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Foliar diagnosis as a guide to olive fertilization

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Abstract

A long term experiment was established in olive orchards of Andalusia, southern Spain, to compare the fertilization practice based on foliar diagnosis versus the current fertilization practice in the area. Only nutrients whose concentration in leaves taken in July were below the critical levels were applied in the former treatment, whereas in the latter 500 kg ha^{-1} of a 15-15-15 complex fertilizer, and three foliar sprays of micronutrients and aminoacids were applied annually. Four olive orchards were selected. One orchard has a small effective depth (33 cm) limited by a petrocalcic horizon. The second has an effective soil depth of 60 cm, and is representative of broad level uplands on hard calcareous rocks. The third orchard has a deep soil (~90 cm), typical of rolling areas with soft marls; and the fourth is a soil whose effective depth is also large (~90 cm), representative of flat areas on soft clayey sediments, partially decalcified. One homogeneous zone of 2-ha was split into two plots within each orchard, and the treatments were randomly assigned to each plot. After five years, no significant differences were found in yield, fruit weight, oil content or vegetative growth, but polyphenols in the oil decreased in trees subjected to the traditional fertilization. Fertilization cost increased in more than 10 times in the traditional fertilization plots. Results indicate that the current fertilization practice apply more nutrients that needed for a good crop, negatively affect olive oil quality, and increase cost compared with foliar diagnosis.

Additional key words: leaf-nutrient analysis; *Olea europaea;* rational fertilization practices.

Resumen

El diagnóstico foliar como guía de fertilización del olivar

Se estableció un experimento a largo plazo en olivares andaluces, con el objetivo de comparar la fertilización basada en el diagnóstico foliar frente a la convencional en la zona. En el primer caso, sólo se aplicaron los nutrientes que presentaban un nivel en hoja por debajo del nivel crítico, mientras que en el segundo caso se aportó anualmente 500 kg ha^{-1} de 15-15-15 y tres aplicaciones foliares de micronutrientes y aminoácidos. Se eligieron cuatro olivares para el estudio. El primero presentaba una profundidad efectiva de 33 cm limitada por un horizonte petrocálcico; el segundo una profundidad de 60 cm, limitado por una roca dura; el tercero, de mayor profundidad (90 cm) y perfil uniforme y calcáreo; y el cuarto, de 90 cm de profundidad, parcialmente descarbonatado. En cada uno se seleccionó una parcela de 2 ha, que se dividió en dos para asignar, al azar, los tratamientos. Después de cinco años no se encontraron diferencias significativas en producción, en tamaño del fruto, en contenido graso ni en crecimiento vegetativo, pero disminuyeron los polifenoles en el aceite en la parcela fertilizada de acuerdo con los criterios de la zona. Por otra parte, los costes aumentaron en más de 10 veces en estas parcelas. Los resultados indican que la práctica convencional de fertilización aumenta la aplicación de nutrientes, afecta negativamente a la calidad del aceite e incrementa los costes de cultivo, mientras que la fertilización basada en el diagnóstico foliar representa un guía útil para una fertilización racional.

Palabras clave adicionales: análisis foliar; fertilización racional; *Olea europaea.*

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Abbreviations used: CV (coefficient of variation), DW (dry weight), fw (fresh weight), NMR (nuclear magnetic resonance), ppm (parts per million).

Introduction

Predicting the amount of fertilizers required annually to support optimum productivity is not simple. It should be based on judgments of tree nutritional status, crop demand, nutrient availability, and other site-specific variables. However, the actual fertilization practices in olive *(Olea europaea* L.) orchards are based mainly on tradition, repeating the same fertilization program every year, and also testimonial of the neighbors (Fernández-Escobar, 2004). This practice leads to arbitrary application of excessive rates of some fertilizers, mainly fertilizer N, and, at the same time, to a lack of other nutrients that could be necessary at this moment. Also, the excessive application of non-needed fertilizers causes environmental degradation (Giménez *et al.*, 2001), and negatively affects olive oil quality (Fernández-Escobar *et al.*, 2006) and flower quality (Fernández-Escobar *et al.*, 2008).

Under a rational point of view, a nutrient must be supplied only when there are proves that it's needed. For this purpose, leaf-nutrient analysis provides an indication of tree nutritional status and represents an important tool for determining fertilization requirements (Shear and Faust, 1980; Benton-Jones, 1985). Interpretation of the results of leaf analysis is based on the relationship between leaf nutrient concentration and growth or yield. Comparing actual leaf nutrient concentration to reference values allows the diagnosis of nutrient deficiency, sufficiency or excess. Optimum tree nutrition could be achieved combining this information with soil and environmental factors that affect tree growth, and symptoms of nutrient deficiency or excess.

Leaf analysis interpreted as indicated above has proven useful as a guide to fertilizer management of fruit crops (Beutel *et al.*, 1983), and may promote more environmentally responsible use of fertilizers in olive orchards. Despite that, recent studies indicate that leaf analysis is being underutilized in olive growing (Fernández-Escobar, 2008) since few olive growers perform leaf analysis annually.

The aim of the present work was to optimize and rationalize the current fertilization practice in olive orchards by the use of leaf analysis as a diagnostic tool for olive fertilization. The goal was to compare the fertilization practice based on foliar diagnosis versus the current fertilization practice in olive orchards.

Material and methods

A randomized block experiment with two treatments and four blocks was established in 1998 in the area of Antequera, Malaga province, southern Spain. The first treatment consisted of olive fertilization based on foliar diagnosis. A nutrient was supplied only if the annual analysis indicates that leaf nutrient concentration was below the sufficiency level according to the reference values (Fernández-Escobar *et al.*, 1999). Nitrogen fertilization consisted of soil application of urea in April at a rate of 1 kg tree^{-1}, foliar sprays of 3% KNO_3 in April, May, and September, and a foliar spray of 2.5% of urea in September. Potassium fertilization consisted of soil application of K_2SO_4 at a rate of 1 kg tree^{-1}, and two or three sprays of 3% KCl, or 3% KNO_3 when nitrogen was also necessary. No other nutrient was supplied during the experiment. The second treatment consisted of the annual application to the soil of 500 kg ha^{-1} of a NPK (15-15-15) fertilizer applied in March, and three foliar applications of micronutrients (Vitafoliar 2.5%) and aminoacids (Amindor 2.5%) in March, June and October. This is the traditional fertilization program in the area.

