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Effects of 20 fungicides on the infectivity of conidia of the aphid entomopathogenic fungus *Erynia neoaphidis*

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Abstract. The effect of 20 fungicides on the infectivity of conidia of the entomopathogenic fungus, *Erynia neoaphidis*, were assessed in the laboratory. After projection on broad bean leaves, conidia were treated with fungicides applied at their recommended field rate. Afterwards, the infectivity of these inocula was assessed using an aphid bioassay. Four fungicides, carbendazim, kresoxym-methyl, nuarimol and thiophanate-methyl reduced the infectivity of the conidia by less than 25% and can be considered harmless for this aphid pathogen. Propiconazole was a little more toxic, with 37% reduction. Other products reduced infectivity by between 50% and 100%. These are, from the least to the most toxic: flutriafol, prochloraz, epoxyconazole, iprodione, hexaconazole, triadimenol, azoxystrobine, cyproconazole, cyprodynil, flusilazole and tridemorph. Chlorothalonil, fenpropimorph, spiroxamine and tebuconazole totally inhibited infectivity of the fungi. Analysis of the results according to chemical class showed that the benzimidazoles were the least toxic for *E. neoaphidis* and the morpholines the most toxic. Effects of triazoles and strobilurines were variable, with reduction ranging from 37% to 100% for triazoles and from 17% to 68% for strobilurines.

Key words: aphid, Entomophthoraceae, *Erynia neoaphidis*, fungicide, infectivity

Introduction

In many crops it is well known that the Entomophthorales play an important and sometimes determinate role in the regulation of aphid populations comparable to that done by hymenopterous parasitoids and aphidophagous larvae. This is documented, for example, in cereals (Dedryver et al., 1985; Latteur and Oger, 1991), potatoes (Shands et al., 1972; Soper, 1981) and lucerne (Hall and Dunn, 1959).

The use of pesticides is not always compatible with the control exerted by aphid natural enemies. The side-effects of pesticides on natural enemies have been long recognised and studied extensively. The use of non-selective

products can lead to pest resurgence (Ripper, 1956; Pimentel, 1961; Horn, 1983; Borgemeister and Poehling, 1989) and to outbreak of secondary pests, previously controlled by natural enemies (Bartlett, 1956; Bacon, 1960; Eveleens et al., 1973; Naïbo and Foulgocq, 1983).

The effects of pesticides and especially fungicides on Entomophthorales have also been investigated by several authors. Most fungicides tested in vitro by Hall and Dunn (1959), Wilding (1972), Zimmerman (1976), Fritz (1977) and Wilding and Brobyn (1982) inhibited the germination of conidia of Entomophthorales and the growth of the mycelium on artificial medium. Some of them (benomyl, captafol, captan, dinocap, ditalimfos, ethirimol, maneb, mancozeb, sulfur, thiram, ziram), but not carbendazim, copper oxychloride, dimethirimol, dodine, triarimol and zineb, also significantly reduced the infectivity of conidia of Entomophthorales (Wilding, 1972; Delorme and Fritz, 1978; Oncüer and Latteur, 1979; Wilding and Brobyn, 1980).

Results of field trials are less numerous. In wheat, Zimmerman and Basedow (1980) found that benomyl, captafol, thiophanate-methyl, triadimefon and tridemorph had no effects on Entomophthorales. In a crop of wheat treated twice with a mixture of benomyl, maneb and sulfur, a reduction in the percentage of aphids killed by Entomophthorales was observed (Latteur et al., 1981). In field bean, adverse effects of benomyl on Entomophthorales were detected, while captafol, mancozeb, maneb and tridemorph had no detectable effects (Wilding, 1982). Pickering et al. (1990) found that more aphids were killed by entomophthorales in untreated pecan orchard plots than in those treated with the fungicide triphenyltin hydroxide. Nanne and Radcliffe (1971) and Lagnaoui and Radcliffe (1998) demonstrated that the fungicides captafol, mancozeb and metalaxyl used in potato crops interfered with Entomophthorales infection and encouraged green peach aphid, *Myzus persicae* (Sulzer) outbreaks.

