Fertilization stimulate root production in cloudberry rhizomes transplanted in a cutover peatland

Jade Boulanger-Pelletier\textsuperscript{1} and Line Lapointe\textsuperscript{1,2}

\textsuperscript{1}Département de biologie and Centre d’étude de la forêt, Université Laval, Québec, Québec, Canada G1V 0A.

\textsuperscript{2}Corresponding author (e-mail: line.lapointe@bio.ulaval.ca)

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Abstract

Cloudberry has good economic potential for Canada, but crop practices must be improved before commercial production can be established. Transplants usually consist of rhizome segments collected in natural populations. However, the very low root density of these transplants might partly explain their initial slow growth and high mortality. The objective of this study was to determine the effects of mineral fertilization and auxin applications on root initiation and elongation. Three NPK fertilization treatments were applied at planting of bare rhizomes in peatlands, while auxin applications were tested in both greenhouse and field experiments. Roots of fertilized plants were two to four times longer and more numerous than those of control plants after one complete growing season, but fertilization did not lead to early rooting. Rhizome segments produced new shoots before investing in root production, suggesting that rhizome carbohydrate reserves are not sufficient to allow both shoot and root to be produced at the same time. Auxin applications to the rhizomes incurred high mortality and did not stimulate root production in both field and greenhouse experiments. We conclude that fertilizers applied at planting can improve cloudberry initial survival rate, rooting and early shoot growth, which could eventually lead to improved plant cover and fruit yield.

Key words: Adventitious rooting, Auxin, Phenology, Rootless transplant, Transplant survival rate

Introduction
Cloudberry (*Rubus chamaemorus* L.) is a circumpolar species that grows in ombrotrophic peatlands and produces amber-coloured fruits (Resvoll 1928). The berries have an established economic value in Scandinavia (Saastamoinen et al. 2000). In Canada, the fruit is mostly known where it is locally abundant. Commercial cloudberry culture has yet to be developed in both Scandinavia and Canada; all fruits are harvested in natural peatlands. The species is dioecious and the ratio between male and female plants in natural populations is high; furthermore, fruit abortion is frequent, which constrains fruit yield (Dumas and Maillette 1987, Ågren 1988). The number of pickers has been decreasing in recent years due to the harsh conditions of collecting delicate fruits in natural peatlands. Pickers have declined in number also due to low fruit density, despite a growing demand for and the high economic value of this fruit (CEPAF 2008). Cultivation in cutover peatlands where peat extraction has ceased could maintain economic activities on these sites, thereby improving plant density and, therefore, fruit yield per hectare.

Cloudberry, a subshrubby plant (Payette, 2015), propagates mainly through its extended system of rhizomes (Taylor 1971). Rhizome segments that are 20 cm long are commonly used as transplants (Rapp 2004, Bellemare et al. 2009b). Nevertheless, high mortality of these transplants has been recorded in the field, upwards of 70% in some years (Bellemare et al. 2009a). Previous studies have indicated that rhizome segments up to 40 cm long do not contain carbon and nutrient reserves that are sufficient to sustain shoot growth that is comparable to growing shoots attached to an intact rhizome system (Gauci et al. 2009). Furthermore, these rhizome segments are often rootless (Jean and Lapointe 2001), which most likely limits the uptake of nutrients from the soil. In natural peatlands, cloudberry produces few roots along the rhizome, but these are capable of producing a
large root system when grown in containers. Root production in containers, in turn, is
strongly influenced by substrate properties. Rhizomes growing in containers for three
growing seasons produced 14 times more root biomass and 25 times more total aerial
biomass on fibric peat compared to in mesic peat which is more decomposed (Bussières
et al. 2015). Fibric peat exhibits physical properties that favour the growth of cloudberry
(Théroux-Rancourt et al. 2009), together with higher P content than mesic peat.

Mineral fertilization stimulates root growth in many species (Fageria and Moreira 2011).
In rootless cuttings, root initiation must be induced before any root elongation can take
place (Hartmann et al. 2011). The effect of mineral fertilization on the different rooting
phases is not very well known (Blazich 1988). Fertilization appears mainly to affect the
elongation phase of roots without accelerating root initiation (Hartmann et al. 2011), as
has been shown in Petunia. In the latter case, the application of fertilizers to stem cuttings
improves root growth, but only after root emergence has occurred (Santos et al. 2011).
Mineral nutrition can increase root length and root number in rootless cuttings of
Eucalyptus globulus Labill., but rooting time and percent was mostly affected by the
application of auxin (Schwambach et al. 2005). In cloudberry, mineral fertilization
improved root length after one growing season in a greenhouse experiment, but the
timing of root initiation was not determined (Gauci 2008).

