Cloudberry cultivation in cutover peatland: improved growth on less decomposed peat

Julie Bussières[1], Line Rochefort[2], and Line Lapointe[1,3]

[1]Département de biologie and Centre d'étude de la forêt, Université Laval, Québec, Québec, Canada G1V 0A6; [2]Département de phytologie and Centre d'études nordiques, Université Laval, Québec, Québec, Canada G1V 0A6.

[3]Corresponding author (e-mail: line.lapointe@bio.ulaval.ca)

For submission to Canadian Journal of Plant Sciences

Abbreviations: **Fib_Fem**, female clones grown in fibric peat; **Fib_Mal**, male clones grown in fibric peat; **Mes_Fem**, female clones grown in mesic peat; **Mes_Mal**, male clones grown in mesic peat;

Summary (200 words)

Cloudberry cultivation is being seriously considered as a rehabilitation option for industrial peatlands after horticultural peat extraction has ceased. Besides increasing the ecological and economic values of these sites, cloudberry cultivation could improve fruit yield and facilitate fruit harvesting compared to picking in natural peatlands. Previous studies reported slow establishment that was tentatively associated with substrate characteristics. Field and greenhouse experiments were thus conducted to better characterize the impact of different peat substrates in combination with restoration techniques on the growth of male and female clones. Cloudberry grew much better in less decomposed fibric peat (H1-H3) than in more decomposed mesic peat. Restoring the moss layer of the former peat field would thus need to precede cloudberry planting by a few years, in order to plant the rhizomes in a newly formed fibric peat layer. Male clones produced larger leaves and more ramets per rhizome than female clones under common greenhouse conditions, which indicated that differences between sexes are most likely genetic rather than environmental. Furthermore, we found cloudberry clones may be very sensitive to aluminium toxicity. In conclusion, the degree of peat decomposition appears to be one of the key factors determining the success of cloudberry plantations.

Key words (up to 6): Cutover peatland, substrate, peatland restoration, berry production, *Rubus chamaemorus*, dioecious species.

Short title (55 characters): Cloudberry cultivation in cutover peatland

Résumé

La culture de la chicouté est sérieusement évaluée comme une option de réhabilitation des tourbières après récolte de la tourbe à des fins horticoles. Outre le gain en termes de valeur écologique et économique de ces sites , la culture de la chicouté pourrait augmenter le rendement en fruits et faciliter la récolte des fruits par rapport à la récolte en tourbières naturelles. Des études antérieures ont montré une croissance initiale lente qui a été provisoirement attribuée aux caractéristiques du substrat. Des expériences sur le terrain et en serres ont donc été mises en place pour mieux caractériser l'effet de différents substrats combinée aux techniques de restauration, sur la croissance des clones mâles et femelles. La chicouté a présenté une meilleure croissance en tourbe fibrique moins décomposée (H1-H3) qu'en tourbe mésique plus décomposée. La restauration devrait donc précéder la mise en culture de la chicouté de quelques années, afin de planter les rhizomes dans la couche de tourbe fibrique nouvellement accumulée. Les clones mâles produisent des feuilles plus grandes et plus de ramets par rhizome que les clones femelles en conditions communes de croissance. Les différences observées entre les sexes sont donc d'ordre génétique plutôt qu'environnemental. De plus, nous avons observé que les clones semblent particulièrement sensibles à la présence d'aluminium. En conclusion, le niveau de décomposition de la tourbe apparaît comme un des facteurs déterminant le succès de plantations de chicouté.

Mots clés: tourbière exploitée, substrat, restauration, production de petits fruits, *Rubus chamaemorus,* espèce dioïque.

INTRODUCTION

Cloudberry (*Rubus chamaemorus* L.) is a circumboreal berry species, from the Rosaceae family, for which there is a relatively good market in Scandinavia (Saastamoinen et al. 2000). An emerging market is developing in North America (Boxall et al. 2003; Centre d'expertise sur les produits agroforestier 2008), the most popular products being jam, jellies and liqueur. The fruit also contains secondary metabolites that can provide health benefits (Thiem 2003). This species is clonal and produces extensive rhizome systems that support few short shoots or ramets. Cloudberry has low fruit productivity (Kortesharju 1988) and berries commercialized fresh, frozen or processed are picked in natural open Sphagnum-dominated ombrotrophic peatlands (bogs). However, there are fewer and fewer pickers due in part to the hostility of the habitat and the low fruit yield productivity, despite being a highly priced berry (Saastamoinen et al. 2000). Growing dense cloudberry cover in cutover peatland could be an interesting alternative to increase fruit yield and meet market needs. It is also a sustainable rehabilitation option for peatlands after horticultural peat harvesting.

Peatlands are now being restored in North America after peat production activities have ceased (Graf et al. 2012). In areas such as the North Shore of the Saint-Lawrence River (Quebec, Canada), where cloudberry is abundant and where economic context is not always easy, commercially grown cloudberry seems a very sustainable rehabilitation option, with or without restoration. Cutover peatland fields present a good potential for cloudberry production because 1) they have few weeds (Salonen 1987), 2) they have not received any pesticides or chemical fertilizers and are thus suitable for organic production and, 3) they have flat fields and roads in place facilitating management and access to site for pickers. All of these characteristics contribute to reducing the cost of production.

Cloudberry cultivation in cutover peatland has been studied in Scandinavia and North America for more than ten years. Despite the knowledge gained through different projects including how, when and what propagation units to plant (Bellemare et al. 2009a; Théroux-Rancourt et al. 2009), poor results are still observed on some cutover sites. Degree of peat decomposition was suspected as a potential cause of this poor productivity. Fibric peat is the recommended type of peat for cloudberry propagation in beds (Rapp 2004), but the peat left after peat harvesting is usually more decomposed (Graf et al. 2012). An initial greenhouse experiment was conducted over one growing season, testing different degrees of peat decomposition on cloudberry growth (Théroux-Rancourt 2007) and results were not conclusive. Cloudberry was also planted in cutover peatlands in combination with restoration techniques i.e. spreading of sphagnum diaspores covered with straw mulch (Graf et al. 2012). Théroux-Rancourt and collaborators (2009) suggested that rhizomes should be planted two or three years after field restoration as cloudberry growth appears to be affected by the presence of mulch needed during the restoration process.

Cloudberry is dioecious and ramets from male and female clones have slightly different morphology (Ågren 1988b; Korpelainen 1994). They both carry a single flower per ramet and female produces a polydrupe (Jean and Lapointe 2001). In natural peatlands, the male to female ratios are generally high (Ågren 1988b; Dumas and Maillette 1987; Korpelainen 1994) and male ramets have larger leaves and greater rhizome mass than female ramets (Ågren 1988b). Repeated higher cost of reproduction for female plants could, over time, induce differential mortality leading to the common spatial segregation reported for perennial dioecious species, where male plants are more abundant in more stressful environments than female plants (Barrett and Hough 2013). However, spatial segregation between sexes has not been conclusively shown in cloudberry (Dumas and Maillette 1987). Although fruit and seed production are generally costly, explaining the high cost of sexual reproduction in females (Obeso 2002), pollen cost may often be significant (Rameau and Gouyon 1991), reducing the difference in cost production between male and female plants.

