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Leaf proline content and its relation to fruit load and flowering in citrus under field conditions

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Abstract - Citrus species grown in temperate zones have sprouting inhibited in winter and this is retaken in spring when they also bloom. The main factor that defines the vegetative or reproductive destiny of the buds is the presence of fruits in development. Low winter temperatures slow plant metabolism, which has been reported as necessary for the expression of the reproductive program. During the winter period of the annual cycle, osmotically active compounds such as proline are increased as part of the cold tolerance response. We investigated whether the presence of developing fruit and the application of exogenous gibberellins affect the proline level in citrus leaves. In an orchard of adult 'Montenegrina' mandarin trees, leaf proline contents were measured over two rest periods for branches with or without fruits. Branches that bloomed, the ones that did not have developing fruits, had higher proline levels as well as higher proline: chlorophyll and proline: total amino acid ratios than did branches with fruits. However, the application of exogenous gibberellins, which reduced flowering, did not affect proline content during the same periods. We discuss the ways in which proline may be involved in floral induction in citrus.

Index terms: proline, flower induction, nitrogen metabolism, alternate bearing, gibberellic acid.

Conteúdo de prolina foliar e sua relação com a carga de frutos e floração em citros sob condições de campo

Resumo - Espécies de citros cultivadas em zonas temperadas têm brotação inibida no inverno, retornando na primavera quando elas também florescem. O principal fator que define o destino vegetativo ou reprodutivo das gemas é a presença de frutos em desenvolvimento. Baixas temperaturas no inverno retardam o metabolismo das plantas, que tem sido relatado como necessário para o desenvolvimento do sistema reprodutivo. Durante o ciclo anual, no período de inverno, compostos osmoticamente ativos como a prolina são aumentados como parte da resposta de tolerância ao frio. Foi investigado se a presença de frutos em desenvolvimento e a aplicação de giberelinas exógenas afetam o nível de prolina em folhas cítricas. Em um pomar de tangerina 'Montenegrina' adulta, o conteúdo de prolina foliar foi medido em dois períodos de descanso para ramos com ou sem frutos. Os ramos que floresciam, aqueles que não tinham frutos em desenvolvimento, apresentaram níveis mais elevados de prolina, bem como maiores proporções de prolina: clorofila e prolina: aminoácidos totais do que os ramos com frutos. No entanto, a aplicação de giberelinas exógenas, que reduziram a floração, não afetaram o conteúdo de prolina durante os mesmos períodos. Discutimos as maneiras pelas quais a prolina pode estar envolvida na indução floral dos citros.

Termos para indexação: proline, flower induction, nitrogen metabolism, alternate bearing, gibberellic acid.

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Introduction

Knowledge of the floral induction process is the key to understanding the reproductive cycles of woody polycarpic species. The effective management of fruit crops requires knowledge of the factors that determine the productive potential of the following annual cycle. In many fruit species, the presence of developing fruits is a main factor that negatively affects yield potential of the following year, process known as alternate bearing. In citrus, the number of developing fruits and the time they remain on the plant determine the degree of flowering inhibition in the following spring (VERREYNNE and LOVATT, 2009; MARTÍNEZ-FUENTES et al., 2010; DAVEMPORT 1990; SAMACH and SMITH, 2013). Fruit is a powerful sink during its development and limits the development of vegetative structures and roots as well as the plant's ability to accumulate reserves. When there are no fruits in development, photosynthesis in the canopy is reduced. However, environmental factors may limit the increase in the net assimilation rate in response to the demand of the developing fruit (SYRVERTSEN et al., 2003, RIBEIRO et al., 2009; NEUBAUER et al., 2011).

Gibberellin is a well-known endogenous factor to be strongly and negatively correlated with flowering levels in citrus (GOREN and GOLDSCHMIDT, 1970; GRAVINA, 2007; IGLESIAS et al., 2007; KOSHITA et al., 1999). Several studies show that the foliar application of gibberellic acid during the autumn-winter rest period reduces flowering intensity in citrus (GUARDIOLA et al., 1982; MUÑOZ-FOMBUENA et al., 2012). Genetic studies also support the negative effects of developing fruit (SHLIZERMAN et al., 2012) and exogenous gibberellins (GOLDBERG-MOELLER et al., 2013; MUÑOZ-FAMBUENA et al., 2012) on Cs FT2 gene expression. Based on these findings, various approaches to remove fruit from the branches have been investigated to evaluate their effects on floral induction.

Regarding to environmental factors, low temperature and water stress each induce flowering in citrus by increasing CiFT expression (NISHIKAWA et al., 2007; CHICA and ALBRIGO, 2013). Furthermore, the process of acclimatization (developing hardiness) in citrus, which begins in the winter in response to low temperature, is accompanied by increases in proline (Pro) levels (KUSHAD and YELENOSKY, 1987). Increases of this amino acid have also been observed as a response to water stress (GARCIA SANCHEZ et al., 2007; RABE et al., 1990; ZAHER-ARA et al., 2016). Many studies have investigated the role of proline as an osmolite in the plant response to stress (SZABADOS and SAVOURE 2009; SIGNORELLI, 2016). An increase in Pro level protects the plant from stress but can also occur as part of a sequence of signals to modify plant development (MAGGIO et al., 2002; KAVI KISHOR et al., 2015). Pro has also been reported to influence flowering transition in other species (MATTIOLI et al, 2009; SAMACH and SMITH 2013; ZDUNEK-ZASTOCKA et al., 2017)

Nitrogen metabolism is involved in the regulation of the annual cycle of woody species (MILLARD and GRELET, 2010). In citrus, the levels of arginine and aliphatic polyamines are related to flowering (ALI and LOVATT, 1995; LOVATT et al., 1988; SAGEE and LOVATT 1991; MARTINEZ-FUENTES et al., 2010 SYVERTSEN and SMITH, 1983). It has also been postulated that the depletion of carbon skeletons can lead to the accumulation of low-molecular weight nitrogen compounds, involving the carbohydrate budget as an incident factor in citrus flower induction (RABE, 1990). Current approaches have shown that the remobilization of C and N reserves are regulated processes of the general proteins turnover in which the proline constitutes a signal molecule (ZDUNEK-ZASTOCKA et al., 2017) and the concept that stress conditions for plants constitute inductive conditions of flowering is accepted (TROVATO et al., 2008; TAKENO 2016; SINGH et al., 2017). The process of signal transduction in which proline participates in reproductive development and its cross talk with stress is unknown. No reports have been found in citrus.

