

Effect of fruit thinning on the mineral composition of loquat (*Eriobotrya japonica* Lindl.) fruit and its connection with purple spot

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Abstract

This work studies the effect of competition among developing fruits on the mineral concentration of the flesh and rind tissues of loquat fruit and its relation to the incidence of purple spot. When fruit reached 10 mm in diameter, the trees were hand-thinned to 1, 3 or 5 fruits per panicle, using non-thinned trees as control trees. In flesh tissue, K concentration significantly increased and Fe concentration significantly decreased at colour break in response to thinning. In rind tissue, N, K, Mg and Fe concentration diminished at colour break, depending on the thinning intensity, down to 23%, 21%, 27% and 41%, respectively, for one fruit per panicle treatment. Changes in the mineral composition of fruits caused by thinning significantly increased the gradient of concentration of N, K, Ca and Mg between the rind and the flesh tissue. This increase in the mineral gradient correlates positively and significantly with the percentage of purple-spotted fruit.

Additional key words: competition, fruit growth, mineral gradient, physiological disorders.

Resumen

Efecto del aclareo de frutos sobre la composición mineral del fruto del níspero japonés (*Eriobotrya japonica* Lindl.) y su relación con la mancha púrpura

En este trabajo se estudia el efecto de la competencia entre frutos de níspero en desarrollo sobre la concentración de elementos minerales en la piel y en la pulpa y su relación con la incidencia de la mancha púrpura. Cuando los frutos alcanzaron 10 mm de diámetro, los árboles se aclararon a 1, 3 ó 5 frutos por panícula, dejando árboles sin aclarar como controles. Como consecuencia del aclareo, la concentración de K y de Fe de la pulpa aumentó y disminuyó, respectivamente, alcanzando la significación estadística en el momento del cambio de color. En la piel, y en el momento del cambio de color, la concentración de N, K, Mg y Fe disminuyó con la intensidad de aclareo hasta un 23%, 21%, 27% y 41%, respectivamente, para el tratamiento de un fruto por panícula. Estos cambios en la composición mineral del fruto debidos al aclareo incrementaron significativamente el gradiente de concentraciones de N, K, Ca y Mg entre la pulpa y la piel que, a su vez, se correlacionaron positiva y significativamente con el porcentaje de frutos afectados de mancha púrpura.

Palabras clave adicionales: competencia, composición mineral, desarrollo del fruto, desórdenes fisiológicos.

Introduction

According to the FAO's statistics, world loquat fruit production is about 314,000 Mg, China (200,000 Mg)

and Spain (41,500 Mg) being the main producing countries. Most of this production is marketed for fresh consumption and consequently requires high quality. The most important physiological disorder affecting loquat fruit worldwide is purple spot, which reduces external fruit quality and decreases commercial value (Ojima *et al.*, 1976; Liu *et al.*, 1993). In Spain, 10-17% of fruit production is affected by purple spot annually (Gariglio *et al.*, 2003a).

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Symptoms appear as a cellular dehydration that initially affects the deepest rind cell layers and then extends to all rind tissues without damaging the flesh (Gariglio *et al.*, 2002). As the cuticle and its water permeability are not affected by the disorder, fruit water loss to the atmosphere cannot be the cause of purple spot (Gariglio *et al.*, 2002). On the other hand, total sugar concentration in the flesh tissue was about two times higher than that of the rind tissue throughout the fruit growth period, and the disorder appeared at fruit colour break, when the highest fruit growth rate takes place and when the percentage of purple-spotted fruit correlates significantly with fruit flesh sugar concentration (Gariglio *et al.*, 2003b). This apparent dependence of purple spot incidence on sugar availability and the histological evidence, suggest that cell dehydration of the rind tissue may be caused by an osmotic gradient between flesh and rind tissues (Gariglio *et al.*, 2000).

Thinning has proved to be an efficient technique to increase final fruit size in loquat. As for other fruit tree species, the increased availability of carbohydrates due to thinning has been thought responsible for the increase in fruit size (Agustí *et al.*, 2000). However, in loquat a positive and significant correlation has been found between total sugar concentration in the fruit flesh at colour break and purple spot incidence (Gariglio *et al.*, 2003b). Since purple spot appears as a cellular dehydration of the rind cell layers closer to the flesh, thinning has been used to modify the flesh-rind sugar gradient to study its effect on the incidence of purple spot (Gariglio *et al.*, in preparation).

Nevertheless, carbohydrates are not the only solutes in the flesh and their flesh-rind gradient can be aggravated by other compounds, such as mineral elements, the availability of which can also be increased by thinning. In this work the influence of thinning on the mineral content of flesh and rind tissues in loquat fruit and its correlation with the incidence of purple spot are studied.

