DISSERTATION

POTATO MINITUBER BUD DORMANCY RELEASE

Submitted by

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY OKTAY KÜLEN ENTITLED POTATO MINITUBER BUD DORMANCY RELEASE BE ACCEPTED AS FULLFILING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

POTATO MINITUBER BUD DORMANCY RELEASE

Potato minitubers are small pathogen and disease-free seed tubers that are field planted to produce basic seed potatoes. Minitubers have a longer dormancy period than regular tubers. Predictable and consistent dormancy release of potato minitubers is necessary to ensure uniform sprouting and vigorous plant development. Circumstances often require that minitubers be planted soon after they have been harvested from greenhouse benches, creating insufficient storage to satisfy dormancy without additional treatment. There have been few non-hazardous, reliable methods to stimulate early release of potato minituber dormancy in a predictable manner, especially for long dormancy cultivars. Plant growth regulators are important in the regulation and termination of potato minituber bud dormancy. Gibberellins and cytokinins are known to release potato tuber bud dormancy while abscisic acid (ABA) is likely the principal dormancy-inducing plant hormone. This research was based on the primary hypothesis that bud dormancy in potato minitubers can be released by application of plant growth regulators, chemicals and exposure to a magnetic field. The objectives were to explore the effects of different temperature regimes, plant growth regulators, electromagnetic field and chemicals on minituber bud dormancy and to develop a more effective minituber dormancy release protocol.

Alpha-galactosidase is a key catabolic enzyme of the raffinose family oligosaccharides (RFO), involved in seed germination of many species. Its primary

function is to break the terminal-linked moiety from galactose containing oligosaccharides. Reducing sugars (fructose and glucose) are known to increase during low temperature storage, presumably as products of enzymatic hydrolysis of complex storage carbohydrates. Phenolic compounds are relatively stable secondary plant metabolites, thought by some to play a role in defense against predators and diseases, and in some species are reported to change as plants pass from a dormant to non-dormant state. We hypothesized that α-galactosidase, soluble sugars and total phenolics are related to potato minituber dormancy and dormancy release. The objectives were to explore the relationship of these metabolites to potato minituber bud dormancy and emergence from dormancy.

Abscisic acid (ABA), N^6-benzyladenine (BA), Florel (ethrel), ProGibb, kinetin, Pro-BA (ProGibb and BA); Pro-Florel (ProGibb and Florel), BA-Florel (BA and Florel) plant growth regulators, catechin, magnetic field, temperature regimes, minituber sizes, 2,2′-azobis- (2-amidinopropane) hydrochloride (AAPH), and 2,2'-azino-bis(3-ethylbenzothiazolamine-6-sulfonic acid) ($ABTS^+$) (free radical producers) were used as dormancy release agents in this research.

Pro-Florel, ProGibb, and Pro-BA treatments shortened potato minituber bud dormancy and increased the sprout number. Larger minitubers were less dormant than smaller minitubers. From the six different temperature regimes utilized, 15, 17, and 19°C accelerated dormancy break most effectively. The larger minitubers combined with the Pro-florel treatment were the most responsive to shortening potato minituber bud dormancy at 15°C by up to 2 weeks or more. Pro-Florel treatments also significantly accelerated sprout growth, increased plant growth, tuber number, and yield in 2002 and

2003. A combined Pro-florel treatment was most effective in shortening potato minituber bud dormancy and improving tuber yield.

Alpha-galactosidase enzyme activity increased significantly following application of a 4-hour electromagnetic field. Fructose and glucose contents were significantly increased by Pro-florel treatment in Russet Norkotah-Selection 3 (RNK-S3) and Silverton Russet minitubers stored for one week. In general, total phenolic contents were not significantly affected by treatments, cultivars and storage periods. These results suggested that alpha-galactosidase activity and total phenolics levels were not associated with dormancy release because the Pro-florel treatment did not alter their content more or less than any other treatments. However, fructose and glucose levels increased with application of Pro-florel in RNK-S3 minitubers stored one week compared to other treatments. High levels of fructose and glucose could be related to energy requirements during termination of minituber bud dormancy.

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TABLE OF CONTENTS

ABSTRACT iii

ACKNOWLEDGEMENTS vi

LIST OF FIGURES x

LIST OF TABLES xiii

CHAPTER 1: LITERATURE REVIEW 1

1.1 Origin and introduction of potato 1
1.2 Potato minitubers 2
1.3 Dormancy 2
1.4 Potato rest 3
1.5 Potato dormancy 4
1.5.1 Potato minituber dormancy 6
1.6 Potato dormancy release issues 7
1.6.1 Potato minituber dormancy release 8
1.7 Methods to release potato dormancy 9
1.7.1 Bromoethene 10
1.7.2 Carbon-disulphide (CS_2) 10
1.7.3 Cytokinins 11
1.7.4 Electrical current 12
1.7.5 Ethylene 13
1.7.6 Fluridone 16
1.7.7 Gibberellins 16
1.7.8 Rindite 19
1.7.9 Storage gases 20
1.7.10 Temperature 20
1.7.11 Water and humidity 21
1.7.12 Wounding and cutting 21
1.7.13 Mixture of dormancy release agents 21
1.7.14 Chemicals on dormancy release 22
1.8.1 Abscisic acid (ABA) 22
1.8.2 Auxins 23
1.8.3 Brasinolide 25
1.8.4 Chemical agents as sprout suppressants 25

1.9 Field performance of minitubers 25
1.10 Physiological age of potato tubers and minitubers 26
1.11 Importance of this research..... 27

CHAPTER 2: POTATO MINITUBER BUD DORMANCY RELEASE WITH CHEMICALS AND PLANT GROWTH HORMONES 29

2.1 Abstract 29
2.2 Introduction 30
2.3 Materials and Methods 33
2.3.1 Temperature experiment 33
2.3.2 Florel (2-chloroethylphosphonic acid, ethephon, ethrel) experiment .34
2.3.3 Plant hormone experiment 34
2.3.4 N^6-Benzyladenine (BA) and Kinetin experiment 35
2.3.5 Catechin experiment 35
2.3.6 Magnetic field experiment 35
2.3.7 Free radical experiment 36
2.3.8 Tuber size experiment 37
2.4 Results 37
2.4.1 Temperature experiment 37
2.4.2 Florel (2-chloroethylphosphonic acid, ethephon, ethrel) experiment .38
2.4.3 Plant hormone experiment 38
2.4.4 N^6-Benzyladenine (BA) and Kinetin experiment 39
2.4.5 Catechin experiment 39
2.4.6 Magnetic field experiment 40
2.4.7 Free radical experiment 40
2.4.8 Tuber size experiment 40
2.5 Discussion 41

CHAPTER 3: THE EFFECTS OF ABA, FLOREL, AND PROGIBB PLANT HORMONES ON POTATO MINITUBER BUD DORMANCY AND PLANT GROWTH UNDER FIELD CONDITIONS AT SAN LUIS VALLEY, COLORADO 60

3.1 Abstract 60
3.2 Introduction 61
3.3 Materials and Methods 62
3.3.1 Plant materials and treatments 62
3.4 Results 64
3.4.1 Dormancy Release 64
3.4.2 Sprout Number 64

3.4.3 Sprout Growth 65
3.4.4 Emergence Number 65
3.4.5 Plant Height 65
3.4.6 Yield 66
3.4.7 Number of tubers 66
3.5 Discussion 66
3.6 Summary 70

CHAPTER 4: ALPHA-GALACTOSIDASE, SOLUBLE SUGAR CONTENT, AND TOTAL PHENOLICS RELATIVE TO POTATO MINITUBER BUD DORMANCY 94

4.1 Abstract 94
4.2 Introduction 95
4.3 Materials and Methods 99
4.3.1 Plant materials and treatments for alpha-galactosidase studies 99
4.3.1.1 Preparation of PNPG assay 100
4.3.1.2 Alpha-galactosidase assay 100
4.3.2 Plant materials and treatments for soluble sugar studies 101
4.3.2.1 Soluble carbohydrate extraction and analysis 101
4.3.3 Plant materials and treatments for total phenolics studies 103
4.3.3.1 Extraction and determination of total phenolics 103
4.4 Results 104
4.3.1 Alpha-galactosidase activity 104
4.3.1 Soluble sugar studies 105
4.3.1 Total phenolics studies 105
4.5 Discussion 105

REFERENCES 118

LIST OF FIGURES

Figures

2.1 Dormancy release of long dormancy cultivars (RNK-S3, RNK-S8) treated with 24-hour water aeration 47
2.2 Dormancy release of long dormancy cultivars (RNK-S3, RNK-S8) treated with 1.8 10^{-3} M ProGibb 47
2.3 Dormancy release of long dormancy cultivars (RNK-S3, RNK-S8) treated with 50 ppm BA 48
2.4 Sprout number of long dormancy cultivars (RNK-S3, RNK-S8) treated with 24-hour water aeration 48
2.5 Dormancy release of intermediate dormancy cultivars (Atlantic, Centennial Russet, DT-6063-11r, CO-86218-2, Chieftain, Sangre-S14) treated with 250, 500 and 1000 ppm Florel 49
2.6 Dormancy release of long dormancy cultivars (Chipeta, RNK-S3, RNK-S8, Snowden) treated with 250, 500 and 1000 ppm Florel 49
2.7 Sprout number of intermediate dormancy cultivars (Atlantic, Centennial Russet, DT-6063-11r, CO-86218-2, Chieftain, Sangre-S14) treated with 250, 500 and 1000 ppm Florel 50
2.8 Sprout number of long dormancy cultivars (Chipeta, RNK-S3, RNK-S8, Snowden) treated with 250, 500 and 1000 ppm Florel 50
2.9 Dormancy release of RNK-S3 treated with plant hormones 51
2.10 Sprout number of RNK-S3 treated with plant hormones 51
2.11 RNK-S3 emergence number in 2000 52
2.12 RNK-S3 plant height in 2000 52
2.13 Sprout length (cm) of the longest sprout/minituber, 5 weeks after treatment in 2002 and 2003 for the three dormancy groups 53
2.14 Dormancy release of short dormancy cultivar (Silverton Russet) treated with magnetic field 53
2.15 Short dormancy cultivar (Silverton Russet) sprout number treated with magnetic field in 2003 54
2.16 Dormancy release of long dormancy cultivars (RNK-S3, Nooksack) treated with AAPH and ABTS 54
2.17 Dormancy release of short dormancy cultivar (Silverton Russet) 55
2.18 Dormancy release of intermediate dormancy cultivars (RNK, Sangre-S14, Desiree) 55
2.19 Short dormancy cultivar (Silverton Russet) sprout number in 2003 56

2.20 Intermediate dormancy cultivars (RNK, Sangre-S14, Desiree) sprout number in 2003 56
2.21 Long dormancy cultivars (RNK-S3, Nooksack) emergence numbers in 2002 57
2.22 Short dormancy cultivar (Silverton Russet) plant height in 2003 57
2.23 Intermediate dormancy cultivars (RNK, Sangre-S14, Desiree) plant height in 2003 58
2.24 Long dormancy cultivars (RNK-S3, Nooksack) plant height in 2003 58
2.25 2003 San Luis Valley yield based upon production from 5 minitubers/plot (kg/5 minitubers) 59
3.1 Dormancy release of short dormancy cultivar (Silverton Russet) for 2002 71
3.2 Dormancy release of intermediate dormancy cultivars (RNK, Sangre-S14, Desiree) for 2002 71
3.3 Dormancy release of long dormancy cultivars (RNK-S3, Nooksack) for 2002 72
3.4 Dormancy release of short dormancy cultivar (Silverton Russet) for 2003 72
3.5 Dormancy release of intermediate dormancy cultivars (RNK, Sangre-S14, Desiree) for 2003 73
3.6 Dormancy release of long dormancy cultivars (RNK-S3, Nooksack) for 2003 73
3.7 Short dormancy cultivar (Silverton Russet) sprout number in 2002 74
3.8 Intermediate dormancy cultivars (Russet Norkotah, Sangre-S14, Desiree) sprout number in 2002 74
3.9 Long dormancy cultivars (RNK-S3, Nooksack) sprout number in 2002 75
3.10 Short dormancy cultivar (Silverton Russet) sprout number in 2003 75
3.11 Intermediate dormancy cultivars (RNK, Sangre-S14, Desiree) sprout number in 2003 76
3.12 Long dormancy cultivars (RNK-S3, Nooksack) sprout number in 2003 76
3.13 Sprout length (cm) of the longest sprout/minituber, 5 weeks after treatment in 2002 and 2003 for the three dormancy groups 77
3.14 Short dormancy cultivar (Silverton Russet) emergence numbers in 2002 78
3.15 Intermediate dormant cultivars (RNK, Sangre-S14, Desiree) emergence numbers in 2002 78
3.16 Long dormancy cultivars (RNK-S3, Nooksack) emergence numbers in 2002 79
3.17 Short dormancy cultivar (Silverton Russet) emergence numbers in 2003 79
3.18 Intermediate dormant cultivars (RNK, Sangre-S14, Desiree) emergence numbers in 2003 80
3.19 Long dormancy cultivars (RNK-S3, Nooksack) emergence numbers in 2003 80
3.20 Short dormancy cultivar (Silverton Russet) plant height in 2002 81
3.21 Intermediate dormancy cultivars (RNK, Sangre-S14, Desiree) plant height in 2002 81
3.22 Long dormancy cultivars (RNK-S3, Nooksack) plant height in 2002 82
3.23 Short dormancy cultivar (Silverton Russet) plant height in 2003 82

3.24 Intermediate dormancy cultivars (RNK, Sangre-S14, Desiree) plant height in 2003 83
3.25 Long dormancy cultivars (RNK-S3, Nooksack) plant height in 2003 83
3.26 2002 and 2003 San Luis Valley yield based upon production from 5 minitubers/plot (kg/5 minitubers) 84
3.27 Total number of tubers from the 2002 and 2003 San Luis Valley harvest of treated minitubers 85
4.1 Alpha-galactosidase activity in RNK-S3 minituber cultivar for 8 treatments after 0-week storage at 15°C 108
4.2 Alpha-galactosidase activity in RNK-S3 minituber cultivar for 8 treatments after 4-week storage at 15°C 108
4.3 Alpha-galactosidase activity in Silverton Russet minituber cultivar for 8 treatments after 0-week storage at 15°C 109
4.4 Alpha-galactosidase activity in Silverton Russet minituber cultivar for 8 treatments after 0-week storage at 15°C 109
4.5 Alpha-Galactosidase activity in Silverton Russet and RNK-S3 minituber cultivars, on the 5th day after treatments, stored 0-week and 4-week at 15°C 110
4.6 Fructose levels (μM/gdw) in Silverton Russet and RNK-S3 minituber cultivars from 5 treatments, stored 1 and 4 weeks at 15°C 110
4.7 Glucose levels (μM/gdw) in Silverton Russet and RNK-S3 minituber cultivars from 5 treatments, stored 1 and 4 weeks at 15°C 111
4.8 Sucrose level (μM/gdw) in Silverton Russet and RNK-S3 minituber cultivars from 5 treatments, stored 1 and 4 weeks at 15°C 111
4.9 Total phenolics level (mg/gdw) in short dormancy cultivar (Silverton Russet) from 4 treatments 112
4.10 Total phenolics level (mg/gdw) in intermediate dormancy cultivar (Desiree, RNK, Sangree S-14) from 4 treatments 112
4.11 Total phenolics level (mg/gdw) in long dormancy cultivar (RNK-S3) from 4 treatments 113

LIST OF TABLES

Tables

3.1 Dormancy release of short (Silverton Russet), Intermediate (RNK, Sangre-S14, Desiree) and long (RNK-S3, Nooksack) dormancy group cultivars in 2002 86
3.2 Dormancy release of short (Silverton Russet), Intermediate (RNK, Sangre-S14, Desiree) and long (RNK-S3, Nooksack) dormancy group cultivars in 2003 87
3.3 Sprout number of short (Silverton Russet), Intermediate (RNK, Sangre-S14, Desiree) and long (RNK-S3, Nooksack) dormancy group cultivars in 2002 88
3.4 Sprout number of short (Silverton Russet), Intermediate (RNK, Sangre-S14, Desiree) and long (RNK-S3, Nooksack) dormancy group cultivars in 2003 89
3.5 2002 yield based upon production from 5 minitubers/plot (kg/5minitubers) 90
3.6 2003 yield based upon production from 5 minitubers/plot (kg/5minitubers) 90
3.7 Number of small (<50 g wt), intermediate (50-100 g wt) and large (>100 g wt) tubers harvested from 5 plants/replicate in 2002 91
3.8 Number of small (<50 g wt), intermediate (50-100 g wt) and large (>100 g wt) tubers harvested from 5 plants/replicate in 2003 92
3.9 Table 3.9 Potato minituber dormancy grouping table 93
4.1 Alpha-Galactosidase activity in RNK-3 for 8 treatments after 0-week and 4-week storage at 15°C 114
4.2 Alpha-Galactosidase activity in Silverton for 8 treatments after 0-week and 4-week storage at 15°C 114
4.3 Alpha-Galactosidase activity in RNK-3 & Silverton on the 5th day after treatment, stored 0-week and 4-week at 15 °C 115
4.4 Fructose levels (μM/gdw) in Silverton Russet and RNK-S3 minitubers cultivars from 5 treatments, stored 1 and 4 weeks at 15 °C 115
4.5 Glucose levels (μM/gdw) in Silverton Russet and RNK-S3 minitubers cultivars from 5 treatments, stored 1 and 4 weeks at 15 °C 116
4.6 Sucrose levels (μM/gdw) in Silverton Russet and RNK-S3 minitubers cultivars from 5 treatments, stored 1 and 4 weeks at 15 °C 116
4.7 Total phenolics levels (mg/gdw) in dormant and non-dormant minituber cultivars of short dormancy cultivar (Silverton Russet), intermediate dormancy cultivars (Desiree, Sangree S-14, RNK), and long dormancy cultivar (RNK-S3) from 4 treatments 117

Chapter 1

Literature Review

1.1 Origin and introduction of the potato

The potato (*Solanum tuberosum* L.) originated as a cultivated species at high altitude in the Andes in South America. It was first brought to Spain in 1570 by Spaniards and to England around 1590. It is assumed that it was then introduced to the rest of the world from Europe (Burton, 1989; Hawkes, 1992).

Potatoes are grown in more than 130 countries and are one of the main world food crops. In several countries potatoes are grown more than any other food crop except rice. Commercial potato belongs to species, *S. tuberosum*, one of more than 2000 species classified under the genus *Solanum* that belong to *Solanaceae* family. Potatoes are vegetatively produced from tubers, thickened and shortened underground stems. They are mainly produced for human consumption, animal feed, industrial uses and seed tuber production (Hawkes, 1992; Struik and Wiersema, 1999).

While potato is a cool-season temperate climate crop, its adaptation to many field conditions is very well established. The hard work of breeders and agronomists has expanded potato production all around the world particularly in cool climatic regions from sea level to 4000 m in altitude. Potatoes can also be grown at high elevation sites from the equator to about 40° north and south (Hawkes, 1992).

1.2 Potato minitubers

Potato plantlets are propagated *in vitro* from internodal stem or leaf cuttings and planted at high density to produce huge quantities of minitubers in a short time period under greenhouse conditions. Minitubers tend to be small pathogen and disease-free seed tubers that are field planted to produce basic seed potatoes (Lommen, 1993a; 1993b; Bandara and Tanino, 1995). Minitubers can be produced year around at intervals of 3 weeks (4, 7, and 10 weeks) from the same plantlets (Lommen and Struik, 1992). Minituber use for basic seed production can be efficient and may decrease the number of field multiplications and *in vitro* conditions help minimize minituber losses during production (Struik and Lommen, 1990).

1.3 Dormancy

Dormancy researchers have faced fundamental terminology problems (Hemberg, 1985). Rappaport and Wolf (1969) concluded that "...there is not universal agreement on terminology that is understandable, because it is difficult to characterize either induction or termination of arrested growth in unexpected stems by a distinct morphological change, such as occurs during conversion from a vegetative to a reproductive form in plants". There have been numerous reports in the literature on dormancy in general and in potatoes particularly (Emilson, 1949; Rappaport and Wolf, 1969; Hemberg, 1985; Lang et al., 1987; Wiltshire and Cobb, 1996).

Taylorson and Hendricks (1977) defined dormancy as an arrest in development of a seed embryo, buds, or spores under conditions otherwise suited for growth. Lang et al., (1987) defined dormancy as a "temporary suspension of visible growth of any plant

structure containing a meristem". According to Lang et al., (1987), dormancy consists of three subgroups: endodormancy, paradormancy and ecodormancy. Endodormancy is the period in which growth is inhibited by internal physiological features, such as rest period in potato. Paradormancy is the period in which growth is inhibited by external physiological features within the plant, such as apical dominance. Ecodormancy is the period in which growth is inhibited by external environmental features, such as temperature extremes and light exposure of sprouting tubers.

1.4 Potato rest

Rest in potato has been defined as the period during which the buds cannot sprout as a result of ontonogous causes (Stuart and Milstead, 1934; Emilson, 1949; Lindblom, 1970). However, Nooden and Weber (1978) called this period as dormancy.

Potato buds are usually in rest immediately after harvest and even during the last weeks before harvest. In general, there are no basic differences between rest of potato buds and buds of other plants (Hemberg, 1985).

Davidson (1958) concluded that potato tubers have no rest duration because growth and development is a continuous process after harvest. He also pointed out that visible sprout appearance is not necessarily the first stage of dormancy break. Rest period has also been called as deep, innate or spontaneous dormancy by many physiologists to eliminate definition problems (Hemberg, 1985).

1.5 Potato dormancy

There has not been complete agreement on the definition of dormancy in the literature with respect to potato tubers (Wiltshire and Cobb, 1996). The dormant period is described as the time after harvest when buds are not growing for any reason (Emilson, 1949). Wright and Whiteman (1949) defined the dormant period as "the total period in which potatoes remain without sprouting within a given storage condition irrespective of their rest period". The dormant period is also defined as "the time when the tuber does not sprout because of unfavorable external conditions" (Lindblom, 1970). If tubers are unable to sprout at favorable temperatures, they are considered as dormant (Coleman, 1987).

Dormancy is defined as "the absence of consistent bud growth owing to certain chemical and physical conditions within the tuber, influenced by a host of factors including the environment" (Burton, 1963). During the European Association for Potato Research annual meeting in 1985, dormancy was also defined as "the physiological state of the tuber in which autonomous sprout growth will not occur, even when it is placed under ideal natural conditions for sprouting (darkness, temperature range 15-20 °C, relative humidity about 90%)" (Reust, 1986). The most acceptable dormancy term is "the entire period during which the buds are unable to sprout as a result of either exogenous or endogenous factors" (Emilson, 1949).

Lang et al., (1987) defined potato tuber dormancy as endodormancy since tuber dormancy occurs due to interior factors. At harvest, potato tubers do not sprout and are endodormant. The period of endodormancy depends on both cultivars and the environmental conditions during tuber development (Burton et al., 1992).

Potato tuber dormancy can include the entire developmental stage starting with tuber formation and ending with tuber sprouting (Wiltshire and Cobb, 1996). This period of potato tuber dormancy can be thus divided into three stages, induction, preservation, and release (Rojas-Beltran et al., 2000). The dormancy period of potato tubers lasts approximately two months or more after harvest (Cho et al., 1983). The duration of the dormancy period is genetically determined and is also affected by environmental conditions prevailing during tuber development. The dormancy period depends on cultivar, maturity of the tuber and soil, and weather conditions during growth, and even in the period just before harvesting if the foliage is still intact (Burton et al, 1992). Extreme weather conditions affect the length of the dormancy period of the tubers (Krijthe, 1962; Burton, 1963). Extremely cold, wet weather has been reported to increase the dormancy period by about 4 weeks. Extremely dry, warm weather reduced it by up to 9 weeks. Under more normal conditions, however, this effect is relatively slight (Burton, 1963).

Potato tubers are metabolically active during dormancy, and the main metabolic activity during potato dormancy is respiration. Although respiration is necessary for cell preservation, it has the negative effect of lowering carbon status and promoting tuber weight loss. Respiration decreases tuber water content and leads to water loss from the lenticels; this metabolic activity decreases dry matter content of tubers (Wiltshire and Cobb, 1996).

Lindblom (1970) suggested that theoretically, there is no need to distinguish rest and dormancy periods in potato tubers even though these terms are practical to use.

Davidson (1958) claimed that potato tubers have no rest period because bud growth is a continuous process after harvest.

There has been disagreement concerning duration of potato tuber dormancy among potato scientists. Currently three suggestions on the time period governing potato dormancy prevail in the literature: 1) planting to sprouting, 2) tuber initiation to sprouting and 3) harvest to sprouting (Cho et al., 1983). In this research, dormancy duration will be defined as from harvest to sprouting for greenhouse grown minitubers.

1.5.1 Potato minituber dormancy

Minitubers are small seed tubers that generally have a longer dormancy period than regular tubers of the same cultivar (Lommen, 1993a; 1993b: 1994). Lommen (1993a) also reported that smaller minitubers have a longer dormancy period than larger minitubers. Struik and Lommen (1990) concluded that the dormancy period of minitubers gradually increased as the weight of minitubers became smaller. On the other hand, Tabori et al., (1999) did not find any significant difference in length of dormancy between different microtuber sizes. They reported that larger microtubers had more sprouts and less loss due to storage deterioration.

The longer dormancy period and smaller size are advantageous for long-term storage of minitubers, however, long-term storage decreases growth vigor of minitubers (Struik and Lommen, 1990). Burton (1973) found that smaller seed tubers lost more weight during the first hours after harvest than regular sized tubers. Small potato seed tubers also lose more weight than regular tubers in storage due to higher surface area to volume ratio. If field planting is only possible once a year, minitubers should be kept in

storage until dormancy release since minitubers have a long dormancy period after harvest (Lommen, 1993a; 1993b).

1.6 Potato dormancy release issues

Postharvest potato disease testing, potato seed multiplication programs, and early potato seed export markets require the use of artificial external dormancy release agents (Coleman and Murphy, 1990). The use of external large-scale dormancy breaking agents (such as, rindite (7 parts ethylene chlorohydrin: 3 parts dichloroethane: 1 part carbon tetrachloride) and carbon disulfide (CS_2)) has been not commercially accepted due to environmental and health safety concerns (Coleman et al., 1992; Kim et al., 1999).

In general, it has been suggested that potato tuber bud dormancy is regulated by a balance of dormancy promoting and inhibiting substances (Hemberg, 1985; Coleman, 1987). Plant hormones have been assigned the most important role in induction, maintenance, release and regulation of potato tuber bud dormancy as with many characteristics of plant dormancy (Rappaport and Wolf, 1969; Hemberg, 1985; Korableva et al., 1989; Cvikrova et al., 1994; Burton et al., 1992; Suttle, 1998). It has been also proposed that potato dormancy is regulated by balance of ABA and GA_3 plant hormones (Hemberg, 1985; Turnbull and Hanke, 1985a; 1985b; Cocucci et al., 1994; Bhargava, 1997). Gibberellins and cytokinins have been implicated in potato tuber bud dormancy release (Smith and Rappaport, 1961; Turnbull and Hanke, 1985a; 1985b; Cocucci et al., 1994), while ABA is implicated to be the principal dormancy-inducing plant hormone (Hemberg, 1985). According to Obhlidalova et al., (1973) as cited in Claassens and Vreugdenhil (2000), both gibberellin and cytokinin levels increased during potato

dormancy release. ABA was proposed as the main part of the inhibitor β-complex (total amounts of acid growth-inhibiting substances) (Hemberg, 1958; 1985). Auxins activate sprout growth after potato tuber dormancy release. In addition to plant hormones, Alam et al., (1994) suggested that dormancy break was also associated with the regulation of protein synthesis.

Appearance of a readily visible sprout (≥2 or 3 mm) has been accepted as a criterion for potato dormancy release. In this research, visible ≥2 mm sprout length was used to identify breaking of dormancy. However, Davidson (1958) suggested that visible sprout could not be accepted as an indication of dormancy break. According to Krijthe (1962), potato tubers lost dormancy when 90% of the tubers had sprouts at least 3 mm long.

Many biochemical changes can be considered indicative of dormancy release such as respiration rate, concentration of plant growth regulators, beginning of nucleic acid synthesis, and cell enlargement (Rlyski et al., 1974), but none are considered definitive.

1.6.1 Potato minituber dormancy release

Potato minituber bud dormancy release is essential before field planting to ensure uniform sprouting and field establishment of vigorous potato plants. While prolonged storage (6-24 weeks) at cool temperatures (5 to 15°C) will break dormancy in most cultivars, there have been few reliable methods or ways to stimulate early release of potato minituber dormancy in a predictable manner, especially for long-dormancy cultivars. Although some chemicals effectively release potato minituber bud dormancy, their use is not widely accepted due to the toxic effects of these chemicals on plants, the

environment and consumers. This is a major problem for the North American and European minituber and seed potato export industry (Coleman et al., 1992). Potato dormancy release methods have been extensively studied since the 1880s (Appleman, 1916), however an effective and environmentally safe potato dormancy release technique is currently not available for the potato industry (Coleman et al., 1992).

Dormancy release of seed minitubers and larger tubers is important for rapid post-harvest disease testing procedures and incorporation into seed multiplication programs (Coleman, 1983). A consistent dormancy release method could allow immediate production from minitubers in the field to save time (Coleman and Coleman, 1986). If it becomes necessary to break dormancy by prolonging storage due to lack of dormancy release agents, many minitubers may become worthless because they loose vigor. Also, with existing methods minituber germination is erratic and unpredictable. Sometimes under field conditions emergence does not occur for months after planting. Each minituber can cost $.50 or more, thus their use becomes very costly when emergence is poor. Dormancy release method(s) can economically provide an opportunity to enhance effective use of minituber seeds for field planting and improve yields (Burton et al., 1992; Struik and Lommen, 1990).