Each block was located in a different orchard. These orchards were selected according to the results of a previous work dealing with the study of the soils in which the olive orchards are established in the area (Parra *et al.*, 2003). Some characteristics of the sites are given in Table 1. The orchards were established on calcareous soils, on gentle slopes with slope gradient varying from 0 to 15 %. Elevation ranged from 560 to 740 m above mean sea level. The climate of the area is Mediterranean subtropical, with a mean annual precipitation ranging from 551–813 mm and a marked summer drought (~30 mm in the June-September period). The mean annual evapotranspiration ranges from 1050 to 1150 mm, and the mean annual temperature ranges from 13.5 to 14.9ºC.

The soils differed widely in aptitude for olive growing and were representative of many olive orchards on calcareous zones of the Mediterranean region. The soil in Casasola has a small effective depth (33 cm) limited by a petrocalcic horizon and is representative of the soils developed on flat old pediments around calcareous massifs. The moderately deep soil in El Pradillo (effective soil depth ~60 cm) is representative of broad level uplands on hard calcareous rocks. San Antonio has a deep soil (effective soil depth: ~90 cm), typical of rolling areas with soft marls. The soil in NoHay, whose

Table 1. Landscape and soil properties of the selected orchards

Orchard	Site characteristics					Soil classification[a]	Rooting depth (cm)	Means values for soil properties in the root zone			
	Slope gradient (%)	Elevation (m)	Mean annual rainfall (mm)	Mean annual temperature (ºC)	Soil parent material			CCE[b] (g kg^{-1})	Clay (g kg^{-1})	Olsen P (mg kg^{-1})	Available K[c] (mg kg^{-1})
Casasola	1-6	635	650	14.5	Coluvial calcareous material	Petrocalcic Palexeralf	33	403	368	12.2	158
El Pradillo	1-3	740	813	13.5	Limestone	Typic Calcixeroll	60	564	298	3.5	211
San Antonio	6-15	560	551	14.9	White marl	Typic Calcixerept	90	595	417	1.5	108
No Hay	<1	585	613	14.6	Coluvial clayey material	Calcic Rhodoxeralf	90	45	577	2.1	486

[a] According to keys to soil taxonomy (Soil Survey Staff, 2006). [b] CCE: calcium carbonate equivalent. [c] Determined in 1 M NH_4OAc pH 7 (Soil Survey Staff, 1984).

effective depth is also large (~90 cm), is representative of flat areas on soft clayey sediments; this soil, in contrast with San Antonio, is partially decalcified and has an argillic horizon. All the soils are well drained and poor in organic matter (data not shown).

Hundred-year-old 'Hojiblanca' trees growing under rainfed conditions at densities of 51 trees ha^{-1} in Casasola and El Pradillo, 29 trees ha^{-1} in NoHay, and 74 trees ha^{-1} in San Antonio, were used in the experiment. Each experimental plot occupied an area of 1-ha. Fifteen trees within each plot were tagged for future measurements.

The nutritional status of the olive trees was determined every year by leaf analysis of a sample of 300 leaves per plot collected in July. Fully expanded, mature leaves from the middle portion of nonbearing, current season shoots were removed for analysis of mineral nutrients. Leaves were collected in paper bags and stored in a portable ice chest. Once in the laboratory, leaves were washed with 0.03% Triton X-100, rinsed in deionized water, dried at 80ºC for 48 h, ground, and stored in an oven at 60ºC until analysis. Nitrogen was determined by the Kjeldahl procedure. For other element determinations, the stored samples were ashed in a muffle furnace at 600ºC for 12 h, and dissolved in 0.1 N HCl. Total P was determined by colorimetry using the method described by Murphy and Riley (1962). Boron was determined in the extract by colorimetry (Greweling, 1976). The remaining elements (K, Mg, Ca, Zn, Mn, and Cu) were measured using an atomic absorption spectrophotometer Perkin-Elmer 1.100 B.

Vegetative growth was determined at the end of the growing season by measuring shoot length on 20 random shoots per experimental tree. Also, the aboveground tree volume, a variable used as covariate to adjust yield, was calculated from height and spread measurements assimilating tree canopy to a spheroid. The effect of treatments on cropping was evaluated by measuring yield per tree at harvest. A sample of approximately 2 kg of fruits per plot was taken at harvest to determine fruit size and oil content. Fruit oil content was determined by nuclear magnetic resonance (NMR) after milling the fruit sample. Another sample of fruits per plot was taken at harvest in 2004 to determine polyphenol content, a minor component of the olive oil of great importance because of its antioxidant effects, and highly correlated to bitterness and oil stability (Gutiérrez *et al.*, 1977; Gutfinger, 1981). Oil was extracted by an Abencor analyzer after mixing the fruit paste at 30ºC during 30 minutes. Polyphenol content, expressed as ppm of caffeic acid, was determined by colorimetry (Vázquez-Roncero *et al.*, 1973).

Analyses of variance were performed on the data to compare the effects of the treatments, with the exception of yield that was analyzed by covariance using the aboveground tree volume as covariate. All percentage values were transformed using the arcsin of the square root before analysis.

Results and discussion

Annual changes in leaf nutrient concentration

Nitrogen was always above the sufficiency threshold of 1.5% in NoHay and Casasola (Fig. 1). Consequently, no N fertilization was practiced in the plots that correspond to the treatment based on foliar diagnosis during the whole period of study in these orchards. On the contrary, deficiency levels (<1.4%) were obtained in 1999 in San Antonio and El Pradillo and, consequently, fertilizer N was applied in 2000 to these orchards. As a consequence of these treatments, leaf N concentration increased in the following years in both places, reaching values above the deficiency threshold of 1.4%. N fertilization was no needed during the rest of the period of study, because in previous works we found no response of olive trees to N fertilization if leaf N concentration was above 1.4% (Sánchez-Zamora and Fernández-Escobar, 2002; García-Novelo *et al.*, 2004). However, the sudden and strong decrease in leaf N concentration observed in 1999 in both treatments of these orchards is unusual. The most plausible explanation is that a mistake occurred during leaf sampling, mixing leaves from the current and one-year growth. N concentration is lower in older than in younger leaves (Fernández-Escobar *et al.*, 1999). No differences at all were observed between treatments in NoHay, a fertile orchard that showed the highest leaf N concentrations during the period of study. In the other places, the traditional fertilization seems to increase tree N, a consequence of the annual application of this nutrient.

