Sustainable development of the Quebec cloudberry industry through domestication

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PROGRAMME DE SOUTIEN À L'INNOVATION EN AGROALIMENTAIRE

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La développent durable d'un industrie via la domestication de la chicouté: des essais de variétés et la micro-propagation.

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FAITS SAILLANTS

Dans un premier volet du projet, portant sur des essais de sélections de la chicouté, la diversité génétique entre les sélections était évidente dès la troisième année au champ. L'écart dans la période de floraison des sélections les plus hâtives et les sélections les plus tardives était de 10 jours. Le nombre moyen de fleurs produites par plant variât de 1 à 28 et les rendements moyens par mètre carré variaient de 0 à 49 grammes. Cette diversité génétique démontre bien le potentiel d'augmentation durable des rendements de la chicouté apporté par l'amélioration génétique et la sélection des variétés performantes. Dans un deuxième volet du projet, portant sur la micro-propagation de la chicouté en vue d'une augmentation rapide des sélections pour des essais en plusieurs régions et des expériences sur la régie de culture, des améliorations dans des protocoles d'initiation et d'enracinement ont été réalisées. La banque *in vitro* de chicouté au Centre de recherche Les Buissons a été augmentée par 17 sélections de chicouté mâles et 50 sélections femelles.

OBJECTIF ET MÉTHODOLOGIE

Les objectifs du projet étaient de démontrer le pouvoir de l'amélioration génétique de la chicouté à améliorer les rendements de chicouté (volet I) et d'améliorer les protocoles de micro-propagation de la chicouté (volet II). Pour démontrer les effets de la génotype de la chicouté sur les rendements, 45 sélections femelles, incluant les deux cultivars de la Norvège, et 15 sélections mâles incluant les deux cultivars de la Norvège ont été plantées dans trois blocs, une plante de chaque dans chaque bloc, dans une tourbière aménagée sur les terrains du Centre de recherche Les Buissons. Des données sur la période de floraison, le nombre de fleurs, la mise à fruit, le nombre de fruit, les rendements et la sénescence ont été prises pendant 3 saisons. Les protocoles de micro-propagation de la chicouté ont été améliorés suivant une série d'expériences portant sur la désinfection des plantes mères et des explants, les milieux d'acclimatisation et des traitements d'enracinement.

RÉSULTATS SIGNIFICATIFS POUR L'INDUSTRIE

Parmi les 45 sélections femelles et 15 sélections mâles mises en essai, il y'a suffisant de diversité génétique pour sortir des cultivars de chicouté performant dans nos conditions (Figure 1). Bien avant d'avoir rempli les parcelles, dans leur troisième année au champ, les sélections ont produit en moyenne 9 gr par mètre. Les cinq meilleures sélections ont produit plus de 30 gr par mètre, ce qui correspond au 300 kg/hectare. Cependant, les effets sur la période de floraison et le nombre de fleurs et fruits produits reliés à l'origine des sélections ainsi que la durée trop courte du projet nous ne permettront pas de faire des recommandations sur des variétés sans avoir fait l'essai des sélections à plus grande échelle en plusieurs régions.

Malgré un surplus de plantes mâles dans les parcelles, il y avait un manque dans la pollinisation et les fruits produits étaient plus petits que prévus. Des différences significatives entre les périodes de floraison des sélections (Figure 1) ainsi qu'un manque de fleurs produit par les

plantes mâles sont peut être à la base de la pollinisation manquante. Il va falloir mieux planifier les plantations dans l'avenir, soit en termes de paires de sélections mâles et femelles compatibles, soit en termes du ratio mâle femelle pour éviter des problèmes de pollinisation.

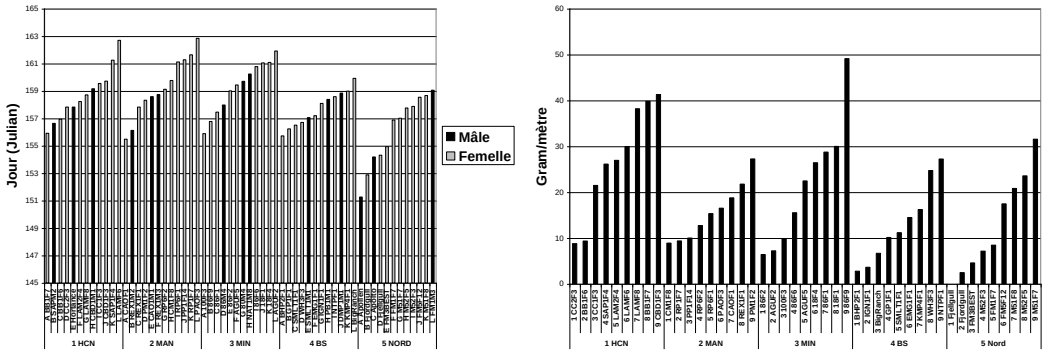

Figure 1. La variabilité dans la période de floraison (à gauche) et dans les rendements de fruits (à droite) entre les quatre cultivars de Norvège et les sélections de chicouté originaire de cinq régions de la Côte-Nord.

La plupart des sélections les plus intéressantes sont maintenant en banque *in vitro* prêt à multiplier pour des fins de production ou autre. Dans les protocoles de micro-propagation développés, des problèmes de contamination, de récalcitrance et d'acclimatation rencontrés dans la culture *in vitro* de la chicouté sont diminués.

APPLICATIONS POSSIBLES POUR L'INDUSTRIE

L'industrie comme telle n'existe pas encore. Les applications des résultats seront plutôt dans les étapes suivantes; le développent des régies de culture pour la chicouté et l'homologation des cultivars productifs de la chicouté. Une source de plantes de chicouté mâles et femelles n'existe pas ni au Québec, ni au Canada. Des pépinières intéressées par des nouveaux types de plantes pouvaient utiliser les protocoles de propagation décrits pour produire des plantes de chicouté.

POINT DE CONTACT

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PARTENAIRES FINANCIERS

L'équipe de recherche tient à remercier le ministère de l'Agriculture, des Pêcheries et de l'Alimentation ainsi que le Ministère du Développement économique, de l'Innovation et de l'Exportation du Québec et la Conférence régionale des élus de la Côte-Nord pour le soutien financier, sans lequel le projet n'aurait pu se concrétiser.

ACTIVITÉS DE DIFFUSION ET DE TRANSFERT AUX UTILISATEURS

Des données préliminaires du projet ont été présentées à la communauté de Blanc-Sablon, le 6 août 2008 par Kristine Naess. Le programme, ainsi qu'une liste des participants, sont présentés en annexe du rapport (Annexe 1).

Les résultats préliminaires du projet ont été présentés aux producteurs et d'autres intervenants intéressés par les petits fruits nordiques lors du Colloque Bioalimentaire Côte-Nord 2009. Le programme du colloque est présenté en annexe du rapport (Annexe 2).

Différentes visites au CRLB a été organisées afin de promouvoir la domestication de la chicouté :

Visite guidée du laboratoire *in vitro* et le chambre de croissance pour Richard Beaudry, producteur potentiel et Laurier Tremblay, agronome au MAPAQ, le 16.01.2009.

Pricilla Griffen, NTFP coordinatrice pour les Coasters sur la Basse-Côte-Nord et Hugh Maynard, consultant pour les Coasters ainsi que Mélodie Desrossiers de la Cooperative Gaia de Pointe-aux-Outardes, sont venus en compagnie avec Stephane Boudreau du MAMROT, et Laurier Tremblay du MAPAQ pour une visite du laboratoire in vitro et de la chambre de croissance le 4.03.2010.

Ashley Morency, agente de développement en agro-alimentaire, CLD Basse-Côte-Nord, est venue pour une visite guidée des projets petits fruits au Centre de recherche Les Buissons le 19.05.2010.

Laurence Simard-Gagnon, étudiante à la maitrise à l'université Laval, est venu pour une visite guidée du champ d'essai le 18.06.2010.

Dr Gilbert Banville, agronome, est venue pour une visite guidée du champ d'essai en fruit le 6.07.2010.

Candy N. F. Keith**,** MSc, du Ontario Plant Propagation Program, New Liskeard Agricultural Research Station, University of Guelph**,** New Liskeard, ON, a fait une visite au laboratoire *in vitro* le 19.10.2010

Un résumé des projets sera présenté sur le nouveau site internet du CRLB qui sera en opération sous peu.

ACTIVITÉS DE TRANSFERT SCIENTIFIQUE

Les résultats préliminaires du projet ont été présentés par Kristine Naess lors du deuxième Labrador Straits Wildberry Conference, L'Anse au Clair, NL le 5 à 6 Septembre, 2007.

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Sustainable development of the Quebec cloudberry industry through domestication: field trials of cloudberry selections and micro-propagation.

Introduction

Wild berries are an abundant natural resource in the territories surrounding many of the communities touched by the crisis in the fishing industry. The cloudberry, which is exclusive to the northernmost regions of Quebec, has a long history of local use. In addition to its distinctive flavor, these berries are rich sources of vitamins, phenolic compounds, soluble and insoluble fibers and minerals. With the rise in popularity of both functional foods and natural foods, the national and international markets for wild berries are in expansion. The communities of the Lower North shore and Minganie have the natural resources to seize upon this expanding market and build a small fruits industry around it. The need for increased research in the field of wild berry crops has been identified as critical to the success of industry development (CEPRO, 2004)

While the demand for cloudberries is growing, supplies are dwindling. The increased demand for cloudberries coupled with increased unemployment has led to increased ATV traffic in the fragile boglands where the cloudberry is found. Just as low yields and yield instability are severe obstacles to industry development, industry development itself may endanger crop production in the wild. Thus the sustainable development of a cloudberry industry is hinged upon its successful domestication as a new crop for the northern regions of Quebec.

Although new crop development is a daunting task involving germplasm collection, breeding, and research in the fields of crop physiology, processing and marketing, these efforts are not made in vain. The initial and crucial stage of a new crop development program is the demonstration of yield improvements without which further support for the program is impossible. The breeding and selection of improved plant materials suitable for cultivation is an important low impact approach resulting in increased yields. Genetic variation in important direct and indirect yield component traits such as fruit numbers and size, flowering precocity and phenology as well as winter hardiness, vegetative vigour and shoot production has been documented among cloudberry populations (Rapp, 1991, Weydahl, 1975). An appreciation of this genetic diversity led to the release of the first cloudberry cultivars in Norway where the domestication and cultivation of the cloudberry has resulted in 25 fold yield increases (Rapp, per com.). The four Norwegian cultivars however have not performed well in preliminary trials under Quebec field conditions, nor have they performed well in comparison to native selections when grown in pots in the growth chamber or outdoors, producing fewer than a third as many flowers as Quebec selected clones. The need for cultivars native to Quebec is thus essential to the success of the domestication program.

In the first part of this project we examine the field behaviour of 43 female and 13 male cloudberry selections gathered from five different regions of the north shore of Québec in comparison with the four Norwegian cultivars. Plant establishment and spread, flowering

and flowering phenology, fruit set and yields as well as plant senescence are followed during three seasons following fall planting.

New crop development also requires the production of sufficient quantities of improved planting materials. Highly efficient propagation techniques are therefore needed for the production of plants from very little starting material. Rhizome cuttings are currently used for propagating cloudberry cultivars both in Norway and in Finland yielding approximately 2000 plants per square meter of greenhouse space (Martinussen et al 2004). This method however, which takes four years from start to finish, requires the availability of significant numbers of mother plants (25 per meter). Tissue culture propagation has several advantages over conventional propagation methods of which high multiplication rates, the possibility of year-round plantlet production in a small amount of space and the rapid multiplication of new and promising cultivars from limited mother plant materials are of particular interest for new crop development programs. With multiplication rates of 3 per subculture and subcultures every 6 weeks it is possible to obtain 2000 tissue culture plantlets starting from five tubes in the course of a year.
Tissue culture propagation methods for cloudberry were developed as early as 1982 (Naess, 1982) and have been improved upon more recently (Theim, 2001, Theim 2002, Martinussen *et al.* 2004, Debnath, 2007). Nonetheless, commercial in vitro production of cloudberry plants has not become a reality based largely on stumbling blocks in two phases of the tissue culture propagation system, namely the initiation phase and the ex-vitro acclimatization phase (Naess *et al.*, 1993).

The cloudberry is an herbaceous perennial in which the growing meristems are located underground. Adequate surface sterilization of buds is extraordinarily difficult, thus the dissection of the bud and explantation of the terminal meristem with a few subtending axillary meristems is necessary (Naess 1982). Nonetheless, contamination problems remain and the small explants are slow to proliferate. Methods used to combat endogenous contaminants include the use of antibiotics and fungicides as pretreatments to *in vitro* culture and their use in the tissue culture medium itself (Pence *et al*., 2002). As fungi are the most frequently encountered contamination problems in cloudberry cultures the use of the systemic fungicide benlate and or the biocide PPM (Plant Preservative Medium) are of particular interest.

A different approach to the establishment of Rubus material in vitro is through the stimulation of de novo meristem regeneration at the edges of cut leaves (Swartz *et al*., 1990). Contamination problems are reduced as leaves are more easily surface sterilized than buds. Proliferation rates are also high due to the juvenile nature of de novo meristems. Regeneration success however is species and often genotype dependant (Graham *et al.,* 1997: Tsoa and Reed 2002) as well as gender dependant (Martinussen and Naess, per obs.), requiring species and genotype specific optimization of the media used. The work involved in developing media can be greatly reduced by using a step-wise screening procedure as practiced by the US Rubus gene bank in Corvallis (Reed 1990).

The substituted urea thidiazuron (TDZ) is commonly used to induce regeneration in woody plant species (Huetteman and Preece, 1993) including Rubus (Fiola *et al*., 1990). Ethylene production may be a negative side effect resulting from the use of TDZ which reduces regeneration in some systems (Murthy *et al.,* 1998). Indeed, the concomitant use of ethylene biosynthesis inhibitors or ethylene competitors leads to significantly increased regeneration in several rosaceous species including apple (Seong *et al.,* 2005), apricot (Burgos et Alburquerque, 2003) and plum. The effects of ethylene inhibitors on cloudberry regeneration have not yet been reported.

More recently a third alternative for the in vitro establishment of cloudberry has been published (Debnath 2007). This method involves the use of the nodes cut from the cloudberry shoot as it elongates in the spring. The cloudberry shoot is annual and no buds or axillary shoots form naturally at these nodes. Nonetheless the method as described is simple and effective.

Once established, the cloudberry is easily propagated in tissue culture with multiplication rates of 4-5 when subcultured at intervals of 6 weeks (Martinussen *et al.,* 2004). However, the acclimatization and rooting of the tissue culture plantlets, which occur simultaneously, constitutes an additional bottleneck in the propagation scheme with top survival percentages of 60-70% (Thiem 2001, Martinussen *et al.,* 2004). The control of fungal contamination during the acclimatization process and the stimulation of adequate rooting and growth both need to be addressed. Decreasing the humidity within the tissue culture vessel stimulates the plantlet to produce the surface waxes needed to survive the transfer *ex vitro*. This can be accomplished through increased ventilation of the tissue culture vessel or through the use of PEG (polyethylene glycol) in the culture medium (Dami and Hughes, 1997). In earlier pilot studies, increasing ventilation proved detrimental to cloudberry cultures (Naess, unpublished results). The range of humidity levels obtainable through the use of differing concentrations of PEG should prove more interesting. Rooting of *in vitro* plantlets, as with other cuttings, is usually promoted with the use of auxins, in particular IBA (indole butyric acid). The cloudberry however responds poorly to most auxin treatments with the exception of IAA (indole acetic acid). The negative response to auxins may be due to toxic side effects of ethylene production which can be counteracted through the use of ethylene biosynthesis inhibitors or inhibitors of ethylene action (Biondi *et al.,* 1990).

In the second part of this project we improve upon cloudberry *in vitro* propagation protocols, concentrating on the *in vitro* initiation step and the rooting and acclimatization step. We examine different methods to reduce contamination at initiation, methods to increase the success of initiation and various pre treatments leading to improved acclimation and rooting in cloudberry.

Field Trials

Materials and methods

Plant material

In the course of the collaborative project "Mise en place d'une culture de chicouté dans un contexte de phytorestauration de tourbières après exploitation" (Line Lapointe *et al.*, 2004-2006), the Centre de Recherche Les Buissons collected cloudberry germplasm from five regions of Quebec spanning the area from Tadousac to Blanc Sablon and reaching as far inland as Fermont. These clones, (9 female and 3 males from each of five regions) were selected in the field based on flower size (pistils per flower), a highly heritable trait effecting fruit size (Rapp, 1989), and flower density, an important yield component.

Plants were potted up and cycled through 6 artificial seasons of growth (growth chamber 13 weeks followed by 13 weeks cold chamber at 4°C) before being planted in the field trials in the fall of 2007.

Field preparation

The field (30 x 8 meters) was prepared in a relatively dry bog on the Manicouagan peninsula. Vegetation was removed and the peat was tilled to a depth of a few inches. Nine beds 1x 8 meters large and 10 cm high were prepared from the tilled peat. A ditch 1 meter deep and 50 cm wide was cut into the peat around the field for use in controlling water levels.