1.7 Methods to release potato dormancy

Several methods and materials have been studied to shorten potato tuber bud dormancy. An ideal dormancy release agent should be environmentally safe, effective, easy to apply, and inexpensive (Coleman, 1983).

1.7.1 Bromoethane (CH_3CH_2Br)

Bromoethane is a highly volatile, clear, and colorless liquid that has been used as an inhalation anesthetic, a mite fumigant and a refrigerant (Coleman et al., 1992). Bromoethane has been studied as a dormancy release agent for a long time. Denny (1926) reported that bromoethane application was effective to break potato tuber dormancy. Bromoethane application significantly shortened potato tuber dormancy, accelerated sprout growth, increased the number of sprouts per tuber, and enhanced yield (Akoumianakis et al., 2000). Application of 100, and 200 ppm for 24-hour at room temperature significantly hastened potato dormancy release and increased the sprout number per tuber (Coleman, 1983). Coleman et al., (1992) suggested that 1 or 2 days application of bromoethane could be used as a commercial dormancy release agent for large-scale release of potato tuber dormancy.

Although bromoethane is an effective dormancy release agent, the usage of bromoethane has not been commercially accepted due to environmental and health safety concerns (Coleman et al., 1992).

1.7.2 Carbon-disulphide (CS_2)

CS_2 is a gaseous, poisonous, very flammable and explosive chemical (Meijers, 1972). Denny (1925, 1926) and Meijers (1972) found that application of CS_2 hastened release of potato tuber dormancy. Although it has been used as a potato tuber dormancy release agent in India, Ceylon and Brazil, it is also commercially unacceptable due to environmental and health issues (Burton et al., 1992).

1.7.3 Cytokinins

Cytokinins, plant growth regulators, induce cell division, morphogenesis (shoot initiation/bud formation) in tissue culture, and the growth of lateral buds, release of apical dominance, and leaf expansion resulting from cell enlargement, as well as stomatal opening in some species (Arteca, 1996).

Cytokinins stimulate tuber initiation of *in vitro* cultured stolons (Palmer and Smith, 1969a). Melis and Van Staden (1984) reported that cytokinins induced potato tuberization. Application of zeatin riboside also induced *in vitro* potato tuberization (Mauk and Langille, 1978). On the other hand, Kumar and Wareing (1974) demonstrated that high concentrations of kinetin inhibited tuber initiation. Woolley and Wareing (1972) found that application of kinetin to a stolon tip resulted in the formation of a leafy shoot. McGrady et al., (1986) also found that cytokinin treatment delayed tuberization. Vreugdenhil and Struik (1989) suggested that cytokinin effects depend on the other hormone levels in stolons. If gibberellin levels were high in stolons, cytokinins induced leafy shoots and if ethylene levels were high and gibberellin levels were low, cytokinins stimulated tuberization (Vreugdenhil and Struik, 1989). Turnbull and Hanke (1985) found that cytokinin level was very high at the beginning of tuberization then declined with tuber development. Hartmans and Van Es (1979) reported that kinetin treatment stimulated potato root growth.

Cytokinins (zeatin, kinetin and benzyladenine) have been reported to shorten potato tuber bud dormancy and decrease the inhibitor β-complex (total amounts of acid growth-inhibiting substances) (Hemberg, 1970; Tsukamoto and Yazawa, 1972; Turnbull and Hanke, 1985a). Application of exogenous cytokinin is effective in dormancy release

only during short periods, after the beginning and before the end of potato dormancy (Turnbull and Hanke, 1985a; Coleman, 1987). Cytokinins did not shorten the potato dormancy period in older dormant tubers that have a low concentration of endogenous cytokinin levels (Turnbull and Hanke, 1985a).

Cytokinins may play an important role in control of potato tuber dormancy (Turnbull and Hanke, 1985b). Cytokinin levels during potato dormancy are relatively low and increase significantly during dormancy break because of high synthesis of zeatin riboside and isopentenyladenosine (Turnbull and Hanke, 1985b; Sukhova et al., 1993). Cytokinin levels increased during the end of the potato tuber dormancy period and their transport from storage tissue to active meristems had been envisaged (Cvikrova et al., 1994). According to Obhlidalova et al., (1973) as cited in Claassens and Vreugdenhil (2000), the level of cytokinins increased during the end of dormancy and decreased again as sprout growth ensued. Suttle (1998) also reported that bioactive cytokinin level in potato buds increased during dormancy release.

Florel (ethrel, ethephon), ethylene releaser, application led to a slight decline in cytokinin levels during potato dormancy and postponed the increase of cytokinin levels about 1 month after dormancy release (Sukhova et al., 1993).

1.7.4 Electrical current

Direct electrical current (DC) and alternating electrical current (AC) have been found to be successful in breaking potato tuber dormancy (Kocasaliskan et al., 1989; Kocasaliskan, 1990) due to damaging potato tuber cell walls (Kocasaliskan et al., 1989; Kocasaliskan, 1990; Wiltshire and Cobb, 1996).

1.7.5 Ethylene (C_2H_4)

Ethylene, a small gaseous phytohormone, has been recognized as a regulator of many physiological responses in plants such as release and regulation of dormancy, regulation of stem elongation, leaf epinasty, flower induction, ripening, senescence and abscission (Abeles, 1972; Arteca, 1996). Ethylene is naturally produced inside the potato tuber and plant (Suttle, 1998). Ethylene production in plants can be induced by both abiotic and biotic stresses (Abeles, 1972; Arteca, 1996; Claassens and Vreugdenhil 2000).

Ethylene plays an active role in potato tuber initiation. Mingo-Castel et al., (1974, 1976) reported that ethylene inhibited tuber initiation of *in vitro* etiolated potato sprouts. Ethylene treatment also inhibited root formation, stolon elongation, and caused thickened stolons (Mingo-Castel et al., 1976; Timm et al., 1986). On the other hand, Garcia-Torres and Gomes-Campo (1973) concluded that ethylene could hasten tuber initiation of *in vitro* etiolated potato sprouts and enhance potato tuber numbers. Suttle (1998) reported that ethylene was produced at low concentration from single node explants during tuberization and tuber development. Ethylene synthesis was highest during the initiation of tuberization then declined. Rex (1992) also reported that 300 g a.i/ha application of florel as a foliage treatment increased the number of tubers, but reduced average tuber weight.

The impact of ethylene application on potato tuber bud dormancy has been studied extensively since the early twentieth century. Ethylene can either shorten or postpone potato tuber dormancy. Rylski et al., (1974) reported that ethylene has a dual

outcome on potato tuber dormancy. Their work showed that duration and concentration of ethylene treatments could impact potato dormancy. Although short time exposure (less than 3 days) ethylene treatments (0.02, 0.2, 2, 20 ppm) induced dormancy release, continuous ethylene treatments (0.02, 0.2, 2, 20 ppm) inhibited dormancy release.

Rosa (1925) reported that ethylene gas treatments (1000, 200, 10 ppm) stimulated potato dormancy release. Alam et al., (1994) reported that application of exogenous ethylene (100 ppm) to partially dormant tubers induced potato dormancy release. Exogenous 10, 100, and 1000 ppm florel treatments were reported to shorten potato bud dormancy (Rama and Narasimham, 1982; Rekha et al., 1983).

On the contrary, Denny (1925) reported that ethylene gas treatments (from 750,000 to 0.2 ppm) were not effective as a dormancy release agent. Applications of florel solutions (2500, 5000 ppm) to potato tubers resulted in prolonging the dormancy period (Korableva et al., 1989; Korableva and Ladyzhenskaya, 1995). Florel treatment (2000 ppm) to potato tubers in soil 2 weeks before harvest resulted in prolonging the dormancy period (Sukhova et al., 1993). Prange et al., (1998) found that 166 ppm continuous ethylene treatment enhanced potato dormancy. Wills and Warton (2003) reported that low levels of ethylene gas 0.005, 0.01, 0.1, 1, and 10 ppm) application prolonged dormancy duration.

Endogenous ethylene level in potato tubers is high at the inception of dormancy, declines and remains at low concentration until the beginning of dormancy release (Korableva et al., 1989). Ethylene can increase the rate of respiration in dormant potato tubers (Reid and Pratt, 1972). Ethylene level is considered important for regulation of

potato dormancy, but high levels in potato are associated with a longer dormancy period (Korableva et al., 1989).

Sprout growth is also affected by ethylene treatment (Ryski et al., 1974; Minato et al., 1979). Ethylene treatment induces thickened sprouts due to inhibition of sprout elongation (Rlyski et al., 1974; Timm et al., 1986; Minato et al., 1979). Minato et al., (1979) reported that 2 ppm ethylene treated potato tubers lost apical dominance. Prange et al., (1998) found that 166 ppm continuous ethylene application resulted in multiple and small sprouts. Florel treatment increased the number of small sprouts (Rama and Narasimham, 1982; Minato et al., 1979; Rekha et al., 1983) and sprout length (Rama and Narasimham, 1982).

Ethylene application stimulated decarboxylation of IAA and activity of IAA conjugation and reduced auxin level that resulted in abnormal sprouting (Minato and Okozawa, 1978; Minato et al., 1979). Minato and Okozawa (1978) concluded that 2 ppm ethylene fumigation is necessary for sprouting but not dormancy release. Sukhova et al., (1993) reported that application of 2000 ppm florel solution to potato tubers in soil before harvest significantly decreased IAA level in potato tubers 3 weeks after application, and then IAA increased to normal level and remained normal. Sukhova et al., (1993) reported that application of florel to the soil before harvest slightly reduced cytokinin levels during potato dormancy until sprouting. Korableva et al., (1989) also reported that florel treated potato tubers were more resistant to *Phytophthora infestans*.

1.7.6 Fluridone ($C_{19}H_{14}F_3NO$)

Suttle and Hultstrand (1995) found that application of fluridone shortened the potato microtuber dormancy period and decreased the level of ABA in microtubers.

1.7.7 Gibberellins

Gibberellins (GAs) are one of the major phytohormones in regulation of potato dormancy, stolon elongation and tuberization. Of the gibberellins, giberellic acid (GA_3) is the most predominant plant hormone in the regulation of potato tuber bud dormancy (Rappaport and Wolf, 1969; Korableva and Ladyzhenskaya, 1995). GA_3 has been commercially approved as a dormancy release agent for potato seed tubers (Claassens and Vreugdenhil, 2000).

Activity of gibberellins in potato tubers is low at harvest, very low during dormancy and significantly high during sprouting or dormancy release (Smith and Rappaport, 1961; Bialek, 1974; Bialek and Bielinska-Czarnecka, 1975). A rise in endogenous gibberellins in buds of potato tubers is associated with dormancy break (Shih and Rappaport, 1971). On the other hand, Suttle (2004) concluded that endogenous GA is not related to potato tuber dormancy release, but it is related to regulation of sprout growth.

Exogenous gibberellins (immersion or foliage-spray) generally shorten potato tuber bud dormancy (Doorenbos, 1958; Lippert et al., 1958; Timm et al., 1962; Rappaport et al., 1965; Rappaport and Wolf, 1969; Rekha et al., 1983; Rama and Narasimham, 1982; Bhargava, 1997; Dogonadze et al., 1999). Application of 10^{-5} M GA_3 hastened potato tuber dormancy (Rappaport et al., 1965). Lippert et al., (1958)

reported that application of 10, 50, 100 and 500 ppm gibberellin to foliage shortened dormancy duration of potato tubers. Rekha et al., (1983) reported that GA treatment shortened potato tuber dormancy. Rama and Narasimham P (1982) found that 10, 100, 1000 ppm GA treatments were affective on dormancy release. Timm et al., (1962) reported that 10 and 100 ppm GA_3 treatments stimulated dormancy release at 25 °C more than 5 °C. GA_3 treatments (10^{-5}, 10^{-6} and 10^{-7} M) shortened potato dormancy duration (Dogonadze et al., 1999). True potato seed dormancy can be broken with 24-h GA_3 (2000 mg dm^{-3}) treatment (Bhargava, 1997). Doorenbos (1958) reported that 25, 50 and 100 ppm GA_3 applications stimulated dormancy release and suggested that immersing tubers into solutions is the best application method.

Higher concentrations of gibberellins can produce unwanted features in potato plants (Timm et al., 1962; Marinus and Bodlaender, 1978). Timm et al., (1962) found that 10 ppm and 100 ppm GA_3 applications to dormant tubers produced abnormal tuber shapes, and decreased total yield. GA_3 treatments (9, 22.5, 45 ppm) caused small leaflets with yellow discoloration and elongated internodes in potato plants (Marinus and Bodlaender, 1978). According to Duda et al., (1971) as cited in Claassens and Vreugdenhil (2000), gibberellins as a dormancy release agent were not accepted in Russia due to poor reliability of breaking dormancy and weakened plant growth.

Rappaport and Sachs (1967) reported that GA application is more effective after wounding or cutting since these phenomena stimulate synthesis of gibberellins and induce sprouting and sprout growth.

Humphries (1958) found that foliage application of gibberellin increased the number of seed tubers. Potato dormancy was broken with 50, 500, 2000 ppm of GA_3 for

5 and 90–minute duration (Rappaport et al., 1957). Application of 5 ppm or lower concentrations of gibberellins promoted emergence and production of normal tubers (Rappaport et al., 1957). GA_3 treatments (10^{-5}, 10^{-6} and 10^{-7} M) were found to reduce level of ABA and ethylene plant hormones in apical meristems of potato tubers (Dogonadze et al., 1999). GA_3 (10^{-7} M) treatment also inhibited synthesis of ABA, 1-aminocyclopropane-1-carboxylic acid (ACC), and ethylene in dormant potato tubers, and hastened potato dormancy duration (Korableva et al., 1989). GA_3 treatments increased the number of sprouts per tuber (Holmes et al., 1970; Rama and Narasimham, 1982; Rekha et al., 1983) and significantly increased sprout length (Rama and Narasimham, 1982).

Dyson (1965) found that 50 and 1000 ppm GA_3 treatments hastened emergence and growth of stems, but postponed leaf and tuber growth. Five, 50 and 100 ppm GA treatments increased the number of stems and seed tubers, however, not total yield (Holmes et al., 1970). Similarly, Mikitzel (1993) reported that applications of 0.5, 1 and 2 ppm GA_3 to the freshly cut potato seed pieces before planting increased stem and seed tuber numbers, but not yield. Foliage application of 50 and 100 g a.i.ha^{-1} GA_3 before tuberization induced stem elongation and growth, increased the total number of smaller tubers and slightly declined or did not change total yield (Bodlaender and Vandewaart, 1989). GA treatments (9, 22.5, 45 ppm) hastened emergence and markedly increased the number of stem and seed tubers (Marinus and Bodlaender, 1978).

Stolon inception, formation and elongation are associated with high activity of gibberellins in potato plants (Smith and Rappaport, 1969: Kumar and Waring, 1974;

Vreugdenhil and Sergeeva, 1999). Gibberellins play a predominant role as an inhibitor for potato tuberization (Vreugdenhil and Sergeeva, 1999).

1.7.8 Rindite

Rindite is a potato dormancy release agent that consists of a mixture of chlorohydrin (7 parts by volume), 1, 2-dichloroethane (3 parts) and carbon tetrachloride (1 part) (Denny, 1945). Rindite acts in the vapor phase and its application to whole tubers hastens dormancy break (Denny, 1945; Lascarides, 1967; McDonald and Coleman, 1988; Kim et al., 1999).

Rindite treatment effectively shortened potato microtuber and tuber dormancy, accelerated sprout growth, increased number of sprouts per tuber (Nasiruddin and Blake, 1997; Kim et al., 1999; Akoumianakis et al., 2000), and enhanced yield (Akoumianakis et al., 2000). Postharvest application of rindite by fumigation shortened the dormancy period of potato microtubers (Kim et al., 1999). McDonalds and Coleman (1988) also reported that rindite treatment could break potato tuber dormancy better than bromoethane treatment. Rindite had been used as a commercial treatment from 1945 to late 1990s (Akoumianakis et al., 2000). Although rindite seems to be potentially suitable for the artificial breaking of dormancy, because of its chemical toxicity to plants and animals, its mutagenicity and carcinogenicity, its use is unacceptable (Kim et al., 1999; Coleman et al., 2001).

1.7.9 Storage gases

Carbon dioxide (CO_2)increases tuberization of isolated *in vitro* cultured potato stolons (Mingo-Castel et al., 1974). Burton (1958) observed that an O_2 reduction and a CO_2 increase in the storage atmosphere of potato tubers shortened the dormancy period. Coleman and McInerney (1997) found that 20 % CO_2 with 40 % O_2 or 60 % CO_2 with 18-20 % O_2 mixtures were efficient to shorten potato tuber dormancy. The mixture of 20 % CO_2 with 40 % O_2 and 50 ppm ethylene application shortened dormancy period similar to bromoethane.

1.7.10 Temperature

Temperature plays an important role in regulating the duration of potato tuber bud dormancy, dormancy release, and subsequent sprout growth. Sprouting and sprout growth can be accelerated with temperatures above 5 °C (Burton et al., 1992). Wurr and Allen (1976) also reported that increasing storage temperature to 15.6 °C shortened the dormancy period compared to low temperature storage (2 °C for 2 weeks), and sprout length was also longer. Similarly, Hartmans and Van Loon (1987) found that higher storage temperature (12 °C) markedly decreased the potato seed dormancy duration compared to low storage temperature (4 °C). Tabori et al., (1999) found that the dormancy duration was greatly prolonged by low storage temperature.

1.7.11 Water and humidity

Potato dormancy can be hastened by storing tubers under moist conditions at 20 to 35 °C (Goodwin, 1966). However, water application is not appropriate if tubers are buried in wet soil or vermiculite (Hemberg, 1985).

1.7.12 Wounding and cutting

Dormancy can be broken, if dormant tubers are peeled (Thornton, 1939). Potato microtuber bud dormancy can be released by cutting (Ewing et al., 1987). The effect of wounding has not been fully understood, but suggested to be related to ethylene synthesis (Rylski et al. 1974).

1.7.13 Mixture of dormancy release agents

Garcia-Torres and Gomez-Campo (1973) reported that a mixture of GA and Florel reduced stolon elongation and caused thicker and short stolons and inhibited root growth. Rekha et al., (1983) reported that a combination of Florel and GA (50 and 50 ppm) application was significantly effective for breaking potato tuber dormancy. Shashirekha and Narasimham (1988) also found that a combination of Florel and GA (60 and 40 ppm) application stimulated dormancy release and enhanced yield more than rindite, thiurea, carbon disulphide (CS_2), GA and Florel treatments.

Humphries (1958) found that foliage application of GA with cytokinins increased the number of tubers. Rex (1992) reported that application of Florel and chloramquat chloride mixture as foliage treatment increased number of tubers, but reduced average tuber weight.

1.7.14 Chemicals on dormancy release

Denny (1925, 1926, 1945) worked on many chemicals to find their effects on potato tuber bud dormancy release. Although ethylene chlorhydrin, thiourea, ethyl bromide, xylol, dichloroethylene, trichloroethylene, sodium bisulphide, sodium thiocyanate and potassium thiocyanate treatments were efficient to release potato bud dormancy, ethylene chlorhydrin was the most effective treatment to break potato tuber bud dormancy. Application of thiurea reduced the dormancy period, but it caused multiple numbers of sprouts per tuber (Denny, 1925; 1926). Denny (1945) also suggested that ethylene chlorhydrin, carbon tetrachloride, ethylene dichloride could be used as an efficient dormancy release agents but were not acceptable because of their adverse environmental impact.

1.8.1 Abscisic acid (ABA)

ABA, is a plant phytohormone that induces the closure of stomata, that regulates seeds to synthesize storage proteins, induces and maintains of dormancy, and inhibits shoot growth and effects of gibberellins in plants (Arteca 1996).

Palmer and Smith (1969b) found that application of ABA inhibited stolon elongation and did not stimulate tuber inception. Xu et al., (1998) found that ABA did not play a major role as a regulator for potato tuber formation. On the other hand, ABA has been considered to play a predominant role in maintaining and releasing potato tuber bud dormancy (Korableva et al., 1989; Wiltshire and Cobb, 1996; Claassens and Vreugdenhil, 2000). Potato bud dormancy is related to the presence of a β–inhibitor

complex (Hemberg, 1958; 1985). ABA was considered a main part of the β–inhibitor complex (Hemberg, 1985). The inception of potato bud dormancy was connected to an increase in the level of ABA. ABA reached its maximum level in the middle of dormancy, and a decline in the ABA content was related to dormancy break (Korableva et al., 1980; 1989; Cvikrova et al., 1994; Suttle and Hultsrand, 1994; Bhargava, 1997). On the contrary, a very low level of free ABA was found in potato tubers when they were stored at 3°C that prolonged potato tuber dormancy (Suttle, 1995; Coleman and King, 1984; Sorce et al., 1996). Bhargava (1997) reported that ABA plays an inhibitor role while GA_3 plays a promoter role for dormancy release in true potato seeds. Korableva et al., (2002) reported that application of 24-epibrassinolide enhanced potato dormancy duration as a result of increased ABA and ethylene levels. Suttle and Hultstrand (1994) concluded that ABA was also involved in microtuber dormancy inception and maintenance.

Korableva et al., (1989) found that 10^{-7} M abscisic acid treatment prolonged potato tuber dormancy. Simko et al., (1997) found that 8 loci were associated with potato tuber dormancy and 3 of them were related to variation in ABA level, showing a relationship between tuber dormancy and ABA content.

1.8.2 Auxins

Auxins are plant phytohormones that can induce cell elongation, root initiation, lateral root elongation in tissue culture, differentiation of phloem and xylem, and production of ethylene in plants (Arteca, 1996).

Auxins do not play a significant role in dormancy release nor control of potato tuber dormancy (De Bottoni et al., 1982; Hemberg, 1985; Korableva et al., 1989), but they affect sprout growth after dormancy release (Wiltshire and Cobb, 1996).

IAA level in the stolons was found to increase before visible tuber initiation (Obata-Sasamoto and Suziki, 1979). Tuber formation has been inhibited by high concentrations of IAA (>5 μM) in induced cuttings (Kumar and Waring, 1974). Hartsmans and Van Es (1979) found that IAA treatment induced root development in potato plants.

Sukhova et al., (1993) found that IAA level was low and constant during the dormancy period and markedly declined after dormancy release in potato tubers. Sorce et al., (2000) reported that both free and conjugated IAA level significantly increased at the end of dormancy at both 3 and 23 °C. Florel application (2000 ppm) to foliage 2 weeks before harvest significantly decreased IAA level 3 weeks after harvest. Fumigated ethylene application decreased auxin levels in potato tubers (Minato and Okozawa, 1978). However, IAA treatment (10^{-7} M) stimulated synthesis of ethylene in dormant tubers (Korableva et al., 1989; Cvikrova et al., 1994; Dogonadze et al., 1999; Suttle, 2003).

Dogonadze et al., (1999) and Rappaport and Wolf (1969) found that high concentrations of IAA prolonged potato tuber dormancy and reduced synthesis of ABA. The methyl ester of NAA was considered as a commercial sprout inhibitor, however, it was subsequently dropped due to storage decay problems (Denny, 1945). On the contrary, Korableva et al., (1989) reported that 10^{-7} M IAA application had no effect on potato tuber dormancy. Although most studies have demonstrated that application of

auxins prolonged or did not affect potato dormancy, Rappaport et al., (1965) reported that low concentrations of IAA and NAA slightly induced potato tuber dormancy release.

1.8.3 Brasinolide

Application of 24-epibrassinolide (EB) to potato tubers enhanced dormancy duration, increased ethylene production and level of ABA (Koreblove et al., 2002).

1.8.4 Chemical agents as sprout suppressants

Many chemicals are commercially available for dormancy enhancement such as, chlorpropham, propham, tecnazene, maleic hydrazide, methylnaphthalenes, and carvone (Wiltshire and Cobb, 1996). Para-coumaric acid (phenolic compound) has also been reported to prolong potato tuber dormancy (Mioduszewska and Bielinska-Czarnecka, 1985).

1.9 Field performance of minitubers

Plants grown from minitubers capture less radiation during plant development than those from regular seed tubers (Marshall and Taylor, 1990). Smaller sprouts at planting can cause delayed emergence (Lommen, 1994). Crop production from minitubers can be unsuccessful due to postponed or decreased emergence due to the length of time required for minituber dormancy release (Lommen, 1993a; 1993b). Minituber yield is lower than from usual seed tubers (Marshall and Taylor, 1990; Ranalli et al., 1994). Heavier and larger minitubers produced more normal emergence, faster plant growth, and higher yields than smaller minitubers (Ahloowalia, 1994; Lommen and

Struik, 1994; Ranalli et al., 1994). Ahloowalia (1994) reported that smaller minitubers produced a higher number of tubers than larger minitubers in the field, but these tubers were much smaller.

Smaller minitubers have low growth vigor with a long storage period. If the growing period is inadequate for growth of smaller minitubers, they may emerge late, resulting in a reduced harvest yield. Additionally, later emergence and longer growth excessively exposes the plants to late season infection of potato viruses leafroll and pvy often rendering the seed unfit for further increase. One of the solutions to increase minituber vigor is appropriate pre-treatment. Field performance of minitubers is weak because they have a slow growth rate at planting and slow growth rate is correlated to the smaller minituber size (Struik and Lommen, 1990).

1.10 Physiological age of potato tubers and minitubers

The status of tubers is associated with their physiological age. The general condition of seed tuber growth at planting is the common definition of physiological age. Cultivar, status during crop development, harvest time, and storage temperature are the important features that affect physiological age (Wiltshire and Cobb, 1996). Electrolyte leakage increases with rising physiological age and at the end of the dormancy period of potato tubers (De Weerd et al., 1995). Storage period affects physiological age of minitubers (Struik and Lommen, 1990). A longer dormancy period at low temperature decreases minituber vigor. Struik and Lommen (1990) found that 'old' minitubers (stored 19 months at 3-4°C) had a lower survival rate than 'young' minitubers (stored 5 months at 18°C) since 'old' minitubers were more affected by stresses and plant

pathogens than 'young' minitubers. Older minitubers produced many weak stems. Although the presence of many stems increases the number of tubers, total harvest was not significantly changed by tuber age. Therefore, 'old' tuber seed produces smaller sized potato tubers. Even though minitubers can survive for a long time in storage, they produce more stems, which results in diseases and disease vectors in the field. Minitubers from a late harvest have shorter dormancy than minitubers that are harvested earlier. Physiological age of minitubers is very important at planting (Lommen and Struik, 1992). Longer storage period (Krijthe, 1962; Bodlaender and Marinus, 1987) and tuber growth conditions (Krijthe, 1962; Van Itterson, 1992) during storage and pre-sprouting (Van Itterson, 1992) can increase minituber physiological age. A longer storage (aging) period (more than 8 months) decreases apical dominance, rooting capability, and sprout vigor of potato seed tubers (Kumar and Knowles, 1993a). Potato seed aging is associated with an increased rate of respiration (Kumar and Knowles, 1996a), lipid peroxidation (Kumar and Knowles, 1993b) oxidative stress (Kumar and Knowles, 1996b), and a decline in protein content (Kumar and Knowles, 1993c). Brierley et al., (1997) found that patatin, protein, level in potato tubers decreased with longer storage period, and total free amides and total amino acids levels increased with longer storage period. Similarly, Kumar et al., (1999) reported that potato ageing decreased patatin level and increased the activities of proteinases.

1.11 Importance of this research

The continuance of a potato production system requires adequate supplies of high quality seed tubers. Pathogen and disease-free minitubers are utilized to produce basic

seed potatoes (Lommen, 1993a; 1993b; Bandara and Tanino, 1995). Postharvest potato disease testing, potato seed multiplication programs and early potato seed export markets require the use of artificial external dormancy release agents (Coleman, 1990). Previous studies have shown that many chemicals are effective to break potato tuber bud dormancy, however most of these methods are not commonly accepted due to environmental and health issues.

While prolonged storage (6-24 weeks) at cool temperatures (5° to 15°C) will break dormancy in most cultivars, there are no reliable methods to stimulate early release of potato minituber dormancy in a predictable manner, especially for recalcitrant cultivars. Also, with existing methods, minituber germination is often erratic and unpredictable. Sometimes, under field conditions, emergence does not occur for months after planting. The cost of minitubers can become counterproductive when emergence is poor.

Chapter 2

Potato minituber bud dormancy release with chemicals and plant growth hormones

2.1 Abstract

Plant growth regulators are important in the regulation and termination of potato minituber bud dormancy. Predictable and consistent dormancy release of potato minitubers is necessary to ensure uniform sprouting for potato plant development. N^6-Benzyladenine (BA), Florel (Ethrel), ProGibb, kinetin, Pro-BA (2.28 10^{-6} M ProGibb and 50 ppm BA); Pro-florel (2.28 10^{-6} M ProGibb and 250 ppm Florel), BA-Florel (50 ppm BA and 250 ppm Florel) plant growth regulators, (±) catechin, electromagnetic field, temperature regimes, minituber sizes, 5 mM 2,2′-azobis- (2-amidinopropane) hydrochloride (AAPH), and 2,2'-azino-bis(3-ethylbenzothiazolamine-6-sulfonic acid) ($ABTS^+$) (free radical producers) were used as dormancy release agents in several experiments.

Pro-florel, ProGibb, Pro-BA plant hormone treatments shortened potato minituber bud dormancy and increased the sprout number. Larger minitubers were less dormant than smaller minitubers. From six different temperature regimes, 15, 17, and 19 °C accelerated dormancy break most effectively. Use of larger minitubers combined with Pro-florel treatment was the most effective in shortening potato minituber bud dormancy at 15 °C by up to 3 weeks faster than the control.

