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Efficacy and crop tolerance of 1,3-dichloropropene applied at low rates in established vineyards for the control of plant-parasitic nematodes

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ABSTRACT

Plant-parasitic nematodes are the most important group of root pathogens affecting vineyards for fresh fruit and wine production in Chile. Due to the low efficacy of presently available nematicides and their high variability of control, new alternatives were evaluated in order to obtain a faster and more reliable control. The aim of this work was to assess the use of the soil fumigant Cordon®, containing 81.4% of 1,3-dichloropropene (1,3-D) plus emulsifiers that allow 1,3-D to be mixed with water and applied at low rate via drip irrigation systems over established vines for the control of *Xiphinema index*, the main nematode species associated with grapevine injury in the country. During the first year of studies, one trial was performed in a group of ungrafted vines cv Chardonnay, applying the treatments twice, one in autumn, and the second one in the next spring, with three Cordon® concentrations, 100, 200 and 400 ppm, equivalent to 3.7, 7.4 and 14.8 L ha^{-1} according to the irrigation system water flow. A second trial was performed the following year to determine limits of crop safety by applying 200, 400 and 800 ppm, equivalent to 12, 24 and 48 L ha^{-1} in two established vineyards, cv Thompson Seedless and Cabernet Sauvignon. Results showed that 200 ppm, equivalent to 7.4 or 12 L ha^{-1} depending of the field, achieved good results considering efficacy and phytotoxicity, with a 93.2% control and no crop damage. Two non-fumigant nematicides, Nemacur® 240 CS (24% Fenamiphos) and Rugby® 200 CS (20% Cadusaphos), used as chemical controls, did not show differences in *X. index* populations when compared to the untreated plants. At rates over 400 ppm of 1,3-D, although no visible foliar injury was observed, the pruning weight of treated plants was reduced, indicating a growth reduction.

1. Introduction

Grapevine (*Vitis vinifera* L.) is an economically important crop in Chile, with a cultivated area of approximately 190,000 ha (Guerrero and Gutiérrez, 2012). Many soil-borne pathogens and pests can damage or completely destroy the new roots that are initiated in spring and after the fruit harvest in late summer. Several genera and species of plant-parasitic nematodes (PPN) have been reported to cause economic damage to grapevines and are commonly found in vineyards; the most frequently occurring species are *Xiphinema index* Thorne and Allen, 1950, *Meloidogyne ethiopica* Whitehead, 1968, *Mesocriconema xenoplax* (Raski, 1952) Loof and De Grisse, 1989, and *Tylenchulus semipenetrans* Cobb, 1913 (Aballay et al., 2009). The presence of PPN continues to be one of the most important problems affecting grapevine root systems, with root damage normally resulting in lower production and, in some cases, total crop loss. Several studies have estimated that PPN cause global losses of US$ 78 billion in agriculture, and an annual yield loss of 12.5% in table grapes (Sasser and Freckman, 1987; Smiley, 2005). The damage caused by nematodes however varies depending on many factors, such as: soil type, cultivar, climate, and crop management (Ferris and Mckenry, 1975).

Currently, the control of PPN in Chile is based on the use of chemical nematicides, mainly carbamates and organophosphates, applied once or twice per year. Biological control is just starting with the assessment of native microorganisms (Aballay et al., 2012), alone or in some cases combined with chemicals. Despite these treatments, nematode populations remain almost unchanged (Valenzuela and Aballay, 1996) in the short term, due to the low residual effect of the nematicides, the loss of efficacy with frequent irrigation, the use of organic amendments, and other soil and application factors. Vineyards affected by PPN eventually exhibit

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destroyed root systems due to their direct damage and/or secondary fungal damage, resulting in the need to replace plants before they are 15 years old, *i.e.*, achieving less than 50% of their potential productive life (Anwar et al., 2002; Montealegre et al., 2009). Even after removing the affected plants, the soil remains infested for many years due to the long-term persistence of PPN in the deeper soil layers (McKenry, 1999).

