Effects of the fungicide Captan on some functional groups of soil microflora

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Abstract

The effects of Captan at rates of 2.0, 3.5, 5.0 and 10.0 kg ha⁻¹ on microbial function were studied in four agricultural soils under aerobic conditions. Parameters monitored included total culturable populations, numbers of aerobic N₂-fixing bacteria, denitrifying bacteria, nitrifying bacteria and nitrogenase activity. Total culturable fungal populations, nitrifying bacteria, aerobic N₂-fixing bacteria and nitrogenase activity were significantly decreased at dose rates of 2.0 to 10.0 kg ha⁻¹. However, the presence of Captan enhanced denitrifying and total culturable bacteria, showing that some microbial groups can tolerate high doses of this fungicide. © 1998 Elsevier Science B.V.

Keywords: Fungicides; Pesticides; Captan; Soil microflora

1. Introduction

Modern agriculture and industry depend on a wide variety of synthetically produced chemicals, including insecticides, fungicides, herbicides and other pesticides. Continual widespread use and release of such synthetics has become an everyday occurrence, resulting in environmental pollution. In this context, the influence of pesticides on the microbial activity of soil microorganisms has been studied by some investigators both in pure culture (Martínez-Toledo et al., 1991; Omar et al., 1992; Sanchez et al., 1994) and in mixed populations (Nayak and Rao, 1982; Sato et al., 1987; Liu et al., 1988). However, it is not possible to reach a general conclusion regarding the effect of these substances on soil because a number of factors (for example, chemical composition of the pesticide, soil type and characteristic of the soil microbial community) influence the effects of these agrochemicals.

Captan (N-trichloromethyl-thio-tetrahydrofuranilimide) is a common agricultural fungicide used to control Botrytis, Fusarium, Fusarium, Pythium, etc., at agronomic doses in the range of 1.5 to 10.0 kg ha⁻¹ (Barbera, 1989). Although sometimes Captan is not directly applied to the soil, it is the eventual sink in one way or another. The possibility that this fungicide may have an adverse effect on soil microflora could be of considerable importance. More so, the benefit of Captan as fungicide could be nullified by detrimental effects on microbial processes having a major influence on plant growth (Omar et al., 1992).
Table 1
Characteristics of agricultural soil samples

<table>
<thead>
<tr>
<th>Soil Type</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<tr>
<td></td>
<td>Mollic xerofluvet</td>
<td>Mollic xerofluvet</td>
<td>Typic xerorthent</td>
<td>Calcixerolic xerochrept</td>
</tr>
<tr>
<td>Texture</td>
<td>Silty loam</td>
<td>Clay loam</td>
<td>Loam</td>
<td>Clay loam</td>
</tr>
<tr>
<td>Sand (%)</td>
<td>10</td>
<td>25</td>
<td>50</td>
<td>30</td>
</tr>
<tr>
<td>Silt (%)</td>
<td>65</td>
<td>45</td>
<td>30</td>
<td>40</td>
</tr>
<tr>
<td>Clay (%)</td>
<td>25</td>
<td>30</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>Total N(%)</td>
<td>0.10</td>
<td>0.13</td>
<td>0.12</td>
<td>0.06</td>
</tr>
<tr>
<td>Organic matter(%)</td>
<td>3.5</td>
<td>3.5</td>
<td>3.8</td>
<td>0.6</td>
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<tr>
<td>pH (H₂O)</td>
<td>8.4</td>
<td>8.4</td>
<td>7.1</td>
<td>8.2</td>
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</tbody>
</table>

Values are means of three separate determinations.

Fig. 1. Number of bacteria (10^6 CFU g⁻¹) in four agricultural soils in the presence of Captan. Values are means of five samples. *: significant vs. control (P < 0.05).
The purpose of the present study was to examine how the fungicide Captan at normal field concentrations affects the microflora of four agricultural soils under aerobic conditions. The interactions established between total number of bacteria, fungal populations, aerobic N₂-fixing bacteria, denitrifying bacteria, nitrifying bacteria and nitrogenase activity were determined.

