Combined use of a resistance inducer (Agro-Mos®) and micronutrients for the control of *Meloidogyne javanica* in soybean

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Abstract

Elicitors of plant resistance are compounds that activate enzymatic processes involved in plant defense. Micronutrients also play an important role in plant responses against pathogens because they function as enzyme cofactors. Despite their well-known benefits, elicitors and micronutrients have been little investigated in nematode control. This study aimed to assess the effects of Agro-Mos® (a commercial biostimulant) and micronutrients (Zn and Mn), alone and combined, on soybean inoculated with *Meloidogyne javanica*. Seeds were sown in trays, treated 15 days after germination, and inoculated with 2000 eggs and juveniles of *M. javanica* at the time of transplanting. Treatments were as follows: 1 L/ha Agro-Mos®, 2 L/ha Metalosate Zinc, 1.5 L/ha Metalosate Manganese, Agro-Mos® + Zn, Agro-Mos® + Mn, and Agro-Mos® + Zn + Mn. Untreated inoculated and uninoculated plants were used as controls. At 60 days after inoculation, plants were harvested and evaluated for vegetative gall parameters. All treatments were effective in reducing *M. javanica* population density in roots compared to the control. Agro-Mos®, Agro-Mos® + Zn, and Agro-Mos® + Zn + Mn were the most effective, reducing total nematode number and population density by 55–78% (*P ≤ 0.05*) in relation to the control. Agro-Mos® + Zn increased shoot dry weight. The results show that balanced fertilization can be used as part of an integrated nematode control strategy.

Keywords: Resistance inducer; Manganese; Root-knot nematode; Zinc; Nutrient.

Abbreviations: **B**_boron; **Ca**_calcium; **Cu**_copper; **Fe**_iron; **K**_potassium; **Mg**_magnesium; **Mn**_manganese; **N**_nitrogen; **P**_phosphorus; **S**_sulfur; **Zn**_zinc.

Introduction

Soybean (*Glycine max* (L.) Merrill) is one of the most produced and consumed crops in the world. In Brazil, soybean production is estimated to reach 120.9 million tonnes in 2019/2020 (Conab, 2019). A major limiting factor of soybean yield is nematode infection (Brida et al., 2016). Nematodes of the genus *Meloidogyne* occur worldwide, and are highly specialized, and have the ability to parasitize almost all cultivated plants (Moens et al., 2009). *Meloidogyne* individuals establish feeding sites and induce the formation of galls in the host’s root system, reducing the ability of the infected plant to absorb water and nutrients. Such damage can lead to poor development, leaf chlorosis, and even plant death (Moens et al., 2009; Brida et al., 2016). As a control measure, nematicides may be applied to seeds, but their effects extend only to the early stages of the plant life cycle, without providing protection during root development (Faske and Starr, 2007; Cabrera et al., 2009). An economically viable and simple alternative to control root-knot nematodes is the use of nematode-resistant cultivars (Fischer et al., 2010). However, virulent nematode strains have been shown to overcome plant resistance genes, compromising the efficacy of this control method (Castagnone-Sereno, 2002). Because of the limitations of conventional control practices and the need for integrated management, it is necessary to search for novel, more efficient strategies. Resistance inducers or elicitors have been increasingly used as alternative methods with promising results (Dias-Arieira et al., 2013; Puerari et al., 2013; Puerari et al., 2015). Methyl jasmonate, potassium silicate, and acibenzolar-S-methyl are important growth-promoting and -regulating compounds with low or no toxicity to plants obtained from rhizobacteria and the fungus *Trichoderma* spp. (Puerari et al., 2013; El-Sherif et al., 2015; Chinheya et al., 2017; Kath et al., 2017; Schouteden et al., 2017). These compounds do not act directly on pathogens but activate several resistance mechanisms in plants (Molinari and Baser, 2010).

Nutrition is another key factor in plant defense. Many micronutrients are enzyme cofactors and have important functions in plant metabolism (Epstein and Bloom, 2004). Therefore, micronutrients may improve the efficiency of resistance inducers. The literature lacks information on the combined use of elicitors and micronutrients for controlling nematodes. The aim of this study was to assess the efficiency of Agro-Mos® (a commercial product containing elicitors of plant resistance), alone and in combination with...
micronutrients, in controlling Meloidogyne javanica (Treub) Chitwood in soybean.

Results and Discussion

Total nematode number and nematode population density

Agro-Mos\(^+\), Agro-Mos\(^+\) + Zn, and Agro-Mos\(^+\) + Zn + Mn reduced total nematode number in relation to the control (Table 1), whereas other treatments did not differ from the control. All treatments decreased nematode population density, but Agro-Mos\(^+\), Zn, Agro-Mos\(^+\) + Zn, and Agro-Mos\(^+\) + Zn + Mn were the most effective (Table 1).