Auxins are commonly used to promote adventitious root production in commercial stem
cuttings (Hartmann et al. 2011). Species producing fewer roots are often more sensitive
to auxin applications due to their low endogenous auxin concentrations (Srivastava
2002). Several forms of auxins are used to promote root formation but the most
commonly used formulation that is applied to commercial cuttings is indole-3-butyric
acid (IBA) (Srivastava 2002). Auxin addition has been tested in vitro on cloudberry shoot clusters from meristem cultures, where it has been found to promote rooting while inhibiting the formation of new shoots (Martinussen et al. 2004). Thiem (2001) reported on in-vitro and in-vivo auxin application to rootless cloudberry shoots. This study showed that agar-solidified medium containing auxins (IBA and IAA) promoted rooting of the shoots, while shoots did not root in the medium that did not contain auxins. Direct application of powdered IBA to in-vitro shoots prior to being transplanted into peat moss, led to 39% rooting after eight weeks. Given the absence of a control, the positive effect of auxin applications on in-vivo rooting could not be confirmed (Thiem 2001). To our knowledge, no studies have reported auxin application trials on rootless rhizome transplants in cloudberry, and very few in other rhizomatous species (Balestri and Lardicci 2006, Nivot et al. 2008).

No studies have investigated the timing of adventitious rooting by cloudberry transplants under field conditions. The only known trials on root production in cloudberry transplants are greenhouse experiments (Gauci 2008, Bussières et al. 2015). These rarely reflect what is happening in the field (Mokany and Ash 2008). The objective of this study was to determine the effect of fertilization and auxin applications on root initiation and subsequent root elongation in cloudberry rhizome transplants. Sequential sampling throughout the growing season would allow us to follow root production more closely and determine whether fertilizer and auxin treatments hasten or enhance root production. Earlier rooting might favour transplant survival, whereas enhanced root production during the first growing season may lead to faster rhizome propagation and improved fruit yield after a few years of cultivation.
Materials and Methods

Field Site and Plant Material

The experimental site is located on Lamèque Island, northeastern New Brunswick (47°48'47"N, 64°37'24"W). This region is characterized by a maritime climate: mild winters and cool humid summers. According to the Bas-Caraquet weather station (47°48'08"N, 64°50'00"W), the mean temperature for the months of May, June, July and August are respectively 9 °C, 14 °C, 19 °C and 19 °C, based on records collected from 1993 to 2007 (Environnement Canada 2015).

Two experiments were established in a residual section of the peatland that was owned by Acadian Peat Moss (1979) Ltd. (Coteau Road, NB). The drainage ditches that had been used for the peat extraction were still functional and maintaining a low water table during the summer. Rhizomes for the two experiments were collected from the edge of the main drainage ditch on the experimental site and cut into 20 cm segments following the recommendations of Bellemare et al. (2009a). These rhizome segments were of different ages since long rhizomes that have been growing for many years were collected then cut into different segments. Most of the rhizome segments carry at least one visible dormant bud, sometimes more. Given that most rhizome segments are rootless, all roots that remained were cut to standardize among-rhizome segments.

Fertilization Trials
Experimental Design

The fertilization experiment included three blocks and four treatments (three fertilizers and one control), which were randomly assigned within the blocks. The twelve plots each measured 3 m x 9 m and there were 2 m between the plots within each block. Two meters separated the first two blocks, while the third block was located on the opposite side of a small ditch. Each plot contained six rows of 18 rhizomes, for a total of 108 rhizomes per plot. They were planted at a distance of 50 cm from one another.

The three fertilizer treatments differed with regards to N, P and K concentrations: 6 %-12 %, 6 %-12 % and 12 %-12 % (N, P₂O₅, K₂O). Nitrogen was applied as an equal mixture of ammonium and nitrate. The three fertilizers were applied at rates of 50-100-50 kg ha⁻¹, 50-100-100 kg ha⁻¹ and 100-100-100 kg ha⁻¹, respectively. They contained the same amount of the following nutrients: 2.5 % Ca, 1 % Mg, 0.3 % B, 0.3% S, 0.004 % Zn, 0.004 % Cu and 0.004 % Mn. The fertilizer formulations were prepared by Engrais Chaleur (Petit-Rocher, NB).