2. Material and methods

Soil samples were collected from the top 10 cm of four maize fields near Granada (Spain) with no previous Captan application. Soil samples (1.0 kg) were collected in plastic bags, shipped to the laboratory, and refrigerated (4°C) under field-moist conditions before use. Various soil characteristics were determined using techniques described previously (Martínez-Toledo et al., 1988; SCS, 1975).

Laboratory studies were performed on five replicates. In the first experiment, a 50 g sample of soil (five replicates) from each maize field was placed in a Petri dish and 5 ml distilled water were added to achieve a soil moisture level of 50–60% of field capacity. Each Petri dish was amended with 5 ml of appropriate dilutions of Captan (Sugelabor, Barcelona, Spain) to give final concentrations of 0.94, 1.64, 2.35 and 4.70 mg kg⁻¹ soil, corresponding to 2.0, 3.5, 5.0 and 10.0 kg ha⁻¹, respectively, and then incubated at 20 ± 2°C under aerobic conditions. After 7, 14 and 30 days, microbial populations and nitrogenase activity were determined. Control samples without Captan received equal amounts of distilled water. In the present investigation, technically pure grades of the fungicide were used.

In a second experiment, 50 g samples (five replicates) of the agricultural soils that 30 days before had been previously treated with Captan at concentrations of 2.0, to 10.0 kg ha⁻¹, received a second fungicide application of 2.0, 3.5, 5.0 and 10.0 kg ha⁻¹, and then incubated at 20 ± 2°C under aerobic conditions. After 7, 14 and 30 days of incubation, microbial populations and nitrogenase activity were determined. Control samples without fungicide received distilled water for comparison.

Total culturable microorganisms were counted by a soil dilution plate technique using tryptic soy agar (TSA, Difco) for bacteria and Czapek-Dox agar (Difco) at pH 6.0 for fungi. The inoculated agar plates (three replicates) were incubated at 28°C for 3 days for bacteria and 5 days for fungi, before the colonies were counted. The populations of aerobic diazotrophs were estimated using a standard dilution

Table 2
Summary of analysis of variance for each functional group of microorganisms as affected by soil type, time of sampling (7, 14 or 30 days), dose rate of Captan (0–10 kg ha⁻¹), and single vs. double (SvD) applications

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>TB</th>
<th>TF</th>
<th>TD</th>
<th>ARA</th>
<th>TDN</th>
<th>N I</th>
<th>N II</th>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Soil</td>
<td>P &lt; 0.0001</td>
<td>P &lt; 0.0001</td>
<td>P &lt; 0.0001</td>
<td>P &lt; 0.0001</td>
<td>P &lt; 0.0001</td>
<td>NS</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Time</td>
<td>P &lt; 0.0001</td>
<td>P &lt; 0.001</td>
<td>NS</td>
<td>P &lt; 0.0001</td>
<td>P &lt; 0.0001</td>
<td>P &lt; 0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Dose</td>
<td>P &lt; 0.0001</td>
<td>P &lt; 0.0001</td>
<td>P &lt; 0.0001</td>
<td>P &lt; 0.0001</td>
<td>P &lt; 0.0001</td>
<td>P &lt; 0.0001</td>
<td>P &lt; 0.0001</td>
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<tr>
<td>SvD</td>
<td>P &lt; 0.0001</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.0001</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.0001</td>
<td>P &lt; 0.0001</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td><strong>Interactions</strong></td>
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<td></td>
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<tr>
<td>Soil × Time</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Soil × Dose</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.0001</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Soil × SvD</td>
<td>P &lt; 0.05</td>
<td>NS</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Time × Dose</td>
<td>P &lt; 0.0001</td>
<td>NS</td>
<td>P &lt; 0.0001</td>
<td>P &lt; 0.0001</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Time × SvD</td>
<td>P &lt; 0.0001</td>
<td>NS</td>
<td>P &lt; 0.0001</td>
<td>P &lt; 0.0001</td>
<td>NS</td>
<td>NS</td>
<td>P &lt; 0.001</td>
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<tr>
<td>Dose × SvD</td>
<td>P &lt; 0.0001</td>
<td>NS</td>
<td>P &lt; 0.0001</td>
<td>P &lt; 0.0001</td>
<td>NS</td>
<td>NS</td>
<td>P &lt; 0.05</td>
</tr>
</tbody>
</table>

