Cultivos Tropicales, 2022, vol. 43, no. 2, e04
ISSN impreso: 0258-5936
ISSN digital: 1819-4087

http://ediciones.inca.edu.cu

abril-junio
Ministerio de Educación Superior. Cuba
Instituto Nacional de Ciencias Agrícolas

Original article

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

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**ABSTRACT**

Fungicides not only affect pathogenic fungi, but also other fungi, 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 arbuscular mycorrhizal fungi colonization inoculated on maize under semi-controlled conditions. For this purpose, maize plants were inoculated with *Rhizophagus irregularis* INCAM 11, *Glomus cubense* INCAM 4 strains and with a cluster of different species of arbuscular mycorrhizal fungi, in the presence or not of the systemic fungicide. At 54 days after inoculation, the frequency of colonization and the visual density of the mycorrhizal fungi used were determined. In addition, root length and total fresh and dry mass of the maize plants were determined. The cluster of different arbuscular mycorrhizal fungi species, together with the fungicide, presented the highest visual density. The presence of the fungicide increased root length and total fresh and dry mass of control plants and those inoculated with *Glomus cubense* INCAM 4. The effect of the *Rhizophagus irregularis* INCAM 11 strain on maize growth was not affected by the use of the systemic fungicide.

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

## INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) (*Glomeromycota*) are an integral part of many ecosystems and they 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 the functions they perform once associated with plants. Among them 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 the 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. Therefore, 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 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.

For this, the aim of the present study was to evaluate the effect of a commercial systemic fungicide on the AMF colonization inoculated on 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 5 kg pots, with a substrate of non-sterile brown fluffy soil. The soil had a concentration of 3-5 resident AMF spores per $g^{-1}$ of soil. Some chemical characteristics of the substrate used are shown in Table 1.

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

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

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

Five maize seeds were sown per pot and inoculated using the seed coating technique (9). *Rhizophagus irregularis* (Blaszk., Wubet, Renker & Buscot) C. Walker & A. Schüßler (INCAM 11) and *Glomus cubense* Y. Rodr & Dalpé

(INCAM 4) AMF strains were used (registered at 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 cluster of different resident fungal spores (RPR), isolated from "La Fidelia" farm, belonging to the CCS "Oscar Núñez Gil" from Los Palacios municipality, was employed. The isolates were obtained from a soft brown carbonate soil of Los Palacios that is also part of the INCA strain collection [10]. The aforementioned inoculums had a concentration of 25 $g^{-1}$ spores of fresh soil.

In addition, the commercial systemic fungicide Previcur energy 840sl (F) was used, which is composed of 16 % inert ingredients and as active components has 53 % propyl 3-(dimethylamino) propyl carbamate and 31 % ethylhydrogen phosphonate. 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 not of the fungicide (factor 2) and the following treatments were established, each with seven replicates (Table 2).

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

| Treatments | Designation |
|---|---|
| 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 from Pinar del Río | RPR |
| Cluster of resident strains from Pinar del Río+ fungicide | RPR + F |

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 840sl 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 colonization frequency 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 begins. The total fresh mass of the 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, the samples were dried in an oven at 75 ºC for three days until a constant weight was obtained.