Field Design

As one of the objectives of the project was to demonstrate to the communities involved the effectiveness of cultivation and the importance of plant genotype to cloudberry cultivation we used a replicated block design which allowed the grouping of genotypes in the field based on their previous performance in pots under controlled conditions. Assuming performance in the field is correlated to previous performance in pots, this block design should provide visual evidence of the importance of genotype which would not be apparent with a completely randomized design. The two Norwegian female cultivars were included in the low performance group along with one of the males and the other male was included in the medium performance group.

The complete experimental design has the following configuration:
3 blocks
3 genotype performance level
5 regions of origin: Haute-Côte-Nord, Manicouagan, Minganie, Nord (Manic Cinq, Fermont, Norway) and Blanc Sablon
4 selections (3 females and 1 male per performance level and region of origin).

The selections from each region were sorted into three performance groups, each of which contained three female and one male selection. Four selections from each of the five region of origin sub plots were planted into each performance level bed (1 x 8 meters). Spacing between the selections, which were in 7 inch azalea pots, was 50 cm.

Between each region of origin sub plot, two additional male plants in four inch pots were planted to insure pollination of the selections. These male plants were of various origins. The field plan design is given in Figure 1.

Figure 1. Field plan for trial of cloudberry selections showing region of origin sub plots nested within performance level beds.

Water and fertilization

Water levels were maintained at no more than 50 cm from the surface by pumping water into the ditch surrounding the field as needed. Water was not drained from the field, thus in wet seasons water levels reached the surface of the field. Plants were fertilized weekly with 200 ml all purpose 20-20-20 water soluble fertilizer at 70 ppm the first two years in the field and with 200 ml 15-30-15 water soluble fertilizer at 35 ppm the third year in the field.

Data

Data was collected on shoot numbers, flower numbers, flowering phenology, fruit set, seed set and plant senescence during two seasons following planting. In the third season following planting flower numbers, flowering phenology, fruit set, yields and senescence were recorded.

Shoot numbers were recorded at the end of the season after the harvest.

Cloudberry flowers generally stay open only for a day or two. Flowers were tagged three days a week as they opened or at petal fall. Open flowers were recorded as flowering on the day they were open while those at petal fall were recorded as flowering on the previous day. For analysis of flowering phenology the average flowering day was calculated for each plant and the total number of flowers per plant used as a covariate.

Flowers on plants used as pollinators were recorded as open if pollen was still visible in the flower, thus these flowers were occasionally recorded on two consecutive tagging days. These flowers were used in calculating sex ratios for each tagging day for use later in the analysis of fruit set.

Sterile flowers and flowering shoots damaged by insects were removed from fruit set calculations. Fruit were weighed individually and the number of seed per fruit divided by the number of pistils was used to calculate seed set.

Plant senescence was followed on a weekly basis following harvest. The cloudberry phenology scale (Beaulieu *et al.*,2001) was expanded for this purpose. Thus the senescence scale used starts at a score of 15 indicating that the leaves are still green, followed by 16; over half of the leaves still green, 17; less than half of the leaves still green and 18; all leaves senesced. Notes on leaf spots were also taken but as the season progressed it was difficult to distinguish seasonal senescence from that caused by plant pathogens.

Temperature and water level data were also recorded in the field using a min max thermometer and a hobo data logger for the temperature data. Plastic pipes perforated at the bottom were sunk into the ground at the northern and southern end of the field and water levels in the pipe were recorded at each visit.

Data analysis

Data were analyzed using the SAS GLM or mixed models procedure (SAS, 2000). Repeated measures analysis was used for across year data while single year data was analyzed using a split plot design. Previous year's data were included in the analysis as covariates where appropriate.

Results

Survival and shoot production

All plants among the selections survived field planting while several of the smaller male plants planted between the region of origin sub plots died out over winter. Overall a slight but significant increase in the number of shoots produced per plant was observed in 2008 as compared to the number of shoots produced by the same plants in pots in 2007. By 2009 shoot numbers per plant had almost doubled, reaching an average of 41 shoots per plant. There was a significant interaction effect between year and region of origin on shoot production. Whereas plants from the region of Minganie produced significantly fewer shoots in pots in 2007 than plants from all other regions these plants were producing as many shoots as all others by 2009. Plants from Blanc Sablon also did relatively poorly in pots compared to plants from all other regions except Minganie but produced as many shoots in the field as the plants from other regions by 2009.

By 2009 male selections were producing slightly more shoots than female clones (44 vs 40) though the differences between the two sexes were not significant. There were significant genotype effects on shoot production. An average of 41 shoots per plant was produced. All four Norwegian cultivars produced above average shoot numbers. The least vigorous selections produced fewer than 30 shoots per plant in their second year in the field while the most vigorous selections were producing over 60 shoots per plant by this time.

Flowering phenology

In 2008 the first flowers opened on June 2nd and flowering was completed by June 16. The 2009 flowering season lasted from June 5th through July 1st while in 2010 flowering began significantly earlier, on the 21st of May, and lasted through the 16th of June (Fig. 2). There were significant effects of the region of origin on flowering phenology. Selections from the northern region (NORD), which includes the cultivars from Norway and selections from Fermont and the region of Manic Cinq, flowered significantly earlier than clones from all other regions. Selections from the region of Blanc Sablon (BS) also flowered earlier than selections from regions further south and west. Selections from the region of Minganie (MIN) were the latest to flower, though their flowering season was not significantly later than those of selections from the regions of Manicouagan (MAN) or the Haut-Côte-Nord (HCN) (Fig. 3). There were no significant differences in the average flowering periods of male and female clones. However, male clones flowered for a longer period, thus on average they began flowering two days earlier than female clones. There were significant differences in flowering periods between the selections. Thus there was a 10 day span between the flowering period of the earliest clone, the Norwegian male cultivar Apollen and the latest clone, PAOF3, a female selection from

the region of Manicoagan. Apollen flowered significantly earlier than 31 of the 43 female Québec selections. Likewise, the Norwegian female cultivar Fjellgull flowered significantly earlier than over half of the male Québec selections.

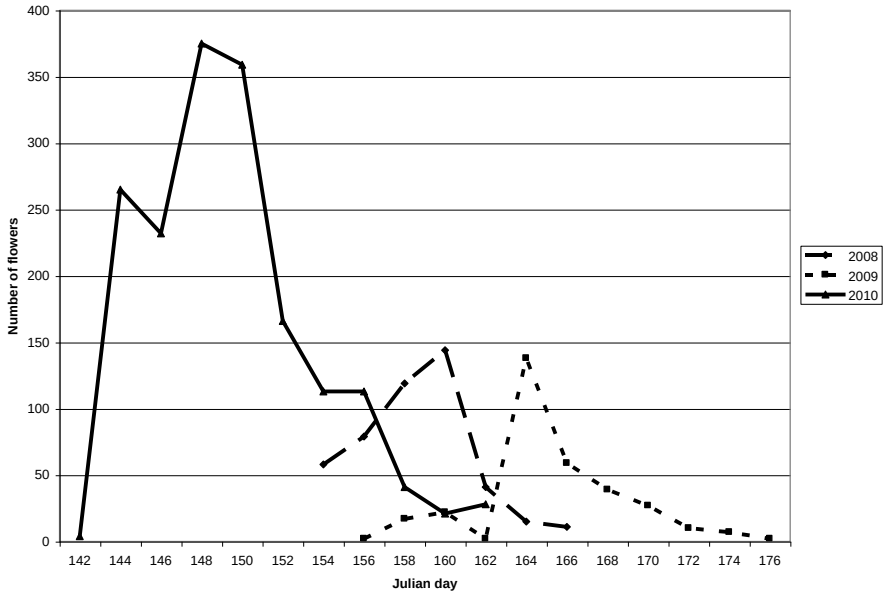

Figure 2. Flowering phenology in the field trial in 2008, 2009 and 2010.

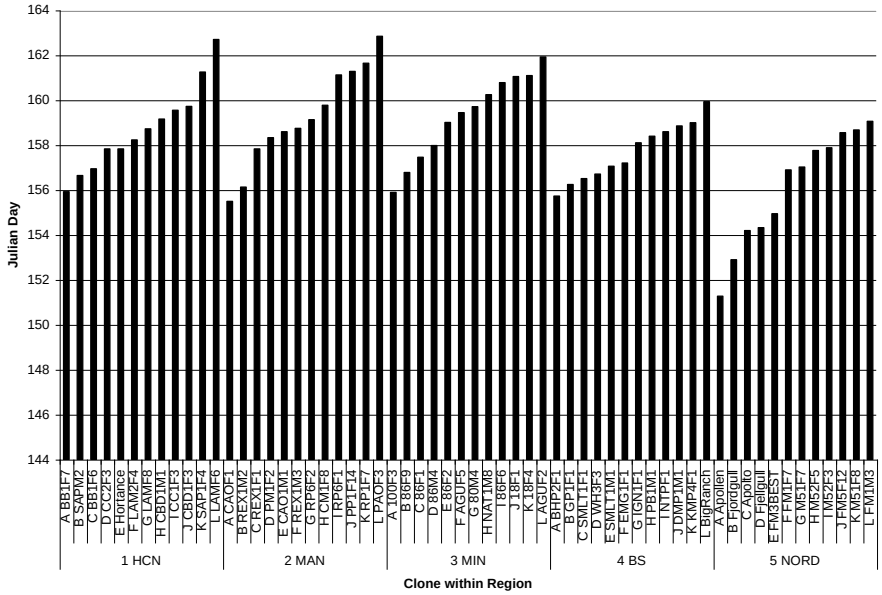

Figure 3. Flowering phenology differed between selections with different regions of origin.

Flower numbers

Flower production in the field varied between years and between planting blocks and was influenced by numerous other factors including the region of origin of the selections. An average of four flowers per plant was produced in the year following fall planting while flower numbers a year later dropped to 2 per plant. In 2010, two years after planting, flower numbers ranged from 0 to 47 per plant and averaged 9 per plant. By this time only eight of the 180 plants in the trials did not flower. Four of these were among the Norwegian cultivars, Apollen, Apolto and Fjordgull.

No block effect was noticed on flower numbers in 2008, however by 2009 block 3 was producing significantly greater numbers of flowers than the other two blocks and by 2010 twice as many flowers were produced in block three than in the other two blocks. Block 3 is wetter than the other two blocks which might explain the marked effect of planting block on flower production, however other factors such as snow accumulation or light exposure may also account for the observed differences.

In 2008 there was a correlation between flower production and the performance group to which the selection had been assigned based on previous performance in pots under controlled growth chamber conditions. By 2009 and again in 2010 however, the correlation between performance group and flower production was lost (Fig. 4). The only region of origin for which earlier performance under controlled conditions was correlated to performance in the field was the northern region of origin containing the Norwegian cultivars and clones from the region of Fermont.

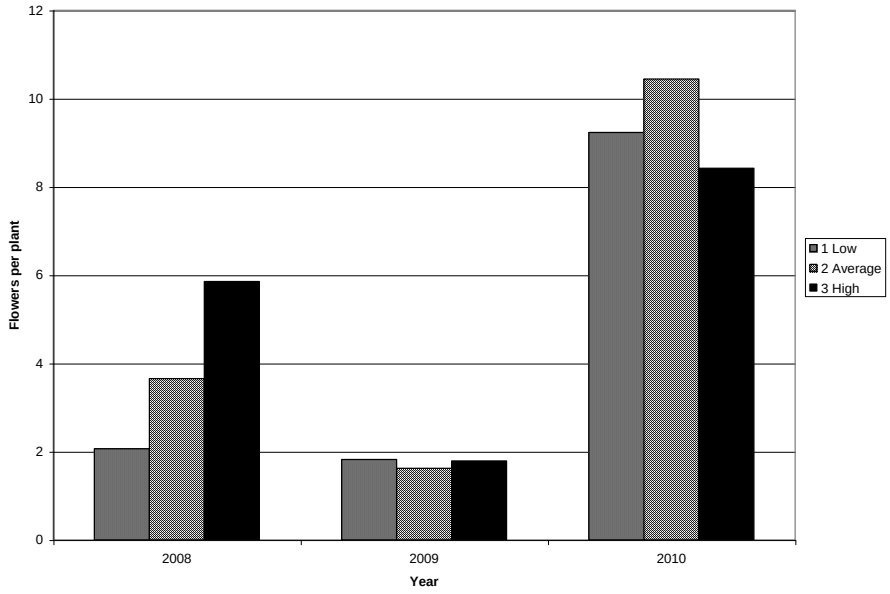

Figure 4. Loss of correlation between performance group and flower production with years in the field.

Region of origin had a significant effect on flower production in the field (Table 1). Clones selected from the regions of Blanc Sablon and Minganie produced the most flowers in the field while those from the Northern regions were the least productive. Clones selected from Manicouagan were not as productive as might be expected considering the plants were field trialled in their region of origin.

Table 1. The effects of region of origin on flower production in the selections.

Region of Origin	**Female selections**	**Male selections**	**Average (across gender)**
Haute-Côte-Nord	5.7 a	4.7 b	5.2 ab
Manicouagan	2.9 b	5.9 b	4.4 bc
Minganie	6.0 a	5.3 b	5.6 ab
Blanc Sablon	3.4 b	9.8 a	6.6 a
Northern regions	3.2 b	3.2 b	3.2 c
Average (across regions)	4.2	5.8	5.0

Means in columns followed by the same letter are not significantly different at p=0.5

There was also a significant interaction effect between region of origin and gender. Although male clones from Blanc Sablon outperformed all others, female clones from the same region did not produce many flowers. Among the female selections, those from the regions of Haute-Côte-Nord and Minganie produced the most flowers.

By the third season in the field, significant clonal differences in flower production were evident for all five regions of origin (Table 3). Differences between average flower production in female selections for a particular region and that of the highest performer for the same region were four to six-fold.

Fruitset

Several late spring frosts occurred during the flowering season in 2008 and half of the flowers produced were killed. Of the 185 female flowers produced, only 38 set fruit.

There were also late spring frosts in 2009 and in 2010 but the damage done was minimal, especially in 2009 when these frosts occurred only early in the season (Fig. 5). On average temperatures during the 2009 and 2010 cloudberry flowering seasons were favorable for pollinating insects with highs above 20°C.

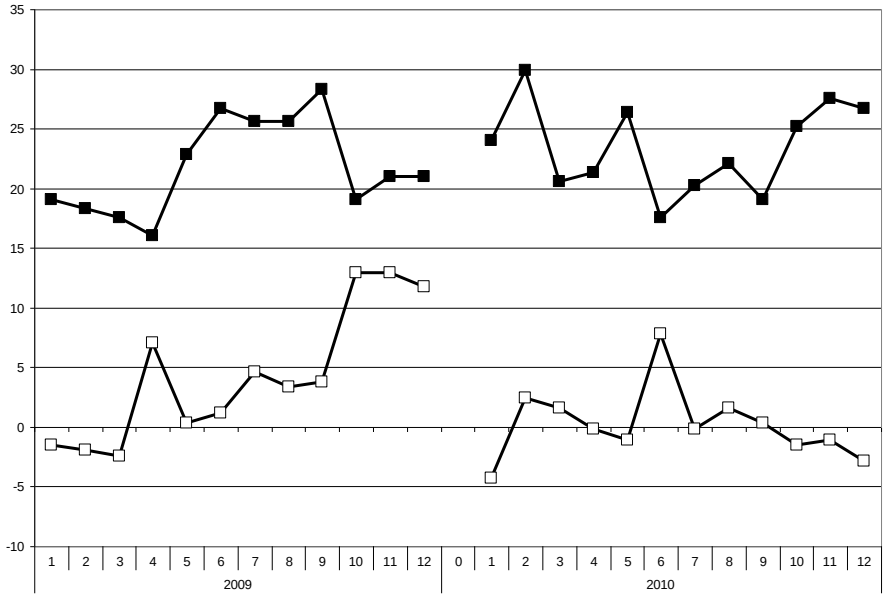

Figure 5. Temperature maxima (solid squares) and minima (open squares) recorded during the 12 flower tagging periods in 2009 and in 2010.

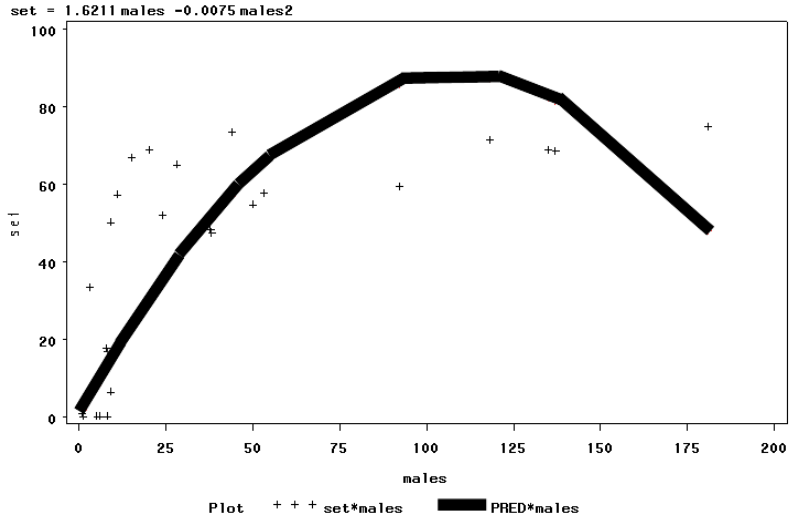

Figure 6. Quadratic no intercept regression curve estimating the correlation between the number of male flowers in the field and fruit set.