2.2 Introduction

Potato (*Solanum tuberosum* L.) tubers and minitubers are generally in dormancy and do not sprout for a period of time after harvest. Minitubers are small virus and disease-free seed tubers produced in climate controlled insect proof greenhouses from *in vitro* plantlets that are used to produce basic seed stock potatoes (Lommen, 1993a; 1993b; Bandara and Tanino, 1995). Minitubers have a longer dormancy period than regular seed tubers and there is considerable genetic variation for dormancy release among cultivars (Emilson, 1949; Lommen and Struik, 1992; Lommen, 1993a; 1993b; Lommen, 1994). For normal potato tubers that are sold as table stock, a longer dormancy period is desirable since most of the fall tuber yield must be stored for a long period for human consumption. Dormancy release or short dormancy duration is desirable in potato minitubers so that they produce a consistent emergence and uniform yield. Small-sized minitubers and cultivars with long dormancy periods are problematic, causing decreased growth and vigor (Struik and Lommen, 1990). Dormancy release of minitubers is also important for rapid post-harvest disease testing procedures and incorporation into seed multiplication programs (Coleman, 1983).

Although potato dormancy release methods have been extensively studied since the 1880s (Appleman, 1916). ProGibb is effective, safe and currently available. There are few effective and environmentally safe potato dormancy release techniques are currently available for the potato industry (Coleman et al., 1992).

Temperature plays an important role in the duration of potato tuber bud dormancy, dormancy release, and subsequent sprout growth. Sprouting and sprout growth can be accelerated with temperatures above 5°C (Burton et al., 1992). Wurr and

Allen (1976) also reported that increasing storage temperature from 2°C (2 weeks storage) to15.6°C shortened the dormancy period of potato tubers, and increased sprout growth. Similarly, Hartmans and Van Loon (1987) found that higher storage temperature (12°C) markedly shortened the potato seed dormancy duration compared to low storage temperature (4 °C). Tabori et al., (1999) also reported that the dormancy duration was greatly prolonged by low storage temperature.

Cytokinins (zeatin, kinetin and benzyladenine) can play an important role in control of potato tuber dormancy (Turnbull and Hanke, 1985a; 1985b). Cytokinin levels during potato dormancy are relatively low and increase significantly during dormancy break because of high synthesis of zeatin riboside and isopentenyladenosine (Turnbull and Hanke, 1985b; Sukhova et al., 1993). Cytokinin levels increased during the end of the potato tuber dormancy period and their transport from the storage tissue to active meristems had been envisaged (Cvikrova et al., 1994). Suttle (1998) reported that bioactive cytokinin level in potato buds increased during dormancy release. Cytokinins have been reported to shorten potato tuber bud dormancy and decrease the inhibitor β-complex (total amounts of acid growth-inhibiting substances) (Hemberg, 1970; Tsukamoto and Yazawa, 1972; Turnbull and Hanke, 1985a). Application of exogenous cytokinin is effective in dormancy release only during short periods, after the beginning and before the end of potato dormancy (Turnbull and Hanke, 1985a; Coleman, 1987). Cytokinins did not shorten the potato dormancy period in older dormant tubers that have a low concentration of endogenous cytokinin levels (Turnbull and Hanke, 1985a).

Ethylene application to overcome potato tuber bud dormancy has been studied extensively since the early twentieth century. Ethylene has a dual outcome on potato

tuber dormancy and either shortens or postpones potato tuber dormancy. Duration and concentration of ethylene treatments can control the effects on potato dormancy (Rlyski et al., 1974).

Activity of gibberellins in potato tubers varies during tuber life being low at harvest, very low during dormancy and significantly high during sprouting or dormancy release (Smith and Rappaport, 1961; Bialek, 1973; Bialek and Bielinska-Czarnecka, 1975). A rise in endogenous gibberellins in buds of potato tubers is associated with dormancy break (Shih and Rappaport, 1971). Exogenous gibberellins (immersion or foliage-spray) generally shorten potato tuber bud dormancy (Doorenbos, 1958; Lippert et al., 1958; Spicer and Dionne, 1961; Timm et al., 1962; Rappaport et al., 1965; Rappaport and Wolf, 1969; Rama and Narasimham, 1982; Bhargava, 1997; Dogonadze et al., 1999).

Catechins are flavonoids, mostly found in green tea leaves. They have antioxidant activity. They may also have anticarcinogenic, anti-inflammatory, anti-atherogenic, thermogenic and antimicrobial activities. They have not been studied as dormancy release agents according to our search.

Direct electrical current (DC) and alternating electrical current (AC) have been found to be successful in breaking potato tuber dormancy (Kocasaliskan et al., 1989; Kocasaliskan, 1990) maybe due to damage of potato tuber cell walls during insertion of 2 needles to apply electrical current (Kocasaliskan et al., 1989; Kocasaliskan, 1990; Wiltshire and Cobb, 1996).

Five mM 2,2′-azobis- (2-amidinopropane) hydrochloride (AAPH) and 2,2'-azino-bis (3-ethylbenzothiazolamine-6-sulfonic acid) ($ABTS^{+}$) are free radical producers.

While they had not been studied as dormancy release agents we hypothesized that free radical activity may serve to chemically release minituber dormancy.

The goal of this study was to compare the impact of several plant hormones, temperature regimes, chemicals and use of electrical current on minituber bud dormancy release and sprout number production with several potato cultivar minitubers.

3.3 Materials and Methods

Minitubers for these experiments were obtained from either the certificated seed producer Martinez Farms, Alamosa, CO or from the Colorado State University, San Luis Valley Research Center, Center, CO and stored under dark conditions at 5±1 °C prior to application of experimental treatments. Chemical and growth regulator treatments were applied by immersing the minitubers in treatment solutions that were also subjected to constant aeration with an aquarium pump, in the dark at specified temperatures. Cultivar selection varied for each experiment depending upon availability from each supplier. Data were taken weekly on sprout number and dormancy release percentage for five weeks. Analysis of variance (ANOVA) and Bonferroni's Method of Multiple Comparisons (SAS) were used for statistical analysis. Microsoft Excel software was used to create graphs.

3.3.1 Temperature experiment

Two potato minituber cultivars with long dormancy characteristics, Russet Norkotah-Selection 3 (RNK-S3) and Russet Norkotah-Selection 8 (RNK-S8) obtained from Martinez Farm, Alamosa, Colorado, March, 2000 were subjected to 13,15, 17, 19,

and 21 °C constant temperatures, as well as 11-21 °C diurnal temperatures. Producers did not provide harvest dates of minitubers.

Growth regulator applications of 50 ppm N^6-benzyladenine (BA); 2.28 10^{-6} M ProGibb; water, and control were applied at each temperature treatment.

3.3.2 Florel (2-chloroethylphosphonic acid, ethephon, ethrel) experiment

Ten potato minituber cultivars that represented different dormancy characteristics were selected: intermediate (Atlantic, Centennial Russet, DT6063-1R, CO86218-2R, Chieftain, Sangre-S14) compared to those with long dormancy (Chipeta, RNK-S3, RNK-S8, Snowden Russet) obtained from Martinez Farm, Alamosa, Colorado, March, 2000. Producers did not provide harvest dates of minitubers.

Treatments of 250, 500 and 1000 ppm florel (plant growth regulator, ethylene releaser), and control were applied.

3.3.3 Plant hormone experiment

RNK-S3 potato minitubers obtained from Martinez Farm, Alamosa, Colorado, March, 2000. Producers did not provide harvest dates of minitubers.

Minitubers were treated with 50 ppm BA (N^6-benzyladenine); 250 ppm Florel; 2.28 10^{-6} M ProGibb; Pro-BA (2.28 10^{-6} M ProGibb and 50 ppm BA); Pro-florel (2.28 10^{-6} M ProGibb and 250 ppm Florel), BA-Florel (50 ppm BA and 250 ppm Florel), water, and control. Treated minitubers were planted at Colorado State University, Agricultural Research, Development and Education Center (ARDEC), Fort Collins, CO,

on May 31, 2000 to test field performance. Individual minitubers were planted 6 inches deep spaced 12 inches apart. Data were taken for emergence number and plant height.

2.3.4 N^6-benzyladenine (BA) and Kinetin experiment

Long dormancy (Chipeta) and intermediate dormancy (Atlantic, Sangre-S14) potato minituber cultivars obtained from Martinez Farm, Alamosa, Colorado, March, 2000. Producers did not provide harvest dates of minitubers.

Minitubers were treated with 40 ppm BA (N^6-benzyladenine); 20 ppm Kinetin; 50 ppm Kinetin, and control.

2.3.5 Catechin experiment

Six potato minituber cultivars, Silverton Russet, Sangre-S14, Desiree, Nooksack, Russet Norkotah (RNK), and RNK-S3 obtained from the San Luis Valley Research Center (SLVRC), January, 2002 were selected to represent different dormancy periods: [short (Silverton Russet), intermediate (RNK, Sangre-S14, Desiree), and long (RNK-S3, Nooksack)]. Storage duration until treatment application varied for each cultivar as follows; 75 days for Silverton Russet, 68 days for Sangre-S14, 64 days for Desiree, 72 days for RNK, and 47 days for RNK-S3 and Nooksack.

Treatments were 10, 20 and 30 ppm catechin, and control.

2.3.6 Magnetic field experiment

Six cultivars, Silverton Russet, Sangre-S14, Desiree, Nooksack, RNK, and RNK-S3 obtained from the San Luis Valley Research Center (SLVRC), January 2002 and 2003

were selected to represent different dormancy periods: [short (Silverton Russet), intermediate (RNK, Sangre-S14, Desiree), and long (RNK-S3, Nooksack)]. Storage duration until treatment application varied for each cultivar as follows; 75 days for Silverton Russet, 68 days for Sangre-S14, 64 days for Desiree, 72 days for RNK, and 47 days for RNK-S3 and Nooksack in 2002 and 77 days for Silverton Russet, 72 days for Sangre-S14 and Nooksack, and 68 days for Desiree, RNK and RNK-S3 in 2003.

Treatments were 1, 2, and 4-hour electromagnetic field, and control in 2002 and 4 and 24-hour electromagnetic field, and control for 2003. For these treatments, minitubers were placed in test tubes that were encircled with insulated 18 gauge copper wire to create a magnetic field when connected to a DC generator. A DC current of 25 mAmps, 25 volts was applied for 1, 2, 4 and 24 hours under dark conditions at 15 °C.

2.3.7 Free radical experiment

Three potato minituber cultivars, Silverton Russet, Desiree, and RNK-S3 obtained from the San Luis Valley Research Center (SLVRC) January 2003 were selected to represent different dormancy periods: [short (Silverton Russet), intermediate (Desiree), and long (RNK-S3)]. Storage duration until treatment application varied for each cultivar as follows; 75 days for Silverton Russet, 68 days for Desiree, and 47 days for RNK-S3.

These minitubers were treated with 5 mM 2,2′-azobis- (2-amidinopropane) hydrochloride (AAPH), 2,2'-azino-bis(3-ethylbenzothiazolamine-6-sulfonic acid) ($ABTS^{+}$) (free radical producers), and control by soaking minitubers in AAPH, and ABTS for 5 minutes at room temperature.

2.3.8 Tuber size experiment

Six potato minituber cultivars, Silverton Russet, Sangre-S14, Desiree, Nooksack, RNK, and RNK-S3 obtained from the San Luis Valley Research Center (SLVRC) January 2003 were selected to represent different dormancy periods: [short (Silverton Russet), intermediate (RNK, Sangre-S14, Desiree), and long (RNK-S3, Nooksack)]. Storage duration until treatment application varied for each cultivar as follows; 71 days for Silverton Russet, 66 days for Sangre-S14 and Nooksack, and 62 days for Desiree, RNK, and RNK-S3. Minitubers were grouped by their weight as large (11.52± 1.3), intermediate (3.74±0.6), and small (1.81 ± 0.3).

3.4 Results

3.4.1 Temperature experiment

Minitubers (kept at 15, 17, 19 and 21°C) started to break dormancy 3 weeks after treatment in the water application (Fig 2.1). Minituber bud dormancy was significantly shortened by 15°C temperature compared to 13 and 11-21°C diurnal temperatures 3 weeks after treatment in water application (Fig 2.1). Constant 15°C temperature attained 40% dormancy break, while 13 and 11-21°C diurnal temperatures were completely dormant 3 weeks after treatment and similarly 15°C temperature 40% increased dormancy release compared to 13°C temperature regime 4 weeks after treatment (Fig 2.1). ProGibb treated minitubers (kept at 15, 17, 19 and 21°C) started to release dormancy 2 weeks after treatment (Fig 2.2). Minituber bud dormancy was significantly shortened by 19°C temperature compared to 13°C temperature 3 weeks after treatment in ProGibb application (Fig 2.2). BA treated minitubers (kept at 13, 15, 17 and 19°C)

started to release dormancy 2 weeks after treatment (Fig 2.3). Minituber bud dormancy was significantly shortened by 15°C temperature compared to 11-21°C diurnal temperature 3 weeks after treatment in BA application (Fig 2.3). Constant 15°C temperature shortened dormancy (30 to 40%) compared to 13 and 11-21°C diurnal temperatures 3 and 4 weeks after treatment (Fig 2.3).

Sprout number was not significantly changed among 6 temperature regimes with application of control, WA, ProGibb and BA, however, sprout number was significantly increased by application of ProGibb compared to control and WA treatments 3 weeks after treatment at 15 °C (Fig 2.4). ProGibb application also slightly increased sprout number overall from 2 to 5 weeks after treatment compared to the rest of treatments at 15°C (Fig 2.4).

3.4.2 Florel experiment

Dormancy release was significantly shortened by all florel applications in long-dormancy cultivars and 250 and 500 ppm florel applications in intermediate-dormancy cultivars compared to the control 3 weeks after treatment (Fig. 2.5, 2.6).

Florel applications significantly increased sprout number during 3 weeks after treatment and slightly increased 4 and 5 weeks after treatment in both intermediate and long dormancy cultivars (Fig. 2.7, 2.8).

3.4.3 Plant hormone experiment

Potato minituber bud dormancy was significantly shortened with all treatments compared to the control from week 1 to week 4 after treatment and Pro-florel and Pro-BA

applications shortened bud break almost 60 to 70% compared to control from 1 week to 3 weeks after treatment (Fig. 2.9). Sprout number was significantly higher in all plant hormone applications compared to water and control treated minitubers (Fig. 2.10). Pro-florel and BA-Florel applications had the highest sprout number from 1 week to 5 weeks after treatment (Fig. 2.10).

Emergence number was significantly increased with Pro-florel application compared to the other treatments except ProGibb, Pro-BA and florel for all data dates (Fig. 2.11). Pro-florel application significantly increased plant height compared to the other treatments except ProGibb for all data dates (Fig 2.12).

2.4.4 BA and Kinetin experiment

Dormancy release and sprout number were not significantly affected by treatments in either dormancy cultivar, although 50 ppm kinetin application slightly increased sprout number during week 4 and 5 after treatment in the intermediate dormancy group.

2.4.5 Catechin experiment

Catechin treatments did not significantly affect dormancy release, sprout number, nor sprout length compared to the control treatment in all dormancy group cultivars with minor exceptions in the intermediate dormancy group and 30 ppm catechin application significantly increased sprout length in intermediate dormancy group compared to control (Fig. 2.13).

2.4.6 Magnetic field experiment

Neither dormancy release nor sprout number were significantly changed by electromagnetic field applications (1, 2, and 4-hour magnetic field) in all dormancy cultivars tested in 2002. Dormancy release and sprout number were only significantly changed by 24-hour magnetic field application in short dormancy cultivar 3 and 4 weeks after treatment in 2003 (Fig. 2.14, 2.15). Control minitubers had approximately 40% less dormancy release compared to was shortened 24-hour magnetic field application 3 and 4 weeks after treatment in 2003 (Fig. 2.14).

2.4.7 Free radical experiment

No significant differences were found for dormancy release, sprout number, nor sprout length between treatments except in the long dormancy group for dormancy release 5 week after treatment (Fig 2. 16).

2.4.8 Tuber size experiment

Large size minitubers had a significantly shorter dormancy period in the short dormancy group 3 weeks after treatment and in the intermediate dormancy group 2 and 5 weeks after treatment compared to small size minitubers (Fig 2.17, 2.18). Large size minitubers shortened bud break 50% 3 weeks after treatment and 80% 4 weeks after treatment compared to small size minitubers in short dormancy cultivars (Fig 2.17). Dormancy release was not detected in long dormancy cultivars 5 weeks after treatment. Large size minitubers significantly increased sprout number in the short dormancy group

3, 4 and 5 weeks after treatment and in the intermediate dormancy group 2, 3 and 5 weeks after treatment compared to small size minitubers (Fig 2.19, 2.20).

Emergence number was not significantly increased by tuber sizes in short and intermediate dormancy groups. In the long dormancy cultivars, large size minitubers significantly increased emergence number 66 days after planting (DAP) compared to intermediate size minitubers (Fig 2.21). Large size minitubers produced taller plants 42 and 66 DAP in short (Fig 2.22), 66 DAP in intermediate (Fig 2.23) and 84 DAP in long (Fig 2.24) dormancy cultivars compared to small size minitubers. Plant emergence started 66 DAP only from larger size minitubers in short dormancy cultivars (Fig 2.22).

Large size minitubers significantly increased yield compared to small size minitubers in the intermediate dormancy cultivars (Fig 2.25). Yield was increased by larger size minitubers approximately 4 kg/5 plants compared to small size minitubers in intermediate dormancy cultivars (Fig 2.25). In short and long dormancy cultivars, large size minitubers slightly increased yield compared to intermediate and small size minitubers (Fig 2.25). In long dormancy cultivars, yield was increased 1.7 kg/5 plants compared to intermediate size minitubers and in short dormancy cultivars 2.5 kg/5 plants compared to small size minitubers (Fig 2.25).

3.5 Discussion

Potato tubers and minitubers are generally dormant at harvest and do not sprout. Previous studies show that increasing storage temperature shortened potato dormancy. Sprouting started 1 week earlier with 15, 17, 19 and 21°C temperatures compared to 13 and 11-21 °C diurnal temperatures in water and ProGibb applications (Fig 2.1, 2.2).

Dormancy release percentage was increased approximately 40 to 60% with 15°C temperature compared to13 and 11-21 °C diurnal temperatures during week 2 and 3 (Fig 2.1, 2.2) in water and ProGibb applications. From the 6 different temperature regimes, 15, 17, and 19°C accelerated dormancy break most effectively. These results were supported by Wurr and Allen (1976).

Storage temperature did not have a predominant effect on minituber sprout number based on lab results because different temperatures did not increase sprout number. However, ProGibb application increased sprout number from 0.3 to 1.2 sprout/minituber from 2 weeks to 5 weeks after treatment compared to rest of treatments at 15 °C (Fig 2.4). Previous storage tests revealed that temperatures above 21°C prolonged dormancy (data not shown). Thus we concluded that 15°C storage temperature was appropriate for subsequent minituber bud dormancy release studies. Florel applications were effective only 3 weeks after treatment in both intermediate and long dormancy cultivars for sprouting and sprout number, even though sprout numbers were slightly higher in other weeks after treatment (Fig. 2.5, 2.6, 2.7, 2.8). Sprout number was increased in range of 0.2 to 1/minituber from 3 to 5 weeks after treatment (Fig. 2.7, 2.8). Dormancy release was shortened from 10 to 30% compared to control 3 weeks after treatment. These results revealed that there were not significant differences between florel concentrations in case of sprouting and sprout number. These results were similar to previous studies (Rosa, 1925; Rylski et al., 1974; Rama and Narasimhan, 1982; Rekha et al., 1983). Plant growth regulators play a predominant role in potato dormancy and dormancy release. ProGibb has been used commercially as a dormancy release agent. In this study, plant hormones significantly shortened bud break (ranging 25 to 80%) and

increased the sprout number compared to the control from 1 to 5 weeks after treatment (ranging 1.7 to 4 sprout/minituber) (Fig. 2.9, 2.10). Plant growth regulator applied plants started to emerge earlier (approximately 20 days) than controls and increased emergence number/plant (ranging 0.1 to 0.6) compared to the control 64 days after planting (Fig. 2.11). Plants treated by plant hormones also grew faster than controls except BA-florel (ranging 5 to 25 cm/plant) (Fig. 2.12). These results have shown that combination of growth regulators Pro-florel shortened dormancy period, increased sprout number and emergence number, and increased plant height. Emergence number was significantly increased with Pro-florel application compared to other treatments except ProGibb, Pro-BA and florel for all data dates (Fig. 2.11). Pro-florel application significantly increased plant height compared to the other treatments except ProGibb (Fig 2.12) for all data dates. These results suggested that Pro-florel and Pro-BA applications were effective as dormancy release agents among plant hormones and were comparable to or better than the currently available treatments. These results were support previous studies on plant growth regulators.

Dormancy release and sprout number were not significantly affected by BA and kinetin treatments in both intermediate and long dormancy, although 50 ppm kinetin application slightly increased sprout number during 4 and 5 week after treatment in the intermediate dormancy group. These results were contradictory to previous studies for cytokinin effects on potato dormancy break because most of previous studies shown that cytokinins were effective to release potato dormancy.

Catechin treatments did not significantly affect dormancy release compared to control in any of the dormancy cultivars. Catechin did not significantly increase sprout

number except in the intermediate dormancy cultivars during week 5 after treatment. While 30 ppm catechin application did significantly increase sprout length 2.1 cm vs. 1.5 cm (control) in the intermediate dormancy cultivars (Fig. 2.13), from these results, it can be concluded that catechin applications produced only a minor response and were not very effective as a dormancy release agent.

Dormancy release and sprout number were not significantly changed by magnetic field applications (1, 2, and 4-hour) in all dormancy groups in 2002. Dormancy release and sprout number were significantly changed by the 24-hour magnetic field application, but only in the short dormancy cultivars 3 and 4 weeks after treatment in 2003 (Fig. 2.14, 2.15). These marginally responsive results suggested that the magnetic field treatments we applied were not effective enough for application with longer dormancy group cultivars. Previous studies suggested that electrical current was effective to release potato bud dormancy, however, their methods was not commercially suitable because they required insertion of two needles in opposite ends of the potato tubers (Kocasaliskan et al., 1989; Kocasaliskan, 1990).

No significant differences for dormancy release, sprout number, and sprout growth were detected for free radical producers except in the long dormancy group for week 5 after treatment (Fig 2. 16). AAPH application significantly shortened the dormancy period in long dormant cultivars during week 5 after treatment (Fig 2. 16). Once again, this marginal response at five weeks was considered too slow to be an effective dormancy release agent.

Previous studies revealed that heavier minitubers shortened bud break, hastened sprout growth and emergence in the field and increased regular more emergence,

increased ground cover, and increased yield (Lommen, 1993a; Lommen, 1993b; Lommen, 1994; Lommen and Struik, 1994). Large size minitubers decreased sprouting at least 1 week compared to small and intermediate size minituber after treatment and significantly shortened bud break ranging from20 to 80% in both short and intermediate dormancy groups 2 or 3 weeks after treatment (Fig 2.17, 2.18). (Fig 2.17, 2.18). Large size minitubers also produced significantly more sprouts (ranging 0.3 to 1/minituber) in the short dormancy group during week 3, 4 and 5 after treatment and in the intermediate dormancy group during week 2, 3 and 5 after treatment compared to small size minitubers (Fig 2.19, 2.20).

Emergence number was not significantly increased between tuber sizes in short and intermediate dormancy groups during all data dates. Only in long dormancy cultivars, large size minitubers significantly increased emergence number (0.5 emergence/minituber) 66 DAP compared to intermediate size minitubers (Fig 2.21). Large size minitubers significantly increased plant height 42 and 66 DAP in short (ranging 15-20cm/plant) (Fig 2.22), 66 DAP in intermediate (ranging 10-15cm/plant) (Fig 2.23) dormancy cultivars compared to small size minitubers and 84 DAP in long (25cm/plant) dormancy cultivars compared to intermediate size minitubers (Fig 2.24). Large size minitubers also significantly increased yield (4kg/5 plant) in the intermediate dormancy group compared to small size minitubers (Fig 2.25). In short and long dormancy cultivars, large size minitubers slightly increased yield compare to intermediate and small size minitubers (Fig 2.25). Yield increase was 2.5kg/5 plants in short dormancy cultivars between large and small size minitubers and was 1.7kg/5 minituber between large and intermediate size minitubers. These findings agree with

previous dormancy release studies using regular tubers or minitubers. It was thus concluded that larger (heavier) minitubers are the best choice to improve rapid emergence. Because more regular or consistent emergence of minitubers produce more uniform plants in height and vigor, and a more uniform yield in an earlier time frame within the growing season. It may be most efficient to examine greenhouse minituber production practices to increase minituber size, as an important strategy to optimize this aspect of seed production, relative to problems with prolonged dormancy cultivars.

In general, shortening minituber bud dormancy release is important to potato seed growers since a more consistent emergence crop of minitubers will produce more uniform plants in height and vigor and a more uniform yield in an earlier time frame within the growing season. This can have enormous impact on quality, size and disease considerations. The later the crop or the more immature the plants in the late season, the greater the risk of vector borne virus disease such as potato leafroll virus potato Y (PVY). Also, by producing a more uniform tuber size and quality, the seed grower can expect to have a more consistent storage period within the next year's crop derived from higher quality by uniformly, physiologically aged seed. This advantage will make the grower more productive in the future with potentially produce less diseased plants and better yields. Therefore, based upon this research, growers should be very selective in how they choose minitubers and the relative size in short or intermediate dormancy cultivars. The nearer the minitubers are to harvest from the greenhouse (within 2 months) the more grower should insist on the larger sizes and recognize that a dormancy break system must be used.

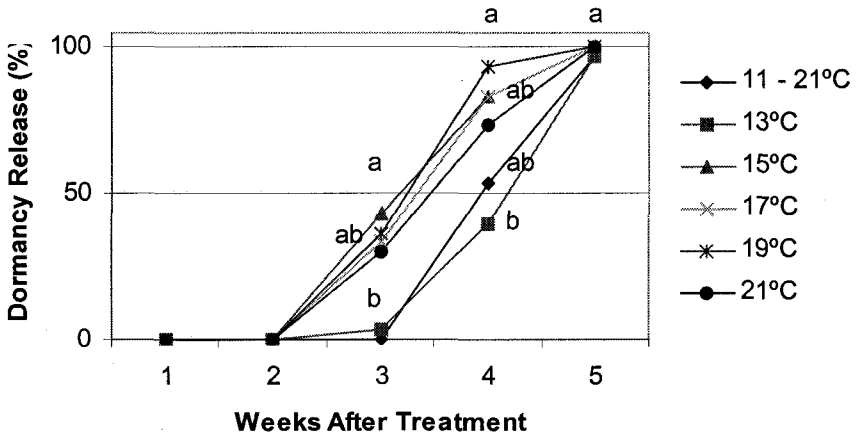


Fig. 2.1 Dormancy release of long dormancy cultivars (RNK-S3, RNK-S8) treated with 24-hour water aeration for freshly harvested greenhouse produced minitubers held at in the dark at 95 to 100 % relative humidity for five weeks. Data are percent of minitubers with sprouts (≥2 mm) from six storage temperatures. For each data point n=30. Treatments with the same letter are not significantly different at $p<0.05$ by Bonferroni's Method of Multiple Comparisons (SAS).

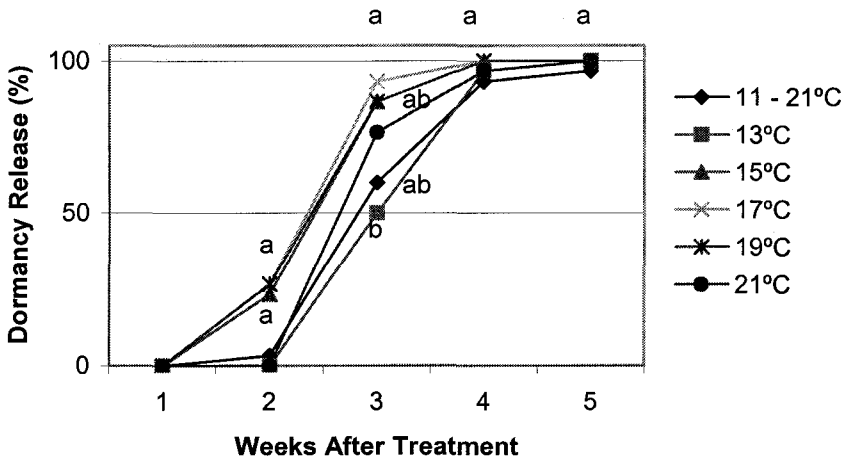


Fig. 2.2 Dormancy release of long dormancy cultivars (RNK-S3, RNK-S8) treated with 2.28 10^{-6} M ProGibb for freshly harvested greenhouse produced minitubers held at in the dark at 95 to 100 % relative humidity for five weeks. Data are percent of minitubers with sprouts (≥2 mm) from six storage temperatures. For each data point n=30. Treatments with the same letter are not significantly different at $p<0.05$ by Bonferroni's Method of Multiple Comparisons (SAS).

