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CCC and Prohexadione-Ca Enhance Rhizome Growth and Lateral Bud Production in Rhubarb (*Rheum rhabarbarum* L.)

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Abstract Accelerating rhizome growth is crucial to enhancing propagule production in rhubarb (*Rheum rhabarbarum* L.) because the crop is propagated through rhizome divisions. This can be achieved through manipulating source-sink activity. This study tested the hypothesis that synthetic plant growth retardants Prohexadione-Ca and CCC enhance rhizome growth in rhubarb. Two different concentrations of these plant growth retardants and GA_3 (positive control) were foliarly applied on the cultivar German Wine at three stages of shoot growth under greenhouse conditions. Both Prohexadione-Ca and CCC favorably enhanced rhizome growth through suppressing shoot growth. CCC at 3000 mg L^{-1} produced the best results and the effect was apparent when applied at 12 weeks after shoot emergence. The rhizome diameter, fresh weight, and the number of viable buds were enhanced significantly in plants sprayed with CCC 3000 mg L^{-1}. Both Prohexadione-Ca and CCC were equally effective in enhancing dry mass and starch allocation preferentially toward the rhizome. Prohexadione-Ca- and CCC-induced rhizome growth enhancement could possibly be due to their known role as GA biosynthesis inhibitors or through increasing photosynthetic efficiency and preferentially reallocating carbohydrates to the rhizome.

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Introduction

Although the individually quick-frozen (IQF) rhubarb industry has a high market potential in North America (Robinson and Comeau 2005) and Europe (Schrader 2002), the inadequate availability of propagules and their high cost have limited rhubarb production and restricted industry expansion. Being a herbaceous perennial, Rhubarb (*Rheum rhabarbarum* L.) is generally propagated by rhizome divisions and, thus, enhancing the rhizome growth process is critical for enhancing propagule generation. Our knowledge of the physiological mechanisms and hormonal relationships involved in rhubarb rhizome growth and subsequent growth of the new propagule is still in its infancy.

Plant growth and developmental processes, including that of storage organs, are influenced by photosynthesis, carbon allocation, and source-sink activity (Huber and others 1985). It is also well established that photoassimilate partitioning plays an important role in determining plant architecture, resulting in balancing shoot and root growth of the plant depending on its reproductive or food storage strategy (Bidel and others 2000). In rhubarb, the size of the rhizome (which is the "sink" in this case) is a significant contributing factor to the number of viable propagules that can be generated from one plant. Under limited photoassimilate availability, a competitive hierarchy exists among different sink tissues and the photoassimilate supply depends largely on the relative sink strength and activity (Wardlaw 1990). This hierarchy is also controlled indirectly by other factors including mineral nutrients and plant hormones (Wardlaw 1990). Altering sink

size or activity can also result in changes in the photosynthesis and photosynthate allocation patterns (Gifford and Evans 1981; Paul and Foyer 2001). Therefore, redirecting photoassimilates (enhancing sink activity of the rhizome) toward the rhizome is critical to promote its growth.

One good example to demonstrate that preferential allocation of photoassimilates can enhance the rhizome yield in rhubarb is the result obtained when flower stalks, occasionally produced in older plants, are removed—enhanced rhizome development (Raffelson and others 2002). In a similar herbaceous perennial, *Hosta* sp., the number of propagules (crown divisions) obtained per plant increases when the inflorescence is removed at pre- or full-bloom stage (Leclerc and others 2005). In addition to physical manipulations, storage organ growth can also be altered through the application of plant growth retardants such as Paclobutrazol, Chlormequat Chloride (CCC), Prohexadione-Ca, uniconazole, morphactins, ancimidol, and diaminozide, which are potent antigibberellins (Singh and others 1987; Rademacher 2000; Leclerc and others 2006).

Cycocel® [Chlormequat Chloride or (2-chloroethyl) trimethyl ammonium chloride referred to as CCC] and Apogee® (Prohexadione-Ca or calcium-3-oxido-5-oxo-4-propionylcyclohex-3- enecarboxylate) are two plant growth retardants that are known to inhibit the biosynthesis of gibberellins (GAs). CCC inhibits at an early stage (Rademacher 2000) and Prohexadione-Ca, a 2-oxoglutarate mimic, inhibits GA biosynthesis at a much later stage (Rademacher 2000). CCC belongs to the onium-type antigibberellin, which acts at an early stage in the pathway blocking the synthesis of *ent*-kaurene, whereas Prohexadione-Ca belongs to the cyclohexatriones group and blocks several later 2-oxoglutarate-dependent steps in the GA biosynthesis pathway, including 3-beta hydroxylation, which "activates" 3-deoxy GAs such as GA_9 or GA_{20} to form GA_4 and GA_1, respectively (Rademacher 2000). Prohexadione-Ca can target the hydroxylation reactions at both the 2β and 3β positions of the gibberellane skeleton (Hisamatsu and others 1998). It is also known that 3β hydroxylation leads to the formation of biologically active GA_4 and GA_1 from their precursors, whereas 2β hydroxylation will yield inactive GAs from the biologically active forms (Graebe 1987; Hisamatsu and others 1998). Thus, depending on the balance of the already existing late-stage GA biosynthesis within the plant, Prohexadione-Ca can either promote accumulation of active GAs in the system (blocking 2β hydroxylation more strongly than 3β hydroxylation) or reduce endogenous GAs (Hisamatsu and others 1998). Previous studies on other herbaceous perennials have shown that both CCC and Prohexadione-Ca are effective in enhancing growth and generation of vegetative propagules (Hicklenton and Reekie 2001; Leclerc and others 2006). Gibberellic acid (GA) has been found to substitute for part of the cold units required to break the dormancy of rhubarb rhizomes and induce sprouting (Schrader 2002). However, the effect of GA and antigibberellins in rhubarb rhizome growth has not been explored before. Given this history, we decided to test the efficacy of two plant growth retardants—CCC and Prohexadione-Ca—in promoting rhizome growth and production of propagules in rhubarb. The objective of this study was to understand the role of GA biosynthesis inhibitors, CCC and Prohexadione-Ca, on rhubarb rhizome growth.