Fruit set in 2009 was 56% and 67% in 2010. There were no significant differences between years nor were there any significant interaction effects between year and other factors effecting fruit set. Data for 2009 and 2010 are therefore combined for the rest of

the analyses. Fruit set per tagging day was not correlated with average maximum or minimum temperatures recorded for the two or three day period between tagging days. The number of males in the field on the tagging day however had a significant quadratic effect on fruit set (Fig.6). Based on this correlation, estimated optimal fruit set would occur with 100 males in the field. However, despite planting additional male plants between each region of origin subplot in the field (see field plan, figure 1), the number of male flowers in the field was fewer than 50 at most times (17 of 24 tagging days) and fruit set was less than optimal.

Fruit set was significantly better in block three (71%) than in the other two blocks. There was also an effect of the region of origin on fruit set. Fruit set in selections from the region of Manicouagan (54%) was poorer than that from selections from the other four regions. An analysis of fruit set among the selections was performed only for the 14 selections producing 9 or more flowers per replicate. Among this set of selections, fruit set ranged from a low of 32% in a selection which produces many flowers on leafless shoots, to a high of 82% (Table 2).

Table 2. Fruit set in selections producing 9 or more flowers per replicate.

Clone	**N**	**Fruit set (%)**	
86F9	3	82	a
LAMF8	3	82	a
BB1F7	3	82	a
AGUF5	3	73	ab
M51F7	3	68	abc
CBD1F3	3	66	abcd
M52F5	3	65	abcd
100F3	3	65	abcd
86F6	3	64	abcd
18F1	3	63	abcd
LAMF6	3	59	bcd
EMG1F1	3	52	cd
LAM2F4	3	49	de
AGUF2	3	33	e

Means in columns followed by the same letter are not significantly different at p=0.5

Yields

By the second year in the field, in 2009, thirteen of the selections still were not producing fruit on any of the three replicate plants. Six of these selections originated in Manicouagan. By 2010, only one selection, the Norwegian cultivar Fjellgull, did not produce fruit on any of the three replicate plants.

As seen earlier with flower numbers, previous performance in pots under growth chamber conditions was not correlated with yields in the field. Yields were influenced by year, planting block and region of origin of the selections. While less than two fruit per plant were produced in 2009, by 2010 an average of 7 fruit per plant were obtained. In both 2009 and 2010 significantly greater numbers of fruit were produced in block three than in the other two blocks (Fig.7). There was also a significant block effect on abortion of fruit in development. In 2010 fully 25% of fruit set in block 1, the driest of the three blocks, aborted before maturity while less than 5% of fruit in the wettest block aborted. Selections originating from the regions of the Haute-Côte-Nord and Minganie were more productive than selections from the other three regions in the field (Fig. 8).

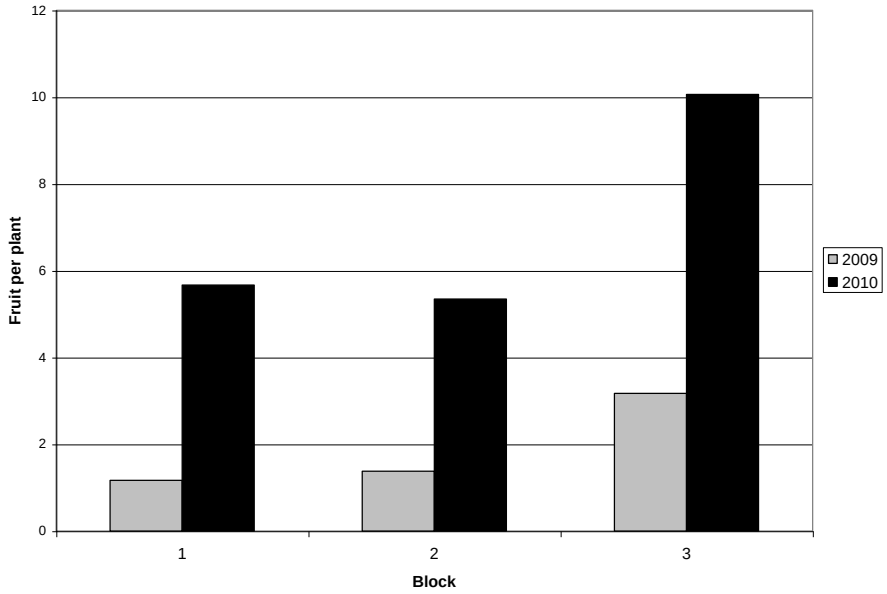

Figure 7. The effect of block on fruit production per plant in 2009 and 2010.

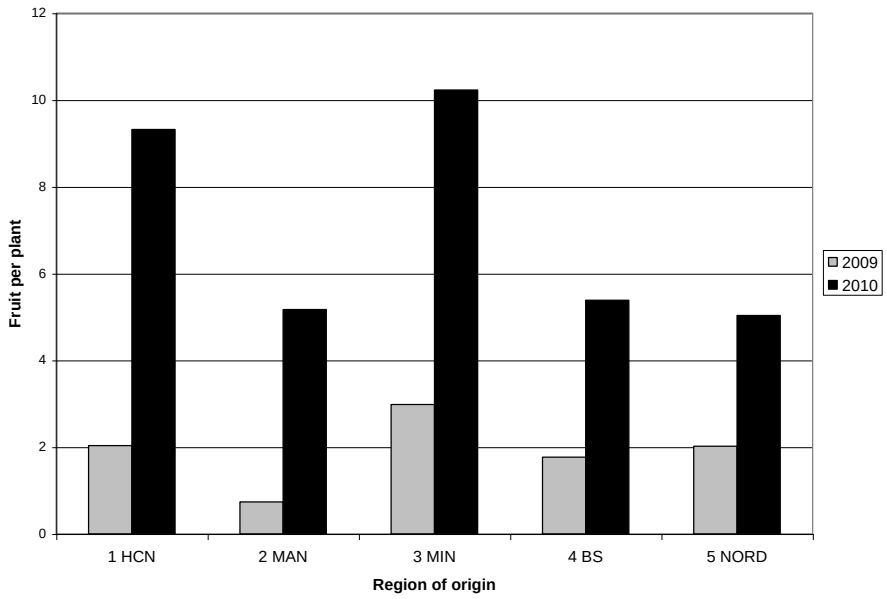

Figure 8. The effect of region of origin on fruit production in 2009 and 2010.

By 2010 there were also significant differences in fruit production between the selections despite the small number of replicates (Table 3). From 1 to 21 flowers per plant set fruit of which 0 to 20 fruit developed to maturity. Fruit abortion was particularly noticeable in the selection Big Ranch from the area of Blanc Sablon on which very few fruit matured. Otherwise, fruit abortion did not vary significantly between the selections.

Fruit size in 2010 was small averaging only 0.79 grams and varied from 0.66 grams for fruit on selections from the region of Minganie to 0.9 grams on fruit on selections from the Nord region. Fruit weight was correlated to the number of druplets in the fruit which in turn was correlated with the number of pistils per flower. Seed set however, which averaged 68%, varied widely, thus selections with the greatest number of pistils per flower did not necessarily produce the largest fruit (Table 3). Fully 28% of the fruit produced had four or fewer druplets due to poor pollination.

In 2010 yields averaged 16 gr/m^2 in the field. Yields varied from 10 gr/m^2 in the driest block to 30 gr/m^2 in the wettest block. Yield variations between selections from the different regions of origin ranged from a low of 14 gr/m^2 for selections from the region of Manicouagan to a high of 27 gr/m^2 for selections from the region of the Haut-Côte-Nord. Yields between the 45 selections in the trial ranged from a low of 0 for the Norwegian cultivar Fjellgull to a high of 49 gr/m^2 for the selection 86F9 from the region of Minganie (Table 3). Within the top ten producing selections there was at least one selection from each of the five regions of origin.

Table 3. Variation in important yield factors between 45 female cloudberry selections from five regions of origin.

Region	Selection	Flowers per plant	Fruit set per plant	Fruit developped	Pistils	Grams per fruit	Yields (gr/m2)
Blanc Sablon	BHP2F1	1 d	1 c	1 c	8 c	0.68 NS	2 NS
	BigRanch	6 abc	6 b	2 bc	14 ab	0.60 NS	2 NS
	EMG1F1	9 abc	5 b	5 ab	10 bc	0.68 NS	14 NS
	GP1F1	6 bcd	5 bc	4 ab	11 bc	0.75 NS	10 NS
	IGN1F1	4 cd	2 bc	2 bc	9 c	0.45 NS	2 NS
	KMP4F1	12 ab	6 ab	4 bc	14 ab	1.38 NS	16 NS
	NTPF1	13 a	10 a	9 a	9 c	0.73 NS	27 NS
	SMLT1F1	5 cd	4 bc	4 bc	8 c	0.85 NS	11 NS
	WH3F3	8 abc	6 ab	6 ab	18 a	0.87 NS	25 NS
Haute-Côte-Nord	BB1F6	8 cd	5 cd	4 cd	10 def	0.46 d	9 d
	BB1F7	20 abc	16 ab	14 ab	10 ef	0.73 bcd	40 a
	CBD1F3	25 a	17 a	16 a	13 ab	0.72 bcd	41 a
	CC1F3	7 cd	6 cd	6 cd	8 f	0.50 cd	14 cd
	CC2F3	3 d	2 d	2 d	14 a	1.31 a	9 d
	LAM2F4	23 ab	11 abc	10 abc	11 cde	0.75 bcd	27 abcd
	LAMF6	21 abc	11 abc	9 bc	11 cde	0.84 bcd	30 abc
	LAMF8	10 bc	9 bcd	8 bc	12 bcd	1.11 ab	38 ab
	SAP1F4	6 cd	5 cd	5 cd	13 abc	0.97 abc	17 bcd
Manicouagan	CAOF1	5 b	4 bcd	4 bc	17 a	0.95 NS	19 NS
	CM1F8	1 b	1 d	1 c	12 ab	0.93 NS	3 NS
	PAOF3	15 a	7 ab	5 b	7 b	0.64 NS	11 NS
	PM1F2	17 a	10 a	9 a	10 b	0.60 NS	27 NS
	PP1F14	8 b	6 abc	4 bc	10 b	0.41 NS	7 NS
	REX1F1	4 b	2 cd	1 bc	8 b	1.18 NS	7 NS
	RP1F7	5 b	3 bcd	3 bc	12 b	0.58 NS	9 NS
	RP6F1	7 b	5 bc	5 bc	12 b	0.96 NS	15 NS
	RP6F2	7 b	4 bcd	4 bc	8 b	0.48 NS	8 NS
Minganie	100F3	10 bc	6 bc	5 b	11 NS	0.48 NS	10 b
	18F1	20 ab	14 ab	11 ab	10 NS	0.69 NS	30 ab
	18F4	5 bc	4 bc	3 b	10 NS	0.69 NS	9 b
	86F1	15 abc	11 bc	10 ab	10 NS	0.59 NS	29 ab
	86F2	2 c	1 c	1 b	11 NS	1.57 NS	2 b
	86F6	11 bc	8 bc	6 b	10 NS	0.74 NS	16 ab
	86F9	28 a	21 a	20 a	11 NS	0.69 NS	49 a
	AGUF2	15 abc	5 bc	4 b	11 NS	0.49 NS	7 b
	AGUF5	19 ab	15 ab	10 ab	9 NS	0.64 NS	22 ab
Nord	FM1F7	6 bc	5 bc	5 bc	9 c	0.65 NS	8 bc
	FM3BEST	4 bc	2 bc	2 bc	15 a	0.56 NS	3 c
	FM5F12	5 bc	3 bc	3 bc	11 bc	1.02 NS	12 bc
	Fjellgull	2 c	1 c	0 c			0 c
	Fjordgull	1 c	1 c	1 c	9 bc	0.70 NS	2 c
	M51F7	9 b	7 ab	7 ab	15 a	1.44 NS	32 a
	M51F8	7 bc	3 bc	3 bc	12 bc	1.27 NS	14 bc
	M52F3	3 bc	2 bc	2 c	11 bc	0.70 NS	2 c
	M52F5	16 a	12 a	10 a	12 b	0.76 NS	24 ab

Means in columns within region followed by the same letter are not significantly different at p=0.5

Senescence

As seen earlier with flowering phenologie, the origin of the selections had an influence on fall senescence. Selections from the regions of the Haute-Côte-Nord and Manicouagan, the selections experiencing the shortest provenance transfer, were the first to senesce (Fig. 9). By mid august several plants from these regions no longer had any leaves. Selections from the regions of Minganie and Blanc Sablon were the last to senesce and presumably remained photosynthetically active into the month of September.

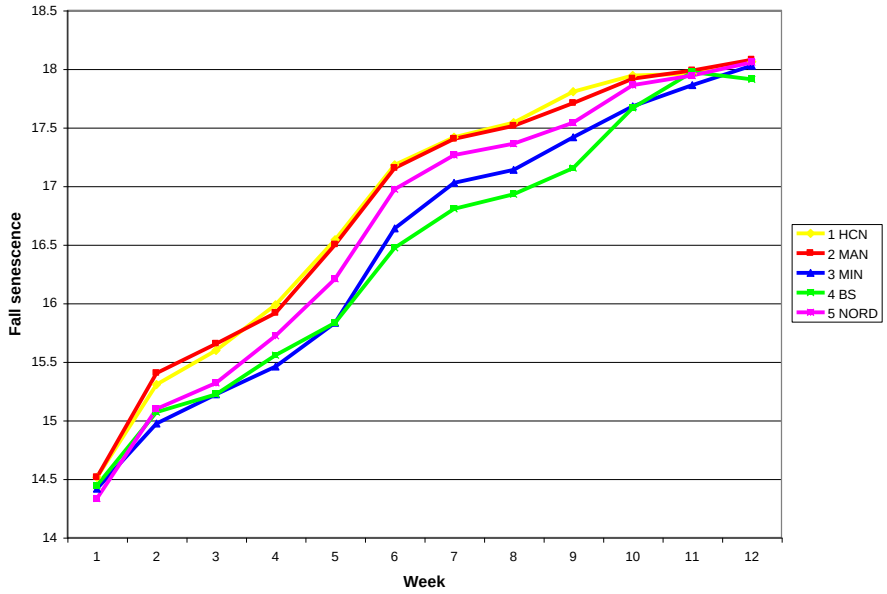

Figure 9. Fall senescence of selections from the five different regions of origin on a scale of 14 to 18 where 14=no fall coloration, 15=1/4, 16=1/2, 17=3/4 of leaves with fall coloration and 18= all leaves senesced.

Discussion

By the third and fourth year of the project genetic diversity among the selections was evident for all the factors examined. Average flowering period between the earliest and latest selections varied by 10 days, flower numbers per plant ranged from 1 to 28 and yields per square meter ranged from 0 to 49 grams. This diversity demonstrates the great potential plant breeding and selection offer in terms of obtaining sustainable increases in cloudberry yields.

The two male and female Norwegian cultivars did not perform well under our conditions. These cultivars were the earliest to flower in the field and were too early to serve as pollinators for most of the native selections or to be pollinated by native male cloudberry selections. An early flowering period subjects these cultivars to a greater risk of frost damage. The Norwegian cultivars also produced very few flowers. Forty of the 43 female selections from Québec produced more flowers than either of the female cultivars in the trial and all 13 of the male selections from Québec flowered more prolifically than the two male cultivars from Norway. The Norwegian cultivar Fjellgull was selected from seedling populations originating north of the 70^{th} parallel and the other three Norwegian cultivars were selected from seedling populations originating near the 69^{th} parallel. Although they do not appear to be adapted to conditions in Manicouagan they may nonetheless be adapted to areas with a shorter cooler season such as Blanc Sablon in north eastern Québec or Fermont in northern Québec.

The existence of cloudberry ecotypes has been documented by both Juntilla *et al*., (1983) in Norway and Kortesharju (1993) in Finland. In both cases ecotypes from more southern latitudes break dormancy and flower later than do those from more northern latitudes. Wallenius (1999) also found that cloudberry plants in North Eastern Finland required fewer accumulated degree days to reach flowering than did those in South Eastern Finland. The latitudes of origin of the genotypes analyzed by Juntilla *et al*., (1983) span over 20 degrees with differences found between regions only 4 degrees apart.

Region of origin also had significant effects on field performance in the cloudberry selections and cultivars trialed here. As was seen in Scandinavia, cloudberry selections from the more northerly regions, Nord and Blanc Sablon, flowered earlier than selections from further south. The latest selections to flower however were not those from the most southern region of origin but rather those from the region of Minganie. Fall senescence also varied with the region of origin of the selections but was not always correlated with flowering phenology. Thus, selections from the region of Blanc Sablon, which were among the earliest to flower, were the latest to senesce in the fall. Significant climatic differences between the regions exist which could account for the evolution of cloudberry ecotypes despite the relatively short distances between the regions of origin of the cloudberry selections trialed here. Due to ecotype effects, secondary selections of plants collected in the wild or of seedlings from controlled crosses should be made only following field trial of the plants at several locations with a potential for commercial cloudberry production and differing significantly in climatic conditions.