There is substantial research on the hindering effects of developing fruit and gibberellic acid application on flowering induction in citrus. However, research that links these factors with nitrogen metabolism regarding to citrus flowering is scarce. In our study, we select a mandarin with great sensitivity to alternate bearing and late harvest, which implies a strong fruit hegemony. 'Montenegrina' mandarin is a mutation of Mediterranean or willowleaf mandarin (*Citrus deliciosa* Tenore), (SCHÄFER et al., 2001).

The aim of this study was to determine whether leaf Pro content during the winter rest period is related to spring bloom intensity by comparing bearing and non-bearing branches with and without foliar gibberellin application.

Materials and methods

The study was conducted in a commercial orchard of seven-year-old 'Montenegrina' mandarin trees (*C. deliciosa* Tenore) grafted on 'Carrizo' citrange rootstocks (*Poncirus trifoliata* L. Raf. x *C. sinensis* L. Osb). The orchard was located in Kiyú (34°39'S, 56°48' W), San José, Uruguay, and the trees were cultivated under drip irrigation. In Year 1, a complete block design was used with 3 replications where each tree was considered as a block. The experimental plot consisted of two similar individual branches: one used to collect leaf samples, and one used to measure bloom intensity. Branches with a diameter of 2 cm in the base were selected, of medium vigor and without biotic or abiotic damage, which had sufficient annual shoots to account for at least 100 total nodes. Two treatments were evaluated: 1) branches with fruits, and 2) branches without fruits. Given the significant results of the presence of fruits on proline leaf levels in the first year, in the second year, the known negative effect of gibberellic acid winter sprays on flowering was included as a new factor. The effect of gibberellic acid depends on time application, so four different application dates were included as four new treatments. For these reasons, in Year II, a balanced incomplete block design was used. Each tree was an incomplete block, to which two of the six treatments were applied. The experimental plot was the same as in Year I. Each treatment had 3 replications, resulting in a total of 45 trees. The six treatments were: 1) branches with fruits, 2) branches without fruits, 3) branches without fruits + Gibberellic acid (GA_3), on May 23rd, 4) branches without fruits + GA_3 on June 6th, 5) branches without fruits + GA_3 on June 20th, and 6) branches without fruits + GA_3 on July 11th. The absence of fruit was achieved by manual thinning prior to the end of the physiological fruit drop period), stage 74 BBCH Scale (AGUSTI et al., 1997). The fruit-bearing branches had between three and six fruits per 100 nodes. GA_3 application was at 100 $mg.L^{-1}$ on each date, and the GA_3 solution was acidified to pH 4-4.5 with phosphoric acid according to GREENBERG and GOLDSCHMIDT (1989). The GA_3 solution was sprayed locally on the branch, using a manual sprayer with a non-ionic wetting agent at a rate of 2.5% v/v.

Flowering evaluation

Branches including the last three flushes of growth were tagged to measure bloom. On these branches, we counted the total number of flowers and the total number of nodes, in order to eliminate the effect of the different number of nodes between branches and work with the variable flowers per 100 nodes (MARTINEZ-FUENTES et al., 2010). The intensity of flowering was measured in full bloom, stage 65 of BBCH scale, (AGUSTI et al., 1997). Full bloom dates were October 15th in the Year I and October 20th in the Year II. The flowering lasted 20 days in both years

Climatic data

The climate data were obtained from the INIA Las Brujas Experimental Station. http://www.inia.uy/gras/Clima/Banco-datos-agroclimatico

Proline, Amino Acid and Chlorophyll Analyses

Proline, total amino acid (AA) and total chlorophyll (Chlo) contents were measured in tissue discs collected from adult leaves with a fresh weight (FW) of at least 0.5 g, and the contents were expressed as $\mu mol.g^{-1}$ FW. Three repetitions per treatment per date were taken. Following their collection, the leaf discs were immediately frozen in liquid nitrogen in the field and were maintained at -80°C until processing. Sampling was conducted during the winter rest period (April to July) every two weeks for two consecutive years ('off' and 'on' years). For the GA_3 application treatments, Pro content was measured only fourteen days after application. Borsani et al. (1999) describe the extraction and quantification methods for Pro and AA. Chlo was measured according to the Wellburn (1994) protocol. The three compounds were expressed in fresh base, and the Pro/Chlo and Pro/AA ratios were calculated.

Statistical Analysis

Linear mixed models were used for all analyses. In Year I, the complete blocks were treated as fixed effects. For the two treatments considered, time-course of the quantified leaf metabolites was evaluated with a repeated measures analysis to analyze the main effects (treatment and date) and their interaction.