Soil fumigants and some rootstocks tolerant to PPN are alternative approaches under replanting conditions (Schneider et al., 2006), though these strategies are not extensively used by most growers because the chemicals are fairly expensive when used before planting and because rootstocks are typically not tolerant to all nematodes, *e.g., T. semipenetrans* or *M. xenoplax* (Aballay et al., 2009; Téliz et al., 2007).

The possibility of using soil fumigants over established vine orchards has not been completely assessed after 1,2-dibromo-3-chloropropane (DBCP) was removed from the market. Soil fumigants are currently used only as pre-plant treatments. However, lower use rates have rarely been assessed as an alternative in the post plant treatment of vineyards and other perennial crops due to the high risk of damaging or killing the plants. In previous reports (Youngson et al., 1981), it has been shown that some formulations of 1,3-dichloropropene (1,3-D) may be applied over established vineyards and trees, but doses and application technique must be carefully adjusted to avoid plant injury.

Drip-applied fumigant efficacy is dependent upon its ability to diffuse from the application area to other portions of the root area, and is also dependent on soil texture and chemical properties of the formulated product. For example, emulsifiable 1,3-D moves largely with the water to which it is applied but its vapor phase could diffuse beyond the area wetted by drip-applied water (Candole et al., 2007).

The aim of this study was to assess the nematicidal and phytotoxic effects of the soil fumigant 1,3-dichloropropene applied through the irrigation system at rates lower than those recommended for soil fumigations, and determine effective rates for PPN control in established vineyards.

2. Material and methods

Two trials were performed to evaluate the efficacy of low rates of 1,3-D on plant-parasitic nematodes control and also to determine the threshold rate at which crop damage occurs.

2.1. Trial 1. Evaluation of different 1,3-D concentrations in PPN control

The first experiment was conducted in Casablanca valley, in a 12 year old vineyard, with non-grafted vines of the cultivar Chardonnay presenting a high infestation of the ectoparasitic nematode *X. index*, average of 530 specimens per 250 cm^3 soil. The block presented a density of 3333 vines per ha (2.0 m × 1.5 m), with drip irrigation system emitters located at 0.7 m and a flow rate of 18.5 m^3 $hour^{-1}$ ha^{-1}. Soil texture ranged from loamy to sandy loam in the first 0.5 m depth (Table 3).

Six treatments, consisting of three concentrations of Cordon® were applied. Cordon® contains 81.4% 1,3-D plus emulsifier that allows the 1,3-D to be mixed with water and applied via drip irrigations systems (813.8 g L^{-1} 1,3-D, formulated as emulsifiable concentrate). Cordon® concentrations evaluated were 100, 200 and 400 parts per million (ppm), plus two non fumigant nematicides, Nemacur® 240 CS (24% Fenamiphos) and Rugby® 200 CS (20% Cadusaphos) applied through the drip irrigation system during the time necessary to reach 0.5 m soil depth, the zone with the major density of new roots. The treatments implemented for Cordon® in this trial, expressed as concentration of the commercial product in part per million and the adjusted dose per hectare, and the controls used are shown in Table 1.

The dose of Cordon® applied per ha to the concentration required, was calculated considering the water necessary to irrigate 0.5 m depth.

Applications were performed twice. The first in autumn, after harvest, with plants showing yellow leaves and entering the winter recess, and the second one during the next spring, at bloom, at the beginning of a new root flush.

Application was performed with a mobile injection system consisting of a Massey Ferguson tractor equipped with a 2000 L tank Parada® and a high pressure hose connected to the irrigation lines, with drippers located at 0.6 m and a flow of 2 L h^{-1}. Solution with the required ppm was prepared in the tank and later injected into the lines for 2 h, the time calculated for the solution to reach the 0.5 m soil depth (Fig. 1). Two days before the applications the soil was irrigated to achieve field capacity condition in the trial area.

After the autumn application the soil was not irrigated again, but after the spring application, the soil was irrigated 25 days after of the treatments.

2.2. Trials to determine potential crop damage

During the second growth season, a trial was performed to determine the effect of different rates of Cordon® (1,3-D) on the growth of the treated plants at two different farms, by measuring the annual growth of the plants.

