amount of membrane surface area available. The average permeation rate for each membrane is shown in Table IV.

TABLE IV. Indoor permeation of 65 % (v/v) of formic acid through five different latex membranes. Membranes were contained in 150-mL beakers

Latex Membrane number.	Permeation rate / ($g.d^{-1}.membrane^{-1}$) * $\bar{x}_{51} \pm SE$
1	0.9 ± 0.1
2	0.6 ± 0.1
3	0.5 ± 0.1
4	0.5 ± 0.2
5	0.4 ± 0.1

*See appendix 3. Page F-H, for original data

The repeatability of this study was evaluated by performing duplicate experiments on the two membranes with the highest permeation rate (number 1 and 2, Table III). In this experiment five membranes of each type were filled with formic acid solution, closed and placed in a beaker as above. Two trials on each membrane type were conducted, making ten experiments on each.

As shown in Table V, the mean permeation rate for each membrane type was constant within experimental error. Differences in permeation rate from the previous study (Table V) can be accounted for by changes in the temperature at which each experiment was conducted. As shown in Section 1.8 a 1 K rise in temperature would be expected to increase the permeation rate by five percent.

TABLE V. Membrane permeation repeatability. Indoor permeation of (65% v/v) formic acid through two different latex membranes. Membranes were contained in 150-mL beakers

Latex Membrane Number	Permeation rate / ($g.d^{-1}.membrane^{-1}$) $\bar{x}_{35} \pm SE$	
	Trial 1	Trial 2
1	1.2 ± 0.3	1.2 ± 0.2
2	1.0 ± 0.2	1.0 ± 0.2

3.3 Membrane structural integrity after and before exposure to (65%, v/v) of formic acid

This experiment was conducted on membranes from all five suppliers and is described in Section 2.3. Breaking forces for the different types of membrane were determined before and after a fifteen-day exposure to formic acid solution. These breaking forces (break points) are given in Table VI.

TABLE VI. Force required to break membranes before and after 15-day exposure to 65% (v/v) formic acid.

Latex membrane Number.	Force to Break Point[†] / N	
	Before exposure to acid	After exposure to acid
1	21	19
2	34	35
3	22	23
4	26	19
5	33	16

The repeatability and reproducibility of measurement was assessed by evaluating eight samples of membrane 1 on a different testing day: 25 ± 5 N ($\bar{x}_8 \pm SE$).

† See appendix 4. Page I, and J for original data.

The standard deviation for this experiment, using eight samples of each membranes was ± 5 N, it seems higher because of cut error (cut by hand using scissors). Thus a difference in break point of ten newtons or higher is considered to be significant. Only membrane five showed any noticeable deterioration. This observation is consistent with that made earlier.

3.4 Membrane thickness as a variable affecting permeation

This experiment was conducted using membranes of varying thichnesses but of the same composition as number 1 (Table III). As shown in Figure 8 the permeation rate of formic acid through membranes decreased as the membrane thickness increased.

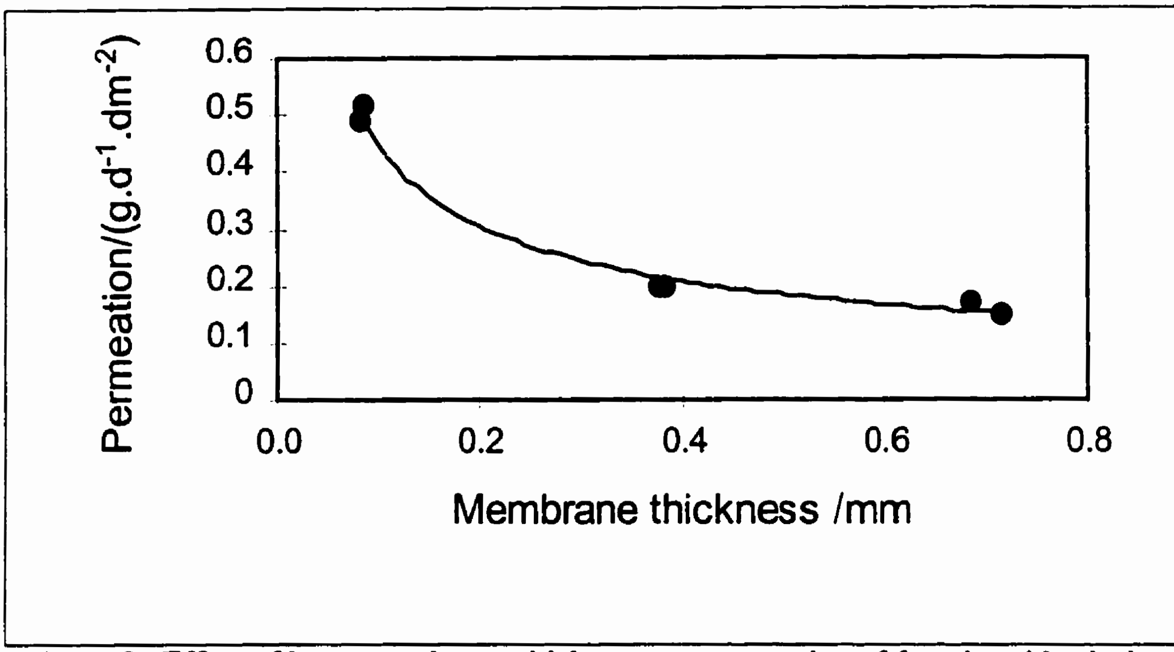


Figure 8. Effect of latex membrane thickness on permeation of formic acid solution.

Three factors contribute to the overall rate of permeation (Figure 9), these being (1) rate of entry into the membrane from solution (**R_1**), (2) rate of permeation of the membrane itself (**R_2**) and (3) the rate of evaporation from the outer surface of the membrane (**R_3**).

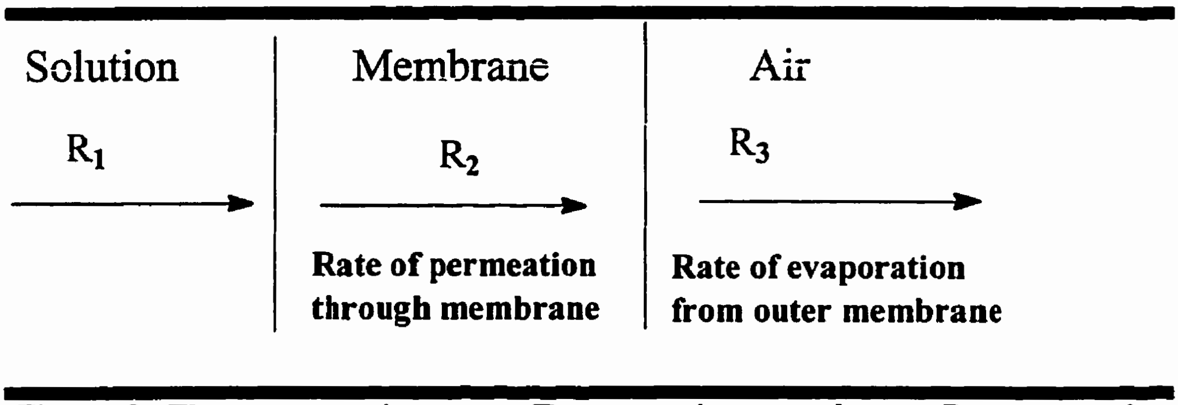


Figure 9. Three permeation rates (R_1= entry into membrane, R_2= permeation though membrane and R_3= evaporation from outer membrane)

