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INSECTICIDES AND MYCORRHIZAE - CHLORFLUAZURON

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ABSTRACT. Insecticides are applied to crops to control insect pests, indirectly increasing crop production. These chemicals can have negative effects on other organisms in the environment that may negate the production gains. Newer insecticides tend to be more specific. Chlorfluazuron (Jupiter®) targets larvae of Lepidoptera and Coleoptera by interfering with chitin synthesis. Thus, killing beneficial Hymenoptera is unlikely. The effects of this insecticide on Glomalean fungi which form (vesicular-) arbuscular mycorrhizae were investigated. Spore germination of *Gigaspora gigantea* and *G. rosea* was severely inhibited in high concentration. However when the insecticide was sprayed on corn (*Zea mays*) seedlings, there was not a significant reduction in previously established mycorrhizae.

INTRODUCTION

Glomalean fungi form (vesicular-) arbuscular (VA) mycorrhizae, beneficial mutualisms with the roots of most crop plants. However, there have been few studies on the effects of pesticides on these fungi and the formation of VA mycorrhizae (Trappe *et al.*, 1984; Persad-Chinnery *et al.*, 1992).

Chlorfluazuron (N-[4-3-chloro-5-trifluoromethyl-2-pyridyloxy)-3,5-dichlorophenyl]-N'-(2,6-difluorobenoxyl) urea) is a benoxylurea insecticide, chitin synthesis inhibitor marketed under trade names including Aim, Atabron, Helix and Jupiter. It is one of the new generation of more environmentally friendly insecticides that selectively target insect groups. It is effective against larvae of Lepidoptera and Coleoptera. It does not affect adult insects, however, their eggs fail to hatch. It is not effective against aphids, whiteflies or mites (Thompson, 1995).

What effects does this insecticide have on plants colonized by VA mycorrhizal fungi and established mycorrhizae? In using this insecticide are farmers losing the benefits of their crops being mycorrhizal? Mycorrhizal plants have been shown to have enhanced nutrient uptake, especially of phosphorus (Smith and Read, 1997), and show fewer incidences of micronutrient deficiencies (Abbott and Robson, 1984; Persad-Chinnery and Chinnery, 1996). Increased growth (Smith, 1980; Gianinazzi-Pearson *et al.*, 1989; Nielsen, 1990), plant disease resistance (Morandi *et al.*, 1984; Feldmann *et al.*, 1989; Caron, 1989), drought tolerance (Mosse and Hayman, 1971; Davis *et al.*, 1992), and reproduction (Koide *et al.*, 1988) have been reported.

This paper reports the effects of Jupiter® on the germination of Glomalean spores of the genus *Gigaspora* and VA mycorrhizae established with corn (*Zea mays* L.).

METHODS

Spore germination tests: Mycorrhizal inocula were obtained from the International Culture Collection of Arbuscular and VA Mycorrhizal Fungi (INVAM), West Virginia University. Spores of *Gigaspora gigantea* (Nicol. & Gerd.) Gerdemann and Trappe (MA453A-6) and *G.*

rosea Nicolson and Schenck (FL105-9) were extracted by wet-sieving and decanting, and surface decontaminated with sodium hypochlorite.

The germination tests were established in 24 well tissue culture plates. (Bellco Biotechnology). The growth area of each well was 2 cm^2. One spore was placed into each well and 1 ml of solution added. The concentrations of Jupiter® 120EC (Ciba-Geigy) used were the manufacturer's recommended rate and twice this (0.0005 and 0.001 ml ml^{-1} sterile distilled water). Ten spores constituted a replicate and five replicates of *G. rosea* and three of *G. gigantea* were established. The culture plates were sealed with parafilm and incubated in the dark at ambient temperature.

Observations were made using a dissecting microscope and the development of a germtube was used to determine germination.

Effects on corn mycorrhizae: A nutrient poor growing medium of equal parts sieved top soil, sieved sand and vermiculite was prepared. This was transferred to forty 15cm plant pots. Five holes were made in each pot and approximately one teaspoon of mycorrhial inoculum and a washed corn "seed" placed in each. The inoculum was collected from a garden bed at Mount Standfast, St.James, Barbados. Previous examination had shown that this contained large numbers of Glomalean spores from the genera *Gigaspora*, *Glomus* and *Scutellospora* as well as root fragments.

Three weeks later, the corn plants were thinned to one plant per pot and 24 pots were selected at random. A randomised complete block design with eight replicates were arranged in a shade house after treatment with insecticide. The plants were sprayed to run-off with 0.0007 or 0.0014 ml ml^{-1} Jupiter® or water. These concentrations were chosen to be equivalent to the recommended rate of 2 teaspoon per gallon of water and twice this.