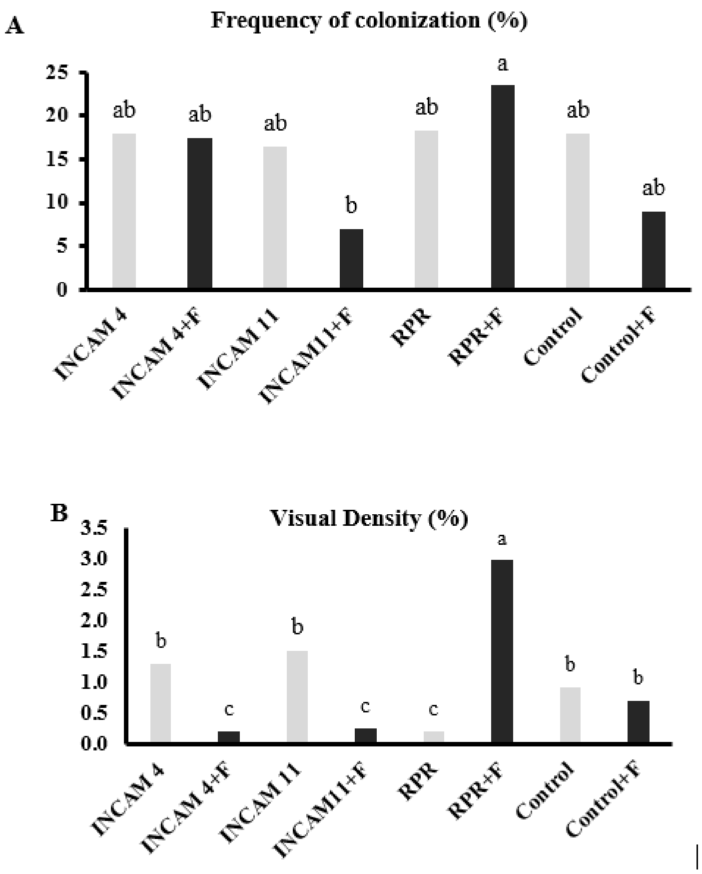
Data were analyzed using a double classification analysis of variance. If significant differences were found in the factors evaluated, the interaction was split, fixing one of the factors, strain (factor 1) or fungicide (factor 2). For this purpose, a mean comparison test was used. The STATISTIC version 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 1 A and B, the variables of colonization frequency and visual density behaved differently in relation to treatments evaluated.

No significant differences were found in the frequency of colonization 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 1 A).

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 fungicide presence, with respect to treatments with these strains without fungicide (Figure 1 B). However, the plants inoculated with RPR in the presence of the fungicide reached the highest visual density values compared to the rest of treatments.



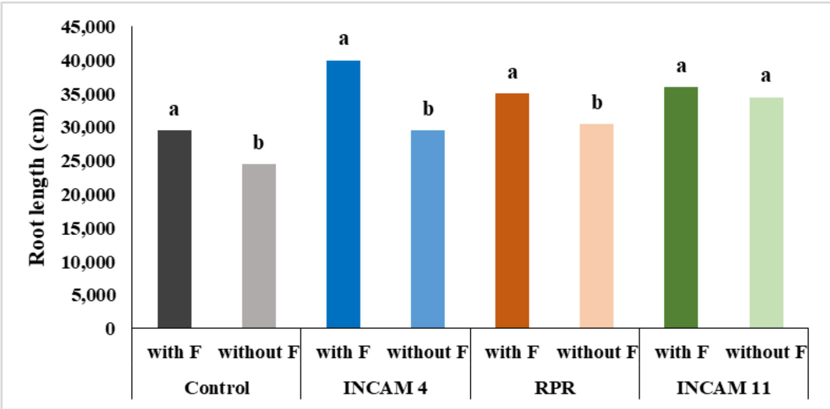

INCAM 11, *Rhizophagus irregularis*; INCAM 4, *Glomus cubense*; RPR, cluster of resident strains, Pinar del Río; F, Fungicide

Means with equal letters in each bar do not differ significantly for $p \leq 0.05$.

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

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 fungicide use, both in control and inoculated plants, increased the root length and the fresh and dry mass of the corn plants (Figure 2 and 3 A,B).

