Carbon allocation during fruiting in *Rubus chamaemorus*

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Received: 17 December 2008 Returned for revision: 16 February 2009 Accepted: 27 April 2009 Published electronically: 10 June 2009

• **Background and Aims** *Rubus chamaemorus* (cloudberry) is a herbaceous clonal peatland plant that produces an extensive underground rhizome system with distant ramets. Most of these ramets are non-floral. The main objectives of this study were to determine: (a) if plant growth was source limited in cloudberry; (b) if the non-floral ramets translocated carbon (C) to the fruit; and (c) if there was competition between fruit, leaves and rhizomes for C during fruit development.

• **Methods** Floral and non-floral ramet activities were monitored during the period of flower and fruit development using three approaches: gas exchange measurements, 14C labelling and dry mass accumulation in the different organs. Source and sink activity were manipulated by eliminating leaves or flowers or by reducing rhizome length.

• **Key Results** Photosynthetic rates were lower in floral than in deflowered ramets. Autoradiographs and 14C labelling data clearly indicated that fruit is a very strong sink for the floral ramet, whereas non-floral ramets translocated C toward the rhizome but not toward floral ramets. Nevertheless, rhizomes received some C from the floral ramet throughout the fruiting period. Ramets with shorter rhizomes produced smaller leaves and smaller fruits, and defoliated ramets produced very small fruits.

• **Conclusions** Plant growth appears to be source-limited in cloudberry since a reduction in sink strength did not induce a reduction in photosynthetic activity. Non-floral ramets did not participate directly to fruit development. Developing leaves appear to compete with the developing fruit but the intensity of this competition could vary with the specific timing of the two organs. The rhizome appears to act both as a source but also potentially as a sink during fruit development. Further studies are needed to characterize better the complex role played by the rhizome in fruit C nutrition.

**Key words:** Allocation pattern, 14C labelling, carbon translocation, carbon reserves, cloudberry, defoliation, fruit production, gas exchange, *Rubus chamaemorus*, source–sink relationship, flowering.

**INTRODUCTION**

There are many causes for fruit abortion in plants. Some causes are external such as insufficient pollen load, damage during fruit formation or water stress (Stephenson, 1981). There are also internal causes such as a lack of resources within the plant leading to competition between the different sinks, i.e. between organs that import and use assimilates (Stephenson, 1981). In cloudberry, a herbaceous perennial peatland plant species that produces an edible polydrupe, external causes of fruit abortion have been identified: insufficient pollen load under adverse meteorological conditions and spring frost that kills most flowers (Kortesharju, 1995). Both factors lead to very early abortion. However, abortion during fruit development is also frequently observed in this species, suggesting a lack of resources to sustain complete fruit development. Fruit formation can be costly in terms of carbon (C) needs. Several sources can help to meet fruit demand: the C fixed by leaves and fruit pericarp (Blanke and Lenz, 1989; Wardlaw, 1990; Lebon et al., 2005), the C translocated from storage organs (Chapin et al., 1990) and, in the case of clonal plants, the C translocated from other ramets (Newell, 1982). Thus, the presence of a developing fruit can modulate both the activity of the sources and the activity of the other sinks.

Leaf photosynthetic rates ($P_n$) can be modulated by the number of sinks and/or sink activity, in other words by growth rate of the sinks or rate of C accumulation within the sinks. Under high light conditions, the presence of fruit increases $P_n$ in several species such as *Agropyron repens*, *Malus domestica*, *Coffea arabica* and *Prunus persica* (Reekie and Bazzaz, 1987; Palmet et al., 1997; Franck et al., 2006; Li et al., 2007). However, in other species, an increase in fruit sink activity does not result in increased $P_n$ (Primack et al., 1994; Proietti, 2002; Egli and Bruening, 2003). In such species, the leaves might already be photosynthesizing at maximum capacity due to the presence of strong sinks in which case plant growth is source-limited; or the plant might have accumulated large amounts of photosynthates in storage organs earlier in the season (Herold, 1980; Wardlaw, 1990), and thus $P_n$ cannot increase because of a low translocation rate between the leaves and the developing fruit.