Of these three factors, the rate of permeation through the solid membrane would appear to be the slowest. Membrane thickness also plays a role in determining the rate at which formic acid reaches the membranes surface, and thus its eventual rate of evaporation into the atmosphere. As the membrane thickness increases, the permeation rate tends to a constant limiting value (Figure 9). This suggests that thinner, less durable membranes would still provide the most satisfactory rates of formic acid vapour delivery.

3.5 Outdoor membrane-permeation of 65% (v/v) formic acid- no honey bees present

Table VII shows the rate of permeation of formic acid solution through the five different latex membranes studied (Table III). Each one was filled with a solution of formic acid, placed in a beaker, and then placed in the middle of the hive as described in Section 2.5. Table VII also shows the rate of permeation of formic acid through three membranes (number 1,2,and 3 Table III) filled with formic acid, but suspended from the roof of the hive.

TABLE VII. Mean permeation of latex membranes filled with 65% (v/v) formic acid: five placed in beakers and three hung in an outdoor hive without honey bees*.

Latex Membrane No.	Permeation / ($g.d^{-1}.dm^{-2}$) In beakers $\bar{x}_{50} \pm SE$	Permeation / ($g.d^{-1}.dm^{-2}$) Hung $\bar{x}_{50} \pm SE$
1	0.4±0.2	0.6± 0.3
2	0.2±0.1	0.5± 0.3
3	0.2±0.1	0.3± 0.2
4	0.2±0.2	----
5	0.2±0.1	----

* Starting 30 May 1997.

† Surface area estimated to be 1.5 ± 0.2 dm^2. Higher variability of measurement in Table 5 compared to Tables 2 and 3 is due to the larger daily temperature variations.

Temperature of 16 ± 5 °C ($\bar{x}_{50} \pm SE$) for 12 days.

See appendix 6 page N-Q, for original data.

The results of this experiment confirm that the different rates of permeation are in the same order as found in laboratory studies (Section 2.2), and are therefore consistent. It is no surprise to note that the permeation rate of formic acid through membranes held in a beaker is less than through those suspended from the roof of the beehive. Presumably the greater air circulation around the membrane, in the hive would cause formic acid to evaporate faster once it reached the outside surface.

The optimum amount of formic acid vapour that should be delivered to a given beehive within a one-day period to control Varroa jacobsoni has been estimated at 10 g. d^{-1} **(15).** Using the data in Table VII and the theory developed in Section 1.8, it can be calculated that 5.4 membranes of the same type as number one (Table III) would be required to produced the required amount of formic acid vapour. (Appendix) at an estimated outdoor mean hive temperature of 28 °C.

3.6 Outdoor membrane-permeation study in hives with honey bee colonies present

Figure 10 shows a typical rate of mass loss of formic acid solution from a membrane throughout the fifteen-day study period. The decreasing slope, i.e. decreasing rate of permeation with respect to time could be due to one of three reasons: (1) a decreasing in-hive mean day temperature, (2) a decreased membrane surface area with time, caused by membrane degeneration, or (3) an increasing propolis or wax residue deposited by honey bees.

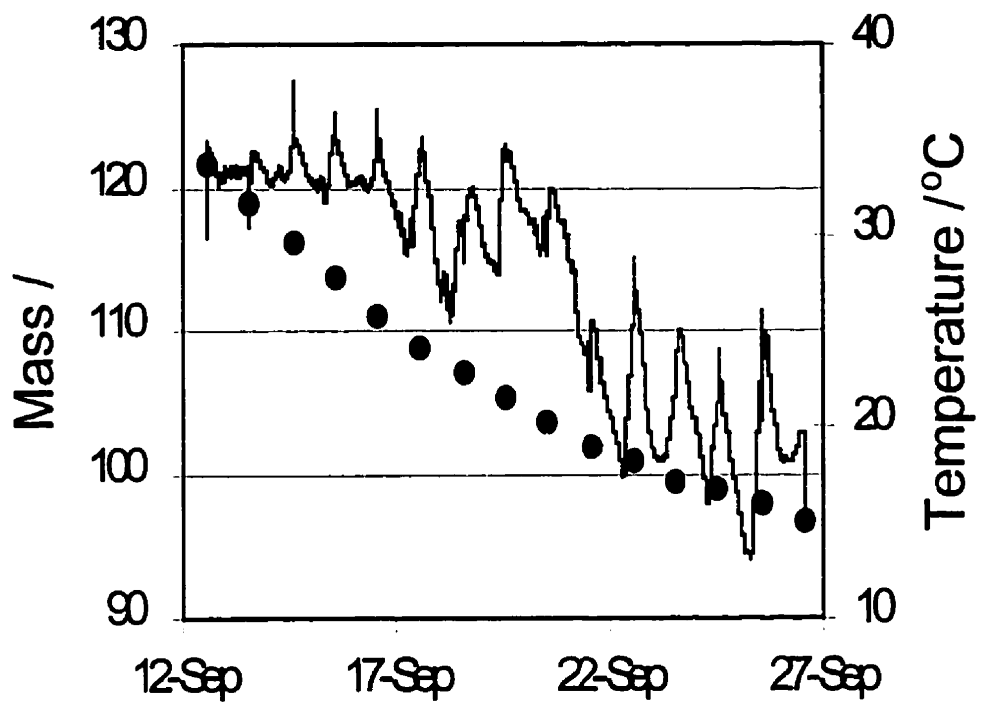


Figure 10. Typical graph of change in mass of formic acid solution (65%, v/v) (•) for one of six latex membranes hung inside an outdoor hive with honey bees -hive A. In-hive temperature indicated with solid line (—).

The results of permeation rate studies conducted in each of three hives with six filled membranes (Section 2.6) are illustrated in Table VIII. In hive A, the average rate of loss of formic acid from the membrane surface area over the fifteen-day period was 2.0 ± 0.3 g. d^{-1} ($\bar{x}_6 \pm SE$). This not only indicates a reasonably good precision of the experiment (15%) given the many uncontrollable variable in a field study, but also that five filled membranes could produce the ten grams of formic acid vapour per hive per day required for optimum effect.