Materials and Methods

Plant Culture and Treatment Application

Test plants were obtained from clonally propagated 7-year-old mother plants of the variety German Wine. Uniformly sized rhizome bits weighing 60.0 g were planted in 30-cm pots filled with the commercial peat-based potting mixture Promix BX (Premier Horticulture, Quebec, Canada). Plant growth retardant (PGR) treatments were applied as foliar sprays (100 ml per plant). Treatments included Prohexadione-Ca (700 and 1400 mg L^{-1}), CCC (1500 and 3000 mg L^{-1}), GA_3 (25 and 50 mg L^{-1}), and a control (distilled water + surfactant). All concentrations are based on active ingredient percentage (a.i.). GA_3 was included as a positive control to monitor the rhizome growth in the presence of exogenous GA and in the absence of endogenous GA (antigibberellins).

Synthetic plant growth retardants Cycocel (CCC) and Apogee (Prohexadione-Ca) were obtained from BASF Canada Inc. (Mississauga, ON, Canada) and GA_3 was purchased from Sigma-Aldrich (St. Louis, MO, USA). The PGR sprays were made once at 8 or 12 weeks after shoot emergence (WK8, WK12) and once both at the 8th and 12th weeks (WK8 + 12). In all the treatments, the surfactant Tween® 20 (Sigma-Aldrich) (0.1% v/v) was added to the spray solutions to improve adsorption efficiency. The potted plants were maintained in a greenhouse at Nova Scotia Agricultural College, Truro, NS, Canada (45°22′ N, 63°16′ W) at 20°/15°C (day/night) under 16-h photoperiod from January to June 2007. The natural daylight was supplemented with light from high-pressure 400-W sodium bulbs. The plants were watered every third day until the soil was saturated.

Shoot Growth and Photosynthesis

Shoot clump numbers were measured before and after 1 week of each spray treatment. (In rhubarb, a "shoot clump" refers to the bunch of "offshoots" that arise from a single bud on the rhizome. Thus, because the buds are emerging from the underground rhizome, which itself is a stem modification, the shoots are called "offshoots.") For comparing the canopy

volume of plants in the different treatments, expressed as leaf area index (LAI), the LAI-2000 Plant Canopy Analyzer (LI-COR Biosciences, Lincoln, NE, USA) was used. Although the LAI-2000 is normally utilized in field situations, the purpose here was to compare canopy volumes and present them in terms of the LAI values. The intention was to get an idea about the canopy volume and light interception rate and compare these between the treatments.

Net leaf photosynthesis (Pn) was measured using the LCA-4 Portable Photosynthesis System (ADC Bioscientific Ltd., Herts, UK). For measuring Pn, a fully expanded, actively growing leaf with leaf lamina completely exposed to light was chosen and tagged in each plant in all replications under the different treatments. Net photosynthesis of the tagged leaves was measured using the LCA-4, a week before and a week after treatments were applied. The Pn (μmol m^{-2} s^{-1}) measurements taken at 1000–1300 photosynthetically active radiation (PAR), 25–30°C leaf surface temperature, and 20–30% relative humidity (RH) were taken between 8:00 a.m. and 11:00 a.m. Three readings were used for calculating the average Pn value for each leaf. For the WK8 + 12 treatment, at every stage of application a new leaf was selected and tagged to measure Pn in an attempt to avoid any leaf senescence or aging effect. The measurements were taken a week before and after each spray to evaluate the changes in leaf canopy and Pn. For comparing the shoot growth before and after treatment application, the percentage reduction or increase of these parameters (relative to appropriate control plants) was calculated and compared.

Measurements on Rhizome Growth at Harvest

Plants were harvested in June 2007 after 5 months of growth. Observations were made on the number of offshoots and the number of healthy buds on the rhizome for each treatment. The offshoots were carefully separated from the rhizome and the fresh weight was measured. Similarly, the rhizomes were cleaned and their fresh weight was also determined.

Estimation of Dry Mass in Shoot and Rhizome

Plants from two of the four replications were used to determine the dry mass after 72 h at 80°C. The dry mass ratio between rhizome and shoot was also determined. The shoot and rhizome samples in the remaining two replications were subjected to starch and sugar analysis.

