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Original article

# Effect of a systemic fungicide on the establishment of Arbuscular Mycorrhizogenic Fungi in maize (*Zea mays* L.)

## Efecto de un fungicida sistémico en el establecimiento de Hongos Micorrizógenos Arbusculares en el cultivo de maíz (*Zea mays* L.)

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**ABSTRACT:** Fungicides not only affect the pathogens fungi, but also to others, including those that are beneficial for plant growth, such as arbuscular mycorrhizal fungi. The aim of the present work was to evaluate the effect of a commercial systemic fungicide on the colonization of arbuscular mycorrhizal fungi, inoculated in corn crop, under semi-controlled conditions. Corn plants were inoculated with strains *Rhizophagus irregularis* INCAM 11, *Glomus cubense* INCAM 4 and with a conglomerate of different species of arbuscular mycorrhizal fungi, with and without of a systemic fungicide. Colonization frequency and visual density of mycorrhizal fungi were determined at 54 days after inoculation. In addition, the root length, total fresh weight and total dry weight of corn plants were determined. The conglomerate of different species of arbuscular mycorrhizal fungi with fungicide had the highest visual density. The presence of the fungicide increased the root length, total fresh weight and total dry weight of the control plants and those inoculated with *Glomus cubense* INCAM 4. The effect of strain *Rhizophagus irregularis* INCAM 11 on the corn growth was not affected for systemic fungicide.

**Key words:** mycorrhiza, pesticide, root length.

**RESUMEN:** Los fungicidas no solo afectan a los hongos patógenos, sino también a otros hongos, incluidos aquellos que son beneficiosos para el crecimiento de las plantas, como los hongos micorrízicos arbusculares. El objetivo del presente trabajo fue evaluar el efecto de un fungicida sistémico comercial en la colonización de hongos micorrizógenos arbusculares, inoculados en el cultivo del maíz, en condiciones semicontroladas. Para ello, se inocularon plantas de maíz con las cepas *Rhizophagus irregularis* INCAM 11, *Glomus cubense* INCAM 4 y con un conglomerado de diferentes especies de hongos micorrízicos arbusculares, en presencia o no del fungicida sistémico. A los 54 días de la inoculación se determinó la frecuencia de colonización y la densidad visual de los hongos micorrízicos empleados. Además, se determinó la longitud de la raíz y la masa fresca y seca total de las plantas de maíz. El conglomerado de diferentes especies de hongos micorrízicos arbusculares, junto al fungicida, presentó la mayor densidad visual. La presencia del fungicida incrementó la longitud radical y la masa fresca y seca total de las plantas control y las inoculadas con *Glomus cubense* INCAM 4. El efecto de la cepa *Rhizophagus irregularis* INCAM 11 en el crecimiento del maíz no se afectó con el empleo del fungicida sistémico.

**Palabras clave:** longitud de la raíz, micorriza y pesticida.

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## INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) (*Glomeromycota*) are an integral part of many ecosystems and are considered particularly advantageous because they are associated with most of the vascular plants studied (1,2). These fungi are obligate biotrophs and colonize roots of their host plants, obtaining sugars. Instead, host plants receive mineral nutrients and water, absorbed and transported through a fine network of extended extraradical hyphae from roots to the surrounding soil (3).

AMF use is feasible for any agricultural production system because of functions they perform once associated with plants. Among them it is the increase in the absorption of mineral nutrients and water, from an increase in the volume of soil explored. They confer greater plant resistance to toxins and pathogen attack and increase the translocation and solubilization of essential elements (2,4). AMF contribute to reducing adverse effects of abiotic stress, such as the presence of heavy metals and soil salinity, as well as biotic stress, caused by numerous pathogens (5,6).

On the other hand, in current cropping systems, fungicides are used to control or eliminate fungal phytopathogens. However, these products could also affect the survival of indigenous microorganisms, including those that are beneficial to plant growth, such as AMF. The expected effect of mycorrhization on host plant growth and development may be negatively affected by the use of fungicides in agricultural systems (7).

Systemic fungicides are those that, when applied to the soil, can be absorbed by roots and transferred to other parts of the plant. They are persistent substances and their action on mycorrhizal fungi can negatively influence the development of their vegetative and reproductive structures, because they are designed to destroy fungal chitins, as well as other specific proteins and enzymes produced by these fungi (8). This inhibits or delays the symbiosis between these fungi and plants, diminishing the positive effects that AMF have on the plant, such as phosphorus absorption (8). In Cuba, there is no documented evidence showing the effect of systemic fungicides on AMF-plant symbiosis.

