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**Original article**

# Assessing delayed and acute toxicity of five formulated fungicides to *Osmia lignaria* Say and *Apis mellifera*[1]

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**Abstract** – The delayed and acute toxicity of benomyl (Benlate®), captan (Captan 50WP), iprodione (Rovral®), propiconazole (Orbit™), and neem oil (Trilogy®) to two crop pollinators, *A. mellifera* and *O. lignaria*, was evaluated. Survival after contact and oral single exposure to high doses of the pesticides was compared to survival of controls with the dosing vehicle. $LD_{50}$ values at 24, 48 and 72 h from exposure were determined. Contact and oral exposure to benomyl and iprodione did not affect survival of any of the two species. Contact exposure to neem oil affected survival of *A. mellifera*. Orally administered propiconazole showed delayed and acute toxicity to both species. Captan severely limited survival of *O. lignaria*. The tested fungicides seemed to be safe to both bee species at the recommended rates, with the exception of captan to *O. lignaria*. To our knowledge, this is the first complete contact and oral toxicity test on an *Osmia* species.

***Osmia lignaria* / *Apis mellifera* / toxicity / fungicide / $LD_{50}$**

## 1. INTRODUCTION

Many insecticides are hazardous to bees and special restrictions or recommendations, which differ among countries, limit their use on crops during bloom. With few exceptions, other pesticides (herbicides, plant growth regulators and fungicides) are considered relatively safe to bees (Atkins et al., 1981; Fell et al., 1983; Johansen et al., 1983; Mayer and Lunden, 1986; Johansen and Mayer, 1990; Bohmont, 2001; Devillers, 2002) and their use during bloom is not restricted.

In 2001–2002, we conducted pollination studies in commercial cherry orchards (Central Valley, California) with populations of the solitary bee, *Osmia lignaria* Say (Hymenoptera, Megachilidae), that has been developed as an orchard pollinator in the U.S. (Torchio, 1985; Bosch and Kemp, 2001). Females of this species will nest in artificial nesting cavities that they provision with pollen and nectar and seal with a mud plug. During these studies, we made several observations that indicated possible side effects of "fungicide sprays" on *O. lignaria* females. The sprays consisted of tank mixtures of the fungicides benomyl, captan, iprodione or propiconazole with surfactants/stickers and foliar fertilizers. The morning after the sprays, homing, nesting and foraging behaviour of well-established females was affected. The intensity of the observed side

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effects varied according to the kind of mixtures and the time of application.

Bee losses after fungicide treatments have been suggested for the honey bee, *Apis mellifera* L. (Hymenoptera, Apidae) (Brasse, 2001; Oomen, 2001; Fletcher and Barnett, 2003; Rivera et al., 2003). In this species, some fungicides have been reported to increase larval mortality and to cause malformations in adults when exposed as larvae (Atkins and Kellum, 1986; Mussen, 2003; Thompson, 2003). Recently, concern has increased over the synergistic effects of ergosterol biosynthesis inhibiting (EBI) fungicides and pyrethroid insecticides (Pilling and Jepson, 1993; Meled et al., 1998; Belzunces et al., 2001; Thompson and Wilkins, 2003). Several fungicides have been shown to be repellent to *A. mellifera* workers when dissolved in sucrose solution (Solomon and Hooker, 1989).

In both Europe and the USA, the evaluation of side effects on non-target organisms is required for the registration of plant protection products. For pollinating insects, official guidelines recommend a series of laboratory, semi-field and field studies on *A. mellifera* (US EPA, 1996a, b; OECD, 1998a, b; OEPP/EPPO, 1992, 1993, 2001a, b). Notwithstanding the extreme value of tests on *A. mellifera*, a plant protection product non-toxic to *A. mellifera* may be hazardous to other bee species, due to biological, behavioural and ecological differences (Torchio, 1973; Johansen et al., 1983; Thompson, 2001; Tasei, 2002).

Given the increasing importance of *Osmia* spp. as commercial orchard pollinators (Maeta and Kitamura, 1974; Torchio, 1985; Sekita and Yamada, 1993; Bosch, 1994; Bosch and Kemp, 2002; Ladurner et al., 2003a; Maccagnani et al., 2003) and the possibility that effects similar to those observed during our California pollination study occur in other bee species, we initiated studies to evaluate the potential side effects of fungicides on *O. lignaria* and *A. mellifera.* We wanted to determine whether five formulated fungicides that are commonly applied during bloom showed delayed and/or acute toxicity to *A. mellifera* and *O. lignaria*. We conducted laboratory assays where the two species were exposed to variable doses by contact and oral applications.

## 2. MATERIALS AND METHODS

### 2.1. Bees

In May 2002, wintering *O. lignaria* females, reared at the Bee Biology and Systematics Laboratory, Logan, Utah, were incubated at 25 °C until emergence from their cocoons. Unfed females were then transferred to a screened flight cage ($40 \times 30 \times 30$ cm) to allow them to deposit meconium. Females were starved overnight and then exposed to a specific fungicide treatment the next morning, approximately 24 h after emergence.