First Trial

Rhizomes were collected in November 2012, returned to the laboratory and maintained in a cold room in damp moss (4° C) until planting in spring 2013. The site was tilled in spring 2013 before plot establishment. Fertilizers were incorporated into the uppermost 6 cm of peat using a rake; control plots were also raked. Rhizomes were then planted at a depth of 5 cm according to Bellemare et al. (2009a).

Second Trial
A new set of rhizomes were transplanted in 2013 due to low transplant survival rate in spring 2013 (first trial). Rhizomes were collected in October 2013 after leaf senescence was completed and transplanted immediately into the same plots used in the first trial. Rhizome transplants remaining from 2013 were replaced with new ones. Following soil analyses in spring 2014, we elected to reapply the same quantity of fertilizer as in 2013, given that nutrient concentrations in the peat did not significantly differ among treatments. The second fertilizer application occurred on June 11. Because of the presence of transplants in the plots, it was impossible to incorporate fertilizers into the peat. Therefore, fertilizers were applied in five trenches (each 5 cm deep) between the rows and covered with peat. We did not broadcast the fertilizers because this would favour growth of competing species (Rapp and Steenberg 1977). The effect of fertilization on growth was also recorded during the second growing season of the second trial in July 2015 on the remaining rhizomes (i.e. those remaining after the sampling of 2014).

Plant Measurements and Samplings

The date of emergence and the presence of a shoot were recorded for each transplant in both trials. The number of shoots were recorded again at the beginning of the second growing season (2015) in the second trial. Leaf diagonal (D, cm) was measured and leaf area was then calculated with the following equation;

\[
\text{Leaf area (cm}^2\text{)} = 0.5242 \exp^{0.7158\times D}
\]

This equation was estimated by measuring D with a ruler, while the corresponding leaf area was determined using a LICOR 3100 area meter (LICOR Biosciences Lincoln, NE)
for leaves having a D < 4 cm (Théroux-Rancourt et al. 2009). The diagonal was measured in 2013 on the transplants in the first trial and in 2014 and 2015 on the transplants in the second trial.

To determine total length of the root system, transplants were dug up carefully to ensure all roots were collected. In 2013, 6 transplants per plot were harvested when possible; only three and five surviving transplants were collected respectively in two of the plots. The low survival from the first trial permitted a single harvest at the end of the summer (6 August). For the second trial, six transplants were collected in each plot on three different dates (10 July, 28 July and 4 October 2014). Each time, the six harvested transplants were selected randomly from the pool of living transplants. The number of shoots and leaves was counted on each harvested rhizome. All underground material was frozen until they could be analyzed using WinRhizo (Regent Instruments Inc., Quebec, QC). This program is an image analysis system designed specifically for roots.

Leaves present on the transplants that were collected in June and July 2014 were subjected to nutrient analysis. Leaves from June and July 2014 were pooled to obtain enough material for nutrient analysis. Leaves were oven-dried at 65 °C for 48 hours, then ground with a mortar and pestle. Concentrations of C, N and S were obtained following high-temperature combustion with a LECO CNS-2000 (St-Joseph, MI). The rest of the tissues were digested in perchloric-nitric acid prior to quantifying their nutrient concentrations by plasma emission spectroscopy (ICP-OES) (Barnhisel and Bertshe 1982).

Soil Analyses
Physical and chemical soil analysis were performed in all plots of the fertilization experiment. Three samples were taken from each plot at 0-20 cm depth, then composited. Bulk pH was measured in a 1:10 soil:solution with CaCl$_2$ (0.01 M). Organic matter content (%) was obtained by combustion at 550 °C for 24 h. Nutrient concentrations were determined by Macro-Kjeldahl for total N, by ascorbic acid method for total P and by inductively coupled plasma-optical emission spectrometry (ICP-OES) for K, Al, Fe, Zn, Ca and Mg after Mehlich-3 extraction (Mehlich 1984).

Two soil cores were collected in each plot in 2014, using a cylinder (8 cm diameter, 7 cm long); ends of the cores were then covered with nylon mesh. The first set of cores was oven-dried at 105 °C for 48 h and weighed to estimate bulk density ($D_b$, g cm$^{-3}$). The remaining cores were used to determine water retention curves using a tension table (Topp and Zebchuk 1979). Different pressure head were applied (-1, -2, -5 and -10 kPa). Pressure head of -1 and -2 kPa were applied for 24 h, whereas pressure head of -5 and -10 kPa were applied for 48 h. In the latter case, the cores were weighed before applying a new pressure head.