Potassium is the main nutritional problem of rainfed olive orchards (Fernández-Escobar, 2004). Studies carried out in Andalusia, southern Spain, reported low and deficiency levels of K in rainfed orchards established on calcareous soils (Fernández-Escobar *et al.*, 1994), even when they are established on soils with high K content (Parra *et al.*, 2003). Therefore, it is easy to understand that only in the more fertile orchard of NoHay leaf K concentration was always above the sufficiency level of 0.8% (Fig. 2). In the other orchards, K concentration fluctuates between the sufficiency and the deficiency level of 0.4%. Since K is difficult to correct in deficiency trees (Restrepo *et al.*, 2008), fertilizer K application is needed when leaf K concentration drops below 0.8%. Consequently, K was supplied in plots of foliar diagnosis treatment in 2000, 2002, and 2003 in San Antonio and El Pradillo, just the year that follows the detection of low K values, and every year in Casasola, because in

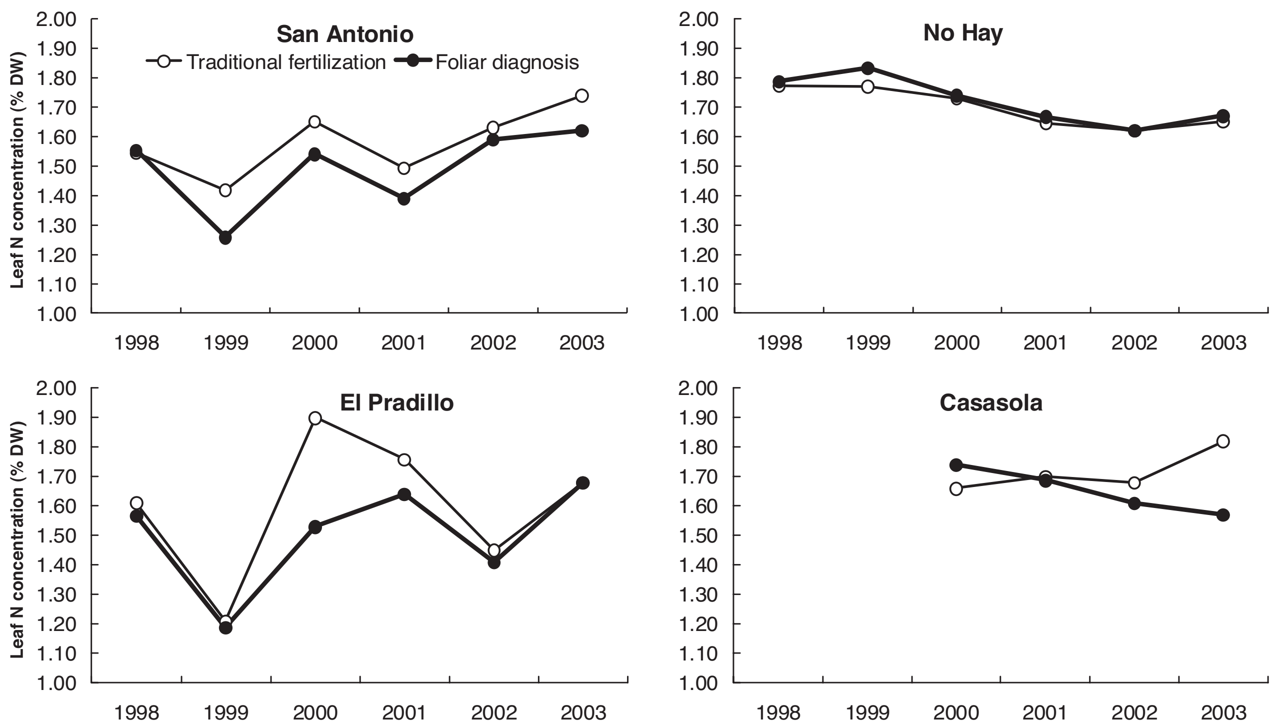

Figure 1. Annual changes of leaf N concentration in four different olive orchards. DW: dry weight.

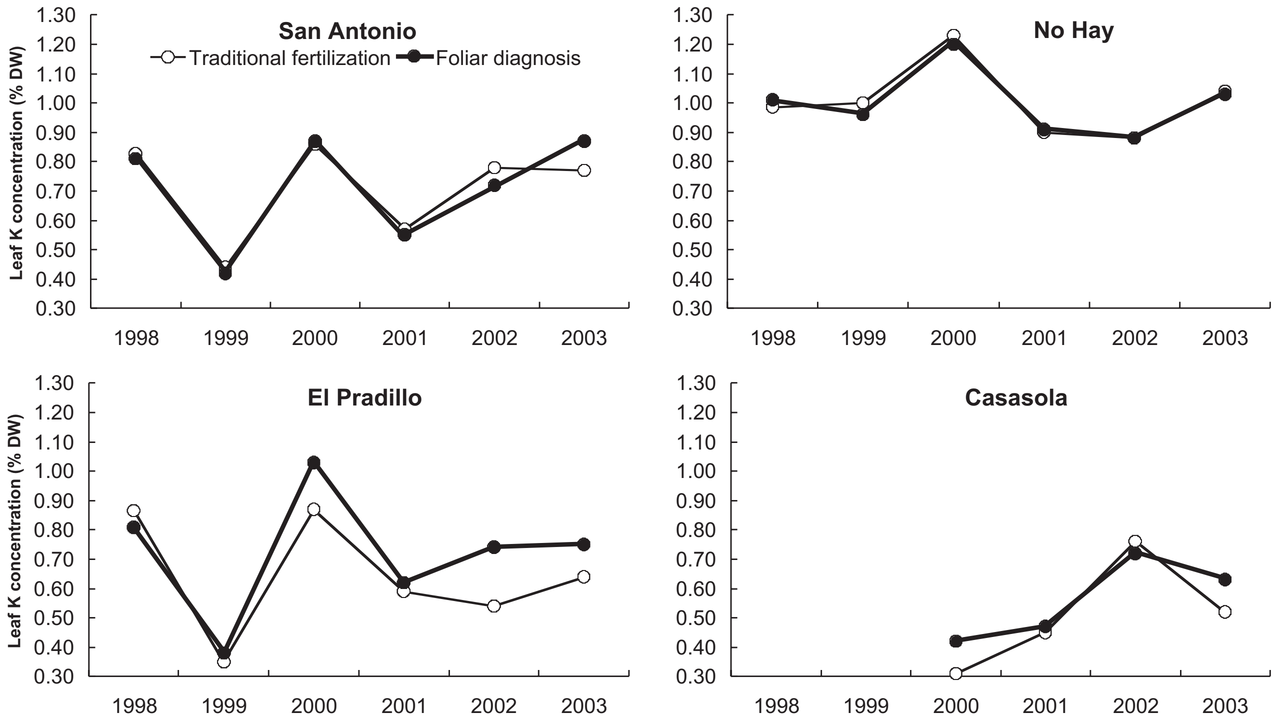

Figure 2. Annual changes of leaf K concentration in four different olive orchards.

