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Pyraclostrobin impairs metabolic activity and phosphorus uptake of the extraradical mycelium of *Rhizophagus intraradices* more severely than iprodione

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Abstract

Pesticide residues are widespread in agricultural soils and may adversely affect arbuscular mycorrhizal (AM) fungi, key symbionts involved in plant phosphorus (P) acquisition. Most studies to date have focused on pesticides effects either on spores (asymbiotic phase) or on the mycorrhizal plant as a whole. Here, we investigated the effects of two fungicides with contrasting modes of action—pyraclostrobin (quinone-outside inhibitor) and iprodione (dicarboximide)—applied specifically to the extraradical mycelium (ERM) of *Rhizophagus intraradices* MUCL 49410 associated with *Medicago truncatula*. To this end, a bi-compartmented pot system was developed, allowing fungicide application at the recommended field dose directly to the ERM, while preventive root exposure. Treatments were applied for 30 days (T1) or 3 days (T2) to assess time-dependent responses.

Pyraclostrobin markedly reduced ERM biomass (by up to 75 %), hyphal alkaline phosphatase activity, and root colonization, particularly arbuscule abundance, indicating severe impairment of mitochondrial function. In contrast, iprodione slightly increased ERM biomass and the proportion of metabolically active spores, and enhanced Pi uptake after prolonged exposure, suggesting a compensatory or mild hormetic response.

Overall, pyraclostrobin exerted pronounced inhibitory effects on AM fungal structures and function, whereas iprodione showed neutral to mildly stimulatory effects under comparable conditions. These contrasting responses likely reflect differences in fungicide mode of action and exposure duration. Our findings demonstrate that AM fungal sensitivity to fungicides is compound-specific and underscore the importance of integrating functional and physiological endpoints into pesticide risk assessments frameworks.

Key words: Extraradical mycelium, Iprodione, Mitochondrial activity, Phosphorus uptake, Pyraclostrobin, *Rhizophagus intraradices*

1. Introduction

Large-scale assessments demonstrate that pesticide residues are pervasive in agricultural soils and can significantly influence belowground biodiversity through off-target effects. A recent survey across European ecosystems (woodlands, grasslands and croplands in 26 EU countries) identified pesticide residues as one of the strongest drivers of soil biodiversity after intrinsic soil properties, reporting negative impacts on several microbial functional groups involved in nutrient cycling, among which arbuscular mycorrhizal (AM) fungi (Köninger *et al.*, 2026). In a parallel study covering the period 2013-2019, Wolfram *et al.* (2026) revealed increasing trends in total applied pesticide toxicity across several organism groups, including soil microorganisms, and emphasized the need for greater adoption of organic agriculture and a transition toward less toxic pesticides to help achieve the United Nations target of reducing pesticide-related risks by 50% by 2030.

AM fungi are key components of terrestrial ecosystems and play a central role in plant nutrition (in particular Phosphorus – P). By developing an extensive extraradical mycelium (ERM), they explore soil volumes beyond the root depletion zone, accessing soil pores that are inaccessible to roots, and enhance the uptake of inorganic phosphate (Pi), which is subsequently transferred to the host plant (Smith and Smith, 2011; Ezawa *et al.*, 2018; Ferrol *et al.*, 2019). In some ecosystems, AM fungal ERM can comprise up to 30% of the total microbial biomass (Olsson and Wilhelmsson, 2000), and some AM fungi have been shown to deliver 60%–100% of the total P acquired by plants (Smith *et al.*, 2003, 2004).

In agricultural systems, AM fungi are routinely exposed to plant protection products, raising concerns about potential impacts of pesticides on their functional traits and ecological performance. A growing body of evidence indicates that fungicides can affect multiple stages of AM fungal development and functioning, including spore germination, hyphal growth, root colonization, and nutrient transfer (e.g., Zocco *et al.*, 2008, 2011; Rodríguez-Morelos *et al.*, 2023; Papadopoulou *et al.*, 2025, Roshanfekrrad *et al.*, 2025). The recognition of these effects has prompted calls for more comprehensive regulatory testing strategies that evaluate pesticide impacts on AM fungi under ecologically relevant conditions, considering both structural and physiological endpoints (Sweeney *et al.*, 2022). The magnitude of these effects depends on the

active ingredient, the formulation, the mode of action, the exposure duration, and the dose of application (Papadopoulou *et al.*, 2025; Roshanfekrrad *et al.*, 2025; Mallmann *et al.*, 2026). Indeed, the impact of fungicides at the recommended pesticide dose (RPD – the concentration expected in soil upon application) for agricultural use in Europe is a major indicator of toxicity. To evaluate this, Roshanfekrrad *et al.* (2025) developed a high-throughput *in vitro* germination assay to determine EC_{50} values and assess whether pesticides exert fungicidal or fungitoxic effects on AM fungal spores. Nineteen pesticides, including six fungicides, were evaluated on the spore germination of four AM fungi. Among them, iprodione and pyraclostrobin were of particular concern, as both inhibited spore germination in various *Rhizophagus* species at concentrations below the RPD. Pyraclostrobin was deleterious to all tested AM fungi, while iprodione affected one out of four tested AM fungi at this concentration. For *Rhizophagus intraradices* MUCL 49410 (used in the present study) the mean EC_{50} values were 6.42 mg/L for iprodione and 0.8 mg/L for pyraclostrobin, corresponding to approximately 0.5 and 0.3 times the RPD, respectively.

The ERM plays a central role in the uptake and transport of Pi from the soil to the host plant. It is particularly vulnerable chemicals because it develops in direct contact with treated soil compartments where fungicide residues may accumulate. Its physiological activity relies on membrane integrity, efficient mitochondrial activity, and a continuous ATP supply to sustain nutrient uptake and translocation (Smith and Smith, 2011; Ezawa *et al.*, 2018). Consequently, disruption of respiratory processes directly compromises P acquisition and transfer to the host plant. Functional studies have demonstrated that mitochondrial respiratory activity is closely linked to symbiotic efficiency and fungal development within host roots (Mercy *et al.*, 2017). Quinone outside inhibitor (QoI) fungicides, such as pyraclostrobin, a broad-spectrum strobilurin fungicide, inhibit electron transfer at the cytochrome bc1 complex (complex III) of the mitochondrial electron transport chain (Bartlett *et al.*, 2002) in a range of foliar and soil-borne pathogens (e.g. *Septoria tritici*, *Puccinia* spp., *Kabatiella zeae* and *Pyrenophora teres*) in crops such as cereals, fruits, and vegetables. This inhibition reduces ATP synthesis and may enhance electron leakage, leading to increased production of reactive oxygen species (ROS). Excessive ROS accumulation can damage lipids, proteins, and nucleic acids, ultimately impairing membrane integrity and metabolic performance (Moller *et al.*, 2007). In contrast, the dicarboximide fungicide iprodione, commonly applied to control diseases caused by *Botrytis*,

Monilinia, *Rhizoctonia*, and *Sclerotinia* species and related pathogens in fruit crops, vegetables, ornamentals, and turf, mainly acts by disrupting osmotic regulation through histidine kinase signaling pathways involved in cell wall integrity (Leroux *et al.*, 2002).

As AM fungi are non-target organisms, the specific molecular targets of pyraclostrobin and iprodione in these symbionts remain unclear. Although these fungicides were developed to control plant pathogens, their modes of action may nonetheless trigger non-specific cellular stress responses in AM fungi, including membrane disruption and oxidative stress (Yaakoub et al., 2022). However, the extent to which these widely used fungicides affect the metabolic activity of the ERM of AM fungi and its capacity to transport phosphate to the host plant remains largely unexplored.

In the present study, we investigated the effects of iprodione and pyraclostrobin on the metabolic activity and Pi uptake of the ERM of *R. intraradices* MUCL 49410 associated with *Medicago truncatula*. A bi-compartmented pot system incorporating a central in-growth tube accessible exclusively to the ERM was used to ensure targeted exposure to the fungicides while preventing direct root contact. Mycelial development, metabolic activity and Pi uptake were assessed to determine how fungicide-induced metabolic disturbances affect the functioning of the AM symbiosis.