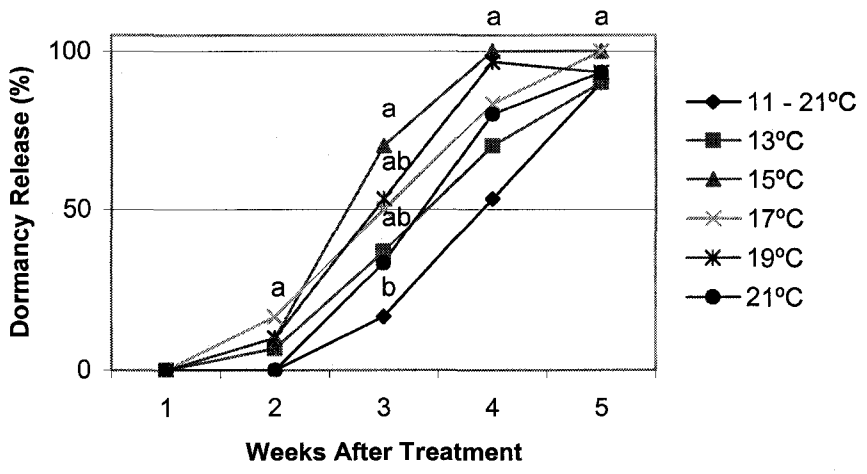


Fig. 2.3 Dormancy release of long dormancy cultivars (RNK-S3, RNK-S8) treated with 50 ppm BA for freshly harvested greenhouse produced minitubers held at in the dark at 95 to 100 % relative humidity for five weeks. Data are percent of minitubers with sprouts (≥2 mm) from six storage temperatures. For each data point n=30. Treatments with the same letter are not significantly different at $p<0.05$ by Bonferroni's Method of Multiple Comparisons (SAS).

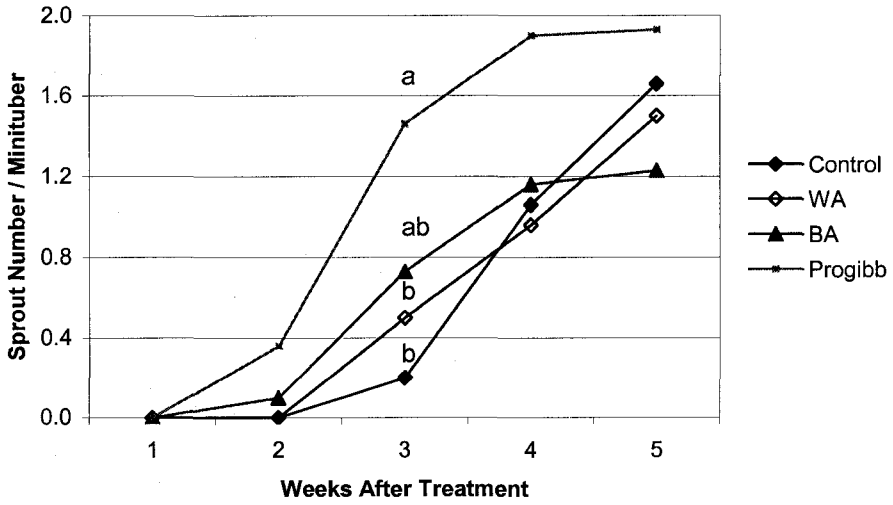


Fig. 2.4 Sprout number of long dormancy cultivars (RNK-S3, RNK-S8) treated with 24-hour water aeration for freshly harvested greenhouse produced minitubers held at 15±1°C in the dark at 95 to 100 % relative humidity for five weeks. For each data point n=30. Treatments with the same letter are not significantly different at $p<0.05$ by Bonferroni's Method of Multiple Comparisons (SAS).

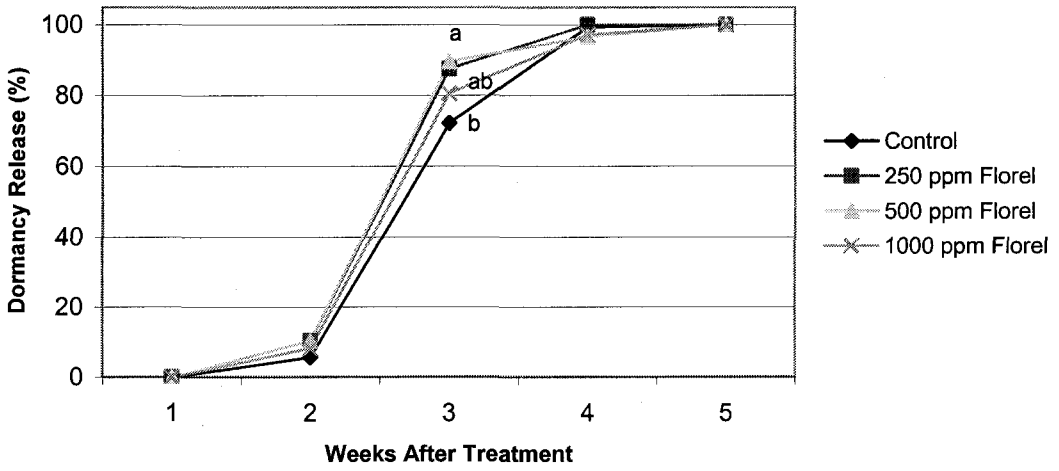


Fig. 2.5 Dormancy release of intermediate dormancy cultivars (Atlantic, Centennial Russet, DT-6063-11r, CO-86218-2, Chieftain, Sangre-S14) treated with 250, 500 and 1000 ppm Florel for freshly harvested greenhouse produced minitubers held at 15±1 °C in the dark at 95 to 100 % relative humidity for five weeks. Data are percent of minitubers with sprouts (≥2mm). For each data point n=144. Treatments with the same letter are not significantly different at $p<0.05$ by Bonferroni's Method of Multiple Comparisons (SAS).

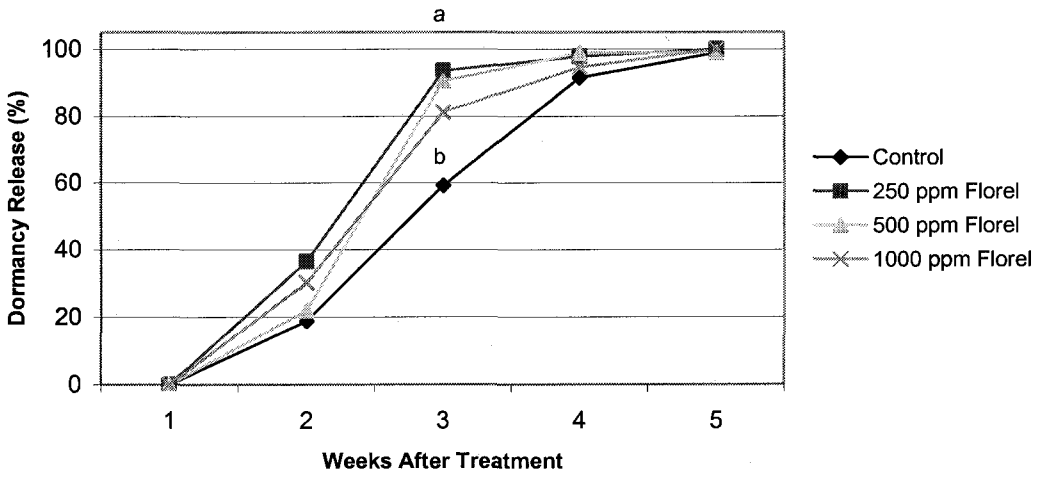


Fig. 2.6 Dormancy release of long dormancy cultivars (Chipeta, RNK-S3, RNK-S8, Snowden) treated with 250, 500 and 1000 ppm Florel for freshly harvested greenhouse produced minitubers held at 15±1 °C in the dark at 95 to 100 % relative humidity for five weeks. Data are percent of minitubers with sprouts (≥2mm). For each data point n=92. Treatments with the same letter are not significantly different at $p<0.05$ by Bonferroni's Method of Multiple Comparisons (SAS).

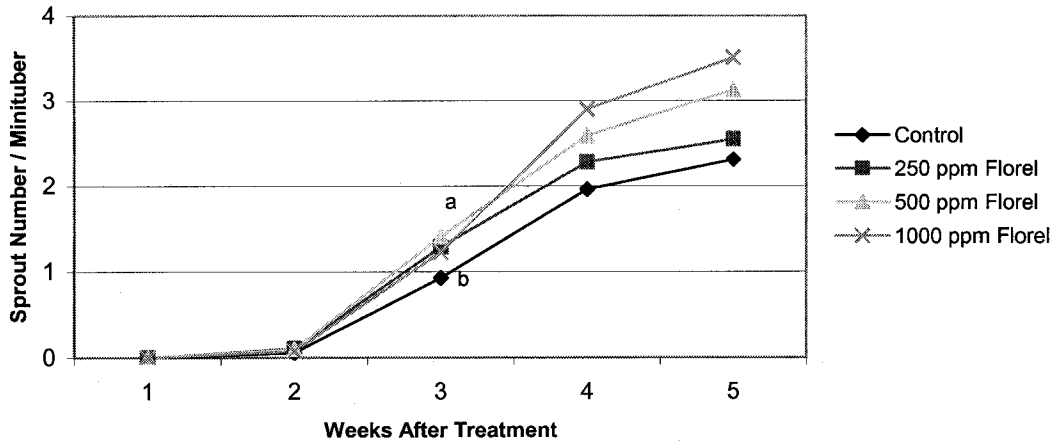


Fig. 2.7 Sprout number of intermediate dormancy cultivars (Atlantic, Centennial Russet, DT-6063-11r, CO-86218-2, Chieftain, Sangre-S14) treated with 250, 500 and 1000 ppm Florel for freshly harvested greenhouse produced minitubers held at 15±1 °C in the dark at 95 to 100 % relative humidity for five weeks. For each data point n=144. Treatments with the same letter are not significantly different at $p<0.05$ by Bonferroni's Method of Multiple Comparisons (SAS). Only the 3 weeks after treatment data point was significantly different as indicated.

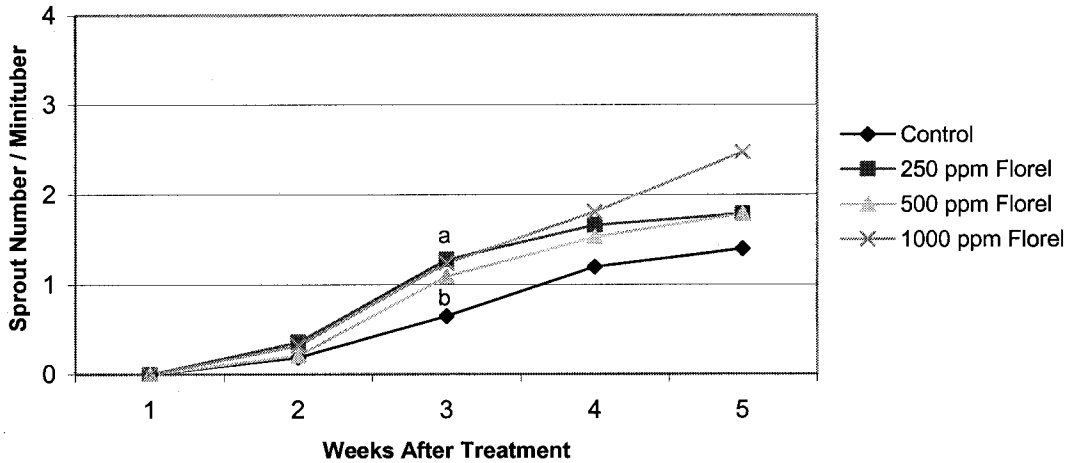


Fig. 2.8 Sprout number of long dormancy cultivars (Chipeta, RNK-S3, RNK-S8, Snowden) treated with 250, 500 and 1000 ppm Florel for freshly harvested greenhouse produced minitubers held at 15±1 °C in the dark at 95 to 100 % relative humidity for five weeks. For each data point n=92. Treatments with the same letter are not significantly different at $p<0.05$ by Bonferroni's Method of Multiple Comparisons (SAS). Only the 3 weeks after treatment data point was significantly different as indicated.

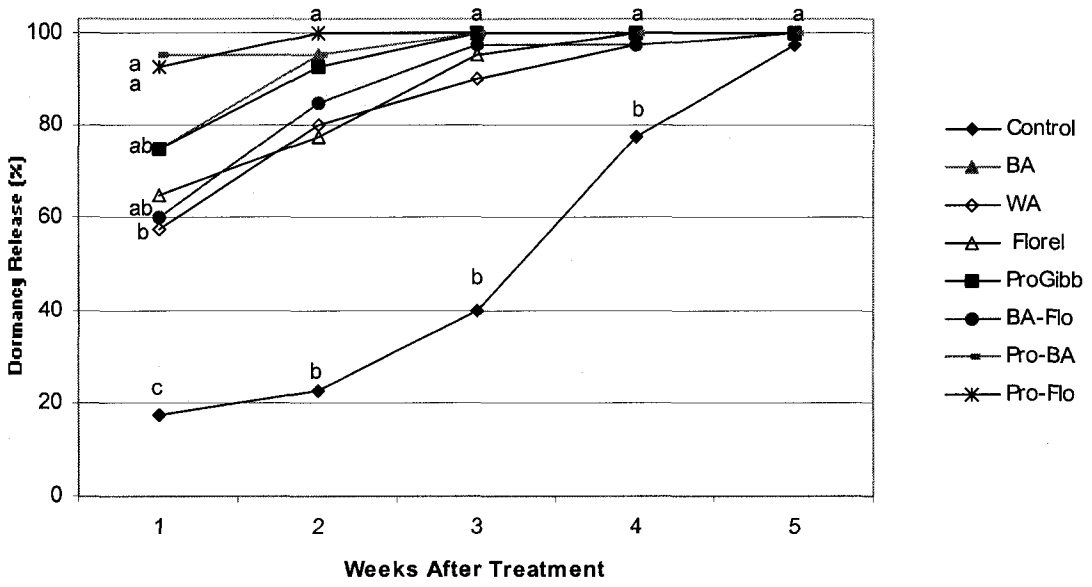


Fig. 2.9 Dormancy release of RNK-S3 treated with plant hormones for freshly harvested greenhouse produced minitubers held at 15±1 °C in the dark at 95 to 100 % relative humidity for five weeks. Data are percent of minitubers with sprouts (≥2mm). For each data point n=20. Treatments with the same letter are not significantly different at $p<0.05$ by Bonferroni's Method of Multiple Comparisons (SAS).

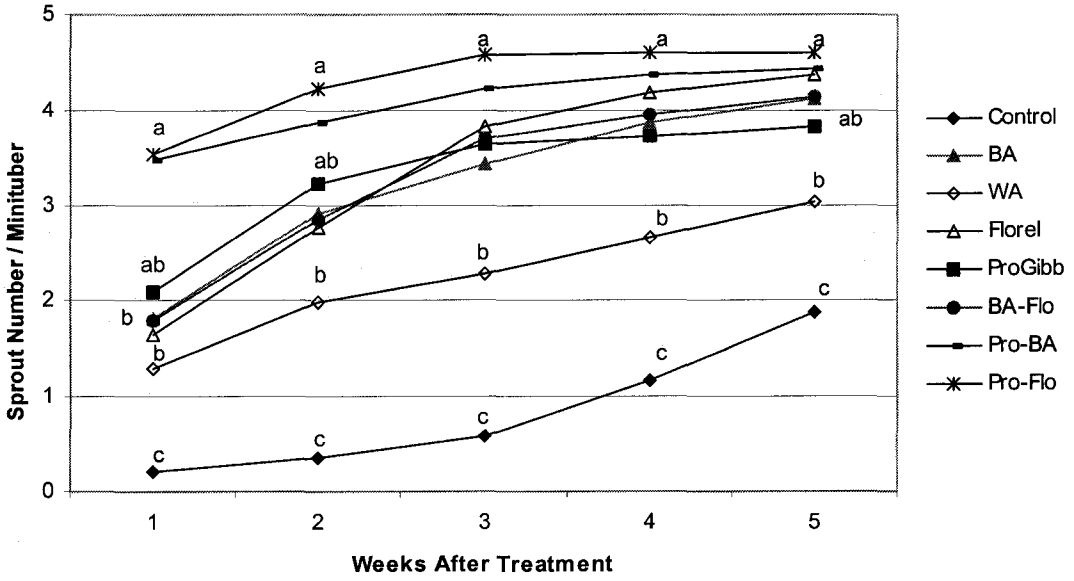


Fig. 2.10 Sprout number of RNK-S3 treated with plant hormones for freshly harvested greenhouse produced minitubers held at 15±1 °C in the dark at 95 to 100 % relative humidity for five weeks. Data are percent of minitubers with sprouts (≥2mm). For each data point n=20. Treatments with the same letter are not significantly different at $p<0.05$ by Bonferroni's Method of Multiple Comparisons (SAS).

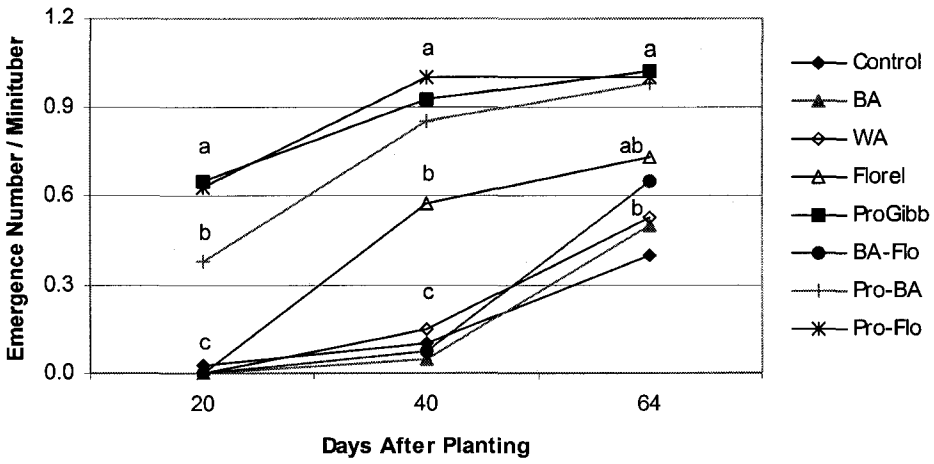


Fig. 2.11 RNK-S3 emergence number in 2000. Emergence number based upon number of shoots per minituber, observed days after planting (DAP) at Colorado State University, Agricultural Research, Development and Education Center, Fort Collins, CO. For each data point n=30. Treatments with the same letter are not significantly different at $p<0.05$ by Bonferroni's Method of Multiple Comparisons (SAS).

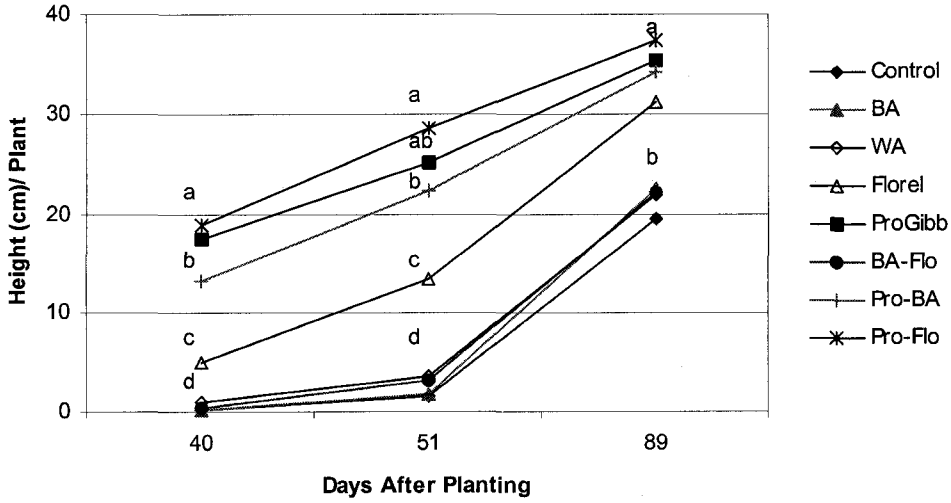


Fig. 2.12 RNK-S3 plant height in 2000. Plant height (cm) observed days after planting (DAP) at Colorado State University, Agricultural Research, Development and Education Center, Fort Collins, CO. For each data point n=30. Treatments with the same letter are not significantly different at $p<0.05$ by Bonferroni's Method of Multiple Comparisons (SAS).

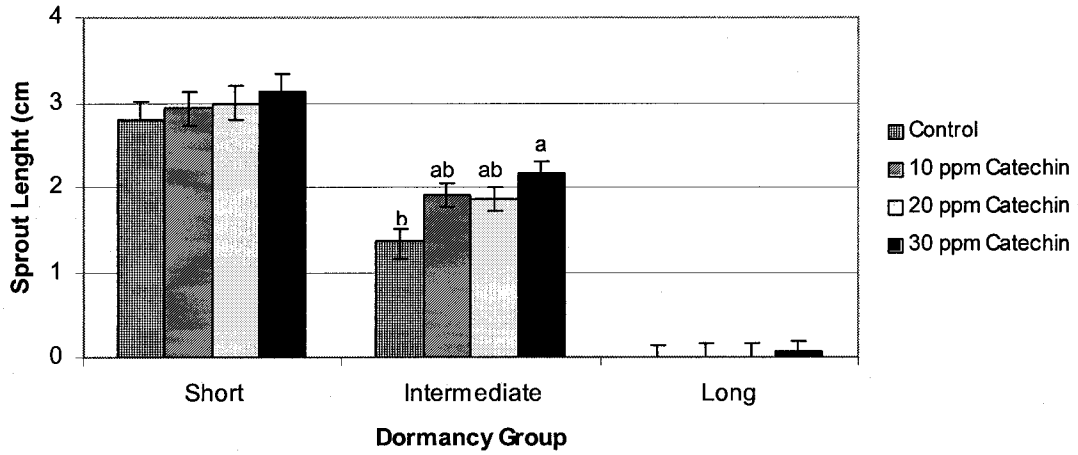


Fig. 2.13 Sprout length (cm) of the longest sprout/minituber, 5 weeks after treatment in 2002 and 2003 for the three dormancy groups. For each treatment in the short dormancy group n=16, in the intermediate dormancy group n=48, in the long dormancy group n=32. Treatments with the same letter within each dormancy group are not significantly different at $p<0.05$ by Bonferroni's Method of Multiple Comparisons (SAS). There are no significant differences between treatments within short and long dormancy cultivars. Potato cultivars are grouped by dormancy period [short (Silverton Russet), intermediate (RNK, Sangre-S14, Desiree), and long (RNK-S3, Nooksack)].

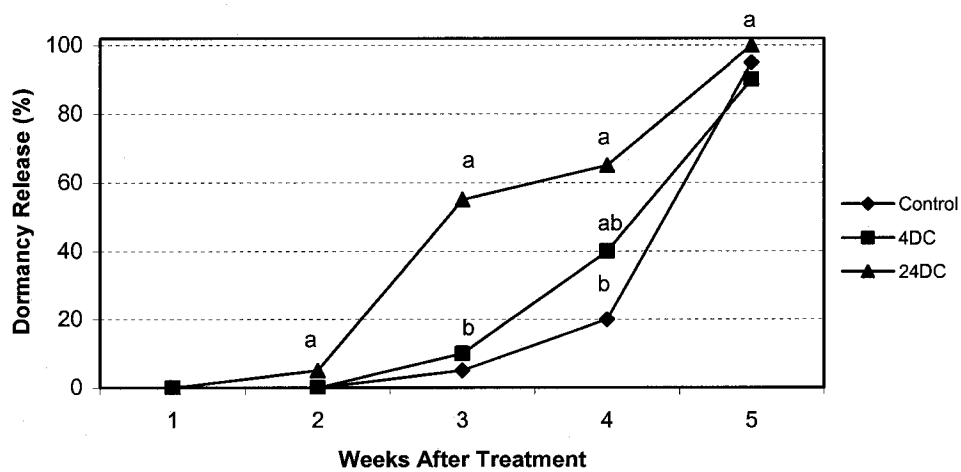


Fig. 2.14 Dormancy release of short dormancy cultivar (Silverton Russet) treated with magnetic field in 2003 for freshly harvested greenhouse produced minitubers held at 15±1 °C in the dark at 95 to 100 % relative humidity for five weeks. Data are percent of minitubers with sprouts (≥2mm). For each treatment in the short dormancy group n=20, in the intermediate dormancy group n=60, in the long dormancy group n=40. . Treatments with the same letter are not significantly different at $p<0.05$ by Bonferroni's Method of Multiple Comparisons (SAS).

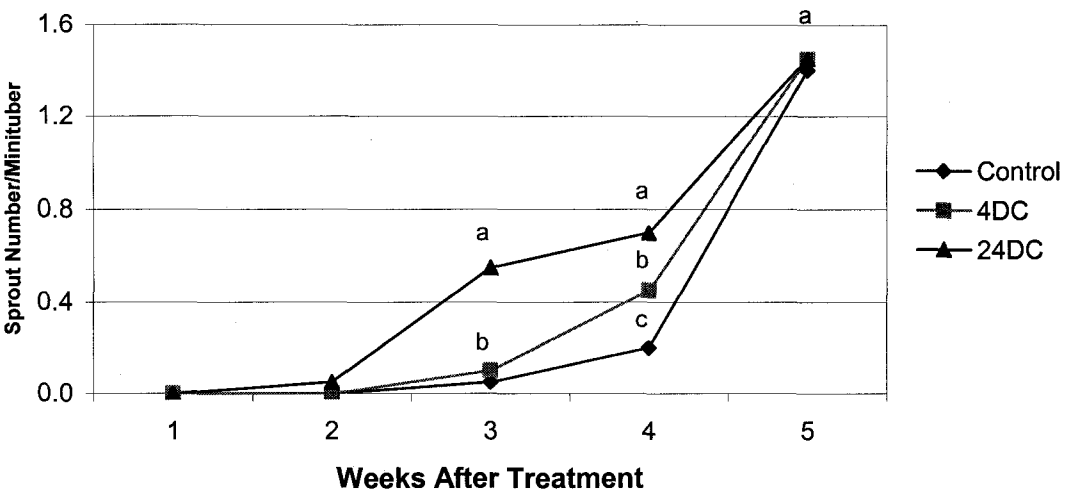


Fig. 2.15 Short dormancy cultivar (Silverton Russet) sprout number treated with magnetic field in 2003 freshly harvested greenhouse produced minitubers held at 15±1 °C in the dark at 95 to 100 relative humidity for five weeks. Sprout number based upon mean number of sprouts (≥2mm). For each treatment in the short dormancy group n=20, in the intermediate dormancy group n=60, in the long dormancy group n=40. Treatments with the same letter are not significantly different at $p<0.05$ by Bonferroni's Method of Multiple Comparisons (SAS).

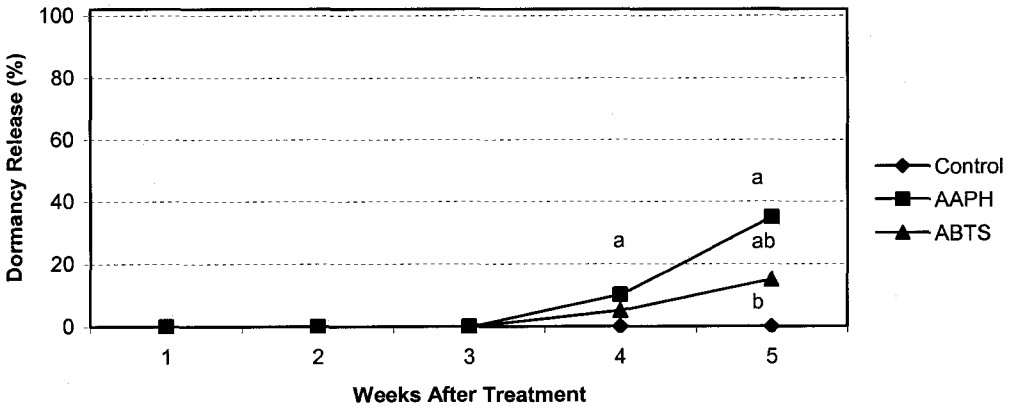


Fig. 2.16 Dormancy release of long dormancy cultivars (RNK-S3, Nooksack) treated with AAPH and ABTS for freshly harvested greenhouse produced minitubers held at 15±1 °C in the dark at 95 to 100 % relative humidity for five weeks. Data are percent of minitubers with sprouts (≥2mm). For each data point n=40. Treatments with the same letter are not significantly different at $p<0.05$ by Bonferroni's Method of Multiple Comparisons (SAS).

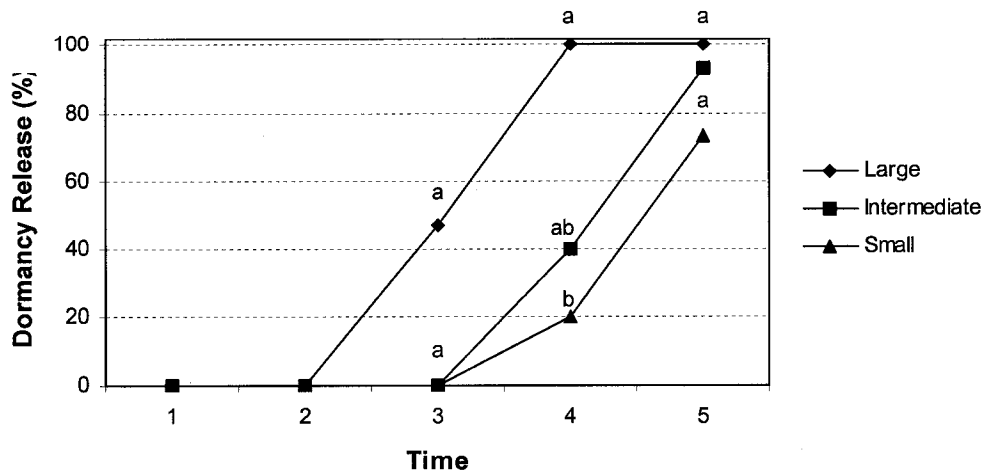


Fig. 2.17 Dormancy release of short dormancy cultivar (Silverton Russet) for freshly harvested greenhouse produced minitubers held at 15±1 °C in the dark at 95 to 100 % relative humidity for five weeks. Data are percent of minitubers with sprouts (≥2mm). For each data point n=20. Treatments with the same letter are not significantly different at $p<0.05$ by Bonferroni's Method of Multiple Comparisons (SAS).