In Belgium and throughout temperate Western Europe, aphids are the main pests of wheat crops in which the foliage often maintains a high humidity favourable for the development of mycoses caused by Enthomophthorales. However, fungicide treatments applied to wheat to control diseases, at the time when aphids are present, can act adversely on the Entomophthorales and facilitate the development of the aphid population. Given that there are few published results on fungicides currently used in cereals it was considered desirable to test the toxicity for the Entomophthorales of these products to complete the development of an IPM programme for aphids in wheat. The experiments were done in the laboratory using an inoculum of conidia of *E. neoaphidis* produced in vivo. This species was selected because it is considered as the most abundant one in cereals (Dedryver et al., 1985). The method was developed in our laboratory and has been used since 1995.

Material and methods

Inoculum

Inocula of conidia were produced from dried mummies of *Acyrthosiphon pisum* (Harris) killed by *E. neoaphidis*. Original inocula was provided by cereal aphids killed by *E. neoaphidis* harvested in a winter wheat field and cultured in the laboratory on the pea aphids (*A. pisum*), reared on French bean (*Vicia fabae*) plants. For the trials, only freshly produced mummies or mummies dried and stored for a maximum of 7 days in a fridge according to specifications made by Courtois and Latteur for optimal conservation (+ 5 °C, RH of about 15% in a desiccator – Courtois and Latteur, 1984). As tests were generally done weekly, mummies produced in the controls were dried and used the following week. By this way, only infectious isolates of *E. neoaphidis* were used during the trials.

Chemicals

The active ingredients used in this study are listed in Table 1, with the commercial name of the formulation used, the mode of action, the chemical class and the dose tested. They are all used in winter wheat crops between the development of the first node and earing. The doses tested corresponded to the maximum recommended field rate for cereals applied in 200 liter of water. For cyprodinil and kresoxym-methyl, which are only used as a mixture in wheat, the formulations used in apple orchards were tested, but at doses corresponding to cereal use.

Course of the experiments

The experiments were done in four stages: production of the inoculum, treatment of the inoculum with fungicides, contact of aphids with treated conidia and incubation of the inoculated aphids.

Conidia were produced from dried mummies of pea aphids (*A. pisum*) killed by *E. neoaphidis*. Before use, dried mummies were placed individually on wetted filter paper (⌀: 25 mm) placed in glass petri dishes (⌀: 20 cm). Number of mummies prepared was adapted to the number of objects of each trials, one mummy producing conidia being used by object. Based on visual inspection, mummies that were not correctly hydrated and that did not project conidia were rejected. Mummies were placed with the ventral surface in close contact with the filter paper. After 1 hour, petri dishes were inverted to prevent the first conidia to be projected from falling back on the filter paper or on other mummies. Two hours later, after primary conidia had been produced,

each filter paper bearing a mummy was placed individually for 90 minutes at the top of a perspex ring cage (h: 2.5 cm, ⌀: 5 cm) so as to project conidia on a field bean leaf (*Vicia fabae*) placed behind the cage. Leaves used for the test were detached from plants that were cultured in a climatic chamber and that were not in contact with pesticide, aphid or entomophtorales before the test. Leaves were placed on moistened filter paper in a plastic petri dish, with the leave underside up. Dishes were held at 20 ± 0.5 °C in the darkness. The leaves were obtained from beans grown without pesticide treatment.

One hour after the period of conidia projection, petri dishes with leaves and conidia were treated with fungicide using a Burgerjon spray tower (Burgerjon, 1956). Spray tower was calibrated to deliver an homogenous deposit equivalent to 200 ± 20 l/ha. Fungicides were diluted in water shortly before application of products. Control units were treated with water.