2. Materials and methods

2.1. Biological materials

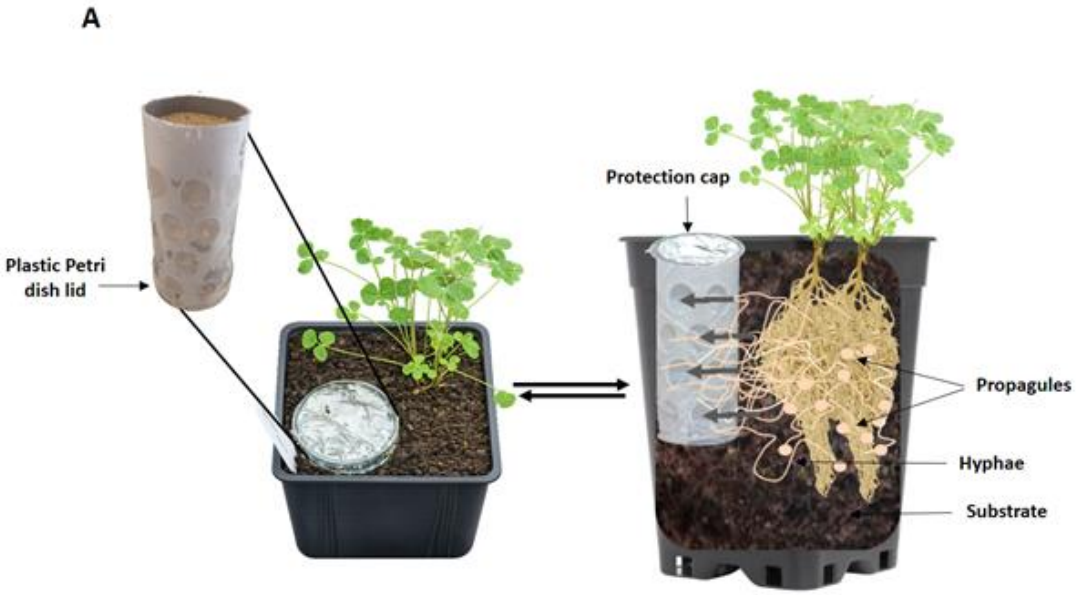
The AM fungus *Rhizophagus intraradices* MUCL 49410 was supplied by the Glomeromycota *in vitro* collection (https://bccm.belspo.be/MUCL-GINCO - GINCO, Belgium). It was first propagated *in vitro* using excised Ri T-DNA transformed roots of carrot (*Daucus carota* L.), clone 3G following Cranenbrouck *et al.* (2005). Subsequently the AM fungus was mass-produced over several months under greenhouse conditions on maize (*Zea mays* L.) grown in 5 L pots containing a sterilized (121°C for 15 min) 1:1:1 (w:w:w) substrate composed of vermiculite (PullRhenen, Netherlands), sand (quartz N°4, Euroquartz, Belgium) and lava stone (DCM, Belgium). The resulting mycorrhizal (M) roots were used as the AM fungal inoculum for the experiment.

Seeds of *Medicago truncatula* L. (cv. Jemalong A17) and *Zea mays* (cv Troïzi) were supplied by the South Australian Research and Development Institute (SARDI, Australia) and by the Centre Indépendant de Promotion Fourragère (CIPF, Belgium), respectively.

2.2. Set-up of the bi-compartmented pot system experiment

2.2.1. Preparation of AM fungal hyphae in-growth tubes

One hundred and ten PVC tubes (10 cm long with inner diameter of 5 cm) were each drilled with 31 holes (1 cm diameter circular cross-section) evenly distributed along the tubes (hereafter named in-growth tubes). The in-growth tubes used in this study were designed following the concept introduced by Johnson *et al.* (2001). The holes were covered on the outside with a 41-µm nylon mesh, fixed with PVC glue. This mesh prevented the roots from penetrating the in-growth tubes, while allowing the hyphae to pass through and proliferate inside the tubes. The bottom of each tube was sealed using a plastic Petri dish lid attached with hot melt glue (Figure 1A).



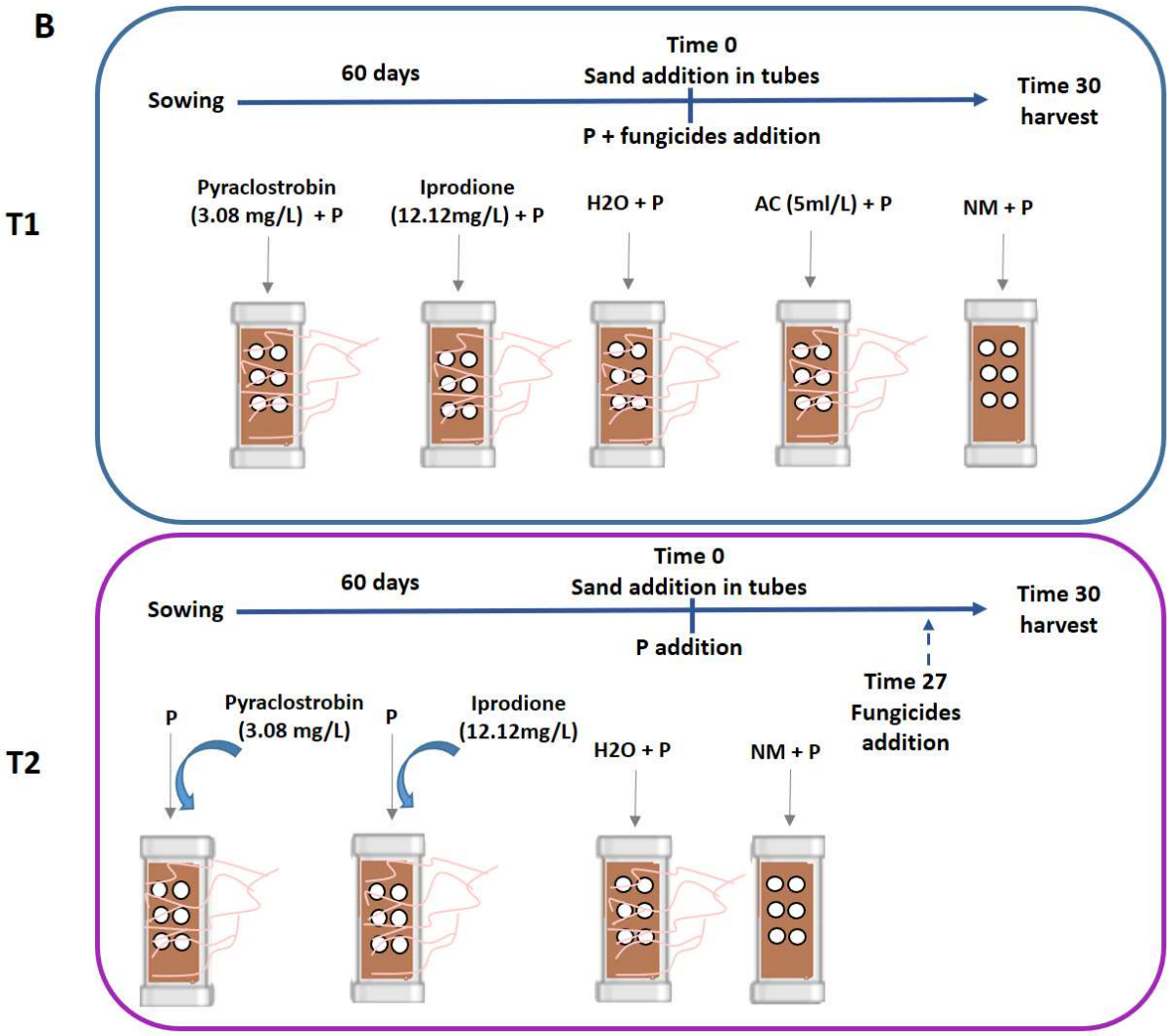


Figure 1. (A) A ten-centimeter-long PVC tube with circular holes 1 cm in diameter, covered on the outside with a 41-µm nylon mesh and sealed at the bottom with the lid of a plastic Petri dish. The tube was placed in a 1 L plastic pot containing a mixture of 50% fine sand (~0.05 mm diam.) and 50% coarse sand (≥ 1 mm diam.) in the presence of a mycorrhizal or non-mycorrhizal *Medicago truncatula* plant. Only the hyphae of the AMF were able to pass through the nylon mesh and grow inside the tube. The plant system was grown in the presence/absence of the fungicides iprodione or pyraclostrobin (B) Experimental design to assess the effect of the fungicides on metabolic activity and Pi uptake capacity of the extraradical mycelium of *Rhizophagus intraradices*. (T1) Iprodione or pyraclostrobin at field concentration (1 time the recommended pesticide dose, RPD) and P (62.45 mg L^{-1}) were applied simultaneously inside the tubes after 60 days of *M. truncatula* growth. (T2) P (62.45 mg L^{-1}) was applied after 60 days of growth of *M. truncatula*, while the fungicides were added 3 days before harvest. Three control treatments: non-mycorrhizal plants (NM treatment), mycorrhizal plants with H_2O in the tube (H_2O treatment) and mycorrhizal plants with acetone in the tube (AC treatment), all watered with the same nutrient solution containing P (62.45 mg L^{-1}), were considered. Twelve replicates were considered for each treatment.

2.2.2. Preparation of the pot system

The empty in-growth tube was placed in the corner of a 1 L pot (Figure 1A). The pot was filled with a mixture of 50% fine sand (~0.05 mm diam.) and 50% coarse sand (≥ 1 mm diam.) supplied by Wendlandkies (Lüchow, Germany). The bottom of the pot was covered with a filter paper (GLAESERgrow, Ulm, Germany), surmounted by a layer (~1 cm) of expanded clay (0.5 – 2.5 mm diam.) supplied by CNBlähton consulting (Randersvej, Denmark) to maintain humidity. All were sterilized 2 times 6 hours at 120°C in a dry oven. With the exception of the NM treatment, the sand mixture was added halfway up the pot and covered with 500 mg of AMF-colonized maize roots sliced into ± 1 mm long fragments, representing approx. 20 000 propagules. The pot was then completely filled with the remaining sand mixture. On the opposite corner to the in-growth tube, four *M. truncatula* seeds were sown at a depth of 1 cm (covered with the sand mixture and finally topped with expanded clay (0.5 – 2.5 mm diam.) to retain soil moisture. Germination occurred after a few days and only two homogenous plants were kept per pot (Fig 1A).

The pots were randomly placed in the greenhouse set to a photoperiod of 16 h of light/8 h of dark, a temperature of 20°C, a relative humidity of 50%, and a photosynthetic photon flux density of 96 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Plants were watered with 50 mL of a 90% reduced P Hoagland solution (Hoagland low-P - Garcés-Ruiz *et al.*, 2017). The pH of the solution was adjusted to 5.6 before use. The Hoagland low-P solution was applied at sowing and once every following week. The plants were also watered with 50 mL of deionized water when needed.