In two of the three hives, B and C, some of the filled membranes were damaged by either beekeeper handling or the destructive work of honey bees. Over the fifteen days during which the experiment was conducted, hive B required the replacement of two membranes, while three membranes were replaced in hive C. An examination of all the latex membranes used indicated that in hive C, they all showed structural damage of one sort or another during the experiment and should have been replaced. The mass loss of formic acid per day in hive C is shown in figure 11 where irregularities are observed for at least three of the membranes studied.

The unusual and apparently significant honey bee activity in hive C could possibly be attributed to the unusually high minimum temperature observed for this hive. This in itself could be a reason for the damaged membranes in this hive.

Table VIII. Mean permeation of latex membranes filled with 65% (v/v) formic acid and hung in outdoor hives with honey bees.

Probe	Temperature / °C			Permeation / (g.d^{-1}.membrane^{-1})
	$\bar{x} \pm SE$*	Minimum	maximum	$\bar{x}_6 \pm SE$
Hive A	27 ± 6	13.0	38.2	2.0 ± 0.3
Hive B	27 ± 6	10.6	38.1	2.3 ± 0.2
Hive C	27 ± 3	20.7	37.3	4 ± 2
Outdoor	15 ± 6	1.3	36.9	---

*Data logger acquisition at 1 measurement every 72 seconds for 15 days between 12 and 26 September 1997.

See appendix 7. Page R-T, for original data.

The results of this field study clearly show that while latex membranes filled with formic acid solution can deliver formic acid vapour to the hive at an acceptable rate, the structural integrity of a given membrane is of concern. Beekeeper safety must still be addressed. Solutions to these concerns may include better containment of membranes when placed in the hive, or the use of formic acid gel formulation as remedies.

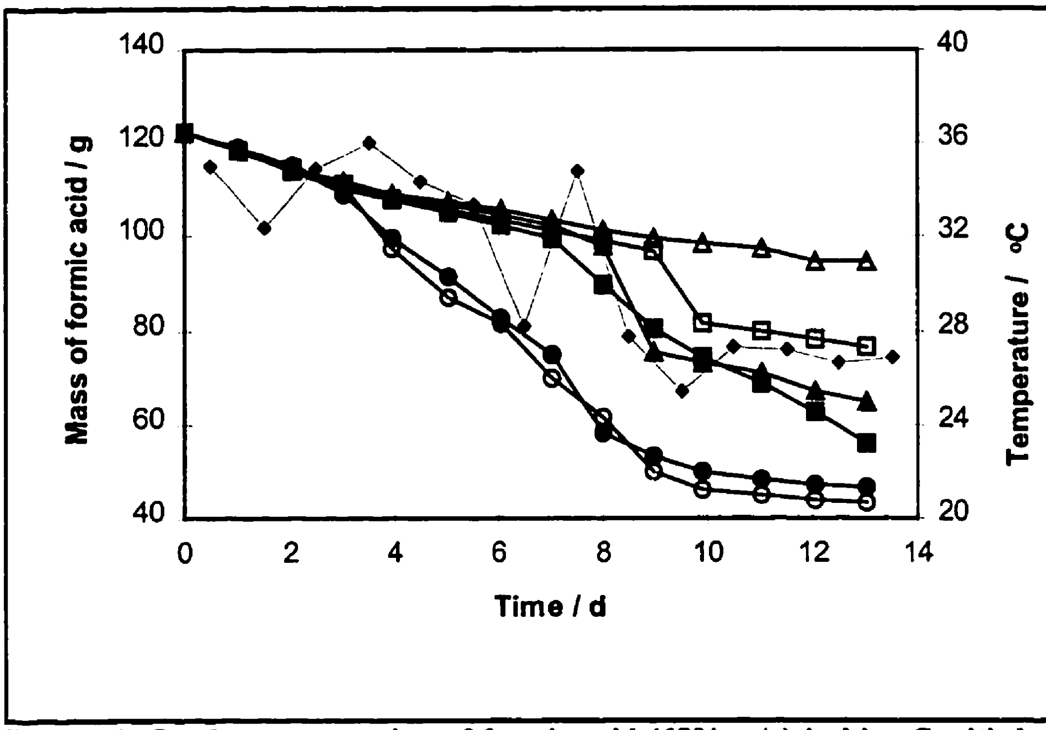


Figure 11. Outdoor permeation of formic acid (65%, v/v) in hive C with honey bee colonies present. Two of six membranes (o,•) were damaged, and therefore replaced with other filled membranes, and (♦) is the temperature / °C

3.7 Absolute measurement for the permeation and diffusion of formic acid and water

When formic acid (65%, v/v) permeates a latex membrane, the material reaching the outer surface could be water, formic acid, or both. This study was undertaken to determine which of these alternatives actually does permeate the membrane fastest.

Figure 12 shows that as the concentration of formic acid in solution increases the permeation rate through the latex membrane also increases. This would indicate that formic acid permeates the membrane at a faster rate than water. In contrast, experiments conducted with an open delivery system indicate that water vaporizes at a faster rate than formic acid (Figure 13)

These results are significant because they indicate that the active ingredient in Varroa mite control not only passes through latex membranes but does so much faster than water vapour. This leads to the conclusion that a closed latex membrane delivery system is a much more effective way of introducing formic acid vapours into beehives than a conventional open delivery system (See appendix 8. Page U-W, for original data).

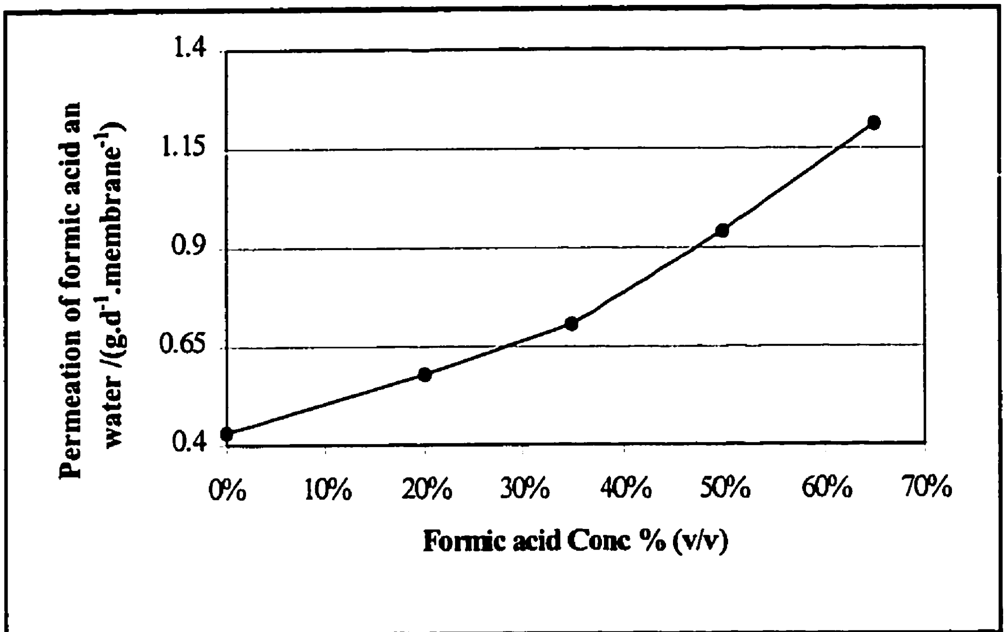


Figure 12. Permeation of formic acid having different concentration through latex membranes (closed system).

