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Testing the ability of vivianite to prevent iron deficiency in pot-grown grapevine

I. Díaz, V. Barrón, M.C. del Campillo, J. Torrent *

Departamento de Ciencias y Recursos Agrícolas y Forestales, Universidad de Córdoba, Edificio C4, Campus de Rabanales, 14071 Córdoba, Spain

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ABSTRACT

Synthetic vivianite (ferrous phosphate octahydrate) has been reported to reduce the iron (Fe) deficiency symptoms in different crops growing on calcareous soils. We investigated the effectiveness of vivianite in grapevine by means of a 3-year (2002–2004) pot experiment with a Fe chlorosis-susceptible rootstock ('110 Richter') grown on a calcareous soil poor in available Fe. There was one treatment in which a suspension of vivianite was injected into the soil at the beginning of the experiment, one treatment with Fe chelate (FeEDDHA) applied yearly, one treatment with one initial application of both vivianite and FeEDDHA, and one control (no Fe fertilizer added) treatment. The concentration of chlorophyll per unit leaf area was estimated with a portable chlorophyll meter (readings in SPAD units). The vines fertilized with vivianite had longer shoots and higher number of leaves, and exhibited higher SPAD values than the control vines. The differences in SPAD value and pruning wood weight between the vines fertilized with Fe and the control were significant through the 3 years. There were no significant differences in SPAD value and pruning wood weight between the vines fertilized with Fe chelate and vivianite. Our results suggest in summary that vivianite is an interesting alternative to other Fe fertilizers used to prevent Fe chlorosis in grapevine judging by its effectiveness and long-term fertilizing effect. Moreover, it is not easily leached from the soil, easy to prepare, and environmentally safe.

1. Introduction

Iron (Fe) deficiency is commonly observed in grapevine cultivated in calcareous soils, its typical symptoms being chlorosis (yellowing) in the interveinal tissue of the youngest leaves and growth depression (Bavaresco et al., 1993; Gruber and Kosegarten, 2002). Severe Fe deficiency results in inhibited leaf expansion (Nikolic and Römheld, 2002), leaf necrosis and, eventually, plant death (Reyes et al., 2006).

The use of tolerant rootstocks originated from American *Vitis* species (Bavaresco et al., 2005) is recommended to prevent Fe deficiency. However, the problem is likely to persist in highly calcareous soils, thus making it necessary to apply Fe fertilizers. Among these, Fe chelates (e.g. FeEDDHA) and ferrous sulfate are the most commonly used, the former being substantially more effective than the latter but more expensive and easily leached from the soil. One other Fe fertilizer that has proved to be effective is ferrous phosphate [an analogue of the mineral vivianite ($Fe_3(PO_4)_2{\cdot}8H_2O$)]. Injecting a suspension of synthetic vivianite into the soil was effective to prevent Fe chlorosis in pear trees (del Campillo et al., 1998), olive trees (Rosado et al., 2002), and kiwifruit (Rombolà et al., 2003). The effectiveness of vivianite is attributed to its high content in Fe (~30%) and its capacity to dissolve incongruently in calcareous media to produce poorly crystalline lepidocrocite (γ-FeOOH) (Roldán et al., 2002)—it should be recalled in this respect that poorly crystalline Fe oxides constitute the main sources of available Fe to plants growing in calcareous soils (Loeppert and Hallmark, 1985; del Campillo and Torrent, 1992; Yanguas et al., 1997; de Santiago and Delgado, 2006). Because vivianite is slowly soluble, and the resulting lepidocrocite particles are not easily leached from the soil, it can be considered as a slow-release fertilizer which effect lasts several years (del Campillo et al., 1998; Rosado et al., 2002); this is an obvious advantage over Fe chelates. The purpose of this work was to assess the short- and long-term effectiveness of vivianite in correcting Fe chlorosis in grapevine and compare its effect with that of a commonly used Fe chelate (FeEDDHA). To this end, we conducted a 3-year pot experiment.