After 60 days of cultivation, the in-growth tubes were filled with 300 g of the same sand mixture as described above allowing the hyphae to extend from the pot into the in-growth tubes.

2.3. Addition of fungicide and phosphate to the in-growth tubes

2.3.1. Preparation of fungicides solution and addition to the in-growth tubes

Iprodione and pyraclostrobin were used at their recommended field concentration (i.e., 1 time the RPD – see Roshanfekrrad *et al.*, 2025). One hundred mg of each fungicide was dissolved in 10 mL acetone to prepare the stock solutions. Working solutions at RPD were then prepared as follows: iprodione at 12.12 mg L^{-1} and pyraclostrobin at 3.08 mg L^{-1}. Stock solutions of iprodione (553 µl) and pyraclostrobin (123 µl) were prepared in 1 L deionized water. The in-growth tubes were then drenched with 50 mL of the respective fungicide solution.

2.3.2. Preparation of the phosphate solution and addition to the in-growth tubes

A KH_2PO_4 solution was prepared by dissolving 232 mg L^{-1} KH_2PO_4 in deionized water to provide a final inorganic P (Pi) concentration of 62.45 mg L^{-1}, corresponding to 10 times the concentration used in standard Hoagland solution. The solution was applied once in the in-growth tubes, at the time the sand was added, i.e. 60 days after growth of *M. truncatula* in the pots. Each in-growth tube received 50 mL of the high-Pi solution.

2.4. Experimental design

Iprodione and pyraclostrobin solutions (at RPD) were applied to the sand in the in-growth tubes at two distinct times (T1 and T2). At T1, the fungicides were applied simultaneously with the Pi to the in-growth tubes, after 60 days of growth of *M. truncatula* (defined as T0 of the experiment - Figure 1B). At T2, the fungicides were added 3 days before harvest (so after 87 days of growth of *M. truncatula*) (Figure 1B). Consequently, at T1, AM fungal hyphae proliferated in the in-growth tubes in the continuous presence of fungicides (i.e., for 30 days), whereas in T2, hyphae proliferated in the in-growth tubes for 27 days before fungicides addition, resulting in a reduced contact period of 3 days.

Three controls were included at T1: non-mycorrhizal plants (NM treatment), mycorrhizal plants with H_2O applied to the in-growth tubes (H_2O treatment) and mycorrhizal plants with acetone applied to the in-growth tubes (AC treatment), all fed with the same amount of Pi (62.45 mg L^{-1}). At T2, the AC treatment was omitted because no differences in plant and fungal parameters were observed between H_2O treatment and AC treatment in T1 (see supplementary results). Twelve replicates were implemented for each treatment.

2.5. Harvest

The in-growth tubes were collected 30 days after Pi addition. The sand was transferred to an Erlenmeyer flask containing 400 mL of deionized water and gently agitated with a spoon to suspend the mycelium. The resulting suspension was passed through a 40 µm sieve to recover the mycelium which was surface-dried on a tissue paper and weighted (mycelium fresh weight; MFW) before transfer to a 2 mL microtube containing Alkaline Phosphatase (ALP) solution for subsequent ALP activity analysis (Lucic-Mercy *et al.*, 2024). Five mL of the suspension were further collected and transferred into 15 mL Falcon tubes for Pi concentration measurement by Inductively Coupled Plasma – Optical Emission Spectroscopy ICP-OES (ICAP 6500, Thermo-Scientific, USA) by the

Mineral and Organic Chemical Analysis (MOCA)) platform of the Earth and Life Institute in UCLouvain.

The shoots and roots of *M. truncatula* plants were separated. Shoots were oven-dried at 70°C for 3 days to determine shoot dry weight (SDW). Roots were carefully washed, surface-dried with tissue paper, and then oven-dried for root dry weight (RDW) determination, following the same procedure as for shoots. The entire root system was subsequently stained to assess root colonization (see method below).

2.5.1. Assessment of spore and hyphal metabolic activity

To assess AM fungal metabolic activity, two analyses were performed. Firstly, six replicates of fresh hyphae were stained with ALP and then with iodonitrotetrazolium salt (INT) solutions following the protocols described by Walley and Germida (1995) and Lucic-Mercy *et al.* (2024). Spore metabolic activity was then evaluated based on spore staining patterns. ALP staining indicates active fungal phosphate metabolism, reflecting the hydrolysis of organic P (Po) sources, a key step in Pi acquisition. This vital staining may be linked to the cytochrome c oxidase (COX) pathway (Lucic-Mercy *et al.*, 2024). This solution turns into an insoluble, dark blue-black precipitate at the enzyme site localized within the spores as well as in the hyphae, arbuscules and vesicles, indicating P-solubilizing activity. INT is a marker of a specific mitochondrial activity (Walley and Germida, 1995; Mukerji *et al.*, 2002), potentially related to the alternative oxidase (AOX) pathway and may be linked to stress or dormancy (Mercy *et al.*, 2017). This solution turns into insoluble formazan crystals (red precipitate) at active respiration sites, localized within the spores as well as in the hyphae, arbuscules and vesicles. By counting red and black spores under binocular, the ratio of spores stained by INT and ALP was calculated for the spore metabolic activity.

Secondly, the ALP activity in the hyphae was determined by ImageJ software. After dispersion of the hyphae between glass slides and coverslips and observation under microscope (Olympus CX33). Pictures were taken at 20 X magnification using an integrated UA510CA camera. The exposure time was 7 ms. The same parameters (light, magnification, brightness and exposure time) were carefully maintained for all samples. For each sample (i.e., replicate), 40 pictures of stained hyphae and spores were taken at random. The evaluation of the viability with ALP was done via analysis of the pictures with the Fiji extension of the ImageJ software (Schindelin *et al.* 2012). The

spores were first removed from the pictures because they always react positively to the applied threshold (Figure 2 A, B). The pictures were then transformed into black and white (16-bit command – Figure 2 C) and then inverted to have the hyphae (previously black) in white on a black background (invert command) (Figure 2 D). The threshold was chosen based on the coloration corresponding to the activity of the AM fungus and the presence of formazan resulting from the enzymatic activity (Figure 2 E and F). Then, the area of the red pixels was calculated by comparing the ratio of the pixeled area in red corresponding to the molecules demonstrating the activity of the AM fungus and the total pixeled area in red (hyphae) between different treatments.

AM fungal viability was calculated with the following formula:

$$Relative\ Red\ Area\ (\%) = \frac{Red\ Area}{Total\ Area} \times 100$$

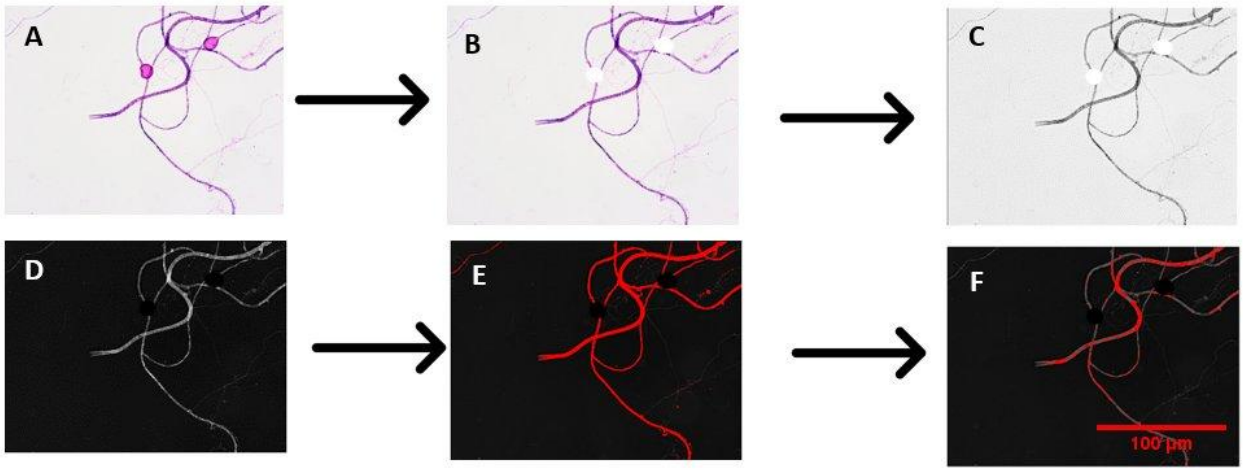


Figure 2. Steps for analyzing pictures taken under the microscope with ImageJ software. (A) Initial picture taken from the mycelium samples by camera, (B) picture after removal of the spores, (C) Picture transformed into black and white, (D) Picture after inversion of the shades of white and black, (E) Changing the pixels corresponding to the hyphae to red, (F) Red pixels corresponding to alkaline phosphatase activity in the hyphae of *R. intraradices* (which makes the hyphae red, only on the active spots).

2.5.2. Root colonization

Dried root samples were cleared in 10% KOH for 3 days at room temperature, then rinsed thoroughly with water. Roots were subsequently stained in a solution containing 5% black ink (Parker, USA) and 5% acetic acid and incubated overnight at room temperature. Roots were then carefully washed 3 to 5 times with water to remove excess of ink. For each sample, 30 pieces of 1-cm long root fragments were mounted on slides. Frequency (F%) and intensity (M%) of root colonization as well as arbuscules (A%) and vesicles/spores (V%) abundances in roots were

assessed according to a modified protocol from Trouvelot et al. (1986), using the Mycocheck Advanced Excel spreadsheet (Lucic-Mercy et al., 2024).