C reserves can be used to support C fruit demand (Wardlaw, 1990; Watson, 2008). Reserves often build up in perennials toward the end of the season (Chapin et al., 1990), but they can also be stored earlier in the season, and used during flowering and fruit formation (Wardlaw, 1990; Kool et al., 1996).
The extent to which C reserves are used to support fruit development under normal growth conditions in perennial species appears to vary between growth habits (Wardlaw, 1990; Chapin et al., 1990).

In cloudberry, the investment in rhizomes is large as these remain functional for many years whereas the allocation to fruit production is small (Mäkinen and Oikarinen, 1974; Dumas and Maillette, 1987). Rhizomes are slender. There is an average of one ramet every 1.2 m of rhizomes, and the majority of those are non-florals (Jean and Lapointe, 2001). Each ramet carries one to three leaves 2–5 cm long and 3–7 cm wide (Taylor, 1971), and floral ramets carry a single flower. The species is dioecious and female ramets thus carry a single fruit composed of 1–14 drupelets (Jean and Lapointe, 2001). Since ramets are often distant from each other, the amount of C translocated towards the fruiting ramets may be small (Chapman et al., 1991). Jean and Lapointe (2001) have shown that a non-floral ramet near a floral ramet which was defoliated, contributes to the development of the fruit. Fruit production in cloudberry could be partly supported by the translocation of carbohydrates from non-floral ramets to floral ramets, but the impact of the distance on the amount of C translocated still remains to be tested. Study on another perennial Rubus species, the raspberry (R. idaeus), suggests very limited C translocation from primocanes (vegetative) to fruiting canes (Fernandez and Pritts, 1993). The C reserves of the rhizome could also contribute to fruit development as longer rhizomes support larger fruits (Jean and Lapointe, 2001). However, since these reserves are low in early spring and replenished early in the season, there might also be some competition between rhizomes and fruit for the newly fixed C. Finally, cloudberry flowers open before the leaves unfold whereas fruit formation is initiated before leaves reach maturity. That suggests a possible competition between the leaves and fruit for resources. Such competition early in the season has already been reported for R. ussuriensis, a non-invasive species, but not in R. discolor, an invasive species for which the two phases are temporally separated ensuring more resource availability during fruiting (McDowell and Turner, 2002). The short growing season between flowering and fruit harvesting in cloudberry could indeed exacerbate competition for C between the different developing sinks. All these factors could explain the low fruit yield in cloudberry (Agren, 1988a).

The main objectives of the present study were: (a) to determine if plant growth is source limited in cloudberry; (b) to quantify the amount of C in the fruit that comes from the floral and the non-floral ramets during fruit development; and (c) to determine if there is competition for C between developing leaves, developing fruit and the rhizome. Source limitation of plant growth was tested by comparing the photosynthetic rates of floral and non-floral or deflowered ramets during fruit development. If plant growth is source limited, reducing the number of sinks would not induce a decrease in photosynthetic rates. $^{13}$C labelling of either a floral or a non-floral ramet in clones carrying at least one fruiting ramet was used to estimate the proportion of the C translocated to the fruit that comes from the non-floral ramet and the influence the distance between ramets has on this proportion. Competition between the different sinks was assessed by modulating the strength of the different sinks. Therefore, plants were either defoliated, deflowered, or their rhizome length reduced by cutting it at some length from the ramet. Shorter rhizomes were expected to represent a weaker sink than control rhizomes due to their smaller C storage size. The evolution of biomass of the three organs along with rhizome carbohydrate content was assessed to estimate the sink strength of each of these organs throughout the growing season. This study should further our understanding of the role of the different C sources during fruit development in clonal species such as cloudberry, and the complex role that the rhizome plays in the source–sink relationship in clonal species.

