

Phenotyping primocane fruiting trait in raspberry (*Rubus idaeus*)

M. Gambardella¹, E. Contreras¹, J. Alcalde¹ and D. Neri²

¹Departamento de Fruticultura y Enología, Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile, Santiago, Chile; ²Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria, Centro di Ricerca per la Frutticoltura, Roma, Italy.

Abstract

In raspberry (*Rubus idaeus*), two groups of cultivars are known: once fruiting, where fruit occurs annually on floricanes, and repeat fruiting (remontant), where a second fruit crop occurs on primocanes in the late summer or fall. Primocane fruiting extends the harvest, resulting in higher yields. The development of primocane fruiting cultivars is a primary objective of current raspberry breeding programs. The phenotypic identification of this trait is not easy because there is a strong genotype/environment interaction, influenced by temperature and photoperiod. In addition, there is a gradual expression of the character. The objective of this research was to generate descriptive data to make the selection of this character in segregating populations more efficient. A description of the degree of primocane fruiting was performed for five raspberry genotypes, ranging from high primocane fruiting to no primocane fruiting. The genotypes studied in order of increasing primocane fruiting were: 'Meeker', 'Tulameen', 'Heritage', 'Autumn Bliss', and UC103 (advanced selection). Plant height was measured, together with number of nodes, number and development of lateral shoots, and number of fruits per lateral shoot. Days from planting to first flower were also measured. With these values, a 1-5 scale was developed that identified the different degrees of primocane fruiting. Additionally, the effects of a chill treatment (2°C for 30 days) applied to plants in the early stages of development in the same genotypes were studied. This treatment was effective only in 'Heritage'. These data have promoted progress in determining the type of inheritance of primocane fruiting in raspberry cultivars.

Keywords: primocane, floricanes, inheritance, remontancy, chilling

INTRODUCTION

The great dynamism showed by the raspberry crop in recent years has intensified research efforts aimed at developing techniques that allow off-season production and the expansion of the area under cultivation to warmer regions (Oliveira et al., 1996; Heide and Sønsteby, 2011). Studies about the flowering period have been based mainly on the environmental characteristics that affect this character; however, it has been observed that it is not only affected by the environment, but that there is also great diversity among cultivars and a strong genotype/environment interaction. This often results in a complex behavior, which is unpredictable and difficult to manage. As is generally known, red raspberry cultivars are divided into two groups. Floricane: are non-remontant cultivars with a biennial life cycle of the shoot, in which in the first year only a shoot with vegetative growth is produced, and in late summer and early autumn buds enter dormancy. Therefore, flowering and fruiting occur during the second year, when the shoot, or cane, already lignified restarts its development after the winter, and flowering and fruiting take place during the spring. Primocane: are remontant cultivars, which are characterized by their ability to flower both in the shoot of the season and in the cane or lignified shoot from the previous season. Thus, two crops are produced: in autumn and spring. Spring production comes from the lignified shoots that were developed in the previous season, while the autumn fruit comes from the shoots of the same season. Hence, remontancy can be defined as the ability of a plant to flower in late summer or in autumn on shoots that develop in the same growing season



(Keep, 1961; Heide and Sønsteby, 2011).

Currently, there are several breeding programs for this species, in which the remontancy trait is a priority (Hall et al., 2009). One of the main advantages of remontant cultivars is that they allow extending the harvest period and therefore can be more productive than non-remontant ones. Moreover, remontant cultivars are well suited for hot climate regions, with limited accumulation of chilling hours, which would affect the development of dormant buds in non-remontant cultivars (Pritts, 2008). In Chile there are about 12,000 ha cultivated with raspberry, distributed between 34.70° and 37.47° South latitudes, in foothill environments, where climate is cold in winter and hot in summer. Despite the importance of this crop, in the country there is an almost mono-varietal situation, 'Heritage' being the main cultivar grown. This cultivar is very hardy and well adapted to growing conditions in the area. It is also considered as remontant, as it fructifies both in spring and autumn. However, it is an ancient cultivar with very small fruit, and as the crop is cultivated northward with warmer climates, its remontant capacity decreases.

