OTHER CONTRIBUTIONS — OTRAS COLABORACIONES

RESEARCH PAPERS — TRABAJOS DE INVESTIGACION

EFFECT OF CARBOFURAN AND OTHER PESTICIDES ON VESICULAR-ARBUSCULAR MYCORRHIZAE IN PEANUTS [EFECTO DEL CARBOFURAN Y OTROS PESTICIDAS SOBRE LAS MICORRIZAS VESICULO ARBUSCULARES DEL MANI]

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ABSTRACT

A variety of pesticides applied preplant were evaluated in the field for their effect on development of vesicular-arbuscular mycorrhizae (VAM) on 'Florunner' peanuts 30 days after planting. Only the biocide sodium azide and the nematicide carbofuran caused significant decreases in VAM development; the effect of carbofuran was confirmed in the greenhouse.

INTRODUCTION

The role of vesicular-arbuscular mycorrhizae (VAM) in increasing nutrient uptake and water absorption by the host plant has been well established (2, 3). Plant pathologists however, are only now becoming aware of the possible effects of pesticides to these beneficial symbionts. Recently Bird *et al.* (1) reported that the nematicide DBCP (2, 4-dibromo 3-chloropropane) significantly increased endomycorrhizal development in cotton roots. They hypothesized that DBCP either controlled nematodes that could limit mycorrhizal development such that mycorrhizal development was favored, or that the nematicide altered the physiological condition of the roots. In a preliminary report, Pfleger and Stewart (7) noted that 2 soil fungicides decreased numbers of chlamydospores of some *Glomus* spp. Nesheim and Linn (5) reported reductions in greenhouse-grown corn endomycorrhizae when treated with some soil fumigants or fungicides. These studies indicate that VAM may be affected, but they have never been confirmed under field conditions. This study was designed to determine if peanut VAM are affected by soil fungicides under field conditions, and to confirm the results with greenhouse tests.

MATERIALS AND METHODS

Plots were established in a field with a long history of peanut *(Arachis hypogaea* L.) culture. The soil type was a Dothan sandy loam with no significant populations of pathogenic nematodes. Pesticides were applied to the soil either 21 days before planting or at planting time. Formulations, rates and methods of application were according to current recommendations (Table 1). Treatments were arranged in randomized complete blocks and were replicated eight times.

Thirty days after planting 4 plants were removed from each plot and the feeder roots were examined for development of endomycorrhizae by a procedure modified from Phillips and Hayman (6). Two randomly selected feeder roots from each plant were placed in 5 ml of 10% KOH for 15 min at 95C. Roots then were transferred into 5 ml of saturated chloral hydrate containing 0.01% acid fuchsin and were autoclaved 30 min at 121 C. Roots were removed, mounted in glycerin, and examined for VAM develop-

Table 1. Pesticides and application methods

Pesticide						
Common Name	Chemical Name	Formulation	Rate/ha (a.i.)	Usual Use	Method[1]	Application date
DBCP	2, 4-Dibromo-3-chloropropane	86% EC	7.0 liters	Nematicide	Chisel in furrow	Planting
Ethoprop	0-Ethyl *S*-*S*-dipropyl phosphorodithioate	10% G	3.4 kg	Nematicide	Banded incorp.	Planting
Fensulfothion	*0*-*0*-dimethyl-0-0(4-(methyl-sulfinyl)phenyl) phosphorothioate	15% G	3.4 kg	Nematicide	Banded incorp.	Planting
Carbofuran	2, 3-dihydro-2, 2-dimethyl-7-benzofuranyl methylcarbamate	10% G	3.4 kg	Nematicide	Banded incorp.	Planting
Sodium azide	Sodium azide	8% G	40 & 54 kg	Biocide	Broadcast incorp.	21 days preplant
Metham sodium	Sodium N-methyldithiocarbamate	4 L	93.5 liters	Biocide	Broadcast incorp.	21 days preplant
Vorlex	20% Methylisothiocyanate + 80% dichloropropenes and dichloropropanes	9.6 L	93.5 liters	Biocide	Broadcast incorp.	21 days preplant
PCNB + ethazole	Pentachloronitrobenzene + 5-Ethoxy-3-trichloromethyl-1, 2, 4-thiodiazole	10%+2.5%G	2.2 + 0.4 kg	Soil fungicide	In furrow	Planting

[1] Banded applications were made in a 30 cm band in the planting area; in-furrow application effectively treats only a 4-5 cm region in the seed planting zone.

ment under a light microscope. Ratings were on a 0-5 scale, with a 0 rating for no visible mycorrhizae and a 5 rating for extensive development of mycorrhizae with vesicles and arbuscules present. Data are reported as the means of the eight roots examined from each test plot.

Field observations were confirmed in the greenhouse using the systemic nematicide carbofuran. In order to approximate field conditions, a natural Dothan sandy loam with a peanut history was used. Carbofuran was incorporated into the upper 10 cm of a 15-cm-deep soil contained in a 15-cm diam plastic pot. Florunner peanut seed were planted 8-cm deep and were watered and grown at a mean temperature of 28 C. Roots were removed after 30 days and examined as in the field studies.

Results of both greenhouse and field tests were statistically analyzed and means were compared using Dunnet's test (8).

RESULTS AND DISCUSSION

Field data indicated that most of the soil pesticides tested had little or no effect on the VAM fungi in peanuts (Table 2a); however, both sodium azide and carbofuran significantly reduced VAM development. Most biocides would be expected to have some effect on development of endomycorrhizae, thus the activity of azide was not unexpected. Carbofuran, however, is an insecticide-nematicide for which little or no nontarget activity has been reported. The fact that carbofuran was equally effective in decreasing development of VAM on peanuts in the greenhouse (Table 2b) supports the field observations. Data not presented here indicated that mycorrhizae levels in the peanut feeder roots had returned to normal levels in all treatments when field plots were re-examined 120 days after planting.