Fifteen days after chemical treatment, the roots were harvested and washed under running water to remove all soil. They were cut into approximately 1 cm lengths and cleared overnight in 2.5% KOH, washed in several changes of tap water, rinsed in distilled water, stained overnight in 0.1% chlorazol black E (w/v lacto-glycerol) and destained in glycerol.

Forty root segments from each plant were mounted, ten at a time, between two microscope slides and gentle pressure applied. They were then viewed under a compound microscope at x40 magnification and mycorrhizal colonisation scored as none, low or high. Low and high scores were summed to estimate percentage colonisation of each plant's roots.

Statistical analysis: All percentage data were arcsine transformed before analysis of variance.

RESULTS

Spore germination: The effects of chlorfluazuron on the germination of *G. gigantea* and *G. rosea* are shown in figure 1. For both species, the final germination percentage in 0.001 ml ml^{-1} concentration was significantly less than in either the lower concentration or the control. Germination in 0.0005 ml ml^{-1} was not significantly different from the control.

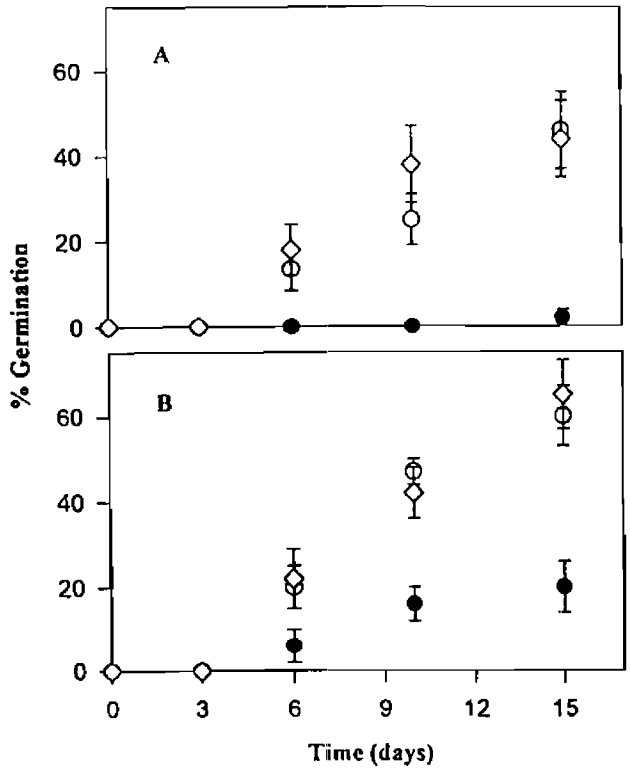

Fig. 1. Germination of spores of *Gigaspora rosea* (A) and *G. gigantea* (B) in different concentrations of Jupiter. (◇ = 0.0000, ○ = 0.0005 and ● = 0.0010 ml ml^{-1}) (means and standard errors).

Corn mycorrhizae: Percentage mycorrhizal colonisation is shown in figure 2. Analysis of variance failed to show a significant difference between treatments ($F_{2,14} = 3.07$, $P = 0.0786$). Duncan's multiple range test at $\alpha = 0.10$, showed the mean % colonisation in the 0.0014 ml ml^{-1} treatment to be significantly different from the control. However, the 0.0007 ml ml^{-1} treatment was not significantly different from either the control or the higher concentration.

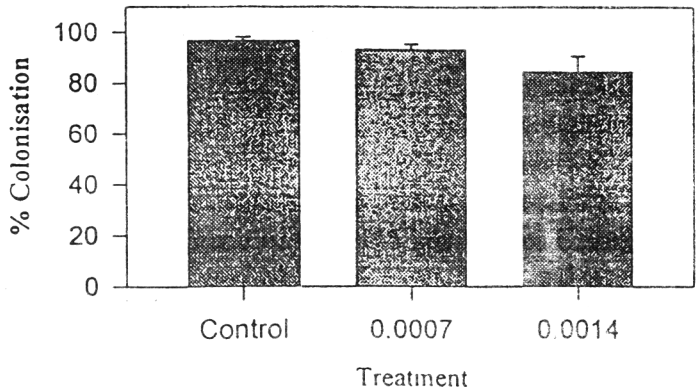

Fig. 2. Relationship between percent. mycorrhizal colonisation and concentration (ml ml^{-1}) of Jupiter applied (means/standard errors of eight replicates).

Figure 3 shows a trend of reduced mycorrhizal colonisation and intensity of colonisation with increasing concentration of the applied insecticide.