Field performance of the cloudberry selections was not correlated with previous performance in pots under controlled conditions. Some of the best performers in the growth chamber were among the worst performers in the field while selections from the regions of Blanc Sablon and Minganie, which were the worst performers in the growth chamber, were high performers in the field. The grouping of selections based on previous performance was therefore not an effective means of obtaining a visual demonstration of the influence of genotype on cloudberry yields. Some of the observed differences between field performance and pot performance were due to ecotype differences. The controlled conditions used in the growth chamber trials did not provide sufficient hours of chilling for selections from the regions of Blanc Sablon and Minganie. In other cases pathogen susceptible selections, which were productive under the sheltered growth chamber conditions, quickly succumbed to plant diseases in the field. Although cycling plants between a cold chamber and a growth chamber is useful for bringing cloudberry plants to size quickly and for physiological studies, data taken on yield factors are not useful in the context of a breeding program even at the earliest stages of plant selection.

Genotype and ecotype were not the only factors affecting yields in these trials. The effect of planting block on flower production, fruit abortion and yields was evident. The wettest planting block was the most productive both in 2009 and 2010. Water levels between 30 and 50 cm are reported as ideal for cloudberry production (Rapp, 2004). In our trial, water levels in the wettest planting block averaged 22 cm below the surface of the bed while water levels in the driest planting block averaged 38 cm below the surface of the bed. Maintaining water levels above 30 cm should perhaps be examined in the early stages of cloudberry production following planting.

After three years in the field the cloudberry plants had still not filled the space allotted to them. While planting distances of 30 cm for 2 inch pots are recommended for commercial production (Rapp, 2004) we planted 7 inch plants with a planting distance of 50 cm. With shorter planting distances a commercial planting may become established and come into full production more quickly.

In Vitro Propagation

Meristem initiation protocol development

Decontamination experiment 1: Stock plant pretreatments

The experiment examines the effects of stock plant pretreatments with the systemic fungicide NOVA (40% myclobutanil) on subsequent in vitro contamination rates of explants dissected from rhizome buds coming out of dormancy.

Materials and methods

Female cloudberry plants of unspecified genotype in four inch pots were used as mother plants in the experiment. There were 10 plants per treatment and block (treatment day, 2 days). The plants were treated to a root drench of 200 ml solution containing 0, 20, 40 or 80 mg myclobutanil per litre (0, 100 mg, 200mg or 400 mg Nova (40%) dissolved in water) 1, 2 and 3 weeks after removal of the plants from the cold chamber. Plants were maintained in a growth chamber, 18 hours photoperiod at 18°C for 12 weeks. At the end of the growth period, observations on foliar leaf spots and mildew were made using a scale of 0= clean leaves, 1= less than 1%, 5= 1-5%, 10, 15, etc for increasing percentage of leaf discoloration. Plants were then put into plastic boxes and incubated in a cold chamber at 4° C for 12 weeks to satisfy dormancy requirements.

Buds (1 per plant) at the surface of the soil were collected and surface sterilized using a quick dip in 70% ethanol followed by 15 minutes in 20% household bleach. Buds were rinsed three times in sterile distilled water before meristem extraction. Meristems, with a few subtending nodes were dissected from the buds in a laminar flow hood and placed on filter paper bridges in test tubes containing a liquid meristem initiation medium (Table 4).

Table 4. Modified Murashige and Skoog liquid medium used for cloudberry initiation.

NH_4NO_3	1.65 g	Thiamine-HCl	0.4 mg
KNO_3	1.9 g	A. Nicotinique	1 mg
$MgSO_4.7H_2O$	370 mg	Pyridoxine.HCl	1 mg
KH_2PO_4	170 mg	BAP	2 mg
$CaCl_2.2H_2O$	440 mg	Myo-Inositol	100 mg
$MnSO_4$	15 mg	Sulfate d'adénine	80 mg
$ZnSO_4.7H_2O$	8.6 mg	Sucrose	20 g
H_3BO_3	6.2 mg	Agar	1 g
KI	0.83 mg		
$NaMoO_4.2H_2O$	0.25 mg	pH	7.5
$CuSO_4.5H_2O$	0.025 mg		
$CoCl_2.6H2O$	0.025 mg		
FeSO4.7H20	27.85 mg		
$Na_2.EDTA.2H_2O$	37.25 mg		
NaH_2PO_4	152.2 mg		

After a month, non contaminated explants were transferred to the same medium solidified with 7 g agar in 1 oz tablet jars. Contamination and survival rate of the explants was followed for two months. The experiment was repeated once.

Results

All three doses of the fungicidal treatment had significant effects on the percentage of foliar leafspot observed on the plants at the end of the growth season (Table 5). However, there was a significant block by treatment interaction effect, thus treatment effects were significant only for one of the two blocks (treatment days) in the experiment.

Table 5. Percentage of foliar leafspot observed on cloudberry plants following root drench treatments with the systemic fungicide myclobutanil (Nova, wettable powder, 40%).

Treatment:	**1: Control**	**2: 20 mg a.i.**	**3: 40 mg a.i.**	**4: 80 mg a.i.**
Block 1	15.5 a	5 b	2.5 b	1.7 b
Block 2	6.3 a	3.9 a	4.2 a	3.4 a
Average	10.8 a	4.4 b	3.4 b	2.6 b

Means in a row followed by the same letter are not significantly different at p=0.05

Table 6. The effect of stock plant treatments with myclobutanil on contamination and survival of meristems *in vitro*.

Treatment	**Fungal hyphae**	**Contaminated**	**Dead**	**Surviving**
1: Control	24%	62%	23%	15%
2: 20 mg myclobutanil	26%	65%	18%	17%
3: 40 mg myclobutanil	8%	40%	22%	38%
4: 80 mg myclobutanil	13%	60%	30%	10%
Average	17%	57%	23%	20%

Fungal hyphae were observed within 17% of the buds at dissection and meristems dissected from these buds were either quickly covered with hyphae or died within the following week (Table 6). While fungal hyphae were observed on only 8% of the buds from treatment 3, the differences between treatments were not statistically significant (p=0.09). Overall contamination rates were high, averaging 57% across experiments and treatments. There were significant treatment effects on the outcome of the experiment (contaminated, dead or surviving) with the most promising treatment, 40 mg myclobutanil, producing a survival rate of 38%.

Discussion

While the observations on foliar disease scores at the end of the growth season suggest that the systemic fungicide was indeed absorbed and distributed to the growing shoots, a reduction in contamination and positive treatment effects on meristem survival in vitro were only obtained at the intermediate dose of myclobutanil used. In order for treatments to be effective they must be applied to the growing plant. However, buds for initiation of meristems in vitro should be taken at the end of the dormancy period, thus the period between treatment and initiation *in vitro* is long and may allow for re-infection of the

buds during the dormancy period or before. With improved timing of stock plant treatment the method may provide better results but as shown below, explant pretreatments are a better alternative in reducing the contamination rate at the *in vitro* initiation step of cloudberry micro propagation.

Decontamination experiment 2: Explant pretreatments

The experiment examines the effects of pre-treating explants dissected from rhizome buds coming out of dormancy to a solution containing the biocide PPM (plant preservative mixture) and the systemic fungicide NOVA (40% myclobutanil) on subsequent contamination rates *in vitro*.

Materials and methods

Male and female cloudberry plants in four inch pots of unspecified genotype were used in the experiment. Rhizome buds were collected from the plants following a 12 week chill period at 4°C to remove dormancy. The buds were surface sterilized using a two step procedure in which the buds were first washed in a solution containing 5 mls Tween 20 and 1 ml household bleach per liter for a period of 1 hour. The buds were then transferred to an acidified bleach solution containing 200 mls household bleach per liter acidified with 6.5 ml concentrated HCl. The buds (5) were maintained in the acidified bleach solution for 20 minutes before transfer to 10 mls sterile distilled water in 1 oz tablet jars. Buds were kept refrigerated until explant dissection the same or following day.

In a pre-screen test of PPM at 0, .05, 0.1 and 0.2% in factorial combination with NOVA at 0, 10, 50 and 100 mg per liter none of the solutions proved phytotoxic to the treated ex-plants. The four PPM concentrations in factorial combination with NOVA at 100 mg per liter were chosen for the experiment.

Five buds per treatment (8), treatment day (2 days) and experiment (2) were used.
Explants consisting of the terminal meristem and 2 to 3 subtending nodes were dissected from the surface sterilized buds and incubated in 5 ml liquid meristem initiation medium (Table 4) containing the treatment products for 24 hours on a shaker at 150 rpms. The explants were then transferred to filter paper bridges in test tubes containing 12 ml liquid meristem initiation medium without the treatment products. Explants were maintained in the growth room under fluorescent lights at 18 ° C, 18 hour photo period for four weeks before transfer to a solid medium in 1 oz table jars. Contamination and explant survival was followed for two months.

Results

The number of clean explants per treatment and treatment day was significantly increased using a pre-treatment solution containing the biocide PPM at all three concentrations used (Table 7). While only 30% of control explants remained uncontaminated by the end of the experiment, 72 % of the explants incubated in a 0.2% PPM solution were still clean at this time. NOVA at 100 mg per liter had no significant effect on contamination rates. There were no significant differences between the two experiments or interaction effects between experiment and treatments on contamination rate.

Table 7. The effect of pre-treatments with the biocide PPM (Plant Preservative Medium) on contamination rates in cloudberry *in vitro* initiation.

Treatment	**Clean explants (%)**
PPM 0%	30 b
PPM 0.05%	58 a
PPM 0.1%	58 a
PPM 0.2%	72 a

Means followed by the same letter are not significantly different at p=0.05

Discussion

Although the explant pre-treatment method described here is rather tedious, the use of 0.2% PPM in the pre-treatment solution effectively reduced contamination rates. The reduction in contamination was greater than that obtained through stock- plant pretreatments and was stable across the two experiments. Explant pre-treatments in a liquid initiation medium containing 0.2% PPM has therefore been retained as a component of the cloudberry tissue culture protocols developed in the course of the project.

Rejuvenation experiment 1: The use of thidiazuron (TDZ)

The experiment examines the effect of an explant pre-treatment with the substituted urea thidiazuron (TDZ) on the success of in vitro initiation of male and female cloudberry selections.

Materials and methods

Twenty two female and 13 male cloudberry selections, including the four Norwegian cultivars, were used in the experiment. The plants were maintained in a cold chamber at 4°C for 12 weeks to satisfy dormancy requirements. Rhizome buds were then collected from the plants and surface sterilized following the two step procedure described earlier. Explants containing the terminal meristem and a couple of subtending nodes were dissected from the buds and incubated in a liquid meristem initiation medium (Table 4) which included thidiazuron at 0, 1, 5 and 10 μM and the biocide PPM at 0.2%. Explants were incubated in the treatment solutions for 24 hours on a shaker at 150 rpm. They were then transferred to filter paper bridges in test tubes containing 12 ml of meristem initiation medium and incubated in a growth chamber under fluorescent lights, 18°C, 18 hour photoperiod. Surviving explants were transferred to a solid initiation medium in 1 oz tablet jars after 4 weeks. They were subcultured monthly until large enough for transfer to test tubes after which they were subcultured at 6 week intervals for the duration of the experiment. There were 10 replicates (clones) per treatment and the experiment was repeated once.

Results

While there were no treatment effects on contamination rate there were significant effects of the region of origin of the clones on contamination rates. Forty-one % of buds from clones originating in Blanc Sablon were contaminated and lost from the experiment, while contamination rates for clones from the other regions ranged from 6 to 25%. Of

non contaminated buds, 45% were successfully initiated *in vitro*. There were no treatment effects on the success rate. However, male clones, with a success rate of 61% were more easily initiated in vitro than female clones with a success rate of 36%. A multiplication rate of 3 was obtained after four to five subcultures. There were no effects of either treatment or gender on this variable.

Discussion

Only 45% of non contaminated explants were successfully initiated *in vitro*. As no benefit was obtained through the use of the substituted urea thidiazuron (TDZ) in the *in vitro* initiation step of cloudberry micropropagation, the focus of additional in vitro initiation experiments was switched to the examination of the use of gibberellins in this step.

Rejuvenation experiment 2: The use of gibberelic acid (GA_3)

The experiment examines the effect of the use of gibberelic acid in the liquid initiation medium on the *in vitro* initiation success rate in cloudberry micropropagation. An initial screen of four concentrations was carried out on two female clones to determine an appropriate range of treatment concentrations. Two experiments were then carried out on male and female clones from five regions of the north shore of Québec.

Materials and methods: Initial screen

Two female cloudberry clones from the region of Blanc Sablon were used in the experiment. Plants were maintained 12 weeks in a cold chamber at 4° C to satisfy dormancy requirements. Twenty rhizome buds per clone and medium were then gathered and surface sterilized as mentioned above. Explants were dissected from the buds, incubated 24 hours in the liquid initiation medium containing 0.2% PPM, and inoculated onto filter paper bridges in test tubes containing 12 mls of the treatment media. The liquid initiation medium (Table 4) was amended with 0, 1, 2.5 or 5 μM GA_3. Explants were incubated on the liquid medium in a growth chamber under fluorescent lights at 18°C, 18 hour photoperiod for one month and then transferred to a solid medium in 1 oz tablet jars. Explant size was scored at two months to evaluate the treatment media using the following score:

0=dead, 1=single leaf, necrosis on bud, 2= couple of leaves, no elongation of petioles 3=several large leaves, some proliferation, 4=large leaves, axillary shoots with long petioles, 5=large leaves, several axillary shoots.

Results: initial screen

Despite the use of the improved protocols for decontamination of the buds and explants, the contamination rate was relatively high at 54%. Both clones benefited from gibberelic acid in the initiation medium with the highest scores being obtained at the 2.5 and 5 μM concentrations (Table 8). There was no phytotoxic effect of GA_3 at the 5μM concentration, thus the same four concentrations of GA_3 were used in the next experiment.

Table 8. The effect of GA_3 in the initiation medium on explant scores*.

Clone	0 µM GA_3	1 µM GA_3	2.5 µM GA_3	5 µM GA_3
Colinda	2.1	3.7	4.0	4.0
Marie-Claire	0.7	1.6	2.4	2.4

*0=dead, 1=single leaf, necrosis on bud, 2= couple of leaves, no elongation of petioles 3=several large leaves, some proliferation, 4=large leaves, axillary shoots with long petioles, 5=large leaves, several axillary shoots.

Materials and methods: full experiment

Twenty-one male and 63 female clones from five regions of the north shore were used in the experiment. Plants were maintained in a cold chamber at 4° C for 12 weeks to satisfy dormancy requirements before the start of the experiment. Rhizome buds were then collected and treated as in the initial screen. Four buds from each pot were used, one per treatment. There were 122 buds per treatment. Following an initial sub-culture on solid medium in 1 oz table jars the explants were sub-cultured to test tubes as they increased in size. The status (alive or dead) of the explants at the end of the experiment and the number of subcultures needed to reach a multiplication rate of three per subculture were used in the evaluation of the treatment media.

Results: full experiment

Once again, contamination rates were relatively high at 53%. Buds taken from clones from the regions of Blanc Sablon, Minganie and the northern region had higher contamination rates than those taken from clones from the regions of Manicouagan and the Haute-Côte-Nord. Contamination rates were also correlated with the media; while 62% of explants on the control medium were contaminated, only 42 % of explants on the medium with 5 µM GA_3 were contaminated. The high rate of contamination and the non random distribution of the contamination relative to region of origin and treatment medium complicate the analysis of the results with respect to explant rejuvenation.

Among non contaminated explants 61% of the explants were successfully initiated *in vitro*. There was a significant effect of gender on success rate; those taken from male clones survived at significantly higher rates than those taken from female clones (respectively 73% vs 59%).

Among the treatment media examined, the success rate was highest on media containing 5 µM GA3 and lowest on media containing 1 µM GA3 (respectively 71% vs 54%). These differences were not significant. There was however a significant interaction effect between the treatment media and the region of origin of the clones. While GA_3 in the medium had no significant effect on initiation success of clones from the regions of the Haute-Côte-Nord, Blanc Sablon or the northern region, a positive effect of GA_3 in the medium was observed for clones from the regions of Minganie and Manicouagan (Fig. 10).

