Effect of low temperature in the first development stage for five red raspberry genotypes

Elida Contreras 1*, Javiera Grez1, José A. Alcalde1, Davide Neri2, Marina Gambardella 1

1Department of Fruticulture and Oenology, Faculty of Agronomy and Forestry, Pontificia Universidad Católica de Chile, Macul, Santiago, Chile
2Department of Agricultural, Food and Environmental Sciences, Faculty of Agriculture, Marche Polytechnic University, Ancona, Italy

*Corresponding author: edcontre@uc.cl


Abstract: In raspberry, the expression of the primocane fruiting trait is influenced by the environment. Although there are several factors that influence the expression of this character, it is well known that low temperatures that occur during the growth season of the primocane, are important and affect the flowering. In this study, plants in their early stages of development were exposed to low temperatures (2°C) for one month, in a dark cold chamber. The following genotypes primocane and floricane were used: ‘UC103’ , ‘ Autumn Bliss’ , ‘Heritage’ , ‘Meeker’ and ‘Tulameen’ . Flowering and growth were recorded until the end of the season and the morphology of the meristem was characterized in this moment. Interaction between cold and genotype was detected in all parameters studied. In ‘Heritage’, a slight primocane, growth and flowering were favoured by exposure to cold. Thereby, low temperature affects flowering, but this effect depends on primocane fruiting degree of each genotype, slight primocane the cold favored flowering and growth. However, strongly primocane the cold had no effect on flowering and growth.

Keywords: flowering; induction; primocane fruiting; vernalization

The red raspberry (Rubus idaeus L.) has different flowering habits, and two groups of cultivars can be identified: primocane and floricane. The primocane cultivars can bloom both on lignified canes in the early summer and on the new growing shoots in late summer and autumn. In contrast, floricane cultivars bloom only on lignified canes and produce fruit once during the early summer. Thus, commercially, the primocane cultivars are called annual raspberry, whereas the floricane cultivars are called biennial raspberry (Heide, Sonstbye 2011).

Currently, primocane cultivars are of greater economic importance because this character extends the harvest; therefore, these cultivars can be more productive than floricane. Primocane cultivars are also better adapted to warm climate regions (Pritts 2008). Thus, several breeding programs are focused on obtaining new cultivars of the primocane type.

The expression of the primocane fruiting trait or autumn fruiting is strongly influenced by the environment, and depending on the geographic location of cultivation this character is expressed in different degree. The percentage of the buds that differentiate into flowers on the growing shoots determines the degree of primocane fruiting (Gambardella et al. 2016. Some cultivars have been observed with an intermediate behaviour; thus, some cultivars classified as floricane can flower...
at the tip of the shoot by the end of the growing period, before the winter rest, when the genotype is exposed to different environmental conditions. Studies have described this behaviour in the cultivars ‘Glen Clova’ (Dale, Daubeney 1987) and ‘Glen Moy’ (Ourecky 1976; Carew et al. 2000).

The aim of several studies has been to understand the physiological basis of the different flowering habits of raspberry by identifying the environmental factors that lead to floral induction. Temperature and photoperiod are the primary environmental factors that influence the induction process (Heide, Sønsteby 2011). However, a genetic component with great diversity among cultivars and a strong genotype x environment interaction has been discovered. The process of floral induction/differentiation is flexible in raspberry, resulting in complex, scarcely predictable and difficult to handle behaviour (Neri et al. 2012). This flexibility leads to a different response of the flowering habit that is dependent on the environmental conditions of cultivation; therefore, raspberry cultivars cannot be definitively classified as primocane or floricane (Carew et al. 2000), based on exposure to only one environment.

