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Original article

Activity of oxalic and citric acids on the mite *Varroa destructor* in laboratory assays

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Abstract – The toxicity of oxalic and citric acid to *Varroa destructor* Anderson and Trueman was studied with a laboratory assay. A contact test was used for the bioassay. Capsules were sprayed with solutions of the acids in a Potter tower and allowed to dry. Slight but significant differences in toxicity of oxalic acid were found among mites from different brood stages; the median lethal density for mites from pupae with white eyes was 1.49 μg/cm^2. Citric acid was less toxic than oxalic acid. The addition of sucrose to oxalic acid made the material more hygroscopic and the mortality of the mites increased at 75% R.H. Glycerol showed a similar synergic activity irrespective of relative humidity.

***Varroa destructor* / bioassay / oxalic acid / citric acid / sucrose / glycerol / synergism**

1. INTRODUCTION

In recent years, resistance to acaricides has become a major problem in the control of *Varroa destructor* Anderson and Trueman. Increased tolerance to the most widely used synthetic active ingredients has been observed (reviewed in Milani, 1995). This renewed the interest in substances of natural origin, such as essential oils and their components (Imdorf et al., 1999) or organic acids, especially formic acid and oxalic acid (Bolli et al., 1993; Imdorf et al., 1997; Mutinelli et al., 1997). In *Varroa destructor*, resistance to these newer acaricidal substances has not been detected. This is not surprising since selection pressure for resistance has been low, due to variable efficacy and limited use of these substances.

However, in principle, there is no reason to believe that resistance to these acaricides could not develop if selection pressure increased, i.e. if the products were repeatedly applied as the only acaricide over large areas. Even a small increase in the tolerance

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of the mite could make them useless for control because of the narrow margin of safety. In this sense, the safety margin is the difference between the amount of material that provides mite control compared to the amount of material that causes damage to the bees. Resistance to low molecular weight substances has been repeatedly reported. Widespread resistance to phosphine, PH_3, in stored-product insects is a well known example (Chaudhry, 1997) and slight increases in tolerance were observed even with extremely unspecific biocides such as methyl bromide (Rajendran, 1992).

Among substances of natural origin used against *V. destructor*, the risk of resistance is higher for oxalic acid. High efficacy in the absence of capped brood, ease of use of the application by trickling, extremely low cost, independence from the temperature and shortage of alternatives might make treatments with this acid widely used in the future and thus increase selection pressure for resistance.

A baseline tolerance curve, for populations that have not been treated with oxalic acid, would be useful for comparisons. The availability of data on the toxicity of coumaphos to mites from untreated populations (Milani and Della Vedova, 1996) has made it possible to detect resistance to this organophosphorous acaricide (Spreafico et al., 2000), before bee losses were reported. On the other hand, little is known of the mode of action of oxalic acid, in particular, why a solution of oxalic acid and sucrose, applied by tricking (Mutinelli et al., 1997), is more effective than a solution of the acid alone, used in the same way.

This paper reports the dose-mortality curve for oxalic acid. Analogous data for citric acid, which was also taken into consideration for the control of *V. destructor*, are given. Bioassays was used to study the effect of the addition of sucrose to oxalic acid to investigate why the addition of this sugar appears to have a synergic effect.

2. MATERIALS AND METHODS

2.1. Organic acids tested

Investigations were carried out on oxalic and citric acids. The former was also tested in mixtures with sucrose and glycerol. All the reagents were analytical grade. Solutions were made in deionized water (conductance < 10 $\mu S \cdot cm^{-1}$).

2.2. Origin and collection of the mites

Mites were sampled in August-October from an infested experimental apiary kept in Udine (Friuli, north-eastern Italy) without acaricide treatments. In that region, organic acids had seldom been used against *V. destructor*. Mites were collected from bee brood (Milani, 1995); mites from brood of different ages (spinning larvae, *l5*; pupae with white eyes, *pw*; pupae with dark eyes and white body, *pd*) were assayed separately. Phoretic mites were not included because their response has been shown to be rather variable and the mortality in the controls higher (Milani, 1995).

