Paclobutrazol effects on soil microorganisms

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Abstract

The side effects of paclobutrazol, a plant growth regulator, on soil microbial community and activity were assessed in soil samples from Petrolina (PE), Pernambuco State and from Lins (SP), São Paulo State, in Brazil. The first experiment was carried out with soils from mango orchards of Petrolina, subjected to frequent field applications of paclobutrazol. A second experiment was conducted with soils from Petrolina and Lins with application of paclobutrazol under greenhouse conditions. For orchard soils, plate counting of soil microorganisms was carried out, while for the greenhouse experiment the parameters evaluated were: microbial biomass C, living hyphal length, dehydrogenase activity, and paclobutrazol dissipation.

The paclobutrazol addition to soils of mango orchards in Petrolina, affected negatively the soil microbial community. The average values for total number of bacteria, fungi and actinomycetes were reduced by 58, 28, and 28%, respectively, compared to the paclobutrazol unamended soil. For the greenhouse experiment, the paclobutrazol application in the soils from Petrolina influenced negatively the dehydrogenase activity and the living hyphal length, but not the microbial biomass C. The addition of this substance to the Lins soils had no effect on the microbial parameters evaluated.

Keywords: Plant growth regulator; Side effects; Microorganisms; Hyphal length; Microbial biomass C

1. Introduction

Paclobutrazol [(2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazol-1-yl) pentan-3-ol] is a plant growth regulator largely utilized to increase grain production and Eucalyptus seed production (Hampton and Hebblethwaite, 1985). It is also utilized in mango cultivation for growth control, and to reduce pruning and tillage. It is usually applied directly into the soil, where it may remain active for many years, and can severely affect the growth and development of subsequent crops or even interact, in a harmful way, with soil microorganisms (Chand and Lembi, 1994; Jackson et al., 1996). It also has some fungicidal activity against mildew and rusts (Deas and Clifford, 1984; Jackson et al., 1996).

Little information is available at present, regarding the effects of this growth regulator on the soil microbial community, despite the extensive literature about the effects of xenobiotics on soil microorganisms (Vischetti et al., 1997; Trasar-Cepeda et al., 2000; Pascual et al., 2000; Omar and Abdel-Sater, 2001). The toxicity of xenobiotics has been examined individually in a variety of soils under a variety of conditions and there is growing evidence that soil biological parameters may have potential for use as early and sensitive indicators of soil ecological stress or restoration (Dick, 1999).
The application of paclobutrazol in the Brazilian northeastern semi-arid region has become a common practice in mango orchards, making it necessary to assess its impact on the microbial community. The soil microbial community can be assessed at many different levels: total microbial biomass, enumeration of total bacterial and/or fungal populations, measurement of functional activity or specific soil enzyme activities. Then, the aim of this work was to determine the effects of paclobutrazol on microorganisms in Brazilian soils, by measuring different microbiological parameters, in field and greenhouse conditions.

2. Materials and methods

2.1. Study sites and soil sampling

To verify the effect of paclobutrazol application on soil microbial populations, two Brazilian regions were selected: one characterized as semi-arid tropical (Petrolina, Pernambuco State: 28 °C mean annual temperature and 400 mm mean annual precipitation) and another characterized as mesothermic tropical (Lins, São Paulo State: 25 °C mean annual temperature and 1300 mm mean annual precipitation).

2.1.1. Field studies

In Petrolina, an assay was conducted in an experimental site (sandy loam texture; organic matter: 9.3 g dm⁻³), of the Nilo Coelho Irrigation District (farm # 1069) that had been cultivated with mango for 3 years. The paclobutrazol application occurred in November 1998, with the concentration of 8 g a.i. g⁻¹ of soil. The compound was diluted in water (1 g paclobutrazol l⁻¹ of water) and applied as a drench around the collar of the tree trunk, at 15 cm depth. The soil samples were collected at 0–20 cm depth in 3 and 30 November 1998, 27 January 1999 and 26 March 1999. Five mango trees were randomly sampled. Each soil sample consisted of a mixture of five subsamples taken around each tree. In the laboratory these samples were stored at 4 °C until analysis. For controls, five soil samples from five mango trees without paclobutrazol application were collected. The number of soil microorganisms was estimated by the plate counting technique (fungi–Martin’s medium, actinomycetes–starch-casein-agar, bacteria–nutrient–agar medium (Tuite, 1969)). Each bulk sample was repeated five times, on the following dilutions: 10⁻⁴ (bacteria) and 10⁻² (fungi and actinomycetes).