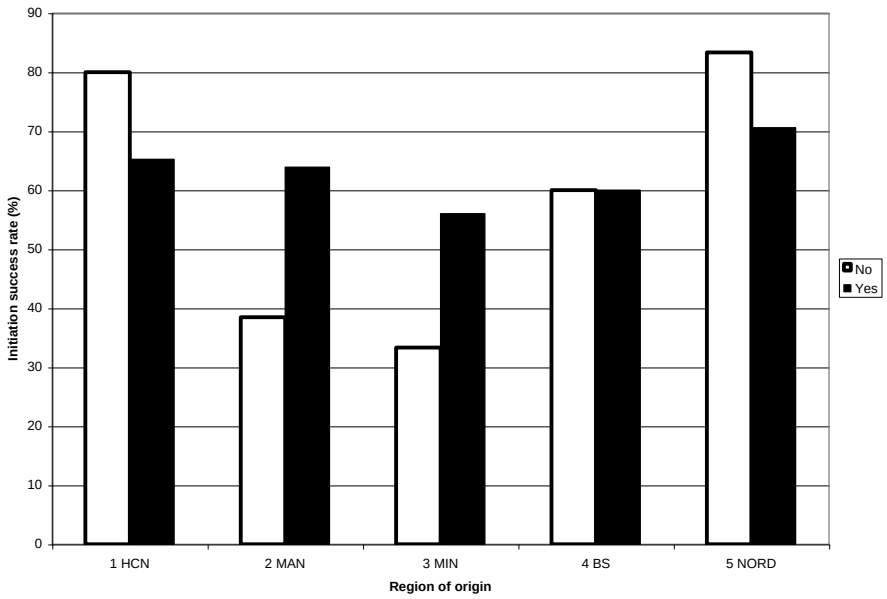

Figure 10. The interaction of region of origin and GA_3 in the medium on initiation of cloudberry selections *in vitro*.

For clones successfully initiated in vitro, the number of subcultures required before a multiplication rate of 3 was obtained averaged 4.7. There were no significant effects of the treatment media, gender or clonal region of origin on this variable.

Discussion

In vitro initiation of cloudberry clones was improved on media containing GA_3 for clones originating in the regions of Minganie and Manicouagan, clones which were in general the most difficult to initiate in the experiment. Although GA_3 had no beneficial effect on clones from the other regions in this experiment, the observed negative effects of GA_3 in the medium for clones originating from the northern regions and from the Haute-Côte-Nord region were not significant. Clone gender was a factor in this experiment as in previous experiments; however, there were no gender by treatment interaction effects. Thus the inclusion of GA_3 at 5 μM as a standard component of the initiation medium should improve the overall success rate of *in vitro* initiation of male and female cloudberry clones from varying regions.

Leaf regeneration protocol development

The use of silver thiosulfate in regeneration media and stock plant pre-treatments with the thidiazuron containing herbicide "Take Down" are examined in the use of leaf regeneration as an alternative method of initiating cloudberry clones *in vitro*.

<u>Leaf regeneration media</u>

In a preliminary screen of 0, 5, 10 and 20 μM thidiazuron (TDZ) in factorial combination with 0, 5, 10, and 50 μM silver thiosulfate (STS) on leaf regeneration in three female

cloudberry clones, none of the treatment media proved toxic though all leaf pieces on media without TDZ died within a couple of weeks. Based on the results of the screen, the following concentrations were chosen for the follow-up experiment: 5, 10 and 20 μM TDZ in factorial combination with 0, 10 and 50 μM STS.

Materials and methods

Two female cloudberry clones were used in the experiment. Leaves from stage 4-6 shoots (Beaulieu scale, Beaulieu *et al.*, 2001) were gathered and surface sterilized as follows: Leaves were dipped briefly in 70% ethanol, and then agitated in a 10% solution of household bleach containing 2 drops Tween 20 per liter for 15 minutes. Leaves were then transferred to three rinses in sterile distilled water. Leaf pieces, $1cm^2$ in size containing a major leaf vein, were inoculated upper leaf surface down onto the media in 5 cm Petri plates. There were 10 replicates per treatment and clone. The plates were incubated at room temperature in the dark for three weeks, transferred to fresh media and incubated an additional three weeks under fluorescent lights in a growth chamber at 18°C with an 18 hour photo period. Leaf regeneration was scored using the following arbitrary scale: 0=necrotic, 1=green, 2=callus formed, 3=nodules formed, 4=shoot regeneration.

The leaf regeneration medium used contains the modified MS macro and micro salts shown in Table 4, 100 mg/l inositol, 30 g/l sucrose, 80 mg/l adenine sulphate and plant tissue culture tested agar (Sigma) at 6 g/l. The medium is adjusted to pH 5.7.

TDZ dissolved in acetone (1mg/ml) is added to the appropriate concentration immediately after autoclaving while the medium is still hot. Staba vitamins (10 mls/l 100 X stock; Table 9) and ascorbic acid (25 mg/l) are filter sterilized into the medium once it has cooled to 50°C. The four concentrations of STS are also filter sterilized into the medium at this time.

Table 9. Staba vitamins (Staba (1969)).

Product	**Per half litre 100 X Stock**
Cyanocobalmin	100ul 75mg/100 ml stock
Thiamin	50 mg
Nicotin acid	100 mg
Pyridoxine	100 mg
Ca Pantothianate	50 mg
Biotin	50 mg
Glycine	100 mg
Folic acid	25 mg
Riboflavin	25 mg
Cholinecloride	50 mg

Results

None of the leaf pieces were contaminated in the experiment. However, only 9 of the 177 leaf pieces inoculated on the media showed signs of regeneration and of those only 3 actually produced shoots. There were no differences in regeneration score between the 9 media tried, nor was there a genotype effect on regeneration.

Discussion

The regeneration potential of the cloudberry clones used in this trial does not appear promising. On the same media, regeneration rates of 30% are obtained with blackberry leaves as compared to a regeneration rate of less than 2% observed here. Nonetheless, there are potential advantages of leaf regeneration as an *in vitro* initiation technique for cloudberry. The speed of the manipulations and the lack of contamination with leaf regeneration techniques as compared to meristem initiations merit additional efforts in developing protocols which might work. The pre-treatment of stock plants with thidiazuron has improved the regeneration potential of subsequently harvested leaves in other species. In the following trial, stock plant pre-treatments with the thidiazuron containing herbicide "Take Down" were attempted in an effort to increase the regeneration potential of cloudberry leaves.

Stock plant pre-treatments

The experiment examines the effects of stock plant pre-treatment with thidiazuron on leaf regeneration potential of several female cloudberry clones.

Materials and methods

Nine female cloudberry clones were used in the experiment, however only three of these clones were used in factorial combination with treatment concentrations as only one of each of the other clones was available for the experiment. Spray solutions containing 0, 50, 100 or 200 μM thidiazuron were prepared by diluting the herbicide TakeDown (42.4% thidiazuron) with water to the appropriate concentration. Two drops tween 20 were added to the solutions as a surfactant. Plants with stage 4-6 shoots (Beaulieu scale, Beaulieu *et al.*, 2001) in eight inch pots were sprayed to runoff with the treatment solutions. There were five plants per treatment. Leaves were harvested from the treated plants after 1 week and surface sterilized. Leaves were dipped briefly in 70% ethanol and agitated in a 10% solution of household bleach containing 2 drops tween 20 per litre for 10 minutes. Leaves were then passed through three rinses in sterile distilled water. Leaf pieces were cut from the leaves and incubated on leaf regeneration medium containing 10 μM TDZ as described above.

Results

Morphological differences between leaf blades from plants treated with varying concentrations of TDZ did not differ noticeably after one week but leaf petioles were larger on treated plants than on control plants. Bud development was disturbed on plants treated with the highest dose of TDZ and these plants declined the season following treatment.

Leaf pieces in only one of the plates in the experiment were contaminated. Shoot regeneration was obtained from 22% of the leaf pieces in the experiment with

respectively 7, 40, 20 and 25% shoot regeneration observed on leaves from plants sprayed with 0, 50, 100 or 200 μM TDZ. Treatment differences however were not statistically significant. Shoot regeneration was obtained from 6 of the 9 female clones in the trial. Leaf regeneration scores differed significantly between the clones (Table 10). Most leaf pieces cut from the leaves of the Norwegian cultivar Fjellgull died, while at least some callus formation was observed on leaf pieces from most of the other clones.

Table 10. The effect of genotype on cloudberry leaf regeneration.

Clone	**N**	**Regeneration score***	
Fjellgull	12	0.33	a
BHP2F1	3	0.94	ab
DMP1F1	6	1.0	ab
Trisha	12	2.0	bc
CAO4F4	12	2.4	c
WH3F3	3	3.0	cd
EMG1F1	3	3.1	cd
NHM1F1	3	4.2	d
Big Ranch F1	3	4.2	d

Means followed by the same letter are not significantly different at p=0.05

*Leaf regeneration was scored using the following arbitrary scale: 0=necrotic, 1=green, 2=callus formed, 3=nodules formed, 4=shoot regeneration.

Discussion

Despite the small scale of the experiment, some shoot regeneration was obtained from most of the clones in the experiment. Stock plant pre-treatments with TDZ appeared to improve the regeneration potential of cloudberry leaves. However, clonal differences in regeneration potential obscured potential treatment effects. Conclusions on treatment effects can not be made without conducting an expanded experiment with all clones in factorial combination with the treatments. Nonetheless, a tentative recommendation can be made for stock plant pre-treatments with 50 μM TDZ the week before leaf harvest as this treatment did not noticeably harm the stock plants and appeared to improve leaf regeneration.

Meristem vs nodal explants

Initial screen

In vitro initiation protocols designed for cloudberry by Debnath (2007) were tried in an initial screen using a single cloudberry clone.

Materials and methods

The medium used for the screen is given in Table 11, medium 8. Cloudberry plants growing in five inch pots were used for the screen. Elongating stage 3 to 4 shoots (Beaulieu scale, Beaulieu *et al.,* 2001) were cut at the soil line. Leaves were cut from the shoots and the shoots were washed in a solution containing 5 mls tween 20 and 2 mls household bleach per liter for 2 minutes. Shoots were then agitated in a 15% household

bleach solution for 20 minutes and briefly rinsed in 70% alcohol before transfer through three rinses in sterile distilled water.

Single node stem segments were cut from the shoots and placed on 15 ml medium in standard test tubes. Nodes were numbered from the top of the shoot down and scored as either leaf nodes or budscale nodes. A total of 68 nodes were inoculated on the medium. Nodes were subcultured to fresh medium after 4 weeks. Two additional 6 week subcultures were performed before final survival scores were taken.

Results

Seventy three percent of budscale nodes and 43% of leaf nodes were contaminated and lost from the experiment. Of the remaining 7 budscale nodes, 1 produced a shoot at the node. Of the 42 leaf nodes in the experiment respectively 15/24 and 9/18 nodes from the top node and second from top node were clean. Seven of 15 clean top node explants produced shoots while none of the 9 second from the top node did so.

Discussion

Only 26% of non-contaminated nodes produced shoots in the experiment and the overall contamination rate using nodes was as high as that suffered when using meristems dissected from the winter bud. Nonetheless, using nodes is so much easier and quicker than dissecting meristems from buds and thus merits further attention. An expanded experiment comparing the use of nodes vs meristems on 8 different media representing the major media differences between our standard medium and that used by Debnath (2007) was therefore run.

Full experiment

The medium used by Debnath (2007) differs substantially from that used in our lab. The pH is lower, different gelling agents are used and the salts also differ. In this experiment 8 media designed to test these differences are used on both nodal and meristem cloudberry explants.

Materials and methods

The eight media used are given in Table 11. Of these, medium 8 represents that used by Debnath (2007) and medium 1 that used in our lab.

Materials and methods: meristem explants

Buds for meristem extraction were collected from potted plants previously chilled for 13 weeks to break dormancy. Two female and two male clones from each of five regions of origin were used in the experiment. There were 10 replicates per medium (across clone). Buds were surface sterilized and extracted meristem explants inoculated onto the liquid media as described earlier. Explants were transferred to agar solidified media in 1 oz tablet jars after 4 weeks. Further subcultures to fresh media were conducted at 6 week intervals. Once shoots started proliferating the shoot clumps were transferred to agar solidified media in tubes. Final data on survival and growth were taken after 6 subcultures.

Materials and methods: nodal explants

Shoots were gathered from the potted plants once they reached stage 4 on the Beaulieu scale (Beaulieu *et al.*, 2001). Leaves were removed from the shoot and the shoots were surface sterilized as described for nodal explants above. Only the top node was used in the experiment as this was the node showing the lowest contamination and highest success rate in the preliminary screen. Nodes were inoculated onto the 8 media and subcultured as described for meristem explants above. Final data on survival and growth were taken after 6 subcultures.

Table 11. Eight initiation* media used to compare the Debnath (2007) method of cloudberry initiation with standard protocols used in our lab. Shading is used to differentiate the media.

			Medium							
			1	2	3	4	5	6	7	8
Stock solution	Compound	Gram/L	per litre	per litre	per litre	per litre	per litre	per litre	per litre	per litre
1	NH_4NO_3	82.50								
	KNO_3	95.00	20 mL	20 mL	20 mL	20 mL	0 mL	0 mL	0 mL	0 mL
	$MgSO_4.7H_2O$	18.50								
	KH_2PO_4	8.50								
2	$CaCl_2.2H_2O$	44.00	10 mL	10 mL	10 mL	10 mL	0 mL	0 mL	0 mL	0 mL
3	$MnSO_4$	1.51								
	$ZnSO_4.7H_2O$	0.860	10 mL	10 mL	10 mL	10 mL	7,5 ml	7,5 ml	7,5 ml	7,5 ml
	H_3BO_3	0.620								
4	KI	0.083	10 mL	10 mL	10 mL	10 mL	7,5 ml	7,5 ml	7,5 ml	7,5 ml
	$NaMoO_4.2H_2O$	0.025								
5	$CuSO_4.5H_2O$	0.025	1 mL	1 mL	1 mL	1 mL	0,75 ml	0,75 ml	0,75 ml	0,75 ml
	$CoCl_2.6H2O$	0.025								
6	FeSO4.7H20	2.78	10 mL	10 mL	10 mL	10 mL	7,5 ml	7,5 ml	7,5 ml	7,5 ml
	$Na_2.EDTA.2H_2O$	3.73								
8	NaH_2PO_4	15.22	10 mL	10 mL	10 mL	10 mL	5,0 ml	5,0 ml	5,0 ml	5,0 ml
9	Thiamine-HCl	0.100								
	A. Nicotinique	0.250	4 mL	4 mL	4 mL	4 mL	0 mL	0 mL	0 mL	0 mL
	Pyridoxine.HCl	0.250								
11	BAP	0.250	8 mL	8 mL	8 mL	8 mL	8 mL	8 mL	8 mL	8 mL
12	Thiamine-HCl	0.600								
	A. Nicotinique	0.400					1 mL	1 mL	1 mL	1 mL
	Pyridoxine.HCl	0.400								
13	Glycine	0.100					1 mL	1 mL	1 mL	1 mL
	Casein hydrolysate		0	0	0	0	0,040 g	0,040 g	0,040 g	0,040 g
	NH_4NO_3		0	0	0	0	0,412 g	0,412 g	0,412 g	0,412 g
	$Ca(NO_3)_2 \cdot 4H_2O$		0	0	0	0	0,330 g	0,330 g	0,330 g	0,330 g
	KNO_3		0	0	0	0	0,248 g	0,248 g	0,248 g	0,248 g
	K_2SO_4		0	0	0	0	0,105 g	0,105 g	0,105 g	0,105 g
	$MgSO_4 \cdot 7H_2O$		0	0	0	0	0,278 g	0,278 g	0,278 g	0,278 g
	$(NH_4)_2SO_4$		0	0	0	0	0,038 g	0,038 g	0,038 g	0,038 g
	Calcium glucomate		0	0	0	0	1,30 g	1,30 g	1,30 g	1,30 g
	Myo-Inositol		0,100 g	0,100 g	0,100 g	0,100 g	0,100 g	0,100 g	0,100 g	0,100 g
	Sulfate d'adénine		0,080 g	0,080 g	0,080 g	0,080 g	0,015 g	0,015 g	0,015 g	0,015 g
	Sucrose		20 g	20 g	20 g	20 g	25 g	25 g	25 g	25 g
	Agar		1 g		1 g		1 g		1 g	
	Agar purifiée			3,5 g		3,5 g		3,5 g		3,5 g
	Gelrite			1,25 g		1,25 g		1,25 g		1,25 g
	pH		5.7	5.7	4.5	4.5	5.7	5.7	4.5	4.5

* Starting with the first sub-culture, media 1,3 5 and 7 are solidified with 7 gr per litre agar.

Results

As seen earlier, contamination rate did not differ significantly between the two explant types used being respectively 52% for the meristem explants and 48% for the nodal explants. There were no significant differences in survival rate between the 8 media used. Neither the pH, the salts or the agar used had an effect on survival rate. However, shoots growing on the Debnath (2007) salts (media 5, 6, 7 and 8) became off colored with advancing subcultures. No differences in survival rate between male and female clones were observed. Explant type and explant type by region of origin were the only significant factors in survival rate (Table 12). While 51% of non-contaminated meristem explants survived, only 17% of non-contaminated nodal explants produced shoots *in vitro*.

Table 12. The effect of explant type and region of origin on cloudberry survival *in vitro*.

Region of origin	**Meristem Explants**		**Nodal Explants**		
	N	Survival (%)	N	Survival (%)	Mean
Haut-Côte-Nord	25	63	17	24	43.5***
Manicouagan	23	21	17	28	24.5 NS
Minganie	13	42	28	22	32.0 NS
Blanc Sablon	8	36	19	0	18.0 *
Nord	13	92	14	12	52.0 ***
	82	50.8	95	17.2	***

NS, *, **, ***: Differences between explant types for each region respectively non-significant, or significant at p=0.05, 0.001 and 0.0001.