2.3 Capsules used in the bioassay

Glass disks (62 mm diameter; Na-Ca glass) were treated to reduce the leakage of ions. They were kept for 3 h in a 1% NaOH solution, rinsed, kept overnight in a 1% oxalic acid solution, rinsed, washed with a 1% acetic acid solution, rinsed with tap water and finally rinsed three times with deionized water. A stainless steel ring (56 mm inner diameter, 2–3 mm height) was glued to each glass disk with four small drops of cyanoacrylate adhesive, to form a half-capsule (Fig. 1), similar to those used in previous research (Milani, 1995).

The interior side of the half-capsules was sprayed with solutions of oxalic and citric acid with a “Potter precision spray tower” (Burkard Manufacturing Co., England). The following operating conditions were used:

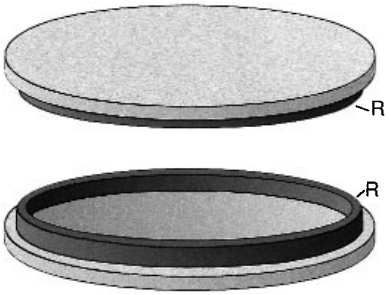

Figure 1. Diagrammatic drawing of the capsule used in the experiments, made of two glass disks and two stainless steel rings (R).

the reservoir was charged with 1 ml of solution; the distance of the sprayed surface from the bottom end of the tube was 11 mm; a nozzle 0.0275 inches was used. Only glasses uniformly sprayed with droplets of the solution were used; those showing a coarse pattern of drops were discarded. The pressure was adjusted (usually in the range 350–500 hPa) until the amount of solution deposited was 1 ± 0.05 mg/cm^2. After drying (which took about 15 minutes), the disks with the glued ring were assembled into a capsule having the inner sides sprayed, and kept together with small pieces of beeswax.

The capsules were used within 60 h of preparation, for not more than three assays. After use, the capsules were washed with Ausilab 201 (Carlo Erba Reagenti), then rinsed twice with tap water and at least three times with deionized water, to remove traces of detergent, and finally dried in an oven.

2.4. Measure of the deposit obtained on glass disks

To determine the amount of solution deposited on the surface of the disk, a preweighed coverslide (24×32 mm) was placed on a glass disk, sprayed and weighed again within 15 s. This measure was repeated three times after adjusting the pressure at the beginning of each series of sprays, and then each time the concentration of the solution sprayed was changed. On average, the solution deposited was 1.016 mg/cm^2 (range: 0.73–1.19) with a standard deviation of 0.08 mg/cm^2, which is somewhat larger than that reported in the literature (e.g., Potter, 1952; also Pye, unpublished data); variations occurred apparently at random, without any definite trend, also in consecutive sprays.

The amount of the active substances deposited (dry deposit) per unit surface was expressed as μg/cm^2. It was chosen to measure the treatment as a surface density, since mite mortality is related to the deposit obtained; the concentration of the sprayed solution does not uniquely identify the deposit, without additional information on the amount sprayed and the operative conditions used. In the conditions chosen here, for practical purposes the "surface density" of the dry deposit in μg/cm^2 was expressed by the same number indicating the concentration in g/l of the solutions sprayed.

2.5. The bioassay

Ten to 15 female *Varroa destructor* were introduced into each capsule; after 4 h they were transferred into a clean glass Petri dish (60 mm dia.) with two or three worker larvae taken from cells 0–24 h after capping (Milani, 1995). To avoid the transfer of acid crystals into the Petri dish, mites were first transferred with a fine brush into a polystyrene dish – without touching the treated surface – and immediately after into the glass Petri dish. The mites were observed under a dissecting microscope 4 h (when transferring into the Petri dish), 24 and 48 h after the beginning of the treatment and classified as mobile, paralysed or dead as in Milani (1995).

Each experiment was replicated with at least three series of capsules; more replications were carried out and more mites were assayed at doses around the median lethal density, as in previous work (Milani, 1995). The assays were carried out at 32.5 °C and

Table I. Treatments carried out, brood stage from which *V. destructor* females were taken, number of replications and mites in assays to determine the dose-mortality curve for oxalic acid (OA) and citric acid (CA) and to study synergic effects of sucrose and glycerol.