2.1.2. Greenhouse study

Two other experiments with soil sampled from Petrolina and Lins, were conducted in greenhouse conditions, utilizing different doses of paclobutrazol. Both soils had no history of paclobutrazol application. The soils from Petrolina and Lins contained, respectively, 9.3 and 12.1 g dm⁻³ of organic matter and a sandy loam texture. The soil sample was collected in the same area utilized as control for the field evaluations of Petrolina, and under 2 year mango trees, in Lins, both at 0-20 cm depth. In the greenhouse, the soil samples were packed into plastic boxes (4 kg) and amended with paclobutrazol in the following concentrations: 0, 80 and 160 µg a.i. g⁻¹ to Petrolina soil, and 0, 8 and 16 µg a.i. g⁻¹ to Lins soil, with three replications for each treatment. Although the recommended paclobutrazol concentration for mango tree ranges from 8 to 10 g a.i. g⁻¹ soil, in the soil from Petrolina, paclobutrazol was applied at concentrations 10 times higher, since the residual concentration of paclobutrazol normally found in the soils with mango cropping, treated with the growth regulator, are very high.

Before the addition of the growth regulator to the experimental units, the soil remained in the boxes for 7 days with the water content corrected to 60–70% of the water holding capacity. The microbiological parameters evaluated after a soil incubation period of 3, 7, 10, 15 and 27 days were: microbial biomass C, living hyphal length, dehydrogenase activity, for Petrolina soil, and microbial biomass C, and dehydrogenase activity for Lins soil. The paclobutrazol residues was evaluated at end of the experimental period.

2.1.2.1. Soil microbial biomass C. Soil microbial biomass C was measured by the fumigation–extraction method (Vance et al., 1987). The soil (50 g) was separated into two equal samples, and one sample was exposed to chloroform for 24 h and extracted with 0.5 M K₂SO₄ (100 ml). The other sample was not fumigated but was extracted, under the same conditions. Organic C in the extracts was determined by dichromate digestion. The soil microbial biomass C
content was calculated using the relation:

\[ C_{mic} = 2.64 Ec \]  

(1)

where Ec is the extracted organic C of the fumigated soil minus the extracted organic C of the non-fumigated soil.

2.1.2.2 Living hyphal length. The living hyphal length was evaluated according to the method proposed by Melloni and Cardoso (1999) and by Nogueira and Cardoso (2000). The soil sample (20 g) was placed in a 500 ml beaker and water (ca. 300 ml) was added under pressure. The resulting suspension was passed through 0.71 and 0.25 mm sieves, mounted above a funnel placed over a 2 l beaker. The sieves were then washed with a strong jet of water (ca. 1.4 l), the resulting suspension was transferred to a blender, and the volume made up to 1.5 l. The blender was operated for 10 s at a low speed to ensure aggregate dispersal, the mixture then allowed to stand for 2 min, and a subsample (500 ml) was passed slowly through a 44 mesh sieve. The retained material was then resuspended in a phosphate buffer solution (pH 7.4, KH₂PO₄ 0.1 M and NaOH 0.1 M; 10 ml). For the determination of living hyphal length, 5 ml of this suspension was mixed with the fluorescein diacetate solution (FDA; 5 ml) (Bloem et al., 1995), except that the FDA (5 mg) was dissolved in acetone (2 ml) Nogueira and Cardoso, 2000). After incubation at room temperature for 5 min, the hyphae were collected by filtration onto a gridded Millipore filter (0.45 μm). The length of fluorescing hyphae was determined using an epifluorescent microscope equipped with blue filter (60 times magnification). Hyphal length was enumerated in 64 squares delineated on the center of the filter. For the calculation of the living hyphal length the Newman’s equation was used (Newman, 1966):

\[ R = \frac{n}{Am} \]  

(2)

where R is the length of extra-radicular mycelium evaluated on the 64 fields of the Millipore filter (mm); A the filter area; n the number of hyphae intersections on the horizontal lines of the grid of reticulated ocular; H the total length of the horizontal lines of the grid.