Material and Methods

Experiments were carried out on 15 to 17 years old 'Algerie' loquat trees (*Eriobotrya japonica* Lindl) grafted onto seedlings, 4 × 3 m apart on a loamy clay with drip irrigation. When fruit reached 10 mm diameter (702 growth stage of the BBCH-scale;

Martínez-Calvo *et al.*, 1999), the whole trees were hand-thinned to 1, 3 or 5 fruits per panicle. Non-thinned trees (9-10 fruits per panicle) were used as a control. Orchards were located at Callosa d'En Sarriá (Alicante, Spain). A randomized complete block design with single-tree plot and six replicates was used in the experiment. Experiments were done over three consecutive years (2000-2002) with analogous results, both in the time course and values, so in this report only the results of the most representative year (2002) are presented. Different trees were used each year.

Fruits were periodically sampled for mineral analysis using ten fruits per tree located in every tree quadrant and at a height of 1.5-2.0 m. Fruits were washed and transported at low temperature to the lab where the rind was separated from the flesh with a knife scalpel. Then, both tissues were weighed and dried in an oven (60°C) for 96 h. Afterwards, the tissues were weighed again for dry matter calculation, powdered and stored at low temperature (5-7°C) until analysis. Two analyses were carried out per sample.

Total N of rind and flesh tissues was determined by a micro-Kjeldahl method (Bremner, 1965). Phosphorus was determined by means of the Fiske and Subbarow (1925) molybdenum blue colour test, and cations by flame photometry (Chapman and Pratt, 1961) using a Varian Spectra A-400 atomic absorption spectrophotometer.

Analysis of variance and regression were performed on the data, using the Newman-Keuls' multiple range test for means separation.

Results and Discussion

The concentration of mineral elements decreased during fruit development reaching the lowest value at maturity (Fig. 1). After fruit set, N was the macronutrient present in the flesh of loquat fruit at highest concentration followed by K and Ca. Phosphorus and Mg were 17.3% and 11.4% lower than N, respectively (Fig. 1a). Potassium concentration diminished more slowly than N and P. Consequently, at maturity K was the major mineral component (Fig. 1a); Ca concentration diminished parallel to N and at maturity represented 30%, approximately, of its concentration at set (Fig. 1a). Iron and Cu were the main micronutrients followed by Zn and Mn (Fig. 1b). The concentration of the latter diminished more slowly

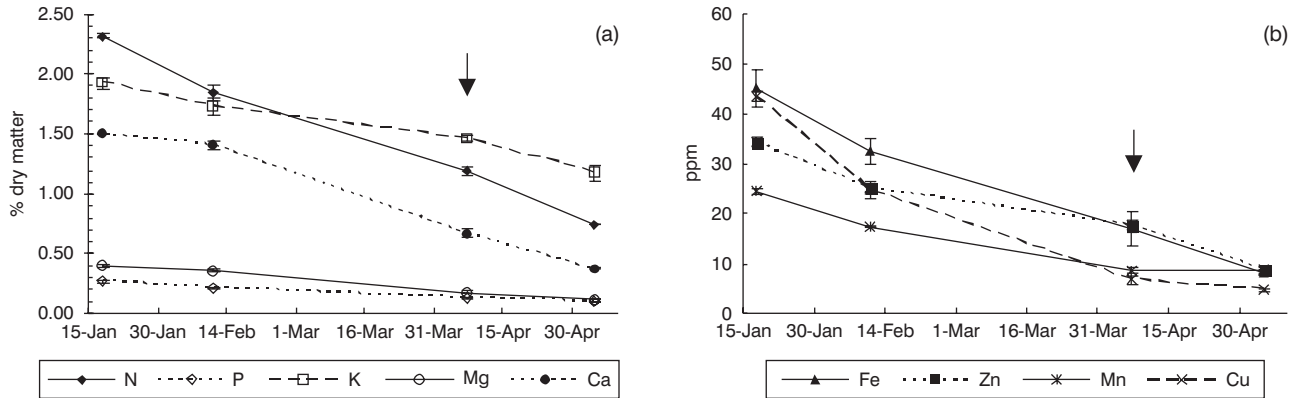


Figure 1. Time course of macro (a) and micro nutrients (b) concentration of the flesh of loquat fruit, cv. Algeria. Data are the means of six replicates per treatment. Values for 2002. Vertical bars represent standard errors. Arrows indicate the temporal onset of fruit colour break.

than that of Fe and reached similar values at maturity; Cu concentration decreased by 90% from fruit set to colour break and it had the lowest concentration at maturity (Fig. 1b).