In the second year, although a partially balanced design that included 6 treatments was used, only treatments one and two were evaluated in all dates during the winter rest period, so the data was analyzed in two ways. First, only treatments 1 and 2 were considered, therefore only the three trees that had both treatments were included (i.e. each treatment was repeated three times), and data for this two treatments was analyzed as if it was a randomized complete block design. For these two treatments, a repeated measures analysis was done, as in the first year. In a second approach, treatments were compared within each date, since in each sampling date only treatments 1 and 2 and the corresponding treatment with GA_3 that had been applied 14 days before were evaluated. In this case, the incomplete blocks were treated as random effects, nested in each complete replication. Mean comparisons of flowering intensity for both years and of metabolite contents of leaves for 'on' years were performed with Tukey tests ($P<0.05$). All of the analyses were performed with SAS [Version 9.2] (SAS Institute, 2005).

Results and discussion

The results confirmed the inhibitory effects of fruit presence in both years, and the application of gibberellic acid (GA_3) during winter rest period in Year II, on subsequent spring flowering (GARCÍA-LUIS et al., 1986; ZHANG et al., 2011) (Table 1 and 2). The magnitude of flowering inhibition by fruit was similar to that reported in studies of other mandarin cultivars (MUÑOZ-FAMBUENA et al., 2011; VERREYNNE and LOVATT 2009, (Table 1). As expected (GOLDBERG-MOELLER et al., 2013) GA_3 application during the winter significantly reduced spring flowering except at the first application date (Table 2). Thus, the present results validate the field experimental strategy of this study.

The proline level in leaves of 'Montenegrina' mandarin during the winter varied between minimum and maximums values of approximately 17 and 45 µmol.g^{-1} FW in Year I and between 30 and 65 µmol.g^{-1} FW in Year II (Figure 1 and Table 3). These values are similar to those reported for various tissues from citrus species (MADEMBA-SY et al., 2003; MORENO and GARCÍA MARTÍNEZ, 1983; KATO, 1986; SHRAMA et al., 2011). Differences between years can be expected considering that the present study was a field study and that many environmental factors determine plant ability to produce this amino acid (KAVI KISHOR, et al., 2015). The highest proline levels measured in Year II can be associated with a greater accumulation of Chilling Hours during the winter compared to Year I (Figure 4) as the acclimation response reported by KUSHAD and YELENOSKY (1987).

The analysis of repeated measures of Pro content and their relations with Chlo and totals AA measured on the leaf based on data from treatment 1 and 2, showed no significant interaction of treatment and sampling date (p-value>0.05). Only treatment main effect was significant on average Pro, Pro/Chlo and Pro/AA levels (p-value <0.05).

Pro levels were higher in leaves sampled from branches without developing fruits than in those from fruit-bearing branches in both studied years. Branches with fruits had 30 and 39% less Pro than did the branches without fruits in Year I and II, respectively (Table 1). Table 3 shows comparisons of proline averages by date of sampling, in the 6 treatments of Year II. Exogenous GA$_3$ did not modify proline levels measured 14 days after its application in any of the dates despite having modified flowering intensity. Leaf proline contents of the branches without fruit treated with GA$_3$ were equal to the leaves of the branches without fruit and without hormone spray. No reports were found that correlate the proline synthesis with the gibberellin signalling that allow us to explain the results obtained.

Chlo content was not affected by treatments and sampling date, which indicated stability in the photosynthetic structures. Total AA content was significantly affected by sampling date but not by fruit presence with p values of 0.0001 and 0.5003 respectively.

Pro/AA and Pro:Chlo ratios were significantly affected by developing fruit, being higher in leaves from shoots without fruits (Table 1 and Figure 2 and 3). The findings that a high Pro:AA ratio was maintained during the study period and that no effect of treatment on AA content was observed suggest that the detected Pro did not come from proteolysis. *De novo* synthesis of Pro has been reported in response to drought (BORSANI et al., 1999; DÍAZ et al., 2005). If the observed Pro was the result of proteolysis, then the amino acid contents should have also increased. However, studies of the enzymatic activity are necessary to elucidate to what extent the proline contents in tissues and plants are regulated by biosynthesis, degradation and intra as well as intercellular transport processes (LEHMANN et al., 2010). The proportion of Pro with respect to the total amino acids was higher than 50% in all cases, which is higher than the values reported by MADEMBA-SY et al. (2003).

Analyzing the time course of the variables studied from date to date, we can observe that the differences between treatments disappeared at the end of the study period for Pro (Figure 1) and Pro:AA ratio in Year I (Figure 3) and in both years for Pro:Chlo (Figure 2). However, the period between May 4th and July 6th for Year I and the period from May 25th to July 11th in Year II, the variables were always higher in the leaves of the fruitless branches. Within that period, there was a tendency to reduce values with average reductions between 22% and 42% for Pro, 11% and 54 % for Pro:Chlo and 13 and 36% for Pro:AA as difference between maximum and minimum for both treatments. At the end of winter and specifically in mid-July in our conditions, the increase in temperatures determine the release of the ecodormancy. Then, the differentiation process of the meristems in new floral and / or vegetative structures, and changes in the C and N metabolism begins. Difference between thermal regimes and annual radiation explain the variations in the beginning of the sprouting and flowering (MICHELOUD et al., 2018).

In summary, we conclude that the level of flowering in the spring is positively correlated with leaf Pro content in the previous winter. Higher levels of Pro in the leaves during the winter were found for branches that did not bear fruit and had higher flowering levels in the following spring than for fruit-bearing branches. These results demonstrate that Pro is involved in floral induction in citrus, as has been reported in other species (MATTIOLI et al, 2009; SAMACH and SMITH 2013; ZHANG et al., 2015; SINGH et al., 2017). If Pro is part of the chain of signals that allow floral expression in citrus, we can conclude that its effect in this study occurred prior to the negative effect of exogenous gibberellins or independently, which inhibited flowering but did not modify Pro level.