The first study was carried out in a 9 year old vineyard cv Thompson Seedless, at the Experimental Station of the University of Chile, in La Pintana district. This location had non grafted vines for table grape production, planted at a density of 814 vines ha^{-1}, irrigated through drip irrigation lines, with drippers located at 1 m and with a water flow of 12 m^3 ha^{-1} h^{-1}. The block was infested with PPN, mainly the species *X. index*, with an uneven initial population and *M. ethiopica,* with a scarce density. Final populations for this trial were not considered.

The second study was established in a wine grape vineyard, located in Alhué district, an important area for vineyards and fruit tree orchards. The vineyard consisted of 13 year old ungrafted vines cv Cabernet Sauvignon, at a density of 3333 plants ha^{-1}, under a drip irrigation system, with drippers located at 0.6 m and a water flow of 13.3 m^3 ha^{-1} h^{-1}. The soil presented an infestation with the same species and infestation level as in the previous field.

Table 1
Treatments implemented to evaluate Cordon® concentrations, doses determined, and commercial doses of the chemical controls.

Cordon® concentrations and controls	Concentrations of active ingredient (%)	Dose L/ha
Cordon® 100 ppm	1,3-D (81.4)	3.7
Cordon® 200 ppm	1,3-D (81.4)	7.4
Cordon® 400 ppm	1,3-D (81.4)	14.8
Nemacur 240 CS	Fenamiphos (24)	17
Rugby 200 CS	Cadusaphos (20)	15
Untreated control	Water	

Table 2
Treatments implemented in both vineyards to assess phytotoxicity of three Cordon® concentrations.

Cordon® concentrations and controls	Concentrations of active ingredient (%)	Dose L/ha
Cordon® 200 ppm	1,3-D (81.4)	12
Cordon® 400 ppm	1,3-D (81.4)	24
Cordon® 800 ppm	1,3-D (81.4)	48
Untreated control	Water	

Table 3
Physical and chemical properties of treated soils.

Zone	Sand	Silt %	Clay	pH	E.C dS m^{-1}	O.M. %	Textural class
Casablanca	63	18	19	6.7	0.33	1.9	Sandy loam
La Pintana	36	38	26	7.5	3.0	3.0	Loam
Alhué	64	19	17	6.1	0.5	1.1	Sandy loam

Soil textures in both fields correspond to loam and sandy loam respectively (Table 3).

The treatments implemented in both vineyards, are presented in Table 2.

Doses per ha were estimated considering the amount of water required to reach the zone with the major amount of roots, for both places it was 0.5 m depth. The time necessary to deliver the water was different for both fields, considering the number of drippers per ha and their flow.

Applications in both fields were performed twice a year, the first one after harvest, and the second in spring, during bloom at the beginning of the new root flush.

Treatments were applied 3 days after irrigation, injecting the solution prepared in a 2000 L tank, in the same way as in the previous trial, over a period of 3 and 4.5 h for table grapes and wine grapes respectively to reach the required depth of major root density.

2.3. Assessments

To evaluate the treatment efficacy, nematode populations were determined prior to product application and 30 days after application (daa). Soil samples taken to determine infestation level were collected using a shovel to dig 25–35 cm deep, zone with a high feeder roots, despite they may reach up to 0.5 m. About 10 subsamples were taken at random to make 1-L pooled sample, kept in plastic bags and stored at 8 °C until they were processed about three weeks later. Nematodes were extracted from a 250 cm^3 soil volume combining the sieving and decanting method with a Baermann's funnel (Hooper and Evans, 1993; Southey, 1986) using sieves of 710 μm, 250 μm, 150 μm and 45 μm. The final suspension was decanted on a filter paper during 48 h, to obtain optimal recovery of *Xiphinema* spp. adults and fourth juvenile stage, the soil samples suspended in water were sieved through the 750 and 250 μm sieves only, and then filtered through a nylon sieve of 90 μm for 24 h (Brown and Boag, 1988). Genera and species identification was made using a dissecting microscope (Carl Zeiss Stemi 2000 C) at 50–90× magnification.

Fig. 1. General view of the vineyard cv Cabernet sauvignon in autumn treated with both 1,3-D and non fumigant nematicides, applied through the drip irrigation system.