Taking into account above aspects, the objective of the present study was to evaluate the effect of a commercial systemic fungicide on the AMF colonization inoculated in corn crops, under semi-controlled conditions.

## MATERIALS AND METHODS

Experiments were conducted at the National Institute of Agricultural Sciences (INCA). Seeds of maize cultivar "Raul Hernandez", obtained from the Department of Genetics and Plant Breeding of INCA, were used. The experiment was carried out in pots of 5 kg capacity, with a substrate of non-sterile Brown Mellow soil. The soil had a concentration of 3-5 resident AMF spores per $g^{-1}$ soil. Some chemical characteristics of the substrate used are shown in Table 1.

Five maize seeds per pot were sown and inoculated by the seed coating technique (9). The AMF strains *Rhizophagus irregularis* (Blaszk., Wubet, Renker & Buscot) C. Walker & A. Schüßler (INCAM 11) and *Glomus cubense* Y. Rodr. & Dalpé (INCAM 4) (registered in the National Mycological Herbarium of Canada, Ottawa, with code DAOM 241198). Both fungal strains belong to the collection of arbuscular mycorrhizal fungi of the National Institute of Agricultural Sciences of Cuba. In addition, a conglomerate of different spores of resident fungi (RPR), isolated from the farm "La Fidelia", belonging to the CCS Oscar Núñez Gil from Los Palacios municipality, was used. The isolates were obtained from a brown carbonate soil of Los Palacios, which is also part of the INCA stock (10). The aforementioned inocula had a concentration of 25 spores $g^{-1}$ fresh soil.

The commercial systemic fungicide Previcur energy 840 sl (F), which is composed of 16 % inert ingredients and has 53 % propyl 3-(dimethylamino) propyl carbamate and 31 % ethylhydrogen phosphonate as active components, was also used. The experiment was conducted using a completely randomized design with a bifactorial arrangement. The factors studied were AMF strains (factor 1) and the presence or absence of the fungicide (factor 2) and the following treatments were established, each with seven replicates (Table 2).

Five days after seedling emergence, seedlings were thinned and one seedling per pot was maintained. Seedlings were maintained under semi-controlled growing conditions for 54 days, at 30 ºC, 80 % relative humidity and natural photoperiod. Fifteen days after plant emergence, the commercial systemic fungicide Previcur energy 840 sl was applied at a rate of 10 mL per pot with a fungicide concentration of 10 mg $L^{-1}$.

Fifty-four days after the experiment was established, visual density and frequency of colonization were determined, as previously described in the literature (11). Variables characterizing plant growth such as root length, total fresh mass and total dry mass of the plant were determined. To determine root length, a graduated ruler was used and measured from the root apex to the stem beginning. The total fresh mass of plants was determined on a technical balance (Sartorius), after washing and drying roots and aerial part with filter paper. To determine the total dry mass, samples were dried in an oven at 75 ºC for three days until a constant weight was obtained.

The data were analyzed using a double classification analysis of variance. If significant differences were found in

**Table 1.** Chemical characteristics of soil used in the experiment

| Substratum | $Ca^{2+}$ (cmol $kg^{-1}$) | $Mg^{2+}$ (cmol $kg^{-1}$) | Organic matter (%) | pH ($H_2O$) |
|---|---|---|---|---|
| Mellow brown | 33 | 5.0 | 1.81 | 7.3 |

pH: Potentiometry; Organic Matter (OM): Walkley Black; Exchangeable cations ($Ca^{2+}$ and $Mg^{2+}$): Complexometry

**Table 2.** Treatments established in the developed experiments

| Treatments | Denomination |
|---|---|
| Control | Control |
| Control + fungicide | Control + F |
| *Rhizophagus irregularis* | INCAM 11 |
| *Rhizophagus irregularis* + Fungicide | INCAM 11+ F |
| *Glomus cubense* | INCAM 4 |
| *Glomus cubense* + Fungicide | INCAM 4+F |
| Cluster of resident strains of P. del Rio. | RPR |
| Cluster of resident strains of P. del Rio. + fungicide | RPR + F |

the factors evaluated, the interaction was split, fixing one of the factors, strain (factor 1) or fungicide (factor 2). For this purpose, a mean comparison test was used. The STATISTIC version 21 program was used for the statistical processing of the data and Microsoft Excel 2010 was used for their representation.