A question that arises from these conclusions is through which mechanism the absence of developing fruits in branches increases the synthesis of Pro. Possible ways to approach this question include studying the processes of energy dissipation in photosynthesis, the loading and unloading of phloem, or in contemporary sinks and Pro metabolism. DIAZ et al. (2005) reported increases in Pro synthesis when there was an excess of reducing power that was not used in the carboxyl phase of the photosynthesis. The absence of developing fruits determines increases in the contents of starch in the leaves (MARTINEZ-FUENTES et al., 2010), a process that negatively regulates the net assimilation of carbon. In these conditions, excesses of reducing power and energy of the photochemical phase of photosynthesis could induce a change in the proline synthesis (DIAZ et al., 2005).

The relationship C / N has been reported as a factor linked to the expression of reproductive programs in annual species (CORBESIER et al., 2002), however, the way in which both Carbon and Nitrogen are quantified and their fractions differ and have not been considered. In citrus, it has been proposed (LOVATT and MARTINEZ) that high levels of N as nitrate or ammonium in the leaves, are associated with a high flowering as well as levels of polyamines. RABE (1990) postulates the absence of carbon skeletons as a condition for the increase of nitrogenous compounds of low molecular weight since the floral induction in citrus responds to stress, water or low temperatures. In our results, it was verified that the presence of fruit was imposed on the inductor effect of cold winter (stress), very similar between the years of the study. Therefore, the absence of sink and the inhibition of photosynthesis could be another condition of stress, not so the insufficiency of carbon skeletons. Whatever the mechanism by which the fruit inhibits floral induction involving proline, our results add to the scant evidence that N metabolism is altered during the floral induction period. Proline was reported as a signal compound in reproductive processes and its synthesis is increased under stress conditions. Therefore, it is feasible that it constitutes a link between floral inductions at the time citrus perceive low temperatures, being also regulated by the presence of fruits in development. Further studies with this approach should be carried out considering genetic evidence of the environmental regulation of the annual cycle of woody polycarpic species, (TAN AND SWAIN, 2006, GYLLENSTRAND et al., 2007, NISHIKAWA et al., 2007). The opposition between vegetative and reproductive development is the result of the plant's ability to adapt to the environment (HANKE et al., 2007). Under this optics, we could possibly think about a change in the partition of assimilated and / or N, due to the presence of fruits in the branches, as a regulating process of floral expression.

Although more evidence is needed to clarify the role of Pro in citrus floral induction, this study provides evidence that fruit effects on the flowering induction process involve changes in leaf Pro content during the winter rest period.

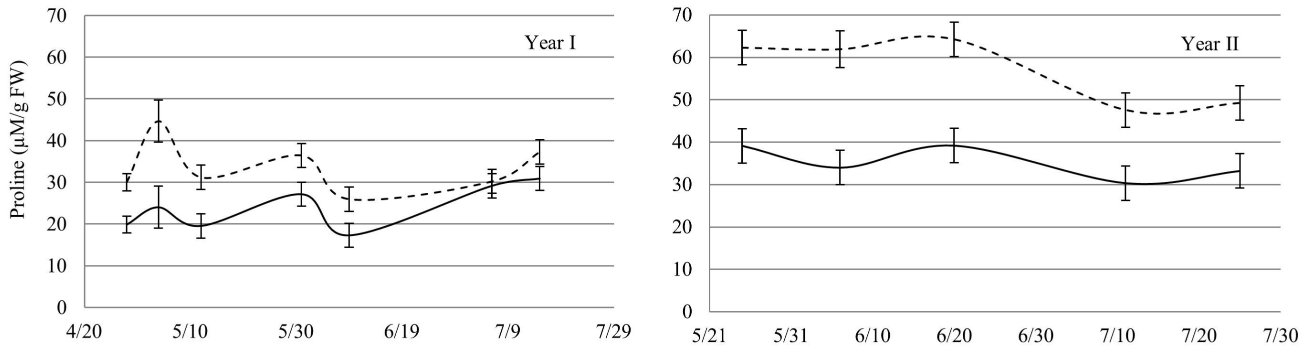

Figure 1. Time course of leaf Pro contents from May to July in Year I and Year II (Without fruit: dotted line, with fruit: continuous line).

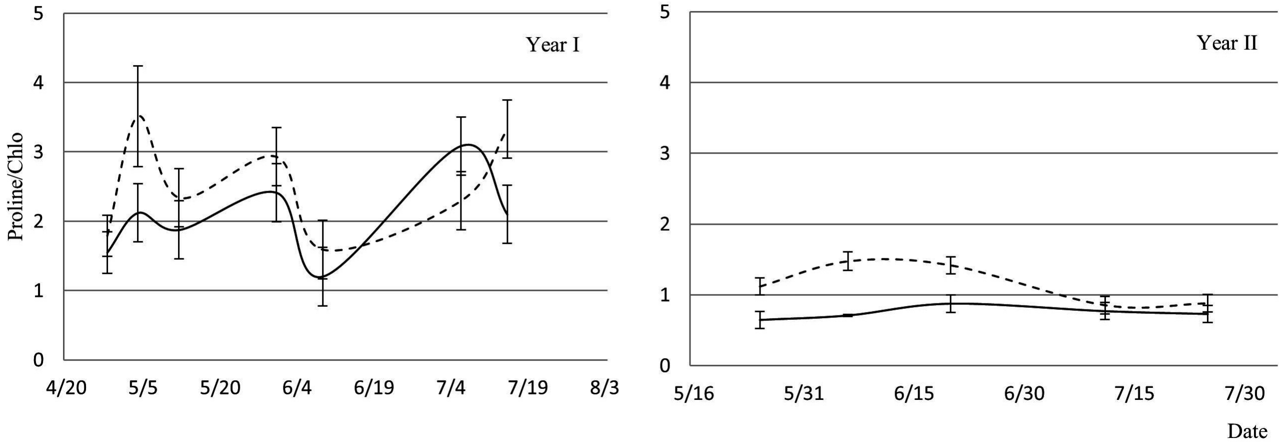

Figure 2. Time course of leaf Pro:Chlo contents from May to July in Year I and Year II. (Without fruit: dotted line, with fruit: continuous line).