In order to assess plant damage, vigor from plants treated was measured through pruning weight (k $vine^{-1}$). Data were obtained comparing pruning weight obtained in winter season after applications (year 2) with data obtained the year previous to the beginning of this study (year 1). Plants were pruned during dormancy, according to the needing of farmers, cutting canes of the last season, and fresh weight was registered.

2.4. Experimental design and statistical analysis

For both trials, the treatments were replicated 4 times and experimental plots distributed according to a completely randomized design, in different rows. Each plot corresponded to a four plant area for table grapes (12 m^2) and eight plants for wine grapes (8 m^2), located in the same row.

To evaluate the effect on nematode populations of the different treatments, the reproduction rate (R) was used, which relates the final with the initial populations, Pf/Pi (Oostenbrink, 1966). Final population corresponds to the one obtained 30 days after applications (daa). Previous to calculating R and performing the analysis of variance (ANOVA), the nematode density data were normalized using a log (x + 1) transformation (Noe, 1985). If significance at $P < 0.05$ was detected, treatment means were compared according to Tukey's multiple range test. Data of pruning weight were analyzed in the same way, in both cases with the statistical package MINITAB® V16.

Control percentages were calculated considering population variation pre and after application in treated plants with respect to the variation in the control, according to the following formula.

$$\text{Control } (\%) = (1 - (\text{R treatment}/\text{R control}))*100$$

3. Results

Data from studies 1 and 2 are presented in Tables 4–6, for nematode population variation and plant growth responses.

Assessments were made mainly on *X. index*, because it was the dominant species present in the field. *M. ethiopica* and *Pratylenchus thornei* Sher and Allen, 1953 were also present, but were not detectable in all the plots.

Table 4
Variation and mortality of *X. index*, post harvest and spring applications, efficacy study. Data correspond to populations detected previous and 30 days after applications, reproduction rate and control percentages per season and considering the population variation in the whole year.

Treatments	N° nematodes 250 cm^{-3} soil				Reproduction rate (Pf/Pi)			Efficacy (%)		
	Post harvest		Spring		Post harvest	Spring	Both seasons	Post harvest	Spring	Both seasons
	P.i.	P.f.	P.i.	P.f.						
Cordon® 100 ppm 3.7 L ha^{-1}	568.5[b]	381.8	341.5[b]	280.3	0.7 ab[a]	0.8 a[a]	0.5 ab[a]	68.6	34.0	77.3
Cordon® 200 ppm 7.4 L ha^{-1}	874.8	236.3	203.8	129.8	0.3 b	0.6 ab	0.1 b	87.4	48.8	93.2
Cordon® 400 ppm 14.8 L ha^{-1}	478.5	109.5	175.0	25.8	0.2 b	0.1 b	0.1 b	89.3	88.2	97.5
Nemacur 240 CS 17 L ha^{-1}	637.5	755.3	1221.3	1218.8	1.2 ab	1.0 a	1.9 a	44.7	19.8	11.9
Rugby 200 CS 15 L ha^{-1}	380.3	853.5	314.8	1374.0	2.2 a	4.4 a	3.6 a	0.0	0.0	0.0
Untreated plots	372.0	796.5	648.8	807.0	2.1 a	1.2 a	2.2 a			

[a] Means within columns followed by the same letter are not significantly different (Tukey's test $P \leq 0.05$).
[b] Data are the average of four replicates.

Table 5
Pruning weight, after autumn and spring applications.

Treatments	Pruning weight g $plant^{-1}$
Cordon® 100 ppm, 3.7 L ha^{-1}	519.2[b] a[a]
Cordon® 200 ppm, 7.4 L ha^{-1}	513.4 a
Cordon® 400 ppm,14.8 L ha^{-1}	505.9 a
Nemacur 240 CS, 17 L ha^{-1}	519.7 a
Rugby 200 CS, 15 L ha^{-1}	550.1 a
Untreated plots	460.8 a

[a] Means within columns followed by the same letter are not significantly different (Tukey's test $P \leq 0.05$).
[b] Data are the average of four replicates.