The above mentioned encouraged the development of a breeding program at Pontificia Universidad Católica de Chile, aimed mainly at replacing the 'Heritage' cultivar with a remontant genotype, more productive in different environments and with larger fruit. This program, based on intervarietal crosses and selection, is being carried out in the town of Santo Domingo (33.6° South latitude), located in an area with a temperate climate and maritime influence. Approximately 30 crosses are performed with 5,000 seedlings being evaluated annually. In these trial plots, a high degree of diversity was verified for the remontancy character, with widely varying responses in flowering habits.

Differences observed in this character are mainly due to different temperature and photoperiod requirements for the processes of floral induction and differentiation. In non-remontant cultivars, flower buds are induced under short days and when temperatures are below 13°C (Williams, 1960). Nevertheless, it has been observed that the effect of the photoperiod disappears when temperatures are lower during the summer. Sønsteby and Heide (2008) found that at temperatures below 12°C, under long-day conditions, buds can also be induced to flower; conversely, under temperatures of over 18°C and long-day conditions, induction is not produced.

Another feature of non-remontant cultivars is that shoots originating from the root undergo a period of "juvenility", since they need to achieve a certain development. It has been noticed that before reaching 15-20 nodes at least, they are not able to respond to low temperatures and short days so that flower induction occurs (Williams, 1960; Sønsteby and Heide, 2008). In these cultivars, growth continues until the end of the season, when growth slows down until it stops. At the same time, the highest apical and lateral buds are induced to flower and then go dormant before flowering takes place (Heide and Sønsteby, 2011). Additionally, for dormancy to come to an end, a period of low temperatures during several weeks is required (chilling).

On the contrary, in remontant cultivars, floral induction and differentiation occurs in the shoot of the season regardless of photoperiod and with high temperatures (28°C, and even 30°C for some cultivars). Sønsteby and Heide (2009) studied the effects of temperature and photoperiod on a remontant cultivar, 'Polka', in order to determine its behavior under protected cultivation, for the production of off-season fruit in Norway. Results indicated that 'Polka' did not need low temperatures for the process of flower induction and formation. The plant flowered at high temperatures (30°C) and days to anthesis were reduced with the increase in these temperatures.

Moreover, in remontant cultivars, no juvenility factor is observed as the response to conditions of floral induction occurs with a stage of development of 5 nodes (Sønsteby and Heide, 2009). Shoot development throughout the season does not stop, and floral induction takes place during the summer, long before days are shortened. Thus, floral development of the apex and higher axillary buds does not stop during the same season, resulting in flowering, fruit set, and fall fruit production.

To these two types of raspberry cultivars, some authors add a third one. This would be an intermediate, so-called "tip flowering" type (Ourecky, 1976; Carew et al., 2000; Dale,

2008; Neri et al., 2012). It is characterized by production of few flowers and fruits on the shoot tip at the end of the first growing season, while the rest of the buds will become flowers and fruits in the second season. This type of behavior can be observed in non-remontant and remontant cultivars, when subjected to certain growing conditions. An example of this was described by Dale and Daubeny (1987) in the non-remontant cultivar 'Glen Clova', which showed flower bud sprouting at the end of the season. These authors named it as "hidden remontant", as it developed flowers on the tip of the primocane at the end of the season.

However, this third type is not sufficient to describe what was observed in the segregating populations from intervarietal crosses evaluated as part of the breeding program of Pontificia Universidad Católica de Chile. In these populations, more gradual differences were observed in flower distribution along the shoot, and the period of beginning of flowering. It is likely that this greater variation in the character is due to the fact that under temperate climate conditions, an increased expression of the differences between genotypes is possible, which are masked when genotypes are cultivated in climates with higher thermal oscillation between day and night.