To better understand how the degree of peat decomposition impacts on cloudberry production, we conducted two substrate experiments: 1) a field experiment in cutover peatland, testing a gradient of peat decomposition and the presence of restoration prior to cloudberry planting on cloudberry establishment, and 2) a greenhouse experiment testing the impact of two peat substrates on the growth of male and female cloudberry clones over three seasons. Based on previous results, we posit that cloudberry will perform better on sites restored a few years earlier, and that less decomposed fibric peat will favour growth of both male and female cloudberry compared to more decomposed mesic peat. The greenhouse experiment also allowed us to determine if differences reported previously between male and female clones in field experiments are still present under common growth conditions.

MATERIALS AND METHODS

Field Experiment

The experiment was set up in 2009 in a coastal cutover ombrotrophic peatland (a former raised bog) in Pointe-Lebel near Baie-Comeau (49°8′N, 68°14′W), Côte Nord region, QC, Canada. Peatland was vacuum- harvested and the operations ceased some years prior to planting or restoration; nevertheless the surface was still devoid of vegetation. Twenty 3.5 × 6 m plots were established to cover a range of increasing peat decomposition from H2 to H5 on a Von Post scale (Payette and Rochefort 2001)) either restored 5 to 6 years prior to planting or unrestored. Restoration was performed following the moss layer transfer technique (Graf et al. 2012), spreading *Sphagnum* diaspores and straw mulch, but without blocking drainage ditches. Therefore, the peat surface was not water logged during the growing season.

Cloudberry rhizomes were harvested in October 2008, in the Pointe-Lebel peatland in a sector that contains mostly female clones. Rhizomes were dug, then cut in 20 cm long sections, then put in plastic bags filled with moist sphagnum. They were cold stratified at 4°C for three months. They were then planted in 10 × 10 cm pots filled with fibric peat and grown for one season in the greenhouse (3 months), then cold stratified for 10 weeks. Fifty rooted cloudberry plants were planted every 50 cm from 11 to 15 June 2009 (Year 1) in each plot. No fertilizer or water was applied throughout the duration of the experiment.

Peat was sampled from 23 to 26 June 2009 in order to estimate its initial degree of decomposition on the von Post scale (Payette and Rochefort 2001), bulk density (dry mass of peat after 24h at 105°C per known volume) and nutrient content (see Greenhouse experiment for complete description of nutrient analysis). Ramets were counted in June of Year 2 to 4, while in Year 1, they were counted in July, to take account of mortality following planting in June. Statistical analyses were performed using Statistix 9 Analytical Software (Tallahassee, FL). Pearson correlations were performed between the number of ramets per plot and nutrients available in peat along with bulk density. Two-sample *T* tests between restored and unrestored plots and ANOVA with polynomial contrasts testing the degree of decomposition were also performed on the number of ramets per plot.

Greenhouse Experiment

This experiment took place at Laval University from June 2010 to December 2011. Both male and female rhizomes were separately planted in either fibric or mesic peat, for a total of 32 containers (4 treatments, $n = 8$). Each experimental unit was 40W × 55L × 30D cm deep, in which four rhizomes were planted (either male or female). Peat was manually harvested from a peat pile in the field, after laboratory tests had confirmed the degree of decomposition: fibric (Fib; H1 to H3 on a Von Post scale) or mesic (Mes; H4 to H6). Male (Mal) and female (Fem) rhizomes, each with an apical bud, were collected at flowering time in mid-June 2010 in the field at Pointe-Lebel and cut 20 cm long prior to being planted 5 cm deep in containers (Bellemare et al. 2009a). These rhizomes were harvested in a different sector of the peatland from the one where we harvested cloudberry rhizomes for the field experiment in autumn 2008. However, there is no reason to believe that they differ in terms of edaphic and climatic conditions they are adapted to. Containers were then transferred in greenhouses for three growing seasons of 13 to 14 weeks each, at 20/15° C and 60-70 / 70% humidity (day/night), with 12 to 16 hours of natural light supplemented when needed with artificial light. Plants were watered two to three times per week, the first season with the nutrient solution proposed by Rudolph et al. (1988), and the other seasons with rain water collected from rainwater harvesting tanks. Excess water was drained from the bottom of the containers, thus avoiding water logging. Between each season, containers were moved to a cold room at 4°C for 14 (between season 1 and 2) and 20 weeks (between season 2 and 3).

Survival was estimated in the middle of the second growing season. It was impossible to properly estimate survival in the third season because of vegetative propagation. Number of ramets and leaf diagonal (an indicator of leaf size) were measured at full expansion (between week 9 and 12). Percent flowering was estimated at bloom, in third growing season, based on the number of ramets in flower within each container. Since we noticed differences in leaf coloration, experimental units were ranked based on the percentage of the leaves that were red at the end of the third season. The total number of ramets produced during the third growing season was divided by four to be reported per rhizome initially planted. At final harvest, at the end of the third growing season, biological material within each container was sorted as rhizomes, roots, petioles and leaves. Samples were oven dried at 70°C and weighed after constant dry weight has been reached (48 h). Four leaf subsamples per treatment (degree of decomposition × sex) were analysed for nutrients. N was extracted by Kjeldahl digest (QuickChem Method 13-107-06-2-D) then quantified using flow injection analysis (FIA QuikChem4000, Lachat Instruments, Loveland, CO). P, K, Ca, Mg, Cu, Fe, Mn, Zn, Al and Na were extracted according to Parkinson and Allen (1975) and their concentration assessed with an inductively coupled plasma optimal emission spectrometer (Optima 4300 DV ICP, PerkinElmer Instruments, Norwalk, CT). Four corresponding subsamples of peat per treatment were extracted following the Mehlich III method (Mehlich 1984) to quantify P, K, Ca, Mg, Al, Cu, Fe, Mn, Zn, B, and Na. Soil subsamples were diluted 1:1 in water to measure pH. Statistical analyses were performed using Statistix 9 Analytical Software (Tallahassee, FL): degree of decomposition and sex were used as fixed factor in the ANOVA and protected LSD comparisons.