**Auxin Experiment**

Auxin trials were performed both in the field and in the greenhouse. Rhizomes were collected in spring 2014 and immediately subjected to one of three treatments: a control and two levels of K-IBA (250 and 500 ppm). The auxin potassium salt (Sigma-Aldrich Canada Co.) was diluted in distilled water prior to use. Ends of the cut rhizomes were dipped for 15 seconds in either the auxin solution or distilled water (control) prior to planting. Auxin concentrations were chosen based on recommendations for softwood
cutting (Hartmann et al. 2001, Balestri et al. 2012) and some preliminary trials we ran on rootless cloudberry rhizomes (unpublished data).

**Greenhouse Experiment**

One rhizome was planted per plastic pot (20 x 18 cm). Peat collected from the field site was used as substrate. Eight pots per treatment were planned for harvest three times during the season, for a total of 72 pots. Pots were randomly positioned on the table. Rhizomes were planted on May 21; the first sampling took place after four weeks, the second sampling after seven weeks, and the final harvest occurred after nineteen weeks of growth. As mortality was high, the number of plants collected varied between 5 and 8 per treatment per sampling time, except for the second sampling where we did not harvest any plants from the higher concentration treatment (500 ppm) to save them for the final harvest. Root length was measured using WinRhizo and leaf area was estimated from leaf diagonal (see Plant Measures and Samplings section for details). Sprouting and survival were monitored throughout the growing season.

**Field Experiment**

Fifteen plots were established in the same cutover peatland as for the fertilization experiment. The five replicates of the three treatments (0, 250 and 500 ppm K-IBA) were randomly distributed among the 15 plots. Fifty rhizomes were planted in five rows in a 5 × 2.5 m plot. Sprouting and survival were monitored during the growing season. Transplants were harvested after 19 weeks of growth to measure root length.
Statistical Analyses

All variables from the fertilization experiment were analyzed using one-way ANOVA and a priori contrasts, on mean per block per treatment. We use a priori contrasts to quantify the effect of fertilization (Control vs fertilized treatments), the effect of a fertilizer containing less N (50-100-100 vs 100-100-100) and the effect of a fertilizer containing less K (50-100-50 vs 50-100-100). Two-way ANOVA was performed on root lengths for the samples that had been collected at the end of the season in the first and second trials, using fertilization treatment and trial as fixed factors. Stepwise linear regression with backwards elimination was performed with root length as dependent variable and different growth measurements and foliar nutrient concentrations as independent variables. All analyses were performed using Statistix (Statistix 10, Analytical Software, Tallahassee, FL).

Root length, leaf area and percent survival from the auxin experiment performed in the field were analysed using one-way ANOVA on mean per plot. Root length and leaf area from the auxin experiment performed in the greenhouse were analysed using a one-way ANOVA with treatments and sampling time as independent factors. $\chi^2$ was run to test survival rates as a function of auxin treatments from the greenhouse experiment. A one-way ANOVA was performed on the root length recorded at final harvest using the condition of growth (greenhouse or field) and the treatments as independent variables. Pearson correlation coefficients were calculated between root length and leaf area of the final sampling of the greenhouse and field experiments.
Results and Discussion

Fertilization Trials

Soil Properties

Soil physical properties after fertilization application did not differ among the 12 plots. Mean organic matter content was 95.6% (± 0.7% SE) and bulk density averaged 0.105 g cm$^{-3}$ (± 0.005 SE). Water retention curves were similar among plots; mean soil porosity (Φ) was 0.96% (± 0.002 SE). Soil pH averaged 3.14 (± 0.006 SE) among plots. Soil nutrient concentrations were quantified one year after the first fertilization and before the second application, and were similar between the treatments and the blocks except for Al and Zn. Aluminum concentration was higher in the control treatment than in the fertilized plots (0.29 ± 0.02 mg g$^{-1}$ vs 0.10 ± 0.01 mg g$^{-1}$; $F_{3,11} = 21.09$, $P < 0.005$), and Zn concentration was higher in the first block (9.4 ± 1.3 µg g$^{-1}$ vs 3.7 ± 0.3 µg g$^{-1}$; $F_{2,11} = 20.2$, $P < 0.01$). The mean concentrations of the other nutrients were 6.7 ±0.08 mg g$^{-1}$ of N, 8.6 ±0.5 µg g$^{-1}$ of P, 0.23 ±0.04 mg g$^{-1}$ of K, 0.26 ±0.02 mg g$^{-1}$ of Ca, 1.4 ±0.1 mg g$^{-1}$ of Mg, and 0.53 ±0.03 mg g$^{-1}$ of Fe.