Two hours after treatment, 20 ± 2 nymphs (N3 + N4) of *A. pisum* were placed on each of the treated leaves with a perspex ring cage (⌀: 25 mm, h: 5 mm) covered by nylon gauze to encourage contact between aphids, treated leaves and treated conidia.

After 18 hours of contact at 18 °C, aphids were placed on young field bean plants grown on vermiculite in plastic pots, with the substrate covered with filter paper to allow easier observation of aphids. During the first 24 hours, a plastic cage without aeration was placed on each plastic pot to maintain high humidity and stimulate the development of the fungi. Plants were kept at 18 ± 2 °C in the dark. After that, closed perspex cages were replaced by ones with two lateral rectangular holes and the top covered with nylon gauze. Light was provided by neon tubes with a 18h/6h day/night photoperiod. L4 and adults aphids killed by Entomophthorales were counted on day 4, 5 and 6 after contact with conidia. Young nymphs produced by the aphids were ignored. On day 6, living adult aphids were counted and the percentage of aphids killed by Entomophthorales was calculated on the basis of the total numbers of adults recovered during the observations (living ones and those killed by Entomophthorales). For each product, there were 8 replicates of one leaf with 18–20 aphids/leaf. Some aphids died during the experiments for reasons other than Entomophtorales infection or they escaped. These were not taken into consideration in the calculation of reduction of infectivity. Thus assessments were made on a mean of 16–18 aphids, sometimes less for fungicides with a degree of toxicity for aphids.

Based on the results of previous experiments, test design (size of cages, duration of experiment, selection of aphid mummies, etc.) were selected to obtain between 60 to 95% of aphids killed by entomophthorales in the water-treated control. If aphid control mortality in a specific experiment was less than 60% or more than 95%, results of this experiment were rejected.

Table 1. List of fungicides (active ingredient, commercial name, dose tested, chemical class, mode of action) tested on the infectivity of conidia of *Erynia neoaphidis*

Active ingredient	Product tested	Dose tested (a.i./ha)	Chemical class	Mode of action
Azoxystrobine	Amistar	250 g	Strobilurine	Systemic
Carbendazim	Bavistin	200 g	Benzimidazole	Systemic
Chlorothalonil	Daconil	1250 g	Unclassified	Contact
Cyproconazole	Alto	100 g	Triazole	Systemic
Cyprodynil	Chorus	250 g	Pyrimidinamine	Systemic
Epoxyconazole	Opus	125 g	Triazole	Contact, systemic
Fenpropimorph	Corbel	750 g	Morpholine	Systemic
Flusilazole	Capitan	100 g	Triazole	Systemic
Flutriafol	Impact	125 g	Triazole	Contact, systemic
Hexaconazole	Sirocco	250 g	Triazole	Systemic
Iprodione	Rovral	200 g	Dicarboximide	Contact
Kresoxym-methyl	Candit	100 g	Strobilurine	Systemic
Nuarimol	Tridal	40 g	Pyrimidine	Systemic
Prochloraz	Sportak	450 g	Unclassified	Translaminar
Propiconazole	Tilt 250EC	125 g	Triazole	Systemic
Spiroxamine	Impulse	750 g	Unclassified	Systemic
Tebuconazole	Horizon EW	250 g	Triazole	Systemic
Thiophanate-methyl	Topsin M	420 g	Benzimidazole	Systemic
Triadimenol	Bayfidan	125 g	Triazole	Systemic
Tridemorph	Calixin	750 g	Morpholine	Systemic

Products were tested in sets of two, plus the control and each product was tested twice. The toxicity of the fungicides were expressed as the reduction in the infectivity of conidia (R), calculated from the formula:

$$R(\%) = (A_t - A_c)/(100 - A_c)$$

where A_t = % of aphids surviving in the treatment and A_c = % of aphids surviving in the corresponding control. This follows Abbott's formula (Abbott, 1925) to correct mortalities, considering the surviving aphids as a measure of the reduction of infectivity of conidia of the fungi. The percentage of reduction of the infectivity of conidia was compared to zero using a mean conformity test (Dagnelie, 1970). The two sets of trials were analysed separately.