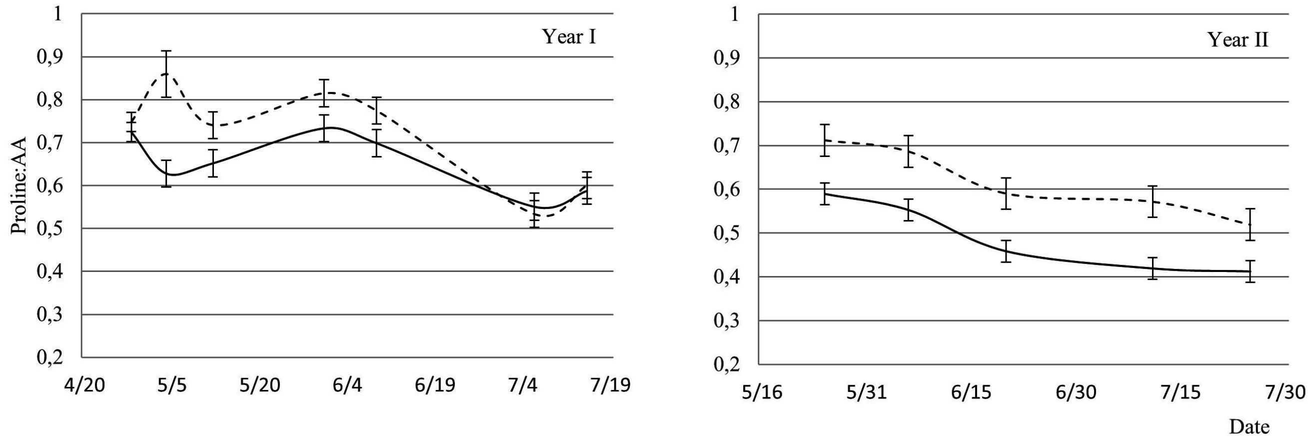

Figure 3. Time course of leaf Pro:AA contents from May to July in Year I and Year II. (Without fruit: dotted line, with fruit: continuous line).

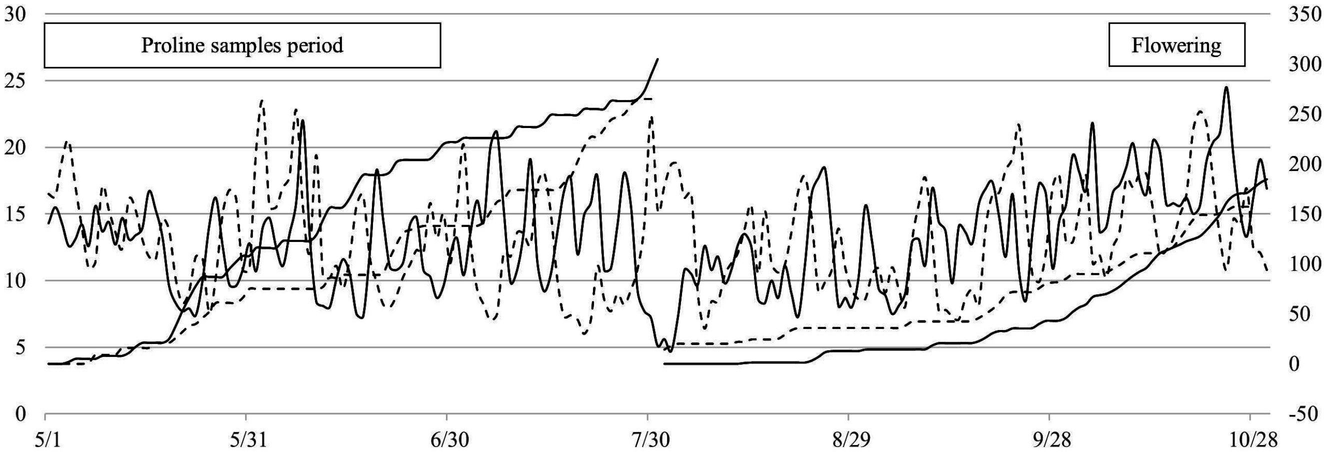

Figure 4. Primary axis: Time course of Average Temperature between May and October. Secondary axis: Accumulated Chilling Hours (∑ < 7,2°C) between May and July and Accumulated Degrees Days (Base Temperature 12.8 ° C) between August and October. Year I (dotted line) and Year II (Continuous line).

Table 1. Mean and standard error of proline leaf content ($\mu m.g^{-1}$ FW), proline:chlorophyll ratio (Pro:Chlo), proline:amino acid ratio (Pro:AA) and flowers per 100 nodes in the following spring for branches with fruits (+ fruit) and branches without fruits (- fruit). Values for two years of experiment.

Treatment	Leaf content			Flowers:100 nodes
	Proline ($\mu m.g^{-1}$FW)	Pro:Chlo	Pro:AA	
Year I				
Branches + fruit	23.99 ± 1.06 a	2.05 ± 1.53 a	0.65 ± 0.01 a	0.17 ± 2.47 a
Branches - fruit	33.68 ± 1.21 b	2.54 ± 1.75 b	0.73 ± 0.01 b	14.61 ± 2.66 b
Year II				
Branches + fruit	35.18 ± 1.81 a	0.75 ± 0.06 a	0.49 ± 0.01 a	0.02 ± 2.07 a
Branches - fruit	57.07 ± 1.84 b	1.15 ± 0.06 b	0.62 ± 0.01 b	16.72 ± 2.24 b

*Different letters indicate in the same column indicate significant differences (P<0.05)

Table 2. Mean number of flowers per 100 nodes for each date of gibberellic acid (GA_3) application in Year II.