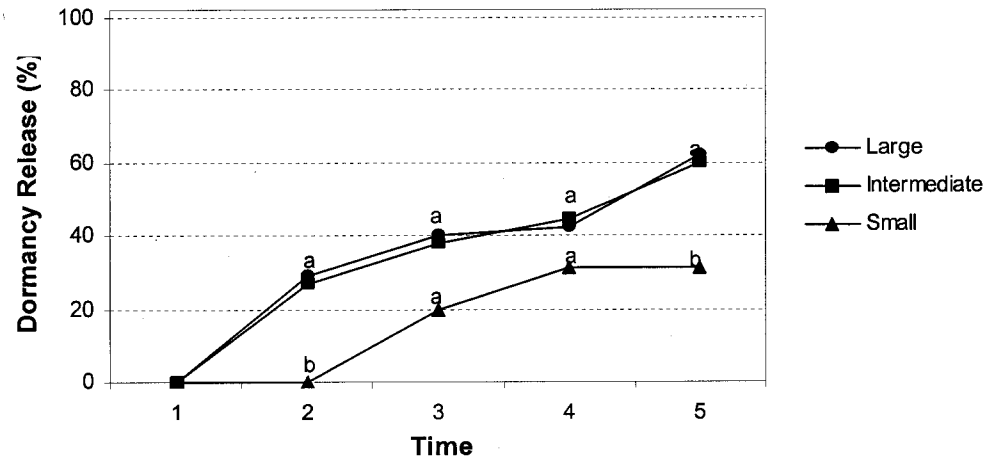


Fig. 2.18 Dormancy release of intermediate dormancy cultivars (RNK, Sangre-S14, Desiree) for freshly harvested greenhouse produced minitubers held at 15±1 °C in the dark at 95 to 100 % relative humidity for five weeks. Data are percent of minitubers with sprouts (≥2mm). For each data point n=60. Treatments with the same letter are not significantly different at $p<0.05$ by Bonferroni's Method of Multiple Comparisons (SAS).

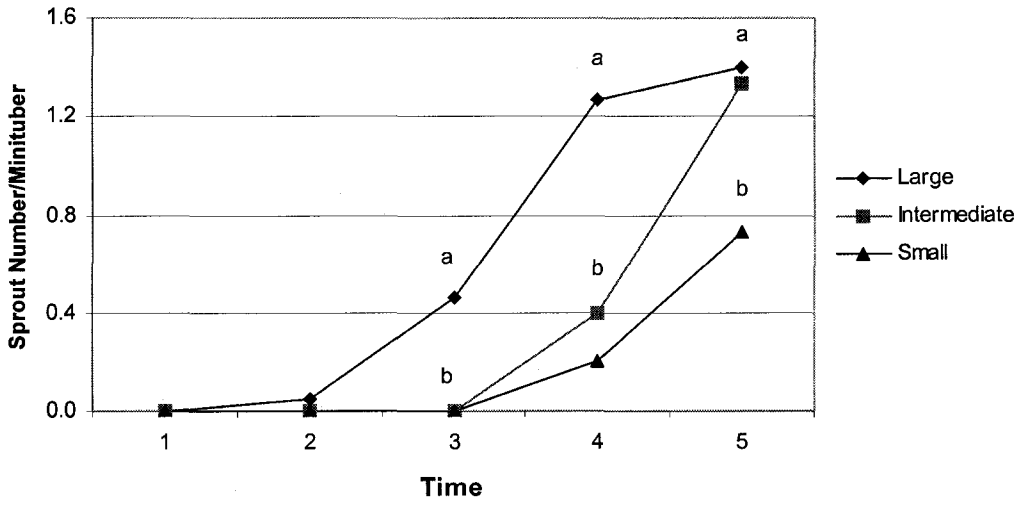


Fig. 2.19 Short dormancy cultivar (Silverton Russet) sprout number in 2003 freshly harvested greenhouse produced minitubers held at 15±1 °C in the dark at 95 to 100 % relative humidity for five weeks. Sprout number based upon mean number of sprouts (≥2mm). For each data point n=20. Treatments with the same letter are not significantly different at $p<0.05$ by Bonferroni's Method of Multiple Comparisons (SAS).

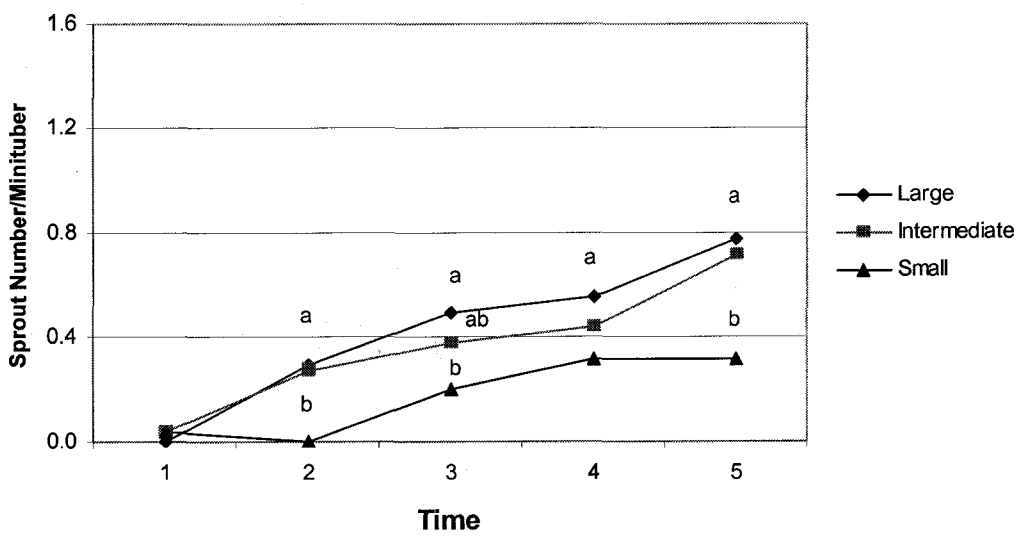


Fig. 2.20 Intermediate dormancy cultivars (RNK, Sangre-S14, Desiree) sprout number in 2003 freshly harvested greenhouse produced minitubers held at 15±1 °C in the dark at 95 to 100 % relative humidity for five weeks. Sprout number based upon mean number of sprouts (≥2mm). For each data point n=60. Treatments with the same letter are not significantly different at $p<0.05$ by Bonferroni's Method of Multiple Comparisons (SAS).

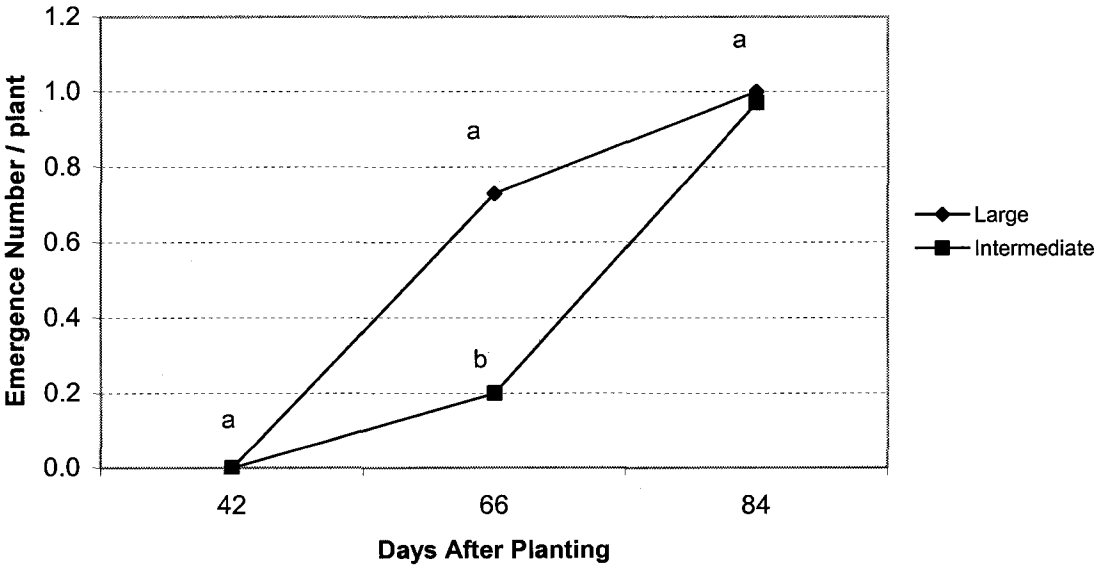


Fig. 2.21 Long dormancy cultivars (RNK-S3, Nooksack) emergence numbers in 2002. Emergence based upon number of shoots per minituber, observed days after planting (DAP) at Colorado State University, San Luis Valley Research Center, CO. For each data point n=40. Treatments with the same letter are not significantly different at $p<0.05$ by Bonferroni's Method of Multiple Comparisons (SAS).

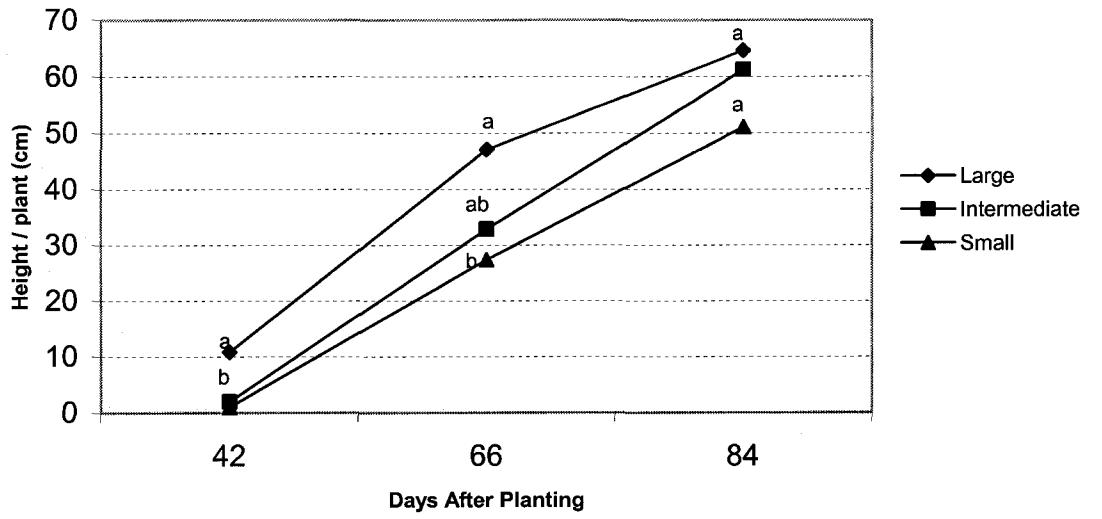


Fig. 2.22 Plant height of short dormancy cultivar (Silverton Russet) in 2003. Plant Height (cm) observed days after planting (DAP) at Colorado State University, San Luis Valley Research Center, CO. For each data point n=20. Treatments with the same letter are not significantly different at $p<0.05$ by Bonferroni's Method of Multiple Comparisons (SAS).

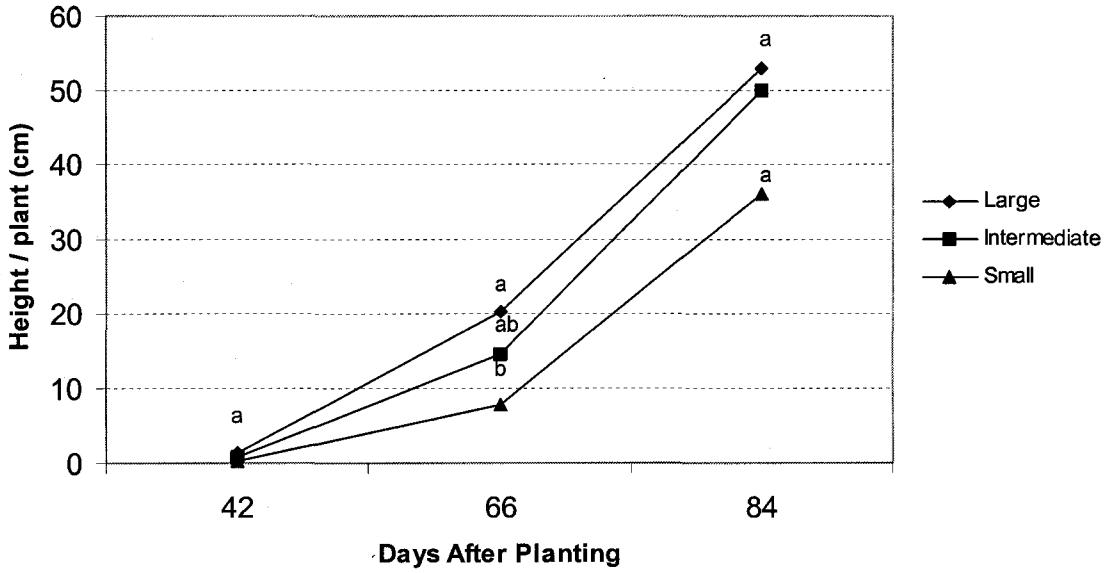


Fig. 2.23 Plant height of intermediate dormancy cultivars (RNK, Sangre-S14, Desiree) in 2003. Plant Height (cm) observed days after planting (DAP) at Colorado State University, San Luis Valley Research Center, CO. For each data point n=60. Treatments with the same letter are not significantly different at $p<0.05$ by Bonferroni's Method of Multiple Comparisons (SAS).

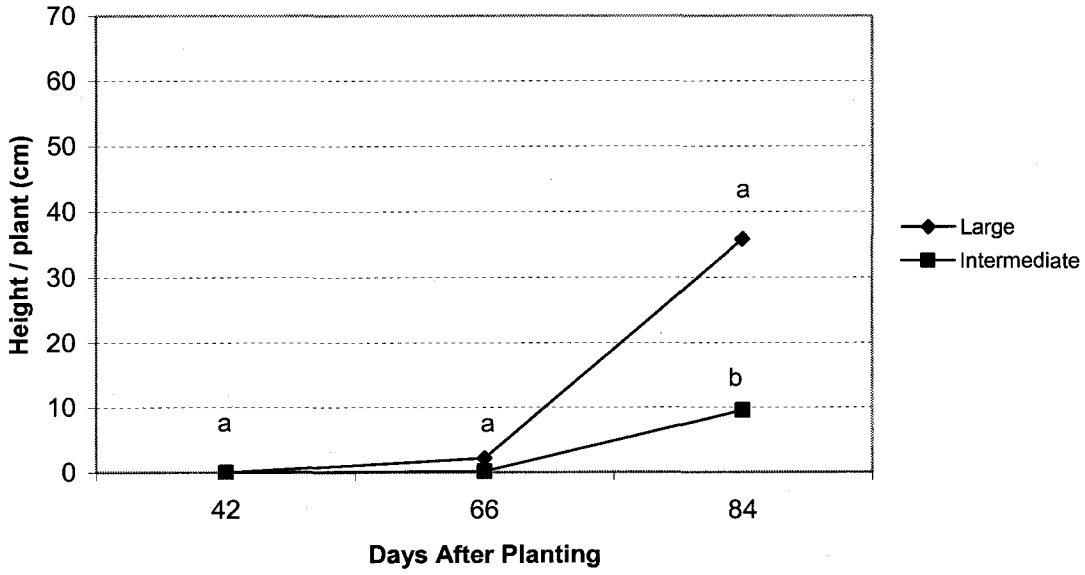


Fig. 2.24 Plant height of long dormancy cultivars (RNK-S3, Nooksack) in 2003. Plant Height (cm) observed days after planting (DAP) at Colorado State University, San Luis Valley Research Center, CO. For each data point n=40. Treatments with the same letter are not significantly different at $p<0.05$ by Bonferroni's Method of Multiple Comparisons (SAS).

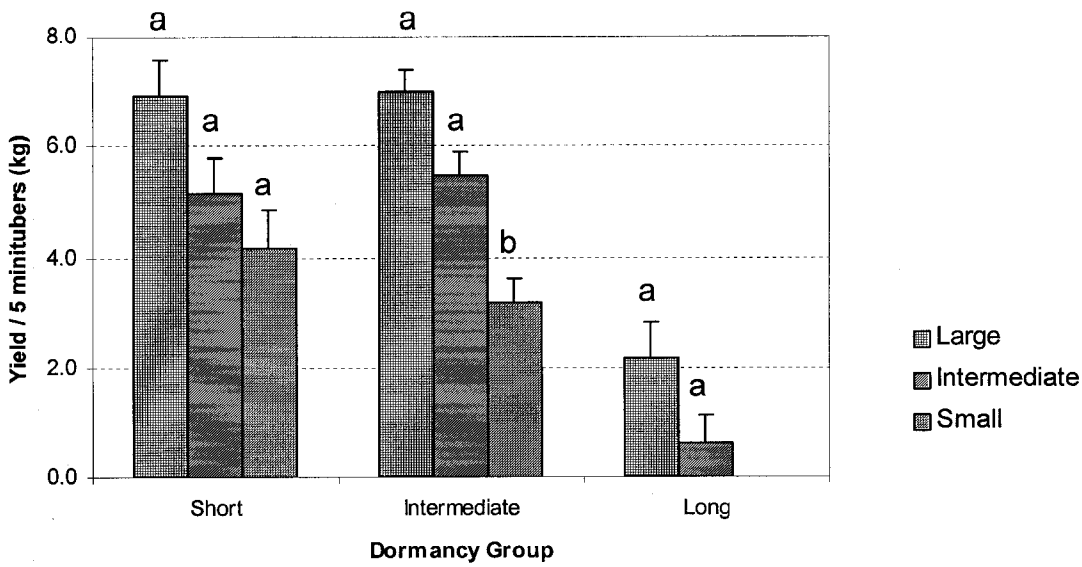


Fig. 2.25 2003 San Luis Valley yield based upon production from 5 minitubers/plot (kg/5 minitubers). Error bars are standard error of means. For each treatment in the short dormancy group n=20, in the intermediate dormancy group n=60, in the long dormancy group n=40. Treatments with the same letter are not significantly different at $p<0.05$ by Bonferroni's Method of Multiple Comparisons (SAS) within each dormancy group. Potato cultivars grouped by dormancy period [short (Silverton Russet), intermediate (RNK, Sangre-S14, Desiree), and long (RNK-S3, Nooksack)].

Chapter 3

The effects of ABA, Florel, and ProGibb plant growth regulators on potato minituber bud dormancy and plant growth under field conditions in the San Luis Valley, Colorado

3.1 Abstract

Plant growth regulators are important in the regulation and termination of potato minituber bud dormancy. Predictable and consistent dormancy release of potato minitubers is necessary to ensure uniform sprouting for potato plant development.

Abscisic acid (ABA), Florel (Florel), ProGibb, and Pro-florel (2.28 10^{-6} M ProGibb and 250 ppm Florel) growth regulators were used as dormancy release agents. Potato (*Solanum tuberosum* L.) minituber cultivars were categorized into three dormancy groups based on their known dormancy duration; group (1) short dormancy group (Silverton Russet); group (2) intermediate dormancy group (Russet Norkotah (RNK), Sangre-Selection 14 and Desiree); and group (3) long dormancy group (Nooksack and Russet Norkotah-selection 3 (RNK-S3)).

Pro-florel treatment shortened potato minituber bud dormancy, increased sprout number, accelerated sprout growth, increased plant growth, tuber number, and yield for all minituber groups in 2002 and 2003.

A combined Pro-florel treatment was most effective in shortening potato minituber bud dormancy and improving tuber yield.

3.2 Introduction

Minitubers are small disease-free greenhouse produced seed tubers used in field planting to produce basic seed potatoes (Lommen, 1993a; 1993b; Bandara and Tanino, 1995). Potato dormancy is desirable in tubers produced for human consumption since they have to be stored for a long time. However, dormancy of minitubers creates problems for potato seed producers. Minitubers have a longer dormancy period than regular tubers (Lommen and Struik, 1992; Lommen, 1993a; 1993b; 1994) and there is considerable genetic variation for dormancy release among cultivars (Burton et al., 1992). The longer dormancy period and smaller size are disadvantageous causing decreased growth vigor (Struik and Lommen, 1990). Dormancy release or short dormancy duration is desirable in potato minitubers because often they have to be planted soon after their harvest. It is also necessary before field planting to ensure uniform sprouting for potato plant development. Although potato dormancy release methods have been extensively studied since the 1880s (Appleman, 1916), few effective and environmentally safe potato dormancy release techniqus are currently available for the potato industry (Coleman, 1992).

Ethylene application to overcome potato tuber bud dormancy has been studied extensively since the early twentieth century. Ethylene has a dual outcome on potato tuber dormancy and either can shorten or postpone potato tuber dormancy. Duration and concentration of ethylene treatments can control the effects on potato dormancy (Rlyski et al., 1974).

Activity of gibberellins in potato tubers is low at harvest, very low during dormancy and significantly high during sprouting or dormancy release (Smith and

Rappaport, 1961; Bialek, 1974; Bialek and Bielinska-Czarnecka, 1975). A rise in endogenous gibberellins in buds of potato tubers is associated with dormancy break (Shih and Rappaport, 1970). Exogenous gibberellins (immersion or foliage-spray) generally shorten potato tuber bud dormancy (Doorenbos, 1958; Lippert et al., 1958; Spicer and Dionne, 1961; Timm et al., 1962; Rappaport et al., 1965; Rappaport and Wolf, 1969; Rama and Narasimham, 1982; Bhargava, 1997; Dogonadze et al., 1999).

ABA maintains or induces dormancy, and inhibits shoot growth and the effects of gibberellins in plants (Arteca, 1996). Bhargava (1997) reported that ABA plays an inhibitor role while GA_3 plays a promoter role for dormancy release in true potato seeds. The goal of this study was to examine the impact of plant growth regulators on minituber bud dormancy release, sprout number, sprout growth, plant emergence, plant height, and yield with several potato cultivars grown for two years at the San Luis Valley Research Center, Center, CO.

3.3 Materials and Methods

3.3.1 Plant materials and treatments

Six potato minituber cultivars, Silverton Russet, Sangre-S14, Desiree, Nooksack, Russet Norkotah (RNK), and Russet Norkotah S-3 (RNK-S3) were obtained from the San Luis Valley Research Center (SLVRC) during January 2002 and 2003 and kept under dark conditions at 5±1 °C until experimental treatments were applied. Storage intervals until treatments were applied varied for each cultivar as followed; 69 days for Silverton Russet, 62 days for Sangre-S14, 58 days for Desiree, 41 days for Nooksack, and RNK-S3, 66 days for RNK in 2002 and 71 days for Silverton Russet, 66 days for Sangre-S14

and Nooksack, 62 days for Desiree, RNK, and RNK-S3 in 2003. These cultivars were selected to represent different dormancy periods: [group 1, short (Silverton Russet), group 2, intermediate (RNK, Sangre-S14, Desiree), and group 3, long (RNK-S3, Nooksack)] based upon characteristics outlined in Table. 3.9).

Treatments were: 10^{-4} M Abscisic acid (ABA); 250 ppm Florel; 1000 ppm Florel; 2.28 10^{-6} M ProGibb; Pro-florel (2.28 10^{-6} M ProGibb and 250 ppm Florel), and control. For the ABA, Florel, ProGibb and Pro-florel treatments, minitubers were soaked and aerated in aqueous solutions with an aquarium plump for 24-hour in the dark at 15°C. In 2003, 1000 ppm florel treatment was eliminated because there was not significant difference between 500 and 1000 ppm florel applications. Data were taken weekly for sprout number and dormancy release percentage for 5 weeks. After 5 weeks storage, the longest sprout length for each minituber was taken for sprout growth data. These minitubers were kept 1 week longer at the same storage conditions, and then transferred to 5°C under light conditions until planting to prevent sprout elongation.

These minitubers were planted at San Luis Valley Research Center (SLVRC), Center, CO in May, 2002 and 2003. A completely randomized block design was used to minimize water usage differences and soil variation in the field plots. Individual minitubers were planted 6 inches deep with 12 inches between plants in row. The distance between each replicate block was 24 inches. Data were taken three times for emergence number and plant height before haulm killing.

Yield data were taken for different tuber sizes, tuber number and total yield for each replicate. These data were taken 2 weeks later in 2002 due to rain at harvest than in 2003.

The analysis of variance (ANOVA) and Bonferroni's Method of Multiple Comparisons (SAS) were used for statistical analysis. Microsoft Excel software was used to create graphs.

3.4 Results

Plant growth regulators exerted significant effects on the duration of minituber bud dormancy, sprout length, sprout growth, emergence number, plant height and yield in 2002 and 2003.

3.4.1 Dormancy Release

Pro-florel treated minitubers shortened the dormancy period compared to other treatments for all dormancy groups in both 2002 and 2003 (Fig. 3.1, 3.2, 3.3, 3.4, 3.5. 3.6). Only, Pro-florel and ProGibb treatments were effective in releasing dormancy duration of long dormancy group minitubers in 2002 (Fig. 3.3). Although 250 ppm florel application shortened the long dormancy group minitubers in 2003, it was significantly lower than Pro-florel and ProGibb treatments (Fig. 3.6). Two years data (2002 and 2003) correlated very well $r = 0.785$ ($p=0.0001$) to dormancy release percentage for all dormancy groups. This suggested that treatment effects were consistent from 2002 to 2003.

3.4.2 Sprout Number

Pro-florel treated minitubers increased the number of sprouts per minituber compared to other treatments for all dormancy groups in both 2002 and 2003 (Fig. 3.7,

3.8, 3.9, 3.10, 3.11, 3.12). Only, Pro-florel and ProGibb treatments increased sprout number for long dormancy minituber cultivars in 2002 (Fig. 3.9). Although 250 ppm florel application increased sprout number, it was significantly lower than Pro-florel and ProGibb treatments in long dormancy group minitubers in 2003 (Fig. 3.12). Two years data (2002 and 2003) correlated very well $r = 0.872$ ($p<0.0001$) to sprout number.

3.4.3 Sprout Growth

Pro-florel treated minitubers significantly increased sprout growth compared to other treatments for all dormancy groups in 2002 and 2003 except short and intermediate dormancy groups in 2003 (Fig.3.13). Florel (250 and 1000 ppm) treated minitubers had a tendency to grow longer sprouts than ProGibb treatments in the short dormancy group in 2002 (Fig.3.13). In long dormancy minituber cultivars, only Pro-florel and ProGibb treatments effectively enhanced sprout growth.

3.4.4 Emergence Number

Emergence number data were taken on 3 different dates in 2002 and 2003. For intermediate dormancy cultivars and the first emergence date, Pro-florel treated minitubers had higher emergence number (Fig. 3.14, 3.15, 3.17, 3.18, 3.19). In general, all minitubers produced approximately 1 emergence/plant on the last data date.

3.4.5 Plant Height

In general, in the short and intermediate cultivars individual plant height was not significantly affected by treatments for three different dates (Fig. 3.20, 3.22, 3.23, 3.24).

Plant height was affected in the long dormancy cultivars (Fig. 3.22, 3.25). Plant height gradually increased with time for all treatments. While Pro-florel application increased the plant height for all dormancy groups, except short dormancy group in 2003, the differences were not significant.

3.4.5 Yield

Although Pro-florel application increased yield for all dormancy groups in 2002 and 2003, Pro-florel treatment significantly increased the yield compared to control and ABA treatments in 2002 (Fig. 2.26). In general, yield of 2003 was higher than yield of 2002 for all treatments since harvest date was 2 weeks later in 2003 than 2002.

3.4.5 Number of tubers

Harvested tubers were categorized into three size classes; small (<50gr), intermediate (50-100gr) and (>100gr). Pro-florel treatment increased total number of tubers for all dormancy groups in 2002 and 2003 except the short dormancy group in 2003 (Fig. 3.27, Table.3.7, 3.8)

3.5 Discussion

Dormancy release was significantly shortened by plant growth regulator treatments compared to the untreated control. Pro-florel treated minitubers started sprouting 2 weeks earlier in the short dormancy cultivar and one week earlier in intermediate dormancy cultivars compared to controls in 2002 and 2003. In long dormancy cultivars, Pro-florel and ProGibb were effectively released for dormancy

release. Pro- florel treated minitubers were attained 50% bud break at least 2 weeks earlier than the control in all dormancy cultivars. Limited previous Pro-florel studies also revealed similar results for sprouting for seed potatoes. ProGibb effects on minituber dormancy supported previous gibberellic acid studies. These results indicated that the combination of ProGibb and florel (Pro-florel) growth regulators, and ProGibb were effective in long dormancy cultivars.

This study has shown that application of florel was efficiently broke bud dormancy in short and intermediate dormancy cultivars. This result agrees with previous studies on florel for dormancy release (Rama and Narasimham, 1982; Rekha et al., 1983; Narasimham, 1988). ABA application effects were not constant between dormancy groups and years. It significantly shortened sprouting in short and intermediate dormancy cultivars in 2002 and significantly prolonged sprouting in intermediate dormancy cultivars in 2003. These results did not reveal the effects of ABA on bud dormancy release like previous contradictory reports. In general, dormancy release percentage was well correlated between 2002 and 2003 $r = 0.785$ ($p<0.0001$).

Sprout number was significantly shortened by Pro-florel treated minitubers compared to the control in all dormancy cultivars both in 2002 and 2003, ranging from 0.2 to 3 sprouts/minituber. Multiple buds were observed on each minituber treated with Pro-florel. This allowed sprouts to grow strong and healthy. ProGibb also significantly increased sprout number/minituber as reported in previous studies (need a reference here). Florel application also increased sprout number (also a reference here). ABA increased sprout number in 2002, but decreased sprout number in 2003 compared to

control. Sprout number was very well correlated between 2002 and 2003 $r = 0.872$ ($p<0.0001$).