In June 2002, *A. mellifera* foragers of different ages from a healthy, queen-right colony were captured in a clear plastic jar as they left the hive in the morning, and brought to the laboratory. Bees were then chilled for a maximum of 30 minutes at 4 °C prior to exposure to fungicide treatments.

### 2.2. Fungicides

We investigated the contact and oral toxicity of five formulated fungicides that are commonly applied to almond and/or cherry during bloom. The fungicides were:

- Neem oil (Trilogy® from Certis USA; a.i. 70% clarified hydrophobic extract of Neem (*Melia azadirach*) oil; 654 g active ingredient (a.i.) /L) has both fungicide and insecticide properties. The neem-based formulated product Trilogy® is recommended as a fungicide and miticide on orchards and other crops. The product is labelled as toxic to bees exposed to direct treatment and should not be applied while bees are actively visiting the treatment area.
- Iprodione (Rovral® from Rhône-Poulenc; a.i. 50% iprodione; 500 g a.i./kg) is a dicarboximide fungicide, registered for application during full bloom of stone fruits and almonds.
- Propiconazole (Orbit™ from Novartis; a.i. 41.8% propiconazole; 431.5 g a.i./L) is a broad spectrum EBI fungicide that controls fungal diseases in stone fruits. It can be applied in full bloom.
- Benomyl (Benlate® from DuPont; a.i. 50% benomyl; 500 g a.i./kg), a systemic benzimidazole fungicide recommended for the control of many important plant diseases, was introduced in 1968 and first used in the U.S. in 1969. The product was withdrawn from sale in the U.S. at the end of 2001.
- Captan (Captan 50WP from Helena Chemical Company; a.i. 48.9% captan; 489 g a.i./kg) is a dicarboximide fungicide used for the control of fungus diseases of fruit and ornamental crops, and for soil treatment for the control of certain seed rots and damping-off diseases.

**Table I.** Fungicide doses administered to *A. mellifera* and *O. lignaria* in contact and oral toxicity tests, and highest recommended field rates of the five fungicides (see Tab. II). The solutions used were at the highest concentration that could be dissolved in the solvent solutions.

| | Contact administration | Oral administration | Highest recommended field rate |
|---|---|---|---|
| Fungicide | µg a.i./bee | µg a.i./bee | µg a.i./ha |
| Neem oil | 196.4 | 196.4 | 12230 |
| Iprodione | 125.0 | 125.0 | 1120 |
| Propiconazole | 104.0 | 65.0 | 125 |
| Benomyl | 125.0 | 125.0 | 1121 |
| Captan | 122.5 | 122.5 | 2192 |

The highest recommended field rates of the fungicides are reported in Table I.

## 2.3. Toxicity tests

In contact toxicity tests, bees were cooled at 4 °C (for a maximum of 30 minutes) until they stopped moving. One µL of test solution was then applied to the dorsal surface of the thorax with a 50 µL-micro-syringe equipped with a repeating dispenser (Hamilton®). The test solution was prepared by dissolving each fungicide in acetone and purified distilled water (50% v/v) to obtain desired fungicide concentrations. Fresh test solutions were used for all tests.

In oral toxicity tests, known amounts of the fungicides were dissolved in a feeding solution (25% v/v sucrose in purified distilled water) to obtain the desired fungicide concentrations. *Osmia lignaria* females and *A. mellifera* workers were individually fed 10 µL of test solution using the flower method devised by Ladurner et al. (2003b). The test solution was pipetted into a plastic ampoule, inserted into the calyx of a flower (cherry, *Prunus avium* L., for *O. lignaria*; morning glory, *Convolvulus arvensis* L., for *A. mellifera*). Flowers and bees were individually housed in holding cages, made of waxed cardboard ice cream cups (8 cm diameter, 5 cm height; Sweetheart Cup Company Inc., Chicago, IL) covered with a plastic Petri dish lid with a wire mesh screen (7 cm diameter, 2 × 1 mm mesh size) to provide aeration. Flowers and bees in holding cages were kept in an incubator (22 °C for *O. lignaria*; 25 °C for *A. mellifera*) under artificial light (two 15W Cool White Sylvania ® fluorescent tubes placed 15 cm above the holding cages) for 1 h.

The feeding solution (25% v/v sucrose in purified distilled water) was the control with dosing vehicle in oral toxicity tests, and 50% v/v acetone in purified distilled water was the control in contact toxicity tests. The reference insecticide, dimethoate (Dimethoate 267 from FMC Corporation; a.i. 30.5% dimethoate; 320 g a.i./L; highest recommended field rate: 561 g a.i./ha), was used as toxic standard (OECD, 1998a, b; OEPP/EPPO, 1992, 2001a).