MATERIALS AND METHODS

Experimental field site

The experimental site was located at Longue-Rive, Québec, Canada (48°57′N, 69°23′W). Mean annual temperature in the area (data from Forestville, 48°44′N, 69°5′W) is 2.6 °C, January being the coldest month with −14 °C and July the warmest with 18 °C based on measurements from 1971 to 2000 (Environment Canada, 2008). Historical total annual precipitation averages 1084 mm, of which 769 mm falls as rain. Experiments were set up on a section of the peatland prepared for peat moss extraction. After removing the above-ground vegetation, ditches were dug creating a sector of homogeneous vegetation, ditches were dug creating a sector of homogeneous peat on either side of the ditch. In the process, deep cloudberry (Rubus chamaemorus L.) rhizomes were brought near the surface while rhizomes of ericaceous species (mainly Kalmia angustifolia, Vaccinium angustifolium and Chamaedaphne calyculata) were turned under too deeply to survive. The development of cloudberry took place without great competition and thus produced more ramets than is common in natural peatland. The substrate in which cloudberry was growing along these ditches was a partly decomposed peat of H2–H3 type on the von Post scale (Parent, 2001).

Ramet development started around 5 June (0 d after the beginning of flowering (DAF)) in 1999, around 10 June (0 DAF) in 2000 and around 11 June in 2004 (0 DAF). Fruit formation was completed around 12 July (37 DAF) in 1999, i.e. when the fruit turned orange and started to abscise, around 15 July (35 DAF) in 2000, and around 25 July in 2004. In 2000, fruit growth was delayed by about 1 week because of several severe frosts in early June.

Experiment 1: assessing leaf photosynthetic activity

Every week throughout the period of fruit development, including 1 week after fruit abscission, eight floral and eight non-floral ramets were randomly selected in 1999. Gas exchange was measured on one of the leaves of each selected ramet with a portable infrared gas analyser (LCA-4 equipped with the broad leaf chamber PLC4B; ADC, Haddeston, UK). These measurements were recorded hourly between 0900 h and 1700 h on cloudless days. Maximum daily photosynthetic rates ($P_{\text{max}}$) were usually observed between 1200 and 1300 h. Air temperature recorded by the portable infrared gas analyser varied between 21.2 ± 0.1 and 28.5 ± 0.2 °C. Stomatal
conductance ($g_n$) and internal CO$_2$ concentration (C$_i$) were recorded at the same time as maximum $P_{n}$. These measurements were repeated in 2000 with the following modifications: only floral ramets were selected and the flowers on half of them were removed at the beginning of the flowering period. This ensured that all measurements were made on female clones, and that the only difference between the two ramets was the presence of a flower. Air temperatures during gas exchange measurements in 2000 varied between $21.3 \pm 0.1$ and $28.9 \pm 0.1$ °C.

Experiment 2: estimation of translocation of photosynthates by $^{14}$C labelling

Experimental design. In 1999, ten floral and non-floral ramets were selected every week throughout the reproductive period (4–45 DAF). The non-floral ramets were dug up to make sure they were connected to at least one identified floral ramet ($^{13}$C NF–F), and the rhizome length between the floral and non-floral ramets was measured. The selected floral ramets ($^{14}$C F) were not dug up before the labelling and could thus have been inter-connected to other floral or non-floral ramets. The leaf diagonal was measured and the individual leaf area was estimated to be $0.4713 \times \text{leaf diagonal}^{2.0021}$. Ramet leaf area is the sum of the individual leaf areas on a ramet. The selected ramets were labelled with $^{14}$C. There were seven labelling dates; the first (4 DAF) occurred when the flower was opening and the two last (37 and 45 DAF) occurred after the fruit harvest.

Each selected ramet (floral or non-floral) was hermetically enclosed in a 3.7-L bag. A small plastic cup containing the sodium bicarbonate solution (total concentration of 60 mM from 10 mg of cold bicarbonate solution and 1 μCi of NaH$^{14}$CO$_3$) was attached to the inside of the bag, at leaf height. Lactic acid was injected, through the bag, into the sodium bicarbonate solution to allow the vaporization of CO$_2$ (final gaseous CO$_2$ concentration: 0.2; final specific activity: 10 μCi mmol$^{-1}$ of CO$_2$). After 2 h of incubation the bags were removed. Labelling occurred between 1100 h and 1400 h on sunny days.

The fruits carried by $^{14}$C F and by $^{14}$C NF–F were harvested as they ripened during the season. Out of the ten $^{14}$C F and ten $^{14}$C NF–F that were labelled each date for the first five labelling dates, only those that carry a mature fruit were analysed further ($n = 4–10$). Rhizomes of $^{14}$C F and $^{14}$C NF–F from the seven labelling dates were harvested in late September 1999, after complete leaf senescence. The whole rhizome system connected to the $^{14}$C-labelled ramet (five per ramet type, per date) were dug and harvested, including the current-year shoots. All samples were stored at $-20$ °C until autoradiography or radiolabelling counts were performed.