TB: total bacteria (g⁻¹); TF: total fungi (g⁻¹); TD: total aerobic diazotrophs (g⁻¹); ARA: ethylene production (nmol h⁻¹ g⁻¹); TDN: total denitrifying bacteria (g⁻¹); N I: total nitrifying bacteria (phase I, g⁻¹); N II: total nitrifying bacteria (phase II, g⁻¹); NS: not significant (P > 0.05).
series and plating on Burk's N-free medium solidified with 1.5% agar as described by Martínez-Toledo et al. (1988). The inoculated agar plates were incubated at 28°C for 3 days and the nitrogen-fixing capability of the colonies was measured in liquid shake cultures. Cells in the exponential phase were harvested by centrifugation at 3000 × g and dispersed in Burk's medium (N-free) containing 0.5% glucose. A total of 5 ml of the suspensions were placed in 12-ml tubes, and the nitrogenase activity was measured as described below.

The most probable number technique (Rodina, 1972) was used to count nitrifying bacteria. A separate analysis was performed for nitrifying phase I bacteria, which oxidize ammonium salts to nitrates, and for nitrifying phase II bacteria, which oxidize nitrates to nitrites. An ammonium sulphate medium was used for nitrifying phase I bacteria and a sodium nitrite medium for nitrifying phase II bacteria, according to Rodina (1972). Inoculated flasks (three replicates per soil sample) were incubated at 28°C and the presence of nitrite–N and nitrate–N in these

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![Soil 1](image1)

![Soil 2](image2)

![Soil 3](image3)

![Soil 4](image4)

Fig. 2. Number of fungi (10^9 CFU g⁻¹) in four agricultural soils in the presence of Captan. Values are means of five samples. s: significant vs. control (P < 0.05).
flasks was determined once a week for up to 8 weeks, as described by Rodina (1972).

Denitrifying bacteria were determined by a most probable number technique in a liquid enrichment (Rodina, 1972). Inoculated test tubes (three replicates) were incubated at 28°C for 7 days and denitrification was detected by the liberation of gas.

The nitrogenase activity in the agricultural soils amended or unamended with Captan was detected by the acetylene reduction technique (Hardy et al., 1968). A 2-g sample of soil was placed in 30-ml tubes and 0.2% (w/w) glucose was added. The soil samples were sealed with rubber stoppers. After 10% of the atmosphere had been replaced by acetylene, the tubes were incubated at 28°C, and 0.5 ml gas samples were assayed for ethylene after 12, 24 and 48 h, as previously described (Hardy et al., 1968), to detect the maximum nitrogenase activity. Immediately before use, acetylene was generated from calcium carbide and water, and ethylene contamination (about 2 nmol ml⁻¹) was always known and accounted for in final calculations. Ethylene was mea-

![Graphs showing soil treatment effects](image)

Fig. 3. Number of aerobic diazotrophs (10⁷ CFU g⁻¹) in four agricultural soils in the presence of Captan. Values are means of five samples. * significant vs. control (*P < 0.05*).
sured by reading peak area and comparing those with a standard prepared from a mixture of ethylene and nitrogen.

Statistical analysis of data obtained through this study was performed using STATGRAPHICS v. 5.0 (STSC, Rockville, MD, USA) software package. Analysis of variance (ANOVA) was conducted on the data for each functional group of microorganisms, with one-way interactions between soil type, time of sampling, dose rate of Captan and single- vs. double-dose effects. Least significant differences between means (Student’s t-test) were calculated at 95% confidence intervals. Pearson’s linear correlation coefficients between parameters are also presented.

3. Results

3.1. Soil type

The characteristics of the agricultural soils, from the Province of Granada, are indicated in Table 1.

Fig. 4. Ethylene production (nmol h⁻¹ g⁻¹) in four agricultural soils in the presence of Captan. Values are means of five samples, s: significant vs. control ($P < 0.05$).
The soils had no record of treatment with Captan fungicide, although the history of Soil 4 before 1986 was unknown.

