Effect of the application of alpha naphthyl sodium acetate (Aponon®) on colonization and production of spores of arbuscular mycorrhiza-forming fungi in lettuce (*Lactuca sativa*)

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The effect of a synthetic auxin, alpha naphthyl sodium acetate (Aponon®), was evaluated on the growth of lettuce plants (*Lactuca sativa*) and their associations with arbuscular mycorrhizal fungi. Percentage colonization, number of arbuscules, coils and vesicles and production of spores of arbuscular mycorrhizal fungi were evaluated. Lettuce plants were inoculated with three species of mycorrhizal fungi *(Glomus clarum, G. intraradices and G. mosseae)*. Three doses of Aponon® (10.6 mg/l, 21.18 mg/l and 42.35 mg/l) were applied and both non-inoculated controls and controls without the application of the synthetic hormone were used. The plants were harvested after 90 days. Plants inoculated with *G. clarum* and with the highest dose of Aponon® resulted in the highest value for colonization. The higher the dose of Aponon®, the lower the colonization percentage in the plants inoculated with *G. intraradices* and *G. mosseae*. The percentage of arbuscules and vesicles showed a similar pattern to that of colonization in the plants inoculated with *G. clarum* and *G. intraradices.* The coils had low values in all treatments. *Glomus mosseae* and *G. intraradices* decreased their spore production as the dose of Aponon® increased, whereas *G. clarum* did not show differences in relation to the dose of Aponon®. The greatest plant biomass was observed in the plants inoculated with *G. mosseae* lacking or with minimal doses of Aponon®.

Key words: alpha naphthyl sodium acetate, AMF, arbuscular mycorrhiza-forming fungi, *Glomus* ssp., *Lactuca sativa*

Introduction

Arbuscular mycorrhizal fungi are soil microorganisms belonging to the phylum Glomeromycota (Schüβler *et al*., 2001); they form symbiosis with more than 80% of plant species, including most agricultural crops and herbaceous and other plants in natural ecosystems (Barea *et al.,* 2005).

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Arbuscular mycorrhiza-forming fungi are obligated biotrophs, and thus cannot reproduce in media without the host plant, and depend on the photosynthates supplied by the plant for their reproduction (Harley and Smith, 1983), or on the radical exudates to be able to develop in axenic culture media (Mugnier and Mosse, 1987). All infective structures of arbuscular mycorrhiza fungi can be used as inoculum sources. Among these are: spores, intraradical or extraradical mycelium, colonized radical fragments and substrate that can contain mycorrhizal structures.

Mycorrhizal colonization increases the uptake of phosphorus and other nutrients from the soil solution by means of the external mycelium (Joner *et al*., 2000) and provides the plant with resistance against biotic and abiotic stress (Jeffries *et al*., 2003). Therefore, arbuscular mycorrhizal fungi have a considerable potential to be used as inoculants to improve horticultural production. Important commercial benefits, such as an increase in the growth and production of plants, thus improving the uniformity of the crop and reducing the phosphorous fertilization, can be derived from their use. Environmental stress, radical damage, and harvest time can also be decreased (Wood, 1992).

The increase in the hormonal levels in mycorrhizal plants can present a significant impact on their growth and development and substantially affect the response of the host plant to the mycorrhizal infection. There are not enough clear evidence to determine whether arbuscular mycorrhizal fungi produce hormones, and if there do, it is not known whether there is a direct transfer to the host or whether the symbiotic association stimulates their production (Cooper, 1986).

The acetic indol acid (AIA), a member of a plant hormone group, is a natural substance produced in the parts of the plant in active growth, which regulates many of the aspects of the plant development. It affects the growth of the stem, leaves and roots and the development of lateral branches and fruit. Auxins influence the growth of these organs by stimulating the lengthening of certain cells and inhibiting the growth of others, depending on the amount of auxin in the plant tissue and its distribution; at low concentrations, they stimulate metabolism and development, and at high concentrations they restrain them. Since rhizogenesis is closely related to cellular division, it is a habitual process performed in horticulture, especially in greenhouses, in order to favour rooting (Azcon-Bieto and Talon, 1996). There are synthetic compounds that cause many physiological responses common to the AIA, and in general, they are considered auxins. From these, the α-naphtaleneacetic acid (NAA), the 2,4 dichlorophenoxyacetic acid (2,4 D) and the 2- methyl-4-

chlorophenoxyacetic acid (MCPA) are the most known (Salisbury and Ross, 1992).