Discussion

In our hands the use of nodal explants for the initiation of cloudberry *in vitro* was not as successful as the use of meristem explants dissected from the bud. We had expected this method to be particularly useful for clones from the regions of Minganie and Blanc Sablon which have a longer dormancy period but this was not the case. No shoots were obtained from nodes of any of the clones from Blanc Sablon in the experiment. None of the media components had significant effects on survival of explants *in vitro*. Less time is required to adjust the media to the lower pH, thus a pH of 4.5, originally proposed by Martinussen *et al*., 2004 and also used by Debnath (2007), has been retained for our standard media. As for the salts and the agar used, our standard medium is less expensive than that used by Debnath (2007) and we see no discoloration of the shoots maintained on this medium as compared to the Debnath (2007) medium.

Acclimatization

Pretreatments with PEG

In the experiment we examine the use of PEG (polyethylene glycol, MW 8000) as an *in vitro* pretreatment to increase the hardiness of cloudberry tissue culture plantlets prior to *ex vitro* rooting.

Materials and methods

A preliminary screen of 4 concentrations of PEG (0, 1, 2 and 4%) in our standard cloudberry rooting medium (Table 11, medium 1 solidified with 7 gr per liter agar and without any BAP) was tried on four cloudberry selections to look for phytotoxicity. The plants were incubated on the media for four weeks. At that time no negative reactions to any of the media were noticed. An attempt at further increasing the PEG concentration was made but at higher PEG concentrations the media did not solidify. The same four media were therefore used in an experiment on 2 female and 2 male clones with 10 replicates per clone and medium. The plantlets were incubated on the treatment media in a growth chamber under fluorescent lights at 18°C, 18 hour photoperiod for one month. They were then treated to a pulse of 100 μM IAA in aqueous solution for 2 hours and planted into vermiculite in individual 4 oz solo cups. Plants were incubated in the vermiculite in a growth chamber under fluorescent lights at 18°C, 18 hour photoperiod for an additional 6 weeks after which contamination and rooting rates were scored. Contamination was scored using a scale of 0-4 where 0=no contamination, 1= 1/4 of the plant contaminated, 2=1/2 of the plant contaminated, 3=3/4 of the plant contaminated and 4= plant completely contaminated. Rooting was scored on a scale of 0-5 where 0=plant dead, 1=no roots, 2=1-3 roots, 3=4-5 roots, 4=6-10 roots and 5=over 10 roots.

Results

Overall 83% of the plantlets showed little or no fungal contamination. Treatment media had no effect on contamination rate; however, genotype did have a significant effect on this factor. One of the male clones, which grows much taller than the other clones, showed a contamination rate of 79% vs only 20 to 35% for the other three clones in the experiment. Rooting was poor with only 22% of the plantlets producing 1 to 3 roots. PEG in the medium had a negative effect on rooting (Fig. 11). While 32% of the plantlets on the control medium produced roots only 11% of the plantlets on the medium with 4% PEG in the medium rooted. Genotype had the strongest effect on rooting in this experiment. Rooting ranged from a high of 59% for the most easily rooted genotype to a low of only 3% for the most difficult clone. Both these extremes were male clones.

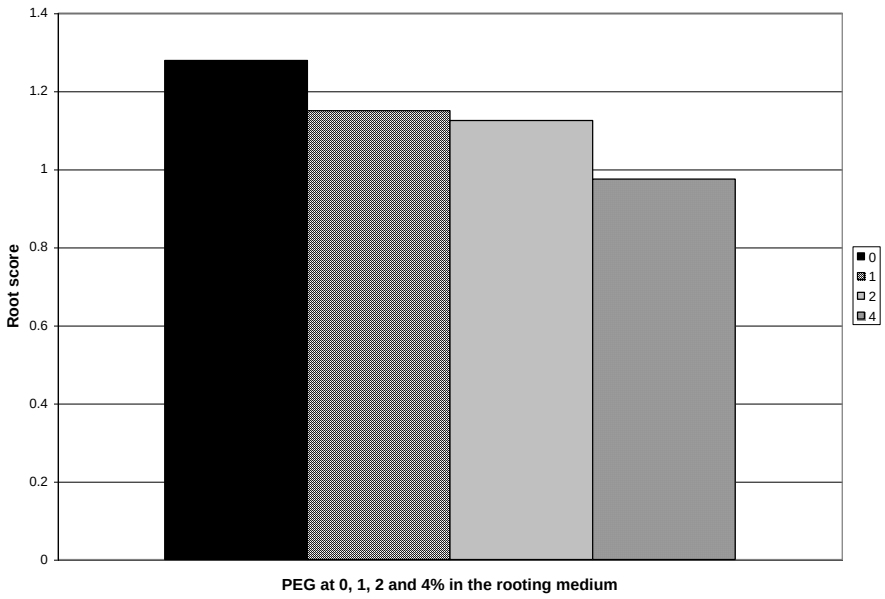

Figure 11. The effect of a polyethylene glycol (PEG) pretreatment on subsequent rooting of cloudberry plantlets.

Discussion

The use of PEG as a pretreatment to increase plant hardiness *in vitro* prior to *ex vitro* rooting does not show promise for increasing rooting in cloudberry plantlets.

Pretreatments with increased sucrose and abscisic acid

In the experiment we examine the use of increased sucrose and abscisic acid (ABA) as an *in vitro* pretreatment to increase the hardiness of cloudberry tissue culture plantlets prior to *ex vitro* rooting.

Materials and methods

Sucrose at 20, 40 and 60 grams per liter in the rooting medium (Table 11, medium 1 solidified with 7 gr per liter agar and without any BAP) in factorial combination with ABA at 0, 1 and 10 μM concentrations were tried. A 10 mM stock solution of ABA was prepared and filter sterilized into the media at the appropriate concentrations after autoclaving. The media were poured into baby food jars, 30 mls per jar. Tissue culture plantlets from several cloudberry seedling populations were used in the experiment. There were 20 replicates per treatment and the experiment was repeated once. The plantlets were incubated on the treatment media in a growth chamber under fluorescent lights at 18°C, 18 hour photoperiod for two weeks. Plantlets were then rooted in sand in 2 inch pots in a growth chamber under fluorescent lights at 18°C, 18 hour photoperiod for six weeks. Flats were covered with domes to maintain humidity during the rooting period. Plants were scored as above for contamination and rooting.

Results

Even a small amount of contamination by the end of the 6 week rooting period had a negative effect on rooting in cloudberry plantlets (Fig. 12). Thirty three percent of the plantlets were somewhat to mostly contaminated in the experiment.

Increasing the sucrose in the medium had a positive effect on subsequent rooting of cloudberry plantlets while ABA in the medium had a negative effect on rooting (Fig. 13). On average 74% of the plantlets on a medium without ABA and with 60 grams per litre sucrose rooted. At high levels of ABA in the media, sucrose concentrations in the media no longer effected rooting percentages. Rooting percentages of 65, 55 and 52% were obtained on media with respectively 0, 1 and 10 µM ABA. Neither increasing sucrose nor the use of ABA in the rooting media had an effect on the contamination rate.

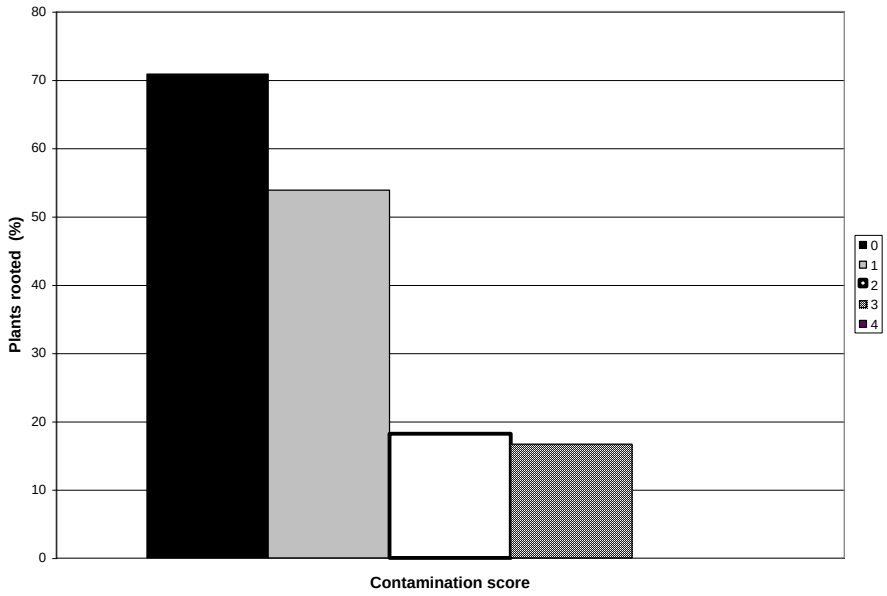

Figure 12. Negative correlation between contamination of cloudberry plantlets *ex vitro* and rooting success. Contamination score: 0=no contamination, 1= 1/4 of the plant contaminated, 2=1/2 of the plant contaminated, 3=3/4 of the plant contaminated and 4= plant completely contaminated

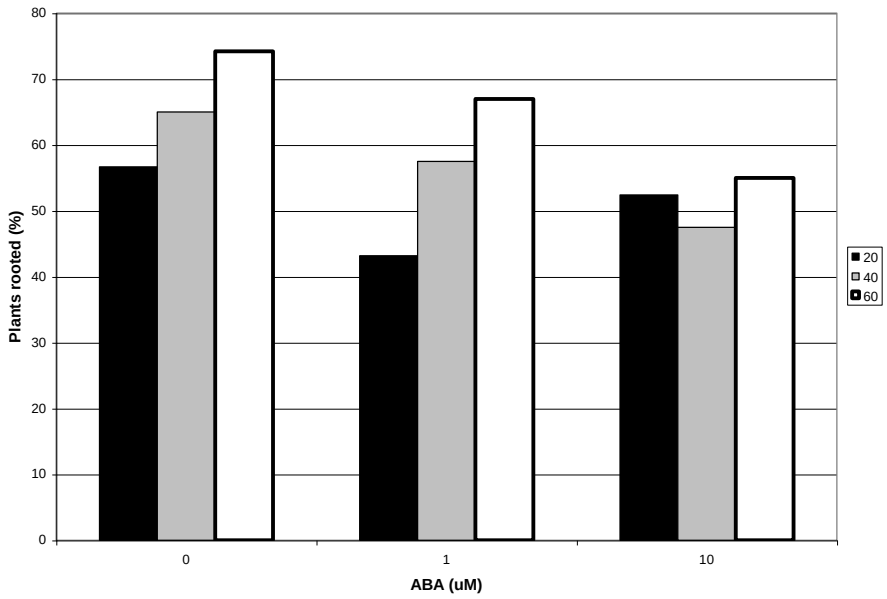

Figure 13. The effect of increasing sucrose levels (20, 40 and 60 grams per litre) and abscisic acid (ABA at 0, 1 and 10 μM) in the medium on subsequent rooting of cloudberry plantlets *ex vitro*.

Discussion

While ABA in the rooting medium does not show promise for rooting cloudberry plantlets, increasing the sucrose concentration from 20 to 60 grams per liter for a 2 week period prior to *ex vitro* rooting was beneficial in this experiment. Contamination of the plantlets once removed from tissue culture continues to be problematic.

Pretreatments with gibberellic acid (GA_3)

The experiment examines the use of gibberellic acid (GA_3) in the rooting medium to stimulate subsequent growth and rooting *ex vitro*.

Materials and methods

The rooting medium (Table 11, medium 1 solidified with 7 gr per liter agar and without any BAP) for the experiment was modified to include 40 grams per liter sucrose and 0, 5, 25 or 50 μM GA_3. GA_3 was dissolved in 95% alcohol and added to the media after autoclaving. The media were dispensed into baby food jars, 30 mls per jar. Ten female and ten male clones, two each from each of five regions of origin, were used in the experiment. There were 10 replicates per medium per trial and four trials per experiment. The experiment was repeated once. Cloudberry plantlets were incubated on the media for two weeks as described earlier. Plantlets were then planted in sand in 2 inch pots in 32 cell flats and the flats were covered with a dome to maintain humidity during the six week rooting period. Contamination and rooting were scored as previously described.

Results

Contamination was not problematic in this experiment. Only 7% of the plantlets were contaminated by the end of the 6 week rooting period.

Both rooting percentage and the number of roots produced per plant were affected by GA_3 in the rooting medium (Fig. 14). There was a slight improvement in rooting at 5 µM GA3 while rooting was significantly decreased at 50 µM GA_3. There was a significant interaction effect between plant gender and GA_3 in the rooting medium. While male plants benefited from GA_3 in the medium at all concentrations tried a significant reduction in rooting percentage and root numbers per female plant was observed at both 25 and 50 µM GA_3 (Fig. 14).

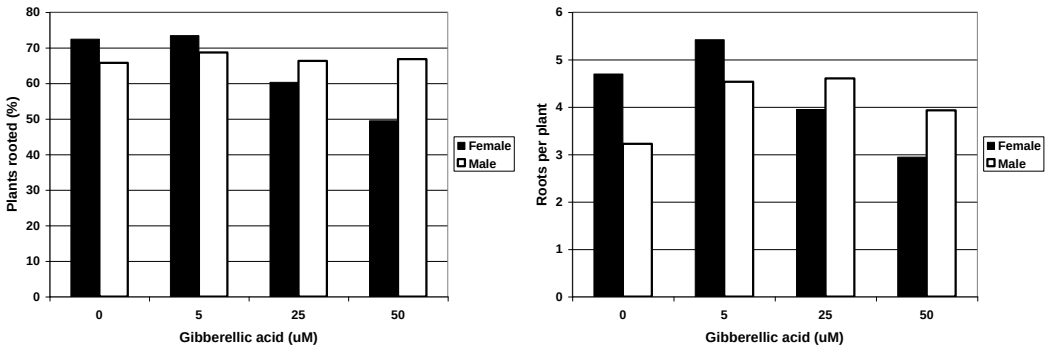

Figure 14. Rooting percentage and number of roots per plant as a function of GA_3 in the rooting medium and plant gender.

Rooting was also affected by both the region of origin of the clones and genotype in the experiment. Clones from the region of the Haute-Côte-Nord and from Norway produced the greatest number of roots per plant while those from the regions of Manicouagan and Blanc Sablon produced fewer roots. While the two female clones from Norway produced more roots than did the two male clones from Norway the opposite was true of respectively female and male clones from the Haute-Côte-Nord (Table 13). Roots per clone ranged from a low of fewer than 1 to a high of 9 by the end of the rooting period.

Table 13. The effect of region of origin and genotype on rooting in cloudberry plantlets.

Region of origin	Clone	Sex	Roots per plant
Haute-Côte-Nord	BB1F6	Female	6.5
Haute-Côte-Nord	LAMF8	Female	2.3
Haute-Côte-Nord	CBD1M1	Male	9.0
Haute-Côte-Nord	SAP1M2	Male	3.2
LS Mean Haute-Côte-Nord			**5.3 a**
Manicouagan	CAO1F1	Female	3.6
Manicouagan	REX1F1	Female	1.8
Manicouagan	RP1F7	Female	1.8
Manicouagan	CAO1M1	Male	3.2
Manicouagan	REX1M2	Male	2.4
LS Mean Manicouagan			**2.6 c**
Blanc Sablon	GP1F1	Female	5.5
Blanc Sablon	NHM1F1	Female	1.0
Blanc Sablon	PB1M1	Male	1.5
Blanc Sablon	SMLT1M1	Male	5.2
LS Mean Blanc Sablon			**3.0 c**
Nord	FM3H1	Female	4.1
Nord	M52F3	Female	3.7
Nord	FM1M1	Male	2.1
Nord	FM1M3	Male	4.2
Nord	FM4M6	Male	5.3
LS Mean Nord			**4.0 b**
Norway	Fjellgull	Female	6.4
Norway	Fjordgull	Female	6.4
Norway	Apollen	Male	3.9
Norway	SRc 12.1	Male	3.9
LS Mean Norway			**5.1 a**

Means in column followed by the same letter are not significantly different at p=0.05

Substantial differences in rooting percentage and root numbers produced per plant were observed between the two replicates of the experiment. While rooting percentage averaged 85% in the first replicate only 45% of the plants rooted in the second replicate of the experiment. No interaction effects between experiment replicate and the other factors examined (contamination, gender or GA_3) were observed.

Discussion

Gibberellic acid at 5 μM in the rooting medium improved subsequent *ex vitro* rooting of both female and male cloudberry plantlets. The observed differences however were minimal compared with differences observed between the two replicates of the experiment. Thus factors not strictly controlled in the experiment such as humidity and light intensity may be of greater importance to rooting success than growth regulator manipulations in the rooting media. Genotype and region of origin were also factors affecting rooting success though no interaction between these factors and GA_3 in the rooting medium were observed. While Martinussen *et al.* (2004) observed better rooting in male cloudberry clones than in female clones the opposite was true in our case.

Rooting conditions however were not the same in the two experiments. Gender effects on rooting ability were not stable across region of origin and most likely reflect the individual genotypes used rather than any effect of gender on rooting per se. The observed gender by GA_3 interaction however was stable across all other factors in the experiment.