First Trial

In the first trial, sprouting was very low, i.e., only 11%. We attributed this low survival to the fact that rhizomes were planted in the spring rather than in the autumn. Keeping the rhizomes in a cold room throughout the winter appears to incur more damage in some years, although good survival rates were reported following spring
planting in other years (Bellemare et al. 2009a; L. Lapointe, unpubl. data). High mortality prevented us from sampling transplants more than once during the growing season. We did a single harvest after two months of growth, as we were concerned that more transplants would die if we delayed sampling until the end of the season. The effect of fertilization was not significant for either root length (Fig. 1; $F_{3,11} = 3.25$, $P = 0.109$) or total leaf area (data not shown; $F_{3,11} = 1.83$, $P = 0.213$).

Second Trial

Survival averaged 51% for the second trial. Fertilization treatments did not affect the percentage of sprouting; indeed, it appears that the rhizome contains carbon and nutrient reserves that are sufficient to sprout regardless of soil nutrient availability. However, mineral fertilization increased cloudberry root length, root number, rhizome mass, number of buds, and total leaf area after one growing season, together with the total leaf area and the number of shoots that were produced per transplant during the second growing season (Table 1). Root initiation was unaffected by fertilization during at least the first 50 days of growth (first and second samplings took place at 34 and 53 days, data not shown). Indeed, root length and root number were very low or negligible during the first two samplings, despite the presence of fully grown shoots (Fig. 2). At first harvest, most plants did not have roots (70 plants out of 88 were rootless), and the mean root number per rhizome varied from 0.15 root for the control to 0.5 root for the 50-100-50 fertilizer treatment (no significant differences among treatments). After one complete growing season, fertilizers had significantly increased the number of roots and they were two to four times longer than those of control plants (Fig. 1). Roots were produced on the
nodes of the existing rhizome. Fertilizer composition did not significantly affect root length. When we compared the root length at the final sampling for the two trials (Fig. 1), we noted significant main effects of trial ($P < 0.001$) and fertilization treatment ($P < 0.001$), as well as the trial $\times$ treatment interaction ($P = 0.008$). The significant interaction indicated that only unfertilized plots exhibited similar root length in the two trials, despite the two additional months of growth during the second trial. Sequential samplings during the second trial showed an important increase in root length in fertilized plots between the second and third harvests (between 8 July and 4 October) (Fig. 2). Indeed, the rate of root elongation in control transplants was constant throughout the season, in contrast with the fertilized transplants, which exhibited a quadratic response (Fig. 2). Therefore, fertilization did not induce early rooting of the cuttings, but stimulated root production and root elongation later on in the season.

Fertilization improved leaf concentrations of N, P, K and S before any significant increase in root length took place (Table 1; Fig. 3). This response suggests that the rhizome is able to absorb some nutrient directly from the soil. Absorption of nutrients through the rhizome has already been reported for *Leymus chinensis* Trin. Tzvelev, a perennial rye grass (Liu et al. 2011). This species can absorb N directly through the rhizome in nutrient-poor environments, with N being preferentially transported to the shoot when uptake takes place in the rhizome. Nitrogen uptake by rhizomes has also been reported for *Carex bigelowii* (Brooker et al. 1999). Rhizome uptake could be a mechanism that has developed in non-mycorrhizal species to enhance N uptake in resource-poor environments (Brooker et al. 1999). Similarly, the rhizome of *Pteridium aquilinum* (L.) Kuhn is capable of taking up $^{134}\text{Cs}$ and $^{85}\text{Sr}$, which are physiological
analogues of Ca and K (Tyson et al. 1990). The seagrass *Zostera marina* L. is able to absorb P through its rhizomes, roots and leaves (Brix and Lyngby 1985). However, what makes cloudberry somewhat unique is that these other species do not develop secondary growth on the rhizome. Therefore, it appears that even woody rhizomes can absorb nutrients directly.

Contrarily to the other nutrients that accumulate to a greater extent in cloudberry leaves following fertilization, Na foliar concentration was higher in control plots. Competition for adsorption sites is likely among nutrients on peat particles (Bolt et al. 1978), causing a decrease in Na absorption in fertilized plants. According to the specific adsorption strength of the different nutrients (Millar and Turk 1943), Na would be replaced by ammonium or K in fertilized transplants.