Experiment	Surface densities used	Brood stage	No. replic. per dose*	Approx. no. mites
Dose-mortality curve, OA	0, 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50 µg/cm^2	*l5*	6	30–60
		pw	5	50–80
		pd	6	50–80
Dose-mortality curve, CA	0, 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50 µg/cm^2	*l5*	4	20–40
		pw	4	30–40
		pd	5	30–70
OA, synergic activity of sucrose	0, 0.1, 0.3, 1, 3, 10 µg/cm^2 sucrose alone; 0, 0.1, 0.3, 1, 3, 10 µg/cm^2 sucrose plus 1 µg/cm^2 OA	*pw*	8	60–80
OA, synergic activity of glycerol	0, 0.1, 0.3, 1 µg/cm^2 glycerol alone; 0, 0.1, 0.3, 1 µg/cm^2 glycerol plus 1 µg/cm^2 OA	*pw*	4	30–50
OA, dose-mortality curve with a fixed concentration of sucrose	0, 0.05, 0.1, 0.2, 0.5, 1, 2, 5 µg/cm^2 OA added with 1 µg/cm^2 sucrose	*pw*	5	30–50
OA, dose-mortality curve with a fixed concentration of glycerol	0, 0.05, 0.1, 0.2, 0.5, 1, 2, 5 µg/cm^2 OA, added with 1 µg/cm^2 glycerol	*pw*	3	30
OA, dependence of synergic effects on the RH	0 (control); 1 µg/cm^2 OA; 1 µg/cm^2 OA plus 0.3 µg/cm^{2}sucrose; 1 µg/cm^2 OA plus 0.3 µg/cm^2 glycerol	*pw*	6	60–90

* Fewer mites were tested when the mortality was close to 0 or 100%, more mites around the median lethal density.

75% R.H., unless otherwise noted. Control capsules were sprayed with deionized water. A few replications in which the mortality in the controls exceeded 30% were discarded.

Dose-mortality curves were obtained for both oxalic and citric acids (Tab. I).

Synergic effect of sucrose and glycerol were studied. Mortality in capsules sprayed with solutions containing different concentrations of the synergists and oxalic acid (Tab. I) was determined; dose-mortality curves for oxalic acid added with a fixed concentration of sucrose or glycerol were also obtained.

Finally, the dependence of the effects of sucrose and glycerol on the relative humidity was studied. Control capsules, capsules treated with oxalic acid alone and capsules treated with a solution of oxalic acid added with sucrose or glycerol were prepared (Tab. I). Mites were kept in these capsules for 1 h; one series of capsules was kept at 42% R.H. a second one at 75% R.H. The treatment was reduced to 1 h, to avoid excessive mortality due to water loss at the lower humidity. Then the mites were transferred into Petri dishes with bee larvae and observed at 24 and 48 h. The relative humidities were obtained in air tight containers with saturated solutions of $Zn(NO_3)_2 \cdot 6\ H_2O$ and NaCl (Winston and Bates, 1960). A 5 mm dia. hole – closed with a nylon net – in one of the glass disks made it possible to obtain equilibrium between humidity in the interior of the capsule and that in the container in a shorter time. Both containers and capsules had been kept for at least 3 h at 32.5 °C, to reach thermal equilibrium; transfer of the mites into these capsules was done as quickly as possible, within five minutes.

2.6 Measure of the relative humidity

The relative humidity (R.H.) of air in a hermetically sealed glass flask in equilibrium with a saturated solution of oxalic acid, citric acid, and a solution saturated both in oxalic acid and sucrose was measured with three resistive RH-8 probes (General Eastern, Woburn, MA, USA) when three successive readings at 1 h interval had stabilized and the average was calculated. The probes were calibrated at 42% and 75% R.H., with an overall error of less than 3% in the interval 40–80% R.H.

Relative humidity was recorded (hourly averages) in four hives housed in Dadant-Blatt colonies in the period November 13–December 3. In northern Italy, treatments with oxalic acid in sugar solution are performed mostly during this period, when colonies are broodless. Two RH-8 probes were introduced into each hive, one at the centre of the nest and the second one just over the top bars, in a central position.

2.7. Statistical analysis

The data of the dose-mortality curves were analysed using the probit transformation; the natural mortality rate was taken into account using an iterative approach (Finney, 1971). The surface densities which kill a given proportion *z* of mites (LDe_z) and their fiducial limits were computed according to Finney (1949).

