Chemical control of *Meloidogyne* spp. in grapevines (*Vitis vinifera*)

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ABSTRACT

Objective: to determine the effect of increasing rates of Mocap® 6EC (ethoprophos-AMVAC) on grape (*Vitis vinifera*) own-rooted cv. Flame and cv Red Globe grafted onto Quebranta rootstock *Meloidogyne* spp. control.

Methodology and Results: two field experiments of increasing rates of 0, 6, 8, 10, and 12 L ha^{-1} of Mocap® 6EC (ethoprophos-AMVAC) using a complete randomized block design with 4 replicates were set up on grape (*Vitis vinifera*) own-rooted cv. Flame and cv Red Globe grafted onto Quebranta rootstock for *Meloidogyne* spp. control. To quantify nematode numbers in soil and roots, and the number of galls in a linear meter root, soil and root samples were taken just before treatment and at 30, 60 and 90 days after product application. In both experiments, at 30, 60 and 90 days after the application, a decreasing linear effect on *Meloidogyne* spp. numbers in soil (P< 0.0001) and roots (P≤ 0.0002) and number of galls (P< 0.0001) was observed as rate increased. The average reduction was of 4.6, 4.9 and 5.2; and 5.5, 6.0 and 6.3 individuals per 100 g of soil, and 5.6, 9.9 and 9.9, and 4.9, 7.7 and 8.2 nematodes per 100 g of roots, and 2.0, 4.0 and 4.3, and 1.8, 3.9 and 4.9 galls per linear meter of root, by every litre of increase on the applied rate, at 30, 60 and 90 days post application, for the experiment at Ica and Lima department, respectively. Differences in biological efficacy among rates were found for soil (P< 0.0001) and root (P< 0.0001) nematode control, and number of root galls (P< 0.0001), increasing the control as the rate increased in both experiments. Efficacy in soil nematode control varied from 51 to 98% and 73 to 99%, in roots it varied from 61 to 85% and 61 to 87%, and in the number of root galls from 55 to 84%, and 58 to 81% for the experiment at Ica and Lima department, respectively.

Conclusions and application of findings: All Mocap® 6EC rates tested reduced *Meloidogyne* spp. in soil and roots, and the number of galls per linear meter of root, with higher reductions as the rate increased. Then the recommended rate is 10-12 L ha^{-1} incorporated in a drip irrigation of two hours.

Key words: chemical control, grapevines, *Meloidogyne*, nematode control.

INTRODUCTION

In Perú, grapevines (*Vitis vinifera*) is cultivated for local consumption and export markets. Besides the constraints of grapes market requirements and demands, there are other factors limiting production. The important abiotic factors constraining yield of grapevines include, edaphic soil condition, mainly due to texture, poor structure, high pH and Na content, and scarcity of rain.

Among the biotic factors, phytonematodes are second after thrips. Worldwide, in most of the grapevine plantations, phytonematodes usually occur in polyspecific communities, consisting mainly of a mixture of *Xiphinema index*, *X. americanum*, *Meloidogyne* spp., *Mesocriconema xenoplax*, *Tylenchulus semipenetrans*, *Pratylenchus* spp. (Australian Wine Research Institute, 2010; Goldammer, 2013). These nematodes are also present in Peruvian grapevine plantations, with *Meloidogyne* spp. as the most abundant (Chávez and Arata, 2004; Alban, 2018). Many of the commercial grapevine rootstocks are susceptible to nematodes including root-knot (*Meloidogyne* spp.). The presence of root-knot nematodes in grape roots induces galls that restrict nutrient and water uptake and growth of the grapevine, as well as facilitate fungal and bacterial infections. Melakeberhan and Ferris (1989) reported a reduction in leaf area of secondary leaves and root mass which ended in the total plant photosynthesis decline. Similarly, Anwar and Van Gundy (1989) and Anwar (1985) found a decrease in root/shoot ratio of *M. incognita*-infected French Colombard plants over longer periods of infection and suggested a greater effect on root than shoot growth. Then, *Meloidogyne* spp. are important pests of grapevine (*Vitis vinifera* L.) which may cause up to 60% yield loss (Nicol and Heeswijck, 1997; Riley and Walker, 2006; Pietsch and Burne, 2008; Australian Wine Research Institute, 2010). Several pest management techniques including resistant rootstocks (McKenry and Anwar 2006; Gutiérrez *et al*., 2011; Ferris *et al*., 2012), plant extracts and bioagents (Mervat *et al*., 2012), organic matter to complement plant nutrition and promote biological control agents (Adb-El-Khair *et al*., 2009; Australian Wine Research Institute, 2010), cover crop management inside vineyards (Quader *et al*., 2001; Addison and Fourie, 2008; Kruger *et. al*., 2015) to reduce pest dispersal, and chemical control (Rajendan and Naganathan 1978; Loubser and Meyer, 1986; Australian Wine Research Institute, 2010) have been tested with varying degrees of success. Application of insecticide-nematicides have been an important component and showed promise as alternatives for integrated grape pest management. Then the objective of this research was to evaluate the effect of a liquid formulation of Mocap® (ethoprophos-AMVAC) on grapevine root *Meloidogyne* spp. control.