### *2.3.1. Delayed toxicity*

We define delayed toxicity as the negative side effects occurring after a period of > 72 h from the application (administration) of a single dose of test substance. To determine whether the products had delayed toxicity effects on the two bee species, three sets of 10 bees of each species were first exposed topically or orally to single high doses of the products. The administered doses of the different fungicides are reported in Table I. We used the highest concentration that could be dissolved/dispersed in our solvent solutions. After single exposure to high doses, each set of 10 bees was transferred to a holding cage (waxed cardboard ice cream cups as those described above), provided with an artificial feeder. The artificial feeder consisted of a 5 mL-LDPE sample vial (Nalge Nunc International), containing a sucrose solution (25% v/v sucrose in water), with a soaked cigarette filter inserted through the lid of the vial. Fresh sucrose solution was provided every 24 h. Holding cages for *A. mellifera* also were provided with a piece of wax foundation comb (4.5 × 6.5 cm) from Cache Valley Honey Company, Logan, UT. Holding cages with bees were kept in an incubator (t = 22 °C, R.H. = 60–80%, L:D = 12:12 h for *O. lignaria*; t = 25 °C, R.H. = 60–80%, L:D = 0:24 h for *A. mellifera*). A lower temperature was used for *O. lignaria*, because this spring-flying bee is active at lower temperatures (Bosch and Kemp, 2001). To establish whether the fungicides had delayed toxicity effects at high doses, mortality counts were recorded every 24 h for 7 days.

### *2.3.2. Acute toxicity*

The U. S. Environmental Protection Agency (1997) defines acute toxicity as "the ability of a substance to cause severe biological harm or death soon

**Table II.** Comparison of survival in treated and control *O. lignaria* and *A. mellifera* after contact and oral administration of single high doses (see Tab. I) of the five fungicides (Wilcoxon Test: df =1).

| | *O. lignaria* | | | | *A. mellifera* | | | |
|---|---|---|---|---|---|---|---|---|
| | Contact administration* | | Oral administration | | Contact administration | | Oral administration | |
| Product | $\chi^2$ | *P* | $\chi^2$ | *P* | $\chi^2$ | *P* | $\chi^2$ | *P* |
| Neem oil | / | / | 2.0339 | 0.1538 | 10.6990 | 0.0011 | 0.1513 | 0.6973 |
| Iprodione | / | / | 1.0000 | 0.3173 | 1.6973 | 0.1926 | 1.4011 | 0.2365 |
| Propiconazole | 1.0000 | 0.3173 | 53.4435 | <0.0001 | 1.2791 | 0.2581 | 29.8306 | <0.0001 |
| Benomyl | / | / | 1.0000 | 0.3173 | 0.3240 | 0.5692 | 3.3600 | 0.0668 |
| Captan | 24.9622 | <0.0001 | 52.8843 | <0.0001 | 0.1145 | 0.7351 | 2.3500 | 0.1253 |

* Survival of *O. lignaria* after contact administration of neem oil, iprodione, benomyl and in the control with the dosing vehicle was 100%.

after a single exposure or, also, any poisonous effect resulting from a single short-term exposure to a toxic substance or dose". A more detailed definition, in relation to honey bees as test organisms, is given by the OECD guidelines No. 213 and 214 (OECD, 1998a, b) which states: "acute contact (oral) toxicity is the adverse effects occurring within a maximum period of 96 h of an application (administration) of a single dose of test substance".

If a product at high doses showed acute toxicity within 72 h from exposure compared to the control with the dosing vehicle, then the contact and oral $LD_{50}$ values (median dose, expressed in µg of a.i. per bee that causes 50% mortality in the species tested) 24, 48, and 72 h after exposure were determined. We tested 5 doses of each product: two between the presumed $LD_{100}$ and $LD_{50}$, one at the presumed $LD_{50}$ and two between the presumed $LD_{50}$ and $LD_0$. For contact exposure of *O. lignaria* to dimethoate, only 4 doses were tested.

### 2.4. Statistical analysis

Survival of *O. lignaria* and *A. mellifera* after single exposure to high doses (highest possible concentration that could be dissolved in our solvent solutions) of the different fungicides was compared to that of *O. lignaria* and *A. mellifera* treated with the dosing vehicle, using survival analysis (LIFE TEST Procedure; SAS Institute Inc., 1989; Allison, 1999).

For both bee species and those products that showed acute toxicity effects at high doses, the $LD_{50}$ values at 24, 48 and 72 h from exposure with 95% confidence limits were determined using Probit analysis (PROBIT procedure; SAS Institute Inc., 1989). For *O. lignaria*, $LD_{50}$ values, with 95% confidence limits, were determined also at 7 days after contact and oral exposure to captan.

For *A. mellifera*, mortality data were corrected for natural mortality using Abbott's formula (Abbott, 1925). No correction of mortality data was necessary for *O. lignaria*, because mortality in the control was 0%. Because the two bee species were tested under different temperatures and kept under different conditions, we analysed the data from each species separately.

## 3. RESULTS

In oral exposure trials, 97.7% of the *O. lignaria* females (n = 871) and 88.2% of the *A. mellifera* workers (n = 755) tested, consumed all the test solution within one hour.