The proportions of dead mites in different experimental groups were compared with the *G*-test.

3. RESULTS

3.1. Dose-mortality curves

The average mortality in the controls ("natural mortality"; Tab. II) was about 15% at 48 h. In some assays, a portion of the mites in the controls performed irregular movements often associated with starvation and loss of water even at 4 h.

Although the effects on *Varroa destructor* were clearly concentration dependent at 4 h, the observations at 24 and 48 h depict a more stable situation, since the proportion of

Table II. Mortality in the controls and percentage of paralysed mites (in treated and control capsules) in experiments to determine the dose-mortality curve for oxalic and citric acids.

Substance	Natural mortality (%)			Paralysed mites (%)		
	4 h	24 h	48 h	4 h	24 h	48 h
Oxalic acid	0	11	13	32.6	1.5	0.6
Citric acid	0	16	17	35.5	8	1.5

paralysed mites is lower (Tab. II) and thus they are reported and discussed mainly in the next sections. In experiments using oxalic acid, the difference between the mortality at 24 and that at 48 h, at a given surface density, did not exceed 8%, except for a single case (15%).

With oxalic acid, the results of the replications were consistent and no heterogeneity was found. All the series of data could be fitted by using the probit regression (Fig. 2). The width of the 95% fiducial interval of the LDe_{50} was 0.4–0.8 times the LDe_{50} (Tab. III). Mites that survived high doses were often found lying on their back or even stuck in this position to the bottom of the treated capsules.

Differences in the susceptibility of mites from different stages were observed (Tab. III). If data of mites from *pw* and *pd* or *l5* are pooled, highly significant heterogeneity χ^2 arises ($P \leq 10^{-3}$). The mites from *l5* tend to be the most susceptible. The regression lines for the three groups of mites are not parallel and the differences disappear at the LDe_{95}.

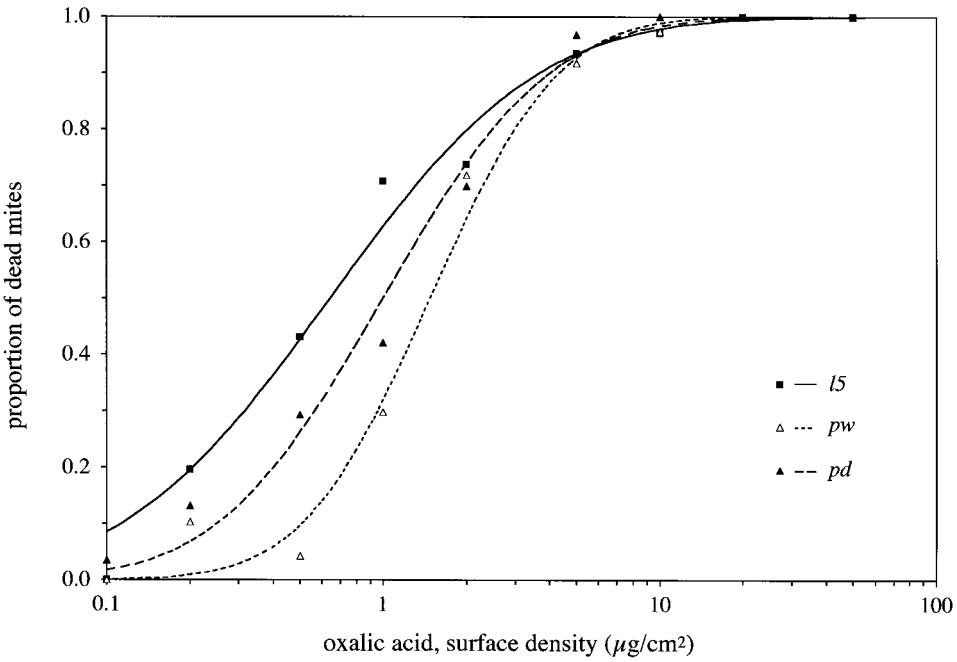

Figure 2. Dose-mortality curve for oxalic acid at 48 h.