MATERIALS AND METHODS

Site description: Two field experiments were conducted in a 10 and 12-year-old grapevine (*Vitis vinifera*) commercial plantation, one in own-rooted cv. Flame and the another on the cv Reb Globe grafted onto Quebranta, both plantations infested with *Meloidogyne* spp. located at the Department of Ica and Lima department Perú, respectively. Plant density was of 1900 plant ha^{-1} with distances of 3 m between rows and 1.75 m between plants. In each experimental site, the grapevines were of almost similar vigour and received the same (fertilization, weed and foliage diseases and pests control) and regular agricultural practices. There was not rainfall during the experimental periods, which means that all water requirement was supplied by drip irrigation. Main daily maximum minimum temperatures were 23.7/15.3°c and 41.2/7.5°c for Ica and Lima department, respectively. The soil for both experiments was taxonomically classified as an Entisol (FAO 2009) with a sandy loam (74% sand, 14% silt and 12% clay) and sandy (98% sand, 2% silt and 0% clay) soil texture with a pH of 7.3 and 7.7 for the experimental area at Ica and Lima department, respectively. Before establishment of the experiment, nematodes were controlled every year with a Rugby (FMC) application after bud burst.

Treatments and application: treatments were added at bud burst in July at Ica and August at Lima and consisted of four increasing rates of Mocap® 6EC (ethoprophos 72%- AMVAC): 6, 8, 10 and 12 L ha^{-1} plus un-treated control using four replicates with six grapevine trees per replicate with plots distributed in a complete randomized block design. The treatment application was done simulating an injection for 2 h into the drip irrigation system of 1.2 mm per hour. There were two drip lines in each grapevine row, with emitters 40 cm apart along the length of the drip hose. The calculated amount of water and the chemical required for the six trees in each plot of each treatment was estimated and mixed with water to have a volume of 75 L. Then, 48 h after an irrigation of 70 mm, when the soil

was about field capacity, the 75 L solution per plot was pumped with a manual knapsack sprayer at 15 bar pressure to a 21 m length drip hose with the same specifications as the hose used in the irrigation system, that was laid out on the soil surface close to irrigation lines with the end of the hose closed. After the injection of the 75 L per plot, 10 L of water was pumped to clean the house.

Soil and root sampling: Before treatment application and thereafter, every month up to 90 days post application, for each treatment, one combined root and soil sample was taken from each replicate, resulting in four root and soil samples per treatment per month. Each combined root and soil sample consisted of roots and soil of four vine trees that were the same in every sampling. At 20 cm from the grape trunk tree, a hole of 30 cm wide, 30 cm length and 30 cm depth, was dug and since there were four trees, each one was sampled at different cardinal point, so each sample included the four cardinal points. Then, in one tree, the hole was north, in the other south, in the another east and in the last one west of the tree, and about 100 g of thin roots ≤ 5 mm diameter and 500 g of soil were collected. The four 500 g soil samples, and the four 100 g of root samples, were homogenized and a 100 g soil sample and 100 g root sample were taken for nematode extraction. From each sampled tree, 3 roots with about 5 mm diameter, from the same dug hole, were taken, cut a 10 cm long, thereafter combined for each replicate, then homogenized and finally 10 root pieces selected to conform one linear meter, where the number of root galls present were counted. In every

sampling the cardinal point was changed in each tree, sampling in each tree, the four cardinal points.

Nematode extraction: In the laboratory, nematode population in 100 g of soil was extracted by gravity-screening method (Ayoub 1980) and from 100 g of roots by the root maceration method (Ayoub 1980) collecting the nematodes in the 0.038 mm sieve.

Statistical analysis: A comparison of the nematode numbers in soil and roots and the number of galls in one meter of roots among treatments was done before treatment application. A regression analysis of those variables on the Mocap® rates was made independently for each evaluation time after application. Since the trend observed in the linear regression analyses was similar, the average of the three evaluations was calculated with the purpose of testing the difference between numbers at 0 days vs the average of the evaluations at 30, 60 and 90 days after application. This was done by means of repeated measurement analyses. Also, a linear regression analysis was done with averages of those three evaluations (30, 60 and 90 days post application) on the Mocap® rates. Comparison among treatments and evaluations, were made with Genmod applying the negative binomial distribution of the residues, and estimation of regression equations were made with Proc Reg, both procedures in SAS. Efficacy of Mocap® rates on the studied variables was calculated following the Abbot (1925) formula, where the average of the treatments was obtained averaging the data of the evaluation at 30, 60 and 90 days after application. Then, treatments efficacy was compared by ANOVA.