3.2. Viable bacteria

The plate-count data indicated that total viable bacteria in each of the four agricultural soils amended with captan were significantly stimulated in the presence of Captan. Our results indicate (Fig. 1) that after the treatment with Captan at concentrations of 2.0, 3.5 and 5.0 kg ha\(^{-1}\), bacterial populations did not increase significantly until 14 days, whereas at a concentration of 10.0 kg ha\(^{-1}\), the populations were significantly higher from the control for days 7, 14 and 30, with the only exception of Soil 1. In addition, our data indicate that bacterial populations appeared to have increased more strongly in agricultural soils previously treated with fungicide (Fig. 1). The interactions of soil type with time of sampling and dose rates of Captan were not significant (Table 2), showing that the effect of Captan on total bacterial populations was similar in the four agricultural soils studied; however, there is a significant interaction between soil type and single vs. double application of the fungicide.

3.3. Fungal populations

The fungal populations in each of the four agricultural soils were significantly decreased in the presence of agricultural concentrations of Captan (Fig. 2). Inhibition was more evident after a first Captan application and the degree of inhibition increased as the concentration of the fungicide increased. The response of the fungal populations to the fungicide was not affected significantly by interactions between soil type and other factors (Table 2).

3.4. Aerobic diazotrophs and nitrogenase activity

The presence of Captan at concentrations of 2.0 to 10.0 kg ha\(^{-1}\) in each of the four agricultural soils negatively affected the populations of aerobic nitrogen fixing bacteria (Fig. 3). The plate-count data indicated that aerobic diazotrophs in soil samples amended with agricultural concentrations of Captan were significantly reduced, particularly after a second fungicide treatment. Moreover, our data indicate that the diazotroph population was negatively affected at the lowest treatment rate after the first treatment. Concomitantly, the presence of 2.0 to 10.0 kg ha\(^{-1}\) Captan negatively influenced nitrogenase activity (Fig. 4). Both parameters are highly and significantly positively correlated (\(r^2 = 0.92\), Table 3), and high and significant negative linear correlation coefficients were also found between total diazotrophic populations or ARA and the dose rate of Captan (Table 3). Diazotrophic microflora and their nitrogenase activity were the only parameters affected by a significant interaction between soil type and dose rate of fungicide (Table 2).

3.5. Denitrifying and nitrifying bacteria

After two applications of Captan to four agricultural soils, the populations of denitrifying bacteria were significantly increased after 14 days, at concentrations ranging from 2.0 to 10.0 kg ha\(^{-1}\) (Fig. 5). However, the presence of Captan in soil samples negatively affects the populations of ammonia-oxidizing bacteria and nitrate-oxidizing bacteria. This negative effect was particularly evident in soil sam-

<table>
<thead>
<tr>
<th></th>
<th>TB</th>
<th>TF</th>
<th>TD</th>
<th>ARA</th>
<th>TDN</th>
<th>N 1</th>
<th>N 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>NF</td>
<td>0.54(^a)</td>
<td>-0.27</td>
<td>-0.33</td>
<td>0.33</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NF</td>
<td>0.48(^b)</td>
<td>0.76(^b)</td>
<td>0.89(^a)</td>
<td>0.87(^a)</td>
<td>0.42(^a)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NF</td>
<td>-0.51(^b)</td>
<td>0.75(^a)</td>
<td>0.87(^a)</td>
<td>0.86(^a)</td>
<td>0.42(^a)</td>
<td>0.93(^a)</td>
<td></td>
</tr>
<tr>
<td>DOSE</td>
<td>0.61(^b)</td>
<td>0.68(^a)</td>
<td>-0.75(^a)</td>
<td>-0.38(^a)</td>
<td>0.27</td>
<td>-0.68</td>
<td>-0.64</td>
</tr>
<tr>
<td>TIME</td>
<td>0.29</td>
<td>-0.03</td>
<td>-0.03</td>
<td>0.06</td>
<td>0.39(^a)</td>
<td>0.02</td>
<td>-0.02</td>
</tr>
<tr>
<td>SvD</td>
<td>0.08</td>
<td>-0.20</td>
<td>-0.20</td>
<td>-0.04</td>
<td>0.42(^a)</td>
<td>0.20</td>
<td>-0.17</td>
</tr>
</tbody>
</table>

\(^aP < 0.0001\)
Fig. 5. Number of denitrifying bacteria (10^3 g⁻¹) in four agricultural soils in the presence of Captan. Values are means of five samples. s: significant vs. control (P < 0.05).