In primocane cultivars floral induction, differentiation and floral development occur early in the season and are favoured at temperatures higher than 20°C (Vasilakakis et al. 1980; Lockshin, Elfving 1981; Sønsteby, Heide 2009); thus, flowering occurs in the summer and fruits are produced in the early autumn. By contrast, in floricane cultivars, buds of the shoot of the season are induced to flower under short-day conditions and when temperatures are below 13°C and therefore bloom the next spring. Nevertheless, when days are long and temperatures are low, below 13°C, floral induction may also occur (Sønsteby, Heide 2008). In this case, the effect of temperature is greater than that of photoperiod, and these cultivars are not strictly dependent on day length.

Although low temperatures (< 18°C) are a prerequisite for floral induction in floricane cultivars, also a positive effect of cold (2°C) has been observed in some primocane cultivars, such as ‘Heritage’ (Takeda 1993) and two genotypes of primocane blackberry (Rubus spp.), studied by López-Medina and Moore (1999). In both cases was observed an increase of flowering in plants subjected to cold. In other primocane cultivars, such as ‘Polka’, flowering and growth improves their response with exposure to high temperatures (Sønsteby, Heide 2009). However, some primocane cultivars show a vernalization-type, because the flowering was stimulated at low temperature, (Sønsteby, Heide 2009; Carew et al. 2001).

The vernalization process defined by Chouard (1960) as “the acquisition or acceleration of the ability to flower by chilling treatment” it could be of the primary differences between the two groups of raspberry cultivars, in relation to the requirement of cold, as a stimulus for floral induction. So, the cold requirements for flower induction could be an important factor for the differences in the expression of primocane fruiting trait for each cultivar. Thus, the purpose of this study was to evaluate the response of flowering and growth in plants subjected to cold treatment at early stages of development using five raspberry genotypes with different degrees of primocane fruiting.

**MATERIAL AND METHODS**

Five raspberry genotypes were selected according to the degree of primocane fruiting trait, based previous observations in the field. The primocane cultivars were ‘Heritage’ ‘Autumn Bliss’, and UC103 selection from the breeding program of Universidad Católica de Chile, and the floricane cultivars were ‘Meeker’ and ‘Tulameen’. In our experiment the primocane fruiting trait was evaluated according to the classification of “degrees of primocane fruiting” described by Gambardella et al. (2016).

Plants were obtained from roots through etiolated shoots and were established in 6.5-l pots with a substrate mixture of 30% peat and 70% coconut fibre in a greenhouse at temperatures that ranged from 25 to 27°C, and without artificial light. The plants were obtained during September and, on October 1, 2014, 12 plants of each genotype were subjected to a cold treatment. Plants were placed in a cold chamber at 2°C in the dark for 30 consecutive days with a total of 720 artificial chilling hours. The control plants were obtained during October, without the cold treatment, under the same propagation condition of plant for cold treatment. The objective was to obtain plants that were at the same stage of development, with an average of four expanded leaves at the establishment of the trial on November 6, 2014.

After treatment in the cold chamber, the trial was established in pots in the locality of Santo Domingo 33°38′S, 71°39′W, which has a Mediterranean
climate with maritime influence, with an average summer temperature of 20°C and an average winter temperature of 7°C. The maximum, minimum and mean temperatures during the test period are presented in Table 1. In relation to the photoperiod, the light hours during the trial evaluation period ranged from 10–15 hours (Fig. 1). Fertilization was applied weekly through irrigation at doses used in commercial production systems.

Days to the first visible floral button, number of floral lateral shoots, number of flowers per plant, height and number of nodes were recorded weekly. These evaluations were conducted from the establishment of the trial in November 2014 to the end of the season in April 2015. Moreover, after the plants entered winter rest on April 30, 2015, the state of differentiation of buds was determined with a stereomicroscope (63× magnification). Classification of buds as vegetative or reproductive was performed according to the scale published by Neri et al. (2012).

The experimental design consisted of completely randomized blocks with four replications, with an experimental unit of three plants. All evaluated parameters, except the meristems, were analyzed using a factorial analysis, with genotype and cold treatment as the factors. For the comparison of averages among genotypes according to treatments, the Tukey test was performed, with a level of significance of \( P = 0.05 \). The Statistix-9 program (Thomas, Maurice 2009) was used for statistical analyses.