RESULTS

Field Experiment

Soil properties in the different sites varied in terms of degree of decomposition on the Von Post scale (H2 to H5), but also in terms of bulk density (mean: 0.078 g cm^{-3}; range: 0.056 to 0.128 g cm^{-3}), and pH (mean: 3.75; range: 3.4 to 4.1). Survival during Year 1 varied greatly among plots with a 52 to 98% survival. Five weeks after planting, cloudberry establishment was independent of restoration application (two-sample *T* test; $P = 0.26$), degree of peat decomposition (ANOVA, $P = 0.80$), bulk density (correlation, $P = 0.40$), pH (correlation, $P = 0.79$), or nutrients (correlations). No difference in number of ramets per plot was detected in the three following years (2 to 4) either as a function of restoration application, bulk density, pH, or nutrient availability. The number of ramets decreased with an increase in the degree of peat decomposition but only in the second year ($P = 0.03$; Fig. 1; note that restored and unrestored plots were pooled to test for the degree of decomposition). Four years after planting, ramet density was still very low and the density even dropped below the initial density at planting, with an average of 46 ramets per plot. Nevertheless, two plots had 4 times more ramets than the average, both in unrestored, weakly decomposed peat (H2 and H3), with a bulk density of 0.058 and 0.072 g cm^{-3}, and a pH of 3.9. Yet, an ANOVA performed on unrestored plots only, with degree of peat decomposition as fixed factor, did not indicate statistical differences in the number of ramets per plot in Year 4 ($F = 0.68$, $P = 0.54$).

Greenhouse Experiment

Vegetative Growth and Flowering

In the second growing season, survival was good (93%) as only 9 out of the 128 rhizomes had died: 1 in Fib_Fem, 7 in Mes_Fem and 1 in Mes_Mal. Male clones grew bigger than female ones (Table 1; Fig. 2). Each male rhizome initially planted produced 34% more ramets compared to females after three growing seasons with, on average, the same number of leaves per ramet. Male clones also produced leaves that were 11% larger compared to female clones. Percent flowering was similar for both sexes. Biomass produced by male clones was higher than for female clones except for leaf biomass and consequently for total aerial biomass in mesic peat, where male and female clones did not differ, and produced on average, only 0.25 ± 0.4 g of aerial tissues per container (Fig. 3).

Cloudberry grew better, and consequently produced more flowering ramets, in fibric than in mesic peat (Table 1; Fig. 4). Cloudberry clones grown in fibric peat produced five times more ramets than in mesic peat (Fig. 5): respectively 6.2 compared to 1.2 ramets produced per rhizome initially planted. Each of the ramets produced in fibric peat had on average two leaves, and each leaf had an area 3 to 4 times larger (diagonal of 5.3 cm) than the single leaf produced on average by ramets in mesic peat (2.5 cm). Plants produced more leaf, petiole, rhizome and root biomass in fibric peat than in mesic peat (Table 1; Fig. 3). Belowground/aerial biomass ratio was decreased by growing plants in fibric rather than in mesic peat. Twice the proportion of ramets flowered in fibric (44%)

compared to mesic (19%) peat. As expected, no fruit was produced in greenhouse, due to the lack of pollination (Pelletier et al. 2001).

Nutrients

Male and female leaves differed in their mineral composition for seven out of 11 elements tested (Table 2, Fig. 6). Female leaves had higher concentrations of nitrogen and lower concentrations of sodium and zinc than male leaves. Female leaves also exhibited higher concentrations of phosphorus and lower concentrations of calcium and magnesium than male leaves, but only for plants grown in fibric peat (Table 2). This led to reduced N:P ratios in female compared to male plants grown in fibric peat and to increased N:P ratios in female compared to male plants grown in mesic peat.

Nutrient profile of cloudberry was clearly different after three years of growth on the two different peat substrates. Differences were recorded for all nutrients, except for Cu (Table 2; Fig. 6). Nitrogen (which is not presented; Fib_Fem : $21.4 \pm 1.0 \times 10^3$ (SE) $\mu g\ g^{-1}$, Fib_Mal : $16.8 \pm 1.4 \times 10^3$ $\mu g\ g^{-1}$, Mes_Fem : $13.0 \pm 0.9 \times 10^3$ $\mu g\ g^{-1}$, Mes_Mal : $9.8 \pm 0.7 \times 10^3$ $\mu g\ g^{-1}$), phosphorus, calcium, magnesium, iron, manganese and zinc foliar concentrations were all increased when plants were grown in fibric peat compared to those grown in mesic peat. For calcium and magnesium, foliar concentration was increased after three years of growth in fibric peat, even though concentration of these nutrients was clearly lower in fibric than in mesic peat. In contrast, potassium, aluminium and sodium concentrations were reduced in leaves of cloudberry grown in fibric peat compared to those grown in mesic peat, even though there were more of those nutrients in fibric compared to mesic peat at the end of the experiment. Leaf N:P ratio was lower in fibric peat (11.6 ± 0.6 (SE) for female and 14.7 ± 1.3 for male) than in mesic peat (25.2 ± 1.2 for female and 21.6 ± 2.6 for male). After the third growing season, pH was on average 3.40 ± 0.03 (SE) in fibric peat and 4.20 ± 0.02 in mesic peat ($F = 489.8$, $P < 0.001$).

Plants grown in mesic peat had more leaves colored in red at the end of the third season than plants grown in fibric peat. After ranking the 32 units, average rank was 28 for male clones grown in mesic peat, which was higher than for female clones in mesic peat (18), which was also higher than for both female (11) and male (9) clones grown in fibric peat.

DISCUSSION

Effect of Substrate

To better understand how the degree of peat decomposition impacts on cloudberry production, we conducted two substrate experiments, one in the field and one under controlled conditions in a greenhouse. Even if the degree of peat decomposition had no significant effect on cloudberry propagation in the field experiment, the greenhouse experiment showed contrasting results consistent with our hypotheses. Indeed, cloudberry grew bigger and produced much more ramets and flowers in less decomposed peat (fibric) than in more decomposed one (mesic). In mesic peat, growth was so low that it was equivalent to virtually no propagation: having a single ramet per initial rhizome planted following three growing seasons is not sustainable. Ramets flowered more frequently in fibric than in mesic peat, which is consistent with the generally better healthiness and larger plant size of the cloudberry grown in fibric peat. These results are also consistent with a larger scale experiment where rooted ramets were planted in fibric peat in unrestored sites, and the number of ramets doubled every year at least up to 7 years after the planting (J. Boulanger-Pelletier, unpublished data). The degree of peat decomposition appears to be one of the key factors in the planting success of cloudberry. Less decomposed fibric peat presents better physical properties for plants grown in containers than more decomposed mesic peat (Caron and Rivière 2003). Mesic peat has better water retention capacity which in some cases can be deleterious to plant growth, as it can reduces soil aeration. However, in both field and greenhouse experiments, drainage was sufficient to avoid water logging conditions. The large difference between cloudberry clones grown in the two types of peat is probably also due to differences in chemical properties. Leaf nitrogen and phosphorus concentration of plants grown in fibric peat was in the range reported from natural sites (Ågren 1987; Hébert-Gentile et al. 2011). The reduced leaf nitrogen and phosphorus concentrations observed in plants grown in mesic peat could explain their high production of anthocyanins, which is a typical symptom of deficiencies of these nutrients (Close and Beadle 2003). Furthermore, female plants had on average higher nitrogen and phosphorus concentration in their leaf tissue than male plants in mesic peat, and they also had on average diminished percentage of red tissue in their leaves. Leaf N:P ratios in fibric peat (12 – 15) suggest that both N and P co-limit plant growth whereas in mesic peat, N:P ratios were strongly biased in favour of N (22 – 25), suggesting significant P limitation (Aerts and Chapin 2006). Previous studies on natural sites in Europe have reported slight to intermediate N limitation in cloudberry based on leaf N:P ratios (N:P ratios of 7 – 14 (Ågren 1988b; Marks and Taylor 1972;; Sæbø 1968; 1970), whereas in Canada, it appears that the two nutrients are co-limiting (Hébert-Gentile et al. 2011). Concentrations of the other nutrients were in the same range as those reported previously (Hébert-Gentile et al. 2011), except for calcium and manganese which were much higher, and iron and zinc which were lower in the present study. Lower iron and zinc concentrations could partially explain enhanced manganese absorption (Thomine et al. 2003).