4. Discussion

There have been constant pressures on authorities controlling the registration and approval of agricultural chemicals, to include measurements of side effects on the soil microflora in their requirements. The Environmental Protection Agency in the United States for example, in guidelines for registering pesticides (Barbera, 1989) required data from ‘Studies of effects on microbial functions…’ in soils. Nitrification is one of the functions specified. To encourage uniformity of presented data and aid interpretation and comparison, the guidelines recommended the use of certain methods. These methods use the normal conditions of laboratory experiments in soil microbiology, usually a single soil temperature of
Fig. 6. Number of nitrifying (phase I and II) bacteria (× g) in four agricultural soils in the presence of Captan. Values are means of five samples. All values are significant vs. control (P < 0.05).

20–30°C and a moisture content of about 60% of field capacity.

The presence of Captan in each of the four agricultural soils studied here increased the number of total bacteria, in particular after a second fungicide treatment. In this sense, it has been reported that pesticides are metabolized by different soil bacteria, indicating that these compounds could be utilized as carbon sources (Cook, 1987). In the absence of data on the mineralization and biotransformation of Captan in agricultural soils, the proliferation of bacterial flora in treated soils may be associated with the transformation of Captan by the bacteria in soil.

The fungicide Captan significantly reduced the total culturable fungi in each of the four agricultural soils. Similar results had been reported for other fungicides (Pozo et al., 1994). However, the effects obtained in our studies may be influenced by the degree of soil disruption and also by the species of fungi present in the soils.
Our results show that Capitan, applied at agricultural concentrations, negatively affected nitrifying bacteria and aerobic diazotrophs, an effect that is strongly correlated with dose rate of the fungicide (Table 3). However, the presence of Capitan in each of the four agricultural soils enhanced the number of denitrifying bacteria. Thus, it is possible that frequent applications of Capitan may result in soil levels of fungicide that have a negative effect on the nitrogen economy and also on the microbial equilibrium of the soil. Similar results for other pesticides were reported by several authors (Tu, 1977; Yeomans and Bremner, 1985; Pozo et al., 1994).

Several studies (Wingfield et al., 1977; Lal, 1982; Pozo et al., 1995) on soil microflora showed that soil characteristics may modify the effect of pesticides on microbial numbers and their biological activity. However, the patterns of Capitan effect on soil microorganisms and nitrogenase activity in each of the four agricultural soils studied were similar. The experiment did not go on long enough to show whether this was a prolongation of a lag period or a total inability to degrade Capitan. In this context, the results obtained must be interpreted with caution and not used to predict the likely effects in soils following agricultural use of Capitan.

The nitrogen fixation and plant-promoting abilities of aerobic diazotrophs such as Azotobacter, Azospirillum and Rhizobium have aroused interest in its use as a bacterial fertilizer (Dart, 1986). However, a number of factors, including chemical compounds applied to the soil and plants, influence the ecology and biological activity of these microorganisms (Balandreau, 1986). In this context, the negative effects due to Capitan on diazotrophic bacteria and nitrogenase activity in agricultural soils could be of primary importance. Thus, it has been observed that pesticides like 2,4-D, 2,4,5-T, Trifluralin, Simazine, Lindane, Toxaphene, BMC and Thiram decrease nodulation and nitrogen fixation in legumes (Aggarwal et al., 1986).

The effects due to Capitan fungicide on soil microflora and especially on microorganisms involved in N-cycling of agricultural soils deserve a great deal of attention. Application of this fungicide to an agricultural soil may affect the composition of the microbial communities and thus disturb the fertility of the soil, especially the N-budget. In this context, rotational use and rational use of Capitan could be suggested as an important way of preventing environmental hazard.

References


