SUSCEPTIBILITY OF THE EGGS OF THE PEST SLUG
DEROCEAS RETICULATUM TO CONTACT WITH
METAL SALTS

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ABSTRACT

Laboratory research is reported in which eggs of the pest slug Deroceras reticulatum were incubated on
filter paper moistened with solutions of cupric sulphate, aluminium sulphate, ferric chloride and zinc
sulphate at different concentrations. After four or more days exposure, the median lethal doses (LD50)
were below 10 μg metal ion/cm² for the four metals tested. Copper showed the highest toxicity with LD50
values below 5 μg metal ion/cm².

INTRODUCTION

A number of terrestrial gastropod species are important pests in agricultural and horticultural
crops worldwide (Godan, 1983, 1999; Port & Port, 1986; South, 1992). Damage is caused by
gastropods due both to feeding and to contamination with faeces and slime, leading to deterioration
in the quality of the harvest and financial loss.

A variety of strategies have been developed or are being investigated for reducing gastropod
damage to crops, including culture methods (Glen, Milson & Wiltshie, 1990; Hammond, 1996; Speiser & Hochstrasser, 1998) and a bio-
control method using parasitic nematodes (Glen, Wilson, Hughes, Carpegg & Hajjar, 1996), but the
fight against pest gastropods relies mainly on chemical control, with metaldehyde and carbamates as the active substances most commonly used (Garthwaite & Thomas, 1996). The major use of these chemical molluscicides is as stomach-poisons via incorporation into baits (Henderson & Martin, 1992).

According to Mendis, Bowen, Liddell & Symondson (1996), who have developed monoclonal antibodies against the eggs of Deroceras reticulatum (Müller) and Arion ater L., as diagnostic tools for the detection of potential natural predators of mollusc eggs, among the many methods proposed for controlling gastropod pests in agriculture, the eggs of the animals have received very little attention. In particular, we have very few data on the effect of chemicals on the viability of terrestrial gastropods (Stringer & Morgan, 1969, 1970, 1972, cited by Godan, 1983) (Ryder & Bowen, 1977), although the use of contact-action molluscicides against the slugs has been investigated (Henderson, Briggs, Coward, Dawson & Pickett, 1989; Henderson & Martin, 1990; Davis, Van Schagen, Widmer & Craven, 1996).

Here we report laboratory research on the effect of simple salts of copper, aluminium, iron and zinc, metals of known molluscicidal activity (Henderson & Martin, 1990), on the viability of Deroceras reticulatum eggs. The study was based on a paper-contact toxicity test with continuous exposure of the eggs to the chemicals as a first step for the evaluation of the susceptibility of the eggs to contact with chemicals.

MATERIALS AND METHODS

The eggs

The structure of the egg and the embryonic development of Deroceras reticulatum were described in
detail by Carrick (1938), and histochemistry of the egg was described by Bayne (1966, 1968).
The eggs for testing were obtained from D. reticulatum which were collected from gardens of the South
Campus of the University of Santiago, Spain. The animals were kept in plastic boxes 25 × 25 × 15 cm
with perforated walls and lids, and with the floor covered with wet filter paper. Refuges were provided
by small pieces of black polyethylene tubes and food
consisted of lettuce, carrots, cabbage, runner beans, potatoes and mushrooms, supplemented with powdered calcium carbonate. The cages were placed in a climatic room at 17°C day/15°C night with a 12D:12L photoperiod, and were cleaned and the food replaced twice weekly. Breeding in *D. reticulatum* takes place whenever environmental conditions are suitable (Carrock, 1938; South 1989) and the cages were inspected for eggs every day. The slugs laid their eggs directly on the filter paper, mainly in those places covered by pieces of food. The eggs were collected, cleaned with distilled water and incubated on wet filter paper inside Petri dishes in the darkness at 18°C.