## RESULTS

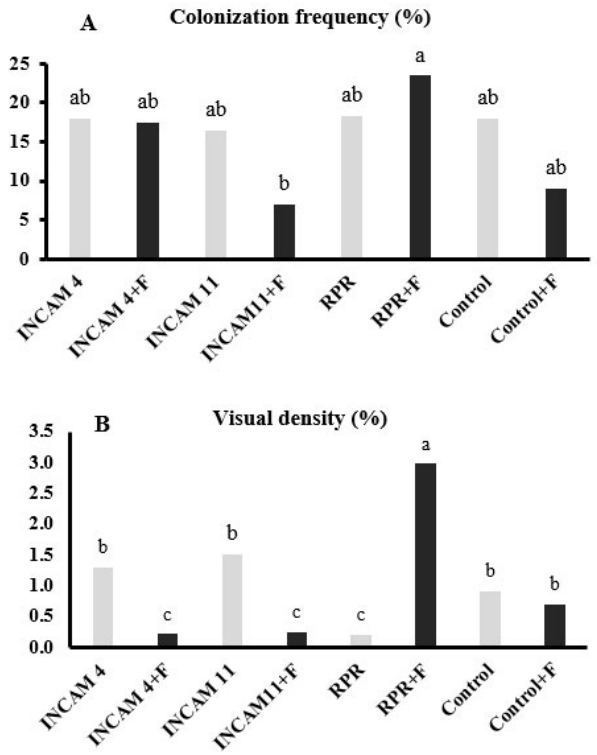
As can be seen in Figure 1A and B, the variables of colonization frequency and visual density behaved differently in relation to treatments evaluated.

No significant differences were found in the colonization frequency between plants inoculated with and without the fungicide and control plants. Nor were differences identified between plants not treated with the fungicide and those treated with the fungicide. With the exception of the treatment with RPR and that with INCAM 11 strain inoculation, both in the presence of the fungicide, between which differences were observed (Figure 1A).

When visual density was analyzed, there was a significant decrease in its values in the treatments inoculated with INCAM 4 and INCAM 11 in the presence of the fungicide, compared to treatments with these strains without fungicide (Figure 1B). However, plants inoculated with RPR in the presence of the fungicide reached the highest visual density values compared to the rest of the treatments.

On the other hand, when the effect of inoculation and the use of the fungicide was analyzed, it could be seen that, in most of the treatments, the use of the fungicide, both in control and inoculated plants, increased the root length and the fresh and dry mass of the corn plants (Figure 2 and 3A,B).

However, among the plants inoculated with INCAM 11 strain, no significant differences were observed in any of the variables measured, related to the growth of maize plants. Nor were significant differences observed between the two groups of plants inoculated with RPR in the fresh mass of the plants.




INCAM 11, *Rhizophagus irregularis*; INCAM 4, *Glomus cubense*; RPR, resident fungi cluster, Pinar del Río; F, fungicide

Means with equal letters in each bar do not differ significantly for p≤0.05

**Figure 1.** Mycorrhizal performance values evaluated through colonization frequency (A) and visual density (B) in roots of maize plants, 54 days after inoculation

## DISCUSSION

It has been reported that mycorrhizal functioning and its action on plants can be affected by the application of fungicides, depending on soil conditions (6). Such effects depend on the active chemical ingredient of these products and the species of mycorrhizal fungus (12). The increase in root length and fresh and dry mass of corn plants treated with the fungicide can be indirectly attributed to some factors. Among them are the effect of the fungicide in reducing root exudates and as a stressor of the host plant. Both events favor the functioning of the mycorrhizal symbiosis, with a consequent effect on growth (13,14).

The diversity of AMF species present in the inoculant from Pinar del Río (RPR) may be a factor that explains the high values of visual density and number of spores when treated with the fungicide. In this sense, it has been demonstrated that a greater diversity of AMF has greater tolerance to the stress imposed by fungicides, due to the

fact that some species are more resistant to certain fungicides than others (15,16).

Visual density is considered to more clearly reflect mycelial occupancy and, indeed, symbiotic efficiency itself because it expresses the percentage amount of fungal structures and exchange between symbionts. This includes colonization levels and exchange structures observed during evaluation (17). Thus, it can be deduced that the species present in the RPR conglomerate were the most efficient in the conditions in which the experimental work was carried out, presenting the highest levels of visual density.