Autoradiography and liquid scintillation counting. For each of the seven dates of labelling, the two longest rhizomes were dried at $70$ °C for 48 h and placed in contact with an X-ray film for 2 months before being developed. As rhizomes were very long, they had to be cut at different lengths to fit one or two X-ray films. The position of the rhizomes and ramets was carefully drawn on transparencies in order to be able to redraw the whole clone on top of the autoradiography. After autoradiography was completed, 3-cm sub-samples were taken on each branching segment of the rhizome. The distance between each sub-sample and the labelled ramet was noted. The berries were dried at 70 °C for 48 h; both fruits and rhizome sub-samples were then weighed.

All the fruit and rhizome sub-samples were counted for $^{14}$C activity. They were subjected to a wet combustion procedure modified from the one described by Clifford et al. (1973). Briefly, 5 mL of 0.25 N NaOH were added to a scintillation vial of 20 mL and a smaller vial of 5 mL was inserted into the larger one. In the smaller vial, 2 mL of chromic acid were added to the sample. Samples were incubated at 60 °C for 3 h. The contact between acid and samples at high temperature induced the vaporization of $^{14}$C as $^{14}$CO$_2$. The gaseous $^{14}$CO$_2$ was solubilized and trapped by the NaOH. To prevent the escape of gaseous $^{14}$CO$_2$, the scintillation vials were tightly closed with Teflon caps. They were then placed at 4 °C overnight to capture all $^{14}$CO$_2$ in the NaOH solution. Samples were prepared for $^{14}$C counting by mixing 5 mL of the NaOH solution with 15 mL of a commercial scintillation cocktail (Ecolume, ICN) in a 20 mL glass scintillation vial. The samples were counted in a scintillation counter (LKB-Wallac, 1217 Rackbeta) after 4 d in the dark to suppress chemiluminescence and the counts corrected with a quench curve.

Plots of becquerel (Bq) as a function of distance along each rhizome segment were drawn and total radioactivity per rhizome was estimated based on the integral of the curve for each rhizome segment using Sigmaplot 8.02 software (Systat Software, Inc, Point Richmond, USA).

Experiment 3: dry mass and rhizome carbohydrate accumulation

Experimental design. Six hundred floral ramets were randomly selected at flower bud stage (0 DAF) in 2004 in the same field site and 60 of these were harvested. Afterwards, half of the remaining ramets were deflowered (270 deflowered plants). Ramets were assigned to pre-established harvesting dates (30 ramets per ramet type per date). Every fifth day, control and deflowered ramets were harvested and the flower or fruit, leaves and 10 cm of the subtending rhizome were cut. Only control ramets with an undamaged flower or a developing or mature fruit were harvested ($n = 13–30$).

On the same site, 150 floral ramets were selected randomly at flowering (0 DAF) and their rhizome was dug and cut 40 cm away from the floral ramets. Thirty of these 40-cm rhizome ramets were then harvested. One week later, when the leaves were unfolding, half (for a total of 60) of the 40-cm rhizome ramets were defoliated. Ramets were assigned to pre-established harvesting dates (15 ramets per ramet type per date). Every tenth day, 40-cm rhizome ramets and defoliated 40-cm rhizome ramets were harvested. Only ramets with an undamaged flower or a developing or mature fruit were harvested ($n = 3–12$).

For each harvest, ramet leaf area was estimated and flower or fruit, leaves and rhizome were weighed and dried at 60 °C for 24 h. The gain of dry mass of each organ was obtained after subtraction of the mean initial value (at 5 DAF). This was not done for the control and deflowered ramets as only the first 10 cm of rhizome was harvested on these rhizomes.
Rhizome starch concentration. Starch concentration in the rhizomes was estimated by the method described in Jean and Lapointe (2001). Four samples were analysed per ramet type, per date, unless fewer were available (n = 3 or 4). Briefly, samples were ground with a Polytron (Kinematica, Switzerland) after 20 min of maceration in a mixture of methanol : chloroform : water, (12 : 5 : 3, v/v/v) at 65 °C. Samples were centrifuged, the pellet was resuspended in water and then heated at 100 °C for 90 min to gelatinize starch. It was then incubated at 55 °C for 1 h in the presence of amylloglucosidase. Reducing sugars were then measured colorimetrically at 415 nm after reaction with p-hydroxybenzoic acid hydrazide.