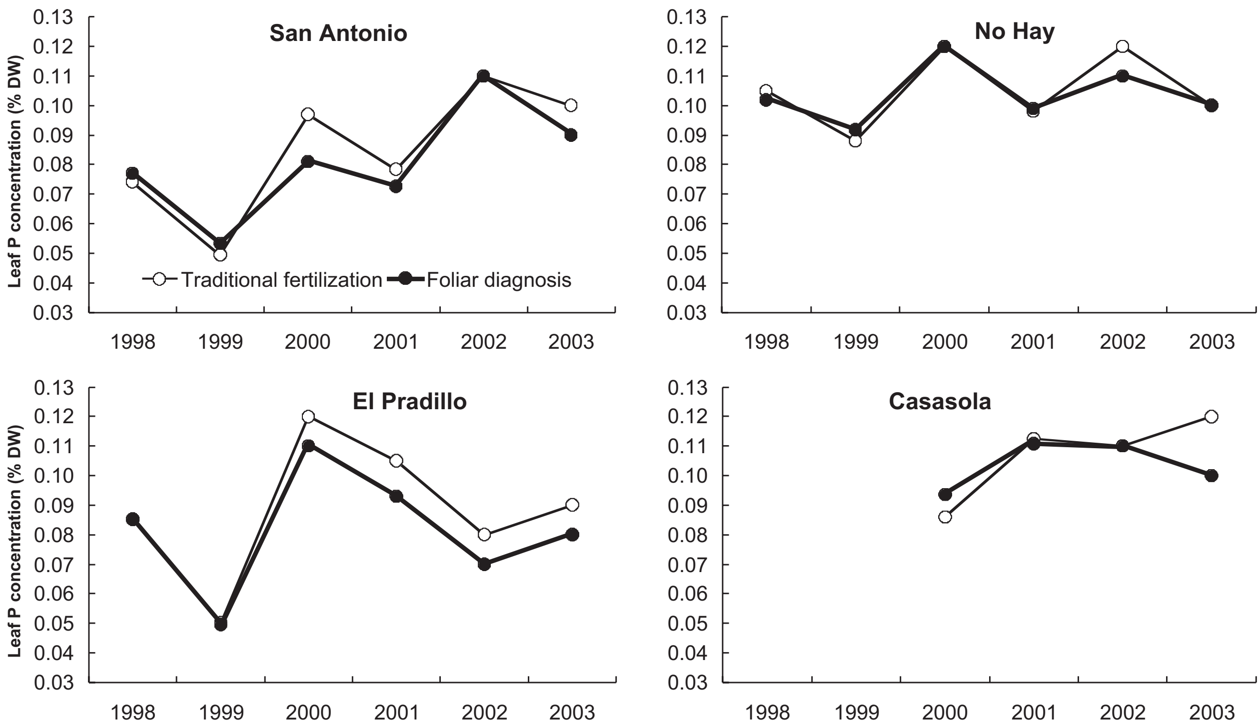

Figure 3. Annual changes of leaf P concentration in four different olive orchards.

this orchard leaf K concentration was always below the sufficiency level of 0.8%. Trees growing in San Antonio and El Pradillo only received foliar sprays of potassium. In Casasola, additional potassium was applied to the soil in 2001 and 2002. A strong decrease in leaf K concentration was also observed in 1999 in San Antonio and El Pradillo in both treatments, as occurred with N. No differences at all were found between treatments in San Antonio and NoHay, and little differences in El Pradillo and Casasola, showing lower K concentration in the traditional fertilization treatment in some years. In spite of the frequent K application in both treatments, in none of these orchards K level reached values above the sufficiency level of 0.8% at the end of the experiment, indicating the difficulties to correct K deficiencies in rainfed olive orchards, as has been mentioned before. Factors as tree nutritional status and water conditions seems to influence the uptake of K by the olive tree (Restrepo *et al.*, 2008). Anyway, K levels were maintained above the deficiency threshold of 0.4%.

Phosphorous was always above the deficiency threshold of 0.05% in all orchards (Fig. 3), reaching values above 0.07%, the normal level found in olive orchards of Andalusia (Fernández-Escobar *et al*., 1994). The exception was in 1999, where a decrease in leaf P concentration was observed in San Antonio and El Pradillo as was found with N and K. Since no deficiencies were found along the experiment, no fertilizer P was applied in plots that correspond to the treatment based on foliar diagnosis. Little differences were observed between treatments, although in some orchards higher values were obtained in the tradition fertilization treatment where fertilizer P was applied.

Leaf Ca concentration was always above the deficiency level of 0.3% in all orchards (Fig. 4) and also above the sufficiency in most of the years within each orchard. No difference seems to be appreciate between treatments. The same tendency was observed with Mg. Leaf Mg concentration was always above the sufficiency level of 0.1% (Fig. 5), with the exception of Casasola in 2000 in the traditional fertilization plot. A mistake probably occurred in leaf sampling or leaf analysis in this orchard since Mg concentration increased in 2001 without fertilizer Mg application. Taking into account the calcareous origin of the soils, the situation of both nutrients could be considerer normal.

Olive trees require high level of B (Hartmann *et al.*, 1966) mainly during flowering and fruit development (Delgado *et al.*, 1994). In this experiment, leaf B concentration was always above the critical level of 14 ppm and in many cases above the sufficiency level of 19 ppm (Fig. 6). Consequently, no B application was practiced along the experiment in plots of the treatment based on foliar diagnosis. The same behavior was observed with other micronutrients such as Mn (Fig. 7), Zn (Fig. 8) or Cu (Fig. 9). The strong decrease in Leaf Zn concentration observed in 1999 was similar, and probably due to the same fact, to that observed in N, K and P. The higher values of Cu were due to the fact that Cu is used as a fungicide in olive growing.