The good results obtained with Agro-Mos\(^+\) are likely associated with the presence of phosphorylated mannooligosaccharides derived from the cell wall of Saccharomyces cerevisiae, which are elicitors of defense responses in plants (Zanardo et al., 2009). A previous study showed that Agro-Mos\(^+\) effectively controlled M. javanica and Pratylenchus brachyurus (Godfrey) Filipjev & Schuurmans Stekhoven in soybean (Miamoto et al., 2017). Costa et al. (2010) investigated the effects of two Agro-Mos\(^+\) formulations (with and without Cu and Zn) on cacao plant seedlings exposed to Maniliovithora perniciosa (Stahel) Singer and found that Agro-Mos\(^+\) + Cu + Zn was able to reduce disease incidence. Cu and Zn play important roles in enzyme activation, biosynthesis, and hormonal control (Merelato et al., 2002). Zn, both alone and combined with Agro-Mos\(^+\), was also effective in controlling M. javanica (Table 1). Couto et al. (2016) reported that Zn reduced the number of M. incognita (Kofoid and White) Chitwood eggs and gall in tomato plants (Solanum lycopersicum L.) when applied to the shoot. Zn is a component and activator of several enzymes, and its deficiency may lead to loss of membrane integrity, which may increase plant susceptibility to different diseases (Epstein and Bloom, 2004).

Mn, especially in association with Cu, contributes to plant defense against pathogens (Costa et al., 2012). Mn and Cu activate various enzymes, including those involved in superoxide radical detoxification and lignin synthesis (Marschner, 2012). This fact could explain why the combined use of Mn and Agro-Mos\(^+\) (which contains Cu) provided better results than the use of Mn alone (Table 1).

Vegetative parameters

Treatments did not affect plant height or root fresh weight but influenced shoot fresh and dry weights (Table 2). Zn and Agro-Mos\(^+\) + Zn increased shoot dry weight in comparison to other treatments but not in comparison to the uninoculated control (Table 2). Plants inoculated with the nematode, whether treated or not, had lower shoot fresh weight than uninoculated plants (Table 2).

Both Zn and Mn are important nutrients for plant development; however, Zn contributed more to shoot dry weight than Mn (Table 2). Zn is known to activate the tryptophan biosynthesis pathway. Tryptophan is an indoleacetic acid precursor found at high concentrations in shoot apical meristems and developing leaves, contributing to the increase in shoot dry weight (Epstein and Bloom, 2004).

The results of the present study corroborate those of Beraldo et al. (2012), who reported increased shoot dry weight after Zn application in Marandu grass (Brachiaria brizantha (Hochst. ex A. Rich.) Stapf cv. Marandu).

Pearson’s correlation analysis

Pearson’s correlation analysis showed that treatment was positively correlated with Ca (Table 3). The means test revealed that plants treated with Agro-Mos\(^+\) + Zn + Mn and the inoculated control had higher Ca concentrations than uninoculated plants. Plants treated with Agro-Mos\(^+\) alone, however, did not differ from other plants in Ca concentration (data not shown). The high Ca accumulation in plants exposed to nematodes is due to the fact that Ca is a constituent of the middle lamella, which is degraded by extracellular enzymes produced by phytopathogenic agents (McGuire and Kelman, 1986). Thus, Ca plays an important role in the maintenance of cellular integrity and membrane permeability, impairing the action of extracellular enzymes (Marschner, 2012; Taiz and Zeiger, 2010).

Plant height was negatively correlated with P, K, and Ca, whereas root fresh weight was negatively correlated with K and positively correlated with Mn (Table 3). Shoot fresh and dry weights were negatively correlated with P, K, Ca, and B but positively correlated with Mn and root fresh weight. Nutrient concentrations in vegetative parts typically decrease during the reproductive stages, as nutrients are redistributed from mature leaves to new growth areas. In wheat (Triticum spp.), for instance, about 90% of the total P content is remobilized from vegetative parts to the kernel (Marschner, 2012). In white lupin (Lupinus albus L.), up to 50% of micronutrients and 18% of Ca originally accumulated in leaves are remobilized to the fruits (Hocking and Pate, 1978). This may explain the negative correlation between P, K, and Ca and vegetative parameters (Table 3). In contrast, Mn had a positive correlation with vegetative parameters, as the nutrient has low tissue mobility and was applied as foliar spray (Marschner, 2012). Nematode population density was positively correlated with K, Ca, and total nematode number (Table 3) and negatively correlated with Mn, root fresh weight, and shoot fresh and dry weights (Table 3). In addition, nematode population density was not significantly correlated with N, P, or plant height (Table 3). According to Pinheiro et al. (2008), high soil Ca levels are highly correlated with high pH, favoring root development; these conditions contribute to the development of infection sites, thereby increasing M. javanica population density (Table 3).