One of the potential explanations for the poor performance of cloudberry in mesic peat may be the toxic effect of an accumulation of aluminium in plants already deficient in some nutrients. Indeed, Parent and collaborators (2013) concluded that an excess of Al and S in female cloudberry leaves is associated with low fruit yield in natural habitats. Concentration of aluminium in leaves of cloudberry grown in mesic peat was on average more than twice that recorded in leaves of plants grown in fibric peat or in natural stands. However, it is worth mentioning that cloudberry leaf aluminium concentrations are much lower than those recorded in plants grown in acidic mineral soils where soluble aluminium is readily available (Jansen et al. 2002). The pH data confirmed that cloudberry is very well adapted to low pH as it exhibited much better growth in the more acidic fibric peat than in the lesser acidic mesic peat.

Effect of Restoration in Cutover Peatlands

Contrary to our prediction, planting five years after restoration was not better than planting on unrestored sites. Nevertheless, two plots performed well: both in weakly decomposed unrestored peat. Cloudberry might require a thick layer of newly accumulated fibric peat layer to survive and propagate, which would require planting rhizomes 15 to 20 years after restoration took place (Lucchese et al. 2010). This would be the closest thing to natural habitat. Overall, the initial survival in the field experiment was good (36 to 90%) compared to what has been reported in previous work (Théroux-Rancourt et al. 2009). Nevertheless, the technique needs further improvements since the number of ramets dropped with time, despite potential vegetative propagation. The poor survival (around 20%) reported by Théroux-Rancourt and collaborators (2009) was independent of the treatment (unrestored vs. restored a few days prior to cloudberry planting) and also decreased with time. They suspected that the combination of deep planting (10 cm) and application of restoration technique was responsible for the high mortality and low propagation rate. Rhizomes planted deeper might exhaust their reserves before buds reach the ground, whereas the presence of a straw mulch in restored plots might have maintained cold soil temperature in spring (Price et al. 1998), slowing plant growth each year. Although these two factors most likely explain the difference in mortality rates observed between the two studies, the degree of peat decomposition appears to strongly influence the capacity of cloudberry to get established in abandoned peatlands.

Performance of Male and Female Clones

The better growth of male compared to female in greenhouse is consistent with previous work (Ågren 1988b). The higher number of male ramets produced is also consistent with the male biased ratio generally observed between flowering ramets in the field (Dumas and Maillette 1987; Karst et al. 2008; Korpelainen 1994). Male clones would produce more ramets which, with time, would translate into a biased ratio in favour of males. A

1:10 male to female ratio at planting was suggested as being sufficient to insure efficient pollination. We also recommend this ratio in order to avoid an overabundance of male ramets after 15 or 20 years of cultivation. The proportion of flowering ramets was the same in male and female clones in the present study. In natural habitat, Ågren (1988b) reported higher proportion of flowering ramets in male compared to female clones, but this was estimated at a single site. Better growing conditions in the greenhouse than in the field most likely explain the high flowering capacity reported here compared to what has been reported in natural peatlands (Ågren 1988a; Bellemare et al. 2009b); good growing conditions could mask potential male-female differences in terms of flowering capacity.

Female clones had on average smaller leaves than male clones. Ågren (1988b) concluded that the smaller leaves of female clones in cloudberry was due to the investment in fruits, but the present study indicated that the leaf size difference was present even in the absence of fruit production. Furthermore, flower investment during flowering is more demanding in cloudberry male than in female ramets because of pollen production (Rameau and Gouyon 1991). Nitrogen and phosphorus were higher in non-fruiting female than in male leaves (for phosphorus, differences are significant only in fibric peat), whereas no difference in terms of nitrogen and phosphorus concentration between leaves of female fruiting ramets and of male ramets has been reported from a field study (Ågren 1988b). Female ramets might require more nutrients in order to flower, considering that part of the nitrogen and phosphorus appears to be translocated to the developing fruit.

Conclusion

The present data clearly demonstrates that cloudberry should be planted on fibric (H1 to H3) rather than on mesic peat. A low proportion of former cutover peatlands present weakly decomposed peat (Graf et al. 2012). Future research should thus focus on the possibility to plant cloudberry on restored sites that have accumulated a significant moss layer in order to maximise both restored areas in cutover peatlands and cloudberry cultivation success. Field and greenhouse experiments confirmed the necessity of conducting experiments on cloudberry over more than one growing season due to the slow growth of the species. Further studies should also address aluminium assimilation and toxicity in cloudberry as the plant appears to be very sensitive to this element. Greenhouse experiments confirmed that male clones produce larger leaves and more ramets than female clones. The proportion of male to female clones at planting should be low in order to avoid a disproportion of male clones after many years of cultivation.

ACKNOWLEDGEMENTS

We are thankful to Antoine Bernatchez, Valérie Brousseau, Louis Charron, Dominique Manny and Laurence Simard for greenhouse, lab and field work. Special thanks to greenhouse's employees, especially Carole Martinez and François Larochelle, and to Alain Brousseau for foliar analysis, all from Université Laval. We are grateful to Les Tourbières Berger ltée for providing peat for the greenhouse experiment, to Premier Tech ltée for allowing us to establish experiments at their field sites and to Agri Quanta Inc for soil analysis. This project was financially supported by the NSERC Industrial Research Chair in Peatland Management (LR).

REFERENCES

Aerts, R. and Chapin, F. S. I. 2006. The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. Adv. Ecol. Res. **30**: 1-67.

Ågren, J. 1987. Intersexual differences in phenology and damage by herbivores and pathogens in dioecious *Rubus chamaemorus* L. Oecologia **72**: 161-169.

Ågren, J. 1988a. Between-year variation in flowering and fruit set in frost-prone and frost-sheltered populations of dioecious *Rubus chamaemorus.* Oecologia **76**: 175-183.

Ågren, J. 1988b. Sexual differences in biomass and nutrient allocation in the dioecious *Rubus chamaemorus*. Ecology **69**: 962-973.

Barrett, S. C. H. and Hough, J. 2013. Sexual dimorphism in flowering plants. J. Exp. Bot. **64**: 67-82.