Table 2 summarizes the fertilizer applications in plots that correspond to the foliar diagnosis treatment. The most fertile orchard of NoHay did not receive any application during the experiment. The other orchards received K fertilization in most of the years, and N only in 2000. These results emphasize the importance of K nutrition in rainfed olives, as has been reported (Fernández-Escobar *et al.*, 1994; Restrepo *et al.*, 2008).

Olive tree response to fertilization treatments

Non-significant differences in yield were found after five years of experiment between trees subjected to a fertilization program based on foliar diagnosis and those receiving a traditional fertilization (Table 3). These results indicate that if all nutrients are above the deficiency threshold, yield is not affected by the application of fertilizers. There were differences among localities, as expected because its differences in the environment. San Antonio's trees showed a strong alternate bearing phenomenon. The olive is strongly alternate bearing (Hartmann and Opitz, 1977), but the phenomenon was particularly notable in San Antonio probably because in this orchard the mean annual rainfall is lower than in other places (Table 1), being more affected by dry winters. Water deficit during the period of flower differentiation, as occurred in 1999, increase pistil abortion in the olive (Hartmann and Panetsos, 1961) and may explain that in this year pistil abortion in San Antonio reached 100%. In 2002, lack of flower formation was due to the high crop of 2001.

In NoHay, a high variability among trees and even among scaffolds within a tree was observed during the whole period of the experiment, explaining that the average yield was similar to San Antonio. El Pradillo is the orchard that showed the highest annual rainfall, and was the most regular orchard, showing only a slight alternate bearing. Casasola was the less productive

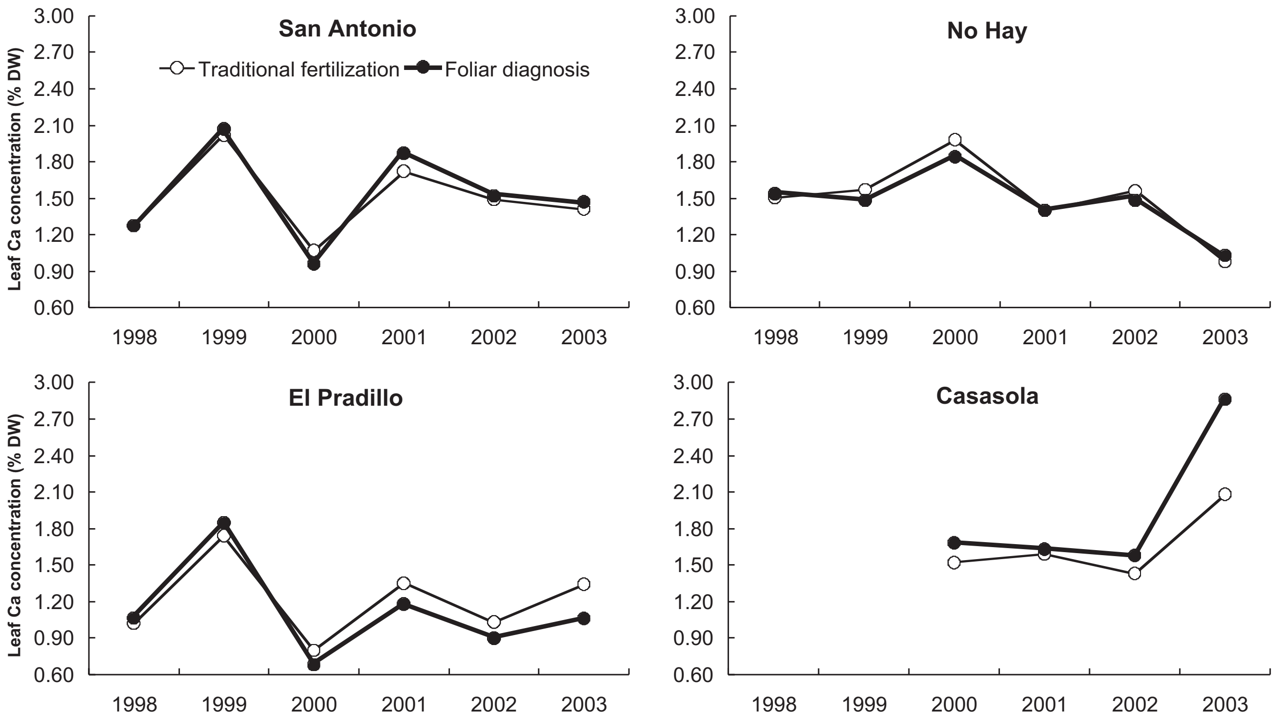

Figure 4. Annual changes of leaf Ca concentration in four different olive orchards.