2.6. Statistical analyses

Data were analyzed separately for each sampling time (T1 and T2). Prior to analyses, normality and homogeneity of variance of the residuals were assessed using the Shapiro–Wilk and Levene (Brown–Forsythe) tests, respectively. The F%, M%, A% and V% were arcsin square-root-transformed. Variables meeting parametric assumptions without transformation (RDW, SDW, ratio of stained spores INT/ALP, proportion of extraradical hyphae exhibiting ALP activity, and Pi uptake) were analyzed using raw data. For normally distributed data, treatment effects were evaluated by one-way ANOVA followed by Tukey multiple comparison test ($p \leq 0.05$) using GraphPad Prism 9.5.1. For MFW, which did not satisfy normality assumptions (Shapiro–Wilk, $p \leq 0.05$), treatments were compared using the non-parametric Kruskal–Wallis test followed by Dunn's multiple comparison test with Bonferroni correction ($p \leq 0.05$).

3. Results

3.1. Preliminary analysis

Because acetone was used to solubilize the two fungicides, we included a control with acetone (AC treatment) and compared RDW, SDW, MFW, spore and mycelium metabolic activity, root colonization and Pi concentration in the in-growth tubes data with the H_2O treatment. In all comparisons, no statistical differences were observed between the two controls (see Figure S1 in supplementary data). Therefore, it was decided to only consider the H_2O control treatments in the analyses below.

3.2. Plant and mycelium biomasses

The RDW and SDW of plants in the pots, as well as the MFW in the in-growth tubes, were evaluated at T1 and T2 under pyraclostrobin and iprodione treatments, as well as under the NM and H_2O control treatments (Figure 3A, 3B, and 3C, respectively). At T1, RDW and SDW were significantly lower in the NM treatment than in the other three treatments, which did not differ

313 significantly from one another. At T2, no significant differences were observed in RDW among
314 the four treatments (Figure 3A), whereas SDW remained significantly lower in the NM treatment
315 compared with the other three treatments, which did not differ significantly from one another
316 (Figure 3B). At T1, fungicide application significantly affected MFW ($p < 0.001$). Post hoc analysis
317 revealed that all treatments differed significantly: iprodione showed the highest value, followed by
318 the H_2O control, while pyraclostrobin resulted in the lowest value. At T2, fungicide application
319 also had a significant effect on MFW ($p = 0.019$). However, iprodione did not differ significantly
320 from the H_2O control, whereas pyraclostrobin remained significantly lower than both the H_2O
321 control and iprodione treatments (Figure 3C).

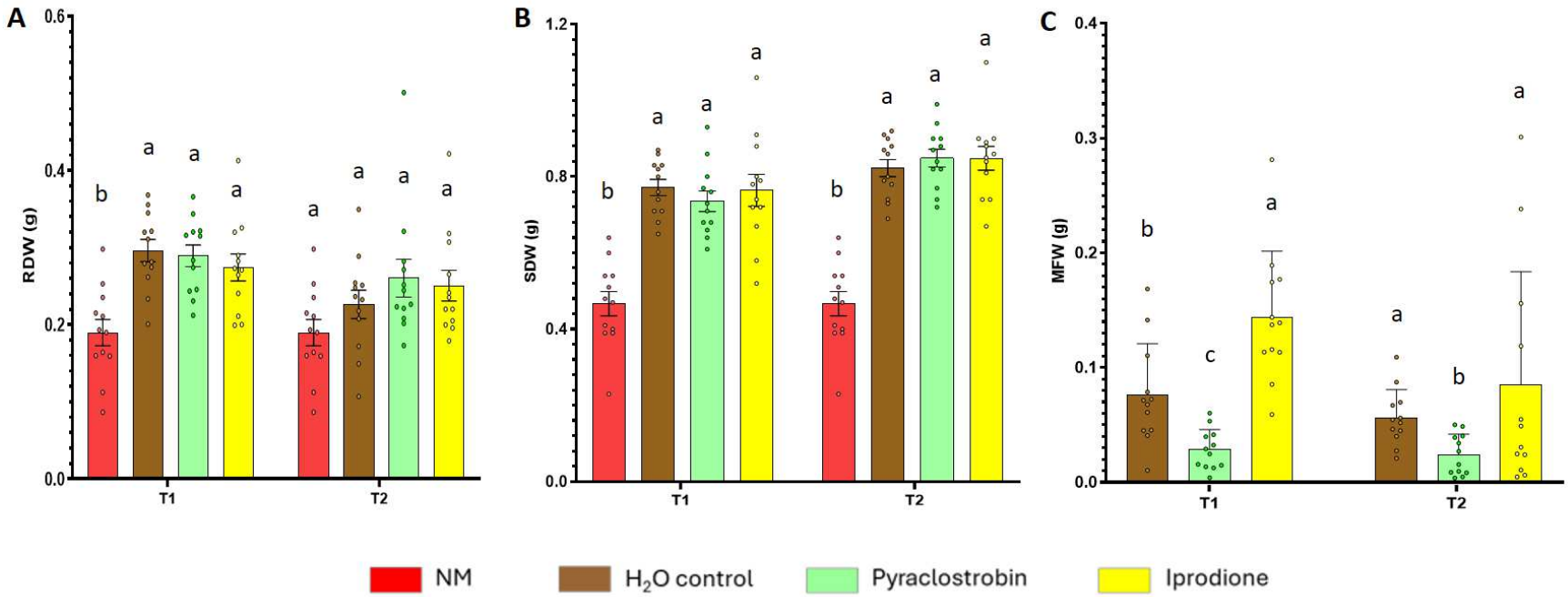


Figure 3. Plant and AM fungal extraradical mycelium (ERM) biomass. (A) Root dry weight (RDW) and (B) shoot dry weight (SDW) of *M. truncatula* plants grown in pots and (C) mycelium fresh weight (MFW) produced within in-growth tubes, measured at T1 and T2 in absence of pesticides (H_2O control) or in the presence of iprodione and pyraclostrobin applied at the recommended pesticide dose. For RDW and SDW, a non-mycorrhizal plant control (NM) was also included. Data are presented as means ± standard errors (n = 12). Different letters indicate significant differences between treatments at T1 and T2 ($P \leq 0.05$, one-way ANOVA followed by a Tukey multiple comparison test). For MFW, boxplots represent the median, interquartile range, and Tukey whiskers (1.5 × IQR). Yellow dot indicates an outlier. Different letters indicate significant differences between treatments within each sampling time according to the Kruskal–Wallis test followed by Dunn's post hoc test ($p \leq 0.05$) (n = 12).

3.3. Spore and hyphae active metabolic staining

Spore and hyphae metabolic activity were evaluated after staining freshly collected mycelium, based on the observation of INT-red and ALP-black staining (Figure 4A). Part of spores were stained in red, whereas others appeared black, reflecting distinct metabolic activities. The ratio of INT/ALP-stained spores of *R. intraradices* in mycelium samples exposed to pyraclostrobin and iprodione treatments, as well as under H_2O control treatment at T1 and T2 is presented in Figure 4B. At T1, the INT/ALP ratio of spores was significantly higher in the iprodione treatments than in the two other treatments that did not differ from one another, whereas at T2, no significant differences were observed between the three treatments.

The percentage of ALP activity in extraradical hyphae was evaluated at T1 and T2 under pyraclostrobin and iprodione treatments, as well as under H_2O control treatment (Figure 4C). At T1, the percentage of ALP activity in extraradical hyphae was significantly lower in the pyraclostrobin treatment than in the other two treatments, which did not differ significantly from one another, whereas at T2, no significant differences were observed between the three treatments.