Bellemare, M., Lapointe, L., Chiasson, G., Daigle, J.-Y. and Rochefort, L. 2009a. Conditions favouring survival of cloudberry (*Rubus chamaemorus*) rhizomes planted in cutover peatland. Mires and Peat **5**: 1-8.

Bellemare, M., Rochefort, L. and Lapointe, L. 2009b. Rhizome sectioning and fertilization increase the productivity of cloudberry in natural peatlands. Can. J. Plant Sci. **89**: 521-526.

Boxall, P. C., Murray, G., Unterschultz, J. R. and Boxall, P. C. 2003. Non-timber forest products from the Canadian boreal forest: an exploration of aboriginal opportunities. J. For. Econ. **9**: 75-96.

Caron, J. and Rivière, L.-M. 2003. Quality of peat substrates for plants grown in containers. Pages 67-93 *in* L.-É. Parent and P. Ilnicki, eds. Organic soils and peat materials for sustainable agriculture. CRC Press, Boca Raton.

Centre d'expertise sur les produits agroforestier 2008. Mise en valeur des produits forestiers non ligneux (http://www.biopterre.com/wp-content/uploads/2011/09/Mise-en-valeur-des-PFNL.pdf). [Online] Available: [2014 July 10].

Close, D. C. and Beadle, C. L. 2003. The ecophysiology of foliar anthocyanin. Bot. Rev. **69**: 149-161.

Dumas, P. and Maillette, L. 1987. Rapport des sexes, effort et succès de reproduction chez *Rubus chamaemorus*, plante herbacée vivace dioïque de distribution subarctique. Can. J. Bot. **65**: 2628-2639.

Graf, M. D., Bérubé, V. and Rochefort, L. 2012. Restoration of peatlands after peat extraction. Pages 259-280 *in* D. Vitt and J. Bhatti, eds. Restoration and reclamation of boreal ecosystems. Cambridge University Press, Cambridge.

Hébert-Gentile, V., Naess, S. K., Parent, L.-É. and Lapointe, L. 2011. Organo-mineral fertilization in natural peatlands of the Quebec North-Shore, Canada: dispersion in soil and effects on cloudberry growth and fruit yield. Acta Agric. Scand. Sect. B-Soil Plant Sci. **61**: 8-17.

Jansen, S., Broadley, M. R., Robbrecht, E. and Smets, E. 2002. Aluminium hyperaccumulation in angiosperms: A review of its phylogenetic signifiance. Bot. Rev. **68**: 235-269.

Jean, D. and Lapointe, L. 2001. Limited carbohydrate availability as a potential cause of fruit abortion in *Rubus chamaemorus*. Physiol. Plant. **112**: 379-387.

Karst, A. L., Antos, J. A. and Allen, G. A. 2008. Sex ratio, flowering and fruit set in dioecious *Rubus chamaemorus* (Rosaceae) in Labrador. Botany **86**: 204-212.

Korpelainen, H. 1994. Sex ratios and resource allocation among sexually reproducing plants of *Rubus chamaemorus*. Ann. Bot. **74**: 627-632.

Kortesharju, J. 1982. Effects of temperature on annual growth, development and cultivation possibilities of the cloudberry (*Rubus chamaemorus*). Research Institute of Northern Finland, University of Oulu.

Kortesharju, J. 1988. Cloudberry yields and factors affecting the yield in Northern Finland. Acta Bot. Fennica **136**: 77-80.

Lucchese, M., Waddington, J. M., Poulin, M., Pouliot, R., Rochefort, L. and Strack, M. 2010. Organic matter accumulation in a restored peatland: Evaluating restoration success. Ecol. Eng. **36**: 482-488.

Marks, T. C. and Taylor, K. 1972. The mineral nutrient status of *Rubus chamaemorus* L. in relation to burning and sheep grazing. J. Appl. Ecol. **9**: 501-511.

Mehlich, A. 1984. Mehlich 3 soil test extractant: A modification of Mehlich 2 extractant. Commun. Soil Sci. Plant Anal. **15**: 1409-1416.

Obeso, J. R. 2002. The cost of reproduction in plants. New Phytol. **155**: 348.

Parent, L.-É., Parent, S.-É., Hébert-Gentile, V., Naess, S. K. and Lapointe, L. 2013. Mineral balance plasticity of cloudberry (*Rubus chamaemorus*) in Quebec-Labrador bogs. Am. J. Plant Sci. **4**: 1508-1520.

Parkinson, J. A. and Allen, S. E. 1975. A wet oxidation procedure suitable for the determination of nitrogen and mineral nutrients in biological material. Commun. Soil Sci. Plant Anal. **6**: 1-11.

Payette, S. and Rochefort, L. 2001. Écologie des tourbières. Les Presses de l'Université Laval, Québec, Québec.

Pelletier, L., Brown, A. O., Otrysko, B. and McNeil, J. N. 2001. Entomophily of the cloudberry (*Rubus chamaemorus*). Entomol. Exp. Appl. **101**: 219-224.

Price, J., Rochefort, L. and Quinty, F. 1998. Energy and moisture considerations on cutover peatlands: surface microtopography, mulch cover and Sphagnum regeneration. Ecol. Eng. **10**: 293-312.

Rameau, C. and Gouyon, P.-H. 1991. Resource allocation to growth, reproduction and survival in *Gladiolus*: The cost of male function. J. Evol. Biol. **4**: 291-307.

Rapp, K. 2004. Cloudberry growers guide. North Norwegian Centre for Research and Rural Development, Tromsø, Norway.

Rudolph, H., Kirchhoff, M. and Gliesmann, S. 1988. Sphagnum culture techniques. Pages 25-34 *in* J. M. Glime, ed. Methods in Bryology, Proceedings of the bryological methods workshop, Mainz. Hattori Botanical Laboratory, Nichinan, Japan.

Saastamoinen, O., Kangas, K. and Aho, H. 2000. The picking of wild berries in Finland in 1997 and 1998. Scand. J. Forest Res. **15**: 645-650.

Sæbø, S. 1968. The autoecology of *Rubus chamaemorus* L. I- Phosphorus economy of *Rubus chamaemorus* in an ombrotrophic mire. Sci. Rep. Agr. Coll. Norway **47**: 1-67.

Sæbø, S. 1970. The autecology of *Rubus chamaemorus* L. II. Nitrogen economy of *Rubus chamaemorus* in an ombrotrophic mire. Sci. Rep. Agr. Coll. Norway **49**: 1-37.

Salonen, V. 1987. Relationship between the seed rain and the establishment of vegetation in two areas abandoned after peat harvesting. Holarctic Ecol. **10**: 171-174.

Théroux-Rancourt, G. 2007. Influence de l'hydrologie, du substrat et de la restauration d'une tourbière abandonnée sur la croissance de la chicouté. M.Sc. thesis, Université Laval, Quebec, Canada, 73 pp .