Barbosa et al. (2010) evaluated the effects of K-based fertilization on Heteroderda glycines Ichinohe populations in resistant and susceptible soybean cultivars and observed that the nutrient stimulated female development in the roots of nematode-resistant plants. According to the authors, K contributes to root development, which increases the availability of feeding sites. These findings are in agreement with the results obtained in the current study (Table 3). The negative correlation between nematode population density and Mn (Table 3) may be explained by two hypotheses. First, high Mn concentrations can reduce nematode population density, as the compound plays a fundamental role in the shikimic acid pathway, which is the major plant defense pathway (Fancelli, 2008). Second, high nematode population densities can impair Mn uptake by the root system, as previously observed by Hurchanik et al. (2004) in coffee plants infected with Meloidogyne konaensis Eisenback, Berbard, & Schmitt. Root fresh weight and shoot fresh and dry weights were negatively correlated with nematode population density (Table 3). This result was expected because uninoculated
Table 1. Total nematode number and population density (number of nematodes/g root) at 60 days after inoculation of soybean with 2000 eggs + J2 of *Meloidogyne javanica*.

| Treatment                  | Total nematode number | Population density |
|----------------------------|-----------------------|--------------------|
| Control                    | 15397 a               | 596 a              |
| Mn                         | 9838 a                | 398 b              |
| Zn                         | 8794 a                | 278 c              |
| Agro-Mos®                  | 3338 b                | 139 c              |
| Agro-Mos® + Mn             | 12288 a               | 367 b              |
| Agro-Mos® + Zn             | 4554 b                | 171 c              |
| Agro-Mos® + Mn + Zn        | 6887 b                | 253 c              |
|                            | 68.35                 | 57.61              |

Means within columns followed by the same letter are not significantly different (P < 0.05) by the Scott-Knott test. Data were √(x + 1) transformed before analysis. CV, coefficient of variation.

Table 2. Plant height, shoot fresh weight, shoot dry weight, and root fresh weight of soybean at 60 days after inoculation with 2000 eggs + J2 of *Meloidogyne javanica*.

| Treatment                  | Plant height (cm) | Shoot fresh weight (g) | Shoot dry weight (g) | Root fresh weight (g) |
|----------------------------|-------------------|------------------------|----------------------|-----------------------|
| Uninoculated control       | 75.42             | 45.96                  | 12.84                | 34.94                 |
| Inoculated control         | 74.28             | 20.87 b                | 7.00 b               | 25.04                 |
| Mn                         | 69.50             | 28.58 b                | 7.69 b               | 28.43                 |
| Zn                         | 61.14             | 26.92 b                | 10.27 a              | 31.36                 |
| Agro-Mos®                  | 72.87             | 29.97 b                | 7.99 b               | 26.87                 |
| Agro-Mos® + Mn             | 74.28             | 32.00 b                | 9.03 b               | 33.00                 |
| Agro-Mos® + Zn             | 74.28             | 25.53 b                | 11.02 a              | 28.40                 |
| Agro-Mos® + Mn + Zn        | 68.42             | 28.53 b                | 7.73 b               | 28.18                 |
| CV (%)                     | 14.89             | 30.80                  | 46.23                | 42.18                 |

Means within columns followed by the same letter are not significantly different (P < 0.05) by the Scott-Knott test. CV, coefficient of variation.

Table 3. Pearson’s correlation coefficients between plant growth, nutrient content, and nematode parameters of soybean at 60 days after inoculation with 2000 eggs + J2 of *Meloidogyne javanica*.