The entire course of the development of the embryo is observable when the egg is immersed in water and viewed under transmitted light (Carrock, 1938). About a week after collection, all the eggs were inspected under a binocular microscope for the selection of those to be used in the paper-contact toxicity tests. Only eggs containing a single living embryo and without foreign inclusions were selected for the tests, and they were used when the embryo achieved the developmental stage IV according to Carrock (1938), which is recognisable by the differentiation of rudiments of the tentacles and posterior sac. The movement of the embryo in the form of a slow and continuous rotation, and the rhythmic contractions and expansions of the still-small posterior sac were the criteria used to determine whether the embryo was alive. Non-motile embryos were considered to be dead.

**Experimental methods**

The chemicals tested were cupric sulphate (pentahydrate), aluminium sulphate (hexadecahydrate), ferric chloride (anhydrinous) and zinc sulphate (heptahydrate). In the paper-contact toxicity tests the eggs were continuously exposed to the chemicals and mortality was assessed each day. The tests were made in glass Petri dishes 10 cm in diameter with the bottom lined with a piece of filter paper 9 cm in diameter. Solutions of the chemicals were prepared in distilled water to give a range of concentrations between 1 mg/cm² and 0.0001 mg/cm² when 1 ml of solution was evenly added to the filter paper. The papers were dried for two hours under a slow stream of air and moistened again with 1 ml of distilled water immediately before the start of the test.

All tests consisted of a control and five doses of the chemical, with four replicates each. Preliminary range-finding tests were made with 5 eggs/dish and the doses of chemicals arranged in a 10-fold geometric series, and definitive tests were made with 10 eggs/dish and a two-fold geometric series of doses. All the eggs were inspected under a binocular microscope every 24 hours to assess whether the embryos were still alive. To avoid immersion of the eggs in water to view them under the microscope, which would produce an undesirable wetting of the eggs, the test-eggs were introduced into a glass tube, 3 mm inner diameter, in this way through the contact zone between the egg and the glass is possible to see the embryo inside the egg producing the same effect as immersion in water.

For each exposure time the data from replicates were pooled and the values of the median lethal doses (LD₅₀), i.e. the calculated doses which produce 50% mortality of the eggs, and 95% confidence limits, were calculated by probit analysis using the SPSS package.

All tests with these chemicals is attributed to the metal mobility (Henderson et al. 1989; Bullock, Coward, Dawson, Henderson, Larkworthy, Martin & McGrath, 1992) the values of the median lethal doses are expressed in terms of concentration of the metal. The LD₅₀ values were considered significantly different when the 95% confidence limits did not overlap (Fisher, Dabrowska, Waller, Bullock, Jackson & Zhang, 1994).

**RESULTS**

Mortality was never observed in the control eggs, which hatched between day 12 and day 18 of the tests. The four salts tested killed the eggs at different doses and after different periods of exposure. The mortality caused by each dose of the different chemicals is given in Table 1. The

<table>
<thead>
<tr>
<th>Dose (mg/cm²)</th>
<th>Cupric sulphate</th>
<th>Ferric chloride</th>
<th>Zinc sulphate</th>
<th>Aluminium sulphate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.000</td>
<td>100 / (1)</td>
<td>100 / (1)</td>
<td>100 / (1)</td>
<td>100 / (1)</td>
</tr>
<tr>
<td>0.160</td>
<td>100 / (1)</td>
<td>100 / (1)</td>
<td>100 / (2)</td>
<td>100 / (3)</td>
</tr>
<tr>
<td>0.080</td>
<td>100 / (1)</td>
<td>100 / (1)</td>
<td>100 / (3)</td>
<td>100 / (7)</td>
</tr>
<tr>
<td>0.040</td>
<td>100 / (3)</td>
<td>100 / (4)</td>
<td>100 / (7)</td>
<td>75 / (23)</td>
</tr>
<tr>
<td>0.020</td>
<td>100 / (15)</td>
<td>100 / (10)</td>
<td>100 / (13)</td>
<td>15 / (20)</td>
</tr>
<tr>
<td>0.016</td>
<td>100 / (9)</td>
<td>100 / (12)</td>
<td>N.T.</td>
<td>N.T.</td>
</tr>
<tr>
<td>0.010</td>
<td>100 / (15)</td>
<td>45 / (16)</td>
<td>30 / (14)</td>
<td>0</td>
</tr>
<tr>
<td>0.008</td>
<td>100 / (18)</td>
<td>37.5 / (21)</td>
<td>N.T.</td>
<td>N.T.</td>
</tr>
<tr>
<td>0.004</td>
<td>0</td>
<td>0</td>
<td>N.T.</td>
<td>N.T.</td>
</tr>
</tbody>
</table>