**RESULTS**

The primocane cultivars showed different degrees of primocane fruiting, according to the number of floral lateral shoots developed from the apex to the base of the shoot, in the case of control plants (Table 2). ‘UC103’ selection had the highest number of floral lateral shoots (18.1), followed by ‘Autumn Bliss’ (10.7) and ‘Heritage’ (3.5). These differences were significant \( P < 0.05 \). The two florican cultivars ‘Tulameen’ and ‘Meeker’ did not develop flowers in local climatic conditions. The studied genotypes had degrees of primocane fruiting between 4 and 1 (Table 2).

The factorial analysis showed that both, cold and genotype factors, induced statistically significant differences \( P < 0.05 \) in each parameter studied. The interaction of these factors also showed statistically significant differences \( P < 0.05 \) for each parameter, except in the number of days to the first visible floral button \( P = 0.0977 \) (Table 3).

For ‘UC103’ selection and ‘Autumn Bliss’, the cold treatment did not significantly affect the number of floral lateral shoots and number of flowers. In Table 2, Number of floral lateral shoots developed and evaluation of "degree of primocane fruiting" (1–5) according to the scale of Gambardella et al. (2016)

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>No. of differentiated nodes</th>
<th>Degree of primocane fruiting</th>
</tr>
</thead>
<tbody>
<tr>
<td>UC103</td>
<td>18.1a</td>
<td>4, highly primocane</td>
</tr>
<tr>
<td>Autumn Bliss</td>
<td>10.7b</td>
<td>3, moderately primocane</td>
</tr>
<tr>
<td>Heritage</td>
<td>3.5c</td>
<td>2, slightly primocane</td>
</tr>
<tr>
<td>Meeker</td>
<td>–</td>
<td>1, florican</td>
</tr>
<tr>
<td>Tulameen</td>
<td>–</td>
<td>1, florican</td>
</tr>
</tbody>
</table>

*all data correspond to averages of four replications, each represented by three plants; different letters within the column indicate significant differences \( P < 0.05 \) according to the Tukey test
contrast, for ‘Heritage’, the values of both parameters were significantly higher in cold-treated plants than in those without cold storage.

Although a genotype × cold interaction was not detected for the number of days to the first visible floral button, an effect of the cold was observed in ‘Heritage’. The treated plants of this cultivar had significantly earlier onset of flowering than the control plants (Table 3).

### Table 3. Effect of cold on flowering of the three primocane genotypes

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Treatment</th>
<th>No. of floral lateral shoots</th>
<th>No. of total flowers</th>
<th>No. of days to the first visible floral button</th>
</tr>
</thead>
<tbody>
<tr>
<td>UC 103</td>
<td>no cold storage</td>
<td>18.3a</td>
<td>96.4a</td>
<td>99.8bc</td>
</tr>
<tr>
<td></td>
<td>cold storage</td>
<td>18.7a</td>
<td>92.8a</td>
<td>94.8ab</td>
</tr>
<tr>
<td>Autumn Bliss</td>
<td>no cold storage</td>
<td>10.7b</td>
<td>46.3b</td>
<td>97.3abc</td>
</tr>
<tr>
<td></td>
<td>cold storage</td>
<td>12.4b</td>
<td>72.5ab</td>
<td>88.8a</td>
</tr>
<tr>
<td>Heritage</td>
<td>no cold storage</td>
<td>3.4c</td>
<td>8.3c</td>
<td>148.0d</td>
</tr>
<tr>
<td></td>
<td>cold storage</td>
<td>11.8b</td>
<td>51.7b</td>
<td>110.8c</td>
</tr>
</tbody>
</table>

*all data correspond to averages of four replications, each represented by three plants; different letters within the columns indicate significant differences (P < 0.05) according to the Tukey test; **data adapted from Gambardella et al. (2016)*

Fig. 2. Plant architecture and state of floral differentiation at the entry into winter rest, according to genotype and cold treatment.
served. In ‘UC103’ selection, in plants with and without cold, a similar number of nodes and few differentiated buds were observed at the entry of winter rest. ‘Autumn Bliss’ developed fewer floral lateral shoots than ‘UC103’ but a larger proportion of differentiated buds with and without cold. In this genotype, plant architecture was similar under different conditions. In ‘Heritage’, plants without cold had fewer nodes, little development of floral lateral shoots, and fewer differentiated buds than plants that received cold treatment.