### 3.1. Delayed toxicity

Oral administration of single high doses of propiconazole significantly reduced survival of both *O. lignaria* and *A. mellifera* (Tab. II; Figs. 2, 4). Irrespective of the mode of application, high doses of captan did not affect *A. mellifera*, but significantly reduced survival of *O. lignaria* (Tab. II; Figs. 1, 2). Contact exposure to single high doses of neem oil affected survival of *A. mellifera*, but not of *O. lignaria* (Tab. II; Figs. 1, 3). However, neem oil was not acutely toxic to *A. mellifera* within the first three days from exposure, and it therefore was excluded from further investigations. For both bee species, survival rates after oral and contact exposure to single high doses of benomyl and iprodione were comparable to those in the control with the dosing vehicle (Tab. II; Figs. 1–4).

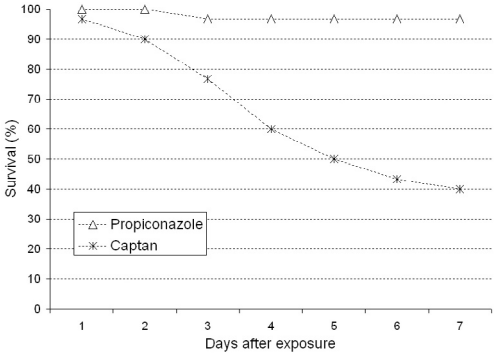


**Figure 1.** Survival rates (%) for *O. lignaria* after contact application of single high doses of propiconazole (104.0 µg a.i./bee) and captan (122.5 µg a.i./bee). Survival after contact application of single high doses of neem oil (196.4 µg a.i./bee), iprodione (125.0 µg a.i./bee), benomyl (125.0 µg a.i./bee) and the control with the dosing vehicle (50% v/v acetone in water) was 100%.

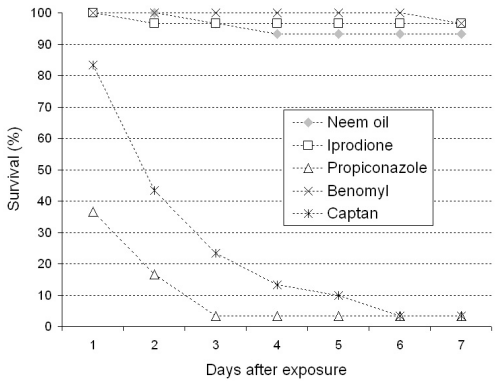


**Figure 2.** Survival rates (%) for *O. lignaria* after oral administration of single high doses of neem oil (196.4 µg a.i./bee), iprodione (125.0 µg a.i./bee), propiconazole (65.0 µg a.i./bee), benomyl (125.0 µg a.i./bee), and captan (122.5 µg a.i./bee). Survival in the control with the dosing vehicle (25% v/v sucrose in water) was 100%.

### 3.2. Acute toxicity

The contact and oral $LD_{50}$ values with 95% confidence limits for *O. lignaria* and *A. mellifera* at 24, 48 and 72 h after exposure to the different products are reported in Tables III and IV, respectively. As expected, dimethoate was toxic to both species.

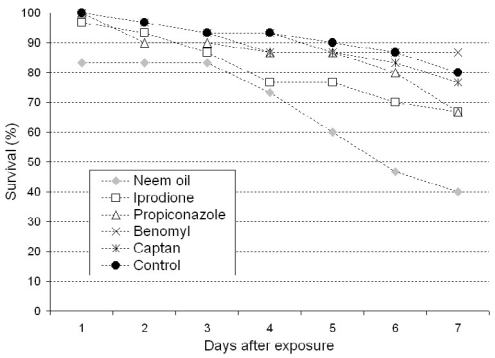


**Figure 3.** Survival rates (%) for *A. mellifera* after contact application of single high doses of neem oil (196.4 µg a.i./bee), iprodione (125.0 µg a.i./bee), propiconazole (104.0 µg a.i./bee), benomyl (125.0 µg a.i./bee), captan (122.5 µg a.i./bee), and the control with the dosing vehicle (50% v/v acetone in water).

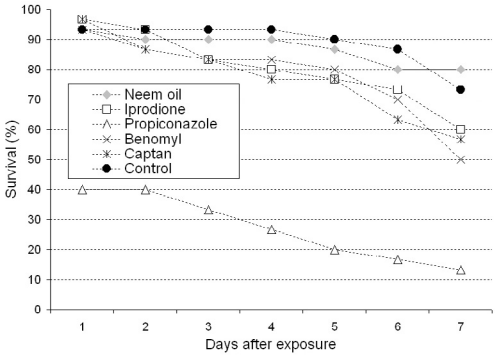


**Figure 4.** Survival rates (%) for *A. mellifera* after oral administration of single high doses of neem oil (196.4 µg a.i./bee), iprodione (125.0 µg a.i./bee), propiconazole (65.0 µg a.i./bee), benomyl (125.0 µg a.i./bee), captan (122.5 µg a.i./bee), and the control with the dosing vehicle (25% v/v sucrose in water).

Propiconazole showed acute oral toxicity to *O. lignaria* and *A. mellifera*: at 72 h, the oral $LD_{50}$ reached 33.30 and 57.25 µg a.i./bee for *O. lignaria* and *A. mellifera*, respectively.