Treatment	Flowers/100 nodes
Branches + fruit (T1)	0.00 a
Branches - fruit (T2)	16.30 c
Branches - fruit + GA3 23 May (T3)	12.40 bc
Branches - fruit + GA3 6 June (T4)	10.20 b
Branches - fruit + GA3 20 June (T5)	9.10 b
Branches - fruit + GA3 11 July (T6)	9.40 b

*Different letters in the same column indicate significant differences (P<0.05)

Table 3. Mean proline leaf content ($\mu m.g^{-1}$ FW) during winter rest period in Year II. For each sampling date, treatments evaluated were T1, T2 and the GA_3 treatment in which GA_3 spray was performed 14 days before each sampling date.

Treatment	Sampling date				
	May 23rd	June 06th	June 20th	July 11th	July 25th
Branches + fruit (T1)	34.0 b[1]	39.1 b	39.5 b	28.5 b	33.2 a
Branches - fruit (T2)	62.3 a	60.4 a	64.5 a	46.9 a	49.3 a
Branches - fruit + GA_3 May 23rd (T3)		63.3 a			
Branches - fruit + GA_3 June 6th (T4)			59.9 a		
Branches - fruit + GA_3 June 20th (T5)				40.7 a	
Branches - fruit + GA_3 July 11th (T6)					46.1 a

[1] Different letters in the same column indicate significant differences (P<0.05)

Conclusions

Although more evidence is needed to clarify the role of Pro in citrus floral induction, this study provides evidence that fruit effects on the flowering process involve changes in leaf Pro content during the winter rest period. This relationship is independent of the inhibitory effect of exogenous gibberellins on citrus flowering.

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References

AGUSTI, M.; ZARAGOZA, S.; BLEIHOLDER, H.; BUHR, L.; HACK, H.; KLOSE, R.; STAUSS, R. Adaptation of the BBCH scale for the description of Citrus fruits' phenological stages. **Fruits**, Paris, v.52, p.287–295, 1997.

ALI, A.G.; LOVATT, C.J. Relationship of polyamines to low-temperature stress-induced flowering of the 'Washington'navel orange (Citrus sinensis L. Osbeck). **Journal of Horticultural Science**, London, v.70, n.3, p.491-498, 1995.

BORSANI O.; DÍAZ, P.; MONZA, J. Proline is involved in water stress responses of Lotus corniculatus nitrogen fixing and nitrate fed plants. **Journal of Plant Physiology**, Stuttgart, v.155, p.269–273, 1999.

CHICA, E.J.; ALBRIGO, L.G. Changes in CsFT transcript abundance at the onset of low-temperature floral induction in sweet orange. **Journal of the American Society Horticultural Science**, Alexandria, v.138, p.184-189, 2013.

CORBESIER, L.; BERNIER, G.; PEERILLEUX C,. C : N Ratio increases in the phloem sap during floral transition of the long-day plants sinapis alba and *Arabidopsis thaliana*. **Plant and Cell Physiology**, Kyoto, v.43, n.6, p.684–688, 2002.

DAVENPORT, T.L. Citrus flowering. **Horticultural Reviews**, New York, v.12, p.349-408, 1990.

DÍAZ, P.; BORSANI, O.; MÁRQUEZ, A.; MONZA J. Osmotically induced proline accumulation in Lotus corniculatus leaves is affected by light and nitrogen source? **Journal of Plant Growth Regulation**, New York, v.46, p.223–232, 2005.

GARCIA-LUIS, A.; ALMELA, V.; MONERRI, C.; AGUSTÍ, M.; GUARDIOLA, J.L. Inhibition of flowering in vivo by existing fruits and applied growth regulators in Citrus unshiu. **Physiologia Plantarum**, Oxford, v.66, n.3, p.515-520, 1986.

GARCÍA-SÁNCHEZ, F.; SYVERTSEN, J.; GIMENO, V., BOTÍA, P.; PEREZ-PEREZ, J.G. Responses to flooding and drought stress by two citrus rootstock seedlings with different water use efficiency. **Physiologia Plantarum**, Oxford, v.130, n.4, p.532-542, 2007.

GOLDBERG-MOELLER, R.; SHALOM, L.; SHLIZERMAN, L.; SAMUELS, S.; ZUR, N.; OPHIR, R.; SADKA, A. Effects of gibberellin treatment during flowering induction period on global gene expression and the transcription of flowering-control genes in Citrus buds. **Plant Science**, Amsterdam, v.198, p.46-57, 2013.

GOLDSCHMIDT, E.E.; MONSELISE, S. P. Physiological assumptions towards the development of a Citrus fruiting model. **Proceedings of the International Society of Citriculture**, Lake Alfred, v.2, p.668-672, 1977.

GOREN, R.; GOLDSCHMIDT, E.E. Regulative systems in the developing citrus fruit i. the hormonal balance in orange fruit tissues. **Physiologia Plantarum**, Copenhagem, v.23, n.5, p.937-947, 1970.

GRAVINA, A. Aplicación del ácido giberelico en Citrus: revisión de resultados experimentales en Uruguay. **Agrociencia**, Montevideo, v.11, n.1, p.57-66, 2007.

GREENBERG, J.; GOLDSCHMIDT, E.E. Acidifying agents, uptake, and physiological activity of gibberellin A3 in citrus. **HortScience**, Alexandria, v.24, n.5, p.791-793, 1989.

GUARDIOLA J.L.; MONERRI, C.; AGUSTI, M. The inhibitory effect of gibberellic acid on flowering in citrus. **Physiologia Plantarum**, Copenhagem, v.55, p.136-142, 1982.