‘Meeker’ (Fig. 2) had several differentiated buds along the shoot before entering dormancy, for both cases, with and without treatment whereas ‘Tulameen’ showed similar numbers of differentiated buds and vegetative buds. For ‘Tulameen’, the apex was vegetative for both cases.

In relation with the growth and development, the growth pattern of plants was adjusted to a normal sigmoid curve, with some differences observed among cultivars. As shown in Fig. 3, for ‘UC103’ and ‘Autumn Bliss’, no difference was detected in total shoot height, and the growth rates were simi-

| Table 4. Effect of cold treatment on height and number of nodes in the five genotypes |
|-----------------|-----------------|-----------------|
| Cultivar        | Treatment       | Height (cm)     | No. of nodes |
| UC 103          | no cold storage | 119.7c          | 35.3de       |
|                 | cold storage    | 118.9c          | 36.4cde      |
| Autumn Bliss    | no cold storage | 124.9c          | 35.7de       |
|                 | cold storage    | 118.0c          | 31.7e        |
| Heritage        | no cold storage | 85.2d           | 31.3e        |
|                 | cold storage    | 120.5c          | 42.1bc       |
| Meeker          | no cold storage | 164.4b          | 45.2b        |
|                 | cold storage    | 205.7a          | 53.8a        |
| Tulameen        | no cold storage | 85.0d           | 32.3e        |
|                 | cold storage    | 102.1bcd         | 38.8bcd      |

*all data correspond to averages of four replications, each represented by three plants; different letters within the columns indicate significant differences (P < 0.05) according to the Tukey test; **data adapted from Gambardella et al. (2016)

In relation with the growth and development, the growth pattern of plants was adjusted to a normal sigmoid curve, with some differences observed among cultivars. As shown in Fig. 3, for ‘UC103’ and ‘Autumn Bliss’, no difference was detected in total shoot height, and the growth rates were simi-
as control plants stopped growth earlier. Floricane cultivars showed differences between treated and untreated plants, with taller cold-treated plants. Comparatively, ‘Meeker’ achieved a larger height than that of ‘Tulameen’, cultivar that had a growth rate like that of the three primocane genotypes.

Table 4 shows results for shoot height and number of nodes according to genotype and cold treatment. For both parameters, the interaction was significant. Cold-treated plants of Meeker and Heritage had higher growth rates and therefore were significantly taller by the end of the season than not treated plants. A similar response was observed for the number of nodes in both genotypes. ‘Tulameen’ plants did not show a difference in height, but a significant increase in the number of nodes was observed.

**DISCUSSION**

Low temperature removes the effects of dormancy but may also have a vernalization effect on floral development in some species. The response to chilling treatments could depend physiological stage it has been applied, knowing that cold is necessary for breaking dormancy, as well as for vernalization process (Metzger 1996). The developmental stage when plants are exposed to low temperatures and duration of the exposure will determine whether the result is a stimulus for satisfying the chilling requirement for breaking dormancy or a vernalization effect on flower development.

In these studies, the objective was evaluated the low temperatures as a vernalization effect. For this, plants in their early stages with four development nodes were exposed to low temperatures. Interaction between cold and genotype was found for most of the parameters analysed, related to both flowering and growth. The significant interaction found between low temperature and genotypes was consistent with work by López-Medina and Moore (1999) on blackberry (*Rubus* spp.), in which cold improved flowering and favoured growth in the shoot of the season in 2 of the 3 primocane genotypes studied. This led them to conclusion that the response to low temperatures is genotype dependent.