Sprout growth was accelerated by Pro-florel application for all dormancy groups in both years. ProGibb also significantly increased sprout growth compared to the control for all except the long dormancy cultivars in 2003. Rama and Narasimhan (1982) also reported that gibberellic acid application accelerated sprout growth. Sprout growth was also well correlated between 2002 and 2003 $r = 0.788$ ($p<0.0001$).

Emergence number was significantly increased by Pro-florel application in the long dormancy group 46 and 64 DAP, ranging from 0.5 to 1.0 sprouts/minituber. Pro-florel applied to minitubers caused earlier emergence than other treatments in long dormancy cultivars. Consequently early emergence results in uniform plant growth. At the last data date (84 DAP), Pro-florel treated minitubers had approximately 1 plant/minituber. Thus, Pro-florel application did not produce multiple shoots/minituber that could result in weak or diseased plants.

Plant height was also significantly increased by application of Pro-florel in long dormancy cultivars. Abnormal plant growth was not detected as a result of treatment applications. Earlier plant growth could also allow plants to better resist environmental stresses and pests.

Pro-florel application significantly increased yield compared to control in intermediate (3 kg/5 plants) and long dormancy (4 kg/5 plants) cultivars in 2003. It also increased approximately 1 kg/5 plants for rest of dormancy cultivars in 2002 and 2003 compared to control. Florel application also significantly increased yield in long dormancy cultivars compared to control (1.7 kg/5 plants). Yields in 2002 and 2003 were

different in magnitude but correlated very well r=0.74 ($p<0.0001$) suggesting that treatment effects were consistent from one year to the next (Fig. 2.26). This could be caused by different harvest dates since planting to harvest date was 2 weeks longer in 2003 (123 days) than in 2002 (108 days).

Pro-florel application also increased total tuber numbers for all dormancy groups in 2002 and 2003 compared to the control (Fig. 3. 26).

Dormancy release percentage, sprout number, growth and yield were correlated to reveal if there was any relationship between lab and field data. In both years, there were correlations between dormancy release percentage and yield $r = 0.721$ ($p<0.0001$) in 2002 and $r = 0.758$ ($p<0.0001$) in 2003. On the other hand, sprout number and length were not well correlated to yield nor to dormancy release [sprout number $r = 0.560$ ($p<0.0001$) in 2002 and $r = 0.533$ ($p<0.0001$) in 2003] [sprout growth r=0.574 ($p<0.0001$) in 2002 and r = 0.0.627 ($p<0.0001$) in 2003].

These results suggested that there was a strong relationship between lab and field data in shortening minituber dormancy and consequently increasing yield. The data also suggest that field studies might be circumvented for future dormancy release studies in specific cultivars. Less costly and less time consuming lab studies provided good predictions of field response for most cultivars.

Shortening minituber bud dormancy release is important to potato seed growers since a more consistent emerging crop of minitubers will produce a more uniform plant in height and vigor and a more uniform yield, earlier within the growing season. This can have enormous impact on quality, size and disease considerations. The later the crop or the more immature the plants in the late season, the greater the risk of vector borne virus

disease such as potato leafroll virus potato Y (PVY). Also, by producing a more uniform tuber size and quality, the seed grower can expect to have a more consistent storage period with in the next year's crop derived from higher quality by uniformly, physiologically aged seed. This advantage will make the grower more productive in the future and potentially produce less diseased plants and better yields. Therefore, based upon this research, growers should be very selective in how they choose minitubers. The nearer the minitubers are to harvest from greenhouse production (within 2 months) the more growers should use a dormancy break system such as Pro-florel.

3.6 Summary

Florel can be added as a hormonal treatment to break minituber dormancy. While effective alone, the best results were obtained using ProGibb in combination with 250 ppm florel. A registration label should be sought for application of florel to break bud dormancy in minitubers. The most important response is in application of this treatment combination to release dormancy of long dormant cultivars. In this case, yields of seed tubers increased from 2 to 5 kg/5 minitubers compared to the control treatment. Laboratory data using this procedure provides a good estimate of expected field results.

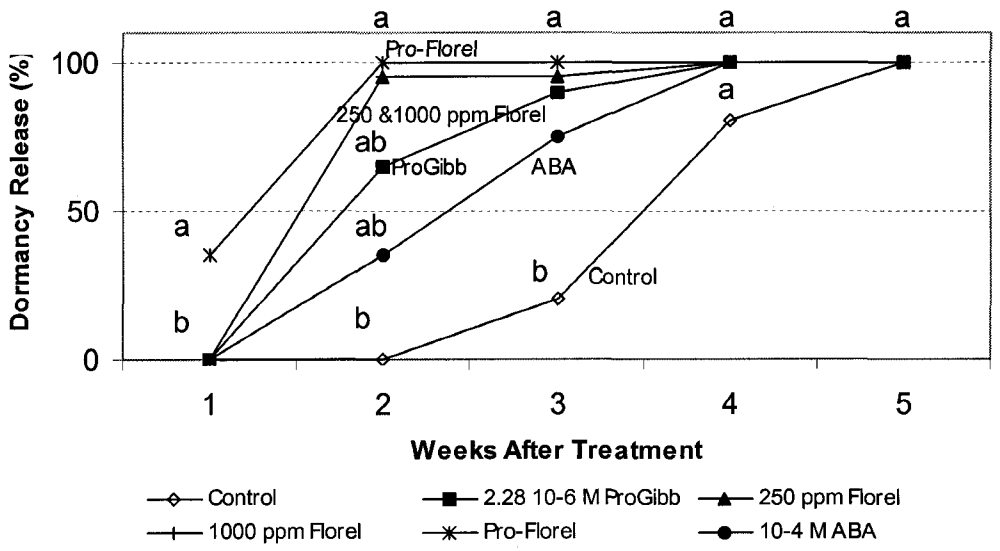


Fig. 3.1 Dormancy release of short dormancy cultivar (Silverton Russet) for 2002 freshly harvested greenhouse produced minitubers held at 15±1 °C in the dark at 95 to 100 % relative humidity for five weeks. Data are percent of minitubers with sprouts (≥2mm). For each data point n=20

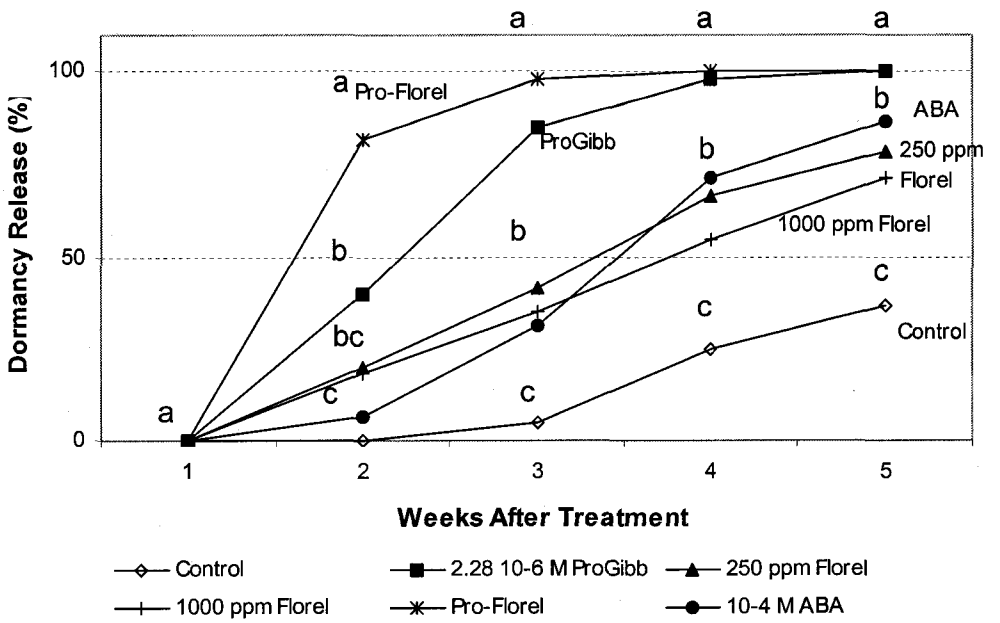


Fig. 3.2 Dormancy release of intermediate dormancy cultivars (RNK, Sangre-S14, Desiree) for 2002 freshly harvested greenhouse produced minitubers held at 15±1 °C in the dark at 95 to 100 % relative humidity for five weeks. Data are percent of minitubers with sprouts (≥2mm). For each data point n=60.

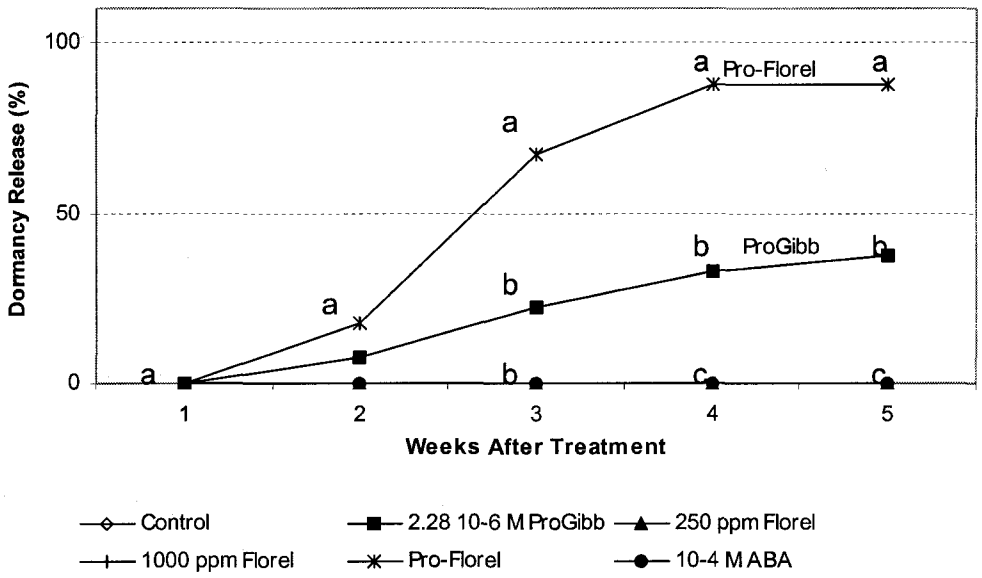


Fig. 3.3 Dormancy release of long dormancy cultivars (RNK-S3, Nooksack) for 2002 freshly harvested greenhouse produced minitubers held at 15±1 °C in the dark at 95 to 100 % relative humidity for five weeks. Data are percent of minitubers with sprouts (≥2mm). For each data point n=40. The control, 250 ppm Florel and 1000 ppm Florel treatments produced no sprouts over the five weeks interval.

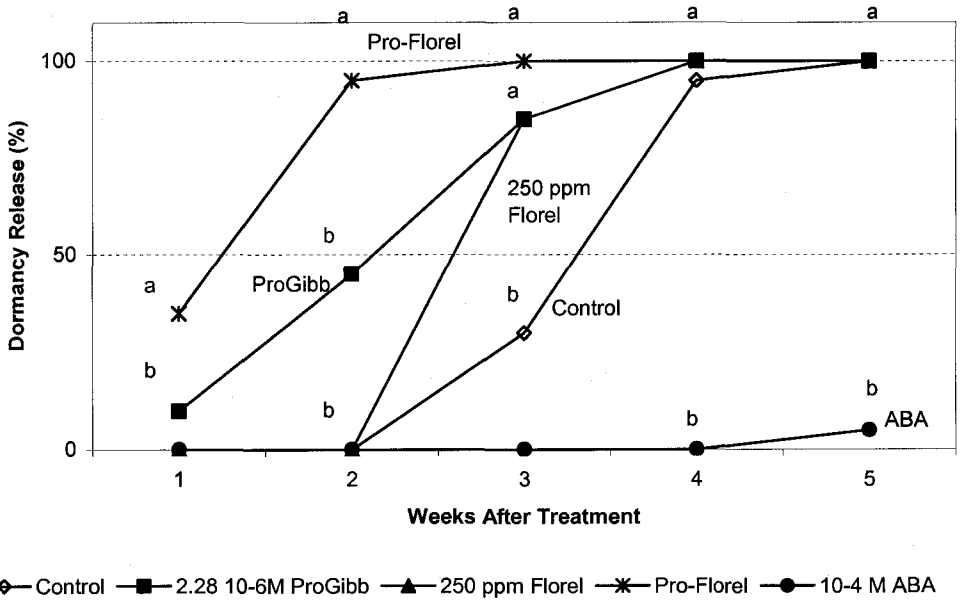


Fig. 3.4 Dormancy release of short dormancy cultivar (Silverton Russet) for 2003 freshly harvested greenhouse produced minitubers held at 15±1 °C in the dark at 95 to 100 % relative humidity for five weeks. Data are percent of minitubers with sprouts (≥2mm). For each data point n=20.

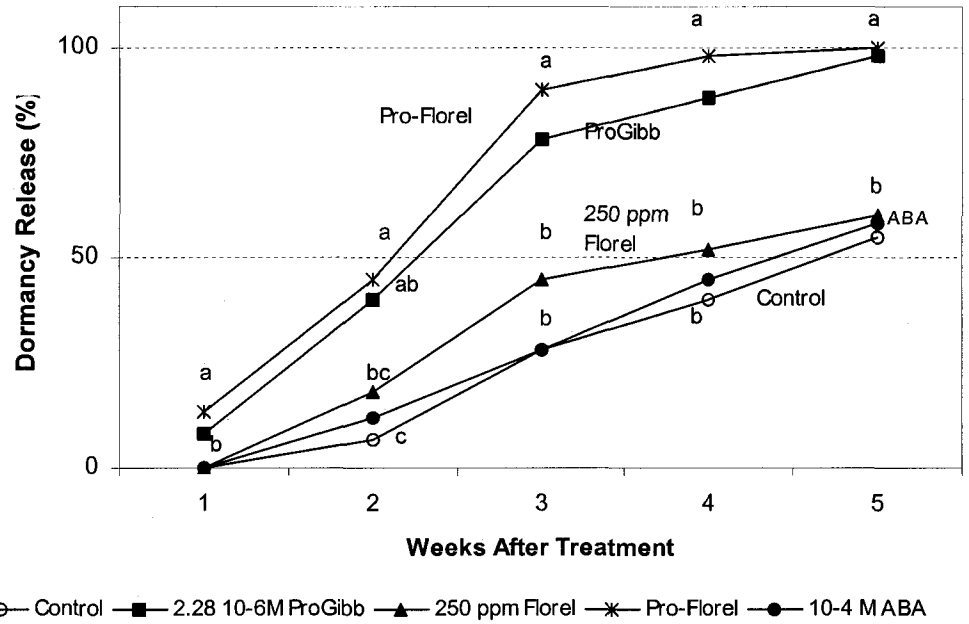


Fig. 3.5 Dormancy release of intermediate dormancy cultivars (RNK, Sangre-S14, Desiree) for 2003 freshly harvested greenhouse produced minitubers held at 15±1 °C in the dark at 95 to 100 % relative humidity for five weeks. Data are percent of minitubers with sprouts (≥2mm). For each data point n=60.

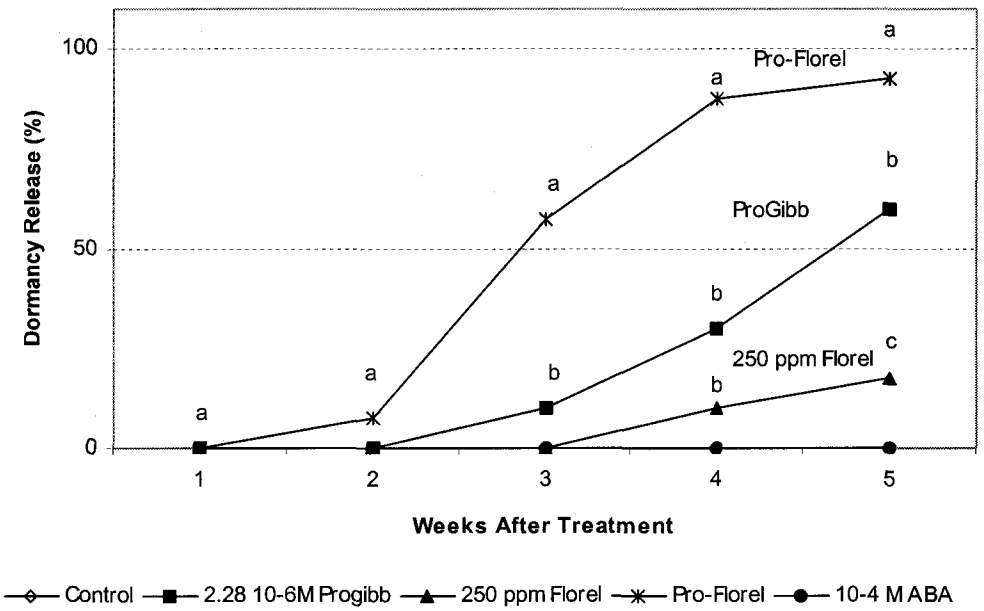


Fig. 3.6 Dormancy release of long dormancy cultivars (RNK-S3, Nooksack) for 2003 freshly harvested greenhouse produced minitubers held at 15±1 °C in the dark at 95 to 100 % relative humidity for five weeks. Data are percent of minitubers with sprouts (≥2mm). For each data point n=40. The control, and ABA treatments produced no sprouts over the five weeks interval.

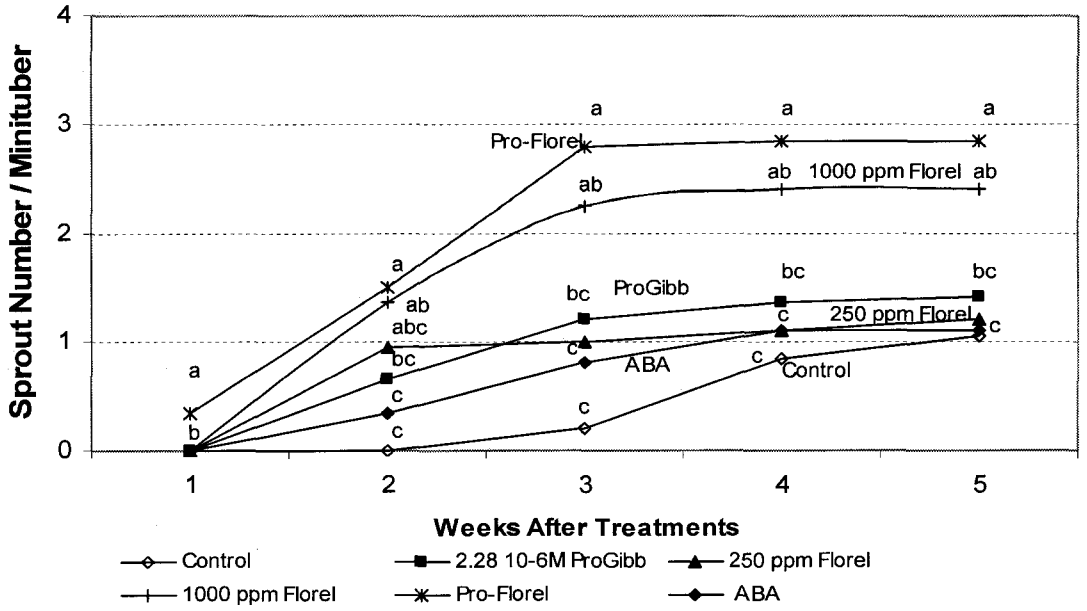


Fig. 3.7 Short dormancy cultivar (Silverton Russet) sprout number in 2002. Sprout number based upon mean number of sprouts (≥2mm). For each data point n=20. Treatments with the same letter are not significantly different at $p<0.05$ by Bonferroni's Method of Multiple Comparisons (SAS).

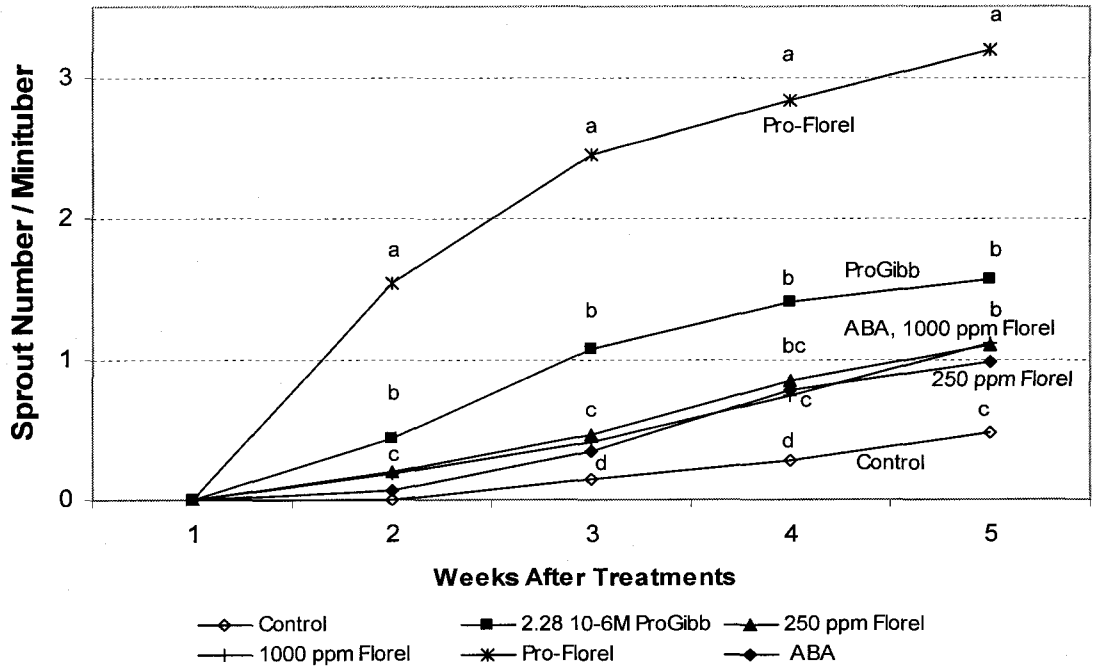


Fig. 3.8 Intermediate dormancy cultivars (Russet Norkotah, Sangre-S14, Desiree) sprout number in 2002. Sprout number based upon mean number of sprouts (≥2mm). For each data point n=60. Treatments with the same letter are not significantly different at $p<0.05$ by Bonferroni's Method of Multiple Comparisons (SAS).

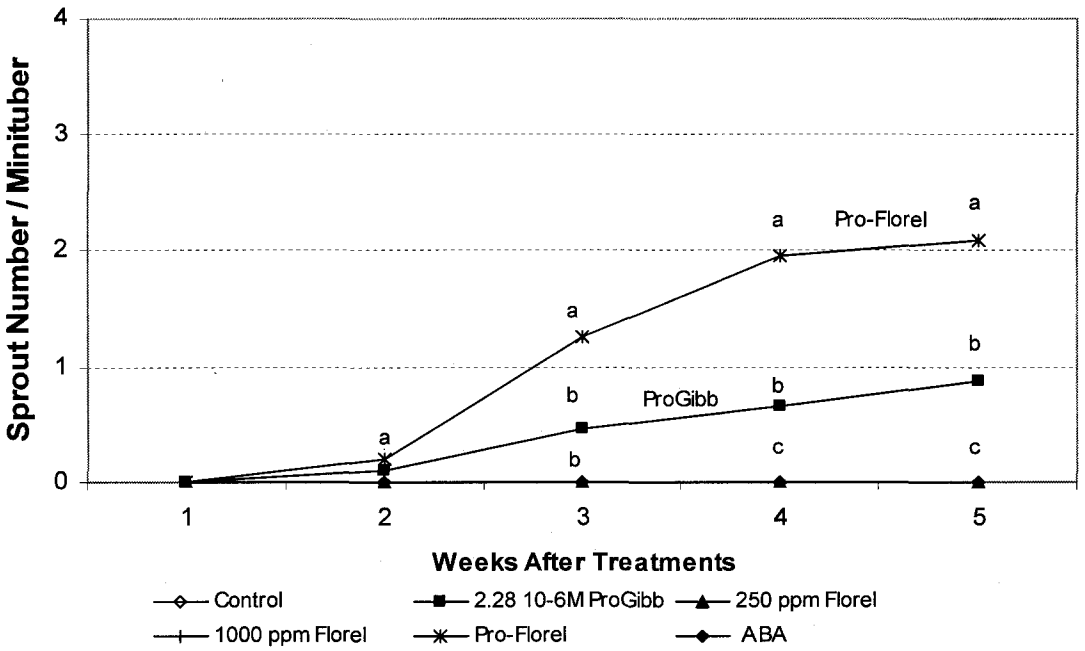


Fig. 3.9 Long dormancy cultivars (RNK-S3, Nooksack) sprout number in 2002. Sprout number based upon mean number of sprouts (≥2mm). For each data point n=40. Treatments with the same letter are not significantly different at $p<0.05$ by Bonferroni's Method of Multiple Comparisons (SAS). The control, 250 ppm florel and 1000 ppm florel treatments produced no sprouts over the five weeks interval.

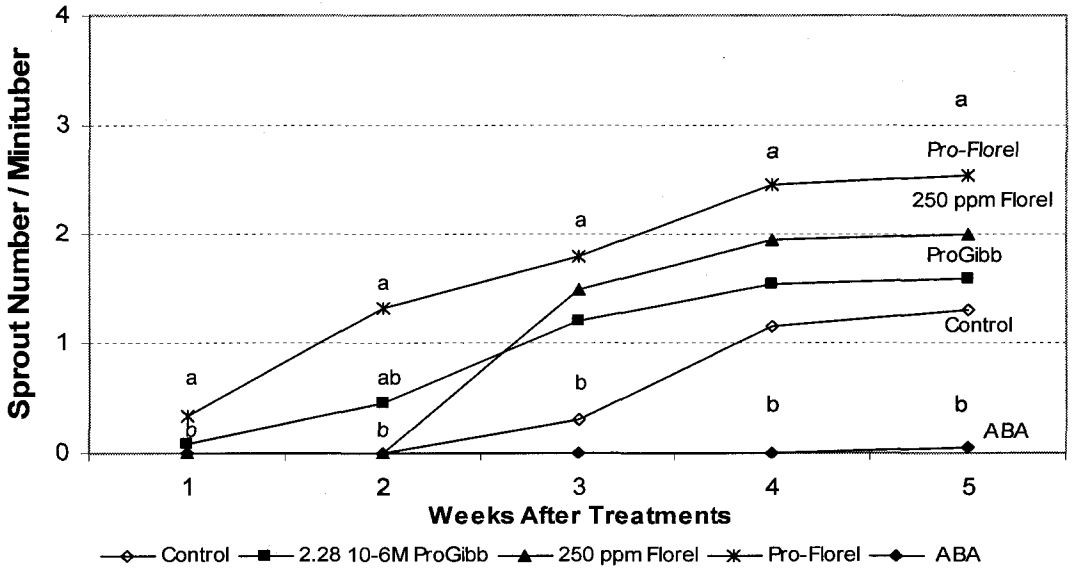


Fig. 3.10 Short dormancy cultivar (Silverton Russet) sprout number in 2003. Sprout number based upon mean number of sprouts (> 2mm). For each data point n=20. Treatments with the same letter are not significantly different at $p<0.05$ by Bonferroni's Method of Multiple Comparisons (SAS).

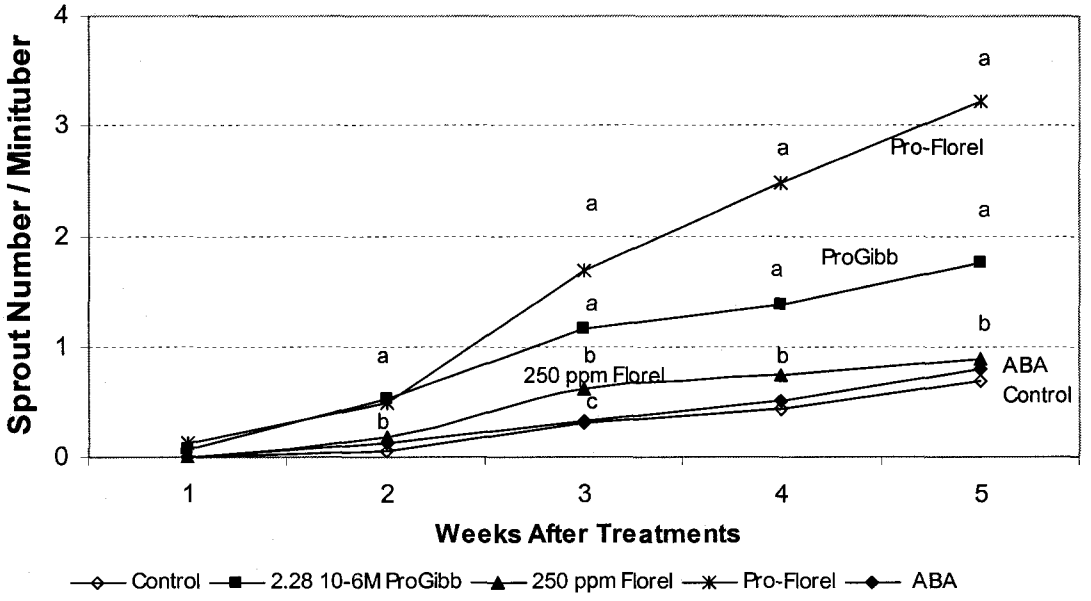


Fig. 3.11 Intermediate dormancy cultivars (RNK, Sangre-S14, Desiree) sprout number in 2003. Sprout number based upon mean number of sprouts (> 2mm). For each data point n=60. Treatments with the same letter are not significantly different at $p<0.05$ by Bonferroni's Method of Multiple Comparisons (SAS).