Rooting

Ethylene inhibitors: preliminary screen

A preliminary screen of 0, 5, 50 and 500 μM Silver Thiosulfate (STS) in factorial combination with 0, 1, 5 and 10 μM Naphthyl acetic acid (NAA) was run to determine if STS was phytotoxic to cloudberry plantlets in the range of concentrations used. NAA, which is a longer lasting auxin than Indole acetic acid (IAA), was used in this screen to provoke epinasty in the plantlets as a possible means of tracking ethylene evolution in the plantlets.

Materials and methods

Sixteen treatment solutions were prepared with STS at 0, 5, 50 and 500 μM in factorial combination with NAA at 0, 1, 5 and 10 μM. Treatment solutions were prepared in water. Cloudberry tissue culture plantlets from two male and two female selections were treated individually in test tubes containing 10 ml of the treatment solutions for two hours before planting in sterilized sand in 4 oz solo cups. There were 5 replicates per treatment (16 treatments) and clone.

Plants were scored for epinasty after 24 hours using a subjective scale of 1 to 3 with 1 indicating no sign of epinasty, 2 indicating moderate epinasty and 3 indicating severe twisting and downturning of the leaves. Plants were incubated in a growth chamber under fluorescent lights at 18°C, 18 hour photoperiod for six weeks after which they were scored for contamination and rooting as previously described.

Results

No phytotoxic effects of the STS at the concentrations tried were observed. NAA at 10 μM provoked higher epinasty scores than were observed in the control treatment without NAA. STS however had no effect on epinasty scores in the trial.

Rooting was poor, especially for the female clones in the trial. Only 7% of the female plantlets rooted vs 37% of the male plantlets. STS in the treatment medium had a positive effect on cloudberry rooting with respectively 13 and 49% of female and male plantlets rooting following treatment in 50 μM STS (Fig. 15). NAA had a negative, though non-significant, effect on rooting (Fig. 15). There was no interaction effect between NAA and STS in the treatment media on rooting.

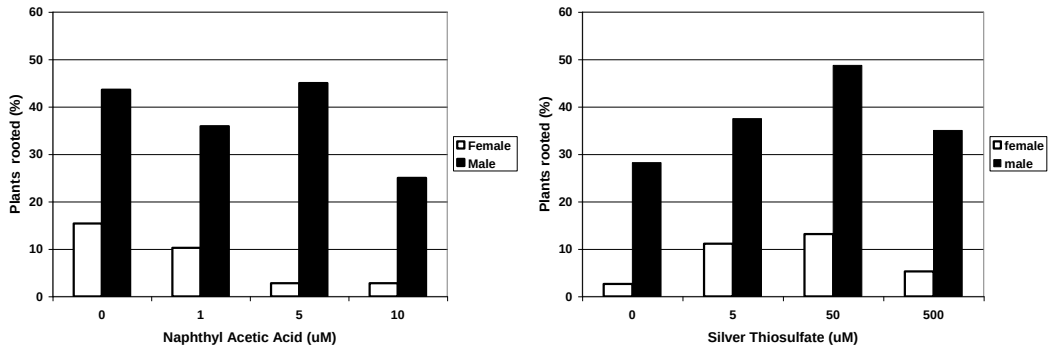

Figure 15. The effects of pretreatments with naphthyl acetic acid (NAA; left) and Silver thiosulfate (STS; right) on subsequent ex vitro rooting of male (black) and female (white) cloudberry plantlets.

Discussion

In the absence of instrumentation to measure ethylene production, scoring tissue culture plantlets for epinasty seems to work as a rough bioassay in this system. In the screen, positive results were obtained with the use of the ethylene inhibitor STS. For the full experiment on different types of ethylene inhibitors, the auxin IAA will be used in place of NAA which had a negative effect on rooting in the trial.

Ethylene inhibitors: full experiment

In this experiment the effects of the ethylene biosynthesis inhibitor aminoethoxyvinyglycine (AVG) and two ethylene action inhibitors; cobalt chloride (CoCl) and silver thiosulfate (STS), were examined in factorial combination with the auxin indole acetic acid on *ex vitro* rooting of cloudberry plantlets.

Materials and methods

The three different ethylene inhibitors were examined in a series of six trials, two trials per type of ethylene inhibitor. A 10 mM stock solution of aminoethoxyvinylglycine (AVG) was prepared by dissolving 25 mg of AVG in 500 ul 1 M NaOH and bringing the solution up to 12.25 ml with distilled water. The stock solution was filter sterilized and stored frozen for later use. A 10 mM stock of cobalt chloride hexahydrate (CoCl) was prepared by dissolving 237.0 mg CoCl in 100 ml distilled water. A 20 mM stock solution of silver thiosulfate was prepared by pouring 20 mls of a 100 mM silver nitrate stock solution into 80 mls of a 100 mM sodium thiosulfate stock solution. A 10 mM stock of indole acetic acid (IAA) was prepared by dissolving 175.2 mg IAA in 10 ml 95% ethanol and bringing the solution up to 100 ml with distilled water. The treatment dosages for each type of ethylene inhibitor, which are based on dosages found in the literature, are given in Table 14.

Table 14. Concentrations of each ethylene inhibitor examined for *ex vitro* rooting of cloudberry plantlets.

Compound	**Treatment dosage (μM)**			
	1	**2**	**3**	**4**
Aminoethoxyvinylglycine	0	5	10	50
Cobalt chloride	0	50	100	500
Silver thiosulfate	0	5	50	500

Each ethylene inhibitor was tested in factorial combination with IAA at 0, 50, 100 and 500 μM. Treatment solutions were prepared in water. Cloudberry tissue culture plantlets from seedling populations were treated individually in test tubes containing 10 ml of the treatment solutions for two hours before planting in sterilized sand in 4 oz solo cups. For each trial there were 10 replicates per treatment (16 treatments). As no phytotoxic effects of any of the ethylene inhibitor dosages tried were observed, no modifications of dosages were necessary for the second trial of each ethylene inhibitor.
Plants were scored for epinasty after 24 hours using a subjective scale of 1 to 3 with 1 indicating no sign of epinasty, 2 indicating moderate epinasty and 3 indicating severe twisting and downturning of the leaves. Plant size was also scored using a subjective scale of 1-4. Plants were incubated in a growth chamber under fluorescent lights at 18°C, 18 hour photoperiod for six weeks after which they were scored for contamination and rooting as previously described.

Data from the six trials were pooled for the analysis. Because of interactions between the ethylene inhibitor type and other factors in the experiment a second round of analyses were run on the two trials of each type of ethylene inhibitor. Contamination, plantlet size and epinasty scores were used as covariates in the analyses.

Results
Despite the lack of contamination problems, rooting percentages were low for the series of trials averaging only 24% and ranging from a low of 0 to a high of 55% between treatments. While there was a dose dependant positive correlation between the amount of indole acetic acid (IAA) in the treatment solutions and subsequent epinasty scores, the negative relationship between IAA in the treatment solution and rooting percentage was not dose dependent (Fig.16). Rooting was best following treatment in solutions containing no IAA and worst following treatments with 50 μM IAA. However, there were significant interaction effects between IAA in the treatment solutions, ethylene inhibitor type and ethylene inhibitor dosage.

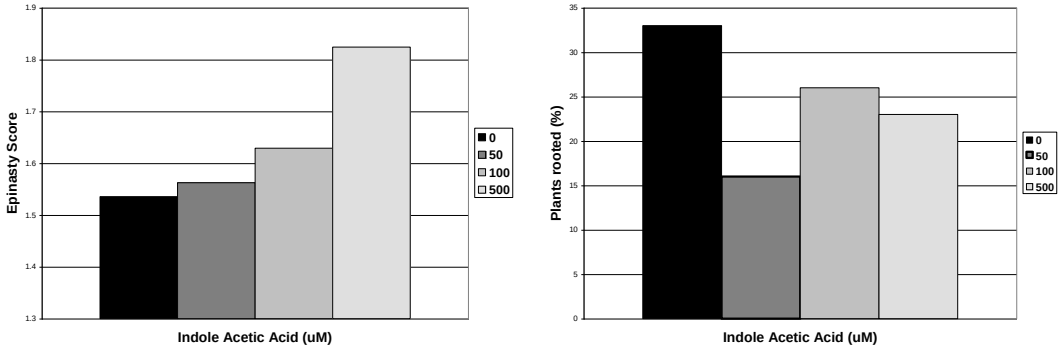

Figure 16. The effect of indole acetic acid (IAA) at 0, 50, 100 and 500 µM on epinasty scores and percentage rooting of cloudberry tissue culture plantlets.

Both silver thiosulfate (STS) and cobalt chloride (CoCl) in the treatment solutions led to reduced epinasty scores in cloudberry plantlets while aminoethoxyvinyglycine (AVG) at the highest dose used led to increased epinasty scores. With all three types of ethylene inhibitors a negative correlation between rooting and epinasty scores were observed (Fig. 17).

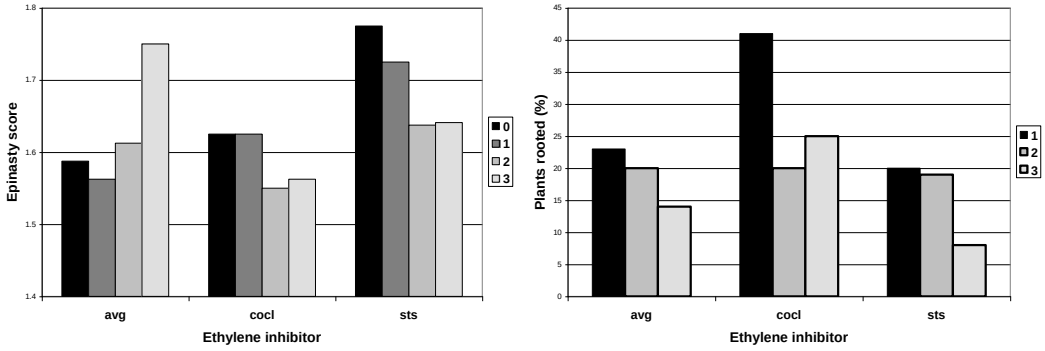

Figure 17. The effect of the ethylene inhibitors aminoethoxyvinyglycine (AVG), cobalt chloride (CoCl) and silver thiosulfate (STS) on epinasty scores (left) and the negative correlation between epinasty score (1, 2 and 3) and rooting percentage in each of the three experiments (avg, cocl and sts; right).

There was no effect of AVG in the treatment solutions on subsequent *ex vitro* rooting of cloudberry plantlets (Fig. 18). In this set of trials, IAA had a positive effect on rooting at the 100 and 500 µM concentrations. Plantlet size was a significant factor affecting rooting in this set of trials with medium sized plantlets rooting more easily than either small or large plantlets (Fig.19).

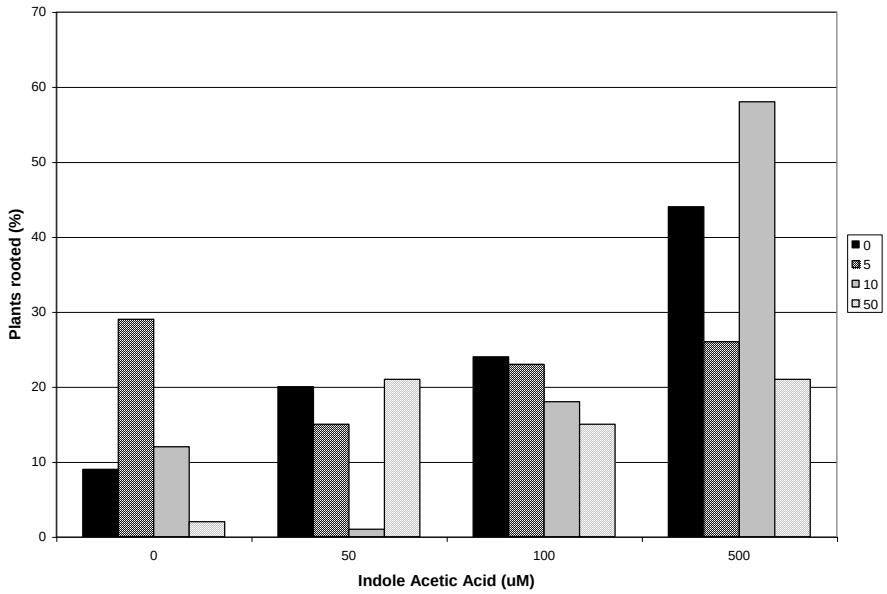

Figure 18. The interaction effect of indole acetic acid (IAA) at 0, 50, 100 and 500 μM and aminoethoxyvinyglycine (AVG) at 0, 5, 10 and 50 μM on *ex vitro* rooting of cloudberry plantlets.

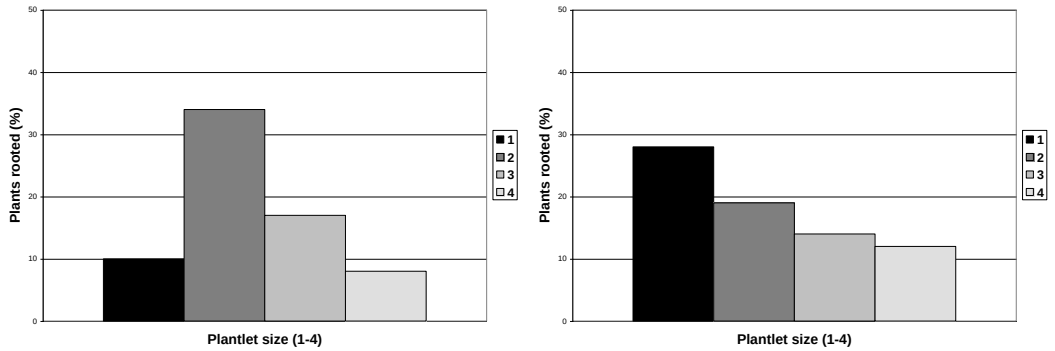

Figure 19. The effect of cloudberry plantlet size (1-4 on an arbitrary scale) on *ex vitro* rooting in trials with aminoethoxyvinyglycine (AVG, left) and silver thiosulfate (STS, right).

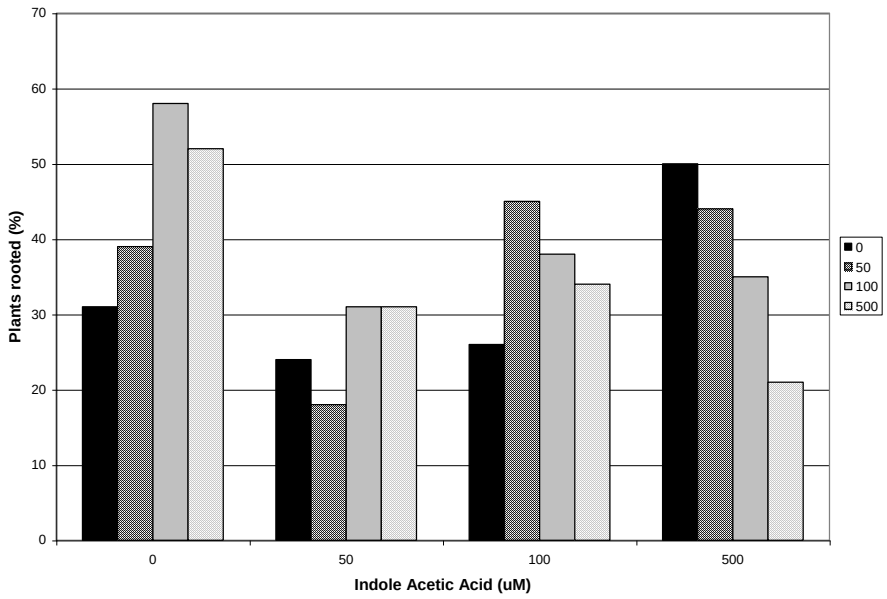

Figure 20. The interaction effect of indole acetic acid (IAA) at 0, 50, 100 and 500 μM and cobalt chloride (CoCl) at 0, 50, 100 and 500 μM on *ex vitro* rooting of cloudberry plantlets.

Cobalt chloride in the treatment solution in the absence of IAA appeared to increase *ex vitro* rooting of cloudberry plantlets while a decrease in rooting was observed in solutions containing increasing concentrations of cobalt chloride in combination with IAA at 500 μM (Fig. 20). These interactions, however, were not significant. Interveinal chlorosis was prevalent on plantlets treated with either 100 or 500 μM CoCl but did not seem to affect rooting of the plantlets. Epinasty score on the other hand was a significant covariate in the analysis. While plantlets showing no signs of epinasty rooted at 40% only 20 % of those showing signs of epinasty rooted.

