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# Phenological and physiological responses to drought stress and subsequent rehydration cycles in two raspberry cultivars

C.G. Morales[a], M.T. Pino[b,*], A. del Pozo[c]

[a] Institute of Agricultural Research (INIA), Raihuen, P.O. Box 34, San Javier, Chile
[b] Institute of Agricultural Research (INIA), La Platina, CP 8831314, Santiago, Chile
[c] Facultad de Ciencias Agrarias, Universidad de Talca, P.O. Box 747, Talca, Chile

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ABSTRACT

Raspberry (*Rubus idaeus* L.) is a deciduous plant with perennial roots, 75% of which are concentrated in the upper level of the soil. Its shallow rooting system requires a regular water supply; a water deficit can affect fructification as well as cane growth and yield for the following season. Despite the demonstrated drought stress impact on the raspberry, there is little information about the phenological and physiological responses to drought stress. The main goal of this study was to evaluate the effects of drought stress on the phenological phases, physiological parameters and yield of two raspberry cultivars: Heritage (remontant type) and Meeker (non-remontant type). All plants were grown in pots under greenhouse conditions, and the following watering treatments were applied: (T1) well-watered, 100% irrigation and (T2) a controlled drought-stress cycle. The volumetric soil water content ($\theta$), phenological phases, leaf net photosynthetic rate ($A$), transpiration rate ($T$), and stomatal conductance (gs) were registered periodically. The free proline and total soluble sugars were also determined. Based on the phenological study, Heritage under drought-stress (T2) showed earlier flowering and a shorter fruit production period in relation to well-watered plants (T1). In Meeker, T2 extended the cane and summer lateral elongation, showing earlier senescence. Leaf gas exchange decreased with drought stress, $A$ declined after 28-day period under drought stress, from 9.2 $\mu molCO_2\ m^{-2}\ s^{-1}$ to 3.0 $\mu molCO_2\ m^{-2}\ s^{-1}$ in Heritage, and from 12.2 $\mu molCO_2\ m^{-2}\ s^{-1}$ to 3.0 $\mu molCO_2\ m^{-2}\ s^{-1}$ in Meeker. In both cultivars, the free proline and total soluble sugars increased with drought stress. The fruit production was also affected in the next season under T2 condition, decreasing in 34 and 38% in relation to well-watered plants.



## 1. Introduction

Raspberry (*Rubus idaeus* L.) is a deciduous plant with perennial rhizomes. The plant's shallow rooting system requires a regular and uniform water supply, particularly in the period from fruit set to harvest (Razeto, 1993; Crandall, 1995). In Mediterranean environments raspberry is grown under irrigated conditions, however water for irrigation is not always available at the time and amount needed by the crop. Furthermore, dry years are occurring more frequently during the last decade probably due to climate change, and the probability of water deficit for raspberry is increasing, particularly in Central Chile (CONAMA, 2008).

*R. idaeus* shows moderate tolerance to short drought stress periods; however, prolonged water deficits result in negative impacts on plant growth and fruit production. The phenological timing and yield for the following season are also affected (Crandall, 1995; Percival et al., 1998; Privé and Janes, 2003). During a water deficit, overall plant development is delayed, and leaf size is reduced; anatomical changes due to modifications in cell size, senescence and, ultimately, plant death are also observed in several species (Añon et al., 2004; Jaleel et al., 2008). The low water availability in the soil decreases photosynthesis and carbohydrate accumulation, limiting overall plant growth (Chaves, 1991; Chaves et al., 2003; Chaves and Oliveira, 2004; Flexas et al., 2004). In addition to affecting stomata closure, drought stress decreases gas exchange in plants by reducing transpiration and the photosynthetic rate (Chaves, 1991; Ekanayake, 1994; Dalla Costa et al., 1997; Deblonde and Ledent, 2001; Glass et al., 2003; Kiziloglu et al., 2006). It has been reported in Heritage that transpiration and photosynthesis are sensitive to water stress, decreasing gradually after two days under drought stress until rehydration (Percival et al., 1998). Other studies in *Rubus* species have concluded that soil water depletion decreases

* Corresponding author. Tel.: +56 2 25779102.
*E-mail address:* mtpino@inia.cl (M.T. Pino).

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leaf cell turgor, reducing stomatal conductance (Stoll et al., 2002; Jaleel et al., 2009). Water content in the leaves, stomatal conductance and transpiration under drought stress are highly correlated (Chaves et al., 2003; Flexas et al., 2004).

Synthesis of compatible solutes such as proline, soluble sugars, glycine betaine and others seems to have a central role in osmotic adjustments, preventing or reducing the loss of turgor. Proline has been associated with drought tolerance and other abiotic stresses in several plant species (McCue and Hanson, 1990; Andrade et al., 1995; Kavi Kishor et al., 1995; Wang et al., 2003; Chaves and Oliveira, 2004; Kavi Kishor et al., 2005; Chaman, 2007). In fact, studies on several fruit species have shown that the free proline concentration increases in leaves with water stress. This phenomenon has been demonstrated in citrus (Nolte et al., 1997), blackberry (Parra et al., 1999), tomato (Claussen, 2005) and olives (Ahmed et al., 2008). A recent study evaluating induced *in vitro* drought-stress effects on raspberries and blackberries reported a sustained increase in free proline content with progressive drought in the majority of evaluated clones (Orlikowska et al., 2009).