Table III. Median lethal density (LDe_{50}), density expected to cause 95% mortality (LDe_{95}) and their 95% fiducial intervals, on *V. destructor* females taken from different bee stages, in the contact bioassay described in the text. Data expressed as $\mu g/cm^2$ active substance.

Active subst.	Bee stage	24 h				48 h			
		LDe_{50}	fid. limits	LDe_{95}	fid. limits	LDe_{50}	fid. limits	LDe_{95}	fid. limits
Oxalic acid	l5	0.68	0.41, 1.00	10.06	6.25, 20.25	0.64	0.40, 0.92	6.01	3.96, 11.14
	pw	1.90	1.49, 2.36	9.43	6.84, 14.98	1.49	1.20, 1.81	5.92	4.42, 9.10
	pd	1.12	0.86, 1.40	7.75	5.56, 12.46	1.02	0.78, 1.27	5.87	4.29, 9.29
Citric acid	l5	9.34	3.69, 20.55*	65.3	26.8, 1765.8*	3.56	1.66, 5.92*	25.9	13.2, 137.7*
	pw	7.13	4.96, 10.45	120.8	58.2, 426.1	2.14	1.53, 2.85*	10.7	7.1, 20.6*
	pd	3.04	1.87, 4.50*	29.5	15.9, 95.7*	2.38	1.77, 3.00	10.1	7.5, 15.6

* A heterogeneity factor was used to compute fiducial intervals.

Citric acid (Tab. III) yielded remarkable variability among replications, often resulting in large heterogeneity χ^2. This makes the treatment of the data and the use of a heterogeneity factor to compute fiducial limits somewhat artificial. Doses required to produce a given mortality were larger than with oxalic acid, but the fiducial intervals were wide, making it impossible to determine the "relative potency" of the acids quantitatively. No conclusion can be made on differences among mites from different brood stages.

3.2. Synergic effect of sucrose or glycerol

In capsules sprayed with sucrose solutions (without oxalic acid), irrespective of the concentration, the mortality did not differ significantly from that of the controls treated with water alone. The same result was obtained with glycerol (in both cases, $P \approx 0.15$, χ^2 test). Thus the average mortality in capsules treated with water or sucrose – or water or glycerol – was used as a joint estimate of the "natural mortality" (at 48 h, 6.1% and 13.4% respectively).

At 48 h, the mortality in capsules treated with oxalic acid added with 1 and 3 $\mu g/cm^2$ sucrose was significantly higher than in capsules treated with oxalic acid alone (Tab. IV). At 10 $\mu g/cm^2$ sucrose, no increase was observed, but the aspect of the deposit was different (possibly as a result of the different viscosity of the solution). With glycerol, a significant increase in the mortality was obtained at 0.3 and 1 $\mu g/cm^2$. The same trend was observed at 24 h, but with higher variability among replications and less clear differences among experimental groups.

These results were confirmed by the dose-mortality curves. If data obtained with oxalic acid added with sucrose or glycerol are pooled with those with oxalic acid alone, significant heterogeneity χ^2 arise ($P \approx 0.02$ and $P < 10^{-3}$ respectively). The LDe_{50} was lower with both synergists but the fiducial intervals partially overlap in the case of sucrose (Tab. V).

3.3. Effect of the relative humidity

No significant differences in the mortality at 42 and 75% R.H. in control capsules (9.3 and 10.3% respectively) nor in capsules treated with oxalic acid alone (Tab. VI) were observed.

At 75% R.H., the deposit in capsules treated with oxalic acid alone was represented by dry crystals, while the mixture of oxalic acid and sucrose or glycerol produced small droplets of solution by absorbing water vapour from the atmosphere. At 42% R.H., the deposit in capsules sprayed with oxalic acid alone or mixed with sucrose was dry,

Table IV. Percent mortality at 48 h of *V. destructor* females from *pw* in capsules treated with oxalic acid (1 $\mu g/cm^2$) added with different concentrations of sucrose or glycerol (data corrected for the "natural mortality") and significance of the differences with respect to the capsules treated with oxalic acid alone.