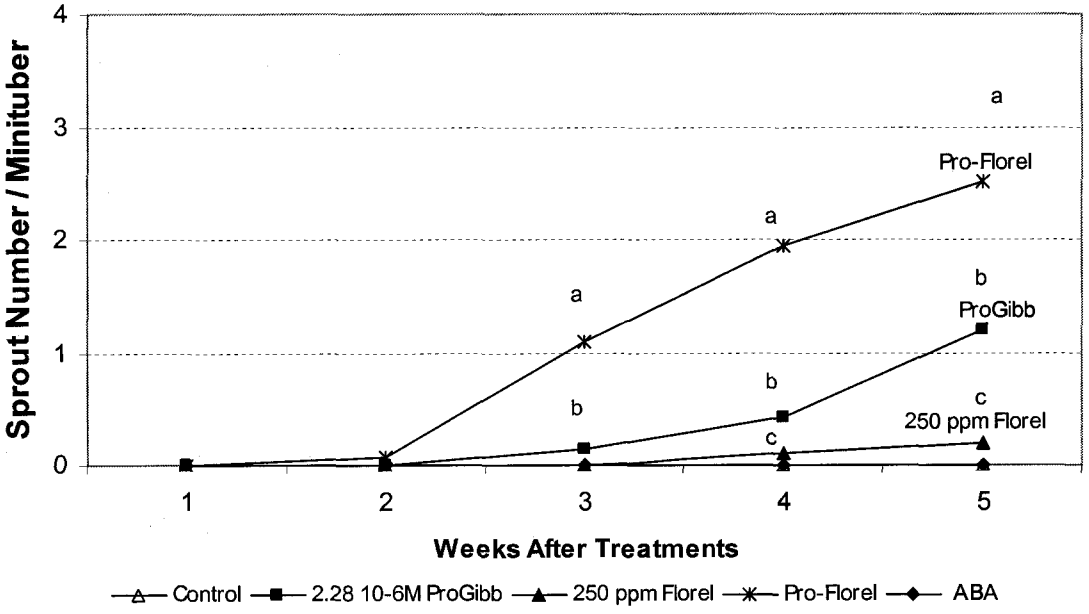


Fig. 3.12 Long dormancy cultivars (RNK-S3, Nooksack) sprout number in 2003. Sprout number based upon mean number of sprouts (> 2mm). For each data point n=40. Treatments with the same letter are not significantly different at $p<0.05$ by Bonferroni's Method of Multiple Comparisons (SAS). The control and 250 ppm Florel treatments produced no sprouts over the five weeks interval.

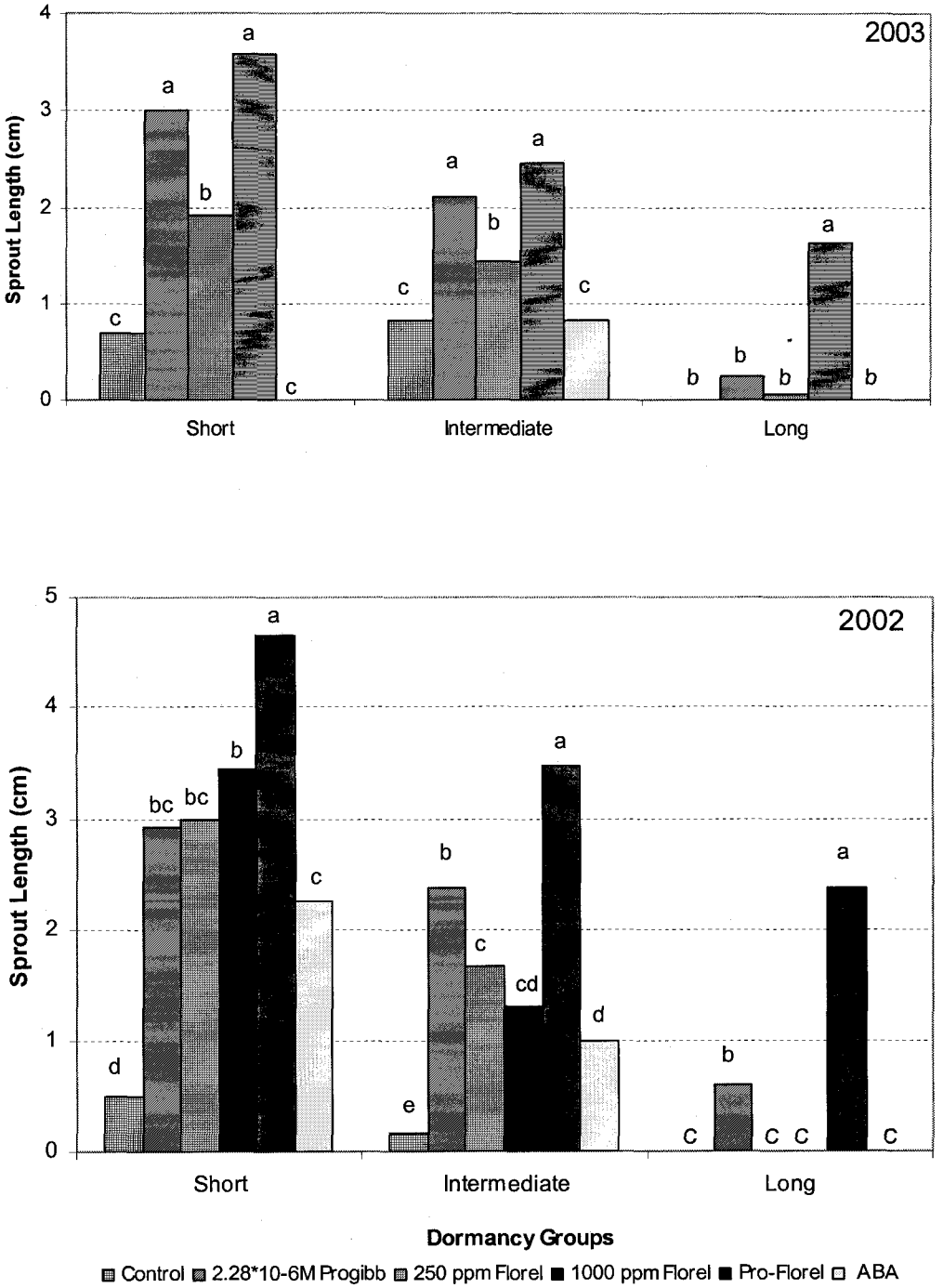


Fig. 3.13 Sprout length (cm) of the longest sprout/minituber, 5 weeks after treatment in 2002 and 2003 for the three dormancy groups. For each treatment n=20. Treatments with the same letter within each dormancy group are not significantly different at $p<0.05$ by Bonferroni's Method of Multiple Comparisons (SAS). Potato cultivars are grouped by dormancy period [short (Silverton Russet), Intermediate (RNK, Sangre-S14, Desiree), and long (RNK-S3, Nooksack)].

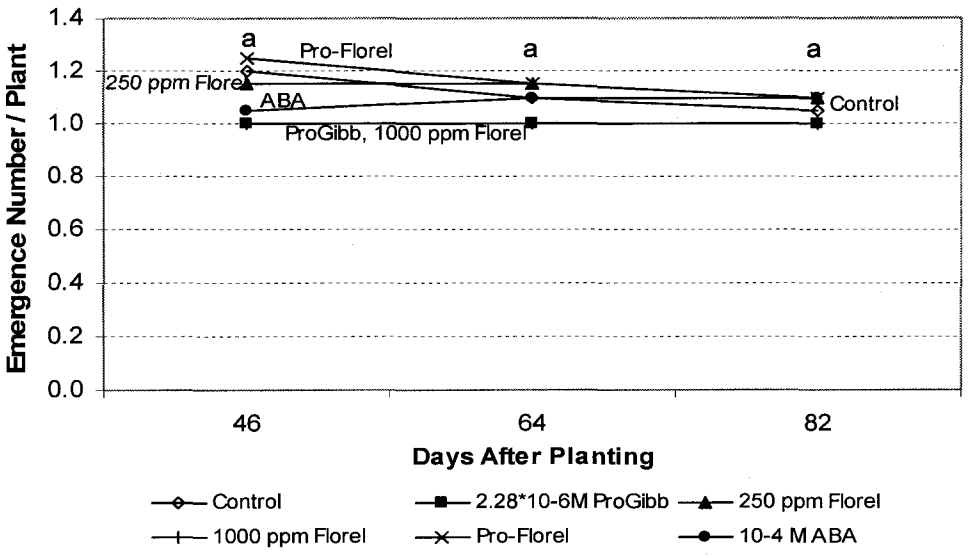


Fig. 3.14 Short dormancy cultivar (Silverton Russet) emergence numbers in 2002. Emergence based upon number of shoots per minituber, observed days after planting (DAP) at Colorado State University, San Luis Valley Research Center, CO. For each data point n=20. Treatments with the same letter are not significantly different at $p<0.05$ by Bonferroni's Method of Multiple Comparisons (SAS). There is no significant differences at 42 and 66 DAP.

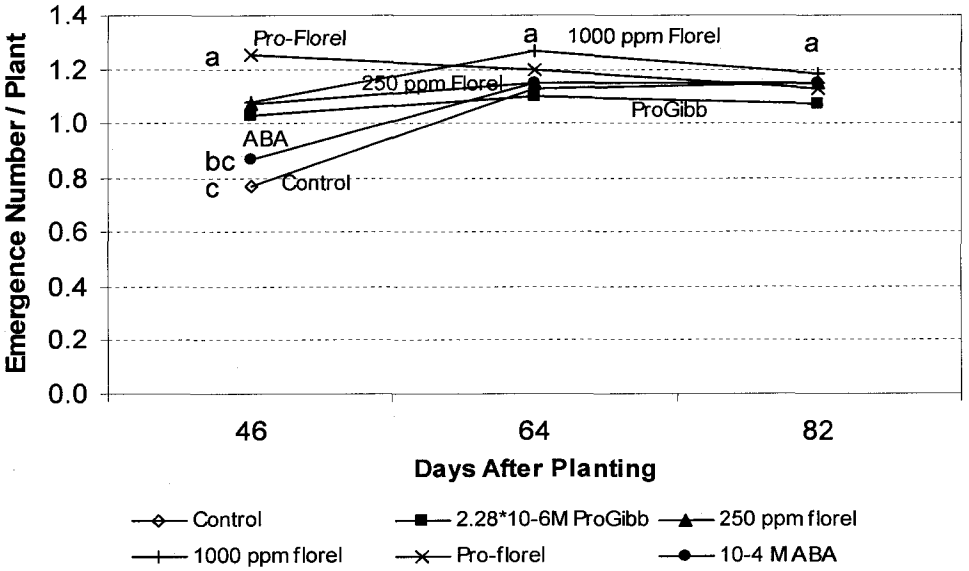


Fig. 3.15 Intermediate dormant cultivars (RNK, Sangre-S14, Desiree) emergence numbers in 2002. Emergence based upon number of shoots per minituber, observed days after planting (DAP) at Colorado State University, San Luis Valley Research Center, CO. For each data point n=60. Treatments with the same letter are not significantly different at $p<0.05$ by Bonferroni's Method of Multiple Comparisons (SAS).

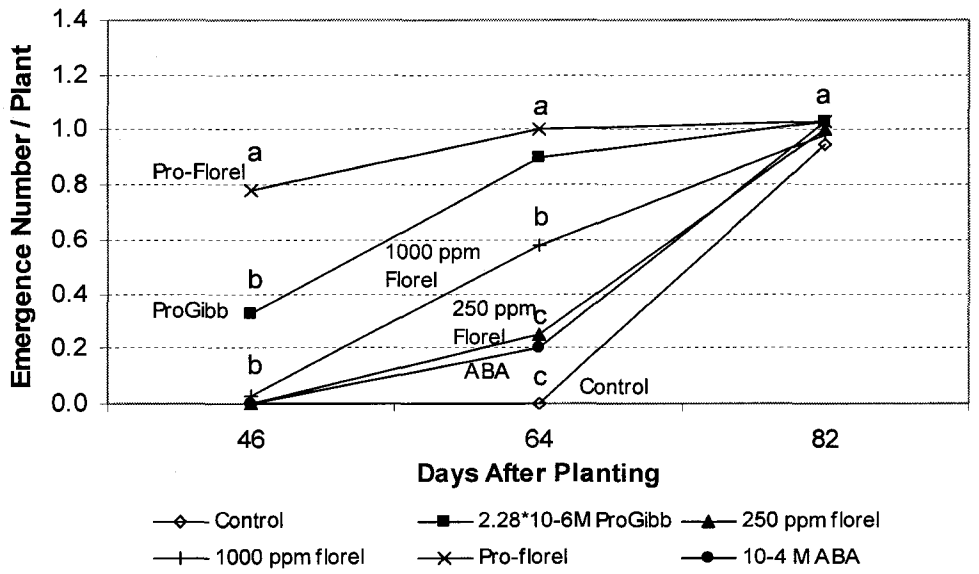


Fig. 3.16 Long dormancy cultivars (RNK-S3, Nooksack) emergence numbers in 2002. Emergence based upon number of shoots per minituber, observed days after planting (DAP) at Colorado State University, San Luis Valley Research Center, CO. For each data point n=40. Treatments with the same letter are not significantly different at $p<0.05$ by Bonferroni's Method of Multiple Comparisons (SAS).

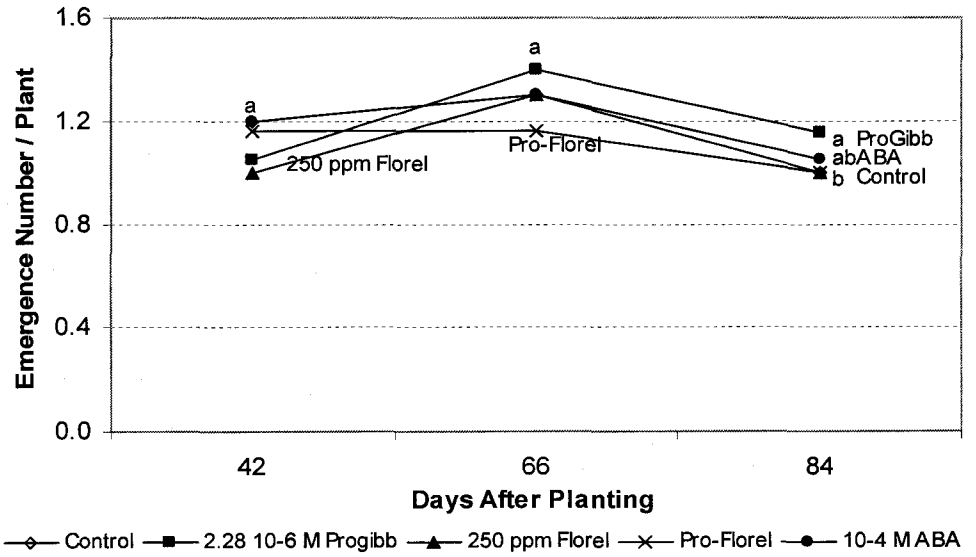


Fig. 3.17 Short dormancy cultivar (Silverton Russet) emergence numbers in 2003. Emergence based upon number of shoots per minituber, observed days after planting (DAP) at Colorado State University, San Luis Valley Research Center, CO. For each data point n=20. Treatments with the same letter are not significantly different at $p<0.05$ by Bonferroni's Method of Multiple Comparisons (SAS). There is no significant differences at 42 and 66 DAP.

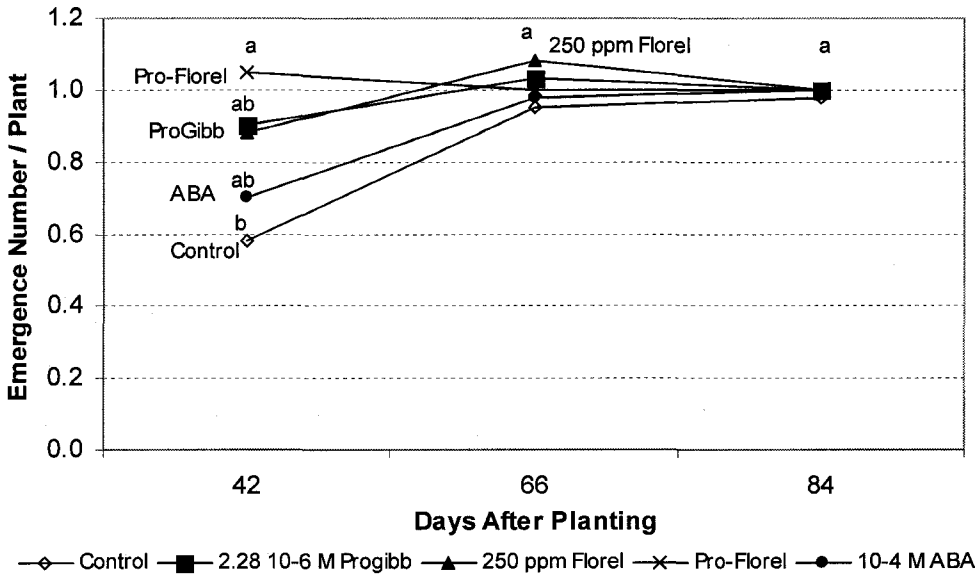


Fig. 3.18 Intermediate dormant cultivars (RNK, Sangre-S14, Desiree) emergence numbers in 2003. Emergence based upon number of shoots per minituber, observed days after planting (DAP) at Colorado State University, San Luis Valley Research Center, CO. For each data point n=60. Treatments with the same letter are not significantly different at $p<0.05$ by Bonferroni's Method of Multiple Comparisons (SAS).

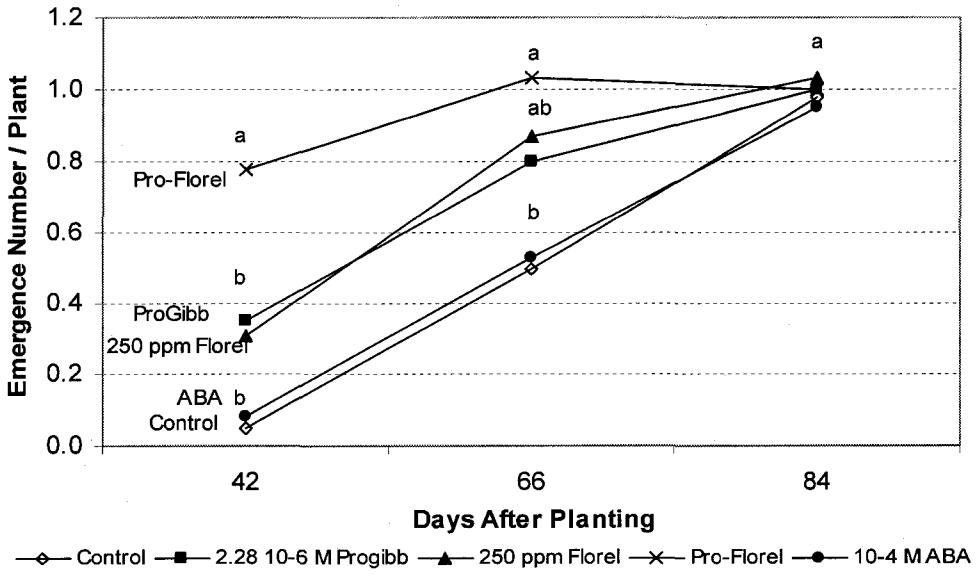


Fig. 3.19 Long dormancy cultivars (RNK-S3, Nooksack) emergence numbers in 2003. Emergence based upon number of shoots per minituber, observed days after planting (DAP) at Colorado State University, San Luis Valley Research Center, CO. For each data point n=40. Treatments with the same letter are not significantly different at $p<0.05$ by Bonferroni's Method of Multiple Comparisons (SAS).

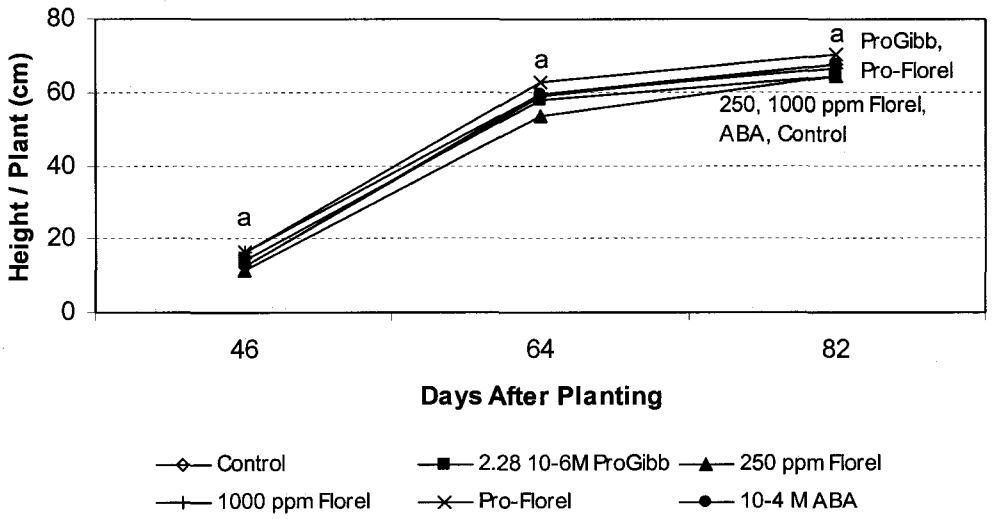


Fig. 3.20 Short dormancy cultivar (Silverton Russet) plant height in 2002. Plant height (cm) observed days after planting (DAP) at Colorado State University, San Luis Valley Research Center, CO. For each data point n=20. Treatments with the same letter are not significantly different at $p<0.05$ by Bonferroni's Method of Multiple Comparisons (SAS).

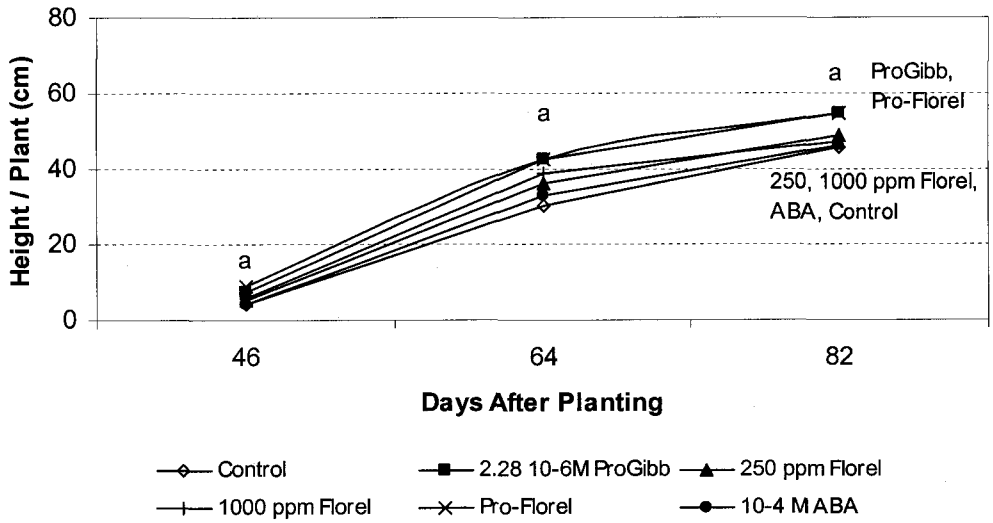


Fig. 3.21 Intermediate dormancy cultivars (RNK, Sangre-S14, Desiree) plant height in 2002. Plant height (cm) observed days after planting (DAP) at Colorado State University, San Luis Valley Research Center, CO. For each data point n=60. Treatments with the same letter are not significantly different at $p<0.05$ by Bonferroni's Method of Multiple Comparisons (SAS).

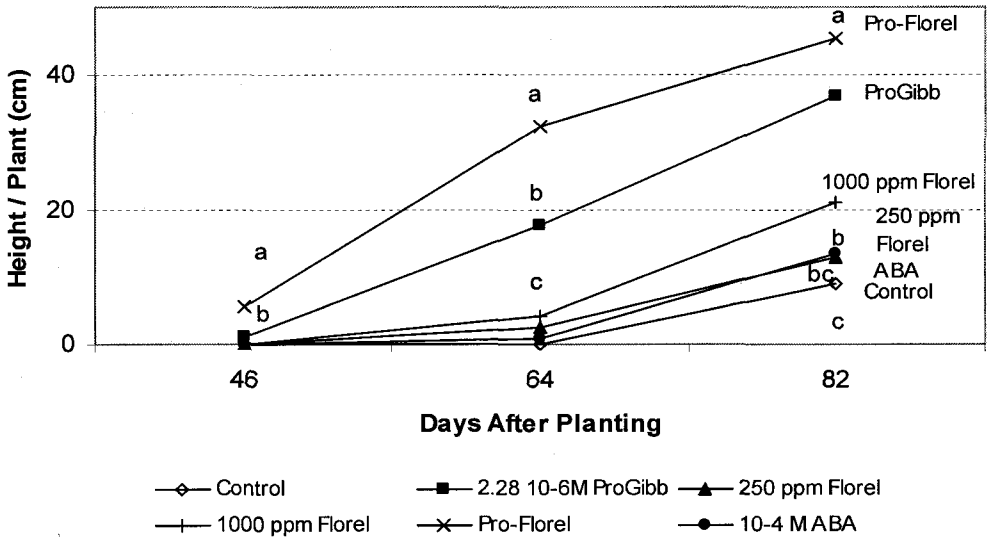


Fig. 3.22 Long dormancy cultivars (RNK-S3, Nooksack) plant height in 2002. Plant height (cm) observed days after planting (DAP) at Colorado State University, San Luis Valley Research Center, CO. For each data point n=40. Treatments with the same letter are not significantly different at $p<0.05$ by Bonferroni's Method of Multiple Comparisons (SAS).

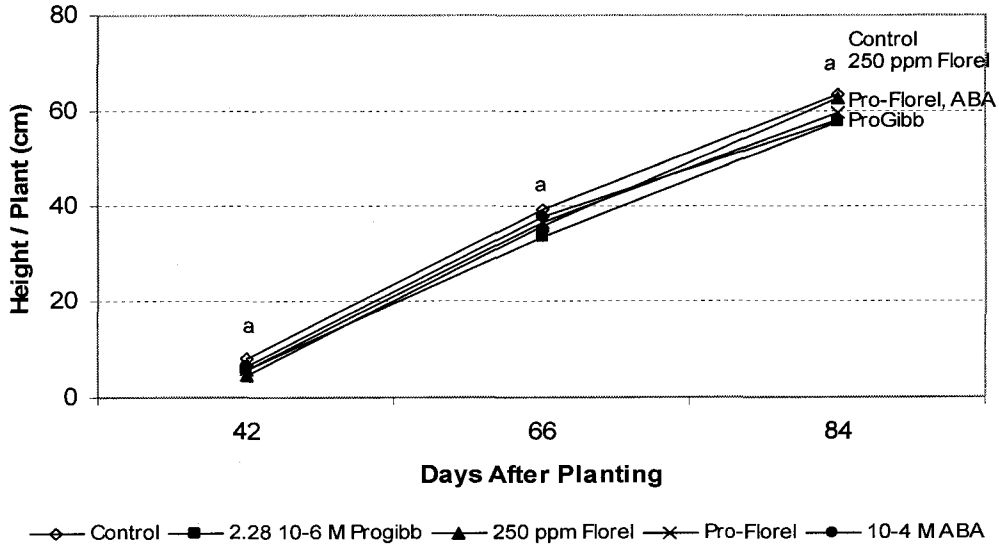


Fig. 3.23 Short dormancy cultivar (Silverton Russet) plant height in 2003. Plant Height (cm) observed days after planting (DAP) at Colorado State University, San Luis Valley Research Center, CO. For each data point n=20. Treatments with the same letter are not significantly different at $p<0.05$ by Bonferroni's Method of Multiple Comparisons (SAS).

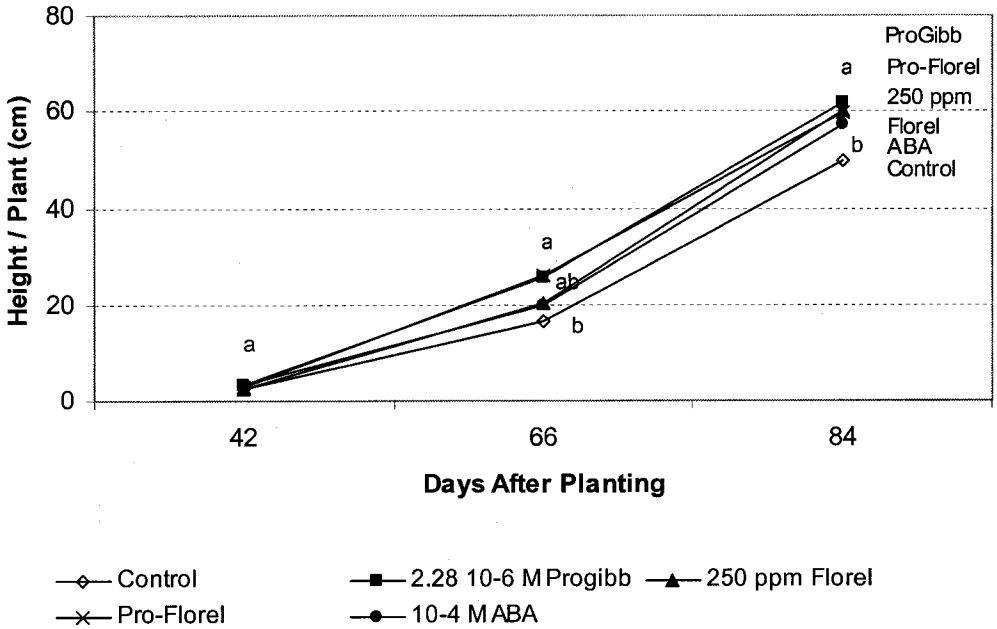


Fig. 3.24 Intermediate dormancy cultivars (RNK, Sangre-S14, Desiree) plant height in 2003. Plant Height (cm) observed days after planting (DAP) at Colorado State University, San Luis Valley Research Center, CO. For each data point n=60. Treatments with the same letter are not significantly different at $p<0.05$ by Bonferroni's Method of Multiple Comparisons (SAS).