Estimation of Total Soluble Sugars and Starch

After measurements of fresh weight, the leaf blade, petiole, and rhizome tissues were quickly immersed in liquid N_2, freeze dried, and ground into fine powder with a mortar and pestle. Total soluble sugars from the leaves, petioles, and rhizomes were estimated following the procedure developed by Farrar (1993). Total soluble sugars were extracted from 100 mg fresh weight of plant tissue. Starch was extracted from 1 g fresh weight of the plant tissue and was determined using colorimetric analysis using iodine with slight modifications to the method followed by Rood and Larsen (1988).

Experimental Design and Statistical Analysis

The pot culture experiment was designed and analyzed in a two-factor factorial design. The different plant growth regulator treatments formed the first factor. There were thus seven treatments including a surfactant control. The stages of application formed the second factor. There were three stages of application. Each treatment combination was replicated four times. The data were analyzed using the statistical software package SAS® (SAS Institute, Cary, NC, USA). The analysis of variance (ANOVA) was carried out using the PROC GLM procedure of the SAS package and the least square means (lsmeans) comparison test was performed to compare the means at the 5% significance level.

Results

Effect of CCC, Prohexadione-Ca, and GA_3 on Shoot Growth and Net Photosynthesis (Pn)

Both CCC and Prohexadione-Ca, at all three stages of application, significantly reduced the number of shoot clumps produced by June 2007 (Fig. 1). Taking into consideration the overall shoot growth (shoot clumps, LAI, and Pn), CCC at 3000 mg L^{-1} was the most effective in reducing the shoot growth. The percentage reductions in the number of shoot clumps, relative to surfactant-treated controls, in CCC (3000 mg L^{-1})-treated plants between a week before and a week after spraying were 54, 57, and 63% at WK8, WK12, and WK8 + 12, respectively (Fig. 1). The highest percentage reduction was observed with CCC (1500 mg L^{-1}) followed by Prohexadione-Ca (1400 mg L^{-1}) at WK12 compared to the other treatments. Application of CCC at WK8 and WK8 + 12 at low concentration (1500 mg L^{-1}) appreciably reduced the number of shoot clumps compared to the high concentration (3000 mg L^{-1}) in WK12. At all three stages, CCC application at a concentration of 3000 mg L^{-1} resulted in more than 50% reduction in the number of shoot clumps, whereas Prohexadione-Ca application (1400 mg L^{-1}) showed a reduction in the number shoot clumps only when applied at WK12. As expected, GA_3 at 25 and 50 mg L^{-1} enhanced the number of shoot clumps significantly compared to the control, but the offshoots produced in the GA_3 treatments appeared unhealthy, with

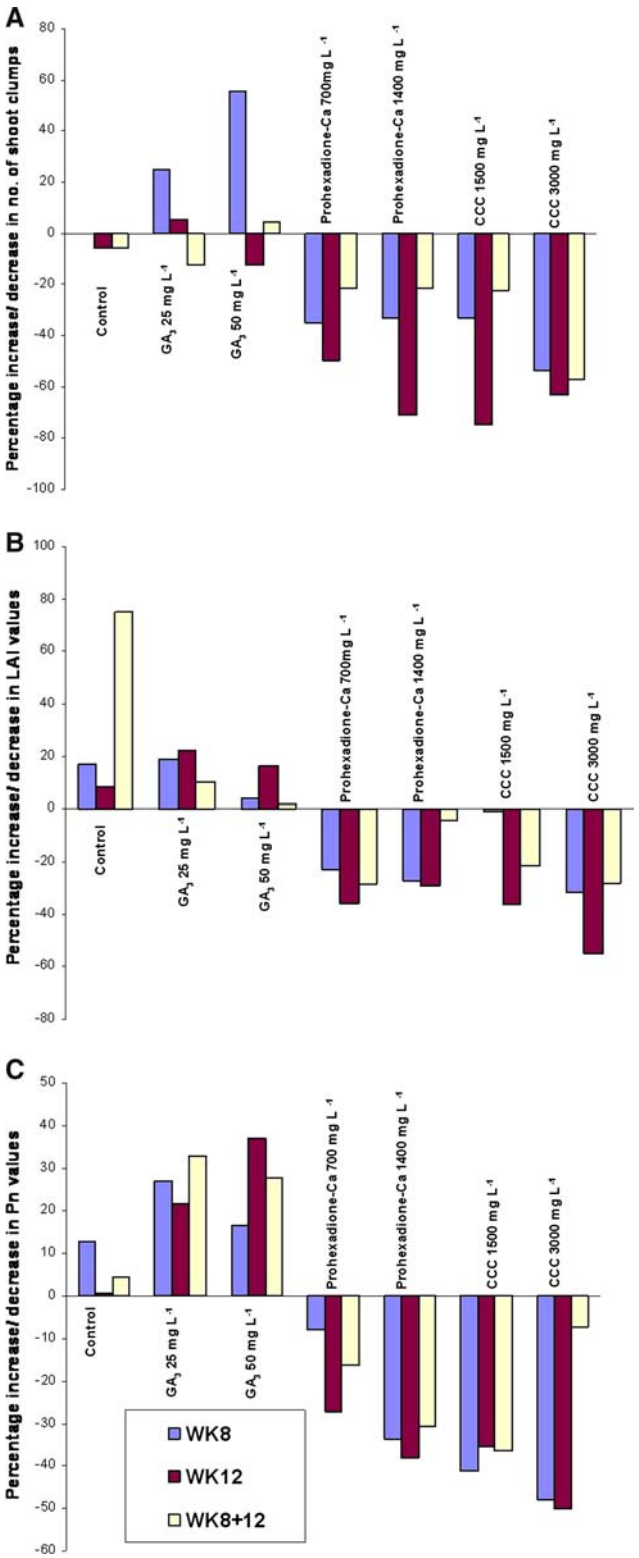

Fig. 1 The percentage increase/decrease in **a** the number of shoot clumps, **b** canopy volume as indicated by LAI values, and **c** net leaf photosynthesis (Pn) values between a week before and a week after applying CCC, Prohexadione-Ca, and GA_3 compared to the control plants at three stages of treatment application