GYLLENSTRAND, N.; CLAPHAM, D.; KÄLLMAN, T.; LAGERCRANTZ, U. A norway spruce flowering locus T homolog is implicated in control of growth rhythm in conifers. **Plant Physiology**, Bethesda, v.144 p.248-257, 2007.

HANKE, M.V.; FLACHOWSKY, H.; PEIL, A.; HATTASCH, C. No flower no fruit – genetic potentials to trigger flowering in fruit trees. **Genes, Genomes and Genomics**, Isleworth, v.1 p.1–20, 2007.

IGLESIAS, D.J.; CERCOS, M.; COLMENERO FLORES, J.M.; NARANJO, M.; RIOS, G.; CARRERA, E.; RUIZ-RIBERO, O.; LLISO, I.; MORILLON, R.; TADEO, F.R.; TALON, M. Physiology of citrus fruiting. Rewiew. **Brazilian Journal of Plant Physiology**, Campinas, v.19, n.4, p.333-362, 2007.

KATO, T. Nitrogen metabolism and utilization in citrus. *In*: JANICK, J. (ed.). **Horticultural Reviews**. Hoboken: AVI Publishing, 1986; v.8, p.81–216.

KAVI KISHOR, P.B.; HIMA KUMARI, P.; SUNITA, M.S.L.; SREENIVASULU, N. Role of proline in cell wall synthesis and plant development and its implications in plant ontogeny. **Frontiers in Plant Science**, New Haven, v.6, p.544, 2015.

KOSHITA, Y; TAKAHARA T.; OGATA, T.; GOTO, A. Involvement of endogenous plant hormones, (IAA, ABA, GAS), in leaves and flower bud formation of Satsuma mandarin , n.*Citrus unshiu* Marc. **Scientia Horticulturae**, New York, v.79, p.185-194, 1999.

KUSHAD, M.M.; YELENOSKY, G. Evaluation of polyamine and proline levels during low temperature acclimation of citrus. **Plant Physiology**, Lancaster, v.84, n.3, p.692-695, 1987.

LEHMANN, S., FUNK, D., SZABADOS, L.; RENTSCH, D. Proline metabolism and transport in plant development. **Amino Acids,** Wien, v.39, p.949–962, 2010.

LOVATT, C. J.; ZHENG, Y.; HAKE K.D. Demonstration of a change in nitrogen metabolism influencing flower initiation in citrus. **Israel Journal of Botany**, Jerusalem, v.37, n.2-4, p.181-188, 1988.

MADEMBA-SY, F.; BOUCHEREAU, A.; LARHER F.R. Proline accumulation in cultivated citrus and its relationship with salt tolerance. **Journal of Horticultural Science and Biotechnology**, Ashford, v.78, n.5, p.617-623, 2003.

MAGGIO, A.; MIYAZAKI, S.; VERONESE, P.; FUJITA, T.; IBEAS, J.I.; DAMSZ, B.; NARASIMHAN, M.L.; HASEGAWA, P.M.; JOLY, R.J.; BRESSAN, R.A. Does proline accumulation play an active role in stress-induced growth reduction? **The Plant Journal**, Oxford, v.31, p.699-712, 2002.

MARTÍNEZ‑FUENTES, A.; MESEJO, C.; REIG, C.; AGUSTÍ, M. Timing of the inhibitory effect of fruit on return bloom of 'Valencia'sweet orange , (**Citrus sinensis**, (L.) Osbeck). **Journal of the Science of Food and Agriculture**, New York, v.90, n.11, p.1936-1943, 2010.

MATTIOLI, R.; COSTANTINO, P.; TROVATO, M. Proline accumulation in plants: not only stress. **Plant Signaling & Behavior**, Georgestown, v.4, n.11, p.1016-1018, 2009.

MICHELOUD, N.G.; CASTRO, D.C.; BUYATTI, M.A. PAOLA MARICEL G.; GARIGLIO, N.F. Factors affecting phenology of different citrus varieties under the temperate climate conditions of Santa Fe, Argentina. **Revista Brasileira de Fruticultura**, Jaboticabal, v.40, n.1, p.e-315, 2018.

MILLARD, P.; GRELET, G.A. Nitrogen storage and remobilization by trees: ecophysiological relevance in a changing world. **Tree Physiology**, Victoria, v.30, n.9, p.1083-1095, 2010.

MORENO, J.; GARCÍA-MARTINEZ, J.L. Seasonal variation of nitrogenous compounds in the xylem sap of *Citrus*. **Physiologia Plantarum**, Copenhagen, v.59 p.669–675, 1983.

MUÑOZ-FAMBUENA N.; MESEJO C.; GONZÁLEZ-MAS MC.; IGLESIAS DJ, PRIMO-MILLO E. and AGUSTÍ M. Gibberellic acid reduces flowering intensity in Sweet Orange [*Citrus sinensis*, L. Osbeck] by repressing CiFT gene expression. **Journal of Plant Growth Regulators**, New York, v.31, p.529-536, 2012.

MUÑOZ-FAMBUENA N.; MESEJO C.; GONZÁLEZ-MAS MC.; PRIMO-MILLO E.; AGUSTÍ M.; IGLESIAS D.J. Fruit regulates seasonal expression of flowering genes in alternate-bearing 'Moncada' mandarin. **Annals of Botany**, London, v.108, p.511-519, 2011.

NEBAUER1, S. G.; BEGOÑA R.M.; GUARDIOLA, J.L.; MOLINA, R.V. Photosynthesis down-regulation precedes carbohydrate accumulation under sink limitation in Citrus. **Tree Physiology**, Victoria, v.31, p.169–177, 2011.