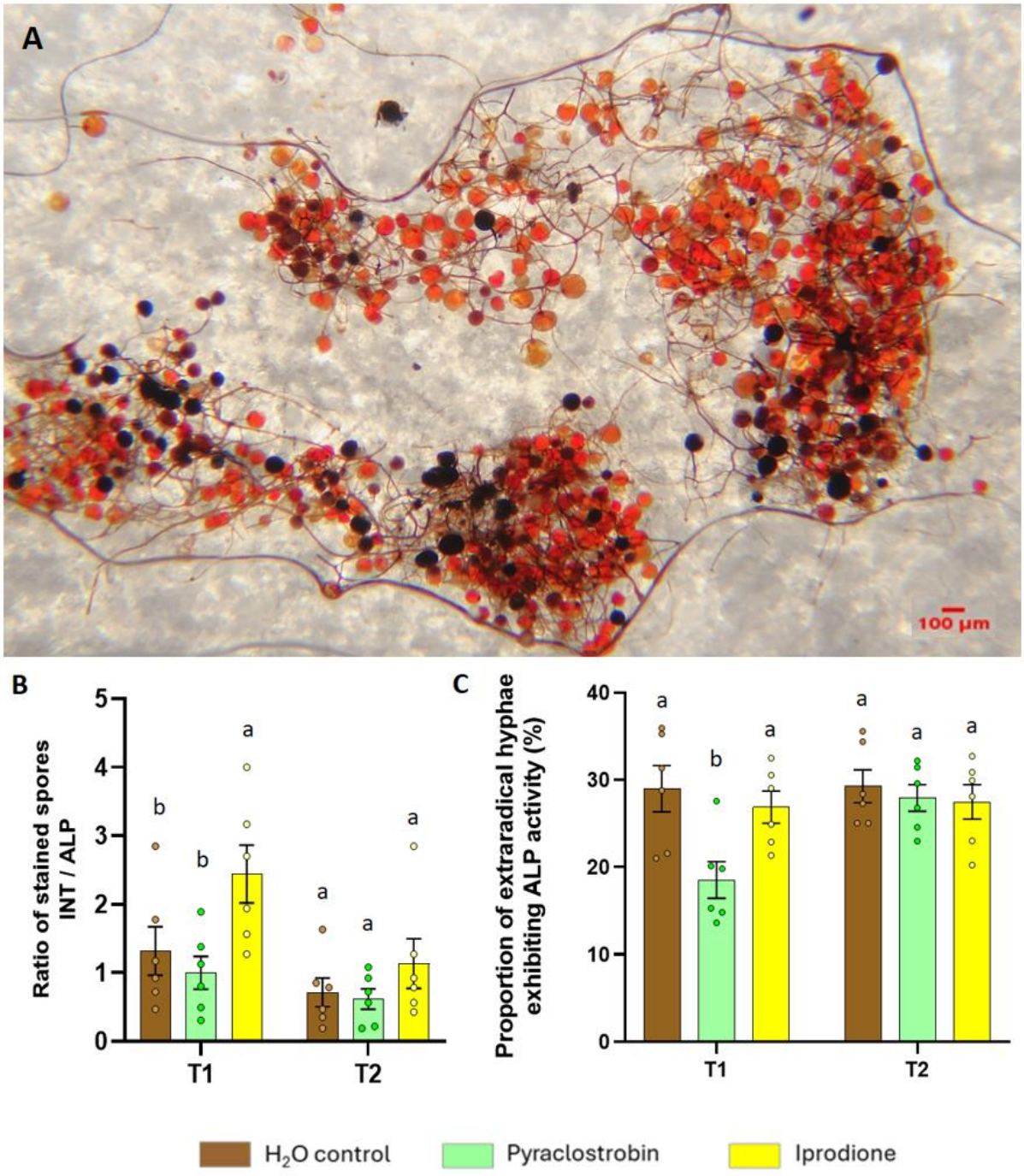


Figure 4. Spore and hyphae metabolic activities in the in-growth tube. (A) Spores of *R. intraradices* MUCL 49410 stained with INT (red spores) and ALP (black spores), (B) INT/ALP-stained spore ratio of *R. intraradices* MUCL 49410, (C) Percentages of extraradical hyphae exhibiting ALP activity (%) measured at T1 and T2 in absence of pesticides (H_2O Control) or in the presence of iprodione and pyraclostrobin applied at the recommended pesticide dose. Data are presented as means ± standard errors (n = 6). Different letters indicate significant differences between treatments at T1 and T2 ($P \leq 0.05$, one-way ANOVA followed by a Tukey multiple comparison test).

3.4. Root colonization

Root colonization frequency (F%), intensity (M%), arbuscules abundance (A%) and vesicles/spores abundance (V%) (Fig. 5A, 5B, 5C and 5D, respectively) by *R. intraradices* MUCL 49410 were evaluated at T1 and T2 under pyraclostrobin, iprodione, and H_2O control treatments. At T1, both F% (Figure 5A) and A% (Figure 5C) were significantly lower in the pyraclostrobin treatment than in the iprodione and H_2O control treatments, which did not differ significantly from one another. At T2, no significant differences in F% were observed between the treatments (Figure 5A). However, A% remained significantly lower for the pyraclostrobin treatments compared to the two other treatments, and the iprodione treatment was also significantly lower than the H_2O control treatment (Figure 5C). At both T1 and T2, the M% was significantly lower in the pyraclostrobin treatment than in the iprodione and H_2O control treatments, which did not differ significantly from one another (Figure 5B). The V% (Figure 5D) was significantly influenced by treatments at both sampling times. At T1, the %V of all treatments differed significantly: iprodione showed the highest value, followed by the H_2O control, while pyraclostrobin resulted in the lowest value. At T2, the %V was significantly higher in the iprodione treatment than in the pyraclostrobin treatment, while it did not differ from the H_2O control treatment. No significant differences were observed between pyraclostrobin and H_2O control treatments.

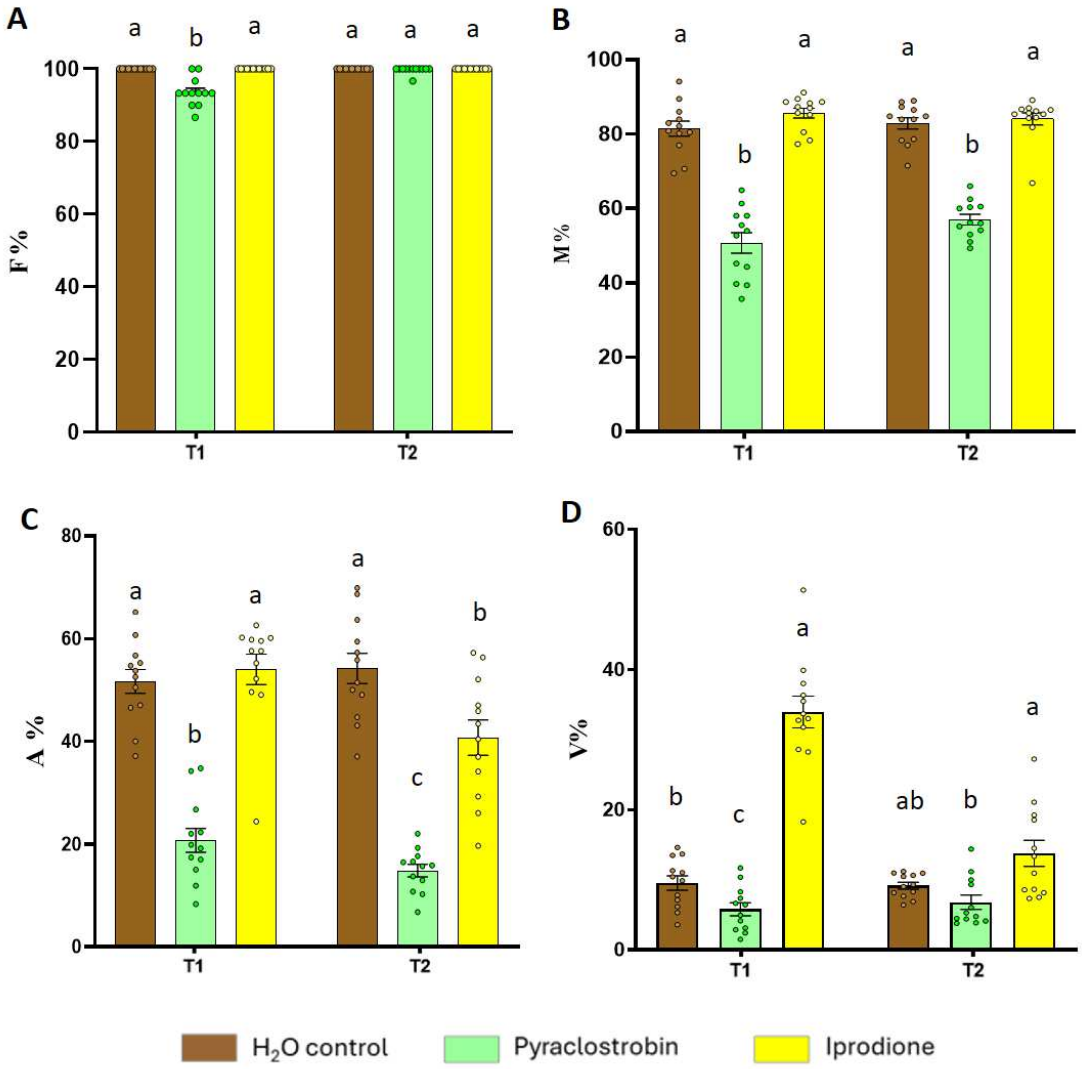


Figure 5. (A) frequency (F%), (B) intensity (M%), (C) arbuscule abundance (A%) and (D) vesicle/spore abundance (V%), measured at T1 and T2 in the roots of *Medicago truncatula* colonized by *Rhizophagus intraradices* MUCL 49410. The extraradical mycelium in the in-growth tube developed either in absence of pesticides (H_2O control) or in the presence of iprodione and pyraclostrobin applied at the recommended pesticide dose. Data are expressed as means ± standard errors (n = 12). Data for F%, M% and A% were arcsine square-root transformed prior to statistical analysis. V% data were log10-transformed prior to statistical analysis. Different letters indicate significant differences between treatments at T1 and T2 (one-way ANOVA followed by a Tukey multiple comparison test, $P \leq 0.05$). Figures present non-transformed means.

3.5. Inorganic phosphorus uptake

Phosphate uptake by the AM fungus *R. intraradices* MUCL 49410 in the in-growth tubes was quantified at T1 and T2 under pyraclostrobin and iprodione treatments, as well as under the NM and H_2O control treatments (Figure 6). At T1, Pi concentration in the in-growth tubes was

significantly lower in the iprodione treatment compared with the NM and H_2O treatments but did not differ significantly from the pyraclostrobin treatment. Likewise, the Pi concentration in the pyraclostrobin treatment was significantly lower than in the NM treatment. In T2, the Pi concentration in the in-growth tubes did not differ significantly between the iprodione, pyraclostrobin and H_20 treatments. However, only H_2O treatment exhibited significantly lower Pi concentrations compared with the NM treatment.