Fertilization enhanced total leaf area produced per transplanted rhizome, but the effect was more apparent during the second growing season (Table 1; Fig. 4). Both the number of shoots and individual leaf size increased following fertilization. Higher foliar concentrations of N and larger leaves of the fertilized plants allow them to fix more carbon through photosynthesis (Evans 1989). This extra carbon gain most likely allowed the fertilized transplants to produce more roots. Indeed, final root length was significantly correlated with total leaf area (*P* < 0.001) (Fig. 5) as reported previously in leafy rose (*Rosa hybrida* L. Madelon®) stem cuttings (Costa and Challa, 2002). Stepwise linear regression indicated that total leaf area (*P* < 0.001) and leaf N concentration (*P* = 0.008) are the most important factors explaining final root length (*r*² = 0.94; *N* = 12). Yet we cannot rule out the possibility that larger root systems were essentially attributable to the presence of fertilizers in the soil (Coutts and Philipson 1977). In phosphorus-poor...
environments, such as peatlands (Damman 1978), plants will not invest in root systems because the construction costs could outweigh the benefits that would be gained from absorbing P (Robinson 1990). Therefore, the application of fertilizers with high levels of P could stimulate root production (Marschner 2012). However, the delay in the production of a larger root system following the application of fertilizers strongly suggests that root production is mainly driven or limited by the availability of carbohydrates rather than by the presence of fertilizers. In leafy rose (Rosa hybrida L. Madelon®) stem cuttings, rooting was delayed by low CO$_2$ concentrations, which led to low photosynthesis and low starch reserves in stems; rooting in rose thus appears to be limited by carbohydrate availability (Costa et al. 2007). The number of roots and root dry weight of leafy cuttings of New Guinea impatiens (Impatiens hawkeri Bull.) and petunia (Petunia x hybrid hort. Vilm.-Andr.) increased with daily light integral (DLI), also confirming the importance of optimizing photosynthesis in order to favor good rooting in leafy cuttings (Lopez and Runkle 2008).

Rhizomes from fertilized plots weighed more than those from control plots at the end of the season (Fig. 6), strongly suggesting that the impact of fertilization on leaf size and most likely on photosynthetic capacity not only allowed the plant to produce more roots but also allowed it to store more carbohydrates in the rhizome during the season. These bigger rhizomes produced more buds at the end of the first growing season, that translated into a higher number of shoots during the second growing season, and which contributed to more leaf area produced per transplant. The larger rhizome mass at the end of the season in fertilised plants most likely mean more nutrient accumulation following leaf senescence and nutrient resorption as previously shown in a fertilisation trial on
cloudberry (Gauci, 2008). These nutrients could be mobilized the following spring along with carbohydrates to stimulate shoot growth. Difficult-to-root olive cultivar had lower sugar content than easy-to-root olive cultivar, also suggesting that the energy reserves in cuttings influence their rooting capacity (Denaxa et al. 2012). It is worth mentioning that two shoots from fertilized plots produced good-sized fruits during the second growing season, which is quite encouraging given that recently transplanted cloudberry very seldom produces fruits.

Fertilizer composition did not lead to significant differences in root, leaf or rhizome growth. The only morphological variable influenced by the fertilizer composition was root number. The 50-100-100 treatment plants produced a fewer number of roots than the two other fertilizer treatments. Cloudberry is usually co-limited by N and P (Hébert-Gentile et al. 2011), but a reduction in N content only negatively affected root initiation and only in the 50-100-100 treatment, not in the 50-100-50 treatment. Phosphorus nutrition appears to be important for cloudberry growth, as plants exhibiting low growth and low fruit yield have lower leaf P concentrations (Gauci 2008, Bussières et al. 2015). A deficiency in P can reduce the photosynthetic rates and, consequently, the growth rate of the plant (Ghannoum and Conroy 2007). A reduction in photosynthesis would most likely lead to a reduction in carbohydrates, which appears to limit growth in cloudberry (Gauci et al. 2009).