Tuset *et al.* (1989) reported a reduction in calcium concentration in loquat fruits from 2.01% to 0.57% from February to May and they hypothesized that purple spot of loquat fruit is caused by a localized fruit calcium deficiency. However, it has also been reported that calcium concentration in the fruit is not the cause of purple spot (Cuevas *et al.*, 2001) and Gariglio *et al.* (2002) concluded that cellular dehydration appearing on loquat purple spot affected tissue was not caused by a localized, epidermal or flesh tissue calcium

deficiency (Gariglio *et al.*, 2002). This conclusion is reinforced by our results since Ca is not the only element which is diluted during fruit development. Thus, Ca concentration was reduced by 75%, whereas N, K, Fe, Zn and Cu were reduced by 65%, 40%, 80%, 73% and 90%, respectively.

Thinning significantly altered the mineral composition of loquat fruit. In flesh tissue, the potassium concentration was significantly increased and Fe concentration significantly reduced at colour break for the highest thinning intensities (three and one fruit per panicle) (Table 1). No trend was clear for Ca, although significant reductions were observed with thinning intensity. N, P, Mg, Zn, Mn and Cu

Table 1. Effect of fruit thinning on the mineral concentration of the flesh tissue of loquat ‘Algerie’ at fruit colour break. Data are the means of six replicates per treatment. Data for 2002. Values expressed as % or mg kg⁻¹ (ppm) of dry matter

	Fruits per panicle				Signif.
	One	Three	Five	Control	
N (%)	1.21	1.22	1.19	1.20	ns
P (%)	0.12	0.14	0.11	0.12	ns
K (%)	1.79c	1.69b	1.41a	1.46a	*
Mg (%)	0.18	0.19	0.16	0.17	ns
Ca (%)	0.48a	0.80c	0.98c	0.67b	*
Fe (ppm)	7.2a	12.2b	15.9c	16.8c	*
Zn (ppm)	18.1	17.6	18.8	17.7	ns
Mn (ppm)	6.8	8.4	9.1	8.6	ns
Cu (ppm)	5.9	5.3	5.9	6.5	ns

*: $P \leq 0.05$; ns: not significant.

Table 2. Effect of fruit thinning on the mineral concentration of the rind tissue of loquat ‘Algerie’ at fruit colour break. Data are the means of six replicates per treatment. Values expressed as % or mg kg⁻¹ (ppm) of dry matter

	Fruits per panicle				Signif.
	One	Three	Five	Control	
N (%)	0.69a	0.68a	1.00b	0.90b	*
P (%)	0.06	0.07	0.07	0.07	ns
K (%)	1.47a	1.55b	1.63b	1.85c	*
Mg (%)	0.08a	0.08a	0.12b	0.11b	*
Ca (%)	1.07	1.00	1.03	1.06	ns
Fe (ppm)	31.6a	45.9b	60.7c	53.2c	*
Zn (ppm)	8.5	10.9	8.6	10.6	ns
Mn (ppm)	9.2	9.4	11.3	9.7	ns
Cu (ppm)	3.0	3.1	4.6	3.4	ns

*: $P \leq 0.05$; ns: not significant.

concentration did not show significant changes due to thinning in flesh tissue.

Thinning intensity also affected the mineral composition of the rind tissue at colour break (Table 2). As the thinning intensity increased, the concentration of mineral elements diminished, particularly for K and Fe, and, to a lesser extent, for N and Mg (Table 2). Their concentration diminished by 21%, 40%, 25% and 28%, respectively, when fruit from one fruit-per panicle treatment were compared with fruit from non-thinned trees (Table 2).

The time course of mineral composition of the rind was also affected by fruit thinning intensity. Nitrogen concentration was reduced during fruit development up to fruit maturity irrespective of the thinning intensity. Values for 1 and 3 fruits per panicle treatments were significantly lower, on average, than those for 5 fruits per panicle and control treatments (Fig. 2). At colour break, differences reached 23% between the two treatment groups, but at maturity there were no significant differences among treatments. As for N, time course of P diminished up to fruit maturity, but no significant differences were observed along the process due to thinning intensity (Fig. 2). K concentration diminished by 40% and Mg by 48% (Fig. 2) one week before fruit colour break in fruit from 1 and 3 fruits per panicle treatments compared with control fruit, and 32% and 30%, respectively, compared with the 5 fruits per panicle treatment.