3.1. Trial 1, nematicide efficacy

X. index population variation is presented in Table 4 for efficacy. Data were ordered to show results obtained with autumn (post harvest), spring, and the combined effect of both applications. Pruning weight data is presented in Table 5.

Data showed that 1,3-D was the most effective chemical for the control of PPN. Autumn and spring applications showed significant levels of control using 200 and 400 ppm in post harvest and 400 ppm in spring, with higher control level in autumn applications. This effect was also cumulative, since the protection period from autumn applications keeps nematode populations at a lower level through the beginning of spring (Table 4). The most effective concentrations considering both periods, were 200 and 400 ppm of Cordon®.

Organophosphate nematicides tested showed no control efficacy under the trial conditions. These were applied in the same way as it is made traditionally, but the final populations were higher than the initial ones in both moments of application, showing that there was no effect of nematicides in reproduction rate. Factors associated to the low mortality caused by non fumigant nematicides are many, and may be associated with their permanent use, some soil properties, or management activities.

Although the pruning weight data are not statistically different with respect to the untreated control, the latter achieved the lowest weight, suggesting weaker plants (Table 5). Vigor of treated plants was numerically higher than the control, representing from 9.8% to 12.6% weight increase.

Values for plants treated with Rugby and Nemacur were also higher than untreated plants, representing a 19.4% weight increase over the untreated plants, which show that these chemicals possibly had some effect on feeding of nematodes, despite the increase of the populations was not suppressed.

3.2. Trial 2, assessment of potential crop damage under different 1,3-D dosages

The studies to determine the threshold rate, over which negative effects of the chemical on vines growth can be detected, are presented in Table 6, showing pruning weight obtained in winter of the last season of studies, presenting data previous to applications, year 1, and those obtained once treatments were performed, year 2.

For the table grape assessed, results show that plants treated with Cordon® at the 200 ppm concentration and dose per ha had the greatest increase in cane growth ($p < 0.05$). With concentrations near at 800 ppm, growth reduction of the vines occurred.

For the wine grape cultivar C. Sauvignon assessed, possible crop injury levels are not clear, since no statistical differences were detected between the control and the Cordon® treatments, although the 800 ppm concentration showed the lowest numerical value for the difference in pruning weight between the two growing seasons evaluated.

4. Discussion

According to trial results, the use of the formulated product Cordon® containing 81.4% 1,3-D plus emulsifiers that allow 1,3-D to

Table 6
Thompson Seedless and Cabernet Sauvignon pruning weight after treatments with Cordon® (1,3-D) in different concentrations and doses per ha, year 1 = winter pre treatments; year 2, winter post treatments.

Treatments	Pruning weight (k $vine^{-1}$)					
	Thompson seedless			Cabernet sauvignon		
	Year 1	Year 2	Difference	Year 1	Year 2	Difference
Cordon® 200 ppm, 12 L ha^{-1}	2.06[b]	4.56	2.50 c[a]	0.37[b]	0.79	0.42 a[a]
Cordon® 400 ppm, 24 L ha^{-1}	4.14	4.70	0.57 b	0.38	0.87	0.49 a
Cordon® 800 ppm, 48 L ha^{-1}	4.03	2.08	−1.96 a	0.46	0.69	0.23 a
Untreated	1.79	1.88	0.09 b	0.30	0.69	0.39 a

[a] Means within columns followed by the same letter are not significantly different (Tukey's test $P \leq 0.05$).
[b] Data are the average of four replicates.

be mixed with water, used at a concentration of 200 ppm, can be an excellent treatment for the control of *X. index* associated to grape vines in Chile. The 400 ppm concentration has also demonstrated good nematode control, but the pruning weights in table grapes, is similar to the untreated plants, possibly indicating some level of phytotoxicity.

According to the irrigation system and root development, it is estimated that the dosages should range between 7.4 and 12 L ha^{-1} to achieve the needed concentration for control. This is a lower amount of product, compared to the organophosphates or carbamates currently used for nematode control programs in vineyards.