Several authors have proved that the type of flowering depends on cultivar and location, indicating that factors such as temperature and light strongly influence the time when flowering occurs. Influence of other abiotic factors, such as water and nutrient availability, has also been observed (Hoover et al., 1989; Privé et al., 1993).

At the time of the selection in segregating populations derived from intervarietal crosses, breeders face the difficulty of having no objective parameters to properly assess the character. In general, plants are classified only according to the two types already described, without considering that there are intermediate behaviors, with a gradual expression of the character. That is, those plants that on the shoot of the season have flowers and fruits, regardless of the quantity and location along the shoot, are classified as remontant. On the contrary, those plants that on the shoot of the season are not able to produce any type of flower are considered non-remontant. The strong environmental influence further interferes in the evaluation of the seedlings. Therefore, a more accurate analysis is necessary, together with the development of tools that help to better understand the responses of each plant, in order to maximize production results.

A common practice among growers is to expose plants to cold temperatures in their early stages of development in order to increase flowering in the shoot of the season in the case of the 'Heritage' cultivar. Apparently, this treatment would have a positive effect, especially when the plantation is established in warmer areas, but it has not been studied whether it is really effective and if it would have an effect on other cultivars.

The goal of this study was to establish a scale of degrees of remontancy by analyzing the expression of the character in selected raspberry cultivars. The selection included cultivars ranging from zero flowering to abundant flowering in the shoot of the season. In these same genotypes, an evaluation was made of the effect of low temperature treatments – applied in the early stages of development – on the flowering habit.

MATERIALS AND METHODS

Five raspberry cultivars with varying degrees of remontancy were chosen, according to descriptions in literature and previous observations in the same field where the trial took place. The cultivars were: 'Meeker', 'Tulameen', 'Heritage', and 'Autumn Bliss' (Jennings, 1988). Selection UC103, from the raspberry breeding program of Universidad Católica, was also included. This selection is remontant and characterized by a very abundant flowering along the shoot of the season.

The trial was established in the town of Santo Domingo, which has a Mediterranean climate with maritime influence, and average temperatures of 20°C in summer and 7°C in winter. Plants were established in a substrate cultivation system with 6.5-L pots and a mixture of 30% peat and 70% coconut fiber. Standard fertilization was applied weekly through irrigation, according to local production system.

To evaluate the effect of low temperatures on the five genotypes, chill treatments were

applied, which consisted in keeping the plants in cold dark chambers at 2°C, for 30 days continuously. This was done when the plants reached an average height of 6 cm. Upon completion of the treatment, plants were established in the field, together with the control plants that did not receive the cold treatment, in both cases, the plants had an average height of 6 cm.

Records of growth rate, by measuring the total height in cm, were kept for each plant throughout the season. Number of nodes, lateral shoots, and fruits per lateral shoot were also counted. Evaluations were carried out weekly from planting, in early November, until the end of the season, in April.

The experimental design was completely randomized, with an experimental unit of three plants, and four replications. The Statistix-9 program was used for the analysis.

RESULTS AND DISCUSSION

The analysis of the growth curve of the genotypes studied (Figure 1) shows that cultivars 'Autumn Bliss', UC103, 'Tulameen', and 'Heritage' have a similar curve, a simple sigmoid curve, with an accelerated growth period and a halt therein approximately in week 15-16 from planting, although there are significant differences in the final height they reach (Table 1). 'Meeker', instead, showed a longer growth period, which only slowed down at the end of the season, coinciding with a sharp drop in temperatures. The differences in height of the shortest cultivars would be caused by a lower average internode length.