For *O. lignaria* exposed to captan, contact $LD_{50}$ values at 24 and 48 h and oral $LD_{50}$ at 24 h could not be determined, because the dose-response relations at the tested doses were not significant ($LD_{50}$ values > 122.5 µg a.i./bee; Tab. III). Higher doses of captan could not be

**Table III.** Toxicity of three products to *Osmia lignaria*: contact and oral $LD_{50}$ values with 95% confidence limits at 24, 48 and 72 h after exposure to different products.

| | 24 h | | 48 h | | 72 h | |
|---|---|---|---|---|---|---|
| Product – Administration | $LD_{50}$ (µg a.i./bee) | 95% confidence limits | $LD_{50}$ (µg a.i./bee) | 95% confidence limits | $LD_{50}$ (µg a.i./bee) | 95% confidence limits |
| Captan – Contact | / | / | / | / | 269.68 | 151.32–2841.84 |
| Dimethoate – Contact | 1.96 | 1.38–16.73 | 1.21 | 1.05–1.57 | 1.02 | 0.92–1.18 |
| Propiconazole – Oral | 67.81 | 52.36–112.44 | 40.04 | 31.91–52.20 | 33.30 | 22.42–48.30 |
| Captan – Oral | / | / | 100.45 | 63.75–245.23 | 47.26 | 32.75–77.44 |
| Dimethoate – Oral | 0.27 | 0.21–0.34 | 0.26 | 0.22–0.30 | 0.25 | 0.21–0.30 |

**Table IV.** Toxicity of two products to *Apis mellifera*: contact and oral $LD_{50}$ values with 95% confidence limits at 24, 48 and 72 h after exposure to different products.

| | 24 h | | 48 h | | 72 h | |
|---|---|---|---|---|---|---|
| Product – Administration | $LD_{50}$ (µg a.i./bee) | 95% confidence limits | $LD_{50}$ (µg a.i./bee) | 95% confidence limits | $LD_{50}$ (µg a.i./bee) | 95% confidence limits |
| Dimethoate – Contact | 0.19 | 0.16–0.23 | 0.16 | 0.14–0.18 | 0.16 | 0.13–0.19 |
| Propiconazole – Oral | 61.67 | 40.69–446.44 | 59.43 | 46.65–94.73 | 57.25 | 48.51–81.59 |
| Dimethoate – Oral | 0.15 | 0.13–0.17 | 0.13 | 0.11–0.15 | 0.11 | 0.08–0.15 |

tested, because additional formulated material could not be dissolved in the solvent solutions. At 72 hours from exposure contact and oral $LD_{50}$ values were 269.69 and 47.26 µg a.i./bee, respectively. However, 7 days after administration, contact and oral $LD_{50}$ values reached 95.26 (95% confidence limits: 79.83 to 134.59) and 10.87 (95% confidence limits: 5.40 to 19.28) µg a.i/bee.

## 4. DISCUSSION

Studies on oral toxicity of pesticides to non-*Apis* species are limited, due in part to the previous lack of effective methods to feed known amounts of pesticides to bee species that do not perform trophallaxis (reviewed in Tasei, 2002; Ladurner et al., 2003b). To our knowledge, this is the first complete acute oral and contact toxicity test on an *Osmia* species.

Contact and oral administration of high doses of benomyl alone did not affect survival of either *O. lignaria* or *A. mellifera*, which is in accord with Atkins and Kellum (1986). However, as mentioned in the introduction, nesting *O. lignaria* females interrupted nesting activities and disappeared from blooming cherry orchards in California following early-morning sprays that included benomyl. Although benomyl alone does not appear to be toxic to *O. lignaria* and *A. mellifera*, further studies on the side effects of this fungicide in combination with recommended adjuvants, fertilizers and other fungicides are warranted.

Contact and oral exposure to iprodione alone was not toxic to the two bee species tested, even when administered at high doses. Mussen (2003) reported death in honey bee brood fed larval diet contaminated with this fungicide. Rivera et al. (2003) found iprodione in corbicular and stored pollen, bees, larvae, and in brood food of *A. mellifera* colonies placed in almond orchards for pollination. Iprodione alone may therefore not affect pollinating activity of *A. mellifera* foragers, but it may reduce colony strength due to brood losses. To our knowledge, the effects of iprodione on

brood of *O. lignaria* have not yet been investigated. We therefore agree with Mussen (2003) who recommends that the use of this fungicide while bees are actively visiting the treatment area be avoided until the completion of further studies to determine the potential effects on brood of both bee species. During our California pollination study, female *O. lignaria* nesting and pollinating activity was interrupted for several days following an evening spray of a mixture of iprodione, surfactants, and fertilizers. The temporary disruption of pollinator function can result in reduced fruit-set and yield (Nyéki and Soltész, 1996; Sanzol and Herrero, 2001), which warrants further investigation on the side effects of field applications of iprodione in combination with the typical adjuvants and fertilizers.