extraordinarily long and slender petioles. This effect was most noticeable in plants sprayed at WK8. In the surfactant-only control plants, no significant difference was observed between the number of shoot clumps before and after spraying at all three stages of treatment application (Fig. 1).

A reduction in canopy volume (LAI values) was observed in plants treated with both of the plant growth retardants irrespective of stage of application (Fig. 1). The percentage reduction in canopy volume was the highest in plants sprayed with CCC 3000 mg L^{-1} at all three stages. The highest percentage reduction in canopy volume was observed in plants treated with 3000 mg L^{-1} CCC (55%) at 12 weeks after emergence compared to the other treatments. At all three stages of treatment application, LAI values increased in both the control and the plants that received GA_3 25 and 50 mg L^{-1} (Fig. 1).

Net leaf photosynthesis (Pn) was reduced 1 week after spraying with CCC and Prohexadione-Ca at all concentrations, irrespective of stage of application (Fig. 1). The maximum suppression of Pn occurred in plants treated with CCC 3000 mg L^{-1} (50%) sprayed at WK12. Like canopy volume, the Pn of the plants sprayed with GA_3 25 and 50 mg L^{-1} (22 and 37%, respectively, at WK12) increased 1 week after treatment application.

Between the two plant growth retardants tested, CCC was comparatively more effective in reducing shoot growth than Prohexadione-Ca, taking into consideration the percentage reduction in shoot growth parameters (number of shoot clumps, LAI, and net photosynthesis). The results indicated that applying CCC at 3000 mg L^{-1} produced the highest shoot growth suppression when applied at W8 and W12. Sprays of CCC and Prohexadione-Ca both at WK8 and WK12 did not produce any additional advantage in suppressing the shoot growth.

Effect of Plant Growth Retardants on Shoot Growth at Harvest

The plant growth retardant (CCC and Prohexadione-Ca) treatments had a significant effect in reducing the shoot height but only at the early stage of application (WK8) (Table 1). CCC 3000 mg L^{-1} produced the highest suppression in plant height when compared to the control when applied at WK8. However, the plant growth retardant treatments applied at any of the three stages did not considerably reduce the number of offshoots at the time of harvest (Table 1). GA_3 treatments appreciably favored shoot growth.

Effect of Plant Growth Retardants on Rhizome Growth

CCC and Prohexadione-Ca significantly enhanced rhizome growth (production) as indicated by increases in rhizome

Table 1 Effect of plant growth retardants on shoot height and number of offshoots at harvest

PGR treatments	WK8		WK12		WK8 + 12	
	Shoot height[a] (cm)	No. offshoots	Shoot height (cm)	No. offshoots	Shoot height (cm)	No. offshoots
Control	21.4a	2.8b	14.5bc	2.8b	16.5b	4.0b
GA_3 25 mg L^{-1}[b]	14.9bc	5.3ab	17.6b	4.0b	16.7b	2.7b
GA_3 50 mg L^{-1}	21.3a	4.8ab	11.9bc	1.3b	23.4a	7.0a
Prohexadione-Ca 700 mg L^{-1}	17.1b	1.8b	18.0ab	2.3b	16.2b	3.5b
Prohexadione-Ca 1400 mg L^{-1}	20.0ab	4.5ab	16.9b	1.0b	12.2bc	2.8b
CCC 1500 mg L^{-1}	16.0b	3.0b	13.1bc	2.0b	14.5bc	2.7b
CCC 3000 mg L^{-1}	10.2c	3.3b	13.7bc	2.0b	11.8bc	1.5b

[a] Mean values within each column followed by the same letter (online) are not significantly different by least square means (LSM) mean separation test at $p \leq 0.05$

[b] GA_3 served as a positive control

Table 2 Effect of plant growth retardants on rhizome width, fresh weight, and number of buds (potential number of viable propagules) at harvest

PGR treatments	WK8			WK12			WK8 + 12		
	Rhizome width (cm)[a]	Fresh weight (g)	No. buds	Rhizome width (cm)	Fresh weight (g)	No. buds	Rhizome width (cm)	Fresh weight (g)	No. buds
Control	5.5cde	67.8d	12.7e	6.4cde	112.9c	12.7e	6.7cd	71.2d	13.5e
GA_3 25 mg L^{-1}[b]	6.1cde	91.4d	10.5e	5.6cde	80.2d	15.7de	3.8def	41.5d	10.5e
GA_3 50 mg L^{-1}	8.9c	109.9cd	15.5de	5.8cde	84.6d	11.5e	5.6cde	102.6cd	18.3de
Prohexadione-Ca 700 mg L^{-1}	13.5b	197.7b	27.3bc	10.0bcd	185.3b	31.0bc	8.6c	153.6bc	24.3cd
Prohexadione-Ca 1400 mg L^{-1}	9.5c	204.8ab	24.0cd	9.6c	218.8ab	36.0b	12.2bc	179.4b	26.5c
CCC 1500 mg L^{-1}	11.9bc	157.4bc	24.0cd	11.8bc	242.6a	39.3b	11.0bcd	178.6b	22.5cd
CCC 3000 mg L^{-1}	11.5b	197.4b	30.3bc	16.6a	257.2a	48.7a	9.5c	196.3b	39.3b

