Enhanced degradation of iprodione in soil after repeated treatments for controlling Sclerotinia minor

C. MARTIN¹, Danielle VEGA², J. BASTIDE² and P. DAVET³

¹SCREAPO, 19 Avenue de Grande-Bretagne, F-66025 Perpignan Cedex. ²GERAP-Université de Perpignan, Chemin de la Passio vella, F-66025 Perpignan Cedex and ³INRA, Laboratoire de Biologie et Pathologie végétale, F-34060 Montpellier Cedex 1, France

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Abstract

Poor field control of lettuce collar rot by iprodione was observed in southern France and was attributed to enhanced biodegradation of the fungicide. Enhanced biodegradation was obtained in vitro after repeated applications of iprodione to non-degrading soils. Normal soils became biodegrading after mixing with degrading soils (3 vol./1 vol.). Activity of the responsible microflora seemed dependent on soil physico-chemical characteristics.

Introduction

Instances of poor field control of collar rot of lettuce (Sclerotinia minor Jagger) by iprodione (isopropylcarbamoyl-1(dichloro-3,5-phenyl)-3-hydantoin) have been observed in Roussillon (South France) since 1983 (Martin, 1983). Similarly, occasional failure of control of white rot of onion (Sclerotium cepivorum Berk.) in England with the same fungicide has been reported (Entwistle et al., 1983). To explain this phenomenon we first tested, and eliminated, the hypothesis of a modification of antagonistic soil microflora, as was suggested for benzimidazoles (Davet, 1979). The hypothesis of development of S. minor iprodione resistant strains was not retained because tolerant strains were very uncommon and their resistance level was low. Field observations and laboratory data led us to the conclusion that the phenomenon was due to enhanced microbial degradation of the fungicide, induced by repeated applications. We present here preliminary results of research in progress, which largely confirm the work carried out under different environmental conditions by Walker (1987a) and Walker et al. (1986).

Methods

Soil samples were collected from the top 10 cm of 46 fields around Perpignan. All were sandy-loam soils (less than 15% clay), with pH (measured in water) between 6 and 7.5, and organic matter content (determined by Anne's method) between 1 and 2.3%. All the samples were passed through a sieve with 2 mm diameter mesh and adjusted to a moisture content of 20% before every trial.

The fungicide used was a commercial wettable powder formulation of iprodione (Rovral, 500 g a.i. kg⁻¹). The product was suspended in water and thoroughly mixed with air-dried soil. Water volume was calculated in order to obtain a final moisture content of 20% (approximately −0.1 MPa) and an iprodione concentration of 50 mg kg⁻¹. Samples were distributed in 10 tightly closed 100 mL vials (25 g per flask) and incu-
bated at 28°C. Subsamples from 2 vials were taken after 0, 2, 3, 7 and 14 days for HPLC determination of fungicide residues or dosage of 3,5-dichloroaniline. Twenty five mL of acetone were added to 25 g of soil and mixed with a rotary agitator during 1 h. Five mL of the acetone solution were pipeted, evaporated to dryness and redissolved in hexane (10 mL). An aliquot of the solution was injected in a 25 cm Spherisorb S₅ NH₂ column. The solvent was a mixture of iso-octane and ethanol (92/8) and the absorbance peaks were observed at 236 nm. We observed that in our samples, as in Walker's (1987b) soils, iprodione degradation was consistently linked with 3,5-dichloroaniline formation (Fig. 1). Therefore it was possible to evaluate iprodione degradation by measuring 3,5-dichloroaniline production, which could be done by a simple colorimetric test. Walker's (1987b) procedure was followed and the dosage was quantified by optical density readings at 530 nm.

Complete or partial sterilization of soil was obtained by heating or by antibiotics. In the first case, soil samples were autoclaved at 100°C during 1 h, 3 times at 24 h intervals. In the second case, we used either cycloheximide (2 mg g⁻¹) or a mixture of tetracycline + ampicillin + chloramphenicol in an ethanol solution, adjusted to obtain a final concentration of 15, 50 and 30 μg g⁻¹, respectively. Samples with antibiotics were incubated 5 days (28°C, 20% moisture content) before addition of iprodione.

**Results and discussion**

In soils with comparable pH and organic matter content, the rate of iprodione degradation was dependent on the number of previous field treatments with this fungicide: for instance (Fig. 1), in soil A with no history of iprodione use, only a small quantity of 3,5-dichloroaniline was detected after a lag phase of 3 days; in soil B, which had received at least 5 applications a year for the previous 4 years, 3,5-dichloroaniline was quickly produced. Similar results were obtained with other soils.

In contrast, no iprodione degradation was observed when the fungicide was added to autoclaved soils (Fig. 4). The formation of 3,5-dichloroaniline was also inhibited in soils amended with a mixture of antibiotics. When cycloheximide was used, a small quantity of the product was detected (Fig. 2). It was possible to restore the degrading capacity of an autoclaved sample by mixing (3 vol./1 vol.) with a non-sterilized sample. Enhanced biodegradation could be observed in 'normal' soils after repeated additions of iprodione: 0.2 mg iprodione were added at 2 day intervals to 20 g soil samples suspended in 100 mL distilled water and incubated at 28°C. After 2 days resting time, the supernatant was eliminated and, after air drying, the soil was analysed following the above mentioned procedure. Five to 7 repeated applications were enough to induce enhanced degradation in the soils we tested. When 1 vol. of such an 'activated' soil was mixed with 3 vol. of a non-sterilized slow-degrading one, enhanced biodegradation was also observed (Fig. 3).

The effect on a slow-degrading soil of 2 different 'activated' soils was compared. Enhanced biodegradation was more important when the 'activated' soil introduced was originally highly degrading (Fig. 3). However, when the same 'activated' soil was mixed with 2 different autoclaved samples (1 vol./3 vol.) 3,5-dichloroaniline production was higher and faster if the receiving