2. Materials and methods

2.1. Plant and soil materials

One-year-old rooted cuttings of *Vitis berlandieri* Resseguier No. 2 × *Vitis rupestris* Martín, '110 Richter' rootstock (110R), which is relatively Fe chlorosis-susceptible (Pouget and Delas, 1989) and rather common in Spanish vineyards, were planted in pots containing about 12 kg of calcareous soil in April 2002. The soil, collected from a vineyard in the Montilla–Moriles wine producing area had pH 8.2, and was clayey and rich in carbonate [550 g kg^{-1} of

* Corresponding author. Tel.: +34 957 218472; fax: +34 957 218440.
E-mail address: torrent@uco.es (J. Torrent).

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calcium carbonate equivalent (CCE) and active lime (Drouineau, 1942) (180 g kg^{-1})]. The contents in the different forms of Fe that are generally used to estimate Fe availability were 3.4 mg kg^{-1} diethylenetriaminepentacetic acid (DTPA)-extractable Fe (Fe_{DTPA}) (Lindsay and Norvell, 1978) and 0.25 g kg^{-1} acid oxalate-extractable Fe (Fe_{ox}) (Schwertmann, 1964). The soil Fe_{ox}/active lime ratio was 14×10^{-4}, which, according to Reyes et al. (2006) implies a high risk of Fe chlorosis in 'Pedro Ximénez/110 Richter' plants.

2.2. Experimental design

The experimental design consisted of six replicates each of the following four treatments: (a) "Vivianite". A single application of 1 g vivianite (equivalent to 0.33 g of Fe) kg^{-1} soil before planting (see below for the preparation method). (b) "FeEDDHA". A yearly application of 0.5 g (2002), 0.7 g (2003), and 1.0 g (2004) of FeEDDHA (6% Fe and 4.8% Fe in the ortho–ortho isomer, Laboratorio Jaer S.A., Barcelona, Spain) per pot, split into monthly applications during the growing season. (c) "Vivianite + FeEDDHA". A single application of 1 g vivianite kg^{-1} soil + 0.5 g FeEDDHA per pot before planting. (d) "Control". No fertilizer application. [It should be noted that: (i) the doses of vivianite and FeEDDHA assayed in this experiment were those that we had found to be effective in other pot-grown crops, and (ii) the (c) treatment was used to supply Fe to the plant before significant dissolution of vivianite occurred.] Pots were randomly arranged and stored in a shadehouse for 3 years with watering to field capacity when water content was near wilting point. A modified Hoagland nutrient solution [2.5 mmol $Ca(NO_3)_2 H_2O$, 2.5 mmol KNO_3, 2 mmol $MgSO_4$, 1 mmol KH_2PO_4, 0.1 mmol KCl, 50 μmol H_3BO_3, 4 μmol $MnSO_4 \cdot 5H_2O$, 4 μmol $ZnSO_4 \cdot 7H_2O$, 1 μmol $CuSO_4 \cdot 5H_2O$, and 0.1 μmol $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ per liter] was applied at a rate of about 5 L per pot per year.

2.3. Synthesis and application of vivianite

Vivianite was prepared in a beaker containing 5 L of continuously stirred water to which 125 g of monoammonium phosphate [$(NH_4)H_2PO_4$] was added until complete dissolution, followed by slow addition of 375 g of ferrous sulfate ($FeSO_4 \cdot 7H_2O$). The resulting thick suspension was initially white but turned rapidly into a greenish blue color typical of partly oxidized vivianite. Continuous stirring was needed to prevent the vivianite particles (2–10 μm in size) from settling on the bottom of the beaker. The prescribed dosis of the suspension, which contained about 50 g vivianite L^{-1}, was immediately injected into the soil in 10 points located at different depths with the help of a syringe.