The aim of this study was to evaluate the application of a synthetic auxin, alpha naphthyl sodium acetate (Aponon®), on the growth of *Lactuca sativa* L. plants, and on colonization percentage, arbuscules, coils and spore production of three species of arbuscular mycorrhiza-forming fungi.

Materials and methods

The experiment was performed according to a factorial 4 × 4 design, with three replications per treatment, and comprised a total of 48 plants. The factors were: a) control without the synthetic hormone and three treatments with different doses of Aponon®: Dose I: 10.6 mg/l (which corresponds to half the commercially recommended dose); Dose II: 21.18 mg/l (commercially recommended dose); and Dose III: 42.35 mg/l (which corresponds to twice the commercially recommended dose); b) control without inoculation and three inoculated treatments. Each treatment was inoculated with an arbuscular mycorrhiza-forming fungus: *Glomus clarum* Nicolson & Schenck LPS culture N° EEA1, isolated from the "Estación experimental Ing. Agr. Hirchhorn" in the locality of Los Hornos (Argentina) (Schalamuk, 2005), *Glomus intraradices* Schenck & Smith LPS culture N° TF28, isolated from a wood of *Nothofagus* in the province of Tierra del Fuego (Argentina) and *Glomus mosseae* Nicolson & Gerdemann LPS culture N° SB1, isolated from sand dunes of San Bernardo (Argentina). The acronym LPS refers to the herbarium belonging to the Instituto Spegazzini from the city of La Plata (Buenos Aires, Argentina).

The inoculum supplied consisted of rhizospheric substrate of *Sorghum vulgare* L., which contained spores, mycelium and root fragments colonized by the fungal species tested. Lettuce seeds were grown in sterile substrate and after 15 days selected by uniformity of size and transplanted to 250 ml pots with 315 g of sterile perlite-vermiculite substrate (1:1); 20 g of mycorrhizal inoculum of each species tested was added. Each pot contained two lettuce seedlings. The doses of Aponon® were applied after the plants had developed their first two leaves. The pots were placed in a greenhouse at 24 ± 1ºC during the day and at 20 ± 1ºC during the night, with a 16 hour photoperiod provided by white-cold incandescent lamps.

Plants were fertilized with a nutritive solution twice a week (Cabello, 1999), and harvested after 90 days. Growth of lettuce plants corresponding to the different treatments was evaluated by determining biomass by means of dry weight at 70ºC until constant weight. Part of the radical system was cleared and

dyed (Phillips and Hayman, 1970). The percentage of arbuscules, coils, vesicles and total colonization was calculated according to the method of McGonigle *et al.* (1990). Spores were extracted by means of the wet sieving and decanting method (Gerdemann and Nicolson, 1963) and centrifuged in a sacarose gradient (Walker *et al*., 1982).

Statistical analysis

One – way ANOVA with LSD was performed to study differences ($P \leq 0.05$) between groups.

Results and discussion

The microscopic observations of the dyed roots did not show colonization in the non-inoculated treatments. In all treatments inoculated with *Glomus clarum*, *G. intraradices* and *G. mosseae,* structures typical of arbuscular mycorrhizal colonization were observed.

The percentage of colonized roots differed between treatments where Aponon® was applied. In plants inoculated with *G. clarum*, the highest colonization value was 70% in the treatment that received the highest dose of Aponon®. The colonization percentage in the treatments inoculated with *G. intraradices* decreased markedly from 71% to 5% as the supplied dose of Aponon® increased. The values found for *G. mosseae* decreased with the application of Aponon® from a maximum of 70% in the control without Aponon® to a value of 45% in the treatment with the commercially suggested dose (Fig. 1). The percentage of arbuscules (Fig. 2) and vesicles (Fig. 3) showed a pattern similar to that of colonization in the treatments inoculated with *G. clarum* and *G. intraradices*. The treatment inoculated with *G. mosseae* and with the intermediate dose of Aponon® presented a higher percentage of arbuscules but no vesicles, while in the other treatments the number of vesicles was low. Gunze and Hennessy (1980) have reported an increase in the development of arbuscules by indol acetic acid, which suggests that auxins influence their formation; these results are in agreement with those in this report in the treatments inoculated with *G. clarum*. The treatments inoculated with *G. clarum* and *G. intraradices* presented low values of coils, while the treatments inoculated with *G. mosseae* showed a tendency similar to that of colonization, being 20% its maximal value in the treatment that did not receive Aponon® (Fig. 4).