DISCUSSION

Very little is known about VA mycorrhizae in the Caribbean (Persad-Chinnery *et al.*, 1995) and even less about the effects of agronomic practises on them (Persad-Chinnery *et al.*, 1992). The aim of this study was to examine the effects of a pesticide which in recent years has become popular in Barbados for controlling a number of pests including Diamondback moth and army worms. Another reason for using Jupiter® in these experiments is its main mode of action - the inhibition of chitin synthesis. Chitin is a component of the cell wall of fungi and in both spore germination and the spread of the fungus in plant roots, *de novo* synthesis of cell wall material is necessary

The spore germination experiments showed a dose effect with the recommended rate not significantly reducing the rate of, or total, germination but twice the rate having an extreme effect. Germination success was greater for *G. rosea* (Fig. 1A) than for *G. gigantea* (Fig. 1B). It is possible that this is related to the relative sizes of the spores. Those of *G. rosea* have a largest diameter in the 200-400 :m range and those of *G. gigantea* are >400- >600 μm (the largest fungal spores known).

The recommended rate did not adversely affect the germination of either species and it would require the application of concentrations of considerably more than this to the crop to obtain anything like this concentration in the soil. Therefore, it can be concluded that under normal circumstances chlorfluazuron will not adversely affect spore germination in crop fields.

Slightly higher concentrations of Jupiter® were used to treat the mycorrhizal corn plants. This was a result of using the alternate 2 teaspoon per gallon of water rather than the 2.5 cm^3 L^{-1} as the recommended rate (Ciba-Geigy, n.d.).

Although not significant at the $\alpha = 0.05$ level, there was a decrease in mycorrhizal colonisation of the roots with increased concentration of the insecticide (Fig. 2). One reason for the failure to obtain significance was the variability among the replicates and, although the blocks showed no significant difference ($F_{7,14} = 1.76$, $P = 0.17$), the error term in the analysis of variance contributed over 17% of the total mean squares.

Fig. 3 shows that the proportion of root segments that were non-mycorrhizal increased and that those highly mycorrhizal decreased with increased concentration of insecticide. However, it is unknown whether this would have affected the final yield of the crop.

Any effect would have resulted from the application of the insecticide to the soil either directly, because of run-off at the time of application or subsequent washing from the leaves during watering. Jupiter® is an insecticide of high persistence on the surface of leaves and is slowly degradable by sunlight (Ciba-Geigy, n.d.). It has no systemic activity (Thompson, 1995). Since Jupiter® will kill beetle larvae, there may be some benefit of the insecticide entering the soil.

More experiments involving other agricultural chemicals are needed so that we can avoid the loss of the myriad benefits of mycorrhizae by prudently using compatible compounds.

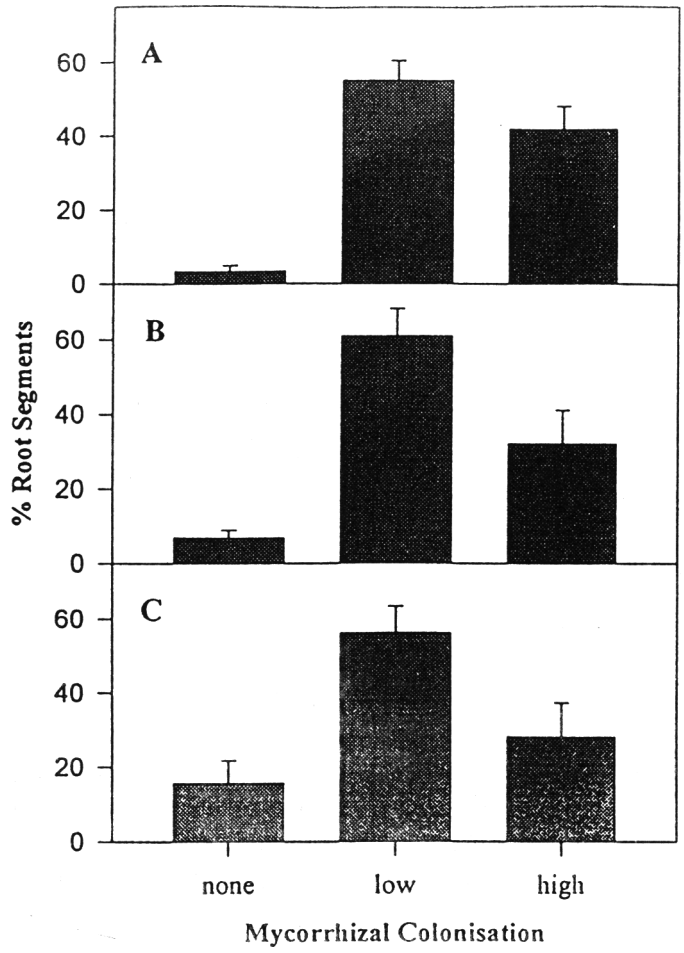

Fig. 3. Variation in intensity of mycorrhizal intensity with concentration of Jupiter applied. (A = control; B = 0.0007 ml ml^{-1} ; C = 0.0014 ml ml^{-1}) (means and standard errors of eight replicates)

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