Ca concentration was reduced by thinning intensity from fruit colour break to maturity (Fig. 2). Fe concentration was reduced from two weeks before

colour break to maturity, 1 and 3 fruits per panicle treatments diminishing by 40%-50%, on average, in comparison with the 5 fruits per panicle or control treatments (Fig. 2). It is interesting to note that, contrary to the other macronutrients, Ca almost doubled its concentration in control fruit during the week following colour break (Fig. 2). Changes in Zn, Mn and Cu concentration due to fruit thinning were similar to those described for Ca (data not shown).

There is no adequate explanation for changes observed in the time course of K and Mg concentration of the rind during the two weeks before colour break (Fig. 2). Fruit from most intense thinning treatments grew faster and became bigger than fruit from control and 5 fruit per panicle treatments, suggesting an advanced peel extension which, in turn, advanced the progress of the concentration of mineral elements.

Thinning significantly altered fruit growth rate (Blumenfeld, 1980; Agustí *et al.*, 2000), sugar concentration and purple spot incidence in loquat fruit (Gariglio *et al.*, 2003b), and there is a strong positive correlation between the proportion of fruits affected by purple spot and total sugar concentration at colour break (Gariglio *et al.*, 2003b). Accordingly, we hypothesized that the rapid increase in sugar concentration that takes place in this fruit should be the main endogenous factor responsible for purple spot, suggesting an osmotic gradient between flesh and rind tissue as the origin of the damage (Gariglio *et al.*, 2003b). Indeed, we observed the existence of a