[a] Mean values within each column followed by the same letter (online) are not significantly different by least square means (LSM) mean separation test at $p \leq 0.05$

[b] GA_3 served as a positive control

Fig. 2 Rhizome growth in the control (*left*) and CCC 3000 mg L^{-1} (*right*) in WK12 treatment at harvest (2 months after treatment application)

width, fresh weight, and number of buds (Table 2) more pronouncedly at WK12. Thus, plants sprayed with CCC 3000 mg L^{-1} at WK12 produced rhizomes with the greatest width (16.6 cm), highest fresh weight (275.0 g), and highest number of buds (49) (Table 2, Fig. 2). Among the treatments, CCC 3000 mg L^{-1} (Fig. 2, Table 2) was the most effective and the effect was more pronounced when sprayed at 12 weeks after shoot emergence. Also, repeating

Table 3 Effect of plant growth retardants on rhizome dry weight, rhizome:shoot ratio, and starch concentration in the rhizome at harvest

PGR treatments	WK8			WK12			WK8 + 12		
	Rhizome dry weight (g)	Rhizome: shoot ratio[a]	Starch conc.[c]	Rhizome dry weight (g)	Rhizome: shoot ratio[a]	Starch conc.[c]	Rhizome dry weight (g)	Rhizome: shoot ratio[a]	Starch conc.[c]
Control	13.1de	1.9e	7.2c	18.2d	1.6e	6.9c	24.45d	1.5e	7.2c
GA_3 25 mg L^{-1}[b]	22.8d	1.4e	4.1c	17.0d	2.8de	7.4c	16.0de	1.6e	9.4bc
GA_3 50 mg L^{-1}	28.9cd	1.5e	4.9c	21.5d	2.6de	5.8c	30.6bcd	2.1de	9.6bc
Prohexadione-Ca 700 mg L^{-1}	45.9ab	4.9d	10.9bc	46.1ab	12.1b	11.8bc	42.2b	8.1c	8.3bc
Prohexadione-Ca 1400 mg L^{-1}	45.8ab	7.6c	16.9a	35.5bc	14.2a	11.5bc	35.5bc	12.4b	13.8b
CCC 1500 mg L^{-1}	48.0ab	8.0c	19.7a	38.2bc	14.7a	10.8bc	46.7ab	12.9a	10.9bc
CCC 3000 mg L^{-1}	54.5ab	14.5a	22.5a	45.0ab	15.0a	11.8bc	28.0cd	14.0a	11.8bc

[a] Mean values within each column followed by the same letter (online) are not significantly different by least square means (LSM) mean separation test at $p \leq 0.05$

[b] GA_3 served as a positive control

[c] Starch concentration (mg/g fresh weight of the tissue)

application of either Prohexadione-Ca or CCC at both WK8 and WK12 gave no added advantage in accelerating rhizome growth (Table 2).

Effect of Plant Growth Retardants on Biomass Allocation Between Shoot and Rhizome

CCC and Prohexadione-Ca significantly favored dry matter allocation toward the rhizome and the effect was more evident when applied at WK12 (Table 3). The rhizome:shoot ratio was the highest in plants treated with CCC 3000 mg L^{-1} at all three stages of application (Table 3), CCC 1500 mg L^{-1} at WK12 and WK8 + 12 and Prohexadione-Ca 1400 mg L^{-1} at WK12. There was no significant difference in the rhizome:shoot ratio between the control and the plants treated with GA_3.

Effect of Plant Growth Retardants on the Total Sugars and Starch in Leaf Blade, Petiole, and Rhizome

There was a significant difference in total starch concentration of the rhizome between the control and the various treatments at the early stage of treatment application (WK8) (Table 3). However, the total soluble sugar concentration in the rhizome did not differ among the treatments when compared to the control at any stage of application (data not shown). Also, there was no significant difference in the total starch and soluble sugar concentrations in the shoot (leaf blade and petiole) between the treatments at any stage of application (data not shown).

Starch concentration in the fresh rhizome was significantly increased by the PGRs. The starch concentration in the rhizome was the highest in the plants treated with CCC 3000 mg L^{-1} and 1500 mg L^{-1} and Prohexadione-Ca 1400 mg L^{-1} when applied at WK8 (Table 3). The effect of PGR treatments in enhancing starch concentration in the rhizomes was pronouncedly evident (relative to controls) when they were applied at an early stage (WK8) of shoot growth.

Also, applications of Prohexadione-Ca and CCC significantly enhanced the dry matter allocation toward the rhizome. There was a substantial increase in fresh (Table 2) and dry weights (Table 3) of the rhizome and the number of potential buds in the plants treated with the two PGRs.

Accompanying this was a significant increase in rhizome starch concentration for plants treated with both growth retardants at WK8.