**Auxin Experiment**

The application of auxins to the rhizome did not induce an early rooting, contrarily to what we initially posited. Auxin application caused high rhizome mortality,
with the highest concentration (500 ppm) causing the highest mortality, followed by the intermediate concentration (250 ppm) in both greenhouse (50%, 75% and 83% survival, for the 500 ppm, 250 ppm and control plants respectively; \( \chi^2 = 6.8, P = 0.03 \)) and field experiments (9%, 35% and 51% survival for the 500 ppm, 250 ppm and control plants respectively; \( F_{2,14} = 23.9, P < 0.001 \)). Transplants from the auxin treatments that survived did not produce longer roots than the controls at the end of the season (Greenhouse: \( F_{2,18} = 0.45, P = 0.64 \) and Field: \( F_{2,14} = 1.06, P = 0.38 \)). Since we did not sample plants from the 500 ppm treatment in the greenhouse at second harvest due to lower survival than in the two other treatments, we only compared root length from the first and last samplings. Roots grew throughout the season and were thus longer at the final harvest than at the initial sampling time (\( F_{1,36} = 32.9, P < 0.001 \)) but their length did not differ between treatments even after including data from the two sampling times (\( F_{2,36} = 0.42, P = 0.66 \)).

Application of auxin on cloudberry shoot clusters from meristem cultures stimulates the production of roots, but inhibits the production of new shoots (Martinussen et al. 2004). The application of auxins may have inhibited the development of auxiliary shoots on the rhizome by maintaining the buds in a state of quiescence, as shown on stem cuttings of other species (Srivastava, 2002). In the fertilization experiment, we showed that root production began after shoot production. Therefore, the inhibition of shoot production by auxin may explain the high mortality rate that was observed in the auxin-treated group. Development of callus was observed at the extremities of rhizomes grown in the greenhouse (pers. obs.). Growth hormones such as auxins can induce the development of callus (Ikeuchi et al. 2013). Such actively growing tissue may have depleted the carbohydrate reserves of the rhizomes incurring their mortality. Age of the
rhizome segment could also have a negative impact on its rooting capacity and response to auxin treatment. Auxin application on mature petiole cuttings of *Hedera helix* L. can induce the formation of callus, which usually does not result in the formation of roots (Wesley 1988). Rooting of rhizome segments of *Posidonia oceanica* (L.) Delile was enhanced by soaking the rhizome in a very low auxin concentration (5 ppm for 24 h). Indeed, prolonged exposure to a very small dose could be more effective in promoting root production along the whole rhizome without causing toxicity (Balestri and Lardicci 2006).

Transplants grown in the greenhouse produced six times more roots than those grown in the field for similar season lengths ($F_{1,33} = 19.7$, $P < 0.001$; Fig. 7). The longer root system in plants that were grown in greenhouse is correlated with higher foliar surface area ($r = 0.725$, $P < 0.001$, $N = 19$), as reported in the fertilization experiment. Growing conditions in the greenhouse, including a higher temperature regime, regular watering and the absence of competition, appear to improve photosynthetic rates and the growth of the plant.

**Conclusion**

Mineral fertilization stimulated root initiation and elongation, and increased the total leaf area of the transplants after a single growing season. Yet fertilization did not promote early rooting. Nutrients appear to be absorbed by the lignified rhizome and used for leaf production well ahead of root production. Once all leaves are fully mature, photosynthates are most likely translocated to the rhizome and roots, which stimulates their growth. The survival of rhizome transplants remains low and variable despite the
overall positive effect of mineral fertilization. As fertilizer composition only had a modest impact by modulating root initiation without statistically affecting total root length nor shoot growth, we recommend using either formulation until trials with new combinations of nutrients further improve cloudberry growth. A long-term follow-up will be necessary to quantify the effects of a larger root system on plant propagation and fruit yield. Auxin, at the concentrations tested, caused high mortality and did not stimulate root growth. Simultaneous greenhouse and field experiments using rhizome transplants from the same source confirm that cloudberry produces many more roots when grown under greenhouse conditions. Fertilization at planting is recommended to improve shoot and root growth of the new transplants. We also recommend planting cloudberry rhizomes in autumn rather than in spring, as spring planting lead to lower survival rates in some years.

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References


Figure Captions

Fig. 1. Effects of different fertilization treatments (50-100-50, 50-100-100, 100-100-100) on cloudberry root number and root length (cm) at the final harvest. Values are means ± SE (N = 3). Final harvest took place after 2 months during the first trial and after complete leaf senescence (i.e. after four months) during the second trial.

Fig. 2. Root length (cm) progression in cloudberry during the first growing season for fertilized and control transplants. Values are means ± SE (N = 3). Data are from the second trial.