The neem-based fungicide, although labelled as "toxic to bees exposed to direct treatment", did not affect *O. lignaria*. To consider neem oil a product of risk for *A. mellifera*, the contact and/or oral Hazard Quotients should exceed 50, which means that the $LD_{50}$ should be lower than 244.6 µg a.i./bee. Contact and oral Hazard Quotients are defined as the ratio between the highest recommended field rate (g a.i./ha), and the contact and oral $LD_{50}$ values (µg a.i./bee). If the Hazard Quotient is below a threshold of 50, the product is considered harmless to bees, but further data and/or restrictions are required if the Hazard Quotient exceeds this threshold (Cluzeau, 2002; OEPP/EPPO, 1993, 2001b). At the highest dose tested (196 µg a.i/bee), neem oil did not show acute toxicity effects on *A. mellifera*, but it had a delayed effect on survival after contact exposure. Neem oil contains many potentially bioactive constituents that vary among crude extracts (Schmutterer, 1995). Several neem-based insecticides were investigated for their anti-feeding effects on adult worker honey bees and for toxicity to *A. mellifera* workers and larvae (Naumann et al., 1994; Naumann and Isman, 1996; Melathopoulos et al., 2000; Peng et al., 2000), and applications were considered relatively safe to honey bee workers and larvae. Therefore, if direct exposure of bees is avoided, neem oil would be compatible with bees during the pollinating season. Nevertheless, possible effects of neem oil on *O. lignaria* larvae should be investigated.

Propiconazole alone had no delayed toxicity effects on *O. lignaria* or *A. mellifera*, when applied topically, but significantly affected survival of both species when administered orally. Propiconazole also showed acute oral toxicity to both bee species, but oral Hazard Quotients were below the threshold value of 50 (4 and 2 for *O. lignaria* and *A. mellifera*, respectively). This fungicide alone should therefore be considered not hazardous to the two bee species. However, to prevent bees from consuming contaminated nectar, we suggest avoiding propiconazole applications when nectar is available on flowers. In the previously mentioned California pollination study, sublethal effects on nesting *O. lignaria* females were observed following an application of propiconazole in a tank mixture with a surfactant and a fertilizer. We therefore recommend assessing the potential side effects of tank mixtures with this fungicide.

Striking differences emerged between the two bee species in their susceptibility to captan. Even at high doses, captan did not cause significant mortality in *A. mellifera*, but it affected *O. lignaria*. At 72 h after administration, contact and oral Hazard Quotients were 8 and 48, respectively, and captan would thus be considered a fungicide harmless to *O. lignaria*. However, at 7 days from exposure the oral $LD_{50}$ reached 10.87 µg a.i./bee and the oral Hazard Quotient 202. Therefore, captan should be considered hazardous to *O. lignaria* females exposed to direct treatment, and should not be applied while bees are actively visiting the treatment area. We noticed that immediately after contact or oral administration of captan, *O. lignaria* females showed behavioural abnormalities, such as inactivity, regurgitation of the ingested sucrose solution, extension of proboscis, abdomen and genitalia (Fig. 5). We did not observe similar effects in *A. mellifera* workers exposed to captan or in *O. lignaria* females exposed to other products. Atkins et al. (1975) did not find any toxicity effects of this fungicide to adult worker honey bees either. However, the fungicide can interfere with the development of exposed honey bee brood, resulting in morphogenic effects in adults (Atkins and Kellum, 1986). Mussen (2003) found that 100% of the *A. mellifera* larvae died when transferred as one-day-old larvae on a diet treated with captan. Honey bee products such as honey, corbicular and stored pollen, nectar, adult bees and brood food of honey bee

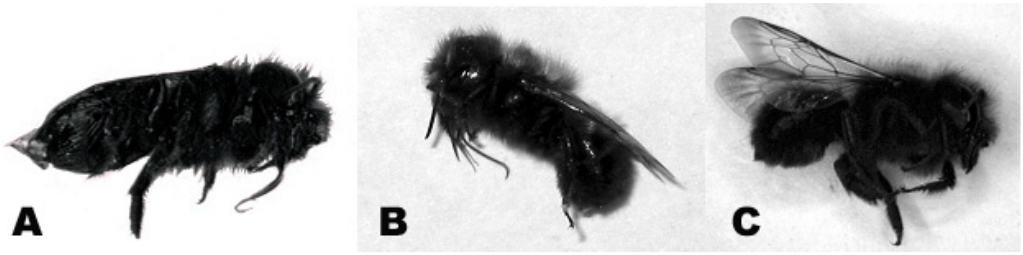


**Figure 5.** Appearance of dead *O. lignaria* females after: (A) single exposure to captan – extended proboscis, abdomen, and genitalia; (B) single exposure to dimethoate – extended proboscis; (C) no exposure (untreated).

colonies placed in apple and almond orchards were found to contain residues of captan (Kubik et al., 2000; Rivera et al., 2003). During our California pollination study, when captan and benomyl were used in tank mixture in a cherry orchard, *O. lignaria* females completely disappeared from the orchard within a few days. To protect adult *O. lignaria* and *A. mellifera* larvae, the use of captan during bloom should be avoided. Possible effects of captan on brood development of *O. lignaria* should also be investigated.

