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# Foliar-applied urea at bloom improves early fruit growth and nitrogen status of spur leaves in pear trees, cv. Williams Bon Chretien

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#### ABSTRACT

The fruit size of early ripening pear varieties is one of the most important attributes for its quality. In pear (*Pyrus communis* L.) the thinning effect of urea is controversial, even if urea sprays increase in general the average fruit size. We therefore tested the hypothesis that foliar-applied urea at bloom also plays an important nutritional role. Trials were carried out in two orchards of the experimental farm of the INTA Alto Valle research station, in the province of Rio Negro, Argentina on mature trees of the cv. "Williams Bon chretien" on seedling. Urea (5%, w/v) application at full bloom had a slight effect of fruit thinning of fruitlets, but increased fruit size and the number of cells per fruit. Sprayed spurs showed larger fruit weight before a significant difference in fruit number per spur was observed. The uptake of N from urea, measured by the aid <sup>15</sup>N techniques, enhanced N concentration and contents of flowers and spur leaves by about 10%. Although we cannot exclude that the thinning effect of urea has contributed to the enhancing of the fruit size and the number of cells per fruits, our data indicated that before the thinning effect of urea occurred, the fruit size already benefited from the foliar N supply due to an enhanced leaf and flower N concentration. Due to the both the thinning and the nutritional effects, the supply of urea early of the season is therefore a powerful and relatively inexpensive tool for enhancing fruit size in pear trees.

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#### 1. Introduction

Argentina is the leading country for export of pear cv. "Williams Bon chretien" (Sánchez, 2007) and fruit size is one of the most important attributes for quality of fruit of this cultivar. Fruit thinning is the most important technique for improving fruit size (Link, 2000). Foliar application of urea has shown a thinning effect in several fruit tree species In peaches and nectarines, a solution of urea at 8–12% concentration significantly reduced fruit set already two weeks after its application and increased fruit weight (Zilkah et al., 1988). In olive, fruit set was reduced by about 50% in treated plants with 6% urea spray 20 days after full bloom (DAFB) (Barattà et al., 1990). In pistachio, urea spray at 5% applied 10 DAFB significantly increased fruit drop and increased nut size (Rahemi and Ramezanian, 2007).

In pear (*Pyrus communis* L.) the information about the thinning effect of urea is rather limited. Sánchez et al. (2007), Sugar (2009) and Curetti (2009) reported that urea spray at 5–7.5% increased the average fruit size and the percent distribution of fruit of larger diameters. In those studies, the thinning effect of

the urea was not consistent among years, although, fruit size was always increased, a fact supporting the hypothesis that foliar-applied urea at that developmental stage may also play a nutritional role.

Urea is the most applied nitrogen (N) fertilizer and it is widely used for foliar application to improve N status in many crops (Sánchez, 2002; Wang et al., 2008). Urea, due to its intrinsic characteristics such as small molecular size, non-ionic nature and high solubility, is usually taken up rapidly through the leaf cuticle (Toselli et al., 2004) although the optimal concentration varies widely according to the species and phenological phase (Tagliavini and Toselli, 2005). The nutrition of plants through aboveground organs is generally regarded as a technique to supply nutrients quickly to a target organ (Tagliavini and Toselli, 2005). Furthermore, in deciduous fruit trees, early leaf growth and flowering predominantly uses N deriving from remobilization from storage organs (Sánchez and Righetti, 1990; Sánchez et al., 1990, 1991; Tagliavini et al., 1997; Sánchez, 2002; Quartieri et al., 2002); in this period, foliar N supply can be beneficial if root uptake and translocation of the newly absorbed N are insufficient to meet plant requirement due to low soil temperature, excess moisture or poor root growth (Weinbaum et al., 1984).

We hypothesized that highly concentrated urea can be taken up by reproductive spurs improving both N status and early fruit

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growth in pear (*Pyrus communis* L.) cv. *Williams Bon Chretien* (hereafter referred as *Williams*). To test that hypothesis, <sup>15</sup>N-labeled urea was applied at full bloom to follow its fate and assess its role during the fruit cell division phase (Harada et al., 2005).

#### 2. Materials and methods

#### 2.1. Experimental site

Trials were carried out in two orchards of the experimental farm of the INTA Alto Valle research station, in the province of Rio Negro, Argentina (39°01′S, 67°40′W, elevation 240 m, 244 mm annual rainfall, 15 °C annual average temperature). The loamy soil had an organic matter concentration of 15 g kg $^{-1}$  at 20 cm depth. Standard agronomic practices were applied including pruning, pest and insect control, irrigation and fertilization. The plot was fertilized at postharvest with 80 kg/ha N applied as urea ( $\sim\!130\,\mathrm{g}$  N per tree).

#### 2.2. Experiment 1

A first trial was undertaken to study the effect of urea on initial fruit set and cell number of fruit at harvest. The trial was conducted with equal design in the growing seasons 2007–08 and 2008–09 on pear trees of cv. Williams grafted on seedling rootstocks, planted at  $4\,\mathrm{m}\times4\,\mathrm{m}$  in 1975. In both years, 20 uniform trees were selected before full bloom. Ten trees received foliar-urea at 5% (w/v) at full bloom (03 October 2007 and 25 September 2008) and the remaining 10 served as a control. Foliar applications were made at early morning and no surfactant was added.