Discussion

Foliar application of Cycocel (CCC) and Apogee (Prohexadione-Ca) was effective in suppressing the number of shoot clumps and reducing other measures of shoot growth while promoting rhizome growth and lateral buds in rhubarb. However, CCC was more effective than Prohexadione-Ca and the effect was dependent on concentration (3000 mg $L^{-1}$was best) and the phenologic stage of the plant at which the CCC treatments were applied (WK12) (Tables 2 and 3). Even though there was a significant reduction in the number of shoot clumps, canopy volume, and net leaf photosynthesis (Fig. 1) in plants treated with CCC and Prohexadione-Ca, larger rhizomes with higher numbers of potential buds resulted from these treatments. Enhanced rhizome growth coincided with a significant increase in both the fresh weight and dry biomass as well as increased starch concentration in the rhizomes (Table 3). Thus, use of these plant growth retardants favored preferential allocation of photoassimilates to the rhizomes.

Suppressing top vegetative growth by chemical manipulation using PGRs has proven to be very effective in

enhancing growth of underground storage organs and propagules in many crop species (Sharma and others 1998; Hussain and others 2006; Leclerc and others 2006; Wang and others 2009). CCC and Prohexadione-Ca are potential GA biosynthesis inhibitors and their use has been shown to facilitate preferential allocation of photoassimilates to underground plant organs at the expense of shoot growth (Rademacher 1989, 2000; Wang and others 2009). CCC belongs to a group called onium compounds and blocks the cyclases copalyl diphosphate esterase and ent-kaurene synthase involved in the early steps of GA metabolism. Prohexadione-Ca (acylcyclohexadiones), a structural mimic of the 2-oxoglutaric acid, blocks both the 3β hydroxylation (activation of endogenous GAs) and the 2β hydroxylation (deactivation) steps toward the later stages in the GA biosynthesis pathway (Rademacher 1989, 2000). Because CCC blocks GA biosynthesis at an early stage in the pathway, the chances of GA being formed through an alternate pathway may have also been blocked. Also, because Prohexadione-Ca can block both the "activation" and "deactivation" steps, the relative strength at which these inhibitions operate upon Prohexadione-Ca treatment would decide the net amounts of growth-active endogenous GAs (GA_4 or GA_1 or both) in the plant. We found that CCC performed comparatively better than Prohexadione-Ca in this study. It appeared that in plants treated with Prohexadione-Ca, the absolute amounts of active GA forms may be appreciably higher (than CCC-treated plants) due to Prohexadione-Ca blocking both the "activation" and "deactivation" steps. These may be the possible reasons for CCC being more effective than Prohexadione-Ca in promoting preferential dry matter allocation toward the rhizome.

CCC and Prohexadione-Ca stimulated underground rhizome growth and enhanced the number of potential buds by suppressing shoot growth (Fig. 1, Tables 2 and 3) possibly through improved photosynthetic efficiency and photoassimilate allocation by manipulating source–sink relationships as indicated by previous studies in other crop species (Singh and others 1987; Sharma and others 1998; Wang and others 2009). Also, in the present study GA_3 favored shoot growth without stimulating rhizome development. Implied in this conclusion is the assumption that high endogenous GA levels maintain a higher level of Pn and promote the allocation of photoassimilate to the shoot. For example, applications of GA_3 have been shown to enhance shoot growth while also stimulating net photosynthesis (Kwan 1996; Hayat and others 2001; Yuan and Xu 2001). Other studies have shown that applied GA_3 can inhibit or delay the formation of underground plant organs like tubers by inhibiting starch accumulation (Golovkov and Tabalenkova 1989; Abdella and others 1995; Vandam and others 1996; Vreugdenhil and Sergeeva 1999). Also, GA_3 application was shown to promote carbon allocation preferentially to the shoots (Yim and others 1997). Thus, a reduction in GA profile *in planta* should result in more dry matter allocation toward roots than shoots.

Despite the significant reduction in net photosynthesis and shoot growth, rhizome growth continued to be enhanced in plants treated with CCC and Prohexadione-Ca. The shoot growth suppression due to PGR treatments was transient (leaf senescence and reduction in Pn observed only within a week after application) and the normal rate of photoassimilation appeared to be regained soon by actively emerging newer shoots. This compensatory photosynthesis and carbon assimilation by newer flushes combined with efficient translocation might have stimulated rhizome growth. Also, as previous studies suggest (Reekie and others 2005; Wang and other 2009), CCC and Prohexadione-Ca might have enhanced photosynthetic efficiency and carbon assimilation in the treated plants. Enhanced photosynthetic activity has been attributed to increased leaf thickness, photosynthetic pigment contents (chlorophyll a and b), and ribulose biphosphate carboxylase oxygenase (Rubisco) activity induced by CCC (Tezuka and others 1989; Wang and others 2009). However, the action of CCC and Prohexadione-Ca could vary according to the crop species and the effect of these PGRs on leaf photosynthetic capacity are largely dependent on the physiologic stage and age of the plants as well as the concentration applied (Rademacher 1989, 2000; Kirillova and others 2003). A time course measurement of photosynthesis of the treated and newly emerged foliage might have given a better understanding of the mode of action of these PGRs in promoting rhizome growth even though the top vegetative growth was compromised.