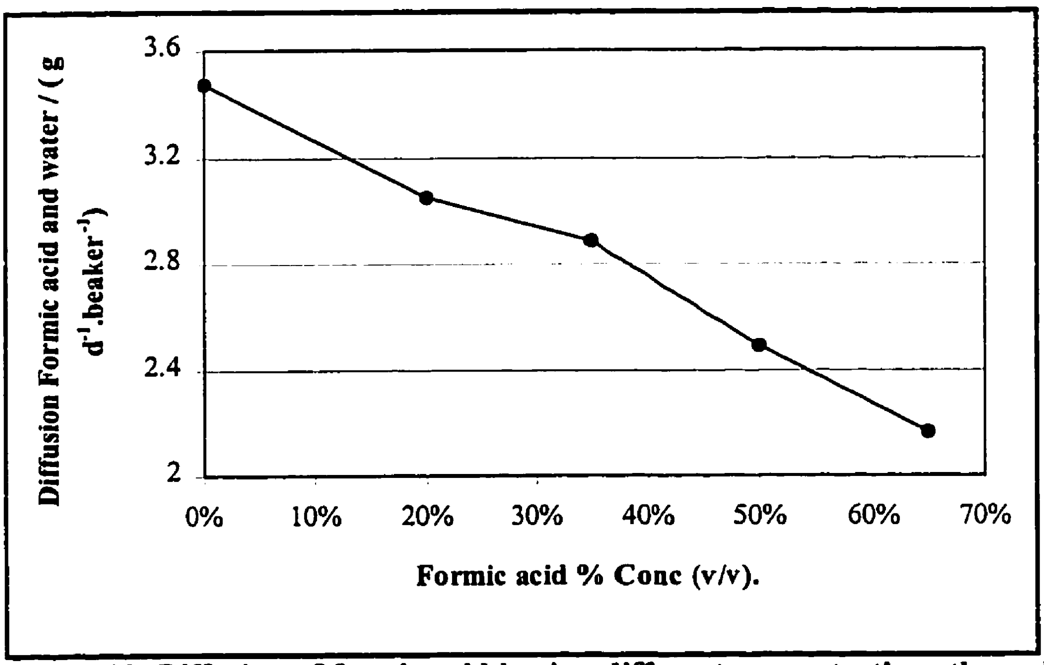


Figure 13. Diffusion of formic acid having different concentrations through latex membranes (open system).

3.8 Radiation effect on permeation and structural integrity of latex membranes

Rubber latex contains emulsoid particles consisting of hydrocarbon aggregates enclosed in protein envelopes, together with fatty acid (2.5-3%), sugars, resins, and a natural antioxidant removable by extraction with acetone **(28)**. Purified latex contains carbon-carbon double bonds, carbon-oxygen double bonds and carbon-carbon single bonds, for example LF (latex), molecular formula $(C_{10}H_5F_{13}O_2)_x$ where x = (1,2,3,....).

When radiation from the fluorescent lamp shines on the outer membrane surface, two events occur. Firstly the radiation causes the temperature of formic acid solution inside the membrane to increase slightly. This has the effect of increasing the rate of permeation of formic acid through the membrane. A comparison of the temperature of membranes kept under light and dark conditions shows this effect (Table IX)

Table IX. Permeation of formic acid though latex membranes in dark and in light.

	In dark	In light
Permeation /(g.d^{-1}.membrane^{-1})		
$\bar{x}_{16} \pm SE$	1.6 ± 0.1	1.8 ± 0.1
The average temperature during this study / °C	25.25	26.31

See appendix 9. Page X-Z, for original data.

Secondly, radiation from the fluorescent lamp affects the structural integrity of latex membranes by causing the membrane surface to gradually deteriorate. This effect is illustrated in Table X where the force needed to break membranes held under light testing conditions is shown to be significantly lower than that required to break membranes held in the dark.

Table X. Force required to break membranes after 15-day exposure to 65% (v/v) formic acid in dark and light

	In dark	In light
Force to Break Point / N ($\bar{x}_{10} \pm SE$)	29 ± 1	24 ± 2

This study shows that one of the main reasons why membranes in the field might leak or break is from structural deterioration through sunlight ultraviolet. This factor is of considerable importance since beekeepers must handle the membranes outside the hive in daylight to charge them with formic acid solution.

4. SUMMARY AND CONCLUSION

The technology for a novel closed system capable of delivering 10-13 g. d^{-1} of formic acid vapour to beehives has been successfully demonstrated.

Various latex membrane types were studied and it was found that formic acid permeated each at a different rate. Using the membrane with the fastest permeation rate, studies showed that factors to be considered in applying this delivery method in a field situation include membrane thickness, the presence or absence of airflow, and the amount of daylight falling on the membrane.

It has been demonstrated that formic acid permeates the membrane at a faster rate than water and that it is possible to deliver an optimum amount of formic acid vapour for Varroa mite control to a beehive in a field situation.

5. FURTHER WORK

One way of promoting safer delivery of formic acid vapour in the beehives safer, would be to use a mixture of menthyl formate and a hydrolyzing agent in the membrane. As the menthyl formate hydrolyzes it would release menthol and formic acid, both agents used in Varroa mite control.

The use of a gel formulation has been used successfully in an open system. This should be investigated in a closed system (inside latex membrane)

Working with six membranes to obtain an evaporation rate of approximately 10 g. d^{-1} formic acid would be more time-consuming and less efficient, possibly, than using a single membrane. Therefore experiments should be conducted using a single latex membrane of 7.5 dm^2 to determine whether this could be placed within a caged frame in the upper-mid section of beehive to obtain similar or better evaporation rate.

Studies on the radiation effects of white light on latex membranes could be extended to obtain more information on the wavelength range that is responsible for latex deterioration.

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7. Appendixes

Appendix 1: Preparation of A mL of (65%, v/v) of formic acid from concentrated acid (90% BDH Chemicals, AnalR B10114).

$$A\,\text{mL of solution} \times \frac{65\text{ mL of formic acid}}{100\text{ mL of solution}} = (A \times 0.65)\text{ mL of formic acid}$$

$$(A \times 0.65) \times \frac{100\text{ mL of formic acid}}{90\text{ mLof formic acid}} = (A \times 0.65) \times 1.11\text{ mL of 90\% (v/v) formic acid}$$

To prepare A mL of (65%, v/v) of formic acid from 90% concentrated acid

Take $\{[A] - (A \times 0.65) \times 1.11\text{ mL of (90\%, v/v) formic acid}\}$ mL of water and placed in container and add $(A \times 0.65) \times 1.11$ mL of 90% (v/v) formic acid .