RESULTS:

Before product application, no difference was found among groups of plots assigned to each treatment for *Meloidoyne* numbers per 100 g of soil (P= 0.4436; P= 0.6377) or 100 g of roots (P= 0.9866; P= 0.9838), nor in number of galls per meter of root (P= 0.8911; P= 0.8359) for the experiment set up at Ica (Fig 1A-C) and Lima (Fig 2A-C) department, respectively. The average nematode population by treatment varied from 29.5 to 39.3 and from 24.3 to 33.8 nematodes by 100 g of soil, and in roots it varied from 109.8 to 116.3 and from 79.8 to 86 nematodes by 100 g of roots, and the number of root galls varied from 18.3 to 40.4 and from 23.8 to 27.8 galls per meter of root for the experiment at Ica and Lima department, respectively. When comparing the nematode population in soil and roots, and number of root galls before treatment application vs the average of 30, 60 and 90 days after product application, an

increase in *Meloidogyne* numbers in soil (Fig 1A and 2A) and roots (Fig 1B and 2B), and galls (Fig 1C and 2C) per meter of root was observed for the untreated trees, while in the Mocap® treated trees, the nematode population and galls was reduced. The increase in the untreated trees was of 25 (72%) and 40.6 (140%) *Meloidogyne* per 100 g of soil (P< 0.0001 Fig 1A; P< 0.0001 Fig 2A), 9 (8%) and 12.9 (16%) individuals per 100 g of roots (P= 0.0387 Fig 1B; P< 0.0001 Fig 2B) and 9.7 (24%) and 19.7 (75%) galls (P< 0.0001 Fig 1C; P< 0.0001 Fig 2C) per meter of roots, for the experiment of Ica and Lima department, respectively. Soil nematodes were reduced (P< 0.0001; P< 0.0001) with all Mocap® rates, such reductions ranging from 10 (33%) to 36 (96%) and 12.5 (58%) to 33.2 (98%) nematodes per 100 g of soil, for the experiment at Ica (Fig 1A) and Lima (Fig 2A) department, respectively.

Nematode numbers in roots were also decreased (P< 0.0001; P< 0.0001) with all Mocap® rates, with reductions varying from 64 (58%) to 98 (84%) and 43.6 (45%) to 73.8 (85%) nematodes per 100 g of roots, for the experiment at Ica (Fig 1B) and Lima (Fig 2B) department, respectively. In parallel, the number of root galls per meter of root was reduced (P< 0.0001; P< 0.0001) with all Mocap® rates, with reductions from 4.8 (18%) to 10.1 (55%) and 8.4 (31%) to 15.3 (64%) galls per meter, for the experiment at Ica (Fig 1C) and Lima (Fig 2C) department, respectively. At 30, 60 and 90 days after the application, a decreasing linear effect (P< 0.0001; P< 0.0001) on *Meloidogyne* numbers in soil was observed as rate increased for the experiment at Ica (Fig 3A, 3D and 3G) and Lima (Fig 4A, 4D and 4G) department, respectively. The average reduction was of 4.6, 4.9 and 5.2; and 5.5, 6.0 and 6.3 individuals per 100 g of soil per litre of increase on the applied rate at 30, 60 and 90 days, for the experiment at Ica and Lima department, respectively. With the highest rate of 12 L ha^{-1}, the number of nematodes was close to zero, in both trials, at the three evaluation times. In parallel, a decreasing linear (P< 0.0001; P≤ 0.0002) effect on *Meloidogyne* numbers in roots was also observed. The average reduction was of 5.6, 9.9 and 9.9; and 4.9, 7.7 and 8.2 individuals per 100 g of roots per litre of increase on the applied rate at 30, 60 and 90 days, for the experiment at Ica (Fig 3B, 3E and 3H) and Lima (Fig 4B, 4E and 4H) department, respectively. The nematode population was close to zero, in both trials, at