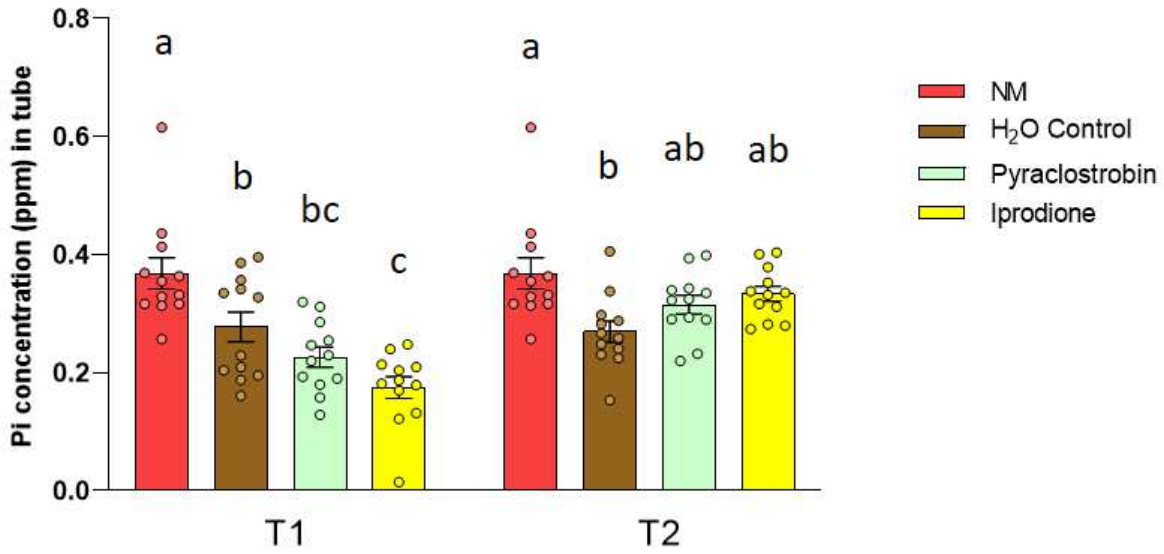


Figure 6. Inorganic phosphorus (Pi) concentration (ppm) remaining in the in-growth tubes colonized by *Rhizophagus intraradices* MUCL 49410 measured at T1 and T2 in absence of pesticides (H_2O control) or in the presence of iprodione and pyraclostrobin applied at the recommended pesticide dose. A non-mycorrhizal plant control (NM) was also included. Data are expressed as means ± standard errors (n = 12). Different letters indicate significant differences between treatments at T1 and T2 (one-way ANOVA followed by a Tukey multiple comparison test, $P \leq 0.05$).

4. Discussion

In this study, we investigated how two widely used fungicides with distinct modes of action – pyraclostrobin and iprodione (FRAC, 2022) – influence the metabolic activity and Pi uptake capacity of the ERM of *R. intraradices* grown in a sand-based substrate. Fungicide application was confined to the in-growth tubes, thereby restricting chemical exposure to the ERM and minimizing direct effects on the host plant. This design allowed us to disentangle plant responses to colonization (biomass and root colonization rates) from fungal physiological responses (spore and

hyphal metabolic activity, Pi uptake capacity), thereby allowing a targeted assessment of fungicides effects on the symbiotic performance. Neither fungicide significantly influenced plant shoot or root biomass. However, pronounced effects were observed on the ERM within the in-growth tubes and on root colonization, particularly following pyraclostrobin exposure. Pyraclostrobin treatment markedly reduced ERM biomass and hyphal metabolic activity, suggesting strong inhibition of mitochondrial respiration and phosphate uptake capacity, consistent with its QoI mode of action. In contrast, exposure to iprodione resulted in increased ERM biomass and enhanced spore metabolic activity, suggesting a mild stimulation of fungal growth and a compensatory adjustment of Pi uptake, potentially mediated by the activation of alternative respiratory or stress-mitigation pathways.

4.1. Impact of pyraclostrobin on mycelium biomass, root colonization and metabolic activities of *R. intraradices*

Pyraclostrobin exerted consistent negative effects on the ERM of *R. intraradices*, impacting root colonization, as well as mycelium biomass and physiological parameters within the in-growth tubes. These effects were evident both after short-term (3 days) and prolonged (30 days) exposure, with ERM biomass reduced by 55% and 75%, respectively. This observation indicates a rapid impairment of fungal development and symbiotic functioning following fungicide application, which persists over time. Comparable findings have been reported for azoxystrobin, another quinone-outside inhibitor (QoI), which has been shown to alter fungal physiology in *Rhizophagus irregularis* and reduce root colonization in potato plants (Rodriguez-Morelos *et al.*, 2021, 2022, 2023).

The significant decrease in ALP activity observed in the ERM within the in-growth tubes, suggests a substantial decline in fungal metabolic activity, likely linked to impaired mitochondrial functioning. Pyraclostrobin inhibits electron transfer at the cytochrome bc1 complex of the mitochondrial respiratory chain (Musso *et al.*, 2020), disrupting oxidative phosphorylation and reducing ATP production. Mitochondrial dysfunction can also promote reactive oxygen species formation, compromising membrane integrity and enzymatic activity in fungal cells (Bartlett *et al.*, 2002; Breitenbach *et al.*, 2015). As mitochondrial respiration underpins AM fungal metabolism, such impairment is likely to limit the energy available for the maintenance of nutrient exchange

structures, including arbuscules. Consistent with this mechanism, we observed a reduced abundance of arbuscules, particularly after 30 days of exposure. The decrease detected after only 3 days was less pronounced, suggesting early or indirect stress responses affecting arbuscules formation. The stronger reduction observed under prolonged exposure therefore likely reflects cumulative metabolic impairment, ultimately compromising symbiotic functioning.

Despite reduction in ERM biomass at both sampling times, and in ALP activity after 30 days induced by pyraclostrobin, Pi depletion in the in-growth tubes remained comparable to that of the H_2O control at both sampling times, showing a slight increase in the short term (day 3) but the opposite trend after prolonged exposure 30 days). This suggests that the ERM within the in-growth tubes at least partially retained its capacity for Pi uptake with no statistically significant difference relative to the control. One possible explanation is that the ERM compensated for reduced biomass by increasing uptake activity per unit of hyphae. Such a response may reflect a transient stimulation of Pi uptake under stress conditions, as reported for AM fungi exposed to sub-lethal doses of fungicides (Zocco *et al*., 2011). This interpretation is consistent with the observation that in our study pyraclostrobin initially elicited a stress-response that allowed maintenance of short-term uptake capacity, whereas prolonged exposure ultimately led to a decrease, although non-significant, in uptake.

4.2. Impact of iprodione on mycelium biomass, root colonization and metabolic activities of *R. intraradices*

In contrast to pyraclostrobin, iprodione induced markedly different responses of the AM fungus. Rather than inhibiting fungal growth, prolonged exposure (i.e., 30 days) led to increased ERM biomass in the in-growth tubes. In parallel, the proportion of metabolically active spores increased, as indicated by changes in the INT/ALP staining ratio, suggesting altered metabolic activity. This change may involve activation of the alternative oxidase (AOX) pathway, which can bypass the cytochrome pathway under stress, allowing partial respiration when the classical electron transport chain is disturbed (Vanlerberghe, 2013). The observed increase in ERM biomass following 30 days exposure to iprodione suggested that fungal activity was stimulated, reflecting compensatory physiological adjustments or mild stress responses (Bedini *et al.*, 2018). Iprodione had little apparent impact on AM fungal metabolism at the applied concentration and may even have

stimulated certain aspects of fungal activity. While vesicle and spore abundance in roots remained low after 3 days exposure to iprodione, a significant increase was observed after 30 days of treatment. This pattern suggest enhanced fungal reproduction and/or a shift in resource allocation toward storage and propagule formation under prolonged iprodione exposure.

After 30 days of exposure, iprodione treatment resulted in significantly lower Pi concentrations in the in-growth tubes compared to the H_2O control treatment, suggesting enhanced Pi uptake by the fungal network over time, whereas no significant differences were observed after short-term exposure (3 days). This effect is likely linked to the iprodione-induced increase in ERM biomass, which may have enhanced the functional capacity of the fungal network for phosphate uptake. Comparable stimulatory responses to low pesticide doses have been documented in AM fungi, where sub-inhibitory concentrations induce hormetic effects, including increased root colonization and hyphal Pi uptake (Jakobsen *et al*., 2021). This suggests that direct stimulation of fungal activity may contribute to the observed pattern. Taken together, these findings highlight the capacity of AM fungi to modulate nutrient acquisition and sustain plant Pi supply under changing environmental or metabolic conditions (Neumann and George, 2010).