Statistical analyses

The gas exchange data (Pₙ, gₛ, and Cᵢ) were analysed by MANOVA with ramet type and date of measurement as factors, followed by individual ANOVAs. Radioactivity in the fruit and rhizome were analysed with a two-way factorial ANOVA with ramet type and labelling date as factors. Their respective dry masses were analysed with the same model. Radio-labelled specific activity of the rhizome was compared with that of the fruit during the fruiting period (five first labelling dates) using a two-way factorial ANOVA with organ type and harvest time as factors. Dry mass of leaves, rhizome and fruit of 40-cm rhizome ramets were compared with a two-way factorial ANOVA with organ type and harvest time as factors. Correlation coefficients were estimated between final fruit dry mass and either ramet leaf dry mass or ramet leaf area using data from expts 2 and 3. Data were transformed when needed so that the residues were normally distributed and the variances were homogeneous between groups. When ANOVAs indicated statistical differences, means of the different groups were compared using LSD tests. All analyses were performed using the GLM procedure of the SAS statistical package (SAS Institute, Cary, NC, USA).

RESULTS

Experiment 1: assessing leaf photosynthetic activity

The MANOVA indicated significant differences between date, ramet type and a significant interaction date × ramet type in 1999; in 2000 the interaction was marginally non-significant (P = 0.068; Table 1) but the individual factors still influenced significantly the gas exchange data. Thus individual ANOVAs for Pₙ, gₛ, and Cᵢ were conducted.

Pₙ evolved differently in 1999 and 2000 (Fig. 1A, B and Table 1). Whereas Pₙ increased during fruit formation in 1999 and decreased following fruit harvest, the response was less variable in 2000. The gₛ followed a similar trend as Pₙ both in 1999 and in 2000 (Fig. 1C, D and Table 1) except after fruit harvest in 1999. In 1999, Cᵢ increased after fruit harvest (Fig. 1E), whereas in 2000 Cᵢ remained relatively constant except for the second data point in the deflowered ramets (Fig. 1F). Ambient CO₂ concentrations were more or less constant during measurements in both years, around 370 μmol mol⁻¹ (data not shown). Thus, changes in Pₙ over time did not seem to be related to water stress since Cᵢ remained fairly constant during the season or even increased when Pₙ was decreasing (last measures of 1999).

In 1999, floral and non-floral ramets exhibited similar Pₙ and gₛ (Fig. 1A and C and Table 1). In 2000, Pₙ was lower in the floral ramets than in the deflowered ramets (Fig. 1B and Table 1) whereas gₛ values did not differ statistically (P = 0.07) but tended also to be lower in floral ramets than in deflowered ramets (Fig. 1D). Cᵢ differed between the two ramet types only for a single date each year (Fig. 1E, F).

Experiment 2: estimation of translocation of photosynthates by ¹⁴C labelling

At 4 DAF, when the flowers were opening, translocation of ¹⁴C was similar in ¹⁴C F and ¹⁴C NF–F (data not shown). At 26 DAF the radiolabelled C from the ¹⁴C NF–F continued to spread to the whole rhizome (Fig. 2A) whereas the radiolabelled C from the ¹⁴C F did not extend beyond the first branching point, so that the labelled rhizome length was 10–30 cm at the most (Fig. 2B). At 37 DAF, just after the fruit was harvested (33

<table>
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<th>Year, Source</th>
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<th>d.f.</th>
<th>Pₙ</th>
<th>P</th>
<th>gₛ</th>
<th>P</th>
<th>Cᵢ</th>
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* The P value reported is from the l.s.d. Significant values are in bold.