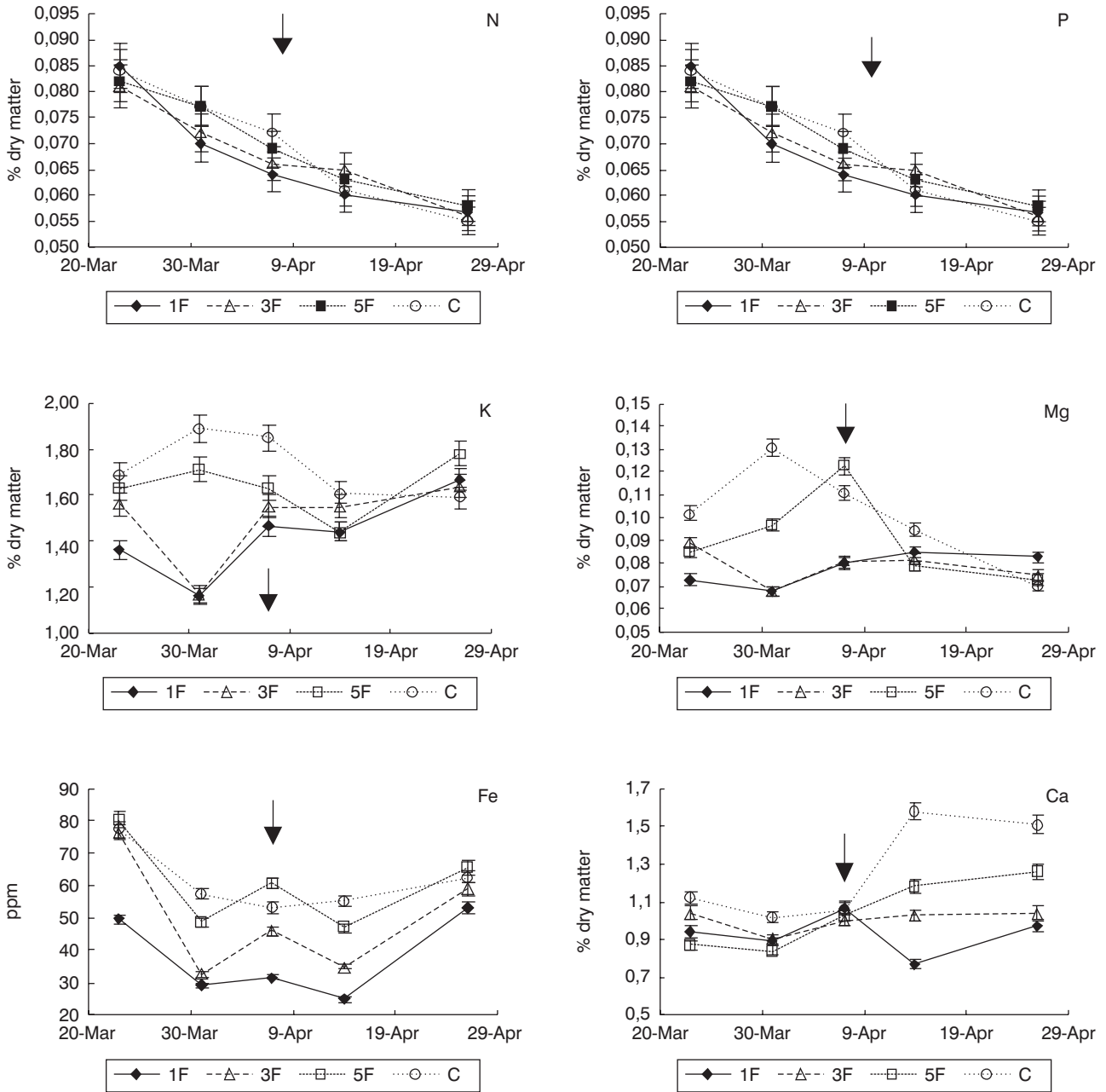


Figure 2. Effect of fruit thinning on N, P, K, Mg, Fe and Ca concentration of the rind tissue of loquat fruit 'Algerie' during fruit development. Data are the means of six replicates per treatment. Vertical bars represent standard errors. Arrows indicate the temporal onset of fruit colour break.

flesh-rind sugar gradient that increased significantly with thinning intensity (Gariglio *et al.*, in preparation). However, sugars are not the only solutes in the flesh affecting flesh-rind relationships; changes in the mineral composition of loquat fruit caused by thinning also modified the concentration gradient between the

flesh and rind tissue at fruit colour break, increasing N, K and Mg content in the flesh with respect to the rind tissue (Table 3).

Although in a fruit tissue the nutrients have a different role, most being part of larger molecules with little or no effect on osmotic potential, these

Table 3. Effect of fruit thinning on the mineral gradient (i.e. differences in concentration) between flesh and rind tissue of loquat 'Algerie' at fruit colour break. Data are the means of six replicates per treatment. Values expressed as % of dry matter

	Fruits per panicle				Signif.
	One	Three	Five	Control	
N	0.52b	0.54b	0.19a	0.30a	*
P	0.06	0.07	0.04	0.05	ns
K	0.32d	0.14c	-0.22b	-0.39a	*
Mg	0.10b	0.11b	0.04ab	0.06a	*
Ca	-0.59a	-0.20bc	-0.05c	-0.39b	*

*: $P \leq 0.05$; ns: not significant.

Table 4. Regression analysis between purple spot-affected fruit (y) and gradient of mineral concentrations between flesh and rind tissue of 'Algerie' loquat fruit at colour break. Data are the means of six replicates per treatment

Mineral gradient concentration	Regression equation ^z	r	
		Value	Signif.
N	$y = -10.42 + 59.16x$	0.65	*
P	$y = -15.00 + 500.00x$	0.42	ns
K	$y = 14.08 + 42.21x$	0.89	*
Mg	$y = -9.39 + 282.44x$	0.60	*
Ca	$y = -1.57 - 45.75x$	-0.68	*

^zy: aggregate purple spot (%); x: mineral gradient concentration between flesh and rind tissue (% of dry matter). r: regression coefficient. *: $P \leq 0.05$; ns: not significant.

gradients of mineral elements concentration caused by thinning may be partially responsible for purple spot incidence, as shown by the significant relationships between the flesh-rind gradient at fruit colour break and the proportion of purple spot affected fruit for the different fruit thinning intensities (Table 4). In these experiments non-thinned trees had 2% of purple-spotted fruit, whereas trees thinned to 5, 3 or 1 fruits per panicle had 6%, 12% and 34% of spotted fruit, respectively. The phosphorus gradient was not affected by thinning, whereas the Ca gradient slightly reduced for higher thinning intensity (Table 3), thus its flesh-rind gradient correlated negatively with purple spot (Table 4). Furthermore, Ca is the only mineral element with a higher concentration in the rind than in the flesh (Table 3) and a lower concentration in the flesh for higher thinning intensity (Table 1). Calcium supply to the fruit is limited by the capacity of its transport system (Marschner, 1989) which, in turn, explains its different accumulation pattern in

developing fruits. The K concentration presents the greatest response to thinning, both by an increased concentration in the flesh (Table 1) and a decrease in the rind tissue (Table 2); thus, its flesh-rind gradient showed the highest correlation with purple spot (Table 4).

In conclusion, these results demonstrate that changes in the mineral composition of loquat fruit caused by thinning significantly modify the gradient of N, K, Ca and Mg concentration between the flesh and rind tissues, with the K concentration showing the highest response to thinning, both by increasing its concentration in the flesh and by decreasing it in the rind tissue. These gradients correlate positively and significantly with the proportion of purple spot affected fruit. Although these correlations do not necessarily indicate the cause of the disorder, our results demonstrate that an association does exist between purple spot incidence and the mineral gradient concentration between rind and flesh tissues.

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