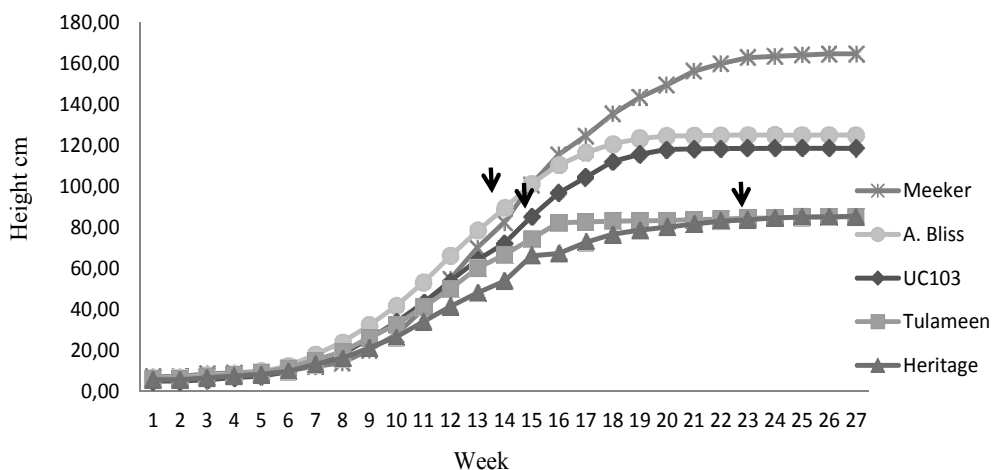


Figure 1. Growth curve of the five genotypes studied. (Arrows indicate the beginning of flowering).

Table 1. Phenotyping of the five genotypes evaluated.

Cultivar	Average height (cm)	No. of nodes	Height/no. of nodes ratio	No. of differentiated nodes
UC103	118.4 b	35.3 bc	3.4 b	18.1 a
Autumn Bliss	124.9 b	35.7 b	3.5 ab	10.7 b
Heritage	85.3 c	31.3 c	2.7 c	3.5 c
Meeker	164.4 a	45.2 a	3.6 a	0
Tulameen	85.0 c	32.3 bc	2.6 c	0

According to some authors, the halt in growth and flower initiation result from the same mechanism of internal induction. The continued growth of shoots would require high temperatures and long photoperiods (Sønsteby and Heide, 2008). In this case, the above would be verified in the 'Meeker' cultivar, in which growth was not halted and there was no flowering. However, in 'Tulameen', the growth curve did halt early, but there was no

flowering. This could demonstrate that environmental factors interact differently depending on the genotype, both in remontant and non-remontant cultivars.

Table 1 also shows the number of buds that developed side shoots with flowers, from the apex to the base of the main shoot. It is noted that among the 5 genotypes, only three had flower development. As expected, selection UC103 was the one with the greater number of differentiated buds (18.1), followed by 'Autumn Bliss' (10.7), and 'Heritage' (3.5), with these differences being significant. 'Tulameen' and 'Meeker' did not develop flowers. It is worth noting the low percentage of buds that flowered in 'Heritage', since this cultivar is normally classified as remontant. Nevertheless, under these conditions of mild temperatures, an erratic behavior, close to tip remontancy, is observed (Ourecky, 1976; Carew et al., 2000; Dale, 2008; Sønsteby and Heide, 2008).

Since the goal of this study was to establish parameters that can be used to differentiate genotypes in segregating populations, where many individuals are usually evaluated, results obtained were transformed to percentage of buds in the main shoot that reached flowering, and on this basis a scale of 1 to 5 was designed. Figure 2 shows a diagram of how flowers are distributed in each of these types of individuals, according to the following "degrees of remontancy": (1) non-remontant, (2) poorly remontant, (3) moderately remontant, (4) highly remontant, and (5) fully remontant, corresponding to 0, 1-20, 21-40, 41-60%, and greater than 60%, respectively. In the five genotypes studied, type 5 was not observed, but in the previous season, the UC103 selection could have classified in this type of flowering habit.