Table 1. Results of two-way MANOVAs and individual two-way ANOVAs for the effect of date and ramet type of Rubus chamaemorus (floral and non-floral ramets in 1999, floral and deflowered ramets in 2000) on Pₙ, gₛ and Cᵢ (n = 5 or 6 in 1999 and n = 8 in 2000)
DAF), a similar pattern to that of the first week (4 DAF) was observed: radiolabelled C moved throughout the whole rhizome without differentiation between \(^{14}\text{C F}\) and \(^{14}\text{C NF–F}\) (Fig. 2C, D). Over the entire labelling period, the C fixed by the non-floral ramets never translocated towards the floral (Fig. 2C, D). Over the entire labelling period, the C fixed by \(^{14}\text{C NF–F}\) was much higher than the activities measured in fruit \(^{14}\text{C F}\). Specific activity did not vary between dates \((F_{6,29} = 1-1, P = 0.36)\) but dry mass of the rhizomes attached to \(^{14}\text{C NF–F}\) was greater than that of the rhizomes attached to \(^{14}\text{C F}\) \((F_{1,29} = 5-3, P = 0.03;\) Fig. 3D).

Specific activity of the fruit in \(^{14}\text{C F}\) \((13649 \pm 2773\text{ Bq g}^{-1})\) was significantly higher than that of the rhizome \((759 \pm 184\text{ Bq g}^{-1})\) during the fruit-bearing period (ramet type \(\times\) organ: \(F_{1,105} = 10-1, P = 0.002\)). In \(^{13}\text{C NF–F}\), rhizome \((322 \pm 118\text{ Bq g}^{-1})\) and fruit specific activity \((653 \pm 170\text{ Bq g}^{-1})\) did not statistically differ during fruiting.

**Experiment 3: dry mass and rhizome carbohydrate accumulation**

**Dry mass accumulation.** Leaf dry mass increased in control, deflowered and 40-cm rhizome ramets up to 25 DAF and then remained fairly constant (Fig. 4A and Table 2). Leaf dry mass did not statistically differ between control and deflowered ramets but both were higher than for 40-cm rhizome ramets.

Fruit growth lasted as long as leaf growth in 40-cm rhizome ramets, i.e. 25 DAF, whereas it continued up to 35 d in control ramets, thus lasting longer than leaf growth in control ramets (Fig. 4B and Table 2). Fruit of defoliated 40-cm rhizome ramets remained small (final mass of 23 \pm 20 mg) compared with the fruit of the foliated 40-cm rhizome ramets \((67 \pm 8\text{ mg})\). Control ramets produced the largest fruits of all treatments \((139 \pm 16\text{ mg})\).

Rhizomes accounted for most of the dry mass whereas leaves had intermediate values and fruit the least dry mass in 40-cm rhizome ramets (data not shown). After having subtracted the initial dry mass of each organ, gain of dry mass was similar for the rhizome and the leaves up to 25th DAF (Fig. 5 and Table 2). Then the rhizomes continued to grow, while leaves slightly decreased (but overall, differences in mass gain were not statistically significant between rhizomes and leaves). The rate of increase of fruit dry mass was similar to those of rhizome and leaf mass between 15 and 25 DAF, but since the initial increase in fruit mass was low (between 5 and 15 DAF), its final mass was also lower than that of the leaf or the rhizome.

There were significant correlations between final fruit mass and ramet leaf area and between final fruit mass and ramet leaf mass (Fig. 6). Data from both exp 2 \(^{13}\text{C F ramets, last labelling date} and exp 3 (control and 40-cm rhizome ramets) were included in these analyses.

**Rhizome carbohydrate accumulation.** At bud opening (5 DAF), rhizome starch concentration was low, \(21 \pm 3\text{ mg g}^{-1}\), in all ramet types. It then increased up to 35 DAF in all ramet
types except in defoliated 40-cm rhizome ramets where, as expected, starch concentration remained relatively low throughout the season at around $28 \pm 11 \text{ mg g}^{-1}$ (Fig. 7 and Table 2). Final starch concentration was significantly higher in the rhizomes of the 40-cm rhizome ramets than in those of the deflowered ramets, and slightly higher than in the rhizomes of the control plants.