Appendix 2: Latex permeation and open diffusion of 65% (v/v) formic acid – ambient temperature.

Time	Mass of 65 % (v/v)of formic acid / g		Formic acid permeation rate / $g.d^{-1}$	
day	Open beaker	Closed beaker	Closed beaker	Open Beaker
0.00	35.30	35.30	-0.32	-2.17
0.28	35.21	34.69	-0.49	-4.88
0.36	35.17	34.29	-0.29	-2.99
0.54	35.12	33.77	-0.37	-3.68
0.87	35.00	32.56	-0.22	-1.32
1.00	34.97	32.38	-0.68	-3.89
1.03	34.95	32.27	-0.28	-1.72
1.25	34.89	31.90	-0.38	-3.98
1.91	34.64	29.25	-0.44	-2.96
2.07	34.57	28.78	0.00	-2.56
2.11	34.57	28.67	-0.62	-3.15
2.15	34.55	28.57	-0.36	-2.01
2.37	34.47	28.12	-0.39	-2.15

2.52	34.41	27.79	-0.36	-2.15
2.94	34.26	26.90	-0.42	-2.06
3.08	34.20	26.60	-0.35	-1.91
3.40	34.09	26.00	-0.34	-2.40
4.07	33.86	24.38	-0.38	-1.71
4.28	33.78	24.02	-0.35	-1.81
4.48	33.71	23.66	-0.32	-1.77
5.33	33.44	22.16	-0.32	-1.57
5.48	33.39	21.92	-0.35	-2.07
5.91	33.24	21.04	-0.37	-1.48
6.07	33.18	20.80	-0.34	-1.60
6.16	33.15	20.66	-0.28	-1.06
6.19	33.14	20.62	-0.39	-1.62
6.40	33.06	20.28	-0.42	-1.74
7.04	32.79	19.17	-0.41	-1.52
7.23	32.71	18.88	-0.42	-1.85
7.40	32.64	18.57	-0.52	-2.20
8.03	32.31	17.19	-0.27	-1.23
8.25	32.25	16.92	-0.46	-2.64
8.38	32.19	16.58	-0.45	-2.08
8.91	31.95	15.46	-0.50	-1.90

9.98	31.41	13.43	-0.46	-1.69
10.16	31.33	13.14	-0.57	-1.75
10.25	31.28	12.98	-0.52	-1.99
11.09	30.84	11.31	-0.51	-2.26
11.42	30.67	10.55	-0.56	-2.11
12.44	30.10	8.39	-0.59	-1.99
13.24	29.63	6.82	-0.61	-1.97
14.12	29.09	5.080	-0.58	-1.64
14.28	29.00	4.82	-0.66	-2.54
14.86	28.61	3.33	-0.63	-1.44
15.09	28.47	3.01	-0.63	-1.26
15.37	28.29	2.65	-0.65	-2.25

Appendix 3: Indoor permeation of 65% (v/v) formic acid ambient- temperature

Time	Permeation of 65% (v/v) formic acid through five different latex membranes				
day	1	2	3	4	5
0.00	110.27	110.27	110.27	110.27	110.27
0.05	110.22	110.23	110.25	110.23	110.24
0.29	109.99	110.05	110.14	110.13	110.10
0.80	109.42	109.74	109.9	109.88	109.85
0.84	109.37	109.73	109.89	109.87	109.84
0.91	109.31	109.69	109.85	109.84	109.82
1.00	109.17	109.63	109.80	109.78	109.74
1.09	109.11	109.58	109.76	109.76	109.72
1.29	108.94	109.46	109.69	109.65	109.61
1.91	108.33	109.12	109.42	109.38	109.33
2.21	108.04	108.96	109.29	109.22	109.17
2.36	107.91	108.89	109.23	109.17	109.14
3.21	107.10	108.42	108.86	108.78	108.72
3.29	107.04	108.37	108.83	108.75	108.68
3.79	106.57	108.08	108.61	108.54	108.45

3.87	106.49	108.02	108.56	108.47	108.41
4.05	106.32	107.92	108.48	108.40	108.31
4.79	105.68	107.51	108.17	108.04	107.98
4.86	105.61	107.46	108.13	108.01	107.94
4.96	105.52	107.40	108.09	107.97	107.86
5.78	104.79	106.96	107.74	107.59	107.50
5.90	104.70	106.88	107.68	107.53	107.45
6.09	104.50	106.76	107.58	107.43	107.34
6.78	103.83	106.30	107.23	107.08	106.96
6.96	103.68	106.18	107.16	107.00	106.88
7.08	103.59	106.12	107.12	106.97	106.83
7.79	102.97	105.71	106.8	106.70	106.50
7.95	102.84	105.64	106.76	106.58	106.44
8.07	102.75	105.57	106.71	106.54	106.40
8.22	102.55	105.44	106.60	106.41	106.29
8.91	101.97	105.05	106.30	106.10	105.96
9.21	101.69	104.87	106.15	105.93	105.77
9.36	101.56	104.79	106.09	105.86	105.72
9.91	101.12	104.51	105.87	105.65	105.47
9.99	101.06	104.44	105.83	105.62	105.45
10.84	100.26	103.94	105.41	105.19	104.97

11.13	100.04	103.79	105.31	105.08	104.87
11.30	99.90	103.72	105.22	105.00	104.78
11.80	99.44	103.45	105.01	104.78	104.57
11.86	99.40	103.41	104.99	104.74	104.53
11.99	99.32	103.35	104.96	104.72	104.50
12.79	98.70	102.95	104.65	104.40	104.17
12.95	98.59	102.86	104.57	104.33	104.12
13.03	98.54	102.82	104.55	104.31	104.09
13.82	97.91	102.37	104.19	103.95	103.73
13.88	97.82	102.36	104.16	103.93	103.69
14.04	97.74	102.28	104.1	103.88	103.60
14.79	97.17	101.91	103.84	103.59	103.33
15.30	96.82	101.66	103.66	103.39	103.12
16.92	95.57	100.82	103	102.72	102.42
17.87	94.95	100.40	102.71	102.39	102.12

Appendix 4- Table A: Membrane structural integrity study after exposure to formic acid

Latex membrane number	Force required to produce stress failure in latex membrane / N — Sample number 1	2	3	4	5	6	7	8	Aver.	Stdev.	c.v.
1	31.3	21.1	25.3	17.3	25.9	28.9	30.0	20.2	25	5	20
2	15.5	37.4	33.7	23.8							
3	23.8	21.8	23.1	25.9							
4	19.1	32.5	17.3	20.4	28.5	29.3					
5	17.2	17.6									

Appendix 4-Table 2: Membrane structural integrity study before exposure to formic acid solution.