Oxalic acid ($\mu g/cm^2$)	Sucrose ($\mu g/cm^2$)	Corrected mortality@48 h	*P*	Glycerol ($\mu g/cm^2$)	Corrected mortality@48 h	*P*
1	0	39.9		0	36.1	–
1	0.1	37.6	N.S.	0.1	48.0	N.S.
1	0.3	52.7	0.06	0.3	60.9	0.01
1	1	67.1	< 0.001	1	73.5	< 0.001
1	3	58.9	0.01			
1	10	30.2	N.S.			

Table V. Median lethal density (LDe_{50}), density expected to cause 95% mortality (LDe_{95}) at 48 h and their 95% fiducial intervals, on *V. destructor* females taken from *pw*, following treatment with oxalic acid alone or oxalic acid added with sucrose or glycerol. Data expressed as $\mu g/cm^2$ active substance.

Treatment	LDe_{50}	fid. limits	LDe_{95}	fid. limits
Oxalic acid alone (Tab. I)	1.49	1.20, 1.81	5.92	4.42, 9.10
Oxalic acid + 1 $\mu g/cm^2$ sucrose	1.17	0.84, 1.53	6.25	3.67, 22.23
Oxalic acid + 1 $\mu g/cm^2$ glycerol	0.60	0.35, 0.94	2.21	1.30, 9.57*

* A heterogeneity factor was used to compute fiducial limits.

Table VI. Percent mortality at 48 h of *V. destructor* females kept for one hour in capsules treated with oxalic acid (1 $\mu g/cm^2$) alone or added with sucrose or glycerol, at different relative humidities (data corrected for the "natural mortality"), and significance of the differences with respect to the capsules treated with oxalic acid alone.

Treatment	48 h			
	42% R.H.	*P*	75% R.H.	*P*
Oxalic acid (alone)	22.3	–	30.0	–
Oxalic acid + sucrose	25.2	N.S.	53.7	0.002
Oxalic acid + glycerol	68.5	$< 10^{-4}$	48.0	0.03

while it was represented by droplets of solution when glycerol was added.

The mortality of mites in capsules treated with oxalic acid added with sucrose or glycerol was significantly higher than that of oxalic acid alone at 75% R.H. In contrast, at 42% R.H. increased mortality was observed only with glycerol, while sucrose did not produce any appreciable effect (Tab. VI). The difference between the mortalities observed with glycerol at 42 and 75% R.H. was also significant ($P \approx 0.02$).

3.4. Measures of relative humidity

At 32.5 °C, the R.H. of air in equilibrium with a solution saturated with oxalic acid or citric acid was respectively 86% and 74%; with a solution saturated both in oxalic acid and sucrose it was 69%. Relative humidities at 22 °C differed by 1.5–2.5% (less than the measurement error). Glycerol absorbs water vapour from the air, irrespective from the R.H.; the higher the R.H., the more diluted the resulting solution (Landolt-Börnstein Tabellen, 1931).

The humidity in the hives was obviously influenced by the outside humidity and temperature. It varied mostly between 50 and 80% in the centre of the nest and exceeded 69% during 20–52% of time. It was less variable over the top bars of the frames, but during part of the time exceeded 69% also in this area.

4. DISCUSSION

The mortality in the controls was higher than in assays with paraffin wax coated capsules, developed for pyrethroid and organophosphorous acaricides (Milani, 1995; Milani and Della Vedova, 1996). The causes of the increase in natural mortality

were not identified. Although a direct comparison is not possible, no increase in the mortality in the controls was observed in routine tests with paraffin coated capsules (Milani, 1995) carried out at the same time.

The mortality in treated capsules indicates that oxalic acid and citric acid have a contact toxicity on *V. destructor*, without excluding other ways of action.

With oxalic acid, the bioassay based has made it possible to establish a "baseline" dose-mortality curve for contact toxicity. The ratio between the width of the 95% fiducial interval of the LDe_{50} and the LDe_{50} was comparable with that obtained with fluvalinate (Milani, 1995), but larger than that with coumaphos (Milani and Della Vedova, 1996). However, to achieve such a ratio, which is indicative of the "precision" of the assay, about twice as many mites were needed. This means that the response of the mites was more variable with oxalic acid than with pyrethroid and organophosphorous acaricides. Variability does not depend uniquely on the error on the amount of substance deposited, but might be caused also by the different sensitivity of different parts of the body of the mite; e.g., mites laying on their back on the treated surface often showed lesser signs of injury. Mites from different brood stages showed a different sensitivity to oxalic acid, as observed with organophosphorous acaricides (Milani and Della Vedova, 1996). The doses toxic to *V. destructor* tare comparable with those effective in field treatment by spraying (Imdorf et al., 1995).