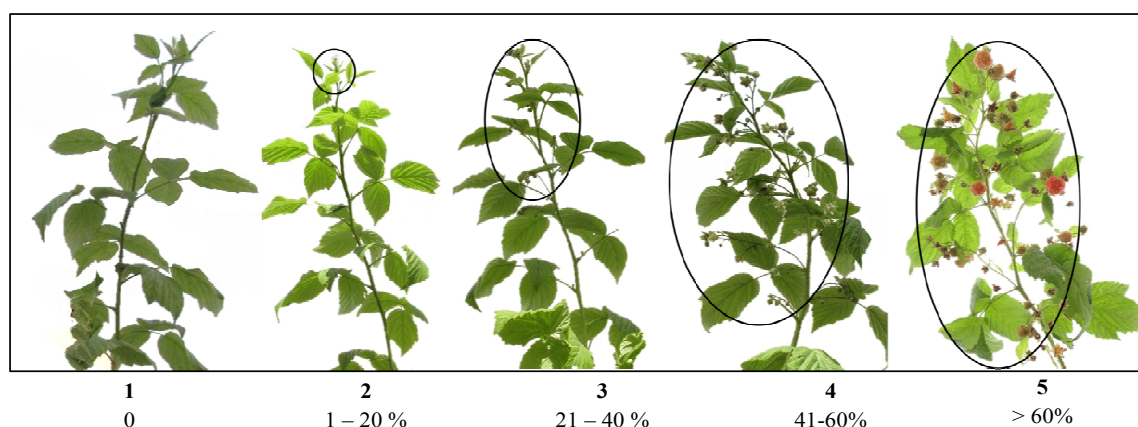


Figure 2. Remontancy scale according to percentage of flowering.

Regarding the effect of the chill treatment, Table 2 presents a comparison of the parameters evaluated in the same genotypes under both conditions, with and without a chill treatment. Results show that only in 'Heritage' there were statistically significant differences in all parameters, that is, height, number of nodes, and degree of differentiation. This cultivar, which according to our classification was at degree 2 (poorly remontant), with the chill treatment could be classified at degree 3. We can assume that the chilling induced a higher vigor to the growing shoots as it is when vernalisation is applied to seeds and the higher number of nodes which increased the fruiting capacity (the remontancy). In the other cultivars, no effect was observed of the cold treatment on the number of buds and so that did not change the number of buds which reached the flowering. However, in 'Meeker', an effect on plant height and number of nodes was observed, while 'Tulameen' only showed a difference in the number of nodes. Thus a higher production would be expected in the next spring in both florican cultivars. A microscopic bud analysis could reveal an effect at the differentiation level.

Table 2. Effect of low temperatures in seedlings on the number of differentiated nodes.

Parameter	Treatment	UC103	Autumn Bliss	Heritage	Meeker	Tulameen
Average height (cm)	Not cold	118.4 a	124.9 a	85.3 b	164.4 b	85.0 a
	Cold	118.9 a	118.0 a	120.5 a	205.7 a	102.1 a
No. of nodes	Not cold	35.3 a	35.7 a	31.3 b	45.2 b	32.3 b
	Cold	36.4 a	31.7 a	42.1 a	53.8 a	38.8 a
No. of side shoots	Not cold	18.3 a	10.7 a	3.5 b	0.0	0.0
	Cold	18.7 a	12.4 a	11.9 a	0.0	0.0

The statistical analysis was performed within the same cultivar to determine the effect of cold. See vertical comparison for each parameter.

CONCLUSIONS

The results obtained in this study showed that there is a gradual expression of the character, because significant differences were found between genotypes studied in relation to percentage of flowering in the shoot of the season. Based on these data, a scale of remontancy was defined, which can be used for the classification of individuals from segregating populations. This classification could help to develop frequency histograms that allow understanding the type of inheritance of this character.

On the other hand, these results show that 'Heritage' is a poorly remontant cultivar, and is responsive to cold treatments applied in the initial stages of plant development. Apparently, cultivars with a high degree of remontancy do not respond to these treatments.

ACKNOWLEDGEMENTS

Thanks to 08CT11PUD-14 Project, "Raspberry Breeding Program" of Innova-Corfo and Comisión Nacional de Investigación Científica y Tecnológica, Scholarship Program, Formación de Capital Humano Avanzado, for financial support.

