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Clover and ryegrass are tolerant species to ammonium nutrition

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Summary

The application of nitrification inhibitors (NIs) together with nitrogen fertilizers in grasslands is an effective alternative to reduce nitrate leaching and nitrogenous gases emissions to the atmosphere. Nevertheless, the use of NIs increases the amount of ammonium available for the plant that, due to its reported toxic effect in plants, can have a direct effect on crop production. Grassland species have traditionally suffered from intensive grazing and urea deposition and, therefore, a tolerance to ammonium nutrition could be expected in these species. Plants of *Trifolium repens* L. var. *huia* and *Lolium perenne* L. var. *Herbus* were grown under two nitrogen nutrition regimes (nitrate or ammonium) and three different nitrogen concentrations (0.5, 2.5 and 5 mmol/L). The effect of nitrogen form was determined on biomass production parameters, gas-exchange and water relations parameters as well as polyamine (PA) and ion tissue contents. Both grassland species showed tolerance to ammonium nutrition due to their capacity to adjust several metabolic processes in a species-specific way. Gas exchange measurements and biomass production (expressed as dry weight (DW)) were unaffected by the nitrogen form or dose in both species except for a decrease in root total DW in ryegrass plants grown under ammonium nutrition. Hydraulic conductance (L_0) increased in ryegrass with increasing ammonium doses but no change due to the nitrogen source was observed

Abbreviations: A, CO_2 assimilation rate; DW, dry weight; J_v, sap flow; L_0, hydraulic conductance; PA, polyamine; Put, putrescine; Spd, spermidine; Spm, spermine; Ψ_w, water potential

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in water potential (Ψ_w) values. Both species, and specially ryegrass, accumulated free ammonium mainly in roots when grown under ammonium nutrition and its translocation to the shoot via xylem was also observed. A clear difference in cations and PAs pattern was observed in each species when comparing both nitrogen nutrition regimes.

Introduction

The application of nitrogen (N) fertilizers has been widely used in agricultural systems over the last years leading to different ecological problems such as nitrate leaching and the release of nitrogenous gases to the atmosphere. Nitrogenous gases are produced during the microbial nitrification and denitrification processes that take place in the soil. Great efforts have been made in order to decrease both nitrate leaching and emission of nitrogenous gases into the atmosphere. In this way, stabilised fertilizers that include nitrification inhibitors (NIs) in their composition have been considered as an alternative from the environmental point of view due to the positive effects observed on diminishing both nitrate leaching and nitrogenous gases emissions to the atmosphere. NIs affect the first step of the nitrification process maintaining N in the NH_4^+ form in the soil for a longer period of time. As a result, application of NIs induces a change in the plant nutrition from NO_3^--based nutrition to NH_4^+-based nutrition. This change might lead to a decrease in biomass production if plants are not adapted to NH_4^+-based nutrition.

It is a well-known fact that the mixed N nutrition including both NO_3^- and NH_4^+ generally guarantees higher yield in crop production than NO_3^- or NH_4^+ alone. Most available crop varieties have been genetically selected for nitric or mixed nutrition and thus, they are not adapted to NH_4^+. Some species develop toxic symptoms when NH_4^+ nutrition is applied (Gerendás et al., 1997; Lasa et al., 2001) and a negative effect on plant growth has been observed under this kind of nutrition (Claussen and Lenz, 1999; Walch-Liu et al., 2000). Although NH_4^+ is an important intermediate in many metabolic reactions, it has been reported that high concentrations of this ion in the soil or in the nutrient solution may lead to an "ammonium syndrome" which may include leaf chlorosis, lower plant yield production and root/shoot ratio, lower cation content, acidification of the rizosphere and changes on several metabolites levels as amino acids or organic acids (Britto and Kronzucker, 2002). Although the toxic effects of NH_4^+ nutrition have often been related to an acidification of the rizosphere or the intracellular medium (Dijk and Grootjans, 1998), some authors indicate that cytoplasmic pH is well regulated under normal conditions and other processes might be responsible for the plant responses to NH_4^+ nutrition (Gerendás and Sattelmacher, 1990). In spite of the information obtained about the appearance of toxic symptoms due to NH_4^+ nutrition, contradictory results have been observed in the different studies. This could be explained on the basis of the specific and the varietal characteristics of each plant and of the experimental conditions. Thus, there is a great range of plant responses to NH_4^+ nutrition; there are some species that are tolerant to high NH_4^+ doses, such as rice (Wang et al., 1993), and some very sensitive species, which are almost unable to live under NH_4^+ nutrition, such as tomato or barley (Magalhaes and Huber, 1989; Britto et al., 2001). Reports about the nitrogen form effect in grassland species are rare and, in general, are focused on high and low availability of nitrate (Castle et al., 2002; MacDuff et al., 2002; Collins et al., 2003). Tolerance to NH_4^+-based nutrition could be expected in these species because intensive grazing process leads to urea deposition in grasslands and, consequently, maintained NH_4^+ availability in the soil. We have previously studied the efficiency of the NIs dicyandiamide (DCD) and 3,4-dimethylpyrazole phosphate (DMPP) in reducing N_2O emissions to the atmosphere when applied to grasslands (Merino et al., 2001, 2002). Both NIs reduced N_2O emissions, although phytotoxicity symptoms due to the use of DCD have also been observed in clover (Macadam et al., 2003). Taking into account the relationship between the use of NIs and the change in plant N nutrition, the aim of this study was to determine the tolerance to NH_4^+ nutrition of two grassland species, *Trifolium repens* and *Lolium perenne*. Biomass production, gas exchange and water relation parameters as well as ion tissue contents pattern were compared under NH_4^+ and NO_3^- based nutrition regimes. In addition, the levels of polyamines (PAs) putrescine (Put), spermidine (Spd) and spermine (Spm) were assayed because of their effect on plant responses to different environmental stresses (Smith 1985; Bouchereau et al., 1999; Hao et al., 2005).