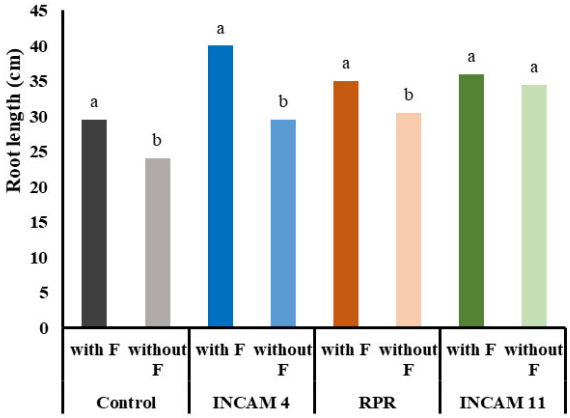
This corresponds with previous studies, which reported that the formation of AMF vesicles in the roots is significantly higher when fungicides such as Captan and Tebuconazole were applied to corn seeds (18). Application of the fungicide Metalaxyl increased AMF colonization of maize plants and may also stimulate fungal mycelium development on *Ananas comosus* (pineapple) plants (14,18). This can occur for several reasons, first a direct action of the fungicide on AMF triggering a positive response by stimulating the production of arbuscules (AMF exchange structure) and also the presence of xenobiotic molecules in the plant that could induce some stress that takes advantage of AMF to express greater colonization and visual density.

Other authors suggest that the dominance of an AMF species in a specific environment can be attributed to various environmental factors. These include soil physicochemical properties, plant morphology, compatibility between host plant and AMF species, and also fungicide application (17). Therefore, the use of the systemic fungicide could explain the low visual density values found in plants inoculated with INCAM 4 strain and the fungicide. These findings coincide with some studies, which corroborate that systemic fungicides can have a suppressive effect on AMF activity and even on the physiology of the plant-AMF interaction and on nutrient and water absorption (17,18).

A study similar to the previous one was carried out with the application of the fungicide Benomyl, which caused a negative effect on some AMF, specifically on the growth of extraradical mycelium, having a direct effect on the length of the germinative tube (19). Similarly, the fungicides Bavistin and Mancozeb had a negative effect on mycorrhizal root colonization and spore number (20).

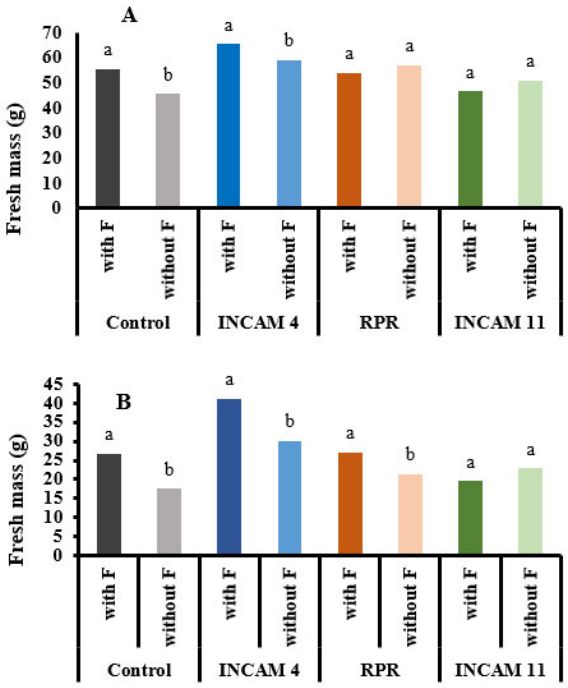
Benzimidazole, another widely used fungicide, has been shown to be detrimental to mycorrhizae and the soil microbiological community (20). In some cases, its use causes a reduction in plant growth and phosphorus uptake (14).

The fungicides Fenhexamide, Dicamba, Benomyl among others, tested in studies (14), significantly reduced the density and length of the extraradical mycelium of the AMF strain *Funneliformis mosseae*. Similarly its viability and ability to explore the surface of plant membranes were negatively affected especially by Phenhexamid.




*Rhizophagus irregularis* (INCAM 11), *Glomus cubense* (INCAM 4), resident fungus cluster, Pinar del Río (RPR), Fungicide (F)
Equal letters, for the same strain and control, do not differ for p≤0.05

**Figure 2.** Root length of maize plants inoculated with arbuscular mycorrhizal fungi strains with and without fungicide application, 54 days after inoculation




*Rhizophagus irregularis* (INCAM 11), *Glomus cubense* (INCAM 4), conglomerate of resident fungi, Pinar del Río (RPR) Fungicide (F)
Equal letters for the same strain and control do not differ significantly for p≤0.05

**Figura 3.** Fresh mass (A) and total dry mass (B) of maize plants inoculated with arbuscular mycorrhizal fungi strains with and without fungicide application, 54 days after inoculation

## CONCLUSIONS

- The systemic fungicide Previcur energy 84 sl caused an increase in corn plant growth.
- An interesting case was the effect of the fungicide on mycorrhizal functioning and growth promotion of maize plants inoculated with the conglomerate of resident AMF species used (PRP).
- The adaptation of these fungal species to the established edaphoclimatic conditions and the effect of the fungicide on increasing visual density acted synergistically to promote the growth of maize plants under the experimental conditions studied.

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