Literature cited

- Carew, J.G., Gillespie, T., White, T., Wainwright, H., Brennan, R., and Battey, N.H. (2000). The control of the annual growth cycle in raspberry. *J. Hortic. Sci. Biotechnol.* 75 (5), 495-503 <http://dx.doi.org/10.1080/14620316.2000.11511275>.
- Dale, A. (2008). Raspberry production in greenhouses: physiological aspects. *Acta Hortic.* 777, 219-225 <http://dx.doi.org/10.17660/ActaHortic.2008.777.32>.
- Dale, A., and Daubeny, H.A. (1987). Flower-bud initiation in the raspberry (*Rubus idaeus* L.) in two environments. *Crop Res.* 27, 61-66.
- Hall, H., Hummer, K., Jamieson, A., Jennings, S., and Weber, C. (2009). Raspberry Breeding and Genetics. In *Plant Breeding Reviews: Raspberry Breeding and Genetics*, Vol. 32, J. Janick, ed. (USA), p.39-353.
- Heide, O., and Sønsteby, A. (2011). Physiology of flowering and dormancy regulation in annual - and biennial-fruited red raspberry (*Rubus idaeus* L.) - a review. *J. Hortic. Sci. Biotechnol.* 86 (5), 433-442 <http://dx.doi.org/10.1080/14620316.2011.11512785>.
- Hoover, E., Luby, J., Bedford, D., Pritts, M., Hanson, E., Dale, A., and Daubeny, H. (1989). Temperature influence on harvest date and cane development of primocane-fruited red raspberries. *Acta Hortic.* 262, 297-304 <http://dx.doi.org/10.17660/ActaHortic.1989.262.43>.
- Jennings, D.L. (1988). *Raspberries and Blackberries: Their Breeding, Diseases and Growth* (London, UK: Academic Press).
- Keep, E. (1961). Autumn-fruited in raspberries. *J. Hortic. Sci.* 36 (3), 174-185 <http://dx.doi.org/10.1080/00221589.1961.11514013>.
- Neri, D., Massetani, F., Zucchi, P., Giacomelli, M., and Savini, G. (2012). Flower differentiation and plant architecture of raspberry, blackberry and current. *Acta Hortic.* 926, 243-250 <http://dx.doi.org/10.17660/ActaHortic.2012.926.33>.
- Oliveira, P., Oliveira, C., Lopez-da-Fonseca, L., and Monteiro, A. (1996). Off-season production of primocane-fruited red raspberry using summer pruning and polyethylene tunnels. *HortScience* 31 (5), 805-807.

Ourecky, D.K. (1976). Fall-bearing red raspberries, their future and potential. *Acta Hortic.* 60, 135–144 <http://dx.doi.org/10.17660/ActaHortic.1976.60.18>.

Pritts, M. (2008). Primocane-fruiting raspberry production. *HortScience* 43 (6), 1640–1641.

Privé, J., Sullivan, J., Proctor, J., and Allen, O. (1993). Climate influences vegetative and reproductive components of primocane-fruiting red raspberry cultivars. *J. Am. Soc. Hortic. Sci.* 118 (3), 393–399.

Sønsteby, A., and Heide, O. (2008). Environmental control of growth and flowering of *Rubus idaeus* L. cv. Glen Ample. *Sci. Hortic. (Amsterdam)* 117 (3), 249–256 <http://dx.doi.org/10.1016/j.scienta.2008.05.003>.

Sønsteby, A., and Heide, O. (2009). Effects of photoperiod and temperature on growth and flowering in the annual (primocane) fruiting raspberry (*Rubus idaeus* L.) cultivar 'Polka'. *J. Hortic. Sci. Biotechnol.* 84 (4), 439–446 <http://dx.doi.org/10.1080/14620316.2009.11512546>.

Williams, H. (1960). Effects of environment on *Rubus idaeus* L. V. Dormancy and flowering of the mature shoot. *J. Hortic. Sci.* 35 (3), 214–220 <http://dx.doi.org/10.1080/00221589.1960.11513985>.