Genotype ‘UC103’, classified as strongly primocane, showed the greatest development of floral lateral shoots compared with the two other primocane cultivars studied (Table 3). However, the cold had no effect on any flowering or growth parameters, being this cultivar insensible to the cold. Sønsteby and Heide (2009) observed a similar response in ‘Polka’, which did not require low temperatures for flower formation; by contrast, the response improved at high temperatures. In ‘UC103’, the proportion of floral lateral shoots and differentiated buds was not modified by cold application, and in both treated and control, plants entered winter rest with a small proportion of differentiated buds (between 9% and 16%), because many of them had previously developed. The early floral differentiation is consistent with that observed by Neri et al. (2012) in primocane genotypes, such as ‘Dolomia’, ‘Erika’ and ‘L03’, on similar dates. The absence of a response to cold suggests that flowering of the primocane fruiting in this genotype is not determined by exposure to cold. Therefore, flowering may be induced early in the season, and with high temperatures plants can develop abundant flowering before winter rest. The response observed in the ‘UC103’ is of great interest, because this genotype could be used to either expand the cultivation in higher temperature growing areas or reduce the effect of high temperatures due to the climate changes that are occurring in many areas of cultivation.

With a similar response to ‘UC103’, the effect of cold in ‘Autumn Bliss’ was not significant for any of the parameters evaluated, related to both flowering and growth. Nevertheless, the total number of flowers was approximately 10% higher in plants subjected to cold treatment than in those without this treatment. Plants treated with artificial cold produced the same number of floral lateral shoots but these shoots had more flowers on average. Carew et al. (2001) studied the effect of temperatures between 6 and 7°C on new shoots from roots from 2 to 10 weeks in ‘Autumn Bliss’. The authors observed a shorter period to anthesis in plants from roots treated with cold, associating this result with a vernalization response. In our studies, plants treated with artificial cold initiated flowering 9 days before control plants; however, the difference was not significant, and plant height was not affected. In combination with the bud analysis, in this study ‘Autumn Bliss’ is not induced of vernalization process and is also not favoured by the cold.

‘Heritage’ showed a response to the artificial cold for all parameter evaluated, and poor development of floral lateral shoots was observed in plants not exposed to cold. The response is consistent with
other studies performed using this genotype, which show that although cold is not mandatory for the beginning of flowering, such treatment accelerates the process and shortens the time to anthesis (Vasilikakis et al. 1980; Takeda 1993). Once induced, high temperatures are better for flower differentiation, as reported by Lockshin and Elving (1981). These authors grew plants of ‘Heritage’ under 16-hour photoperiod and 29/24°C and 25/20°C day/night temperatures. They found that plants in the high temperature treatment flower two weeks earlier and produce more flowers. Because of the diversity of responses of this genotype to temperature, further studies are required.

Results of meristem dissection showed a differentiation in the basipetal direction for the three primocane genotypes, as described for other cultivars of this type (Heide, Sønsteby 2011). The results indicated that floral differentiation of control plants of ‘Heritage’ was later in relation with that in the two other primocane genotypes. In treated plants low temperatures promoted floral induction, because was observed more buds differentiate so, when are favourable the temperatures for flower development, the production of flower will be higher. The above results confirmed that a period of low temperatures was a determining factor in the process of floral induction in this genotype, unlike the process that occurred in the two other primocane genotypes. Despite this promote of flowering, the proportion of differentiated buds was lower than that reported by Neri et al. (2012) in ‘Dolomia’, ‘Erika’ and ‘L03’, that did not undergo artificial cold treatment but were cultivated in an alpine climate with low night temperatures during the spring/early summer period. Of note, in this study ‘Heritage’ showed intermediate behaviour between strongly primocane and florican cultivars, like the behaviour observed by Neri et al. (2012) in the ‘Lagorai’ cultivar.