The number of spores from mycorrhiza-forming fungi in 100 g of dry substrate is shown in Fig. 5. *Glomus clarum* did not show significant

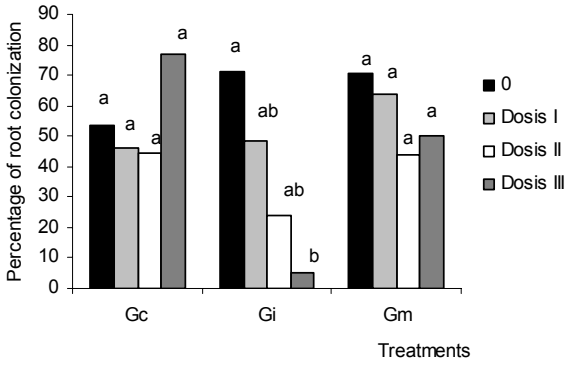

Fig. 1. Percentage of root colonization. Gc, *Glomus clarum*; Gi *G. intraradices*; Gm *G. mosseae.* Different letters indicate significant differences (LSD P≤ 0.05)**.**

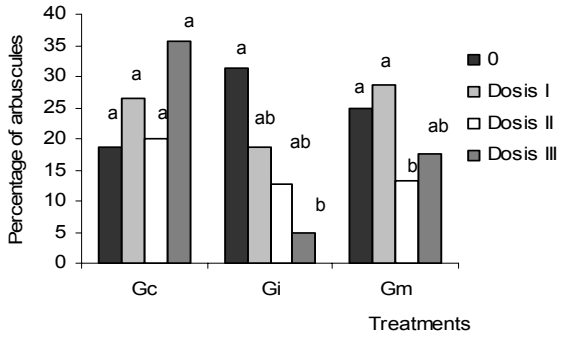

Fig. 2. Percentage of arbuscules. Same notations as in Fig. 1.

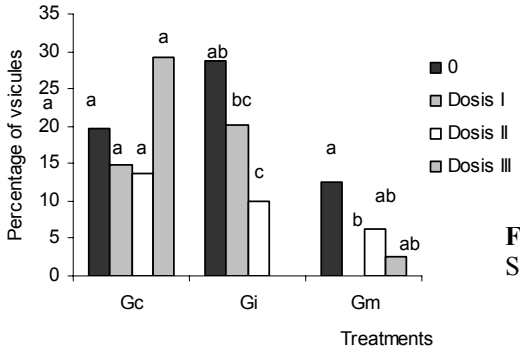

Fig. 3. Percentage of vesicles. Same notations as in Fig. 1.

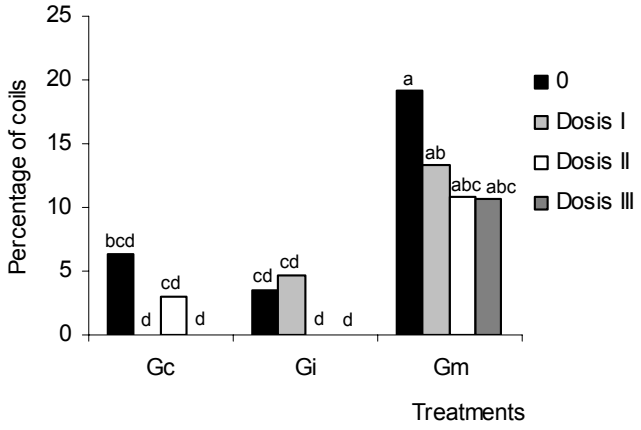

Fig. 4. Percentage of coils. Same notations as in Fig. 1.

