High phosphorus fertilization reduces mycorrhizal colonization and plant biomass of three cultivars of pineapple
Altas dosis de fertilización fosforada reducen la colonización micorrízica y biomasa de la planta de tres variedades de piña

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SUMMARY

A strategy to increase the productivity of pineapple agricultural systems is the excessive application of chemical fertilizers. In the state of Veracruz, Mexico, pineapple fields are being fertilized with P (180 to 240 kg ha^{-1}) to maximize yields so that fertilizer applications keep increasing. We assessed the interaction between chemical phosphorus fertilizers, the establishment of mycorrhizal associations and biomass production of Cayenne, Champaka, and MD2 cultivars of pineapple in a greenhouse experiment. Plants were inoculated with a mycorrhizal consortium and grown with 0, 80, 200, 300, 500, and 600 mg phosphorus kg^{-1} soil additions in 8 kg pots for 8 months. Phosphorus reduced mycorrhizal colonization already at the lowest phosphorus fertilization dose in two of the three cultivars and became minimal for all cultivars from 200 mg P kg^{-1} soil. At the two highest phosphorus fertilization levels, shoot dry biomass and the dry weight of leaf D was reduced in all cultivars. The results show that the farmer´s practice of adding excessive fertilizer as an insurance principle is reaching the levels where fertilization is becoming detrimental for production and mycorrhizal benefits for the crop and the soil are being eliminated.

Index words: *Cayenne, Champaka, MD2, mycorrhiza, phosphorus.*

RESUMEN

Una estrategia para aumentar la productividad de los sistemas agrícolas de piña consiste en la aplicación excesiva de fertilizantes químicos. En el estado de Veracruz, México, los cultivos de piña son fertilizados con fósforo (180 a 240 kg ha^{-1}) para maximizar los rendimientos por lo que las aplicaciones de fertilizantes siguen aumentando. Se midió la interacción entre los fertilizantes químicos fosforados, el establecimiento de asociaciones micorrízicas y la producción de biomasa en plantas de piña de las variedades Cayenne, Champaka y MD2, en un experimento de invernadero. Se inocularon plantas con un consorcio micorrízico y se aplicaron dosis de fósforo de 0, 80, 200, 300, 500 y 600 mg kg^{-1} suelo en macetas de 8 kg por 8 meses. La fertilización con fósforo redujo la colonización micorrízica en todos los tratamientos fertilizados y se volvió mínima para todos los tratamientos a partir de 200 mg P kg^{-1} de suelo. En los dos tratamientos con mayor fertilización, la biomasa seca disminuyó en todas las variedades, así como el peso seco de la hoja D. Los resultados indican que las prácticas de los agricultores, de exceder la fertilización para asegurar una mayor producción, están alcanzando niveles donde la fertilización se está volviendo perjudicial para la producción y además se eliminan los beneficios de la micorrización para el cultivo y el suelo.

Palabras clave: *Cayena, Champaka, MD2, micorriza, fósforo.*

Recommended citation:
Trejo, D., J. Bañuelos, M. E. Gavito, and W. Sangabriel-Conde. 2020. High phosphorus fertilization reduces mycorrhizal colonization and plant biomass of three cultivars of pineapple. Terra Latinoamericana 38: 853-858.
DOI: https://doi.org/10.28940/terra.v38i4.701

Received: January 08, 2020.
Accepted: June 14, 2020.
Published in Terra Latinoamericana 38: 853-858.