Table 1. Percentage mortality and exposure periods for the different doses and compounds tested. Results shown in the table correspond to different tests since each individual test consisted of five doses of the chemical and a control. N.T. = not tested.
relationship between the doses and the exposure periods for which 100% mortality was obtained with the different chemicals is shown in Figure 1. Both cupric sulphate and ferric chloride killed all the eggs at 0.08 mg/cm² after 24 hours exposure. For lower doses, cupric sulphate was the compound which required less time to kill the eggs and also cupric sulphate was the one which caused 100% mortality at the lowest dose (0.008 mg/cm², 18 days exposure). The eggs exposed to the next lower dose of cupric sulphate (0.004 mg/cm²) all hatched successfully although with a delay of a week with respect to the controls.

For ferric chloride the lowest dose which killed all the eggs was 0.016 mg/cm² after 12 days exposure. The doses 0.01 and 0.008 mg/cm² of ferric chloride caused 45% mortality after 16 days exposure and 37.5% mortality after 21 days exposure respectively; in both cases the rest of the eggs hatched successfully with a delay of 5 days with respect to controls.

Zinc sulphate at 0.02 gm/cm² killed all the eggs after 13 days exposure, but the dose 0.01 mg/cm² caused only 30% mortality.

Aluminium sulphate was the least toxic of the compounds tested. The lowest dose which caused 100% mortality was 0.08 mg/cm² after 7 days exposure. The doses 0.04 and 0.02 mg/cm² produced respectively 75% and 15% mortality; in both cases the rest of the eggs hatched successfully with a delay of 5 days with respect to controls.

In terms of concentration of the metallic ion (Table 2), copper was the metal which showed the highest toxicity, with values of LD₅₀ ranging from 11.19 µg/cm² after 24 hours exposure to 1.53 µg/cm² after 18 days exposure. Significant differences existed between copper and all others metals with respect to the values of LD₅₀ calculated for 1, 2, 4, 7 and 10 days of exposure. The values of LD₅₀ for aluminium were between 23.79 µg/cm² after one day exposure and 4.66 µg/cm² after 7 days exposure, and this metal occupied the second place in the ranking of toxicity for exposure periods between 2 and 7 days. For exposure periods of two or more days, iron and zinc showed almost identical values of LD₅₀ in the order of 13.5 µg/cm² after two days exposure and 4.0 µg/cm² after ten days exposure. 1-day LD₅₀ were not significantly different either between iron and aluminium or between zinc and aluminium. Iron and zinc were significantly different for the 1-day LD₅₀, but not for longer periods of exposure.

The median lethal doses were below 10 µg/cm² for the four metals tested after four or more days exposure.
Table 2. Median lethal doses (LD<sub>50</sub>) of the compounds tested, calculated for different periods of exposure. LD<sub>50</sub> values are reported in μg metal ion/cm<sup>2</sup>. For a given period of exposure, LD<sub>50</sub> values followed by the same letter are not significantly different from each other. 95% confidence limits are shown in parenthesis.