Théroux-Rancourt, G., Rochefort, L. and Lapointe, L. 2009. Cloudberry cultivation in cutover peatlands: hydrological and soil physical impacts on the growth of different clones and cultivars. Mires and Peat **5**: 1-16.

Thiem, B. 2003. *Rubus chamaemorus* L. - a boreal plant rich in biologically active metabolites: a review. Biol. Lett. **40**: 3-13.

Thomine, S., Lelièvre, F., Debardieux, E., Schroeder, J. I. and Barbier-Brygoo, H. 2003. AtNRAMP3, a multispecific vacuolar metal transporter involved in plant responses to iron deficiency. Plant J. **34**: 685-695.

Table 1. Analysis of variance testing the effect of the degree of decomposition and sex of the clones on vegetative growth, flowering and dry biomass of aerial and belowground tissues of cloudberry after three growing seasons in greenhouse

	Degree of decomposition		Sex		Degree of decomposition × Sex	
Variable	*F* [z]	*P*	*F* [z]	*P*	*F* [z]	*P*
Number of ramets / rhizome planted [y]	114.3	**< 0.001**	8.3	**0.008**	0.6	0.43
Number of leaves / ramet	236.1	**< 0.001**	0.5	0.51	0.5	0.49
Individual leaf diagonal (cm)	292.4	**< 0.001**	5.8	**0.02**	1.0	0.33
Percent flowering per container	17.7	**< 0.001**	0.3	0.58	0.1	0.81
Aerial biomass [x]	615.1	< 0.001	19.3	< 0.001	6.0	**0.02** [w]
Leaf biomass [x]	572.5	< 0.001	17.9	< 0.001	6.3	**0.02** [w]
Petiole biomass [y]	144.3	**< 0.001**	18.6	**< 0.001**	1.3	0.3
Belowground biomass [x]	280.5	**< 0.001**	23.4	**< 0.001**	0.2	0.7
Rhizome biomass [x]	171.1	**< 0.001**	31.3	**< 0.001**	0.7	0.4
Root biomass [x]	368.4	**< 0.001**	8.1	**0.008**	1.5	0.2
Belowground/aerial biomass [x]	252.7	**< 0.001**	0.8	0.4	0.0	1.0

[z] Df is 1 for degree of decomposition, 1 for sex, 1 for the interactive term degree of decomposition × sex and 28 for error except for aerial and belowground/aerial biomass variables for which df_{error} is 27.

[y] rank transformed

[x] log transformed; all biomass were estimated on a per plant basis

[w] LSD comparisons for interactions for aerial biomass and for leaf biomass is: Fib_Mal a, Fib_Fem b, Mes_Mal c and Mes_Fem c.

Table 2. Analysis of variance testing the degree of decomposition and sex effects on cloudberry leaf nutrient concentration and leaf red coloration after three growing seasons in greenhouse

Variables	Degree of decomposition		Sex		Degree of decomposition × Sex		LSD comparisons for interactions
	F^{z}	*P*	F^{z}	*P*	F^{z}	*P*	
N	55.8	**< 0.001**	14.4	**0.003**	0.55	0.47	
P^{y}	203.8	< 0.001	15.8	0.002	5.8	**0.03**	Fib_Fem a Fib_Mal b Mes_Fem c Mes_Mal c
K	20.2	**< 0.001**	0.1	0.76	1.2	0.28	
Ca	59.4	< 0.001	0.4	0.54	9.5	**0.01**	Fib_Mal a Fib_Fem b Mes_Fem c Mes_Mal c
Mg	126.0	< 0.001	8.0	0.02	11.6	**0.005**	Fib_Mal a Fib_Fem b Mes_Fem c Mes_Mal c
Cu	0.2	0.67	3.6	0.08	6.1	**0.03**	Fib_Mal a

							Mes_Fem ab
							Mes_Mal ab
							Fib_Fem b
Al	17.6	**0.001**	0.1	0.77	4.0	0.07	
Fe [x]	17.2	**0.001**	3.5	0.09	3.2	0.10	
Mn	55.1	**< 0.001**	1.6	0.23	0.5	0.51	
Na	12.1	**0.005**	14.6	**0.003**	0.04	0.84	
Zn	92.5	**< 0.001**	5.9	**0.03**	0.02	0.89	
N : P ratio	149.0	< 0.001	0.1	0.79	15.7	**0.002**	Mes_Fem a
							Mes_Mal b
							Fib_Mal c
							Fib_Fem d
Leaf red coloration[w]	39.2	< 0.001	2.8	0.10	9.1	**0.006**	Mes_Mal a
							Mes_Fem b
							Fib_Fem c
							Feb_Mal c

[z] Df is 1 for degree of decomposition, 1 for sex, 1 for degree of decomposition × sex and 12 for error.
[y] log transformed
[x] rank transformed
[w] based on ranked data

Figure legends

Fig. 1. Number of ramets per plot produced during four years in restored and unrestored cutover peatland as a function of the degree of peat decomposition. Fifty rooted cloudberry plants were initially planted per plot in Year 1. Note that there was no H5 in unrestored plots.

Fig. 2. Vegetative growth and flowering of male and female cloudberry grown in greenhouse. Data (mean ± SE) are combined results of plants grown in fibric and mesic peat after three growing seasons. As each ramet bears a single flower, the percentage of flowering reflects the number of flowering / total number of ramets per container.

Fig. 3. Dry biomass of aerial and belowground tissues produced by surviving female (Fem) and male (Mal) clones of cloudberry grown in fibric (Fib) and mesic (Mes) peat for three seasons in greenhouse. Four rhizomes were initially planted per container. Survival was 97% for Fib_Fem, 100% for Fib_Mal, 78% for Mes_Fem, and 97% for Mes_Mal after two growing seasons.

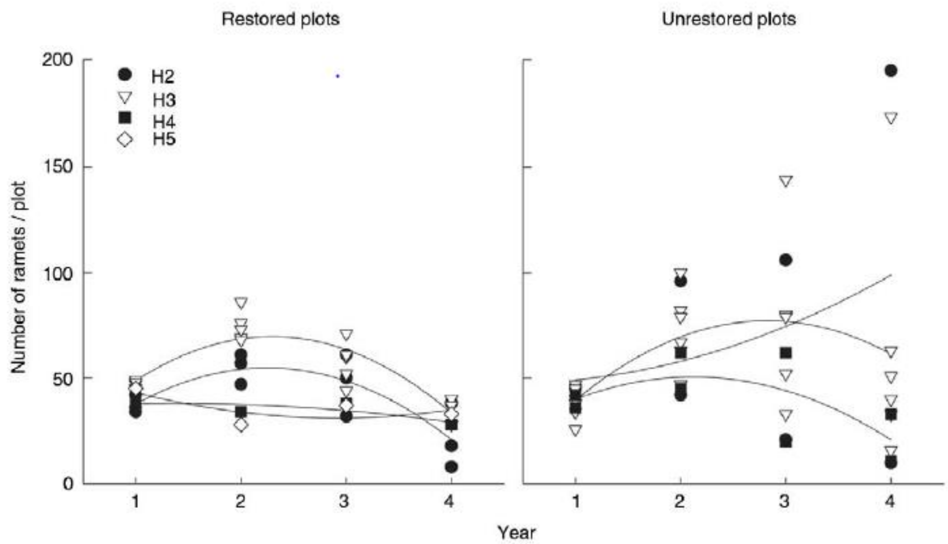
Fig. 4. Cloudberry grown for three seasons in greenhouse. Upper six plates are plants grown in mesic peat (A) and lower six plates in fibric peat (B). Four rhizomes were initially planted in each container.