Similar control results were obtained by Youngson et al. (1981) with the fumigant TELONE II (92% 1,3-D) applied in irrigation water to Cabernet Sauvignon and Carignane grapevine cvs on St. George rootstock, infested with *Meloidogyne incognita* (Kofoid and White,1919) Chitwood, 1949 *Xiphinema americanum* Cobb, 1913 or *Paratylenchus* species. They obtained a nearly 100% control with doses ranging from 56 to 112 L ha^{-1}, but observed phytotoxicity with 224 L ha^{-1}, i.e. much higher rates than in our experiments. One difference may be the irrigation system; broadcast irrigation in the Youngson et al. experiment and drip irrigation lines in our studies, affecting the amount of total water applied. Also there are important differences in the emulsifiers used in current Cordon® formulation, which contribute to improve the mix with water resulting in an even application. These doses may need to increase according to soil factors, like increased organic matter that affects 1,3-D movement and its efficacy (Riegel et al., 2001).

One of the important factors necessary to achieve a high degree control is 1,3-D movement into the soil, which depends directly of its diffusion into the soil with the water as the carrier. According to Candole et al. (2007), the distribution of 1,3-D +chloropicrin were higher at 10 cm below the emitter than at 20 cm away from the emitter which indicated poor 1,3-D+chloropicrin distribution in treated beds, reflected in a higher survival of soil fungi at 20 cm away from the emitter than at 10 cm below the emitter. This effect was not seen in our study, with soil samples obtained 25 cm from emitter and 25–30 cm depth, due may be to differences in soil texture, sandy in the study reported by Candole et al. (2007).

The increase of plant growth under a long lasting nematicide application program is a slow process in grapes, since normally affected plants have a damaged root system and the recovery period usually takes 2–3 years of continuous treatments (Rasky et al., 1981). This means that plants in trial 1 under a continuous program with Cordon® 200 ppm, should produce an increased plant growth that will be reflected in a larger pruning weight.

Standard nematicides tested did not show a significant control of the nematodes present. This may be due to the high number of initial population (Table 4) followed by a fast recovery after application due to their low residual effect. The lack of efficacy or the very short residual effect of carbamates and organophosphates has been reported under different conditions (Aballay et al., 2009; Hafez et al., 1981; Harris, 1986; Walker and Stirling, 2008) and results in a big problem for growers, who must increase dosages and/or the number of applications. Financial constraints make this option not possible for most farmers. However, it should be noted that there is a tendency to show a higher vine pruning weights, possibly due to some nemastatic effect, which is a common answer with non fumigant nematicides (Bunt, 1987; Hafez et al., 1981).

The low effect of non fumigants is due to many factors affecting active ingredients in the soil: the need to cover a big root system, and the post application management, especially irrigation (Aballay et al., 2009; Harris, 1986; Loubser, 1985). In our study, one of the key elements considered especially important in affecting the efficacy of both chemicals, is the accelerated soil microbial degradation, which continues being one of the main problems due to the continuous use of a few number of molecules (Karpouzas et al., 2004; Matthiessen, 2001).

The second trial, performed to check the rate at which the soil fumigant is phytotoxic, showed that grapevines treated with the lowest rate of Cordon® 1,3 D achieved the greatest pruning weight, being statistically significant in table grapes ($p < 0.05$). Wine grape have less cane weight than table grapes, which is normal considering that farmers try to have plants with limited foliage growth to enable berries direct sun exposure for part of the day.

Pruning weight achieved at 400 ppm concentration was similar to the untreated control, meaning that it could be the high concentration limit of Cordon®, where the positive effect on nematode control counteracts phytotoxic damage to root system.

The highest concentration of Cordon®, 800 ppm, produces a significant decrease in pruning weight in table grapes, which probably means a phytotoxic effect. This effect is very clear on tables grapes, where the larger amount of canes produced by vines is clearly affected when compared with wine grapes.

In conclusion, according to the results obtained, the soil fumigant 1,3-D formulated as a concentrated emulsion doses equivalent to 162.8 parts per million, calculating final rates per ha based on volume of the irrigation water per ha to reach the required depth of root system may be a very effective alternative for the control of plant-parasitic nematodes in established vineyards.

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