60 and 90 days after the application of the highest rate. Accordingly, a decreasing linear (P< 0.0001; P< 0.0001) effect on number of root galls was found in the experiment at Ica and Lima department, respectively. The average reduction was of 2, 4 and 4.3; and 1.8, 3.9 and 4 galls per meter of root by every litre of increase on the applied rate at 30, 60 and 90 days, for the experiment at Ica (Fig 3C, 3F and 3I) and Lima (Fig 4C, 4F and 4I) department, respectively. In both trials, the number of galls was close to zero with the highest rate at 60 and 90 days after the application. When the regression analysis was done averaging the data of the three evaluations, the reduction was of 4.9 and 6 nematodes per 100 g of soil (P< 0.0001; P< 0.0001), 8.5 and 6.9 individuals per 100 g of roots (P< 0.0001; P< 0.0001), and 3.5 and 3.2 galls per meter of root (P< 0.0001; P< 0.0001), for the experiment at Ica and Lima department, respectively (Data no shown). Differences in biological efficacy among rates were found for soil (Fig 5A-B) and root (Fig 5C-D) nematode control and number of root galls (Fig 5E-F), increasing the control as the rate increased in both experiments at Ica (P< 0.0001) and Lima (P< 0.0001) department, respectively. Efficacy in soil nematode control varied from 51 to 98% and 74 to 99% (Fig 5A-B), in root nematode control it varied from 61 to 85% and 61 to 87% (Fig 5C-D), and in number of root galls from 55 to 84% and 58 to 81% (Fig 5E-F), for the experiment at Ica and Lima department, respectively.
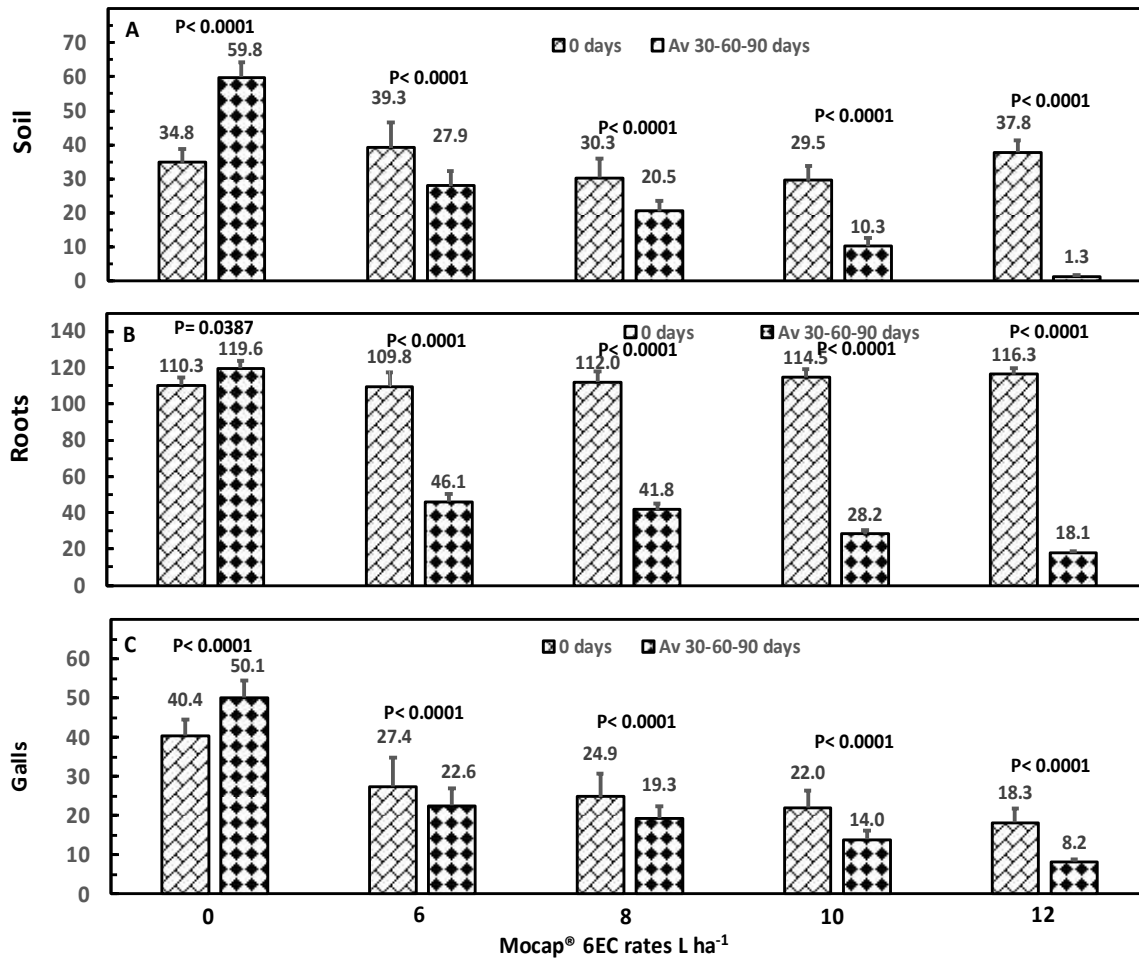
Figure 1A-C. *Meloidogyne* spp. per 100 g of soil (A), 100 g of roots (B) and number of root galls per linear meter of root of about 0.5 mm diameter (C) in grapevines (*Vitis vinifera*) own-rooted cv Flame that were treated with different Mocap® 6EC rates in a sandy loam soil at Ica department, Perú. Each bar is the mean ± standard error of four replicates, and in each replicate the value comes from four sampled trees.