Fig. 5. Vegetative growth and flowering of cloudberry grown in fibric and mesic peat in greenhouse. Data (mean ± SE) are combined results of male and female clones after three

growing seasons. As each ramet bears a single flower, the percentage of flowering reflects the number of flowering / total number of ramets per container.

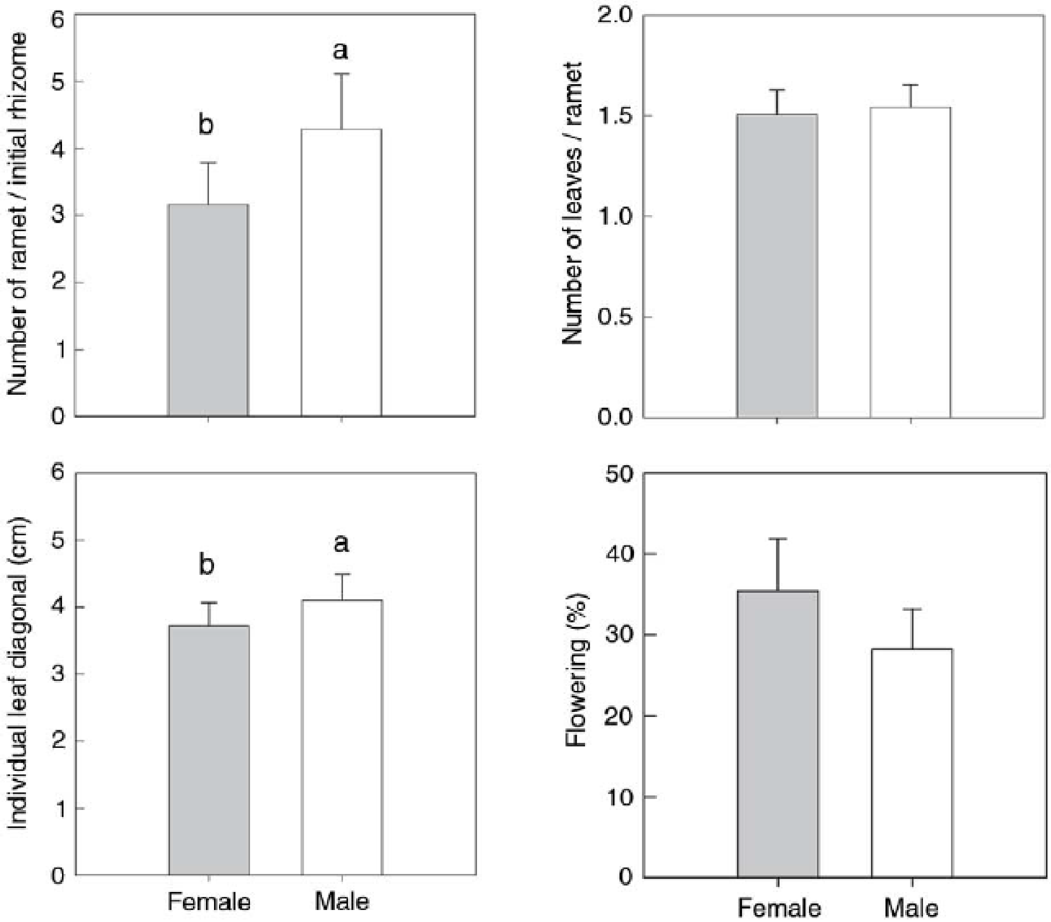
Fig. 6. Soil and foliar concentration of phosphorus, potassium, calcium, magnesium, manganese, copper, iron, zinc, aluminium, and sodium for male (Mal) and female (Fem) clones of cloudberry grown for three seasons in fibric (Fib) and mesic (Mes) peat in greenhouse.

Figure 1



Restored plots
Unrestored plots
H2
H3
H4
H5
Number of ramets / plot
200
150
100
50
0
1
2
3
4
Year

Figure 2



Number of ramet / initial rhizome
Number of leaves / ramet
Individual leaf diagonal (cm)
Flowering (%)
a
b
a
b
Female
Male
Female
Male

Figure 3

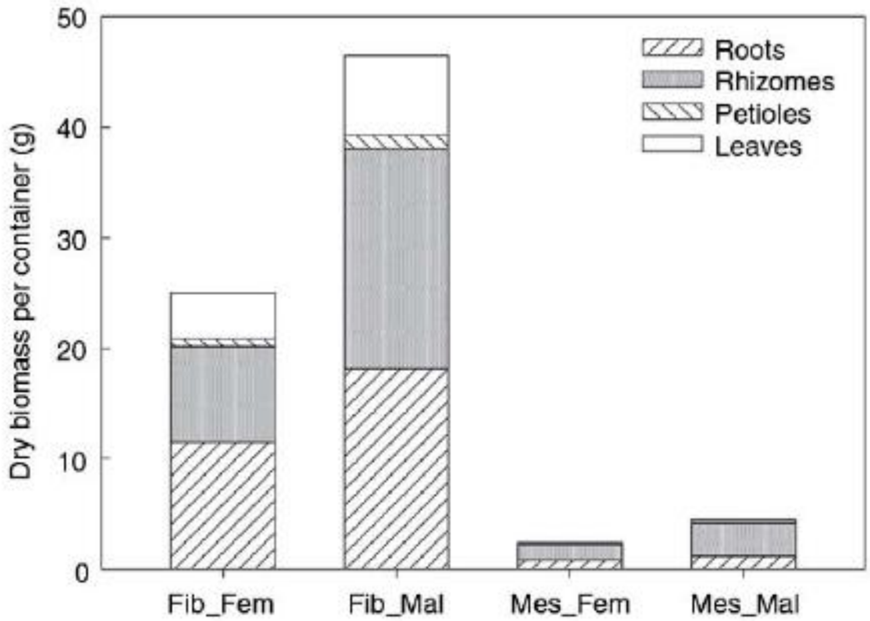

Roots
Rhizomes
Petioles
Leaves
Dry biomass per container (g)
0
10
20
30
40
50
Fib_Fem
Fib_Mal
Mes_Fem
Mes_Mal