The results obtained with citric acid were more variable, indicating that the method used is unsatisfactory for the purpose of comparing the susceptibility of different mite populations. At a given concentration, citric acid is less active than oxalic acid.

Sucrose and glycerol proved to be synergists of oxalic acid – at suitable concentrations – under laboratory conditions. The data on the relative humidity in equilibrium with saturated solutions of these substances explain the mechanism of this synergism: oxalic acid does not absorb water vapour from the atmosphere at R.H. below 86% but when mixed with sucrose it does at R.H. > 69%. Thus at 75% R.H. the deposit of oxalic acid and sucrose is constituted by highly concentrated droplets of a liquid, but not at 42% R.H.; the addition of glycerol has the same effect also at 42% R.H., since glycerol is always hygroscopic. Deposits constituted by droplets of liquid were more active than dry deposits. Effect of glycerol was higher at 42% than at 75%; the solution is more diluted at the higher R.H.

This mechanism explains also the large variability in response of the mites in assays with citric acid. Small variations in the humidity around 75%, the R.H. used in the assays, can cause the deposit to turn from dry to a solution, since the R.H. of air in equilibrium with saturated solutions of citric acid is around 74%.

In the autumn, the relative humidity in the hive exceeded 69% at least for part of the time. Thus the R.H. may be in the range needed to observe the effects described above, although caution is needed to extrapolate these data to other conditions and to different parts of the hive, in particular to the thin layer of air surrounding the bees or combs. These results indicate that the efficacy of a treatment with oxalic acid mixed with sucrose and its side effects on bees, although independent of temperature, might be influenced by relative humidity.

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Résumé – Activité des acides oxalique et citrique sur l'acarien *Varroa destructor* au cours de tests de laboratoire. Parmi les substances d'origine naturelle utilisées

contre l'acarien *Varroa destructor* Anderson and Trueman, l'acide oxalique (OA) présente le risque de résistance le plus élevé. La forte efficacité en absence de couvain, la facilité d'application par goutte à goutte, le prix extrêmement bas, l'indépendance vis-à-vis de la température et le manque d'alternatives peuvent rendre à l'avenir le traitement à l'OA courant et accroître ainsi la pression de sélection pour la résistance. Une courbe de tolérance de base pour des populations non traitées à l'AO serait utile pour faire des comparaisons et détecter d'éventuelles futures résistances.

Un test biologique a été mis au point pour mesurer la toxicité de l'acide oxalique et de l'acide citrique (AC) pour *V. destructor*. Les acariens provenaient de populations du Frioul (nord-est de l'Italie) qui n'avaient jamais été traitées avec ces acides organiques. Ils ont été prélevés sur du couvain operculé (jusqu'au stade de nymphes aux yeux noirs et corps pâle). Des solutions d'acides ont été vaporisées avec une tour de pulvérisateur de précision de Potter sur la surface interne de capsules, constituées de deux disques de verre (diamètre de 60 mm environ) et d'un tube d'acier inoxydable (hauteur 2 à 3 mm). On a laissé sécher les capsules, puis introduit dedans les acariens. Ils y sont restés quatre heures puis ont été transférés dans une boîte de petri de 60 mm de diamètre, où ils ont pu s'alimenter sur des larves d'abeilles. Les observations ont été faites 4 h, 24 h et 48 h après l'introduction. Le test a été effectué à 32,5 °C et 75 % d'humidité relative (H.R.). Les observations faites à 48 h – quand la proportion d'acariens blessés mais non morts était en général négligeable – ont donné les résultats les plus fiables.

Dans les tests à l'AO, des différences faibles mais significatives ont été observées entre les acariens issus de divers stades de couvain (Tab. III, Fig. 2). L'AC a eu une moindre action que l'AO, mais de fortes variations entre les répétitions ont réduit la précision du test (Tab. III).