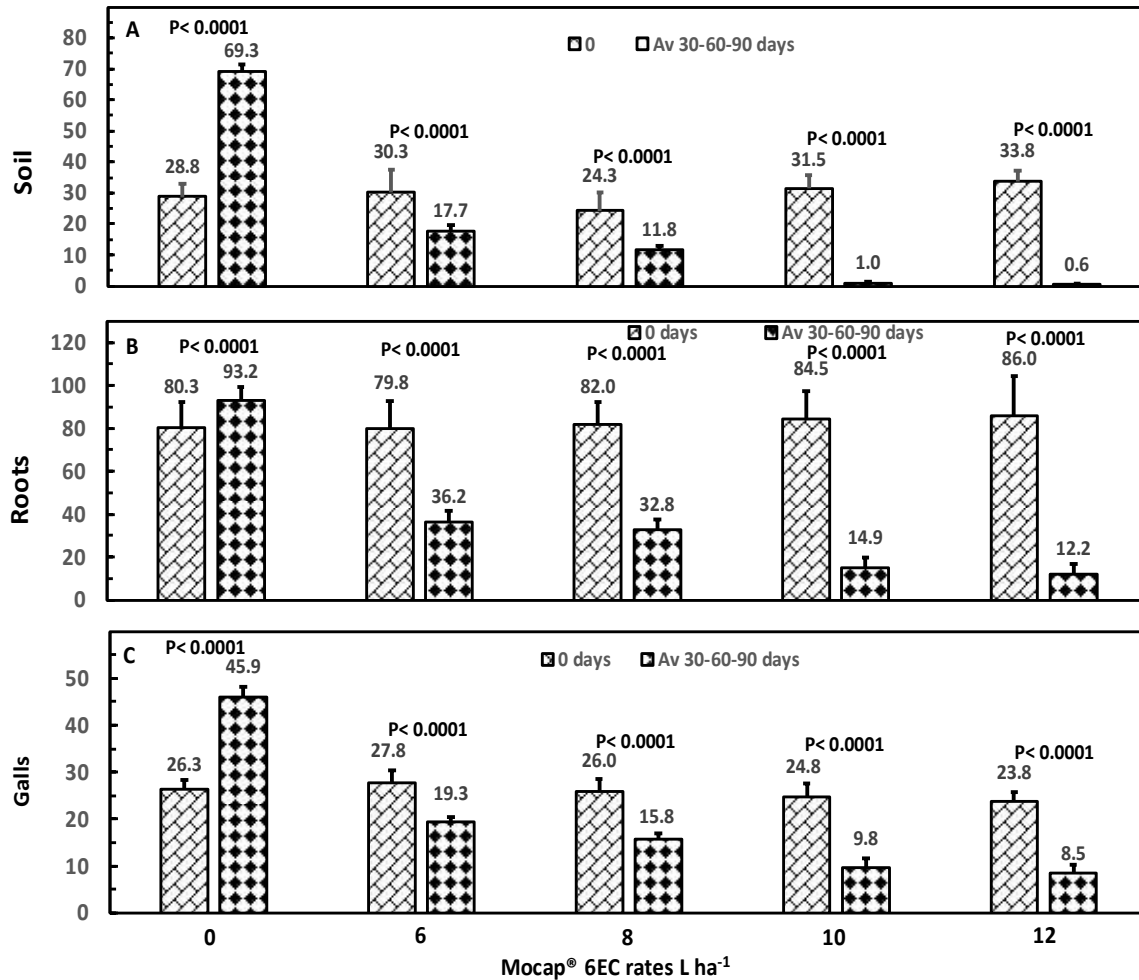
Figure 2A-C. *Meloidogyne* spp. per 100 g of soil (A), 100 g of roots (B) and number of root galls per linear meter of root of about 0.5 mm diameter (C) in grapevines (*Vitis vinifera*) cv Red Globe grafted onto Quebranta that were treated with different Mocap® 6EC rates in a sandy soil at Lima department, Perú. Each bar is the mean ± standard error of four replicates, and in each replicate the value comes from four sampled trees.
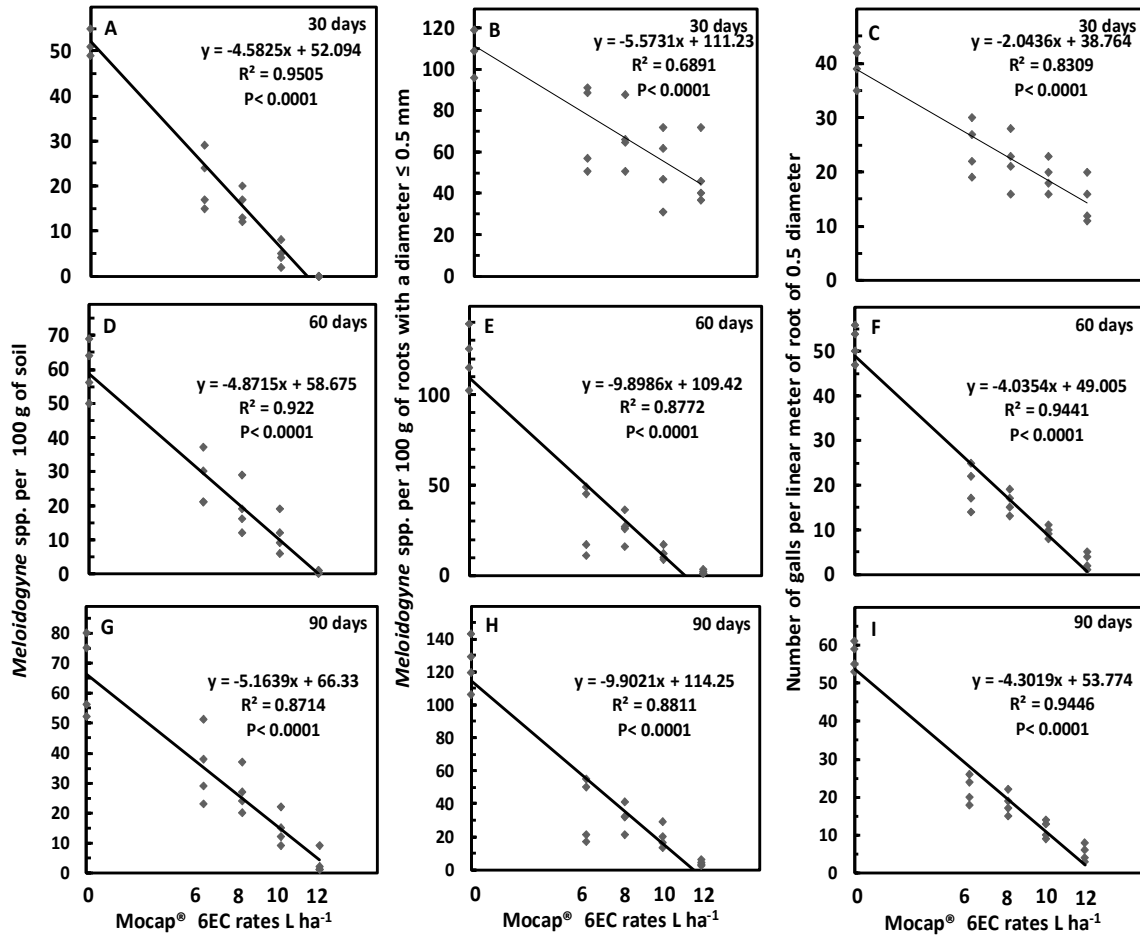
Figure 3A-I. Effect of Mocap® 6EC rates on *Meloidogyne* spp. per 100 g of soil (A, D, G) or 100 g of roots (B, E, H) and number of galls per linear meter of root (C, F, I) in grapevines (*Vitis vinifera*) own-rooted cv. Flame at 30, 60 and 90 days after product application in a sandy loam soil at Ica department, Perú. Data points in each replicate comes from four sampled trees.