Results

The results of trials are listed in Table 2 (Set 1 and 2, mean of two). In the controls, between 64% and 94% of aphids were killed by Entomophthorales, with a mean of 83%. Five products – chlorothalonil, fenpropimorph, fenpropidin, spiroxamine and tebuconazole – totally prevented infectivity of conidia and no aphids exposed to these products were killed by the entomopathogenic fungus. Cyproconazole, cyprodinil, flusilazole and tridemorph severely affected the infectivity with reductions of between 80% and 96%. Others products (azoxystrobine, epoxyconazole, flutriafol, hexaconazole, iprodione, prochloraz, propiconazole and triadimenol) were less toxic, with reductions of infectivity between 38% and 68%. Only four products, carbendazim, kresoxym-methyl, nuarimol and thiophanate-methyl reduced infectivity of conidia of *E. neoaphidis* by less than 25%.

The comparison of the results obtained in the two sets of experiment were similar, demonstrating the robustness of the method developed. Most of the time, the number of aphids that were infected plus those still alive at the end of the experiment was comprised between 15 and 18. However, this number was notably less for the fenpropimorph, the spiroxamine and the tebuconazole treatments during the first set of experiments and for the tridemorphe treatment during the second (Table 2).

Discussion and conclusions

Of the fungicides tested in this study, only three have been studied before for their action on the Entomophthorales: carbendazim, thiophanate-methyl and tridemorph. Results on the first two of these confirm previous ones, demonstrating very little toxicity to these fungi. However, results obtained with tridemorph contrast with those of others. Whereas Zimmermann and Basedow (1980) and Wilding (1982) failed to register an effect of this compound in the field, it was amongst the most toxic in our experiments. This may be explained by the fact that for this product, as for all the systemic ones, the laboratory conditions to which it was submitted were more intense than those in the field.

Table 2. Effects of fungicides on the infectivity of conidia of *E. neoaphidis*. (expressed as% reduction in infectivity relative to controls treated with water). Two sets of experiments, mean of 8 replicates ± sd/set and the mean of the two sets. (n) = average number of aphids used for the assessment, living aphids + aphids killed by Entomophthorales. For the controls, n = mean of 16,8 aphids (range 14.9–18.4)

Active ingredient	% reduction in infectivity of conidia				
	n	Set 1	n	Set 2	mean
Carbendazim	16.0	7,2 a ± 15,6	17.5	3,1 a ± 7,1	5,6
Nuarimol	15.8	27,7 b ± 25,7	16.6	5,2 a ± 16,3	16,4
Thiophanate-methyl	17.1	16,0 b ± 15,2	17.2	18,8 a ± 28,3	17,4
Kresoxym-methyl	18.0	8,9 a ± 16,5	14.2	25,8 c ± 13,2	17,5
Propiconazole	17.8	28,0 a ± 33,1	17.5	46,7 d ± 20,1	37,4
Flutriafol	16.6	58,3 d ± 6,9	15.8	42,6 d ± 19,8	50,5
Prochloraz	15.6	68,3 d ± 20,3	15.5	44,6 d ± 17,6	56,4
Epoxyconazole	16.5	45,0 d ± 11,1	16.3	70,2 d ± 11,5	57,6
Iprodione	16.5	55,5 d ± 20,9	16.8	67,2 d ± 14,2	61,4
Hexaconazole	15.7	66,0 d ± 10,5	17.0	63,8 d ± 12,3	64,9
Triadimenol	16.6	75,9 d ± 14,0	17.0	58,8 d ± 13,0	67,4
Azoxystrobine	15.6	72,4 d ± 8,4	17.1	63,5 d ± 14,0	68,1
Cyproconazole	15.0	83,6 d ± 8,8	14.6	91,4 d ± 11,2	88,0
Cyprodynil	17.3	96,0 d ± 5,3	15.8	94,2 d ± 8,4	95,1
Flusilazole	16.3	97,1 d ± 3,8	16.0	95,7 d ± 5,6	96,4
Tridemorphe	15.6	93,4 d ± 8,2	8.9	100,0 d ± 0,0	96,7
Chlorothalonil	16.4	100,0 d ± 0,0	15.4	100,0 d ± 0,0	100,0
Fenpropimorph	11.4	100,0 d ± 0,0	15.0	100,0 d ± 0,0	100,0
Spiroxamine	11.1	100,0 d ± 0,0	16.1	100,0 d ± 0,0	100,0
Tebuconazole	5.5	100,0 d ± 0,0	16.3	100,0 d ± 0,0	100,0