Table 2a. Effect of soil pesticides on vesicular-arbuscular mycorrhizal development on roots of field-grown peanuts 30 days after planting.

		Subjective Rating[1] Replication								
Treatment	Rate	1	2	3	4	5	6	7	8	X^2
Control	- -	5	5	5	5	5	5	5	5	5.0
Metham sodium	93.5 l	5	5	5	5	5	5	5	5	5.0
Vorlex	93.5 l	5	4	5	5	3	3	5	5	4.4
DBCP	7.0 l	5	5	5	5	5	5	5	4	4.9
Ethoprop	3.4 kg	5	5	5	5	5	5	5	5	5.0
Fensulfothion	3.4 kg	4	5	4	5	5	4	5	4	4.5
Carbofuran	3.4 kg	5	4	1	0	1	1	2	1	1.9**
NaN 3	40.0 kg	4	5	5	4	5	5	5	4	4.6
NaN 3	54.0 kg	1	4	5	4	4	0	3	4	3.1*
PCNB + ethazole	2.2 + 0.6 kg	4	5	3	5	4	5	5	5	4.4

Table 2b. Effect of carbofuran on vesicular-arbuscular mycorrhizal development on roots of greenhouse-grown peanuts 30 days after planting.

Treatment	Rate	Subjective Rating[1] Replication 1	2	3	4	5	6	7	X^2
Control	- -	4	5	5	4	3	3	5	4.1
Carbofuran	3.4 kg	1	3	5	1	3	1	1	2.1*
Carbofuran	6.8 kg	1	1	1	1	1	0	1	0.9**

[1] Methodology of subjective rating:

5 - extensive mycelium, vesicles, and arbuscules
4 - mycelium, arbuscules, and vesicles
3 - mycelium evident with occasional vesicles
2 - some mycelium present; no arbuscules or vesicles
1 - one or two isolated strands of mycelium/root
0 - no evidence of fungal growth

[2] Statistical notation: (*) significantly different from the control at $P > 0.05$ and (**) $P > 0.01$, using Dunnet's test.

Microscopic examination of peanut roots from the field and the greenhouse revealed arbuscules and numerous vesicles, indicating that the fungus involved probably was a species of *Glomus* (4).

Carbofuran has been used successfully as a commercial nematicide in peanuts for the last few years; yield benefits have been substantial. Results of this study indicate that a carbofuran yield response may be affected by losses of VAM; in low nutrition soils yields could be reduced, while in high nutrition soils yields could be increased due to the removal of "parasitic" VAM.

The effect of carbofuran on other fungal species that form VAM on peanuts was not determined. Information is needed on the effect of carbofuran on VAM infections already established when the nematicide is applied. Carbofuran could be useful in determining the parasitic effect of VAM in high nutrition soils under field conditions.

RESUMEN

El efecto de varios pesticidas sobre el desarrollo de las microrrizas arbúsculo- - vesiculares del maní Florunner se estudió 30 dias después de la siembra bajo condiciones de campo. Sólo la azida de sodio, un biocida, y el nematicida carbofuran, causaron reducciones significativas en el desarrollo de las micorrizas; el efecto del carbofuran fué confirmado en estudios de invernadero.

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ACKNOWLEDGEMENTS

The authors acknowledge the technical contributions of Steve L. Brown, and Susan E. Corcoran and the financial support of the Alabama Peanut Producers Association.

DESCRIPCION DE UN NUEVO METODO DE COLORACION Y DESTEÑIMIENTO PARA LA VISUALIZACION EFECTIVA DE ORGANOS INTERNOS DE LOS NEMATODOS [A NEW METHOD FOR THE DIFFERENTIAL STAINING OF INTERNAL ORGANS OF NEMATODES]

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RESUMEN

Se estudió el uso del tinte azul de toluidina al 0.05% (p/v) y un regulador de fosfato (0.05M, pH 4.6) para efectuar la coloración de espécimenes *in toto* de *Hoplolaimus galeatus* y de *Helicotylenchus dihystera.* La inmersión de los nemátodos en el tinte por 7 hras a 60C dió muy buena coloración. Subsiguientemente, cuando los espécimenes se sometieron a un lavado en una solución reguladora de fosfato (0.01 M, pH 4.6) por 5 hras a 55-60C se obtuvo una buena resolución de la región esofágica y del aparato reproductor tanto en las hembras como en los machos. Resultados igualmente satisfactorios se obtuvieron a temperaturas más bajas cuando se aumentó la duración del proceso.

INTRODUCCION

La enseñanza de la taxonomía en nematología se dificulta debido a la clasificación de espécimenes en base a caracteres morfológicos que muchas veces son difíciles de visualizar por el principiante. Las dificultades aumentan cuando solamente se dispone de micoscopios ordinarios de poder resolutivo inadecuado. Sin embargo, la literatura (1, 2, 9) sugiere técnicas de coloración para solucionar estos problemas. Por ejemplo, recientemente hemos sugerido la utilización de técnicas histoquímicas para visualizar detalles morfológicos de la cutícula de nemátodos sin necesidad de usar equipos costosos (6). Este artículo describe un método de teñir los ácidos nucleicos (3, 5) para la mejor visualización de los sistemas reproductivo, digestivo y órganos internos de los nemátodos.