2.1.2.3 Dehydrogenase activity. Soil dehydrogenase activity (a measure of microbial activity) was determined according to Alef (1995). Moist soil (5 g) was weighed into test tubes and mixed with 5 ml of triphenyl tetrazolium chloride (TTC) solution (0.3%). The tubes were sealed and incubated in the dark for 24 h at 37 °C. The controls contained only 5 ml of Tris–HCl buffer 100 mM (without TTC). After the incubation, 20 ml of methanol were added to each tube. The tubes were then shaken vigorously for 1 min and centrifugated at 2000 rpm for 10 min. The triphenyl formazan (TTF) formed by the TTC reduction was measured spectrophotometrically at 485 nm against the blank.

2.1.2.4 Measurement of paclobutrazol residues in each soil. To obtain the soil paclobutrazol residues, a 10 g subsample of each soil was mixed with 80% MeOH (100 ml) in a flask, and shaken (100 rpm, room temperature) overnight. After 24 h, the soil extract was separated by centrifugation (8000 rpm) and the extraction process repeated again (100 rpm, room temperature, 7 h). The extracts were combined, the MeOH removed with a rotary vacuum evaporator (35 °C), and the aqueous residue adjusted to pH 11 with NaOH (6.5N). The resulting aqueous phase was partitioned in methylene chloride (3 ml × 50 ml). The extracts were combined, concentrated to approximately 2–3 ml with a rotatory vacuum evaporator (35 °C), and taken to dryness using a stream of nitrogen. The paclobutrazol residues were redissolved in n-hexane, made to volume and analysed by capillary gas chromatograph using a thermionic nitrogen specific detector, capillary column HP 5 (30 m × 0.32 mm × 0.25 μm) under the followings operation conditions: carrier gas, helium 5 ml min⁻¹, temperature program, 60 °C then 20 °C min⁻¹ to 220 °C (15 min). Duplicate standard solution and samples extracts were injected. Paclobutrazol retention time was found to be 9.9 min. The limit of determinations was reached 0.25 μg g⁻¹ (Ferracini and Silva, 1999).

2.1.2.5 Statistical analysis. The univariate split plot method was utilized to analyse all variables, once that hypothesis of uniformity of the covariance matrix was accepted. Initially an overall analysis of variance (ANOVA) model was used to each dependent variable. The model included the main effects of date,
3. Results and discussion

The paclobutrazol addition in soils with mango cropping, at Petrolina, negatively affected the soil microorganisms (Table 1). Average values of this inhibition were 58, 28 and 28% of controls, for total viable counts of bacteria and actinomycetes and cfu’s of fungi, respectively. Although this methodology only allows the detection of a small fraction of the total microbial community, this approach has been used to assess the differential response of soil communities to single and multiple doses of organic pollutants (Vieira et al., 2001) and heavy metals (Knight et al., 1997).

The introduction of organic pollutants, that can potentially act as toxic substances and nutrient sources, has been shown to preferentially stimulate specific populations (Atlas et al., 1991). In our study, the deleterious effect of paclobutrazol on the fungal community was on average 29% smaller than that observed for bacteria. Since numerous populations of different character coexist and interact with each other to form a microbial community, the differential effect of paclobutrazol on specific groups of microorganisms may alter the microbial population balance, affecting negatively the soil fertility (Pankhurst et al., 1996; Thompson et al., 1999; Smith et al., 2000).

The dehydrogenase activity in Petrolina soil was negatively influenced by the growth regulator (Fig. 1). Analysis of variance showed that both time and dose regimes had a significant effect on this activity and the interaction between these two factors was significant ($P < 0.005$). Within the first week, the dehydrogenase activity decreased 23 and 44%, respectively, for the 80 and $160 \mu g g^{-1}$, when compared to the control. Thereafter, this activity gradually recovered, probably due a higher availability of C not only of the microorganisms which died due to non-adaptation to the new environment conditions, but also to the microorganisms that were killed as a result of the paclobutrazol application. After the 10th day of incubation, the dehydrogenase activity increased in all the treatments until the 15th day. Despite this increase, dehydrogenase activity was still negatively affected by the presence of the paclobutrazol, when compared to the controls, although significant differences among the doses were not observed. After 10, 15, and 27 days of incubation, the average decrease observed in the enzyme activity, due to the application of the paclobutrazol, was 38, 12 and 63%, respectively, compared to the controls. This high inhibition in the last evaluation may be related to the negative effect of the compound associated with a possible stress on the microbial community, such as the depletion of carbon sources. This hypothesis is based on the fact that a decrease was also seen in the control, although to a smaller extent than in the paclobutrazol treatments.