| Treat | N   | P   | K   | Ca  | Mg  | S   | Fe  | Mn  | Cu  | Zn  | B   | Height | RFW  | SFW  | SDW  | N   | N   |
|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-------|------|------|------|-----|-----|
| 1     |     |     |     |     |     |     |     |     |     |     |     |       |      |      |      |     |     |
| N     | .320| 1   |     |     |     |     |     |     |     |     |     |       |      |      |      |     |     |
| P     | .359| .519| 1   |     |     |     |     |     |     |     |     |       |      |      |      |     |     |
| K     | .349| .281| .860**| .731**| .782**| 1   |     |     |     |     |     |       |      |      |      |     |     |
| Ca    | .578*| .199| .594*| .676*| .731**| 1   |     |     |     |     |     |       |      |      |      |     |     |
| Mg    | .428| .432| .594*| .704*| .390*| .754**| .491| 1   |     |     |     |       |      |      |      |     |     |
| S     | .428| .099| .766**| .434*| .399*| .192| .479*| .108| 1   |     |     |       |      |      |      |     |     |
| Mn    | .240| .313| .164| .429| .348| .171| .052| .035| 1   |     |     |       |      |      |      |     |     |
| Cu    | .550| .503| .501*| .319| .378| .336| .020| .494| -.148| 1 |     |       |      |      |      |     |     |
| Zn    | .005| .731**| .523| .321| .286| .598*| .003| .791**| .209| .652*| 1   |       |      |      |      |     |     |
| B     | .504| .292| .800**| .855**| .790**| .594*| .494| .409| -.211| .576| .421| 1   |       |      |      |      |     |     |
| Height| -.060| -.136| -.776**| -.579*| -.638*| -.466| -.471| -.095| .274| -.313| -.356| -.534| 1   |     |      |     |     |
| RFW   | .030| .200| -.308| -.629| -.330| -.228| .132| -.072| .738**| .093| .110| -.363| .303| 1   |     |      |     |     |
| SFW   | -.280| -.251| -.598**| -.779**| -.581*| -.174| .301| .049| .613*| -.152| .149| -.705**| .519| .720**| 1   |     |      |     |
| SDW   | -.369| -.149| -.637**| -.829*| -.645| -.278| -.300| -.070| .707**| -.242| .068| -.731**| .530| .747**| .980**| 1   |     |      |     |
| N     | .195| .110| .375*| .493| .163| .275| .055| -.282| .080| -.036| .383| -.185| -.223| -.517| -.516| 1   |     |      |     |

Means within columns followed by the same letter are not significantly different (P < 0.05) by the Scott-Knott test. CV, coefficient of variation.
plants had higher root and shoot weights than plants exposed to the nematode.

**Materials and Methods**

The experiment was conducted between September and December 2016 in a greenhouse at the State University of Maringá, Umuarama, Brazil. A completely randomized design was used, with eight treatments and eight replications.

**Treatments**

Seeds of soybean cv. Pintado were sown in polystyrene trays filled with potting substrate (Bioplatin®). Ten days after germination, the following treatments were applied: (i) 1 L/ha Agro-Mos® (commercial biostimulant containing phosphorylated mannanoligosaccharides from Saccharomyces cerevisiae, 2.75% S, 2.00% Cu, and 2.00% Zn, density 1.23 g/cm³, pH 2.84, Alittech Crop Science), (ii) 2 L/ha Metalosate® Zinc (6.8% Zn, 15.0% amino acid chelating agent, density 1.21 g/cm³, Albion Plant Nutrition), (iii) 1.5 L/ha Metalosate® Manganese (6.0% Mn, 17.4% amino acid chelating agent, density 1.21 g/cm³, Albion Plant Nutrition), (iv) Agro-Mos® + Zn, (v) Agro-Mos® + Mn, and (vi) Agro-Mos® + Zn + Mn. Untreated inoculated and uninoculated plants were used as controls.

**Experimental procedures**

Fifteen days after germination (five days after treatment), seedlings were transplanted to pots containing 2 kg of a 2:1 mixture of soil and sand, previously autoclaved at 120 °C for 2 h. At transplanting, plants were inoculated with 4 mL of a suspension containing 2000 eggs and eventual second-stage juveniles of *M. javanica*. The inoculum was obtained from a single-species nematode population multiplied in tomato cv. Santa Clara under greenhouse conditions for two months. The experiment was conducted between September and December 2016 in a greenhouse at Santa Clara under greenhouse conditions for two months.

**Materials and Methods**

The leaves of plants treated with Agro-Mos® and Agro-Mos® + Zn + Mn, as well as inoculated and uninoculated controls, were collected and analyzed for N, P, K, Ca, Mg, S, Fe, Mn, Cu, Zn, and B contents.

**Statistical analysis**

Data were subjected to analysis of variance followed by the Scott–Knott test (*P < 0.05*) using the Sisvar software (Ferreira, 2014). When necessary, data were transformed before analysis to meet normality assumptions based on the Shapiro test. Pearson’s correlation analysis was used to identify associations between treatments, nutrient content, plant growth, and nematode parameters. When significant correlations were found between treatments and plant parameters, treatment effects were investigated using Tukey’s test (*P < 0.05*).

**Conclusion**

All treatments decreased at least one of the nematode parameters evaluated. Agro-Mos® + Zn and Agro-Mos® + Zn + Mn were efficient in reducing both total nematode number and population density. Agro-Mos® + Zn had a positive effect on shoot dry weight. The results indicate that balanced fertilization can be used as part of an integrated strategy for the control of *M. javanica* in soybean.

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