Le saccharose et le glycérol n'étaient pas toxiques pour l'acarien *V. destructor*, mais leur ajout à l'AO a augmenté la mortalité (Tabs. IV, V). Avec le glycérol, l'effet a été observé pour une H.R. de 42 % et de 75 % ; avec le saccharose, seulement à l'H.R. la plus élevée (Tab. VI). Le glycérol maintenait le dépôt toujours hygroscopique alors qu'une solution saturée en AO et saccharose n'absorbait l'humidité qu'à la H.R. la plus élevée. Dans ces conditions il y avait formation de gouttelettes au sein de la solution vaporisée sur la capsule.

***Varroa destructor* / acide oxalique / acide citrique / toxicité / synergie / saccharose / glycérol**

Zusammenfassung – Wirkung von Oxal- und Zitronensäure auf die Milbe *Varroa destructor* in Labortests. Von den gegen *Varroa* eingesetzten natürlichen Substanzen ist bei der Oxalsäure das Risiko der Entwicklung einer Resistenz erhöht. Durch die hohe Wirksamkeit in Abwesenheit verdekkelter Brut, die leichte Anwendbarkeit der Träufelmethode, die äuβerst niedrigen Kosten, die Unabhängigkeit von der Temperatur sowie der Mangel an Alternativen könnte die Verwendung dieser Säure in der Zukunft weite Verbreitung finden und hierdurch einen hohen Selektionsdruck in Richtung einer Resistenz der Milben ausüben. Um eine zukünftige Resistenzentwicklung entdecken zu können, ist eine Ermittlung einer Grundlinie der Wirksamkeit auf Milben aus einer bislang nicht mit Oxalsäure behandelten Population von Nutzen.

Für die Messung der Toxizität von Oxal- und Zitronensäure für die Milbe *Varroa destructor* wurde ein Biotest entwickelt. *Varroa* Milben wurden in Friuli (Nordost Italien) aus Völkern gesammelt, die niemals mit organischen Säuren behandelt wurden. Die Milben für den Biotest stammten aus verdeckelten Brutzellen (bis zum Entwicklungsstadium von Puppen mit dunklen

Augen und weiβem Körper). Die Schalenhälften bestanden jeweils aus einer Glasscheibe (Durchmesser ca. 60 mm) und einem Ring aus rostfreiem Stahl (2–3 mm hoch, Abb. 1). Das Innere der Schalen wurde mit Säurelösungen in einem Potter Spray Tower besprüht und danach Zeit zum Trocknen gelassen. Die Milben wurden in diesen Schalen 4 Stunden lang gehalten und dann in eine saubere Petrischale überführt (Durchmesser ca. 60 mm), wo sie an Bienenlarven saugen konnten. Sie wurden 4, 24 und 48 Stunden nach der Überführung kontrolliert. Die Versuche wurden bei einer Temperatur von 32,5 °C und 75 % Luftfeuchtigkeit (RH) durchgeführt. Die Ergebnisse nach 48 Stunden waren am zuverlässigsten, da nach dieser Zeitspanne der Anteil an geschädigten, aber noch nicht toten Milben zu vernachlässigen war.

Bei den Versuchen mit Oxalsäure zeigten sich geringe, aber signifikante Unterschiede zwischen Milben verschiedener Brutstadien (Tab. III, Abb. 2). Zitronensäure hatte eine geringere Wirkung als Oxalsäure. Allerdings reduzierte die groβe Variation bei den Wiederholungen der Versuche die Präzision des Biotests (Tab. III).

Saccharose und Glycerol waren für die Milben nicht giftig, aber ihr Zusatz zur Oxalsäure erhöhte die Mortalität im Biotest (Tabs. IV, V). Bei Glycerol wurde der Effekt sowohl bei 42 % als auch bei 75 % R.H. beobachtet, bei Saccharose nur bei höherer Luftfeuchtigkeit (Tab. VI). Durch Glycerol war der Belag immer hygroskopisch, während eine gesättigte Lösung von Oxalsäure und Saccharose die Feuchtigkeit nur bei der höheren R.H. absorbierte. Dadurch entstand unter diesen Bedingungen eine Tropfenbildung des in die Schalen gesprühte Belags.

***Varroa destructor* / Biotest / Oxalsäure / Zitronensäure / Saccharose / Glycerol / Synergismus**

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