Fruit number was counted in 100 spurs per treatment at 15 and 45 days after application (DAA). The number of fruits per tree was also determined at their harvest (21 January 2008 and 12 January 2009), when 50 fruits per treatment were randomly selected to determine the number of cells. Fruit volume was calculated assuming specific weight of Williams pear fruits at harvest equal to 1.00 g cm<sup>-3</sup> (Westwood, 1962). Transverse sections were taken from the mid-cortical region of each fruit along the equatorial radius and sections were made with a sharp razor blade. Two slices per fruit were prepared, one from each side of the fruit (Westwood et al., 1967; Goffinet et al., 1995). The cell walls at the surface of each slice were stained by immersing for 3 min in 0.5% safranin. Nail polish was applied on the stained section and after drying naturally for  $\sim$ 1 h. The solidified nail polish was exfoliated from the section with tweezers for examination by light microscopy (Sugiura et al., 1995). Both major and minor axes of 10 cells per fruit were measured and cell volume was calculated by Westwood et al. (1967). Finally, cell number was estimated using the formula suggested by Denne (1960).

The experimental design was completely randomized. Statistical analysis was performed separately per each year by analysis of variance (ANOVA) or *t*-test using the statistical program Infostat (2009). In order to analyze spur distributions, a contingency table based on the frequency of absolute values was made; results were also expressed as a percentage of total spurs evaluated.

#### 2.3. Experiment 2

The trial was conducted with the same experimental design in 2006 and 2008 in a pear orchard of cv. *Williams* grafted on seedling rootstock, planted with a spacing of  $4\,\mathrm{m}\times2\,\mathrm{m}$  in 1993 and trained as a trellis. In both years, before full bloom, we selected 12 trees of uniform size and fruit yield (around 50 kg tree<sup>-1</sup>). Thirty reproductive 2–3 year-old spurs on principal branches were randomly

selected and tagged in each tree canopy at bloom. <sup>15</sup>N-labeled urea (10 atom % <sup>15</sup>N) was dissolved to achieve a solution with a concentration of 5% (w/v). No surfactants were added. The solution was sprayed on the tagged spurs of six trees randomly chosen out the 12 selected trees, while the other untreated were used as a control. The amount of solution ( $\sim$ 1.4 ml spur $^{-1}$ ) was sufficient to uniformly wet the leaves (10.7 and 9.8 leaves/spur on average in 2006 and 2008, respectively) and the flower clusters (7.8 and 7.0 flowers/spur on average in 2006 and 2008, respectively), avoiding solution run-off. Immediately after application, treated spurs were covered with plastic bags and the whole trees were sprayed until runoff with a 5% (w/v) solution of non-labeled urea. Foliar applications were made on 27 September 2006 and 24 September 2008. during the morning. Weather conditions over the trial are shown in Fig. 1. Spraying occurred at the beginning of full bloom and after the application, leaves showed no visual symptoms of stress, chlorosis

In 2006, three samplings were made at 2, 7 and 16 days after application (DAA). At 12 DAA, the number of fruits in marked spurs was counted and then hand thinning was performed, leaving two fruits in each spur. In 2008, two samplings were made at 2 and 9 DAA. No hand thinning was done in 2008. Each time, a sample of reproductive spurs per tree was collected (4 in 2006 and 6 in 2008) and the number of leaves and flowers or fruits was counted. Spur-leaves area (LA) was measured using a leaf area meter (Li 3100 Area Meter, Li-Cor Inc., Lincoln, NE, USA). All sampled organs were carefully washed with distilled water to remove residual urea from external surfaces and dried at 70°C to constant weight. Dry mass (DM) was recorded and each organ was ground separately. Total nitrogen and <sup>15</sup>N enrichment of each organ were determined by CF-IRMS (Continuous Flow-Isotope Ratio Mass Spectrometry, Thermo Fisher Scientific). The percentage of nitrogen derived from fertilizer (NFF) present in each organ was calculated according to the following equation (MacKown et al., 1987):

$$NFF = \frac{(Atom\%^{15}N~in~tissue - ^{15}N~natural~abundance)}{(Atom\%^{15}N~in~fertilizer - ^{15}N~natural~abundance)} \times 100$$

with atom %  $^{15}$ N in fertilizer and  $^{15}$ N natural abundance being equal to 10.0% and 0.37% (International Atomic Energy Agency, 1983), respectively. The amount of NFF partitioned into fruit and leaves was calculated as the amount of dry matter × N concentration × NFF.

#### 3. Results

In the experiment 1, foliar application of urea at full bloom caused a slight decrease in the number of fruits per spur at 15 and 45 DAA in 2008–09 and in the fruit number per tree at harvest in 2007–08 (Table 1). At harvest, fruits from treated trees were slightly heavier, with a higher number of cells (+11 to 16%) than control ones, whereas the cell volume was unaffected by foliar urea (Table 1).

In experiment 2, urea sprays increased leaf mass and leaf area (only in 2006) at full bloom, and showed a positive effect on the fruit growth in its early stages in both years (Table 2). There was not significant difference in the number of flowers per spur between sprayed and control trees at full bloom and at petal fall (2 and 7–9 DAA, Table 2). In 2006, at 12 DAA, a higher proportion of sprayed spurs, as compared to control ones, had less than five fruits (Table 3), indicating the beginning of the thinning effect of urea. At 16 DAA, the hand thinning was performed, leaving 2 fruits per spur.

Nitrogen content in leaves, flowers and subsequent fruits showed a substantial increase over time, while nitrogen

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