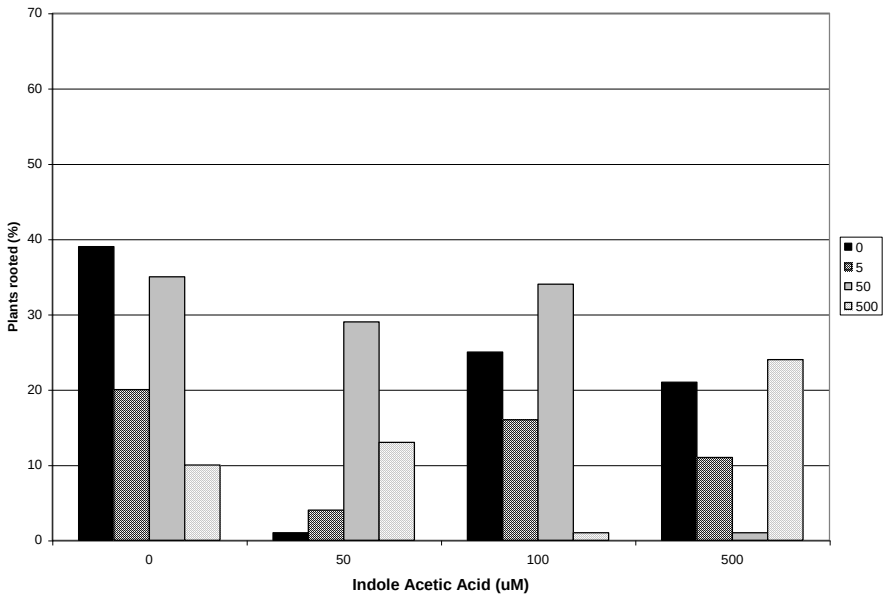

Figure 21. The interaction effect of indole acetic acid (IAA) at 0, 50, 100 and 500 μM and silver thiosulfate (STS) at 0, 5, 50 and 500 μM on *ex vitro* rooting of cloudberry plantlets.

The beneficial effects of STS on rooting of cloudberry plantlets observed earlier (Fig. 15) were not repeated in this second series of trials with STS (Fig. 21). Rooting of plantlets treated with 50 μM STS averaged 25% across IAA concentrations as opposed to 21% for plantlets not treated with STS. As seen earlier in trials with CoCl, plantlet size had a significant effect on rooting (Fig. 21) with smaller plantlets rooting at higher percentages than larger plantlets.

Discussion

While we are unable to analyse ethylene production directly in our lab the dose dependent increase in epinasty scores in response to IAA treatments and the reduction in epinasty scores following treatment with the ethylene inhibitors STS and CoCl indicate that the system is working. The ethylene synthesis inhibitor AVG however was not effective in the system, perhaps because the auxin which stimulates ethylene production was applied at the same time as the AVG.

There was a negative correlation between epinasty scores and rooting in cloudberry, thus a reduction in epinasty score should translate into increased rooting. However, the reduction in epinasty score brought about by the ethylene inhibitors was minimal and not within the range of epinasty scores affecting rooting.

Main effects of the ethylene inhibitors were not significant due to interactions between the IAA and ethylene inhibitors in the treatment solution. There were too few repetitions per treatment and other factors important to rooting, such as plantlet size, were not controlled in the experiment. The interaction results, though statistically significant in many cases, appear to be meaningless. Following another experiment examining the

effects of 50 μM STS or 100 μM CoCl with or without IAA at 100 μM on rooting with 40 replicates per treatment the results were no more promising and this method of rooting cloudberry plantlets was rejected. An experiment examining the effect of plantlet size on *ex vitro* rooting of cloudberry plants follows.

Cloudberry plantlet size and *ex vitro* rooting

Many factors not controlled for in the rooting experiments appear to have greater effects on rooting than the treatment solutions examined. Plantlet size is one of these factors. In all experiments where plantlet size has been noted, larger plantlets root poorly compared to small and medium sized plantlets. This has also been noted previously (Martinussen *et al.*, 2004). The negative effect of large plantlet size on rooting may be due to a lack of optimal contact between the plantlet and the rooting medium. It may however also be inherent to vigorous plantlets which may contain more endogenous cytokinins than do less vigorous plantlets. In this experiment we examine the effect of plantlet size, before and after division, on *ex vitro* rooting in four male cloudberry clones.

Materials and methods

Four male cloudberry clones *in vitro* (160 plantlets each) were sorted into 8 size groups and the size groups 1, 4 and 8 were retained for the experiment. Five plantlets from each size group were weighed and transferred to rooting medium (Table 11, medium 1 solidified with 7 gr per liter agar and without any BAP) in baby food jars without division. The remaining five plantlets in size groups 4 and 8 were divided into clumps approximately equal in size to plantlets in size group 1. A clump from each was weighed and transferred to the rooting medium. Plantlets in size group 1 were not divided- however, in order to simulate the same type of injury that the other plantlets were subjected to a single shoot was cut from the clumps before they were weighed and transferred to the rooting medium. The plantlets were incubated on the rooting medium in a growth chamber under fluorescent lights at 18°C, 18 hour photoperiod for two weeks. Plantlets were then rooted in sand in 2 inch pots in a growth chamber under fluorescent lights at 18°C, 18 hour photoperiod for six weeks. Plantlets, 32 to a flat, were randomized in the flats such that clone effects on contamination were equally distributed between flats. Flats were covered with domes to maintain humidity during the rooting period. Plants were scored as described for earlier experiments for contamination and rooting after four weeks.

Results

Plantlet weights for the three size groups and two treatments are given in Table 15. The sorting method used led to significant fresh weight differences between the groups while the fresh weight of divided plantlets did not differ significantly from those of undivided group 1 plantlets.

Table 15. Fresh weights of cloudberry plantlets sorted into three size groups and divided or left as undivided controls.

Group	Treatment	Weight	
1	Control	0.69	c
1	Divided	0.59	c
4	Control	1.19	b
4	Divided	0.60	c
8	Control	1.90	a
8	Divided	0.64	c

Means in column followed by the same letter are not significantly different at p=0.05

Contamination rate in the experiment was low and was not an important covariate in the analysis. On average 9% of the plantlets showed some signs of contamination at the end of the four week rooting period. Contamination rate was higher among the undivided control group than among divided plantlets at respectively 13 and 6%.

Rooting percentages were low. Only 27% of the plantlets produced roots by the end of the rooting period. Rooting was significantly higher in divided plantlets than in the undivided control plantlets for all size groups (Fig. 22). No effects on rooting of plantlet size, clone or interactions between the various factors examined were observed in the experiment.

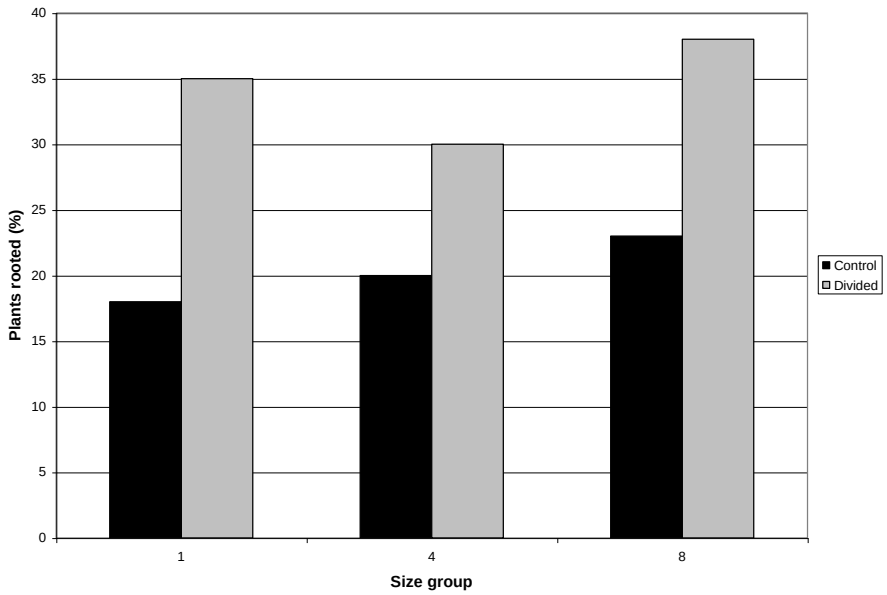

Figure 22. Rooting of cloudberry plantlets as a function of plantlet size group (1, 4 or 8) and division.

Discussion

Considering results from previous experiments, it is surprising that no effects of plantlet size on rooting were observed in this experiment. Rather, the division of the plantlets before rooting or, in the case of the smallest size group, the injury inflicted on the plantlets, appeared to stimulate rooting in these clones relative to the control plantlets which were transferred directly to the rooting medium without division or injury. The best rooting percentage was obtained from the most vigorous plantlets which had been divided. There is thus no evidence from this experiment that endogenous factors related to plantlet vigor affect *ex vitro* rooting of cloudberry plantlets.

Cloudberry *in vitro* propagation protocol retained

Stage l: Initiation

Plant material

Collect cloudberry buds from plants having just completed their dormancy cycle (16 weeks at 4°C) before the bud scales begin to open.

Surface sterilization of buds

Wash buds in a solution of 5 ml Tween 20 and 2 ml household bleach per liter for 1 hour with agitation. Transfer buds to an acidified bleach solution containing 200 mls household bleach per liter and acidified with 6.5 mls concentrated HCl. Agitate for 20 minutes before transferring the buds to sterile water. Refrigerated buds in water until use.

Explant dissection, surface sterilization and initiation

Under a dissecting scope dissect the apical meristem with 2 to 3 subtending nodes out of the buds. Place explants in 5 mls liquid initiation medium (Table 16) containing 0.2% plant preservative mixture (PPM) in 1 oz tablet jars. Incubate 24 hours on shaker bath at 150 rpms. Transfer explants to filter paper bridges in standard 25 mm test tubes containing 10 mls of liquid initiation medium. Incubate under cool white fluorescent lights, 18 hour daylength and 18°C for four weeks.

Stage ll: Multiplication

Transfer the explants to the multiplication medium (Table 16) in 1 oz table jars after four weeks. Subculture to fresh medium in jars every four weeks until the plantlets have 10 or more shoots. Thereafter subculture to multiplication medium in standard 25 mm test tubes every six weeks.

Stage lll: Acclimatization

Divide plantlets into clumps of approximately 10 shoots and transfer to acclimatization medium (Table 16), 30 mls per baby food jar. Incubate two weeks on the medium under cool white fluorescent lights, 18 hour daylength and 18°C.

Stage lV: Rooting *ex vitro*

Rinse the plantlets well and plant into medium grain sand in 2 inch pots, 32 pots per flat. Cover flats with a dome to maintain humidity. Root in a growth chamber 18 hour daylength, 18°C. Cool white fluorescent lights or a mixture of high pressure sodium and metal halide lights have both been used in rooting cloudberry plantlets in the course of this project.

Table 16. Components of cloudberry initiation, multiplication and acclimatization media.

Stock solution	Compound	Gram/L	Medium		
			Initiation per litre	Multiplication per litre	Acclimatization per litre
1	NH_4NO_3	82.50			
	KNO_3	95.00	20 mL	20 mL	20 mL
	$MgSO_4.7H_2O$	18.50			
	KH_2PO_4	8.50			
2	$CaCl_2.2H_2O$	44.00	10 mL	10 mL	10 mL
3	$MnSO_4$	1.51			
	$ZnSO_4.7H_2O$	0.860	10 mL	10 mL	10 mL
	H_3BO_3	0.620			
4	KI	0.083	10 mL	10 mL	10 mL
	$NaMoO_4.2H_2O$	0.025			
5	$CuSO_4.5H_2O$	0.025	1 mL	1 mL	1 mL
	$CoCl_2$.6H2O	0.025			
6	FeSO4.7H20	2.78	10 mL	10 mL	10 mL
	Na_2.EDTA.$2H_2O$	3.73			
8	NaH_2PO_4	15.22	10 mL	10 mL	10 mL
9	Thiamine-HCl	0.100			
	Nicotinic acid	0.250	4 mL	4 mL	4 mL
	Pyridoxine.HCl	0.250			
11	BAP	0.250	8 mL	8 mL	0
12	GA_3	0.350	5 mL	0	5 mL
	Myo-Inositol		0.100 g	0.100 g	0.100 g
	Adenine sulfate		0.080 g	0.080 g	0
	Sucrose		20 g	20 g	60 g
	Agar		1 g	7 g	7 g
	pH		4.5	4.5	4.5

Discussion

Through the series of experiments described here a number of small improvements to our cloudberry *in vitro* propagation protocols have been made. At the initiation step contamination rates have been significantly reduced through the pretreatment of explants in a solution of Plant Preservative Mixture (PPM). The success of initiation has been further improved by the inclusion of 5 μM GA_3 in the initiation medium. Although the success rate differed between genders there was no interaction effect between gender and initiation medium on the success rate, thus the development of gender specific media was not required. Other initiation protocols, for example leaf regeneration protocols or the Debnath (2007) protocols, which are technically much simpler, were nonetheless rejected due respectively to genotype specificity and poor success rate in our hands.

We were not successful in developing direct rooting protocols involving combinations of auxins and the three ethylene inhibitors examined. No synergistic effects of the auxins and ethylene inhibitors used in the treatment solutions were observed. Rooting success was however improved through the inclusion of an *in vitro* acclimatization step in the

protocols. An increase in the sucrose concentration to 60 grams per liter and the inclusion of 5 μM GA_3 in the acclimatization medium both led to improved *ex vitro* rooting of male and female cloudberry clones.

In the course of the project 17 male and 50 female selections have been added to the bank of cloudberry clones maintained at the Centre de recherche Les Buissons. Ten copies of each are maintained allowing us to rapidly increase the material for use in expanded variety trials or cloudberry production projects.

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Charles Jones	Field Worker	Field Trials
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Karin Blais St-Laurent	Technician	Field Trials

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La Conférence régionale des élus de la Côte-Nord

Annexe 1

<u>Rencontre d'information à Blanc-Sablon</u>

Quand : le mercredi 6 août 2008
Durée : de 8h30 à 17h00
Endroit : Bureau Municipale de Blanc-Sablon
Frais de déplacements et de séjours assumés par les participants

8h30 Présentation des participants

8h45 Présentation du projet Domestication par Kristine Naess du Centre de recherche

9h30 Présentation du projet Semi domestication, volet engrais biologique par Valérie Hébert-Gentile étudiante en maîtrise sous la direction de Line Lapointe et de Léon-Etienne Parent de l'université Laval et de Kristine Naess du Centre de recherche

10h30 Pause café

10h45 Présentation du projet Semi domestication, volet brise-vent par Kristine Naess

11h30 Table ronde

12h00 Dîner

13h30 Visite des installations en tourbières

17h00 Fermeture de la rencontre

Prévoir 2 nuitées à Blanc-Sablon
Arrivée à Blanc-Sablon le 5 août en fin d'après-midi

Départ le 7 août tôt le matin

Liste de participants :

David Calderisi	CLD de Blanc-Sablon
Alexandre Dumas	Coasters Association
Réjean L. Dumas	Directeur général Municipalité de Blanc-Sablon
Réginal Hancock	Maire de Forteau, Labrador
Armand Joncas	Maire de Blanc-Sablon
Bruce Moores	Labrador Straits Development Corporation, Labrador
Roberto Stéa	DEC, Sept Iles
Jane White	Fruit Crop Development Officer, Terre-Neuve

Annexe 2

Colloque Bioalimentaire

Côte-Nord 2009

Date: jeudi le 19 novembre 2009

Endroit: Baie-Comeau, Jardin des Glaciers
3 Denonville, dans le quartier St-Georges

Programmation

8h15 Accueil des participants(es)
8h30 Mot d'ouverture, Alain Côté, direction régionale du MAPAQ
8h45 à 11h30 Ateliers par secteur du bioalimentaire :
Un travail situé dans deux salles différentes

Agroalimentaire-secteur petits fruits nordiques	**Pêche et aquaculture**
Mot de bienvenue du porte-parole du Conseil	Mot de bienvenue du porte-parole du Conseil
Bilan annuel	Enjeux et dossiers stratégiques
Enjeux et dossiers stratégiques	Retour sur le plan d'action 2009-2010
Retour sur le plan d'action 2009-2010	Élections des membres au Conseil de l'industrie des pêches
Mot de bienvenue de l'UPA Côte-Nord	
Portrait de la production agricole	

Avant-midi	
Volet Agroalimentaire-secteur Petits fruits nordiques et production agricole	
8h45	Mot du porte-parole du Conseil de l'industrie des petits fruits nordiques
	Bilan de la dernière année :
	Portrait des récoltes et la situation du marché
	Accès des terres publiques : dossier CRRNT et situation par territoire
	UPA Côte-Nord : bilan des dossiers de la dernière année et besoins futurs, Ghislaine Morin, présidente
10h15	**Pause**
10h30	Centre de recherche Les Buissons : présentation de l'équipe recherche et résultats de travaux menés sur le territoire
11h15	Plan d'actions 2009-2010- Projet d'une ressource-filière petits fruits nordiques

Après-midi	
Volet Bioalimentaire	
Les deux groupes sont réunis dans la même salle	
11h30	**Dossier-Appellation d'origine ou géographique**, Rémy Lambert, vice-recteur à la recherche de l'université Laval
12h	**Dîner-Conférence : marketing Six Continents Inc.** ,Gilbert H. Aura, vice-président
13h30	Vitrine régionale des produits : logo ou autres initiatives
13h45	**Mise en place de la Table bioalimentaire Côte-nord**
	Mandats de la Table bioalimentaire Côte-Nord
	Aspect corporatif et composition du conseil d,administration
	Orientations et axes d'interventions
	Élection des postes vacants
16h	**Mot de fermeture du colloque régional**