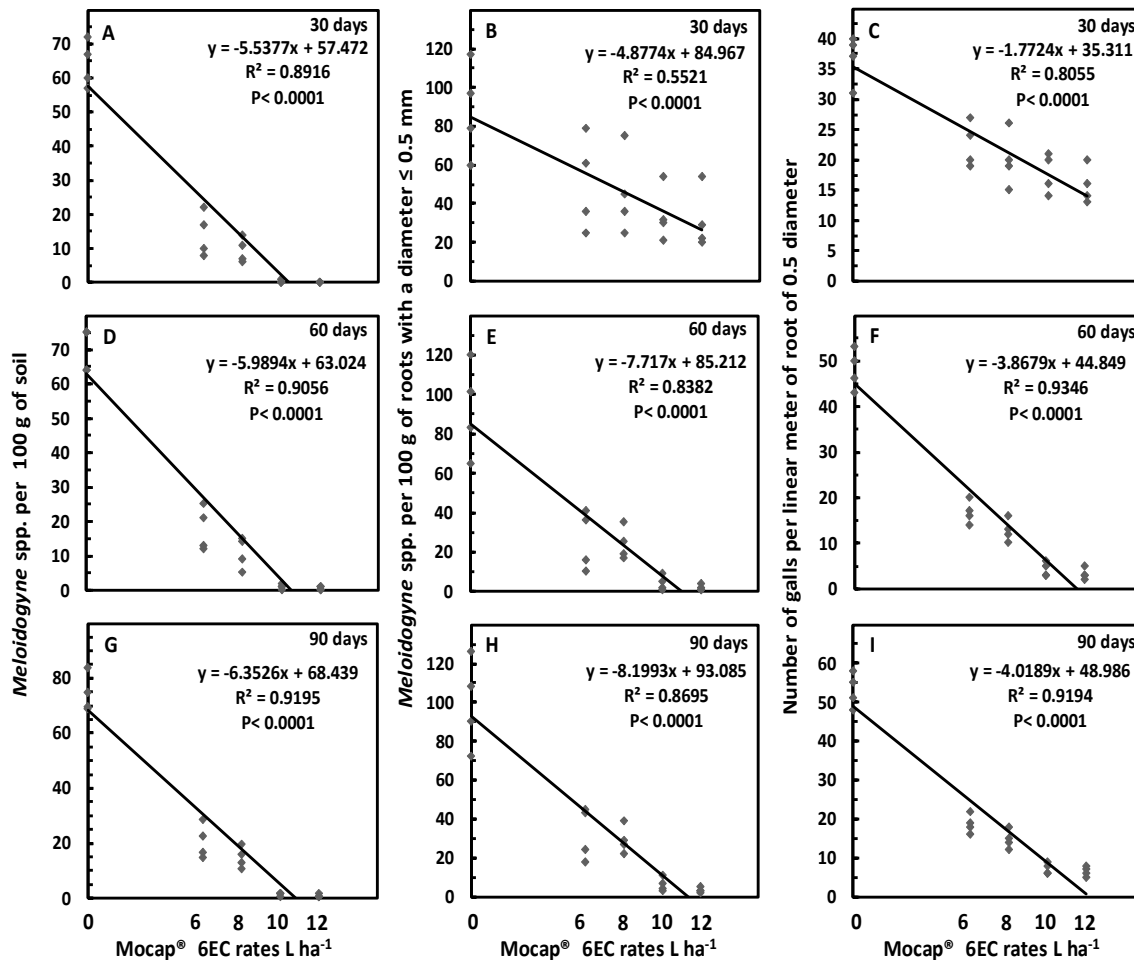
Figure 4A-I. Effect of Mocap® 6EC rates on *Meloidogyne* spp. per 100 g of soil (A, D, G) or 100 g of roots (B, E, H) and number of galls per linear meter of root (C, F, I) in grapevines (*Vitis vinifera*) cv Red Globe grafted onto Quebranta at 30, 60 and 90 days after product application in a sandy soil at Lima department, Perú. Data points in each replicate comes from four sampled trees.
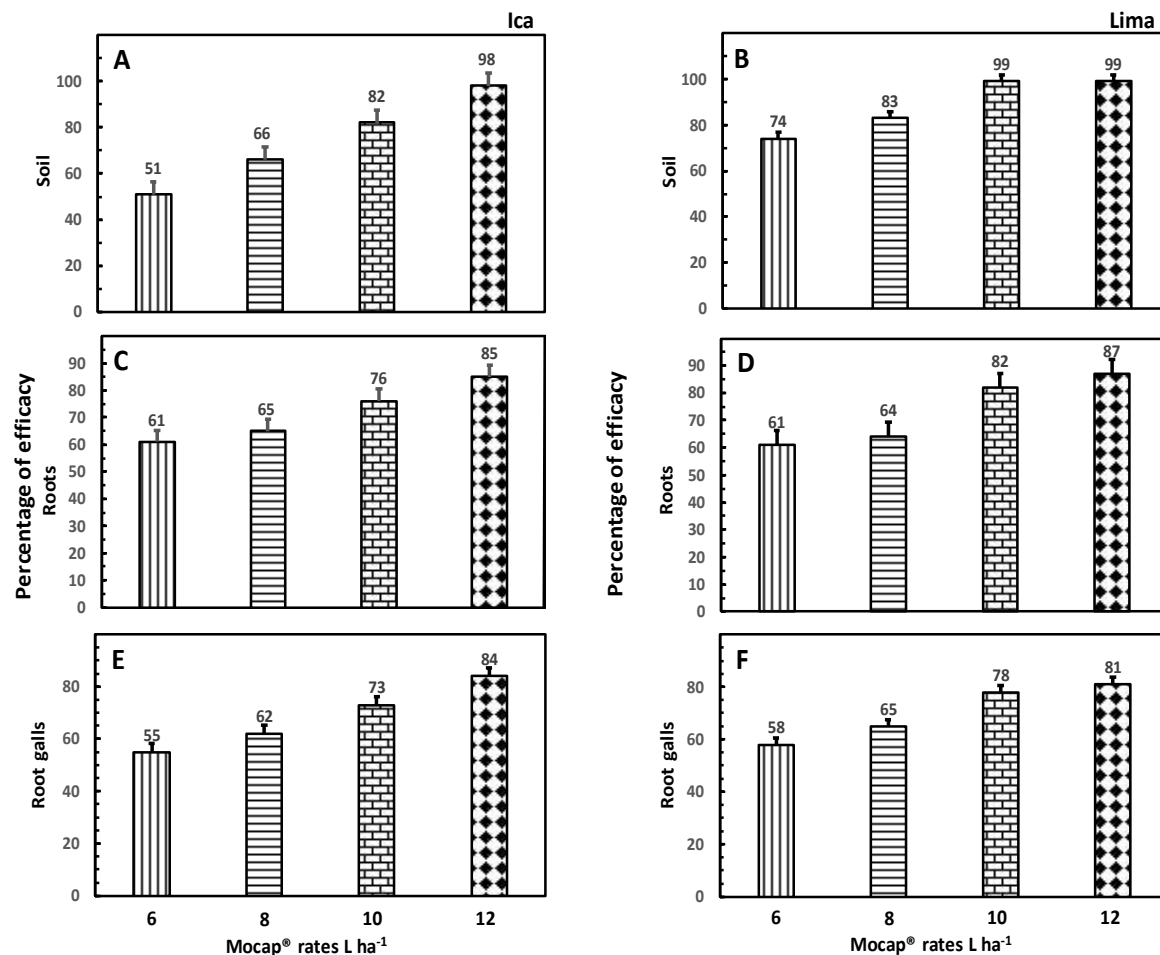
Figure 5A-F. Percentage of efficacy on *Meloidogyne* spp. control in soil (A-B) and roots (C-D) and number of root galls (E-F) per linear meter of root with different Mocap® 6EC rates ha^{-1} on grapevines (*Vitis vinifera*) own-rooted cv Flame and cv Red Globe grafted onto Quebranta at ICA (Sandy loam soli) and Lima (Sandy soil) department, Perú, respectively. Each bar is the mean ± standard error of four replicates, and in each repetition four trees were evaluated.