4.3. Mycorrhizal colonization and physiological responses

The functional inhibition observed with pyraclostrobin aligns with the mode of action of QoI fungicides, which target mitochondrial respiration, as well as with the high energetic costs associated with nutrient transport. In contrast, the increase in Pi uptake under iprodione after 30 days of exposure may reflect a compensatory response of the fungal network, highlighting the potential functional resilience of AM fungi under certain chemical stresses. Our results thus support the view that the impact of fungicides on AM fungi strongly depends on their biochemical targets and on the ability of the AM fungi to compensate for metabolic disturbances (Schweiger and Jakobsen, 1998; Smith and Smith, 2011). These observations highlight the importance of a multi-tier testing approach for AMF. Tier I assays are useful for initial toxicity screening, while Tier II or more integrative assays enable the assessment of functional endpoints under more realistic conditions. For instance, Roshanfekrrad *et al*. (2025) reported that iprodione reduced spore germination of *R. intraradices* in Tier I assays based on EC_{50} values. At 1x the RPD, this effect was mostly fungistatic, prolonging spore inactivity rather than causing mortality, whereas higher

doses (10x and 20x RPD) were more fungicidal. In the present study, the pesticide was applied on the ERM connected to the plant, therefore in the symbiotic phase of the association. Under this condition, iprodione increased ERM biomass and spore metabolic activity of the fungus, illustrating how functional endpoints can reveal compensatory or adaptative responses not captured by single-endpoint assay. Collectively, these findings emphasize that integrating both functional and physiological endpoints is essential for accurately evaluating pesticide effects on AM fungi.

Building on this perspective, our findings indicate that spore germination endpoint alone may underestimate fungicide effects. Our results advocate the inclusion of colonization and functional parameters, encompassing both asymbiotic and symbiotic endpoints in pesticide risk assessment frameworks for AM fungi as suggested by Roshanfekrrad *et al.* (2025) and Papadopoulos *et al.* (2025).

4.4. Differential responses of *R. intraradices* to short-term (3 days) and prolonged (30 days) exposure

The duration of exposure to the fungicide strongly influenced the magnitude of AM fungal responses. Prolonged exposure (30 days) elicited more pronounced physiological responses than shorter term exposure (i.e., 3 days), particularly under pyraclostrobin, indicating that sublethal effects may accumulate over time and remain undetected in short-term assays. This suggests either that brief exposure does not induce measurable impairment or that fungi can temporarily compensate the induced stress.

The observed differences in ERM responses as a function of fungicide exposure duration highlight the ecological relevance of temporal exposure. Pyraclostrobin is moderately persistent in soil, with reported half-lives typically ranging from approximately 13 to 42 days depending on soil type and environmental conditions (EFSA, 2025). In contrast, the U.S. EPA Registration Eligibility Decision (RED) indicates that field dissipation half-lives (DT_{50}) of iprodione are generally shorter, in the range of about 3 – 7 days under typical field conditions, although longer persistence can occur possible depending on soil characteristics and environmental factors. In agricultural systems, repeated fungicide applications during the growing season may increase the likelihood of sustained exposure. For example, in wheat crop, pyraclostrobin formulations (200 g L^{-1}) is often applied up

to twice per season at a rate of 1.25 L ha^{-1}, with a minimum interval of approximately 21 days between applications (Belgian Federal Public Service Health, Fytoweb). Such regimes suggest that ERM or AM fungi in agricultural soils may be exposed to fungicides over periods comparable to those examined in our study, particularly during the active cropping season in temperate European regions. In these conditions AM fungi are most active between March and October as indicated by seasonal crop calendar datasets (Dimou *et al.*, 2018). However, because fungicide applications are primarily aboveground, the concentrations reaching the soil, and thus directly contacting the fungi, are likely lower than the applied doses. Nevertheless, systemic effects of foliar applications can still influence belowground fungi (Bastogne *et al.*, 2025), even if such effects are not visible in aboveground plant traits. Soil processes such as leaching may further reduce local exposure, highlighting a key difference from the controlled conditions of the present study. These factors underscore the importance of considering realistic exposure concentrations and durations, and of integrating structural, physiological, and functional endpoints when assessing the impact of fungicide impacts on AM fungi (Papadopoulos *et al*., 2025).

5. Conclusion

In this study we demonstrated that fungicides – pyraclostrobin and iprodione - are far from a uniform class when it comes to their interactions with beneficial soil fungi. Rather, their effects unfold along a spectrum shaped by biochemical targets and the intrinsic capacity of fungal organisms to buffer metabolic disruption (Schweiger and Jakobsen, 1998; Smith and Smith, 2011). Pyraclostrobin emerged as particularly disruptive. Its effects propagated throughout the fungal network, impairing mitochondrial activity, reducing mycelial growth and arbuscule abundance, and altering metabolic functioning. These findings indicate that fungicides targeting core metabolic pathways can strongly disrupt key structural and metabolic components of AM fungal networks, particularly hyphal development and energy metabolism, while not necessarily affecting functional processes such as Pi uptake, which remained unchanged in this study. In contrast, iprodione followed a distinctly different trajectory. Rather than causing inhibition, it elicited a more nuanced physiological response, including moderate adjustments accompanied by increased ERM biomass and even stimulation of metabolic activity and Pi uptake, without substantially affecting root colonization, suggesting a comparatively compatible interaction.

Taken together, our findings indicate that AM fungal responses are influenced not only by fungicide mode of action but also exposure duration. Persistent, sublethal compounds such as pyraclostrobin can impair ERM function and nutrient cycling, whereas others, like iprodione, appear comparatively benign under similar conditions. Moreover, the potential for adaptive responses or resistance development should be considered, as repeated exposure may alter fungal sensitivity to specific modes of action (Lucas *et al.*, 2015). Moving forward, incorporating both physiological and functional endpoints into pesticide risk assessment will be essential to capture these subtleties, ultimately guiding the development and selection of plant-protection products that are compatible with, rather than disrupt to, AM symbiosis.

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7. References

Bartlett, D.W., Clough, J.M., Godwin, J.R., Hall, A.A., Hamer, M. and Parr-Dobrzanski, B. (2002) 'The strobilurin fungicides', *Pest Management Science*, 58(7), pp. 649–662. Available at: https://doi.org/10.1002/ps.520.

Bedini, A., Mercy, L., Schneider, C., Franken, P. and Lucic-Mercy, E. (2018) 'Unraveling the initial plant hormone signaling, metabolic mechanisms and plant defense triggering the endomycorrhizal symbiosis behavior', *Frontiers in Plant Science*, 9:1800. Available at: https://doi.org/10.3389/fpls.2018.01800

Belgian Federal Public Service Health (n.d.) Fytoweb: Plant protection product details (pyraclostrobin product). Available at: https://apps.health.belgium.be/fytoweb/pages/public/detail.xhtml?product=41967120

Breitenbach, M., Weber, M., Rinnerthaler, M., Karl, T. and Breitenbach-Koller, L. (2015) 'Oxidative stress in fungi: its function in signal transduction, interaction with plant hosts, and lignocellulose degradation', *Biomolecules*, *5*(2), pp.318-342. Available at: https://doi.org/10.3390/biom5020318

Calabrese, E.J. and Baldwin, L.A. (2002) 'Applications of hormesis in toxicology, risk assessment and chemotherapeutics', *Trends in Pharmacological Sciences*, *23*(7), pp.331-337. Available at: https://doi.org/10.1016/S0165-6147(02)02034-5

Cranenbrouck, S., Voets, L., Bivort, C., Renard, L., Strullu, DG., Declerck, S. (2005) 'Methodologies for *in vitro* Cultivation of Arbuscular Mycorrhizal Fungi with Root Organs', In: Declerck, S., Fortin, J.A., Strullu, DG. (eds) *In Vitro Culture of Mycorrhizas. Soil Biology*, vol 4. Springer, Berlin, Heidelberg. Available at: https://doi.org/10.1007/3-540-27331-X_18

582 Dimou, M., Meroni, M. and Rembold, F. (2018) 'Development of a national and sub-national crop
583 calendars data set compatible with remote sensing derived land surface phenology', *European*
584 *Commission Joint Research Centre, Publications Office of the European Union: Luxembourg*.

585 European Food Safety Authority (EFSA), Álvarez, F., Arena, M., Auteri, D., Leite, S.B., Binaglia, M.,
586 Castoldi, A.F., Chiusolo, A., Colagiorgi, A., Colas, M. and Crivellente, F. (2025) 'Peer review of the
587 pesticide risk assessment of the active substance pyraclostrobin', *EFSA Journal*, 23(3), p.e9257.
588 Available at: https://doi.org/10.2903/j.efsa.2025.9257

589 Ezawa, T. and Saito, K. (2018) 'How do arbuscular mycorrhizal fungi handle phosphate? New insight into
590 fine-tuning of phosphate metabolism', *New phytologist*, *220*(4), pp.1116-1121. Available at:
591 https://doi.org/10.1111/nph.15187

592 Ferrol, N., Azcón-Aguilar, C. and Pérez-Tienda, J. (2019) 'Arbuscular mycorrhizas as key players in
593 sustainable plant phosphorus acquisition: An overview on the mechanisms involved', *Plant*
594 *Science*, *280*, pp.441-447. Available at: https://doi.org/10.1016/j.plantsci.2018.11.011

595 FRAC (Fungicide Resistance Action Committee) (2022) 'FRAC Mode of Action Classification and
596 Fungicide Use Guideline'*, CropLife International*. Available at: https://www.frac.info.

597 Garcés-Ruiz, M., Calonne-Salmon, M., Plouznikoff, K., Misson, C., Navarrete-Mier, M., Cranenbrouck,
598 S. and Declerck, S. (2017) 'Dynamics of short-term phosphorus uptake by intact mycorrhizal and non-
599 mycorrhizal maize plants grown in a circulatory semi-hydroponic cultivation system', *Frontiers in Plant*
600 *Science*, 8:1471. Available at: https://doi.org/10.3389/fpls.2017.01471.