Under conditions of this study, control plants of the ‘Heritage’ cultivar, usually classified as primocane, presented a low proportion of floral lateral shoots. This behaviour has been referred to as “primocane fruiting tip” by several authors, although described in florican cultivars (Ourecký 1976; Carew et al. 2000; Sønsteby, Heide 2008). The response observed in control plants was consistent with the response to cold treatment, because at the locality in which the study was conducted, temperatures are moderate (average minimum temperature of 7°C and average maximum temperature of 20°C) with low thermal oscillation. The lack of cold in this cultivar is known to have little effect on flowering, however, in conditions of cold temperatures or greater daily oscillation, this cultivar would be classified as highly primocane.

For the growth response of shoots, only the ‘Heritage’ cold-exposed individuals had an increase in growth rate, like the response observed by López-Medina and Moore (1999) in 2 of 3 blackberry primocane genotypes. Takeda (1993) observed a different effect in ‘Heritage’ plants and found a reduction of growth in response to low temperatures. These antecedents suggest that when plants are exposed to low temperatures is a key factor for the effect on growth, which may be fostered when this exposure occurs in early stages of development. In full growth cold could be a factor inducing dormancy, resulting in a decrease of activity. Takeda (1993) also noted that cold-exposed adult plants had reduced height and number of nodes, suggesting there may be an optimum cold exposure depending on the genotype. Further research in this area is required.

Plants of ‘Meeker’ and ‘Tulameen’, treated and not treated with artificial cold, did not flower on the growing shoot. However, ‘Meeker’ presented more differentiated buds before the entry into winter rest than the ‘Tulameen’, in which the vegetative apex continued to be observed. This characterization indicated that ‘Meeker’ was most likely less florican than ‘Tulameen’ and could be potentially primocane under conditions of greater exposure to cold during the growth period, like of the behaviour of tip flowering observed in others florican cultivars (Ourecký 1976; Dale, Daubeney 1987; Carew et al. 2000; Sønsteby, Heide 2008).

In ‘Tulameen’, the undifferentiated apex at the beginning of the winter rest is consistent with the description by Heide and Sønsteby (2011). They noted that florican cultivars have a differentiation that begins from bud five to ten below the apex, and therefore, the process advances in two directions, basipetal and acropetal.

For the growth response, ‘Meeker’ plants subjected to cold-treatment were taller and had more nodes, whereas ‘Tulameen’ plants only showed more nodes. ‘Tulameen’, despite a growth rhythm that was like that of primocane cultivars, the growth was stopped early in the season, and a cessation of growth did not was accompanied by floral differentiation.
CONCLUSIONS

This study demonstrated that cold applied in early stages of plant development had a favourable effect on flowering of ‘Heritage’, because of the reduction in days to anthesis, the increase in the number of floral lateral shoots, and the increase in the number of flowers per plant, in addition to an increase in growth rate and larger height and number of nodes in the shoot in ‘Heritage’ and ‘Meeker’. The cold was applied to young plants, therefore, this effect of cold could be attributed to vernalization.

Low temperatures stimulated flowering primarily in genotypes with an intermediate behaviour, such as ‘Heritage’, suggesting that a different vernalization requirement between primocane and floricanes cultivars may be involved. These requirements are likely quantitative and would generate gradualness in the expression of primocane fruiting in accordance with the environmental conditions of the locality in which the crop is grown. ‘UC103’ and ‘Autumn Bliss’ require little or no cold to flower and are insensitive to this factor in the flowering process, whereas in ‘Heritage’, the exposure to cold increases degree of primocane fruiting. It is possible that the amount of cold applied to ‘Meeker’ and ‘Tulameen’ was insufficient to observe a difference in the sprouting of flower buds, which should be considered in future studies. A quantitative expression of the primocane fruiting trait in raspberry cultivars can be hypothesized, with higher sensitivity to low temperature in weak primocane types or with an intermediate behaviour.

References


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