INTRODUCTION

Pineapple has become highly dependent on fertilizer application due to the scarce development of its roots that are heavily attacked by nematodes. Phosphorus fertilization doses of up to 400 kg ha^{-1} of P_2O_5 per cycle (240 kg ha^{-1} of phosphorus) are commonly applied under the principle of "more fertilizer, more yield" without considering the soil fertility levels or the P requirements of the crop (Valkama *et al.,* 2009; Uriza-Ávila *et al.,* 2018). Adequate levels of fertilizer could ensure quality effects on the yield (Yousaf *et al.,* 2017). High levels of available P may reduce colonization by arbuscular mycorrhizal fungi (AMF) and thus the mycorrhizal contribution to plant nutrition and soil quality and health (Gianinazzi *et al.,* 2010), whereas an adequate combination of potassium sources could lead to higher yields (Teixeira *et al.,* 2011). Genetic variability of most tropical crops and particularly pineapple cultivars (Rebolledo-Martínez *et al.,* 2002) is another important interacting factor in the response to chemical fertilization and the establishment of mycorrhizal associations. Some studies have shown the advantages of inoculation with AMF in low fertility soils (Barea *et al.,* 2002) and tropical crops (Rodríguez-Romero *et al.,* 2011). Few studies have shown the benefits of inoculation with mycorrhizal fungi in pineapple, most of them evaluate micro-propagated plants during the acclimatization stage (Thamsurakul *et al*., 2000; Rodríguez-Romero *et al.,* 2011; Moreira *et al*., 2019). In Mexico, pineapple production is still based on a strict vegetative process by planting sprouts **(**Uriza-Ávila *et al*., 2018**)** where the high use of P fertilizer conflicts with any potential use of mycorrhizal associations to promote, not only plant nutrition and growth but also biological control, drought resistance, and soil structure and quality. Considering the environmental costs and ecological imbalance associated to the excessive use of chemical fertilizers (MacDonald *et al*., 2011), it is important to find the appropriate fertilization rate to prevent damage due to excessive applications (Malezieux and Bartholomew, 2003) and to allow the expression of natural mechanisms that do not impoverish the soil, improve plant nutrition and promote other environmental services (Gianinazzi *et al*., 2010). This study explored the interaction between the establishment of mycorrhizal associations and the application of P fertilizers and its effect on crop biomass production of three cultivars. Given that genetic variability of cultivars may result in a different response to fertilization (Benin *et al*., 2012) and mycorrhizal inoculation (Malezieux and Bartholomew, 2003), we included three commonly used cultivars in pineapple production in Oaxaca and Veracruz in Mexico. We aimed at providing supporting evidence for an integrated management program that reduces fertilizer application and increases environmental benefits with minimal yield loss.

MATERIALS AND METHODS

The experiment was established in a nursery. Sprouts of the three cultivars commonly grown in Mexico: Cayenne, Champaka, and MD2, were collected from pineapple fields and planted in pots with pineapple field sterile soil until roots developed. The soil, according to the Official Mexican Standard NOM-021-RECNAT-2000 (SEMARNAT, 2002), had a pH of 4.78 and contained 73.20% sand, 8.80% clay, 18.08% silt, soil organic matter 4.50 mg kg^{-1}, and nutrients (mg kg^{-1}): total N 84, K 57, Ca 707, Mg 89, Fe 39, Cu 0.7, Zn 14, Mn 23.7 and available P 51 (Bray P1 method). Additionally, it contained 54 milliequivalents per 100 g soil of exchangeable Al. Sprouts, 20 cm tall on average, were inoculated with 10 g of mycorrhizal inoculum consisting of colonized roots and 150 spores in 50 g powder of a product with an arbuscular mycorrhizal fungi (AMF) consortium called Rizofermic-UV. The consortium contained a mixture of 12 AMF species (*Acaulospora morrowiae, A. spinosa, A. scrobiculata, Funneliformis mosseae, F. geosporus, Gigaspora rosea, Gi. decipiens, Glomus macrocarpum, Gl. aggregatum, Rhizophagus intraradices, Scutellospora pellucida* and *Claroideoglomus etunicatum*), reproduced in *Brachiaria decumbens* pot cultures according to Sieverding (1991).

Plants in the experiment received fertilizer in aqueous solution in similar quantities to those applied by farmers. MULTI/MKP fertilizer 0/52/34 in the following doses: 0 mg kg^{-1} (control with pure water), 80 mg kg^{-1} (P 70 kg ha^{-1}), 200 mg kg^{-1} (P 175 kg ha^{-1}), 300 mg kg^{-1} (P 262 kg ha^{-1}), 500 mg kg^{-1} (P 437 kg ha^{-1}) y 600 mg kg^{-1} (P 524 kg ha^{-1}), were applied in 200 mL applications once a week. In addition to this, all plants received at the same time 200 mL Hoagland nutrient solution to avoid other nutrient deficiencies. Each fertilization treatment was applied to eight replicate pots.

Mycorrhizal colonization was evaluated under a stereomicroscope (Giovannetti and Mosse, 1980) in stained roots (Phillips and Hayman, 1970) sampled from the pots 45 days after inoculating. Plants were harvested 8 months (210 d) after transplanting and dry weights of leaf "D", (Montilla de Bravo *et al.,* 1997), and total above-ground biomass was determined.

Two-way ANOVAs and Tukey´s mean comparisons ($P < 0.05$) were used to explore fertilization effects on mycorrhizal colonization, dry weight of leaf D and total shoot weight, using STATISTICA software version 7.0.