Our contact and oral $LD_{50}$ values for *A. mellifera* at 24, 48 and 72 h after exposure to dimethoate are comparable to those reported in the literature (Gough et al., 1994; Lewis et al., 2001; Devillers, 2002; Tornier et al., 2003) and $LD_{50}$ values at 24 h were within the accepted range reported in the OECD guidelines (contact $LD_{50}$: 0.10–0.30 µg a.i./bee; oral $LD_{50}$: 0.10–0.35 µg a.i./bee; OECD, 1998a, b). Contact and oral $LD_{50}$ values of dimethoate for *O. lignaria* were intermediate between those reported and obtained for *A. mellifera* and those reported for *Bombus terrestris* (L.) (24–72 h contact $LD_{50}$ = 4.1–13 µg a.i./bee; 24–72 h oral $LD_{50}$ = 1.7–4.7 µg a.i./bee; Schaffer et al., 1996; Thompson, 2001; van der Steen, 2001). Contact and oral Hazard Quotients at 24 h from exposure were 286 and 2078 for *O. lignaria*, and 2953 and 3740 for *A. mellifera*.

Our testing procedures provided an adequate assessment of toxicity effects for two reasons: (1) even when fungicides or insecticides at high concentrations were added to the solution and offered to the bees with the flower method (Ladurner et al., 2003b), bees fed rapidly (88.2–97.7% of the bees tested consumed all the test solution within 1 h); and (2) our dimethoate $LD_{50}$ values are consistent with those found by others.

In conclusion, the five tested fungicides, with the exception of the captan and *O. lignaria* combination, seem to be relatively safe to both *O. lignaria* and *A. mellifera* at recommended field rates, and no significant adverse effects on pollinator function should occur when the products alone are applied in the evening or at night. However, given the observations made during our California pollination study, further investigations are needed on possible side effects and/or synergistic effects with other fungicides and products that are commonly added to tank mixtures (surfactants/stickers, foliar fertilizers), to identify plant protection products, compatible with the use of bees for pollination. Finally, because *O. lignaria* females disappeared from orchards when fungicide tank mixtures were applied early in the morning, but resumed normal foraging activity after a few days following evening and night sprays, studies comparing the effects of early-morning and evening sprays may be useful to elaborate adequate recommendations for protecting both the bees foraging on the crop and the growers' yields.

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**Résumé – Évaluation de la toxicité aiguë et des effets toxiques retardés de cinq formulations de fongicides vis-à-vis de *Osmia lignaria* et *Apis mellifera*.** Lors d'études sur la pollinisation de vergers commerciaux de cerisiers, nous avons observé d'éventuels effets non intentionnels des pulvérisations de fongicides sur *Osmia lignaria* Say. Les pulvérisations consistaient en un mélange en cuve de fongicides bénomyl, captane, iprodione ou propiconazole avec des adjuvants et des engrais foliaires. Le matin après les pulvérisations, les comportements de retour au nid, de nidification et de butinage des femelles bien établies ont été affectés. L'intensité des effets non intentionnels observés a varié en fonction de la nature des mélanges et de l'heure du traitement. Nous avons donc estimé au laboratoire la toxicité éventuelle par voie orale et par contact de cinq formulations de fongicides (Tab. I) sur *O. lignaria* et *Apis mellifera* L. Afin de déterminer les effets toxiques retardés, *O. lignaria* et *A. mellifera* ont été exposées à des doses de fongicides uniques mais fortes et leur survie a été comparée à celle des témoins sur 7 j. Pour les produits qui présentaient une toxicité aiguë aux fortes doses, on a déterminé les valeurs de la $DL_{50}$ orale et de la $DL_{50}$ par contact.
Il s'agit, à notre connaissance, du premier test complet de toxicité orale et par contact sur une espèce d'*Osmia*. L'exposition par voie orale et par contact à de fortes doses de bénomyl et d'iprodione n'a affecté la survie d'aucune des espèces (Tab. II, Figs. 1–4). L'huile de margousier (*Melia azadirach* ou « neem tree ») fortement concentrée n'a pas affecté *O. lignaria* (Tab. II, Figs. 1–2). Des applications topiques de cette huile ont montré des effets toxiques retardés pour *A. mellifera*, mais aucun effet toxique aigu n'est apparu (Tab. II, Fig. 3). Le propiconazole n'a pas été toxique par contact pour *O. lignaria* ni pour *A. mellifera* (Tab. II, Figs. 1–3), mais l'a été par voie orale (Tabs. II, III, Figs. 2, 4). Le captane est resté sans effet sur *A. mellifera*, mais a présenté des effets toxiques retardés par contact et par voie orale pour *O. lignaria* (Tabs. II, III, Figs. 1–5). Comme l'on si attendait, le diméthoate a été toxique pour les deux espèces (Tab. III).
Le bénomyl ne semble être toxique pour aucune des deux espèces. Étant donnés les effets observés du captane sur les femelles d'*O. lignaria*, l'utilisation de ce fongicide devrait être évité lorsque cette espèce visite activement la zone à traiter. Le captane peut ne pas affecter l'activité pollinisatrice des adultes d'*A. mellifera*, on sait néanmoins que le captane et l'iprodione tuent le couvain d'abeilles et réduisent donc la force de la colonie. L'huile de margousier pourrait être un fongicide compatible avec les abeilles durant la période de pollinisation, si l'exposition directe est évitée. Pour empêcher les abeilles de consommer du nectar contaminé par le propiconazole, nous suggérons de ne pas utiliser ce pesticide pendant les miellées.
Pour conclure, à l'exception de l'action du captane sur *O. lignaria*, les produits testés devraient être non toxiques pour les deux espèces d'abeilles et la fonction pollinisatrice ne devrait pas être affectée tant que l'exposition directe est évitée. Il est pourtant nécessaire de poursuivre les recherches sur les effets sublétaux et les effets de synergie avec d'autres fongicides et les substances qui sont couramment ajoutées aux mélanges de cuve.