Fig. 3. Effects of fertilization treatments (50-100-50, 50-100-100, 100-100-100) on foliar nutrient concentrations (mg g⁻¹) in cloudberry transplants. Leaf samples collected in June and July were pooled before running the chemical analysis. Values are means ± SE (N = 3). Only nutrients for which at least two treatments differed are presented (see Table 1). Data are from the second trial.

Fig. 4. Effects of fertilization treatments (50-100-50, 50-100-100, 100-100-100) on total leaf area (cm²) produced per rhizome transplant during their first and second growing seasons. Values are means ± SE (N = 3). Data are from the second trial.

Fig. 5. Correlations between total leaf area per rhizome transplant (cm²) and total root length (cm) for the four treatments to which cloudberry transplants were subjected. N = 18. Data are from the first growing season of the second trial.

Fig. 6. A) Effects of fertilization treatments (50-100-50, 50-100-100, 100-100-100) on the number of buds per rhizome at the end of the first growing season and on the number of shoots per rhizome produced during the second growing season in cloudberry transplants. Values are means ± SE (N = 3). Data are from the second trial. B) Effects of fertilization treatments (50-100-50, 50-100-100, 100-100-100) on rhizome mass (mg) at
the end of the first growing season. Values are means ± SE ($N = 3$). Data are from the second trial.

**Fig. 7.** Effects of auxin treatments (K-IBA) on root length (cm) at the end of the first growing season in cloudberry rhizome transplants. Transplants were grown either in the field or in the greenhouse. Values are means ± SE. $N = 6$ for the greenhouse experiment and $N = 5$ for the field experiment.
Table 1. Results of ANOVA ($F$ and $P$ values) and a priori contrasts ($P$ values) comparing the effects of the different fertilization treatments on plant growth and nutrient concentrations in the first and second growing season for cloudberry transplants of the second trial.

<table>
<thead>
<tr>
<th>Year</th>
<th>Variable</th>
<th>$F_{3,11}$</th>
<th>$P$</th>
<th>Control vs 100-100 Fertilized</th>
<th>100 vs 50-100 Fertilized</th>
<th>50-100 vs 50 Fertilized</th>
<th>50-100 vs 100 Fertilized</th>
<th>100 vs 50 Fertilized</th>
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<td>Carbon</td>
<td>1.30</td>
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<td>Phosphorus</td>
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<td>Calcium</td>
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<td><strong>0.043</strong></td>
<td><strong>0.003</strong></td>
<td>0.107</td>
<td>0.103</td>
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<tr>
<td>2014</td>
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<td><strong>0.007</strong></td>
<td><strong>0.000</strong></td>
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<td>0.095</td>
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<td>Number of buds</td>
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<td><strong>0.001</strong></td>
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<td>2015</td>
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<td><strong>0.002</strong></td>
<td><strong>0.005</strong></td>
<td><strong>0.014</strong></td>
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<tr>
<td></td>
<td>per rhizome</td>
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<td><strong>0.031</strong></td>
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<td>Leaf area</td>
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<td><strong>0.001</strong></td>
<td>0.222</td>
<td>0.067</td>
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</table>
Fig. 1. Effects of different fertilization treatments (50-100-50, 50-100-100, 100-100-100) on cloudberry root number and root length (cm) at the final harvest. Values are means ± SE (N = 3). Final harvest took place after 2 months during the first trial and after complete leaf senescence (i.e. after four months) during the second trial.
Fig. 2. Root length (cm) progression in cloudberry during the first growing season for fertilized and control transplants. Values are means ± SE (N = 3). Data are from the second trial.
Fig. 3. Effects of fertilization treatments (50-100-50, 50-100-100, 100-100-100) on foliar nutrient concentrations (mg g$^{-1}$) in cloudberry transplants. Leaf samples collected in June and July were pooled before running the chemical analysis. Values are means ± SE ($N = 3$). Only nutrients for which at least two treatments differed are presented (see Table 1). Data are from the second trial.
**Fig. 4.** Effects of fertilization treatments (50-100-50, 50-100-100, 100-100-100) on total leaf area (cm$^2$) produced per rhizome transplant during their first and second growing seasons; Values are means ± SE ($N = 3$). Data are from the second trial.
**Fig. 5.** Correlations between total leaf area per rhizome transplant (cm$^2$) and total root length (cm) for the four treatments to which cloudberry transplants were subjected. $N = 18$. Data are from the second trial.
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