DISCUSSION

For both experiments, there were no differences among treatments for soil and root nematodes and number of root galls per linear meter of root before the product application. That means that any difference detected later should be attributed to treatment effect. The nematode population consisted mainly of *Meloidogyne* spp. which is one of the most aggressive and damaging nematode in the vines (Nicol *et al*., 1999; Goldammer, 2013) and agrees with the nematodes found by Chávez and Arata (2004), Chang (2014), and Alban (2018) in grapevines of Perú, who reported *M. incognita, M. morocciensis*, *M. arenaria*, *M. ethiopica*, *M. javanica*, and *Meloidogyne* sp. With exception of *M. morocciensis*, the other cited species are common in grapes (Walker and Catherine, sf; Ferris *et al.* 2012, 2013; Goldammer, 2013; Aballay and Vilches, 2015).

Vineyards infected with *Meloidogyne* spp. are reported in Australia (Stirling and Cirami, 1984), Spain (Téliz *et al*., 2007), USA (McKenry, 1992; McKenry and Anwar, 2006), Brasil (Somavilla, 2011), South Africa (Loubser, 1988), France (Boubals, 1979), where they also cause significant economic losses. All Mocap® 6EC rates tested reduced *Meloidogyne* spp. in soil and roots, and the number of galls per linear meter of root, with higher reductions as the rate increased. With 10 L ha^{-1} or more, the nematode population reduction at 90 days post application was under or close to the economic threshold suggested by McKenry and Roberts (1985) of 1 *Meloidogyne* spp., Anwar and Van Gundy (1989) of 50 eggs and J$_2$, Nicol *et al*., (1999) from 7.5 to 50, Dickerson *et al*., (2000) of 1, Quader *et al*., (2002) of 2.5, Vanstone and Lantzke (2006) of 7.5 to 50, Riley

and Walker (2006) of 20 to 200, Pietsch and Burne (2008) of 7 to 50, and Montealegre *et al.*, (2009) of 40 per 100 g of soil. Similarly, a reduction in *Meloidogyne* spp. numbers by 100 g of roots was observed, the population remaining at 90 days after application was close to zero. Good control was observed up to 90 days after product application, but it is known that ethoprophos has a soil half-life of 98 (Jordan *et al.*, 1986) and up to 120 days (Smelt and Leistra, 1992), then a longer control would be expected encouraging the use of 10-12 L ha^{-1}. The nematode population reduction found in these trials agrees with results of Lillo (2006), who testing different Mocap® (ethoprophos) formulations reported good control of the nematode *Xiphinema index* in grapevines cultivated in pots. Also, it is in parallel with the results of Farias (2005) who testing Mocap® for the control of *X. index* in grapevines cv Thompson seedless cultivated in pots, with different percentages (0-2,5-5-7,5-10 and 20%) of organic matter content in the substrate, the product always reduced the nematode population. This study results also agree with Rich *et al.*, (1984) who reported *M. javanica* control with Mocap® in tobacco, and Fortnum *et al.*, (1990) and Crozzoli *et al.*, (1995) who reported *M. incognita* control with Mocap® in bananas and tobacco, respectively, and with Wabere (2016) findings, who reported *Meloidogyne* spp. control with Mocap® applied at planting in tomatoes. Other authors like Cubillos *et al.*, (1980), Sipes and Schmitt (1995), Araya and Lakhi (2004), Castillo *et al.*, (2010), also mentioned the control of other nematodes with Mocap® such as *Helicotylenchus* spp., *Rotylenchulus reniformis* which sometimes are also present in grapevines (Nicol *et al.*, 1999; Aballay and Insunza, 2002; Aballay *et al.*, 2009). Even though, in these experiments yield was not recorded, it is mentioned that when grape nematodes are controlled subsequently increased yield by 20-30% was reported in Australia (Walker, 1989; Edwards 1991) and by more than two-fold in California (McKenry and Ferris, 1979). Physical properties of both soil and the nematicide play an important role in the distribution of the nematicide and consequently in its efficiency on nematode control. At both field trials, the coarse soil texture (high sand content) may favoured product efficacy, since sorption of the active ingredient is the least and pore space is greatest, allowing equal diffusion of the product throughout the

soil profile (Heald, 1987). Even though soil moisture was not really determined, it looks like it did not affect the nematicide efficacy. Efficacy on nematode control varied from 51 to 99% which is between the range of 50 to 90% cited by Van Gundy and McKenry (1977) and Schmitt (1985). This efficacy also agrees with that reported by Araya and Cheves (1997) of 59% controlling *Meloidogyne* spp. in bananas. These fields were irrigated every other day with 40-70 mm which more likely was appropriated for product soil distribution and nematode control. Since these fields were drip irrigated, the water supplied may be adjusted to the product and crop requirement more easily. The application was done simulating an injection in the drip irrigation system, which should be appropriated, since it is known that most of the grapevine roots occurred widen the 60 cm from the trunk, both vertically and horizontally (McKenry, 1984; Loubser and Meyer, 1986) and in addition, it is the place where nematode populations are highest, which corresponds with along the vine rows (Quader *et al.*, 2001; Addison and Fourie, 2008; Essling, 2010; Chang, 2014). It is known that root-knot nematode larvae usually penetrate near the root tip (Loubser and Meyer, 1986). Depending on the ecological conditions, new root growth in grapevines normally occurs at bud burst, and after harvest (Pratt, 1974; Freeman and Smart, 1976; Conradie, 1980; Mckenry, 1984; Loubser and Meyer, 1986). Treatment application was done in July and August, then at the right time, when rooting more likely was occurring. *Meloidogyne* spp. was found occurring at population densities exceeding the levels reported to damage grapevine which can severely affect the production in this field conditions. *Meloidogyne* cause typical alterations in root cell structure and morphology (Wyss, 2002; Téliz *et al.*, 2007; Grove and Perry, 2014) which would negatively constraint the efficacy of nutrient and water uptake and transport by infected roots (Agrios, 2005; Essling, 2010). Consequently, these rootstocks should be considered as good host of *Meloidogyne*, evidence which, furthermore, should be taken into accounts for future field replants. The information obtained highlights the need for an integrated nematode control to avoid yield crop losses, and when replanting, select a tolerant or resistant rootstock cultivar with a proper nematode identification to be a reliable nematode control option.

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