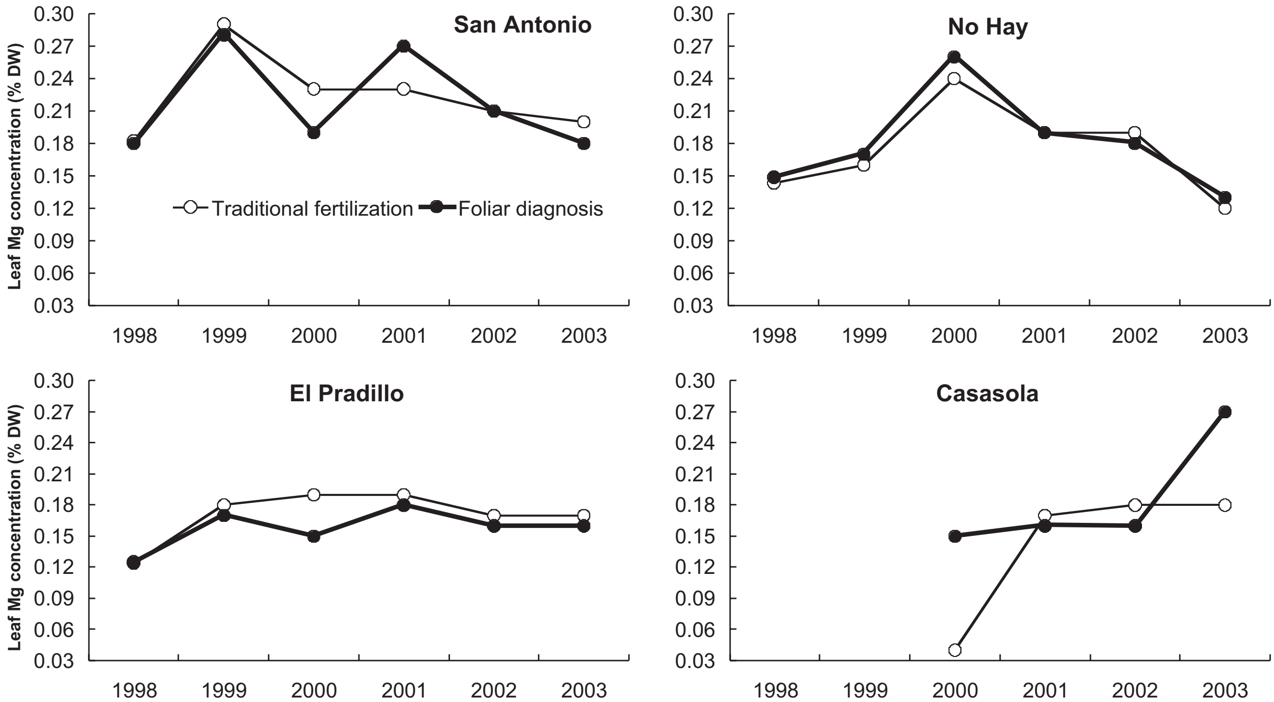

Figure 5. Annual changes of leaf Mg concentration in four different olive orchards

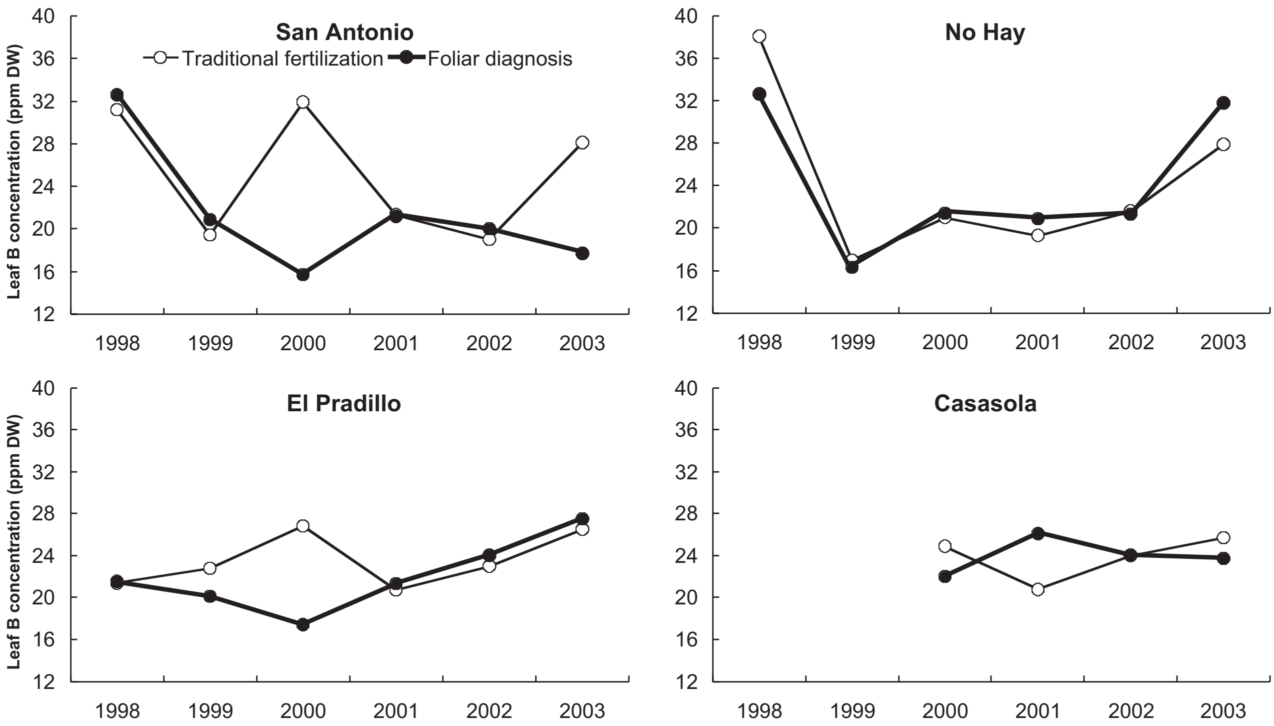

Figure 6. Annual changes of leaf B concentration in four different olive orchards.

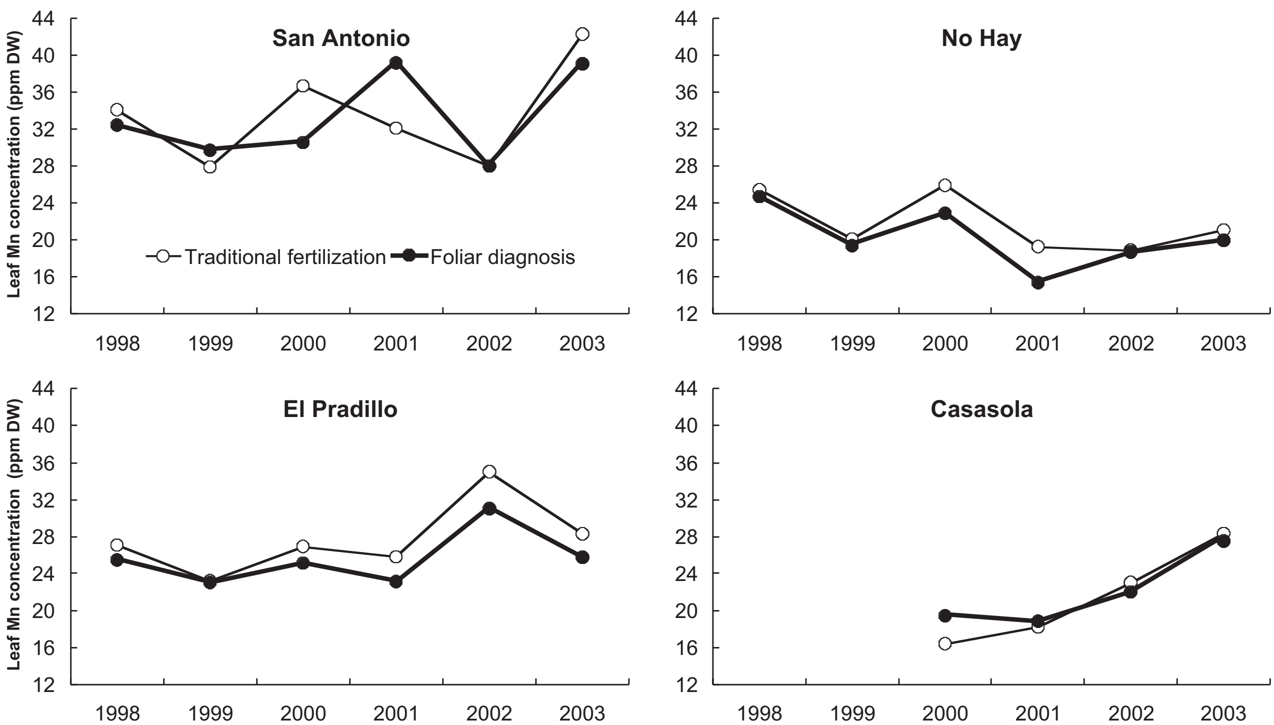

Figure 7. Annual changes of leaf Mn concentration in four different olive orchards.