RESULTS AND DISCUSSION

Phosphorus fertilization reduced mycorrhizal colonization already at the lowest dose but low levels of colonization were maintained in all cultivars up to 500 mg kg^{-1} (Figure 1A-C). Mycorrhizal colonization was eliminated in MD2 and Champaka cultivars with 600 mg kg^{-1} but some root colonization was still observed in Cayenne plants at the highest P fertilization level. In terms of shoot biomass Cayenne plants were less affected by high P fertilization than Champaka and MD2 plants (Figure 1G-I), but all cultivars showed significantly lower shoot biomass at the highest P fertilization dose after 8 months. The dry weight of leaf D, an indicator of final yield, did not respond to P fertilization in MD2 plants but in Champaka and Cayenne the negative effect of the highest P dose observed in shoot weight was also observed in this indicator (Figure 1D-F).

Discussion

Studies on the effect of mycorrhizal inoculation on pineapple plants propagated by sprouts are scarce. In this study, all plants showed association to AMF regardless of pineapple cultivars, however, the response to phosphorus fertilization levels varied among cultivars. AM colonization was strongly reduced in the MD2

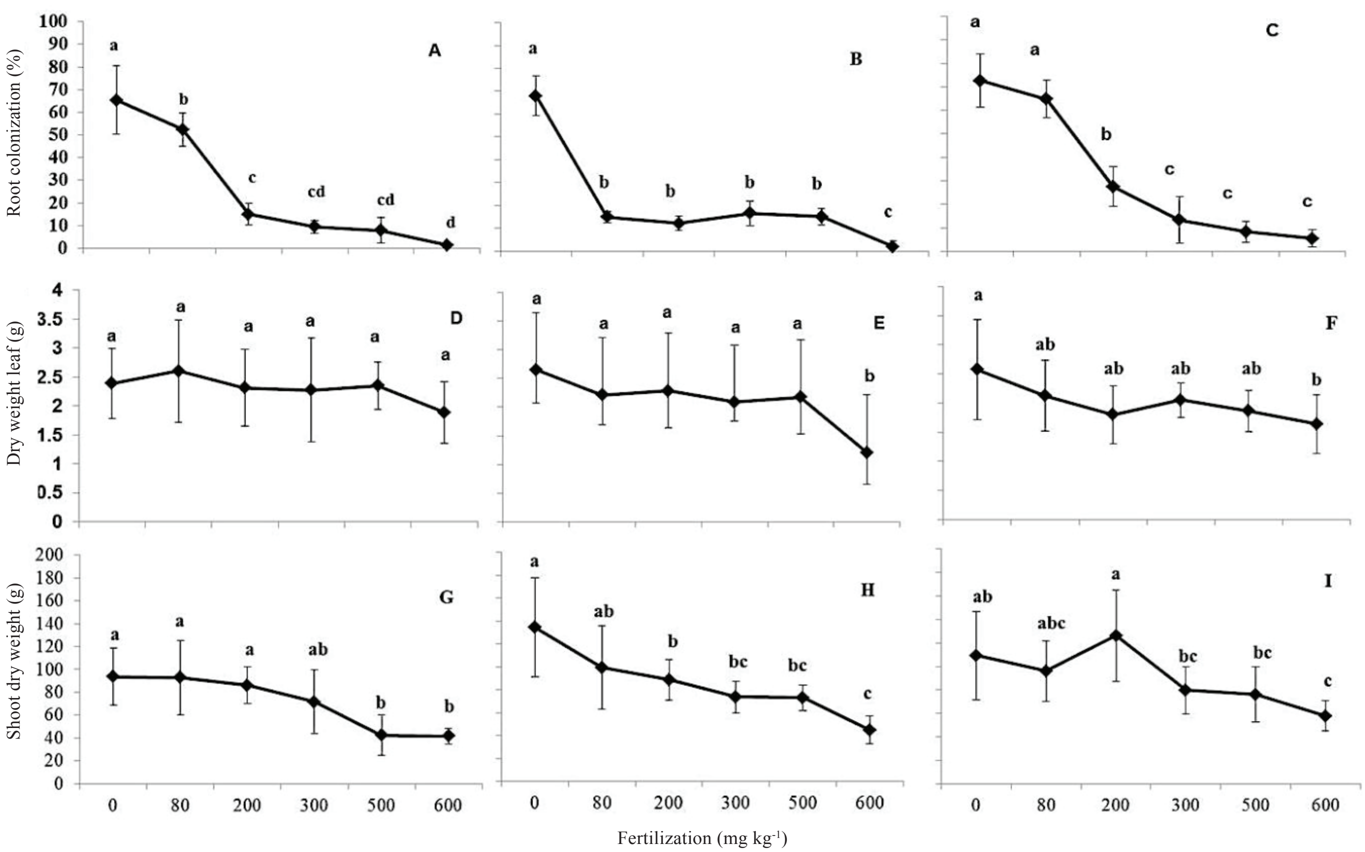

Figure 1. Mycorrhizal colonization percentages in roots, dry mass of the indicator of final yield leaf D, and aboveground dry mass measured in the MD2 (A, D, G), Champaka (B, E, H) and Cayenne (C, F, H) cultivars of pineapple inoculated with AM fungi and grown with increasing doses of P fertilizer, at harvest. Means (+ S. D., n = 8) marked with the same letters do not differ significantly at *P* < 0.05.

cultivar (80% approximately) and the Cayenne cultivar (60%) when 200 mg kg^{-1} of P were applied but in the Champaka cultivar, only 80 mg kg^{-1} of P were enough to cause such a drastic drop in colonization.