Statistical analysis: mean conformity test, (a) not statistically different from 0 at $p = 0.05$ level; (b) different from 0 at $p = 0.05$ level, (c) at 0.01 level, (d) at 0.001 level.

It should be noted that tridemorph, like the majority of the fungicides tested, acts during cell division by inhibiting the synthesis of ergosterol. In the case of *E. neoaphidis*, inhibition of the synthesis of ergosterol occurs inside the aphid, when the aphid had fed on the treated leaf surface for 18 hours. In these conditions, amount of fungicides absorbed by aphids in the experiments are certainly greater than it would be in the field. Furthermore, the quantity of active ingredient intercepted by the leaf surface in the field are considerably less because the leaves are rarely horizontal and are not receiving all the treatment and, secondly, the product is distributed systematically throughout the plant. There is then, in the field, a double dilution

effect which is absent in our laboratory tests. The results obtained should not, therefore, be directly extrapolated to crop conditions: a fungicide which totally inhibits the infectivity of the fungus in the laboratory could allow the development of the fungus infection in the field or not.

These trials can be considered as a worst case study, as are laboratory tests on Beneficial arthropods carried out by the IOBC working group 'Pesticides and beneficial organisms' (Hassan, 1992). As carbendazim, nuarimol, kresoxym-methyl, thiophanate-methyl and propiconazole had no or little effects in the laboratory on infectivity of conidia of *E. neoaphidis*, adverse effects in the field are not expected. For other products, field tests or tests conducted in the laboratory in conditions close to field situation are needed for an adequate assessment of their impact on Entomophtorales. However, results obtained in this study are indicating that potential adverse effects exists and, as no relevant field data are available, their use must be limited if situation where aphid control by entomophtorales are promoted.

Analysis of the results according to the chemical class of the fungicide showed that the benzimidazoles were the least toxic for *E. neoaphidis* and the morpholines (fenpropimorph, tridemorph) the most. Effects observed with triazoles were very variable, with a reduction in infectivity ranging from 37% (propiconazole) to 100% (tebuconazole). Reasons of this variability are unknown. Effects observed with strobilurine were less, with 17% to 68% of reduction of infectivity of conidia of *E. neoaphidis*. Most of fungicides tested act as ergosterol synthesis inhibitor. However, reduction of infectivity of entomophtorales of ergosterol synthesis inhibitor ranged from 16% (nuarimol) to 100% (tebuconazole) and this specific mode of action cannot be correlated with their impact on entomophtorales.

All fungicide formulations tested in this study contained a single active ingredient. There are several questions about the possible effects of mixtures of two or three active ingredient at the same time, as farmers often do. In cereals, most common mixtures include a triazole and a morpholine compound or a strobilurine and a triazole or a morpholine compound, thus at least one, sometimes two compounds that are very toxic for *E. neoaphidis* in the laboratory. Thus, the question is to determine to what extent fungicide applications in cereals are promoting aphid populations. The question is also relevant in crops where repeated applications of fungicides are needed, as in potato, with up to 10 fungicide treatments in a season. To answer these questions there is a need to improve tests with fungicides and Entomophtorales.

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