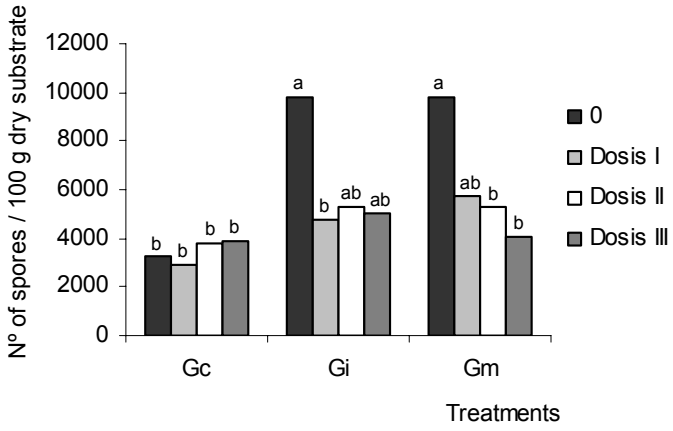

Fig. 5. Number of spores in 100 g dry substrate. Same notations as in Fig. 1.

differences in relation with the dose of Aponon® employed. We found 3,457 ± 475 spores in 100 g of dry substrate. Although these values was not influenced by the application of Aponon®, they were low when compared to the results found in agroecosystems (Schalamuk, 2005). The treatments inoculated with *G. intraradices* and *G. mosseae* presented significant differences in the number of spores; in both cases the greatest production of spores was observed in the treatments that did not receive Aponon® (9,836 and 9,751 spores in 100 g of dry substrate respectively); this demonstrated a deleterious effect of the synthetic hormone on sporulation.

Total biomass of lettuce plants following various treatments is given in Table 1. With the exception of plants inoculated with *G. clarum*, which resulted in maximum values when Aponon® was added, treatments lacking and those with minimal doses of Aponon® resulted in a greater plant biomass.

Table 1. Biomass of *Lactuca sativa* in different treatments with Aponon®. Different letters indicate significant differences (LSD P≤ 0.05).

	Dose of Aponon®	**Biomass (g)**
Without mycorrhizas	0	0.52abc
	I	0.51abcd
	II	0.41abcde
	III	0.34cde
Glomus clarum	0	0.45abcde
	I	0.29e
	II	0.34de
	III	0.28e
G*lomus intraradices*	0	0.48abcd
	I	0.50abcd
	II	0.37cde
	III	0.33de
Glomus mosseae	0	0.57ab
	I	0.58a
	II	0.37cde
	III	0.39bcde

The greatest plant biomass resulted in treatments inoculated with *G. mosseae* lacking or with minimal doses of Aponon®. Although there is no specificity between the arbuscular mycorrhiza-forming fungi and the plants they colonize (Peterson *et al*. 2004), there are certain host plant - mycorrhizal fungus combinations that are more efficient (Sieverding, 1991). In this study it was shown that the most efficient association for growth was between lettuce plants and *G. mosseae*, and that the most efficient association for spore production was between lettuce inoculated with *G. intraradices* without the application of Aponon®.

Arbuscular mycorrhizal fungi are capable of influencing the growth of the host by means of producing hormones. There has been little research on the possible production of growth-promoting compounds by these organisms and the studies are limited because of the incapacity of these fungi of growing in axenic culture media (Cooper, 1986).

Conclusions

In the system studied (arbuscular mycorrhiza –forming fungi - *Lactuca sativa* - different doses of Aponon®) different responses in plant growth, percentage of arbuscules, coils, vesicles, total colonization and sporulation of mycorrhizal fungi were observed.

The application of Aponon® did not affect spore production in *G. clarum,* no did it stimulate plant growth. The highest dose of Aponon® significantly increased the percentage of arbuscules, vesicles and colonization, but this was not reflected in spore production.

Glomus intraradices and *G. mosseae* inoculation of lettuce resulted in the highest values of colonization and resulted in plants with a greater biomass and with a greater radical development. The addition of Aponon® was deleterious for sporulation of *G. intraradices* and *G. mosseae*.

The application of alpha naphthyl sodium acetate (Aponon®) in this system did not produce a significant increase in spore production and thus it is not effective for the reproduction of these microorganisms; however, the high percentage of arbuscules, vesicles and colonization found in the treatments inoculated with *G. clarum* and a double dose of Aponon® could suggest their application in those cases in which colonized radical fragments are used as inoculum source.

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