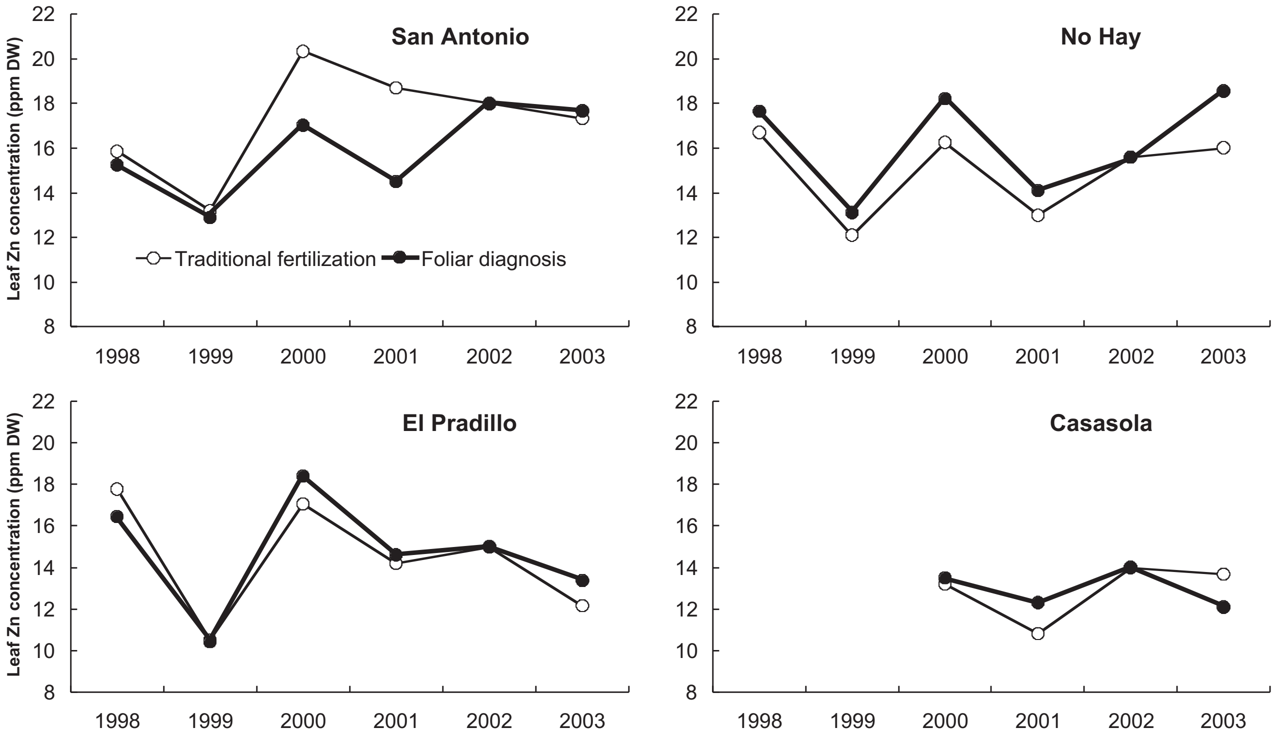

Figure 8. Annual changes of leaf Zn concentration in four different olive orchards**.**

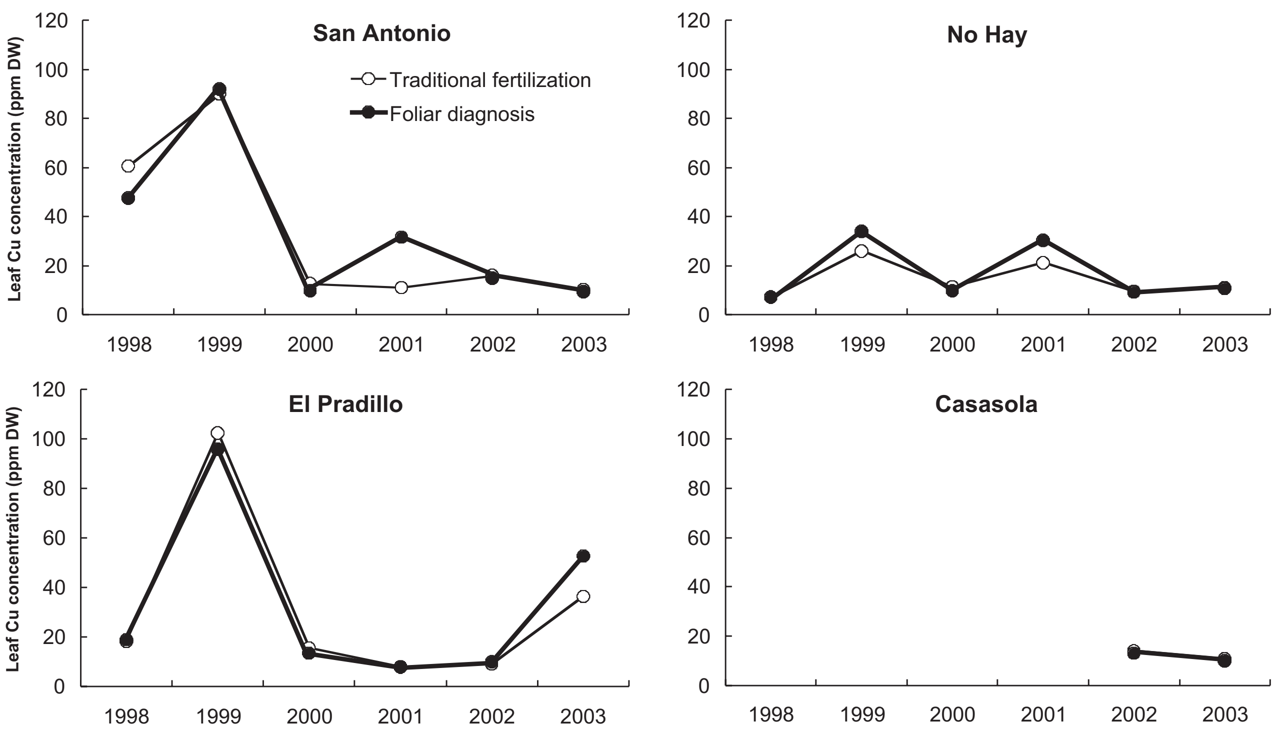

Figure 9. Annual changes of leaf Cu concentration in four different olive orchards**.**