601 Javot, H., Pumplin, N. and Harrison, M.J. (2007) 'Phosphate in the arbuscular mycorrhizal symbiosis:
602 transport properties and regulatory roles', *Plant, Cell & Environment*, *30*(3), pp.310-322. Available at:
603 https://doi.org/10.1111/j.1365-3040.2006.01617.x

604 Johnson, D., Leake, J.R. and Read, D.J. (2001) 'Novel in-growth core system enables functional studies of
605 grassland mycorrhizal mycelial networks', *New Phytologist*, 152(3), pp.555-562. Available at:
606 https://doi.org/10.1046/j.0028-646X.2001.00273.x

607 Köninger, J., Labouyrie, M., Ballabio, C., Dulya, O., Mikryukov, V., Romero, F., Franco, A., Bahram, M.,
608 Panagos, P., Jones, A. and Tedersoo, L. (2026) 'Pesticide residues alter taxonomic and functional
609 biodiversity in soils', *Nature*, 625, pp. 1–7. Available at: https://doi.org/10.1038/s41586-025-09991-z

610 Lucas, J.A., Hawkins, N.J. and Fraaije, B.A. (2015) 'The evolution of fungicide resistance', *Advances in*
611 *Applied Microbiology*, 90, pp. 29–92. Available at: https://doi.org/10.1016/bs.aambs.2014.09.001

612 Lucic-Mercy, E., Mercy, L., Jeschke, A., Schneider, C. and Franken, P. (2024) 'Short-term artificial
613 adaptation of *Rhizoglomus irregulare* to high phosphate levels and its implications for fungal-plant
614 interactions: phenotypic and transcriptomic insights', *Frontiers in Plant Science*, *15*, p.1385245.
615 Available at: https://doi.org/10.3389/fpls.2024.1385245

616 Mercy, L., Lucic, E. and Franken, P. (2017) 'A functional approach towards understanding the role of the
617 mitochondrial respiratory chain in an endomycorrhizal symbiosis', *Frontiers in Plant Science*, 8, p. 417.
618 Available at: https://doi.org/10.3389/fpls.2017.00417

Moller, I.M., Jensen, P.E. and Hansson, A. (2007) 'Oxidative modifications to cellular components in plants', *Annual Review of Plant Biology*, 58, pp. 459–481. Available at: https://doi.org/10.1146/annurev.arplant.58.032806.103946

Musso, L., Fabbrini, A. and Dallavalle, S. (2020) 'Natural compound-derived cytochrome bc1 complex inhibitors as antifungal agents', *Molecules*, 25(19), p.4582. Available at: https://doi.org/10.3390/molecules25194582

Neumann, E. and George, E. (2010) 'Nutrient uptake: the arbuscular mycorrhiza fungal symbiosis as a plant nutrient acquisition strategy', in Koltai, H. and Kapulnik, Y. (eds.) *Arbuscular Mycorrhizas: Physiology and Function*. Dordrecht: Springer, pp. 137–167.

Olsson, P.A. and Wilhelmsson, P. (2000) 'The growth of external AM fungal mycelium in sand dunes and in experimental systems', *Plant and Soil*, 226(2), pp. 161–169. Available at: https://doi.org/10.1023/A:1026565314345

Papadopoulos, C., Roshanfekrrad, M., Tsikou, D., Papadopoulou, K.K., Calonne-Salmon, M., Declerck, S. and Karpouzas, D.G. (2025) 'Developing a toolbox of Tier I tests to assess pesticides toxicity on the asymbiotic and symbiotic phases of arbuscular mycorrhizal fungi', *Ecotoxicology and Environmental Safety*, *303*, p.118892. Available at: https://doi.org/10.1016/j.ecoenv.2025.118892

Rodriguez-Morelos, V.H., Calonne-Salmon, M., Bremhorst, V., Garcés-Ruiz, M. and Declerck, S. (2021) 'Fungicides with contrasting mode of action differentially affect hyphal healing mechanism in *Gigaspora sp*. and *Rhizophagus irregularis*', *Frontiers in Plant Science*, *12*, p.642094. Available at: https://doi.org/10.3389/fpls.2021.642094

Rodriguez-Morelos, V.H., Calonne-Salmon, M. and Declerck, S. (2022) 'Anastomosis within and between networks of *Rhizophagus irregularis* is differentially influenced by fungicides', *Mycorrhiza* 33, 15–21. Available at: https://doi.org/10.1007/s00572-023-01103-x

Rodriguez-Morelos, V.H., Declerck, S. and Calonne-Salmon, M. (2023) 'Azoxystrobin alters the dynamics of short-term phosphorus uptake of mycorrhizal potato plants associated to *Rhizophagus irregularis*', *Journal of Plant Nutrition and Soil Science*, 186(1), pp.95-104. Available at: https://doi.org/10.1002/jpln.202200256

Roshanfekrrad, M., Papadopoulos, C., Calonne-Salmon, M., Schneider, C., Zhang, K., Karpouzas, D. and Declerck, S. (2025) 'Development of a high-throughput spore germination test to assess the toxicity of pesticides on arbuscular mycorrhizal fungi', *Mycorrhiza*, *35*(3), p.38. Available at: https://doi.org/10.1007/s00572-025-01211-w

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B. and Tinevez, J.Y. (2012) 'Fiji: an open-source platform for biological-image analysis', *Nature Methods*, *9*(7), pp.676-682. Available at: https://doi.org/10.1038/nmeth.2019

Schweiger, P.F. and Jakobsen, I. (1998) 'Dose–response relationships between four pesticides and phosphorus uptake by hyphae of arbuscular mycorrhizas', *Soil Biology and Biochemistry*, *30*(10-11), pp.1415-1422. Available at: https://doi.org/10.1016/S0038-0717(97)00259-9

Schuster, M. and Steinberg, G. (2020) 'The fungicide dodine primarily inhibits mitochondrial respiration in *Ustilago maydis*, but also affects plasma membrane integrity and endocytosis, which is not found in *Zymoseptoria tritici*', *Fungal Genetics and Biology*, 142, p. 103414. Available at: https://doi.org/10.1016/j.fgb.2020.103414

Smith, S.E. and Smith, F.A. (2011) 'Roles of arbuscular mycorrhizas in plant nutrition and growth: new paradigms from cellular to ecosystem scales', *Annual Review of Plant Biology*, 62, pp. 227–250. Available at: https://doi.org/10.1146/annurev-arplant-042110-103846

Smith, S.E., Smith, F.A. and Jakobsen, I. (2003) 'Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses', *Plant physiology*, 133(1), pp.16-20. Available at: https://doi.org/10.1104/pp.103.024380

Smith, S.E., Smith, F.A. and Jakobsen, I. (2004) 'Functional diversity in arbuscular mycorrhizal (AM) symbioses: the contribution of the mycorrhizal P uptake pathway is not correlated with mycorrhizal responses in growth or total P uptake', *New phytologist*, *162*(2), pp.511-524. Available at: https://doi.org/10.1111/j.1469-8137.2004.01039.x

Sweeney, C.J., Bottoms, M., Ellis, S., Ernst, G., Kimmel, S., Loutseti, S., Schimera, A., Carniel, L.S.C., Sharples, A., Staab, F. and Marx, M.T. (2022) 'Arbuscular mycorrhizal fungi and the need for a meaningful regulatory plant protection product testing strategy', *Environmental Toxicology and Chemistry*, 41(8), pp. 1808–1823. Available at: https://doi.org/10.1002/etc.5400

Trouvelot, A., Kough, J.L. and Gianinazzi-Pearson, V. (1986) 'Mesure du taux de mycorhization VA d'un système radiculaire. Recherche de méthodes d'estimation ayant une signification fonctionnelle', in Gianinazzi-Pearson, V. and Gianinazzi, S. (eds.) *Physiological and Genetical Aspects of Mycorrhizae*. Paris: INRA Press, pp. 217–221.

U.S. Environmental Protection Agency, (2006) 'Reregistration Eligibility Decision (RED) for iprodione', EPA 738-R-06-004. *Washington, DC: U.S. EPA.* Available at: https://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=200006IN.TXT

Vanlerberghe, G.C. (2013) 'Alternative oxidase: a mitochondrial respiratory pathway to maintain metabolic and signaling homeostasis during abiotic and biotic stress in plants', *International journal of molecular sciences*, 14(4), pp.6805-6847. Available at: https://doi.org/10.3390/ijms14046805

Walley, F.L. and Germida, J.J. (1995) 'Estimating the viability of vesicular-arbuscular mycorrhizae fungal spores using tetrazolium salts as vital stains', *Mycologia*, 87(2), pp. 273–279. Available at: https://doi.org/10.1080/00275514.1995.12026530

Wolfram, J., Bussen, D., Bub, S., Petschick, L.L., Herrmann, L.Z. and Schulz, R. (2026) 'Increasing applied pesticide toxicity trends counteract the global reduction target to safeguard biodiversity', *Science*, 391(6785), pp. 616–621. Available at: https://doi.org/10.1126/science.aea8602

Yaakoub, H., Mina, S., Calenda, A., Bouchara, J.P. and Papon, N., (2022) 'Oxidative stress response pathways in fungi', *Cellular and Molecular Life Sciences*, 79(6), p.333. Available at: https://doi.org/10.1007/s00018-022-04353-8

694 Zocco D., Van Aarle I.M., Oger E., Lanfranco L. and Declerck S. (2011). Fenpropimorph and fenhexamid
695 impact phosphorus translocation by arbuscular mycorrhizal fungi. *Mycorrhiza*, 21:363-374. Available
696 at: https://doi.org/10.1007/s00572-010-0344-0

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