Latex membrane number	Force required to produce stress failure in latex membranes / N									
	Sample number									
	1	2	3	4	5	6	7	8	9	10
1	25.4	23.1	22.2	26.2	19.8	28.7	20.6	23.5	27.3	23.5
2	32.9	26.9	34.2							
3	21.6									
4	26.4	30.8	23.1							
5	33.4									

Appendix 5: Membrane thickness as a variable affecting permeation

Temperature	Time	Latex membranes thickness / mm					
/ ^{0}C	Day	0.07-0.08	0.08-0.09	0.34-0.43	0.34-0.42	0.65-0.72	0.69-0.74
26	0.00	111.93	111.93	111.93	111.93	111.93	111.93
26.8	0.38	111.48	111.44	111.84	111.80	111.69	111.92
27.2	0.92	110.76	110.62	111.67	111.62	111.58	111.92
27	1.13	110.49	110.32	111.60	111.56	111.52	111.74
27	1.38	110.10	109.93	111.51	111.45	111.47	111.69
26	1.93	109.45	109.25	111.36	111.33	111.38	111.60
26	2.13	109.24	108.97	111.31	111.27	111.33	111.55
25.9	2.38	108.92	108.64	111.20	111.19	111.28	111.50
25.1	2.94	108.19	107.88	111.04	111.01	111.17	111.37
25	3.13	107.98	107.66	110.96	110.97	111.12	111.34
25.5	3.38	107.67	107.32	110.85	110.85	111.04	111.25
25	3.93	107.02	106.68	110.66	110.67	110.89	111.11
25.2	4.14	106.78	106.42	110.54	110.59	110.82	111.06
26	4.38	106.47	106.12	110.43	110.47	110.74	110.97
25.2	4.92	105.81	105.47	110.25	110.29	110.61	110.83
25	5.13	105.54	105.21	110.17	110.20	110.51	110.78

25.5	5.38	105.28	104.95	110.09	110.14	110.50	110.74
25	5.92	104.74	104.32	109.90	109.97	110.35	110.60
27	6.09	104.55	104.15	109.83	109.92	110.31	110.58
27.9	6.47	104.04	103.67	109.65	109.75	110.19	110.44
26.5	6.93	103.43	103.02	109.43	109.49	109.99	110.26
27	7.17	103.10	102.65	109.32	109.37	109.89	110.19
27	7.42	102.76	102.31	109.20	109.26	109.80	110.11
26	7.92	102.13	101.62	108.95	109.03	109.60	109.95
26	8.18	101.82	101.29	108.86	108.93	109.54	109.88
25	8.42	101.51	100.98	108.75	108.82	109.47	109.78
24.8	8.94	100.94	100.37	108.56	108.60	109.24	109.65
25	9.14	100.75	100.14	108.49	108.54	109.19	109.57
25	9.38	100.50	99.87	108.38	108.44	109.13	109.50
24	10.14	99.74	99.01	108.10	108.17	108.91	109.30
24	10.34	99.56	98.80	108.02	108.09	108.85	109.25
24	10.92	98.98	98.24	107.82	107.90	108.70	109.11
24	11.17	98.76	97.98	107.75	107.82	108.63	109.05
24.9	11.43	98.50	97.72	107.67	107.75	108.54	108.98
24	11.98	98.03	97.20	107.49	107.58	108.40	108.85
25	12.13	97.92	97.03	107.43	107.53	108.34	108.79
25	12.39	97.66	96.74	107.35	107.42	108.28	108.72

25.5	12.93	97.14	96.23	107.12	107.24	108.09	108.55
25.5	13.14	96.92	96.02	107.05	107.15	108.02	108.50
24.9	13.39	96.63	95.71	106.92	107.04	107.90	108.39
25	13.94	96.07	95.11	106.69	106.81	107.69	108.16
25	14.18	95.86	94.89	106.62	106.73	107.64	108.14
25	14.40	95.62	94.63	106.52	106.64	107.53	108.05
25	14.94	95.07	94.05	106.28	106.43	107.35	107.87
25.3	15.38	94.63	93.60	106.11	106.25	107.17	107.71

Appendix 6 Outdoor membrane-permeation of 65% (v/v) of formic acid in hive no honey bees present.

Appendix 6-Table A. Permeation of (65%, v/v) formic acid through five different membranes /g placed in beakers inside hive no honey bees present.

Time	Temperature	Permeation of 65% (v/v) formic acid through five different membranes /g placed in beakers inside hive				
day	°C	1	2	3	4	5
0.00	24.0	101.83	101.83	101.83	101.83	101.83
0.33	19.0	101.35	101.61	101.72	101.26	101.49
0.79	18.0	101.11	101.45	101.61	101.18	101.41
1.01	19.0	101.01	101.40	101.59	101.17	101.36
1.26	20.5	100.87	101.30	101.51	101.11	101.31
1.72	14.5	100.63	101.10	101.41	100.99	101.21
1.97	16.0	100.52	101.05	101.37	100.97	101.16
2.22	15.0	100.38	100.95	101.31	100.92	101.10
2.75	8.5	100.20	100.84	101.24	100.87	101.03
2.96	22.0	100.11	100.79	101.18	100.83	100.99
3.26	16.0	99.81	100.61	101.05	100.70	100.86
3.75	8.0	99.68	100.51	100.99	100.64	100.80

3.97	21.0	99.60	100.46	100.95	100.60	100.76
4.26	15.0	99.41	100.33	100.87	100.51	100.66
4.75	7.0	99.25	100.24	100.78	100.44	100.60
4.96	20.0	99.22	100.2	100.77	100.43	100.58
5.38	14.0	99.06	100.11	100.70	100.37	100.49
5.75	10.0	98.95	100.02	100.64	100.28	100.42
5.97	11.0	98.92	99.99	100.61	100.27	100.40
6.22	10.5	98.86	99.94	100.58	100.26	100.37
6.75	9.0	98.76	99.88	100.54	100.19	100.30
7.00	16.0	98.68	99.83	100.52	100.18	100.28
7.25	13.0	98.59	99.77	100.46	100.14	100.24
7.75	9.0	98.47	99.71	100.40	100.11	100.17
7.97	20.8	98.36	99.64	100.37	100.06	100.14
8.21	18.0	98.16	99.51	100.23	99.94	100.01
8.75	7.3	97.98	99.45	100.17	99.89	99.95
10.25	25.5	97.47	99.15	99.92	99.65	99.69
10.80	16.0	97.03	98.83	99.71	99.43	99.47
10.96	26.0	96.95	98.79	99.65	99.38	99.42
11.76	18.0	96.47	98.44	99.36	99.13	99.14

Appendix 6-Table B: Permeation of 65% (v/v) formic acid through three different membranes hung between frames inside hive / g no honey bees present.