<table>
<thead>
<tr>
<th>Exposure (days)</th>
<th>Cupric sulphate</th>
<th>Ferric chloride</th>
<th>Zinc sulphate</th>
<th>Aluminium sulphate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30.81&lt;sup&gt;c&lt;/sup&gt;</td>
<td>23.79&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>6.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.37&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.73&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.51&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>4.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.89&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.51&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>(4.07–4.69)</td>
<td>(8.06–9.69)</td>
<td>(8.06–10.14)</td>
<td>(5.0–6.02)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>3.23&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.82&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.66&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>10</td>
<td>2.37&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.0&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>(2.22–2.52)</td>
<td>(3.77–4.31)</td>
<td>(3.52–4.48)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>1.53</td>
<td></td>
<td></td>
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<tr>
<td>(1.43–1.83)</td>
<td></td>
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</tbody>
</table>

DISCUSSION

As stated by Bowen & Mendis (1995) the development of an integrated pest management programme for slug control needs to explore innovative aspects of both biological and chemical control, and in particular there is a need for more effective molluscicide formulations containing lower concentrations of toxic ingredients. Application of molluscicides in the form of baits should be only considered as a short-term crop-protection measure since field populations of Deroceras reticulatum can recover rapidly following bait applications (Glen, Wiltshire & Milson, 1984). On the other hand, sub-lethal poisoning with relatively high recovery rates has been recorded for both metaldehyde and methiocarb (Kemp & Newell, 1985; Wedgwood & Bailey, 1986; Briggs & Henderson, 1987; Bourn, Jones & Bowen, 1988), and Kemp & Newell (1989) found that proprietary molluscicides based on these substances have no effect on the fecundity of sub-lethally poisoned D. reticulatum. Therefore, the slugs can lay their eggs on the soil even when the population is exposed to poisoned baits and the eggs can persist on the soil even if the population of slugs is completely killed. Egg mortality under natural conditions was recorded by South (1989) to be as low as about 10%, and persistence of the eggs on the soil before hatching was recorded to be from five or six months (South, 1989) to nine to ten months (Dmitrieva, 1969, cited by South, 1992). This results in the populations of slugs being 'potentially' present on the soil almost all the time.

Many metals are known to be toxic to slugs and snails and several inorganic metal compounds in the form of dusts or sprays have been recommended as contact poisons for the control of pest gastropods (South, 1992). For example copper sulphate is referred to by Gordon (1983) as the classical molluscicide since copper salts have been used extensively in the control of pest molluscs in the past. Metal-containing molluscicides like Ferlosan (aluminium sulphate) and Nobble (aluminium sulphate, copper sulphate and borax) have been marketed as wettable powders for the control of slugs in gardens and crops, but with limited success (Glen & Orsman, 1988; Glen, Milson & Wiltshire, 1986).

Ryder & Bowen (1977b) demonstrated that copper can be absorbed through the epithelium of the foot of slugs crawling on filter paper saturated with an aqueous copper sulphate solution, and also that copper can be taken up by the slug egg and initially retained in the perivitelline membrane (Ryder & Bowen, 1977a).

Henderson et al. (1989) investigated the toxicity of ten simple metal salts to D. reticulatum confining slugs on dry glass plates coated with the chemicals and showed that copper, zinc, iron and aluminium salts all killed slugs by contact. They found that simple metal salts are ineffective field molluscicides because they are rapidly inactivated on contact with soil by adsorption and hydrolysis, but inactivation is delayed when metals are applied as organic complexes (metal chelates). Henderson & Martin (1990) showed that a broadcast application of the chelate iron(III) 2,4-pentanedionate
applied at a rate of 40 kg a.i. ha\(^{-1}\) was effective in killing slugs by contact action.

Bullock et al. (1992) studied the rate of uptake and toxicity of some common metals in different chemical forms using the slug *D. reticulatum* and concluded that control of terrestrial gastropods by contact molluscicides acquired passively by crawling animals is potentially more efficient than the bait-delivery method commonly used. The results shown here demonstrate that the eggs of *D. reticulatum* are highly susceptible to contact with chemicals and therefore the contact-action strategy against pest slugs has potential not only against the animals, but also their eggs.

ACKNOWLEDGEMENTS

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REFERENCES


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