**DISCUSSION**

Fruit C demand in cloudberry did not stimulate $P_n$. This suggests that $P_n$ was already maximal and could not be increased further. Results from other perennial *Rubus* species are variable, with higher $P_n$ in fruiting than in deflowered canes in the biennial cane raspberry cultivar (Fernandez and
Pritts, 1994), variable through time in presence of fruit in a primocane fruiting cultivar (Privé et al., 1997), not affected by the presence of a fruit in R. discolor or reduced in presence of a fruit in R. ursinus (McDowell and Turner, 2002). Plant growth thus appears to be source limited in cloudberry. In fact, $P_n$ even decreased in fruiting ramets compared with deflowered ramets, as reported previously in this species (Johansson, 1974a), but the reduction in $P_n$ in the floral ramets over time, compared with non-fruit-bearing ramets, could be related to a significant reduction in foliar phosphorus concentration during fruit development (Gauci, 2008). Phosphorus concentration decreased in leaves during fruit development and even more so in fruit aborting ramets than in fruiting ramets (Gauci, 2008). Ramet leaf area is another factor that could influence source strength, besides $P_n$. Fruit mass was indeed correlated with floral leaf area and leaf mass. However, these correlations were weak and suggest that the relationship between fruit and leaf size is complex.

Fruit development in cloudberry appeared to rely extensively on the photosynthates of the floral ramets. During fruit formation, floral ramets translocated C to both fruit and rhizome, but the translocation to the rhizome was restricted to the few proximate centimetres and fruit specific activity was higher than that of the rhizome suggesting that the fruit was a stronger sink for the floral ramet than the rhizome. The presence of a developing fruit thus exerted a strong influence on the pattern of C translocation of the floral ramets as shown in red raspberry (Fernandez and Pritts, 1993; Privé et al., 1994) and in other species such as Malus domestica, Pyrus pyrifolia and Coffea arabica (Hansen, 1967; Teng et al., 2002; Vaast et al., 2005).

Photosynthates of the non-floral ramets were translocated to the whole rhizome system, regardless of the presence of a developing fruit on an adjacent ramet along the same rhizome. Only low levels of $^{14}$C were translocated from non-floral ramets to fruit irrespective of the distance, indicating that current non-floral ramet photosynthates are not actively involved in fruit production. Translocation follows different patterns in different plant species (Ashmun et al., 1982; Price et al., 1992), and some of the limitations most probably rely on anatomical restrictions as already reported in red raspberry (Privé et al., 1994). However, non-floral ramets could play an indirect role in fruit production in cloudberry as already suggested in red raspberry (Fernandez and Pritts, 1996) by allowing C reserves to accumulate during the season while the floral ramet is supporting fruit development. Furthermore, the division of labour between floral and non-floral ramets might partly alleviate C limitation of fruit growth.

![Fig. 3. (A, C, E) Fruit and (B, D, F) rhizome total $^{14}$C activity (A, B), dry mass (C, D), and specific activity (E, F) as a function of the date of $^{14}$C labelling (in DAF) of either the non-floral ($^{14}$C NF–F) or the floral ($^{14}$C F) ramet of Rubus chamaemorus (mean ± s.e). Fruit development began around 7 DAF, and fruit harvest (FH) occurred at 33 DAF. All fruits were harvested at maturity and rhizomes were harvested after complete leaf senescence ($n = 2–4$ for rhizome counts; $n = 4–10$ for fruit counts).](https://academic.oup.com/aob/article-abstract/104/4/703/152812)
There appears to be competition between leaf and fruit during the initial phase of cloudberry fruit development as suggested previously (Ågren, 1988b). Since leaves become a source organ only after they are 30–60% fully expanded (Turgeon, 1989), C from the leaves could not begin to be translocated toward the fruit before 12 DAF, at which time, the first signs of fruit development are visible to the naked eye (pers. obs.). Leaf development continued up to 30 DAF, and fruit abortion could be observed throughout this period (15–30 DAF; Jean and Lapointe, 2001). Since leaf growth rate and final size were not smaller in the fruiting ramets than in defoliated ramets, it appears that the presence of a fruit did not affect leaf C investment. However, smaller leaf mass in fruiting than in defoliated ramets has also been reported in Rubus chamaemorus (Ågren, 1989). Since leaves become a source organ only after they are 30–60% fully expanded (Turgeon, 1989), C from the leaves could not begin to be translocated toward the fruit before 12 DAF, at which time, the first signs of fruit development are visible to the naked eye (pers. obs.). Leaf development continued up to 30 DAF, and fruit abortion could be observed throughout this period (15–30 DAF; Jean and Lapointe, 2001). Since leaf growth rate and final size were not smaller in the fruiting ramets than in defoliated ramets, it appears that the presence of a fruit did not affect leaf C investment. However, smaller leaf mass in fruiting than in defoliated ramets has also been reported in cloudberry (Ågren, 1989). The intensity of the competition early in the season between fruit and leaves might vary depending on the exact phenology and size of the two organs and explain the weak correlation observed between total leaf area of the floral ramet and final fruit size.