Table 2. Nutrients applied to olive trees subjected to a fertilization based on foliar diagnosis

Locality	1999	2000	2001	2002	2003
San Antonio	None	N, K	None	K	K
NoHay	None	None	None	None	None
El Pradillo	None	N, K	None	K	K
Casasola	-	N, K	K	K	K

orchard, showing as San Antonio a strong alternate bearing phenomenon. Lack of soil depth in this orchard may explain the low productivity.

No crop was harvested in 2004. The scarce crop in all orchards was due, in part, to the high proportion of shotberries produced. Shotberries are small, undesirable, partenocarpic fruits produced by a failure in pollination (Fernández-Escobar and Gómez-Valledor, 1985) probably because of the high temperatures reached during the flowering period in this year. In El Pradillo, whose trees are cultivated under a non-tillage system, more grass growth in the traditional fertilization plot that in the foliar diagnosis one, probably because of the soil application of 15-15-15 fertilizer.

Non-significant differences were found between treatments in vegetative growth, fruit weight and oil content (Table 4). Polyphenol content, a component of great importance in oil quality because of its antioxidant effects, significantly decreased in trees subjected to the traditional fertilization at the end of the experiment. Reduction in polyphenols has been associated to an excess of N fertilization (Fernández-Escobar *et al.*, 2006), as could be occurred in the traditional fertilization plot that received annual applications of fertilizer N.

Fertilization cost

High significant differences were obtained in fertilization costs between treatments (Table 5). Excluding the application costs, the cost of fertilization varied from 129.5 € in plots subjected to the traditional fertilization to 11.0 € in plots fertilized according to

Table 3. Yield (kg ha^{-1}) of olive trees subjected to a fertilization based on foliar diagnosis versus those fertilized according to the fertilization practices in the growing area

Locality and treatment	1999	2000	2001	2002	2003	Average[a]
San Antonio						
– Foliar diagnosis	0	2,913	6,986	0	7,637	3,507
– Traditional fertilization	0	3,007	7,326	0	8,249	3,716
NoHay						
– Foliar diagnosis	1,249	6,333	1,836	4,978	-	3,599
– Traditional fertilization	882	8,584	1,150	5,293	-	3,977
El Pradillo						
– Foliar diagnosis	5,141	4,696	5,716	5,330	3,029	4,782
– Traditional fertilization	5,884	4,407	6,564	5,535	3,257	5,129
Casasola						
– Foliar diagnosis	-	-	1,558	0	2,533	1,364
– Traditional fertilization	-	-	1,216	0	2,272	1,163
Whole experiment						
– Foliar diagnosis						3,313 a
– Traditional fertilization						3,496 a

[a]Letters indicate mean separation by F test at $P \leq 0.05$. Coefficient of variation = 5.53%

Table 4. Growth and yield quality of olive trees subjected to a fertilization based on foliar diagnosis versus those fertilized according to the fertilization practices in the growing area. Mean of 1999-2003 period

Treatment[a]	Shoot growth (cm)	Fruit weight (g)	Oil content (% fw)	Polyphenol content[b] (ppm)
Foliar diagnosis	5.02 a	3.32 a	21.98 a	1119.5 a
Traditional fertilization	4.82 a	3.19 a	21.93 a	769.3 b
CV (%)[c]	7.69	3.52	1.05	11.5

[a]Mean separation by F test at P≤0.05. [b]Data from 2004. [c]Coeficient of variation

foliar diagnosis. There were, obviously, differences among localities. In NoHay, the plot based on foliar diagnosis did not receive any fertilizer because all nutrients were always above the sufficiency threshold, so the cost was zero. In Casasola, the orchard which trees were established in poor soil, the cost of fertilization was higher in the foliar diagnosis plot than in the other orchards.

In summary, after five years of experiments we can conclude that the traditional fertilization of olive orchards, based on the annual application of N, P, K fertilizers together with foliar sprays of a mixture of micronutrients and aminoacids, increased in more than 10 times the cost of fertilization without an increase of yield or vegetative growth. On the contrary, this practice negatively affects oil quality, and probably to the environment, because of the excessive use of fertilizers. When fertilization is based on foliar diagnosis, the practice was optimized and serves as a rational guide to olive fertilization since satisfy the nutritional needs of the olive trees, minimize the environmental impact, improve crop quality, and prevent the excessive and systematic use of fertilizers.

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Table 5. Fertilization cost (€ ha^{-1}) of olive trees subjected to a fertilization based on foliar diagnosis versus those fertilized according to the fertilization practices in the growing area[a]

Locality and treatment	1999	2000	2001	2002	2003	Average[a]
San Antonio						
– Foliar diagnosis	0	44.4	9.0	12.9	16.0	16.5
– Traditional fertilization	175.9	125.0	139.3	139.3	146.0	145.1
NoHay						
– Foliar diagnosis	0	0	0	0	0	0
– Traditional fertilization	172.7	179.7	76.2	131.9	138.5	139.8
El Pradillo						
– Foliar diagnosis	0	30.6	6.2	8.9	6.0	10.3
– Traditional fertilization	163.5	107.6	128.6	128.6	131.6	132.0
Casasola						
– Foliar diagnosis	-	11.6	22.9	23.8	11.0	17.3
– Traditional fertilization	-	95.4	90.0	119.1	100.5	101.2
Whole experiment						
– Foliar diagnosis						11.0 b
– Traditional fertilization						129.5 a

[a]The application cost is excluded. [b]Letters indicate mean separation at P≤0.01 by F test. Coefficient of variation = 24.4%

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