There are a number of problems with assessing the significance of the side effect tests in soil contaminated with high pesticide concentrations. It can be difficult to discriminate a real effect from background variation in the measured activity. It is known that at field concentrations and under field conditions, some xenobiotics can sometimes have a short-life inhibitory effect, but there is not enough scientific evidence to assure that xenobiotics generally have a measurable long-term effect on soil microbial populations (Sommerville and Greaves, 1987).

### Table 1

<table>
<thead>
<tr>
<th>Evaluation times</th>
<th>Without paclobutrazol</th>
<th>With paclobutrazol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bacteria ($\times 10^4$)</td>
<td>Fungi ($\times 10^2$)</td>
</tr>
<tr>
<td>3 November 1998</td>
<td>36.4</td>
<td>57.8</td>
</tr>
<tr>
<td>30 November 1998</td>
<td>36.8</td>
<td>71.6</td>
</tr>
<tr>
<td>27 January 1999</td>
<td>25.0</td>
<td>33.8</td>
</tr>
<tr>
<td>26 March 1999</td>
<td>41.4</td>
<td>49.1</td>
</tr>
</tbody>
</table>

Average of five replications per plate.
In Lins soil it was not observed significant effect of the paclobutrazol on dehydrogenase activity (Fig. 2). The statistical analysis demonstrated only variation in relation the incubation time ($P \leq 0.05$).

Studies have shown correlations between enzyme activity and soil structural properties (Miller and Dick, 1995). However, the results obtained for Petrolina soil and Lins soil in relation to the dehydrogenase activity are not related to differences in soil characteristics, since both belong to the same textural class and had similar organic matter contents. For to analyse the absence of harmful effects in the Lins soil, as opposed
to the results observed in the Petrolina soil must be taken into consideration that the dose of paclobutrazol in the Petrolina soil was 10-fold higher than the one applied in the Lins soil. This could suggest that in the recommended doses, paclobutrazol should not cause a deleterious effect in the soil microflora (Gianfreda and Bollag, 1996; Dinelli et al., 1998).

Considering that the activity of this enzyme is a parameter that should be carefully interpreted, once that it is influenced by many factors (Nannipieri et al., 1990), these results also may indicate the presence of a different microflora between the two soils. The composition of the microbial community determines its potential for enzyme synthesis, and thus, any modification of the community due to environmental factors should be reflected on the level of soil enzymatic activities. The possible effect of the soil microflora composition on the dehydrogenase activity has been already cited by others researchers (Beyer et al., 1993).

For Petrolina soil, an inhibitory effect of paclobutrazol was observed on the hyphal length parameter after the 7th day of incubation, without significant differences amongst the pollutant doses (Fig. 3). The average decrease observed in hyphal length was about 79% in relation to the control treatment. This decrease could be related to the fungicide effect of paclobutrazol (Jackson et al., 1996), once that the average recovery of the growth regulator in Petrolina and Lins soils was 85 and 78%, respectively, demonstrating that its content was slowly decreased.

The microbial biomass C measured in the soils studied, did not demonstrate a harmful effect of paclobutrazol on the microbial populations. The statistical analysis showed, in the case of Petrolina soil,
a significant effect only for the period of incubation. Therefore, the results were exhibited only considering the averages for the three doses of the product within each time (Fig. 4). In the Lins soil, a significant effect on the days of incubation was observed, but in this case, it was different for each dose of the compound (Fig. 5).

The decrease in microbial biomass C, in Petrolina soil, over time, in all treatments, can be associated to variations in edaphic factors in arid and semi-arid regions. In these ecosystems, the climatic extremes are such that many of the species in any taxon will be operating very close to their tolerance limits (Whitford, 1996). This fact more than the lower diversity affects the composition of the functional fraction of the soil biota determining alterations so far through the selectivity as through the alternation of species resistant to the stress caused. However, the effect of paclobutrazol could have been hidden by the outbreak of resistant microorganisms after those sensitive to the growth regulator.

The data of this work demonstrated that the problem with the paclobutrazol utilization would be in its application for long periods, once that, due to its low degradation, it could accumulate in soil and harbour the microflora. A microbiological monitoring in areas subject to frequent applications of this growth regulator should be needed.

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References