Time	Temperature	Permeation of 65% (v/v) formic acid through three different membranes hung between frames inside hive / g		
day	°C	1	2	3
0.00	24.0	105.07	105.07	105.07
0.33	19.0	104.17	104.46	104.61
0.79	18.0	103.63	104.03	104.44
1.01	19.0	103.31	103.82	104.35
1.26	20.5	102.76	103.47	104.27
1.72	14.5	102.23	103.11	104.09
1.97	16.0	102.02	102.94	104.01
2.22	15.0	101.72	102.76	103.88
2.75	8.5	101.39	102.50	103.79
2.96	22.0	101.16	102.47	103.67
3.26	16.0	100.47	101.80	103.38
3.75	8.0	100.23	101.63	103.29
3.97	21.0	100.02	101.49	103.19
4.26	15.0	99.61	101.17	103.01
4.75	7.0	99.39	101.04	102.90

4.96	20.0	99.22	100.93	102.84
5.38	14.0	98.91	100.72	102.69
5.75	10.0	98.65	100.53	102.58
5.97	11.0	98.56	100.41	102.57
6.22	10.5	98.42	100.36	102.48
6.75	9.0	98.2	100.21	102.43
7.00	16.0	98.04	100.13	102.33
7.25	13.0	97.74	99.97	102.25
7.75	9.0	97.47	99.79	102.18
7.97	20.8	97.25	99.63	102.06
8.21	18.0	96.79	99.29	101.84
8.75	7.3	96.53	99.11	101.75
10.25	25.5	95.33	98.24	101.15
10.80	16.0	94.36	97.48	100.71
10.96	26.0	94.16	97.32	100.57
11.76	18.0	92.92	96.43	99.97

Appendix 7-Table A. Outdoor permeation of 65% (v/v) of formic acid in hive A with honey bee present.

Time / day	Temperature / °C	Mass of 65% (v/v) formic acid / g 1	2	3	4	5	6
0.00	34.98	122.90	122.90	122.90	122.90	122.90	122.90
0.99	33.37	120.17	120.00	118.95	118.72	119.25	119.86
2.02	34.77	117.44	116.80	115.21	114.65	115.29	116.61
2.99	35.39	115.02	114.40	111.87	110.77	112.05	114.07
3.96	34.57	112.19	111.50	108.42	107.82	109.41	112.24
5.00	34.37	109.96	109.50	105.59	104.25	106.78	110.32
6.03	29.72	108.24	107.80	103.46	101.40	103.94	108.69
7.02	34.57	106.43	105.80	101.24	99.05	101.61	106.56
7.99	29.72	104.71	104.00	98.70	96.40	99.37	104.84
9.00	23.34	102.99	102.30	96.58	94.67	97.75	103.41
9.90	22.33	101.88	101.00	95.26	93.55	96.94	102.40
11.00	22.16	100.56	100.00	94.25	92.84	96.03	101.49
12.00	21.16	99.86	99.10	92.83	91.72	95.12	100.17
13.00	21.33	98.85	97.70	92.23	91.51	94.61	99.86
13.99	22.18	97.63	97.30	91.42	90.29	93.70	98.95

Appendix 7-Table B: Outdoor permeation of 65% (v/v) of formic acid in hive B honey bee present.

Time / day	Temperature / °C	Mass of 65% (v/v) formic acid / g 1	2	3	4	5	6
0.02	34.12	121.90	121.90	121.90	121.90	121.90	121.90
1.00	33.12	118.67	117.68	118.07	117.67	118.18	118.70
2.03	33.72	115.95	113.56	113.84	113.24	114.17	115.50
3.00	34.73	112.93	110.24	110.42	109.41	110.96	112.50
3.98	33.12	110.41	107.03	107.29	106.28	108.34	110.70
5.00	32.34	107.89	104.21	104.27	103.36	105.73	108.70
6.03	29.68	105.97	101.90	101.95	101.05	103.52	107.70
7.02	34.32	103.75	99.19	99.13	98.23	100.41	104.70
7.99	32.54	101.94	96.88	96.71	94.90	96.90	101.70
9.00	27.14	99.52	94.97	95.00	93.29	95.19	100.20
9.90	27.86	97.50	93.06	92.89	91.88	93.28	97.80
11.00	24.35	95.58	91.65	91.48	90.37	91.27	95.70
12.00	18.88	93.67	90.24	90.47	88.86	89.97	93.30
13.00	18.39	91.75	88.84	89.76	87.95	88.06	92.00
13.99	19.25	89.84	88.64	89.46	85.13	86.66	91.20

Appendix 7-Table C. Outdoor permeation of 65% (v/v) of formic acid in hive C honey bee present.

Time / day	Temperature / °C	Mass of 65% (v/v) formic acid / g 1	2	3	4	5	6
0.0	34.98	122.4	122.4	122.4	122.4	122.4	122.4
1.0	32.37	118.8	119.0	118.3	118.5	118.2	118.5
2.0	34.96	115.4	115.2	113.8	114.4	114.6	114.5
3.0	35.99	109.2	110.7	110.3	111.1	111.5	111.6
4.0	34.35	99.4	97.4	107.8	108.0	108.6	109.3
5.0	33.35	91.3	86.9	105.0	105.8	106.6	107.3
6.0	28.23	82.3	81.6	102.6	103.6	104.5	105.5
7.0	34.76	75.0	69.9	99.4	101.2	102.3	103.5
8.0	27.69	58.3	61.4	89.7	98.9	98.2	101.3
9.0	25.41	53.4	49.8	80.6	96.7	96.1	99.7
9.9	27.33	49.7	46.1	74.0	94.5	94.0	98.5
11.0	27.16	48.4	45.1	68.6	93.3	91.3	97.3
12.0	26.63	47.2	44.1	62.6	91.3	87.6	94.6
13.0	26.80	46.6	43.2	56.2	89.9	85.6	94.7
14.0	27.35	45.9	42.6	50.5	88.9	82.8	93.4

Appendix 8. Absolute measurement for the permeation and diffusion of formic acid and water

Appendix 8-Table A. Diffusion of formic acid in open beakers with different concentration

Time		**T/°C**	**Formic acid concentration % (v/v)**							
/ hour	**/ day**		***0%***	***20%***	***35%***	***35%***	***35%***	***35%***	***50%***	***65%***
0.21	0.01	22	142.39	143.28	150.43	147.15	147.96	144.79	148.44	140.47
27.38	1.14	23	138.43	139.77	147.41	144.16	144.66	141.63	145.72	138.47
48.43	2.02	23	135.56	137.21	145.17	141.73	141.66	139	143.35	135.86
72.53	3.02	23	132.4	134.41	142.91	139.3	138.59	136.25	140.84	133.52
94.2	3.93	22	128.77	131.46	140.55	137.11	136.41	134.14	138.68	131.32
118.54	4.94	23	126.4	129.58	138.74	135.04	134.27	132.12	136.55	129.37
142.28	5.93	25	124.08	127.7	136.89	133.03	132.32	130.38	135.07	127.95
167.24	6.97	25	120.94	125.06	134.42	130.45	129.81	128.08	133.24	126.43
190.2	7.93	25	117.61	122.14	131.66	127.38	126.7	125.15	130.7	124.3
214.18	8.92	24	114.14	119.17	128.86	124.09	123.23	121.82	127.87	121.9
238.29	9.93	22	110.48	115.8	125.4	120.47	119.68	118.39	125	119.59
262.47	10.94	23	105.69	111.32	121.32	116.47	116.01	114.92	121.81	117.01
286.02	11.92	27	101.33	107.19	117.25	112.81	112.58	111.69	118.94	114.69