NISHIKAWA F.; ENDO T.; SHIMADA T.; FUJII H.; SHIMIZU T.; OMURA M.; IKOMA Y. Increased CiFT abundance in the stem correlates with floral induction by low temperature in Satsuma mandarin (*Citrus unshiu* Marc). **Journal of Experimental Botany**, Oxford, v.58, p.3915-3927, 2007.

RABE, E. Stress physiology: the functional significance of the accumulation of nitrogen containing compounds. **Journal of Horticultural Science**, Ashford, v.65, p.231-243, 1990.

RIBEIRO, RV.; MACHADO E.C.; SANTOS M.G.; OLIVEIRA R.F. Seasonal and diurnal changes in photosynthetic limitation of young sweet orange trees. **Environmental and Experimental Botany**, Oxford, v.66, n.2, p.203-211, 2009.

SAGEE, O.; LOVATT C.J. Putrescine content parallels ammonia and arginine metabolism in developing flowers of the washington navel orange. **Journal of the American Society for Horticultural Science**, Alexandria, v.116, n.2, p.280-285, 1991.

SAMACH, A.; SMITH H.M. Constraints to obtaining consistent annual yields in perennials. II: Environment and fruit load affect induction of flowering. **Plant Science**, Bethesda, v.207, p.168-176, 2013.

SCHÄFER, G.; PANZENHAGEN, N.; VALTIR, S.I.; IVAR, A.; SCHWARZ, S.; FRANCISCO, K.; OTTO, C. Produção e desenvolvimento da Tangerineira-'Montenegrina' propagada por enxertia e estaquia, no Rio Grande Do Sul. Mandarin cv Montenegrina production and development propagated by cuttings and cuttings and grafting in Rio Grande Do Sul State, Brazil. **Revista Brasileira de Fruticultura**, Jaboticabal, v.23, n.3, p.668-672, 2001.

SHARMA, S.; VILLAMOR J.G.; VERSLUES P.E. Essential role of tissue-specific proline synthesis and catabolism in growth and redox balance at low water potential. **Plant Physiology**, New York, v.157 , n.1, p. 292-304, 2011.

SHLIZERMAN, H.; ZEMACH, M.; WEISSBERG, R.; OPHIR, E.; BLUMWALD, E.; SADKA, a. Alternate bearing in Citrus: changes in the expression of flowering control genes and in global gene expression in ON- versus OFF- crop trees. **PLoS One**, San Francisco, v.7, n.10, p.1-16, 2012.

SIGNORELLI, S. The fermentation analogy: a point of view for understanding the intriguing role of proline accumulation in stressed plants. **Frontiers in Plant Science**, Lausanne, v.7, p.1339, 2016.

SINGH A.; KUMAR SHARMA M.; SENG R.S. Osmolytes: proline metabolism in plants as sensors of abiotic stress. **Journal of Applied and Natural Science,** Uttarakhand, v.9 , n.4, p.2079 -2092, 2017.

SYVERTSEN, J.P.; GONI, C.; OTERO A. Fruit load and canopy shading affect leaf characteristics and net gas exchange of 'spring' navel oranges. **Tree Physiology**, Victoria, v.23, p.899–906, 2003.

SYVERTSEN, J.P.; SMITH JR, M. L. Environmental stress and seasonal changes in proline concentration of citrus tree tissues and juice [Grapefruit, *Citrus paradisi*, rough lemon, *Citrus jambhiri*]. **Journal of the American Society for Horticultural Science**, Alexandria, v.108, n.5, p.861-866, 1983.

SZABADOS, L.; SAVOURÉ A. Proline: a multifunctional amino acid. **Trends in Plant Science**, New York, v.15, n.2, p.89-97, 2009.

TAKENO, K. Stress-induced flowering: the third category of flowering response. **Journal of Experimental Botany**, Oxford, v.67, n.17, p.4925-4934, 2016.

TAN, F. C.; SWAIN, S. M. Genetics of flower initiation and development in annual and perennial plants. **Physiologia Plantarum**, Copenhagen, v.128, n.1, p.8-17, 2006.

TROVATO, M.; MATTIOLI, R.; COSTANTINO, P. Multiple roles of proline in plant stress tolerance and development. **Rendiconti Lincei**, Milano, v.19, p.325e346, 2008.

VERREYNNE, J.; LOVATT C.J. The effect of crop load on budbreak influences return bloom in alternate bearing 'Pixie' mandarin. **Journal of the American Society of Horticultural Science**, Oxford, v.134, p.299-307, 2009.

WELLBURN, A.R. The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. **Journal of Plant Physiology**, Stuttgart, v.144, n.3, p.307-313, 1994.

ZAHER-ARA, T.; BOROOMAND, N.; SADAT-HOSSEINI, M. Physiological and morphological response to drought stress in seedlings of ten citrus. **Trees**, Santa Monica, v.30, n.3, p.985-993, 2016.

ZDUNEK-ZASTOCKA, E.; GRABOWSKA G.; BRANICKI T.; MICHNIEWSKA, B. Biochemical characterization of the triticale TsPAP1, a new type of plant prolyl aminopeptidase, and its impact on proline content and flowering time in transgenic Arabidopsis plants. **Plant Physiology and Biochemistry,** Paris, v.116, p.18-26, 2017.

ZHANG, J.Z.; AI, X.Y.; SUN, L.M.; ZHANG, D.L.; GUO, W.W.; DENG, X.X.; HU, C.G. Transcriptome profile analysis of flowering molecular processes of early flowering trifoliate orange mutant and the wild-type [Poncirus trifoliate , (L.) Raf.] by massively parallel signature sequencing. **BMC Genomics**, London, v.12, p.63, 2011.

ZHANG, L.; BECKER, D.F. Connecting proline metabolism and signaling pathways in plant senescence. **Frontiers in Plant Science**, New Haven, v.6, p.552, 2015.