Significant enhancement in the dry matter allocation toward the rhizome, and therefore an increase in the rhizome:shoot ratio, was observed in response to CCC and Prohexadione-Ca in the treated plants. This is in accordance with the previous findings reported in the literature (Sharma and others 1998; Hicklenton and Reekie 2001; Leclerc and others 2006). For example, strawberry plants treated with Prohexadione-Ca consistently and preferentially allocated more dry weight toward roots than toward shoots, with a reduction in height, leaf area, and specific leaf area (SLA) even though there was an increase in photosynthetic efficiency (Reekie and others 2005). In a recent study using ^{14}C isotope labeling in potato, Wang and others (2009) demonstrated that CCC improved leaf photosynthetic capacity (the maximum photosynthetic rate per unit leaf biomass or per unit leaf area) and promoted preferential photoassimilate partitioning into the tubers. In our study, it appears that CCC and Prohexadione-Ca have suppressed the shoot growth and at the same time enhanced the photosynthetic efficiency of the leaf, promoting both carbon assimilation and allocation of more photoassimilates to the rhizome. The preferential

allocation of photosynthates toward the rhizome, at the expense of suppressed shoot growth, has thus enhanced the dry matter accumulation in the rhizomes.

The increased accumulation of starch in the rhizome (Table 3) upon PGR application was more apparent during the early stage (WK8) of treatment application and disappeared toward the later stage of growth (WK12). Even though there was a significant enhancement in the dry mass allocation toward the rhizome compared to the shoot, there was no significant difference in the concentration of the total soluble sugars in the rhizome tissues between the treatments. It is possible that a major portion of the allocated sugars might have been utilized as structural carbohydrates as seen from the increase in the rhizome fresh weight, number of active buds on the rhizomes, and increased dry matter content in the rhizomes on growth retardant-treated plants.

Even though CCC and Prohexadione-Ca were equally effective in suppressing shoot growth (as indicated by reduction in shoot clump numbers, LAI, and Pn) when applied at WK8 and WK12, the rhizome growth enhancement (indicated by rhizome growth parameters) was appreciably more apparent when they were applied toward the later stage (WK12) of vegetative growth (Fig. 1, Table 2), indicating that the stage of shoot growth is also critical in enhancing the effectiveness of these PGR treatments.

Conclusion

Between CCC and Prohexadione-Ca applied as a foliar spray, CCC (3000 mg L^{-1}) produced rhizomes with the largest diameters, greatest fresh and dry weights, and increased numbers of active lateral buds (potential new shoots) by suppressing shoot growth in rhubarb. Our results indicate that foliar application of the plant growth retardant Cycocel (CCC) promotes both rhizome growth and active bud numbers on the rhizomes in rhubarb when applied once as a foliar spray at an active stage of vegetative growth (12 weeks after shoot emergence), possibly via inhibition of the biosynthesis of endogenous GAs, thereby increasing photoassimilate allocation to the rhizomes.

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Appendix

See Tables 4–6.

Table 4 Number of shoot clumps 1 week before and after spraying plant growth retardants

PGR treatments	Treatment application stage								
	WK8			WK12			W8 + 12		
	No. shoot clumps (±SD)[a]		Percentage increase (+) or decrease (−)[c]	No. shoot clumps (±SD)		Percentage increase (+) or decrease (−)	No. shoot clumps (±SD)		Percentage increase (+) or decrease (−)
	Before spray (x)	After spray (y)	$[(y - x) \div x] \times 100$	Before spray (x)	After spray (y)	$[(y - x) \div x] \times 100$	Before spray (x)	After spray (y)	$[(y - x) \div x] \times 100$
Control	2.8 ± 1.5	2.8 ± 1.27	0	4.5 ± 2.99	4.3 ± 2.94	−5.5	4.3 ± 2.38	4.0 ± 2.63	−5.8
GA_3 25 mg L^{-1}[b]	4.0 ± 2.45	5.0 ± 3.11	+25	4.8 ± 0.82	5.0 ± 0.96	+5.4	2.0 ± 2.22	1.8 ± 1.83	−12.5
GA_3 50 mg L^{-1}	4.5 ± 1.29	7.0 ± 0.82	+55.6	2.7 ± 2.52	2.3 ± 2.50	−12.7	5.5 ± 1.53	5.8 ± 1.53	+4.5
Prohexadione-Ca 700 mg L^{-1}	4.3 ± 2.99	2.8 ± 2.22	−35.3	3.5 ± 1.91	1.8 ± 1.26	−50	3.5 ± 1.91	2.8 ± 1.71	−21.4
Prohexadione-Ca 1,400 mg L^{-1}	1.5 ± 0.58	1.0 ± 1.15	−33.3	2.3 ± 1.29	0.7 ± 0.96	−71.2	3.5 ± 1.15	2.8 ± 0.58	−21.4
CCC 1,500 mg L^{-1}	2.5 ± 1.00	2.0 ± 1.83	−33.3	2.7 ± 0.96	0.7 ± 0.96	−74.9	2.3 ± 1.53	1.8 ± 0.58	−22.2
CCC 3,000 mg L^{-1}	3.3 ± 2.06	1.5 ± 0.58	−53.8	4.8 ± 1.00	1.8 ± 1.00	−63.2	3.5 ± 2.22	1.5 ± 1.26	−57.1