As reported previously, defoliated ramets with a short rhizome could carry a fruit, although this fruit was much smaller than in foliated ramets of similar rhizome length (Jean and Lapointe, 2001). However, Ågren (1989) showed
at flowering in the first study (Jean and Lapointe, 2001) and at early flowering in the second study (Ågren, 1989); that could also explain a differential impact on fruit abortion (Garcia and Ehrléén, 2002; Knight, 2003; Zhu et al., 2004). Rhizome length also influenced fruit size in accord with previous published data (Jean and Lapointe, 2001). However, in the present study, it was shown that shorter rhizomes produced both smaller ramets and smaller fruits. Therefore, the impact of a shorter rhizome might be on both ramet and fruit size via a reduction in C reserves available early in the season, or on ramet size only, with an impact on fruit size via a reduction in leaf area.

However, since the rhizome received significant amounts of C during fruit development, it might behave also as a competing sink for the developing fruit. C storage in the perennial root system has also been identified as a major competing sink for the developing fruits in red raspberry and could partly explain the low fruit yield observed in this other Rubus species (Fernandez and Pritts, 1994). At the beginning of the season, rhizome carbohydrate reserves were low (15 mg g⁻¹). Marks (1978) and Kaurin et al. (1981) also reported significant starch depletion over the winter in cloudberry, followed by a further depletion to sustain the initial growth of the shoot (Marks, 1978; Gauci, 2008). Therefore, rhizome demand for C was high early in the season but, according to the ¹⁴C-labelled pattern, this was maintained throughout the season as reported previously (Johansson, 1974b). Once C reserves were partly replenished, the growth of new rhizomes was initiated (17 DAF; pers. obs.). Therefore, the rhizome remains a large sink throughout the season at least at the clone scale. However, it is still unresolved if the rhizome behaves more like a source or like a sink for the floral ramet, and to what extent, if any, and under which conditions, C reserves participate in fruit development in cloudberry.

**Conclusions**

The floral ramet appeared to be the main C source for the fruit in cloudberry while the non-floral ramets contributed to C reserves in the rhizome. Despite a division of labour between ramet types in cloudberry, fruit abortion is generally high. The growing season being short, leaf development, accumulation of C reserves in the rhizome and fruit development take place simultaneously early in the growth season. Both leaf and rhizome appear to be competitors for the fruit at least in terms of C allocation. The presence of strong sinks maintained Pₙ high throughout the growing season in both floral and non-floral ramets. The role of C reserves in fruit maturation was not clearly defined in this study, but the impact of C reserves on fruit size could be mainly indirect through its impact on ramet leaf size. Thus, the present study supports our hypothesis that fruit yield in cloudberry is strongly source limited, with many sinks competing with the developing fruit. Further studies are required to characterize better the complex role played by the rhizome in the source–sink relationship in cloudberry but also in other clonal species presenting source limitation of their growth.
On more practical issues, selection for increased source capacity or for changes in phenology with leaves beginning and/or completing their development earlier in the season might help increase cloudberry fruit yield in a context of cultivation.

ACKNOWLEDGEMENTS

We thank Julien Beaulieu and Olivier Larouche for their technical assistance, Julie Bussières for improving an earlier version of the manuscript and Tourbière Lambert, Inc. for kindly providing sites for our experimentation. We thank the three anonymous referees for their very helpful comments. This work was supported by the Ministère de l’agriculture, des pêcheries et de l’alimentation (MAPAQ) through the Conseil des recherches en pêche et en agro-alimentaire du Québec (CORPAQ) program.

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