Figure 4

Figure 5

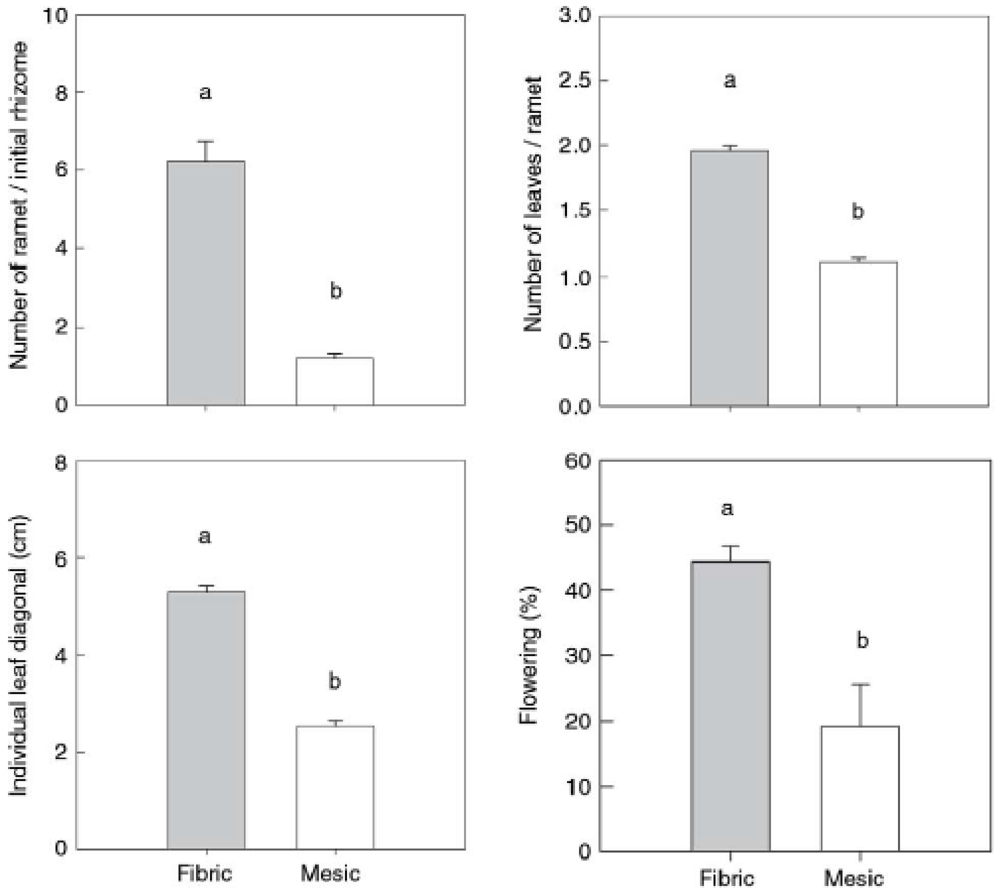
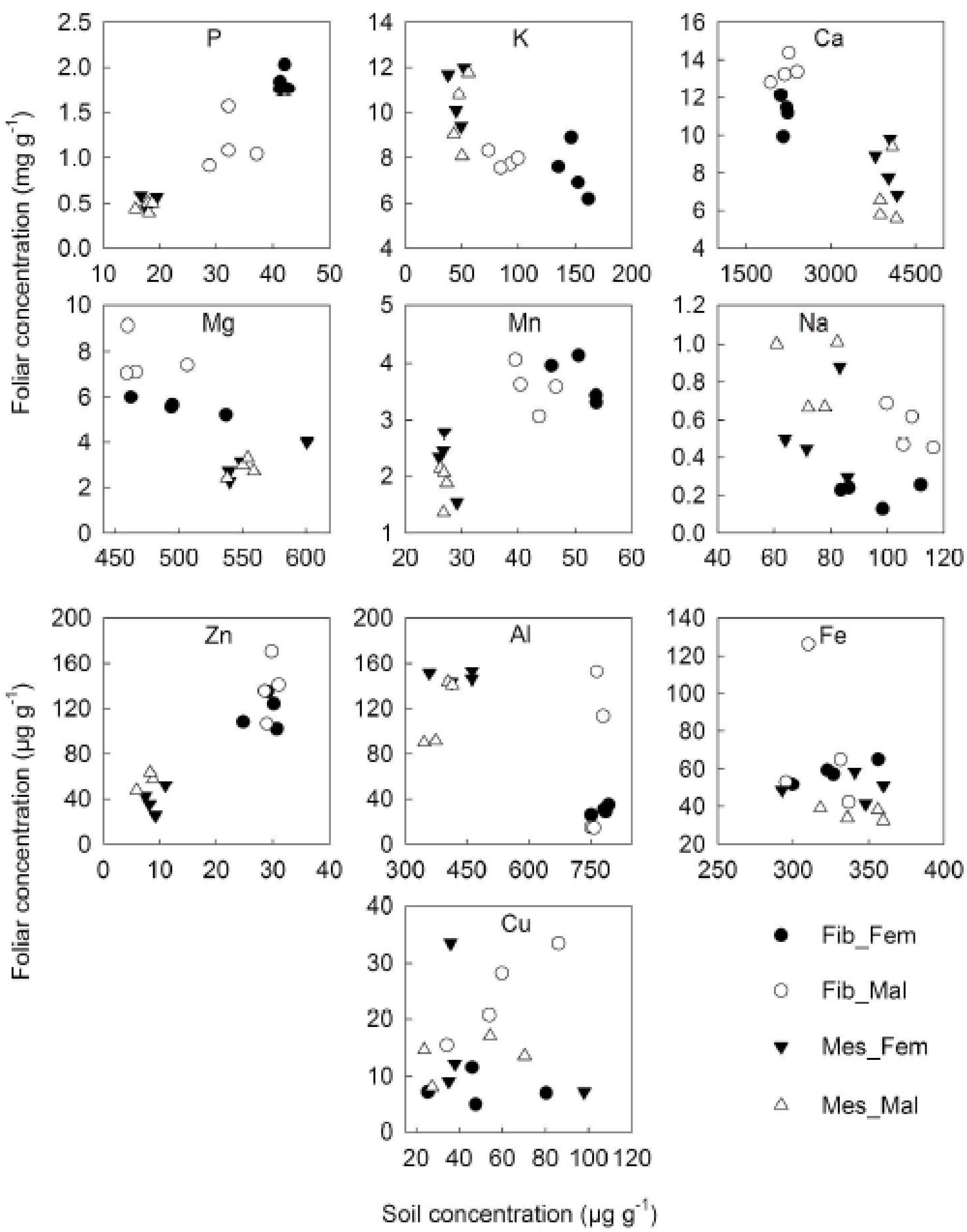

Number of ramet / initial rhizome
Number of leaves / ramet
Individual leaf diagonal (cm)
Flowering (%)
a
b
Fibric
Mesic

Figure 6


P
K
Ca
Mg
Mn
Na
Zn
Al
Fe
Cu
Foliar concentration (mg g-1)
Foliar concentration (µg g-1)
Soil concentration (µg g-1)
Fib_Fem
Fib_Mal
Mes_Fem
Mes_Mal