Raspberries are classified as remontant and non-remontant types, according to their production season. The remontant cultivars flower and bear fruit twice a year, at the beginning of spring (on floricanes from the previous growing season) and at the end of summer (on primocanes from the same year). Non-remontant cultivars bear fruit only once a year on floricanes, from the end of spring until the beginning of summer. In accordance with these productive characteristics, the phenological and physiological responses during drought stress can be expected to differ between the two types of cultivars. Only two studies have addressed these effects in raspberry: those by Percival et al. (1998) and Privé and Janes (2003). In this work, it was hypothesized that remontant and non-remontant raspberry cultivars have different phenological and physiological responses to drought stress.

The main goal of this study was to evaluate the effect of a controlled water deficit on the phenological phases, physiological parameters and yield of the Heritage and Meeker raspberry cultivars.

## 2. Materials and methods

### 2.1. Material vegetal and growth conditions

This study evaluated two raspberry cultivars: Heritage (remontant type) and Meeker (non-remontant type). The plant materials used during this experiment were two-year-old plants and were produced by a nurseryman certified by the Agricultural and Livestock Service (SAG). They were obtained from etiolated shoots. All plants during the growing season were grown in 25 L pots under greenhouse conditions (25 °C ± 3, 16/8 h day/night photoperiod, 400–480 μmol $m^{-2}$ $s^{-1}$ light intensity), spacing 1.5 m × 0.5 m. After summer, the greenhouse temperature acclimation system was turn-off to allow cold acclimation. Plants were grown in a mixture of peat Sunshine Mix#6 (Sun Gro Horticulture Inc., Bellevue, WA), compost and sand (3:3:1), supplemented with 3N, 3P and 1K every season. Plants were watered with well water using drip irrigation, one dropper of 4 L $h^{-1}$ per pot.

### 2.2. Watering treatments

The following watering treatments were applied to plants: (T1) well-watered, with 100% irrigation and (T2) a controlled drought stress cycle consisting of a 28-day period without watering, until the soil water decreased close to the permanent wilting point, a 7-day period of recovery (100% irrigation) and another 28-day period without watering. In the well-watered treatment (T1), the water supplied was equal to the transpiration losses, as determined by differences in pot-weight between successive waterings. The watering treatments were applied from early summer to early autumn, when raspberry plantations are naturally affected by drought stress. Visual phytosanitary inspections did not uncover pests, fungal or bacterial problems.

### 2.3. Soil water status

The soil water content was measured as the volumetric water content ($\theta$), defined as the ratio of the water volume in the soil to the total volume of soil ($m^3$ $m^{-3}$). The $\theta$ was measured 15 cm below the soil surface by using ECH2O probes and Em50 data loggers (Decagon Devices, Inc., Pullman, WA). Data points were automatically recorded every 30 min. Two soil moisture sensors (ECH2O probes) per experimental unit were installed during all experiment.

### 2.4. Phenological phases in raspberry

The phenological stages (sprouting/bud development, lateral cane elongation, flowering, fruiting-ripening, plant senescence or beginning of dormancy) were evaluated using the protocol described by the Centre Technique Interprofessionnel des Fruits et Légumes – Le Francia CTIFL (Granier et al., 2006). The readings were taken every two days from winter (2009) to the autumn of the next season (2010), in all plants under all experimental conditions.

### 2.5. Leaf gas exchange parameters and fruit yield

The leaf gas exchange was evaluated according to Seppänen and Coleman (2003) and Schittenhelm et al. (2004) by using a LI-6400XT Portable Photosynthesis System (LICOR Biosciences, Inc. Lincoln, Nebraska, USA) with an automatic leaf chamber (6 $cm^2$ leaf area, 25 °C constant air temperature, 600 ppm of external leaf $CO_2$ concentration (Ca) and a 1000 μmol saturation point $m^{-2}$ $s^{-1}$ PAR). We evaluated the photosynthetic rate ($A$, μmol$CO_2$ $m^{-2}$ $s^{-1}$), transpiration rate ($T$, mmol$H_2O$ $m^{-2}$ $s^{-1}$), stomatal conductance (gs, mmol $m^{-2}$ $s^{-1}$) and the $A$/Ci ratio ($CO_2$ assimilation rate/intercellular $CO_2$ concentration). gs was also followed with a porometer Decagon Device (Leaf Porometer model, USA). Leaf gas exchange measurements were taken weekly throughout the assay in completely expanded leaves located in the middle portion of the cane in each cultivar, watering treatment and replication. A total of three readings were taken per plant, daily at midday.

The total fruit yield was determined during the second season of evaluation (2010–2011) considering the production of 5 plants for each cultivar, treatment and replication. Yield was express in gr $plant^{-1}$.

### 2.6. Proline and sugar analysis

The total soluble sugars and free proline contents were determined using fully expanded leaves for the two watering treatments (T1 and T2). Samples were taken weekly during the first drought-stress cycle from completely expanded leaves (young and mature) located in the middle portion of the canes for each cultivar, watering treatment and replication. Young leaves and mature leaves were taken from the apical and basal part of the shoot, respectively. A total of three samples were taken for each plant. Leaf tissue was collected, pulverized in liquid $N_2$ and stored at −80 °C. Proline analyses were conducted as in Gilmour et al. (2000), using 20 and 30 mg lyophilized tissue per sample for the T1 and T2 conditions, respectively. Total soluble sugars analysis was performed using the phenol-sulfuric acid method as in Pino et al. (2008). The absorbance was determined at 515 nm for proline and at 492 nm for total