Phosphorus fertilization has reduced mycorrhizal colonization in numerous studies with different plants, where mycorrhizal associations have become superfluous for plant nutrient uptake (Treseder, 2004). Plant root systems are highly plastic in response to environmental conditions and soil nutrients content (Kong *et al*., 2014), pineapple plants have thick and scarce roots that might constrain nutrient uptake even with high doses of fertilizer and this might explain why plants maintained a low level of colonization even with very high doses of P fertilizer.

It is known that when P availability increases plants do not produce the necessary signals for the establishment of the mycorrhizal symbiosis (Balzergue *et al*., 2011). In some plants, the establishment of mycorrhizal associations under high P availability has been detrimental to plant growth because mycorrhizal fungi continue to feed on plant C but without contributing to plant nutrition and become like pathogens (Johnson *et al*., 1997). High phosphorus affected negatively the dry mass of both the entire shoot and leaf D, indicating that final yields most likely would have become lower with the higher P doses. Moreover, for all three cultivars, maximum growth was achieved already at the lowest P dose and one of them, Champaka, showed a clear negative relation between P fertilizer addition and plant biomass. Pineapple has low P requirements (Lin, 2010), and a high sensitivity to excessive P (Uriza-Ávila *et al*., 2018), as observed in this study in the Champaka cultivar. Cayenne and Champaka produced similar biomass and MD2 a little less than these two cultivars, which can be explained because Champaka is a pure clone obtained from Cayenne. MD2 is a hybrid obtained from a different phylogenetic group, and its management is highly demanding of nutrients, mainly K, due to its fast growth and is very susceptible to fungal attack (Barreto-Cruz *et al*., 2015; Valleser, 2019). Excessive fertilizer applications in field production and tested in this work under greenhouse conditions proves again that high P quantities reduce AMF development, as reported in previous research (Balzergue *et al*., 2011), and in these pineapple cultivars may even cause crop growth depressions.

CONCLUSIONS

- The results showed that P fertilization up to 80 mg kg^{-1}, not only inhibit AM colonization but reduce plant growth. Shoot and leaf D biomass reductions suggest that current management with excessive P fertilizer is highly cost-inefficient and possibly leading to yield losses and environmental degradation. These results mean that farmers could greatly reduce their production costs by lowering fertilizer applications to less than half and in this way also reduce soil degradation and groundwater pollution.
- Farmers could safely lower P fertilization to 80 mg kg-¹ soil at no yield cost. The reduction in mycorrhizal development associated to excessive P fertilizer applications most likely reduces not only the mycorrhizal contribution to plant nutrition but also its numerous benefits in promoting healthy soil.
- We suggest additionally that the producers could reduce the P doses applied without risking yields by complementing fertilization with a consortium of arbuscular mycorrhizal fungi inoculation or management of native AMF communities. Such strategy could highly reduce production costs and environmental damage.

ETHICS STATEMENT

Not applicable

CONSENT FOR PUBLICATION

Not applicable

DATA AVAILABILITY

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

COMPETING INTERESTS

The authors declare that they have no competing interests.

FUNDING

This research was partially supported by the Program for Teacher Professional Development (PRODEP): Support for the Integration of Thematic Networks of Academic Collaboration 2008. This work was also supported by the FOMIX 95502 project CONACYT-Veracruz Government.

AUTHORS CONTRIBUTIONS

Conceptualization: Dora Trejo. Methodology: Wendy Sangabriel-Conde. Validation: Dora Trejo. Formal analysis: Dora Trejo. Investigation: Wendy Sangabriel-Conde. Data curation: Jacob Bañuelos. Writing, original draft preparation: Mayra E. Gavito. Writing, review and editing: Mayra E. Gavito. Visualization: Dora Trejo. Supervision: Wendy Sangabriel-Conde. Project administration: Wendy Sangabriel-Conde. Funding acquisition: Dora Trejo.

ACKNOWLEDGEMENTS

We thank Ing. Enrique Contreras for his participation in the project, contributing his experience as a producer cooperator and Ing. Daniel Uriza Avila for his comments on the paper.

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