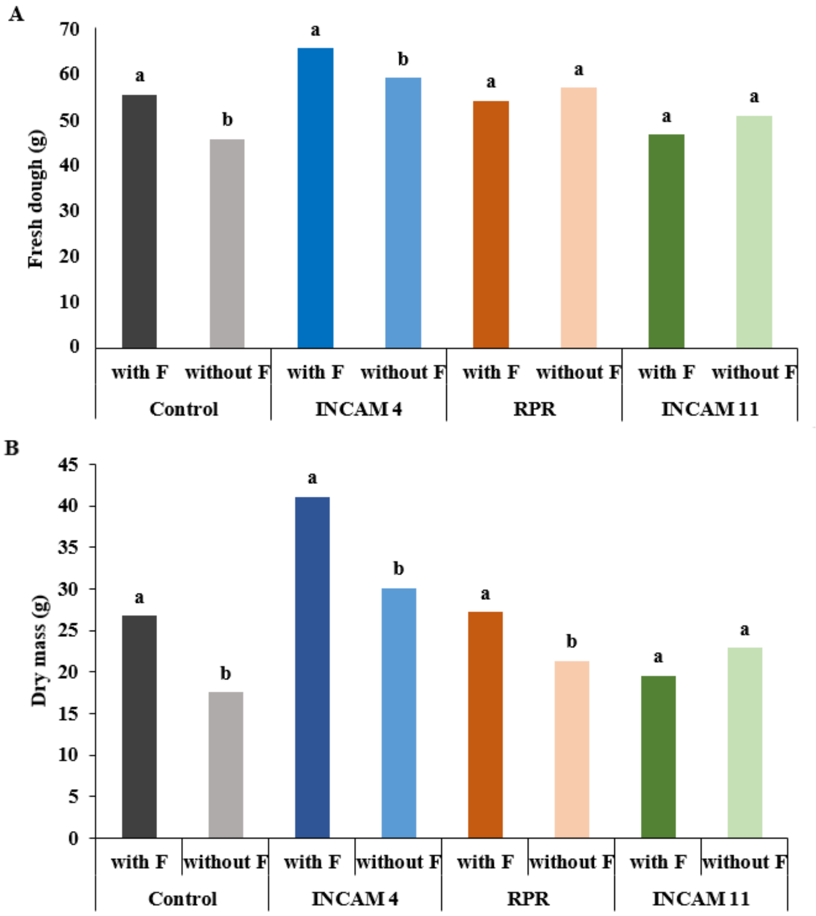
However, among plants inoculated with INCAM 11 strain, no significant differences were observed in any of the variables measured, related to the growth of maize plants. Nor were significant differences observed between the two groups of plants inoculated with RPR in the fresh mass of plants.




*Rhizophagus irregularis* (INCAM 11), *Glomus cubense* (INCAM 4), cluster of resident strains, 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)*, cluster 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

**Figure 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

# 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 [12]. Such effects depend on the active chemical ingredient of these products and mycorrhizal fungus species [13]. 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 fungicide effect in reducing root exudates and as a stressor of the host plant. Both events enhances the mycorrhizal symbiosis functioning, with a consequent effect on growth [14,15].

The diversity of AMF species present in the inoculant from Pinar del Río (RPR) may be a factor that explains high values of visual density and number of spores when treated with the fungicide. In this sense, it has been demonstrated that a greater AMF diversity has better tolerance to the stress imposed by fungicides, due to the fact that some species are more resistant to certain fungicides than others [16,17].

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 levels of colonization and exchange structures observed during evaluation [18]. Thus, it can be deduced that species present in the RPR cluster were the most efficient in conditions in which the experimental work was carried out, presenting the highest levels of visual density.

This corresponds to previous studies, which reported that the AMF vesicles formation in the roots is significantly higher when fungicides such as Captan and Tebuconazole were applied to corn seeds [19]. Application of the fungicide Metalaxyl increased AMF colonization of maize plants and may also stimulate fungal mycelium development on *Ananas comosus* (pineapple) plants [15,19]. This can occur for several reasons, first a fungicide direct action 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 [18]. 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 plant-AMF interaction and on nutrient and water absorption [18,19].

A study similar to the previous one was carried out with the application of Benomyl fungicide, which caused a negative effect on some AMF, specifically on the growth of extraradical mycelium, having a direct effect on the germinative tube length [20]. Similarly, Bavistin and Mancozeb fungicides had a negative effect on mycorrhizal root colonization and spore number [21].

Benzimidazole, another widely used fungicide, has been shown to be detrimental to mycorrhizae and the soil microbiological community. In some cases, its use causes a reduction in plant growth and phosphorus uptake [21].

Fenhexamide, Dicamba, Benomyl fungicides among others, tested in studies (21), significantly reduced the density and the extraradical mycelium length of the *Funneliformis mosseae* AMF strain. Similarly, their viability and ability to explore the surface of plant membranes were negatively affected, especially by Phenhexamid.

# CONCLUSIONS

- The systemic fungicide Previcur energy 840sl caused an increase in corn plant growth.
- An interesting case was the fungicide effect on mycorrhizal functioning and growth promotion of maize plants inoculated with the resident AMF species cluster (PRP) used.
- The adaptation of these fungal species to the established edaphoclimatic conditions and the fungicide effect on increasing visual density acted synergistically to promote maize plant growth under the experimental conditions studied.

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