***Osmia lignaria* / *Apis mellifera* / toxicité / fongicides / DL50**

**Zusammenfassung – Bestimmung der Langzeit- und Akute Toxizität von fünf formulierten Fungiziden bei *Osmia lignaria* Say und *Apis mellifera*.** Bei Untersuchungen von Bestäubungen in kommerziellen Kirschplantagen in Kalifornien beobachteten wir, dass es möglicherweise Nebeneffekte der fungiziden Sprays bei *Osmia lignaria* Say gibt. Die Sprays bestanden aus den Tankmischungen von Benomyl, Captan, Iprodion oder Propiconazol mit Tensiden und Blattdüngern. Am Morgen nach der Sprühung waren Heimkehr-, Nist- und Sammelverhalten von bereits fest ansässigen Weibchen gestört. Die Stärke dieser Nebeneffekte variierte mit der Mischung und der Tageszeit bei der Ausbringung. Deshalb untersuchten wir die mögliche Kontakt- und orale Toxizität der 5 formulierten Fungizide (Tab. I) bei *O. lignaria* und *Apis mellifera* L. im Labor. Zur Bestimmung der Giftwirkung wurde 7 Tage lang das Überleben von *O. lignaria* und *A. mellifera* nach Verabreichung einer einzigen hohen Dosis der Fungizide verglichen mit der Kontrolle mit dem Dosierungsmittel. Bei den Substanzen mit einer akuten toxischen Wirkung nach Verabreichung einer hoher Dosis wurden die $LD_{50}$ Werte nach Kontakt und nach Fütterung bestimmt (toxischer Standard: Dimethoat).
Nach unserer Kenntnis ist dies der erste vollständige Kontakt- und orale Toxizitätstest bei einer *Osmia* Art. Kontakt und Fütterung mit hohen Dosen von Benomyl und Iprodion zeigte bei beiden Bienenarten keine Wirkung (Tab. II; Abb. 1–4). Hoch konzentriertes Neemöl war bei *O. lignaria* wirkungslos (Tab. II; Abb. 1, 2). Durch Kontakt verabreichtes Neemöl zeigte eine Langzeit- aber keine akute Giftwirkung bei *A. mellifera* (Tab. II; Abb. 3). Propiconazol zeigte weder bei *O. lignaria* noch bei *A. mellifera* eine Giftwirkung nach Kontakt (Tab. II; Abb. 1, 3), dagegen war es nach Fütterung bei beiden Arten giftig (Tab. II und III; Abb. 2, 4). Captan war bei *A. mellifera* wirkungslos, dagegen war es ein auffälliges Kontakt- und Nahrungsgift bei *O. lignaria* (Tab. II und III; Abb. 1–5). Wie erwartet war Dimethoat bei beiden Arten toxisch (Tab. III).
Benomyl scheint bei beiden Bienenarten ungiftig zu sein. Nach der jetzt erkannten Wirkung von Captan auf *O. lignaria* Weibchen, sollte dieses Fungizid dort vermieden werden, wo *O. lignaria* aktiv das zu sprühende Gelände besucht. Zwar mag Captan die

Bestäubungstätigkeit der adulten *A. mellifera* nicht beeinflussen, Captan und Iprodion stehen aber in dem Ruf, die Brut der Honigbienen zu töten und führen damit zur Reduzierung der Volksstärke. Neemöl könnte als bienenverträgliches Fungizid während der Bestäubung eingesetzt werden, aber nur bei Vermeidung eines direkten Besprühens. Um Bienen von Propiconazol-kontaminiertem Nektar abzuhalten, schlagen wir vor, dieses Fungizid nicht während der Blüte anzuwenden.

Abschließend sei festgestellt, dass die getesteten Produkte, mit Ausnahme von Captan für *O. lignaria,* für beide Bienenarten sicher sein sollten und die Bestäubungsfunktion nicht beeinträchtigt werden sollte, solange ein direkter Kontakt (Einsprühen) vermieden wird. Trotzdem sind weitere Versuche nötig, um mögliche sublethale und / oder synergistische Wirkungen mit anderen Fungiziden und Substanzen zu erkennen, die normalerweise den Gebrauchsmischungen zugefügt werden.

***Osmia lignaria* / *Apis mellifera* / Toxizität / Fungizide / $LD_{50}$**

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