[a] Data are the mean ± SD (standard deviation) of four replicates

[b] GA_3 served as a positive control

[c] The values indicate percentage increase or decrease after spraying when compared to before treatment application

Table 5 Canopy volume (LAI) 1 week before and after spraying plant growth retardants

PGR treatments	Treatment application stage								
	WK8			WK12			W8 + 12		
	Canopy volume (LAI)[a]		Percentage increase (+) or decrease (−)[c]	Canopy volume (LAI)[a]		Percentage increase (+) or decrease (−)	Canopy volume (LAI)[a]		Percentage increase (+) or decrease (−)
	Before spray (x)	After spray (y)	$[(y - x) \div x] \times 100$	Before spray (x)	After spray (y)	$[(y - x) \div x] \times 100$	Before spray (x)	After spray (y)	$[(y - x) \div x] \times 100$
Control	2.5 ± 0.24	2.9 ± 1.16	+17.1	2.8 ± 0.23	2.9 ± 0.70	+8.3	2.7 ± 0.62	4.0 ± 0.69	+75.2
GA_3 25 mg L^{-1}[b]	1.8 ± 0.34	2.1 ± 0.38	+19.1	2.3 ± 0.68	2.8 ± 0.46	+22.1	2.4 ± 0.16	2.7 ± 1.09	+10.4
GA_3 50 mg L^{-1}	2.8 ± 0.81	2.9 ± 0.65	+3.9	2.8 ± 1.24	3.3 ± 1.13	+16.6	2.4 ± 1.25	2.4 ± 0.40	+2.1
Prohexadione-Ca 700 mg L^{-1}	2.8 ± 1.12	2.1 ± 0.64	−22.8	2.3 ± 0.18	1.5 ± 0.97	−35.8	2.3 ± 0.09	1.7 ± 0.47	−28.7
Prohexadione-Ca 1400 mg L^{-1}	2.6 ± 0.88	1.9 ± 0.74	−27.1	2.8 ± 0.79	1.9 ± 0.68	−28.9	2.7 ± 0.21	2.5 ± 0.30	−4.2
CCC 1500 mg L^{-1}	2.1 ± 0.73	2.1 ± 0.25	−0.9	2.9 ± 0.78	1.9 ± 1.15	−36.1	2.6 ± 0.37	2.1 ± 0.15	−21.5
CCC 3000 mg L^{-1}	3.1 ± 0.85	2.1 ± 0.58	−31.4	2.7 ± 1.00	1.1 ± 0.88	−55.1	3.1 ± 0.49	2.2 ± 0.30	−28.3

[a] Data are the mean ± SD (standard deviation) of four replicates

[b] GA_3 served as a positive control

[c] The values indicate percentage increase or decrease after spraying when compared to before treatment application

Table 6 Net photosynthesis (Pn) 1 week before and after spraying plant growth retardants

PGR treatments	Treatment application stage								
	WK8			WK12			W8 + 12		
	Net photosynthesis[a]		Percentage increase (+) or decrease (−)[c]	Net photosynthesis[a]		Percentage increase (+) or decrease (−)	Net photosynthesis[a]		Percentage increase (+) or decrease (−)
	Before spray (x)	After spray (y)	$[(y - x) \div x] \times 100$	Before spray (x)	After spray (y)	$[(y - x) \div x] \times 100$	Before spray (x)	After spray (y)	$[(y - x) \div x] \times 100$
Control	8.7 ± 3.49	9.8 ± 1.69	+12.7	8.5 ± 2.88	8.6 ± 3.26	+0.6	7.4 ± 2.20	7.7 ± 0.75	+4.4
GA_3 25 mg L^{-1}[b]	7.1 ± 2.48	9.1 ± 1.50	+26.9	6.8 ± 3.37	8.2 ± 1.24	+21.5	7.9 ± 1.48	10.5 ± 1.99	+32.8
GA_3 50 mg L^{-1}	8.7 ± 3.22	10.2 ± 3.49	+16.5	7.2 ± 2.97	4.6 ± 3.07	+37.1	7.0 ± 2.53	8.9 ± 2.29	+27.8
Prohexadione-Ca 700 mg L^{-1}	7.9 ± 1.91	7.3 ± 2.13	−8.0	5.1 ± 1.49	3.7 ± 0.87	−27.2	7.6 ± 1.40	6.3 ± 3.58	−16.1
Prohexadione-Ca 1400 mg L^{-1}	9.4 ± 1.55	6.3 ± 1.52	−33.6	6.8 ± 2.53	4.2 ± 2.08	−38.1	7.9 ± 2.04	5.5 ± 2.22	−30.7
CCC 1500 mg L^{-1}	8.9 ± 3.06	5.1 ± 1.91	−41.2	9.9 ± 1.36	6.4 ± 3.62	−35.6	8.9 ± 1.58	5.7 ± 21.06	−36.2
CCC 3000 mg L^{-1}	6.4 ± 3.43	3.3 ± 2.82	−47.8	9.4 ± 4.69	4.8 ± 4.07	−49.9	6.4 ± 2.75	5.9 ± 1.42	−7.5

[a] Pn are the mean ± SD (standard deviation) of four replicates (μmol m^{-2} s^{-1})

[b] GA_3 served as a positive control

[c] The values indicate percentage increase or decrease after spraying when compared to before treatment application

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