311.36	12.97	25	96.87	103.06	113.27	109.06	108.94	108.4	115.73	112.01
335.3	13.97	27	92.23	98.71	109.34	105.38	105.44	105.07	112.73	109.6

iffusion of formic acid from Open beaker	3.47 ±0.09	3.05 ±0.10	2.84 ±0.11	2.94 ±0.09	2.97 ±0.07	2.78 ±0.07	2.49 ±0.06	2.16 ±0.04

Appendix 8-Table B. Permeation of formic acid through latex membranes with different concentration

Time		Formic acid concentrations							
/ hours	/ day	0%	20%	35%	35%	35%	35%	50%	65%
0.03	0.00	92.02	92.02	92.02	92.02	92.02	92.02	92.02	92.02
18.51	0.77	91.49	91.23	91.17	91.12	91.19	91.29	90.86	90.04
42.31	1.76	91.07	90.56	90.38	90.32	90.39	90.56	89.78	87.86
67.21	2.80	90.51	89.84	89.53	89.43	89.47	89.73	88.65	85.99
90.24	3.76	89.98	89.24	88.86	88.67	88.71	89.05	87.70	84.52
114.14	4.76	89.47	88.64	88.20	87.92	87.98	88.36	86.86	83.25
138.33	5.76	88.99	88.08	87.63	87.27	87.32	87.73	85.94	82.18
162.45	6.77	88.59	87.62	87.14	86.68	86.77	87.22	85.23	81.25
187.06	7.79	88.22	87.17	86.66	86.13	86.27	86.73	84.55	80.36

211.25	8.80	87.87	86.75	86.21	85.56	85.76	86.24	83.87	79.43
235.33	9.81	87.47	86.28	85.69	84.96	85.21	85.69	83.15	78.42
259.33	10.81	87.09	85.81	85.22	84.42	84.71	85.17	82.45	77.44
283.25	11.80	86.73	85.42	84.80	83.92	84.27	84.70	81.87	76.37
307.32	12.81	86.38	85.02	84.37	83.38	83.82	84.23	81.28	75.28
331.24	13.80	86.04	84.61	83.94	82.83	83.36	83.75	80.67	74.18
Permeation of FA through membranes		0.43 ± 0.01	0.58 ± 0.02	0.67 ± 0.02	0.75 ± 0.02	0.72 ± 0.03	0.70 ± 0.02	0.94 ± 0.03	1.21 ± 0.16

Appendix 9 –Table A. Permeation of formic acid though latex membranes in light.

Time / hour	Time /day	Membrane number 1	2	3	4	5
0.0	0.00	118.19	118.19	118.19	118.19	118.19
24	0.99	115.76	115.48	115.52	116.04	115.61
47.47	1.97	113.68	113.42	113.50	114.18	113.55
71.3	2.96	111.57	111.55	111.43	112.29	111.47
96.06	3.99	109.31	109.61	109.49	110.51	109.21
119.32	4.96	107.04	107.74	107.50	108.70	107.04
143.21	5.96	104.78	104.90	105.73	106.97	105.07
167	6.95	102.53	104.01	103.94	105.21	103.12
191.18	7.96	100.74	102.42	102.40	103.73	101.48
215.23	8.96	98.92	100.76	100.84	102.19	99.81
239.4	9.97	97.32	99.36	99.43	100.85	98.33
264.02	10.99	95.67	97.86	98.01	99.48	96.88
287.28	11.96	94.02	96.39	96.57	98.09	95.40
310.23	12.92	92.29	93.77	95.06	96.68	93.90
336.37	14.01	90.26	92.87	93.29	94.86	92.06
359.43	14.97	88.21	90.93	91.53	93.14	90.21
Standard deviation		1.97±0.03	1.75±0.03	1.71±0.03	1.62±0.02	1.81±0.04

Appendix 9- Table-b. Permeation of formic acid though latex membranes in dark.

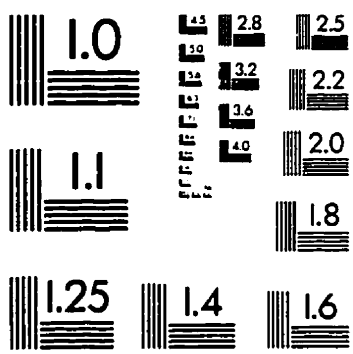
Time /hour	Time /day	Membrane number 1	2	3	4	5
0.0	0.00	116.93	116.93	116.93	116.93	116.93
24	0.99	114.32	114.37	114.57	114.30	114.24
47.47	1.97	112.43	112.49	112.73	112.42	112.28
71.3	2.96	110.68	110.76	111.01	110.67	110.44
96.06	3.99	108.80	108.98	109.21	108.76	108.69
119.32	4.96	106.87	107.24	107.43	106.84	106.97
143.21	5.96	105.03	105.58	105.82	105.03	105.30
167	6.95	103.15	103.91	104.21	103.42	103.68
191.18	7.96	101.61	102.50	102.82	102.00	102.20
215.23	8.96	100.17	101.15	101.50	100.64	100.80
239.4	9.97	98.89	99.97	100.36	99.46	99.54
264.02	10.99	97.57	98.72	99.17	98.23	98.29
287.28	11.96	96.22	97.51	97.96	97.00	96.99
310.23	12.92	94.81	96.25	96.70	95.73	95.64
336.37	14.01	93.15	94.72	95.19	94.23	94.07
359.43	14.97	91.52	93.17	93.61	92.69	92.46

Standard deviation	1.64±0.03	1.52±0.04	1.50±0.04	1.55±0.04	1.56±0.03

Appendix 9 –Table C. Mass need to cut each membrane sample per N

Force needed to cut each sample / N		
Sample #	In dark	In light
1	29.5	20.8
1	31.2	22.8
2	25.8	22.8
2	28.6	24.8
3	29.0	25.7
3	30.7	24.3
4	30.4	26.5
4	28.7	20.4
5	27.5	23.9
Average	29.03	23.55
Stdev	1.67	2.07
c.v.	6 %	8 %

IMAGE EVALUATION TEST TARGET (QA-3)



150mm

6″

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