2.4. Plant analyses

The chlorophyll concentration in young leaves was estimated five times in 2002 and four in 2003 and 2004 from the "SPAD" readings acquired with an SPAD 502 portable chlorophyll meter (Minolta Camera Co., Osaka, Japan). Three youngest fully expanded leaves were selected from each vine and both SPAD units and midrib length were recorded. Previous experiments had shown that the ethanol-extracted chlorophyll content per unit surface was highly correlated with SPAD for the '110R' rootstock leaves ($r = 0.79$; $P < 0.001$). Fifteen leaf petioles from the midshoot per pot were sampled at the end of each annual growth cycle (fall) for mineral element analysis. Petioles were dried at 65 °C for 72 h and digested with nitric/perchloric acid (Zazoski and Burau, 1977). Calcium, Mg, Fe, Mn, Cu and Zn in solution were determined by atomic absorption spectrophotometry, K by flame emission, and P with the Molybdenum Blue color method of Murphy and Riley (1962). All flowers of each plant (collected at full bloom in the springs of 2004 and 2005) were weighed and their mineral nutrients determined as described above. Vines were pruned in winter and pruning wood weighed.

2.5. Statistical analyses

The analysis of variance (ANOVA) was performed with Statistix 8.0 (Analytical Software, Tallahassee, FL, USA). Unless otherwise stated, the word "significant" is used here to indicate significance at the $P < 0.05$ level. Means were separated via the LSD test. For some measurements repeated at different times (Fig. 3) the mean and the standard error for each treatment and time are shown.

3. Results

Iron chlorosis symptoms were observed in '110R' plants grown in the calcareous soil 2 months after planting. This was consistent with the low values of Fe_{ox} (0.25 g kg^{-1}) and Fe_{ox}/active lime (14×10^{-4}).

Shoot length and number of leaves in the first year increased with time, especially over the July–September period (i.e. from 80 to 120 days after planting, Fig. 1A and B). The average shoot length in the Fe-fertilized vines in August, September and October was 77% (66 cm), 128% (112 cm) and 135% (118 cm) higher, respectively, than that of the control vines (Fig. 1A). The number of leaves in the Fe-treated vines in August, September and October was 40% (16 leaves $vine^{-1}$), 69% (27 leaves $vine^{-1}$) and 78% (32 leaves $vine^{-1}$) higher than in the respective control vines (Fig. 1B). Growth of control vines was severely depressed after July; thus, out of six plants, only one in August, two in October, and one in September grew new leaves.

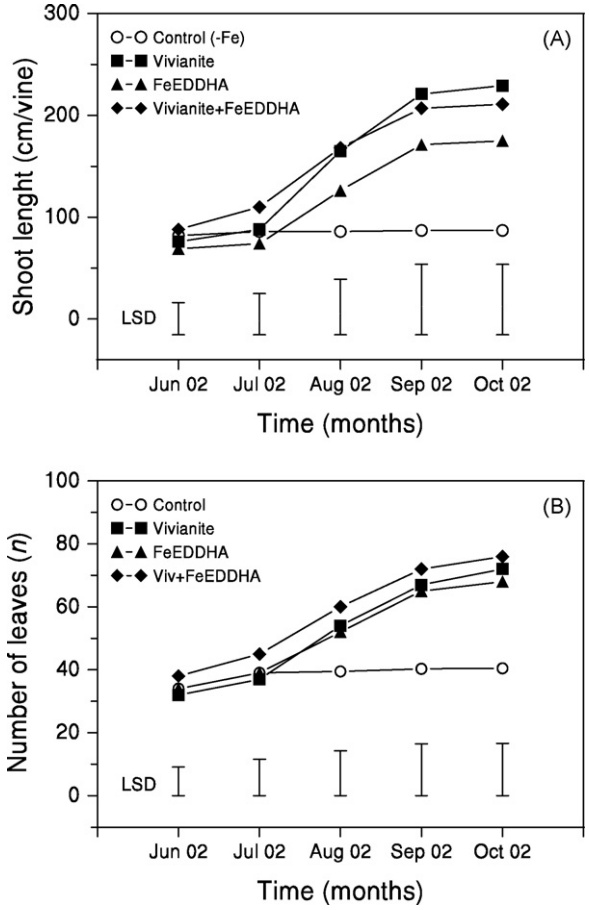

Fig. 1. Shoot length (A) and number of leaves (B) of '110 Richter' rootstocks grown in pots. Bars indicate the least significant difference (LSD) at the $P < 0.05$ level at each time.