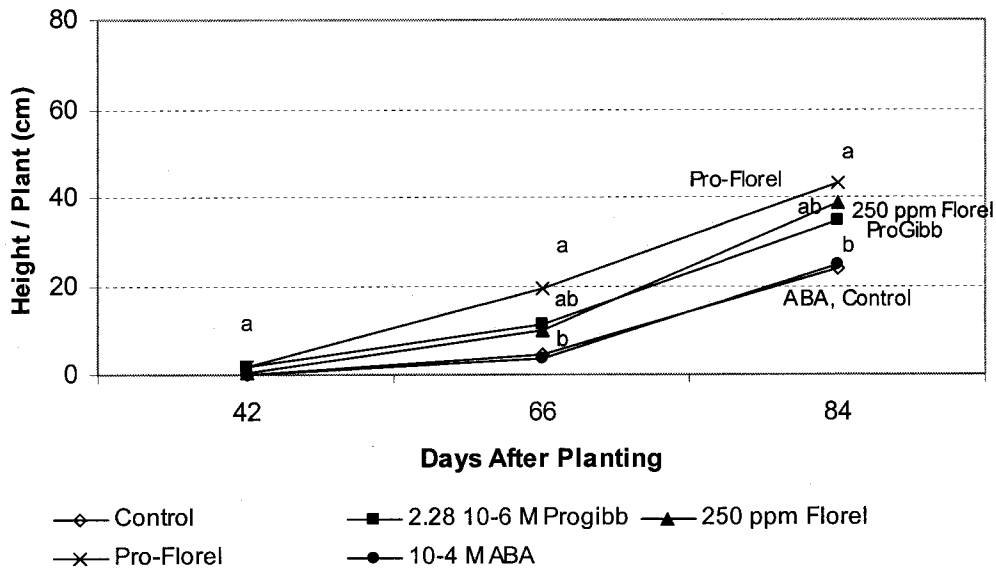


Fig. 3.25 Long dormancy cultivars (RNK-S3, Nooksack) plant height in 2003. Plant Height (cm) observed days after planting (DAP) at Colorado State University, San Luis Valley Research Center, CO. For each data point n=40. Treatments with the same letter are not significantly different at $p<0.05$ by Bonferroni's Method of Multiple Comparisons (SAS).

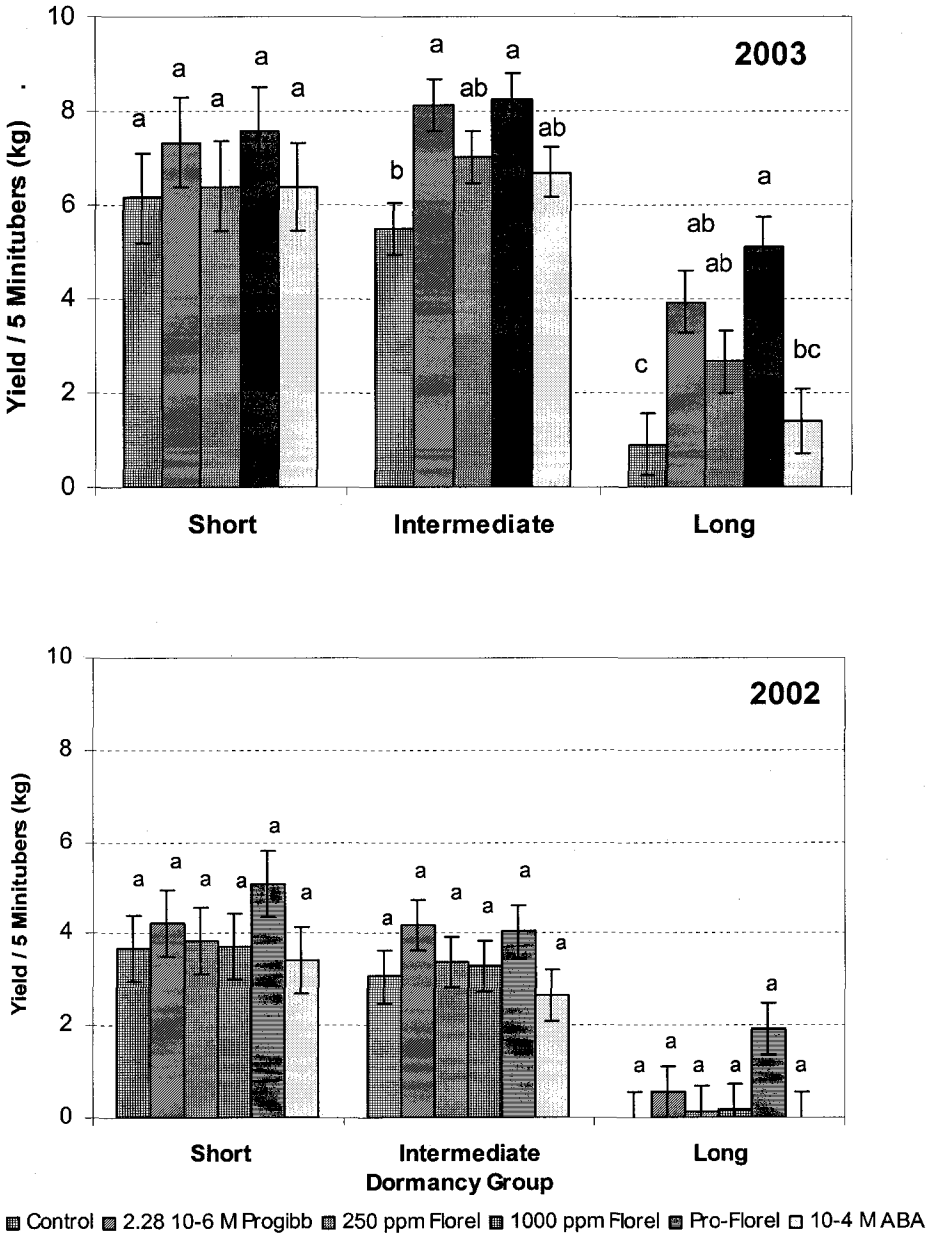


Fig. 3.26 2002 and 2003 San Luis Valley yield based upon production from 5 minitubers/plot (kg/5 minitubers). Error bars are standard error of means. For each treatment n=20. Treatments with the same letter are not significantly different at $p<0.05$ by Bonferroni's Method of Multiple Comparisons (SAS) within each dormancy group. Potato cultivars grouped by dormancy period [short (Silverton Russet), intermediate (RNK, Sangre-S14, Desiree), and long (RNK-S3, Nooksack)]. 2002 and 2003 yields were different in magnitude but correlated very well $r=0.7423$ ($p<0.0001$) suggest that treatment effects were consistent from one year to the seasonal refer to Fig 2002 and 2003 to show yields.

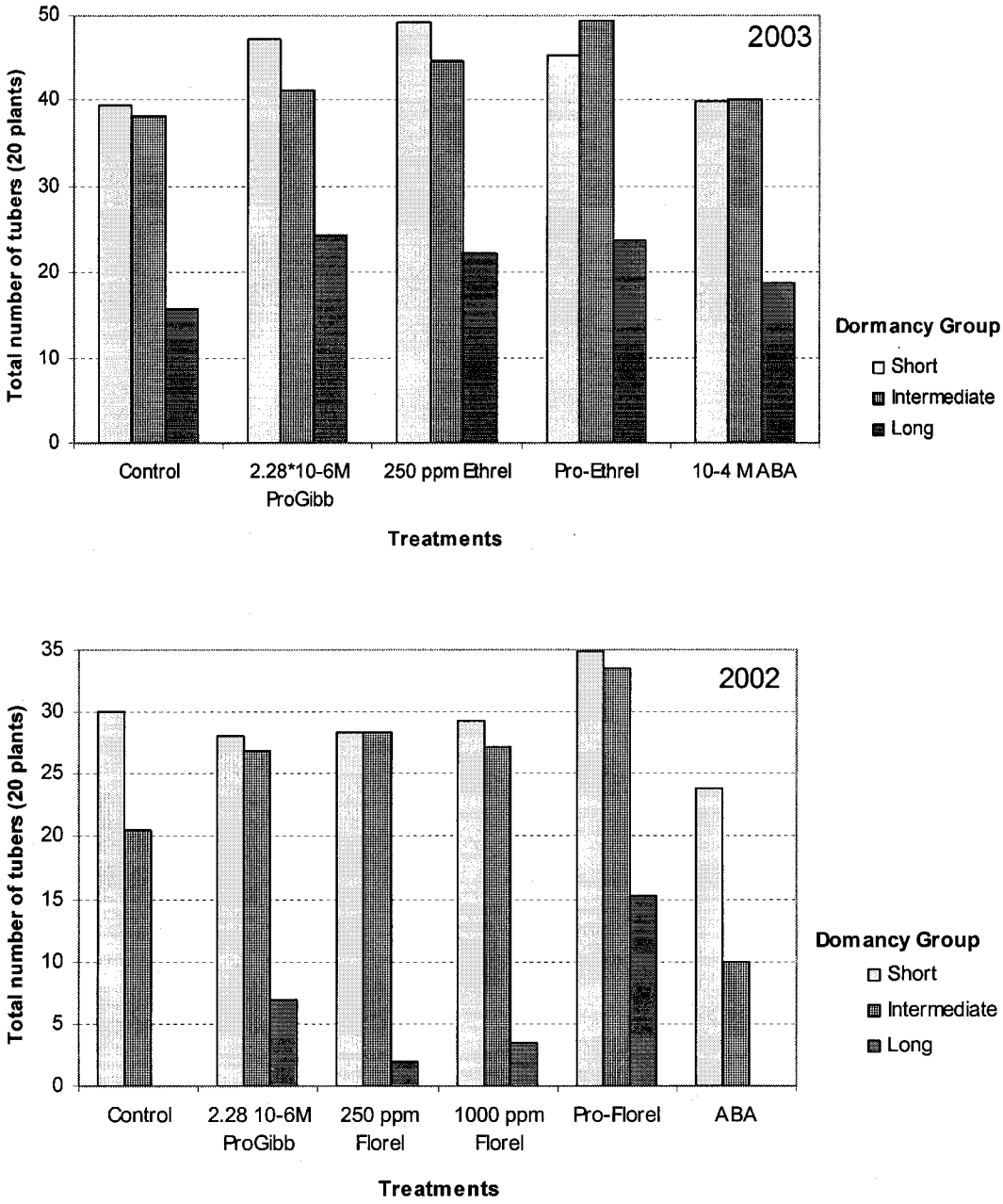


Fig. 3.27 Total number of tubers from the 2002 and 2003 San Luis Valley harvest of treated minitubers. Potato cultivars are grouped by dormancy period [short (Silverton Russet), intermediate (Desiree, RNK, Sangre-S14), and long (RNK-S3, Nooksack)].

Table 3.1 Dormancy release of short (Silverton Russet), intermediate (RNK, Sangre-S14, Desiree) and long (RNK-S3, Nooksack) dormancy group cultivars for freshly harvested greenhouse produced minitubers held at 15±1 °C in the dark at 95 to 100 % relative humidity for five weeks in 2002. Data are percent of minitubers with sprouts (2mm) from four replicates of five minitubers per treatment combination.

	Dormancy Group														
	Short					Intermediate					Long				
	Week					Week					Week				
Treatments	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Control	0^{B}	0^{B}	20^{B}	80^{A}	100^{A}	0^{A}	0^{C}	5^{C}	25^{C}	37^{C}	0^{A}	0^{A}	0^{B}	0^{C}	0^{C}
$2.28\ 10^{-6}$ M ProGibb	0^{B}	65^{AB}	90^{A}	100^{A}	100^{A}	0^{A}	40^{B}	85^{A}	98^{A}	100^{A}	0^{A}	8^{A}	23^{B}	33^{B}	38^{B}
250 ppm Florel	0^{B}	95^{A}	95^{A}	100^{A}	100^{A}	0^{A}	20^{BC}	42^{B}	67^{B}	78^{B}	0^{A}	0^{A}	0^{B}	0^{C}	0^{C}
1000 ppm Florel	0^{B}	95^{A}	95^{A}	100^{A}	100^{A}	0^{A}	18^{BC}	35^{B}	55^{B}	72^{B}	0^{A}	0^{A}	0^{B}	0^{C}	0^{C}
Pro-florel	35^{A}	100^{A}	100^{A}	100^{A}	100^{A}	0^{A}	82^{A}	98^{A}	100^{A}	100^{A}	0^{A}	18^{A}	68^{A}	88^{A}	88^{A}
10^{-4} M ABA	0^{B}	35^{AB}	75^{A}	100^{A}	100^{A}	0^{A}	7^{C}	32^{B}	72^{B}	87^{B}	0^{A}	0^{A}	0^{B}	0^{C}	0^{C}

$^{A\rightarrow B}$Means followed by the same letter within columns are not significantly different ($p<0.05$) based on Bonferroni's Mean Separation test.

Table 3.2 Dormancy release of short (Silverton Russet), intermediate (RNK, Sangre-S14, Desiree) and long (RNK-S3, Nooksack) dormancy group cultivars for freshly harvested greenhouse produced minitubers held at 15±1 °C in the dark at 95 to 100 % relative humidity for five weeks in 2003. Data are percent of minitubers with sprouts (2mm) from four replicates of five minitubers per treatment combination.

	Dormancy Group														
	Short					Intermediate					Long				
	Week					Week					Week				
Treatments	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Control	0^{B}	0^{B}	30^{B}	95^{A}	100^{A}	0^{B}	7^{C}	28^{B}	40^{B}	55^{B}	0^{A}	0^{A}	0^{B}	0^{B}	0^{C}
$2.28\ 10^{-6}$ M ProGibb	10^{B}	45^{B}	85^{A}	100^{A}	100^{A}	8^{A}	40^{AB}	78^{A}	88^{A}	98^{A}	0^{A}	0^{A}	0^{B}	30^{B}	60^{B}
250 ppm Florel	0^{B}	0^{B}	85^{A}	100^{A}	100^{A}	0^{B}	18^{BC}	45^{B}	52^{B}	60^{B}	0^{A}	0^{A}	0^{B}	10^{B}	18^{C}
Pro-florel	35^{A}	95^{A}	100^{A}	100^{A}	100^{A}	13^{A}	45^{A}	90^{A}	98^{A}	100^{A}	0^{A}	8^{A}	58^{A}	88^{A}	93^{A}
10^{-4} M ABA	0^{B}	0^{B}	30^{B}	0^{B}	5^{B}	0^{B}	12^{C}	28^{B}	45^{B}	58^{B}	0^{A}	0^{A}	0^{B}	0^{B}	0^{C}

$^{A\rightarrow B}$ Means followed by the same letter within columns are not significantly different ($p<0.05$) based on Bonferroni's Mean Separation test.

Table 3.3 Sprout number of short (Silverton Russet), intermediate (RNK, Sangre-S14, Desiree) and long (RNK-S3, Nooksack) dormancy group cultivars for freshly harvested greenhouse produced minitubers held at 15±1 °C in the dark at 95 to 100 % relative humidity for five weeks in 2002. Data are mean number of sprouts (2mm) from four replicates of five minitubers per treatment combination.

	Dormancy Group														
	Short					Intermediate					Long				
	Week					Week					Week				
Treatments	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Control	0.0^{B}	0.0^{C}	0.2^{D}	0.9^{C}	1.1^{C}	0.0^{A}	0.0^{C}	0.1^{D}	0.3^{D}	0.5^{C}	0.0^{A}	0.0^{A}	0.0^{B}	0.0^{C}	0.0^{C}
$2.28\ 10^{-6}$ M ProGibb	0.0^{B}	0.7^{BC}	1.2^{BC}	1.4^{BC}	1.4^{BC}	0.0^{A}	0.4^{B}	1.1^{B}	1.4^{B}	1.6^{B}	0.0^{A}	0.1^{A}	0.5^{B}	0.7^{B}	0.9^{B}
250 ppm Florel	0.0^{B}	1.0^{ABC}	1.0^{C}	1.1^{C}	1.2^{C}	0.0^{A}	0.2^{C}	0.5^{C}	0.9^{BC}	1.1^{B}	0.0^{A}	0.0^{A}	0.0^{B}	0.0^{C}	0.0^{C}
1000 ppm Florel	0.0^{B}	1.4^{AB}	2.3^{AB}	2.4^{AB}	2.4^{AB}	0.0^{A}	0.2^{C}	0.4^{C}	0.8^{C}	1.1^{B}	0.0^{A}	0.0^{A}	0.0^{B}	0.0^{C}	0.0^{C}
Pro-florel	0.4^{A}	1.5^{A}	2.8^{A}	2.9^{A}	2.9^{A}	0.0^{A}	1.6^{A}	2.5^{A}	2.8^{A}	3.2^{A}	0.0^{A}	0.2^{A}	1.3^{A}	2.0^{A}	2.1^{A}
10^{-4} M ABA	0.0^{B}	0.4^{C}	0.8^{CD}	1.1^{C}	1.1^{C}	0.0^{A}	0.1^{C}	0.4^{C}	0.8^{C}	1.0^{B}	0.0^{A}	0.0^{A}	0.0^{B}	0.0^{C}	0.0^{C}

$^{A\rightarrow B}$ Means followed by the same letter within columns are not significantly different ($p<0.05$) based on Bonferroni's Mean Separation test.

Table 3.4 Sprout number of short (Silverton Russet), intermediate (RNK, Sangre-S14, Desiree) and long (RNK-S3, Nooksack) dormancy group cultivars for freshly harvested greenhouse produced minitubers held at 15±1 °C in the dark at 95 to 100 % relative humidity for five weeks in 2003. Data are mean number of sprouts (2mm) from four replicates of five minitubers per treatment combination.

	Dormancy Group														
	Short					Intermediate					Long				
	Week					Week					Week				
Treatments	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Control	0.0^{B}	0.0^{B}	0.3^{B}	1.2^{B}	1.3^{B}	0.0^{B}	0.1^{B}	0.3^{B}	0.4^{C}	0.7^{C}	0.0^{A}	0.0^{A}	0.0^{B}	0.0^{B}	0.0^{C}
$2.28\ 10^{-6}$ M ProGibb	0.1^{AB}	0.5^{AB}	1.2^{A}	1.6^{AB}	1.6^{A}	0.1^{A}	0.6^{A}	1.2^{A}	1.4^{B}	1.8^{B}	0.0^{A}	0.0^{A}	0.2^{B}	0.4^{B}	1.2^{B}
250 ppm Florel	0.0^{B}	0.0^{B}	1.5^{A}	2.0^{AB}	2.0^{A}	0.0^{B}	0.2^{B}	0.6^{B}	0.7^{C}	0.9^{C}	0.0^{A}	0.0^{A}	0.0^{B}	0.1^{B}	0.2^{C}
Pro-florel	0.4^{A}	1.3^{A}	1.8^{A}	2.5^{A}	2.6^{A}	0.1^{A}	0.5^{A}	1.7^{A}	2.5^{A}	3.2^{A}	0.0^{A}	0.1^{A}	1.1^{A}	2.0^{A}	2.5^{A}
10^{-4} M ABA	0.0^{B}	0.0^{B}	0.0^{B}	0.0^{C}	0.1^{C}	0.0^{B}	$0.1B^{C}$	0.3^{B}	0.6^{C}	0.8^{C}	0.0^{A}	0.0^{A}	0.0^{B}	0.0^{B}	0.0^{C}

$^{A\rightarrow B}$ Means followed by the same letter within columns are not significantly different ($p<0.05$) based on Bonferroni's Mean Separation test.

Table 3.5 2002 yield based upon production from 5 minitubers/plot (kg/5minitubers). Potato cultivars grouped by dormancy period [short (Silverton Russet), intermediate (RNK, Sangre-S14, Desiree), and long (RNK-S3, Nooksack)].

Treatments	Dormancy Groups		
	Short	Intermediate	Long
Control	3.68 ^{AZ}	3.06 ^{AZ}	0.00 ^{AY}
2.28 10^{-6} M ProGibb	4.23 ^{AZ}	4.18 ^{AZ}	0.54 ^{AY}
250 ppm Florel	3.83 ^{AZ}	3.36 ^{AZ}	0.11 ^{AY}
1000 ppm Florel	3.73 ^{AZ}	3.28 ^{AZ}	0.16 ^{AY}
Pro-florel	5.08 ^{AZ}	4.04 ^{AZY}	1.93 ^{AY}
10^{-4} M ABA	3.43 ^{AZ}	2.64 ^{AZ}	0.00 ^{AY}

$^{A\rightarrow B}$ Treatments with the same letter are not significantly different within columns and $^{Z\rightarrow Y}$ within rows at $p<0.05$ by Bonferroni's Method of Multiple Comparisons (SAS).

Table 3.6 2003 yield based upon production from 5 minitubers/plot (kg/5minitubers). Potato cultivars grouped by dormancy period [short (Silverton Russet), intermediate (RNK, Sangre-S14, Desiree), and long (RNK-S3, Nooksack)].

Treatments	Dormancy Groups		
	Short	Intermediate	Long
Control	6.15 ^{AZ}	5.48 ^{BZ}	0.91 ^{CY}
2.28 10^{-6} M	7.33 ^{AZY}	8.11 ^{AZ}	3.93 ^{ABY}
250 ppm Florel	6.40 ^{AZY}	7.01 ^{ABZ}	2.66 ^{ABCY}
Pro-florel	7.58 ^{AZY}	8.24 ^{AZ}	5.09 ^{AY}
10^{-4} M ABA	6.38 ^{AZ}	6.70 ^{ABZ}	1.40 ^{BCY}

$^{A\rightarrow B}$ Treatments with the same letter are not significantly different within columns and $^{Z\rightarrow Y}$ within rows at $p<0.05$ by Bonferroni's Method of Multiple Comparisons (SAS).

Table 3.7 Number of small (<50 g wt), intermediate (50-100 g wt) and large (>100 g wt) tubers harvested from 5 plants/replicate in 2002 at San Luis Valley from five dormancy breaking treatments. Potato cultivars grouped by dormancy period [short (Silverton Russet), intermediate (RNK, Sangre-S14, Desiree), and long (RNK-S3, Nooksack)].

	Dormancy Groups								
	Short			Intermediate			Long		
Treatment	Small	Intermediate	Large	Small	Intermediate	Large	Small	Intermediate	Large
Control	11.5^{A}	15.5^{A}	3.0^{A}	7.1^{A}	10.1^{A}	3.3^{A}	0.0^{A}	0.0^{B}	0.0^{A}
$2.28\ 10^{-6}$ M ProGibb	5.3^{A}	16.0^{A}	6.8^{A}	7.8^{A}	13.3^{A}	5.8^{A}	4.6^{A}	2.3^{B}	0.0^{A}
250 ppm Florel	7.0^{A}	19.3^{A}	2.0^{A}	14.5^{A}	11.6^{A}	2.3^{A}	1.4^{A}	0.6^{B}	0.0^{A}
1000 ppm Florel	9.0^{A}	17.8^{A}	2.5^{A}	15.3^{A}	8.3^{A}	3.5^{A}	2.8^{A}	0.6^{B}	0.0^{A}
Pro-florel	9.3^{A}	22.5^{A}	3.0^{A}	16.1^{A}	14.4^{A}	3.0^{A}	3.8^{A}	7.6^{A}	3.9^{A}
ABA	8.3^{A}	12.5^{A}	3.0^{A}	12.1^{A}	6.5^{A}	3.4^{A}	0.0^{B}	0.0^{B}	0.0^{A}

$^{A \rightarrow B}$ Treatments with the same letter are not significantly different within columns at $p<0.05$ by Bonferroni's Method of Multiple Comparisons (SAS).

Table 3.8 Number of small (<50 g wt), intermediate (50-100 g wt) and large (>100 g wt) tubers harvested from 5 plants/replicate in 2003 at San Luis Valley from five dormancy breaking treatments. Potato cultivars grouped by dormancy period [short (Silverton Russet), intermediate (RNK, Sangre-S14, Desiree), and long (RNK-S3, Nooksack)].

	Dormancy Groups								
	Short			Intermediate			Long		
Treatment	Small	Intermediate	Large	Small	Intermediate	Large	Small	Intermediate	Large
Control	4.5^{A}	23.5^{A}	11.5^{A}	8.9^{A}	19.3^{A}	10.0^{A}	11.4^{AB}	3.5^{B}	0.8^{B}
$2.28\ 10^{-6}$ M ProGibb	6.3^{A}	32.3^{A}	8.5^{A}	6.4^{A}	19.9^{A}	14.8^{A}	8.0^{AB}	10.8^{AB}	5.6^{AB}
250 ppm Florel	12.3^{A}	28.3^{A}	8.5^{A}	9.5^{A}	24.4^{A}	10.7^{A}	7.8^{AB}	11.6^{A}	2.8^{AB}
Pro-florel	5.5^{A}	25.3^{A}	14.5^{A}	10.6^{A}	23.8^{A}	14.9^{A}	4.8^{B}	9.3^{AB}	9.6^{A}
ABA	8.5^{A}	22.0^{A}	9.3^{A}	10.8^{A}	18.8^{A}	10.4^{A}	13.5^{A}	4.4^{AB}	0.8^{B}

$^{A \rightarrow B}$ Treatments with the same letter are not significantly different within columns at $p<0.05$ by Bonferroni's Method of Multiple Comparisons (SAS).

Table 3.9 Potato minituber dormancy grouping table.

	Dormancy Group					
	Short	Intermediate			Long	
	Silverton Russet	Desiree	Sangre-S14	RNK	RNK-S3	Nooksack
Days to bud break, untreated[z] (7°C) (Holm, 2002)[y]	66	NA	92	96	105	>105
Dormancy release (%) 1 week after treatment[x]	35	0	0	0	0	0
Dormancy release (%) 2 weeks after treatment[x]	100	90	65	90	35	0
Dormancy release (%) 3 weeks after treatment[x]	100	100	95	100	80	55
Sprout number/minituber 5 weeks after treatment[x]	2.9	1.9	3.5	4.3	2.9	1.4
Yield/minituber[x]	1.2	1.4	0.7	1.1	0.8	0.3

[z]Untreated potato tubers. [y]Personal communication (60-80 days = short, 80-100 days = intermediate, >100 days = long) based on historical evaluation at San Luis Valley Research Center, CO. [x]Pro-Florel treated minitubers in 2002

Chapter 4

Alpha-galactosidase, soluble sugar content, and total phenolics relative to potato minituber bud dormancy

4.1 Abstract

Alpha-galactosidase is a key catabolic enzyme of the raffinose family oligosaccharides (RFO), involved in the seed germination whose primary function is to break the terminal-linked moiety from galactose containing oligosaccharides. Reducing sugars (fructose and glucose) often change during storage and their content is associated with potato tuber quality. Phenolic compounds are relatively stable secondary plant metabolites, sometimes thought to be related to defense against predators and diseases and in some species change as plants pass from a dormant to non-dormant state.

Alpha-galactosidase enzyme activity was significantly higher with application of a 4-hour direct current (DC) electromagnetic field. Fructose and glucose contents were significantly increased by Pro-florel treatment in RNK-S3 and Silverton Russet minitubers stored 1 week. In general, total phenolic contents were not significantly affected by treatments, cultivars and storage periods.

These results suggested that alpha-galactosidase activity and total phenolics levels were not associated with dormancy release because the Pro-florel treatment did not alter their content more or less than any other treatments. However, fructose and glucose levels increased with application of Pro-florel in RNK-S3 minitubers stored 1 week

compared to other treatments. High levels of fructose and glucose could be related to energy requirement during termination of minituber bud dormancy.

4.2 Introduction

Alpha-galactosidase (EC 3.2.1.22) is extensively present in monocot and dicot plant species even in plants that do not transport raffinose sugars. Alpha-galactosidase has been studied widely in plants and the description of the various types of α-galactosidases from many plant species has been determined (Keller and Pharr, 1996).

α-Galactosidase has been thoroughly studied in germinating seeds since seeds store huge quantities of raffinose-type sugars as an energy source for germination (Smart and Pharr, 1980). Alpha-galactosidase contributes to the hydrolysis of many types of stored substances such as raffinose-type oligosaccharides, cell wall galactomannans, cell wall polysaccharides and storage glycoproteins (Keller and Pharr, 1996). Alpha-galactosidase hydrolyses the α- (1→6) linkage in raffinose-type sugars to form galactose and sucrose (Bewley and Black, 1994).

The enzyme α-galactosidase can be grouped as acid or alkaline depending on enzyme metabolism in response to pH (Keller and Pharr, 1996). Various acidic forms of α-galactosidase are present in leaves and seeds (Smart and Pharr, 1980; Keller and Pharr, 1996). Due to it's significant function in seed development and germination, acidic forms of α-galactosidase have been the main focus for research studies (Keller and Pharr, 1996). In addition to three acidic forms of α-galactosidase, an alkaline form has also been described in the immature leaves of cucurbits, like *Cucurbita pepo, Cucumis sativus* and *Cucumis maxima*. (Gaudreault and Webb, 1983; 1986). The alkaline form of α-

galactosidase is associated with hydrolysis of transported stachyose in young leaves of cucurbits (Gaudreault and Webb, 1986; Gao and Schaffer, 1999).

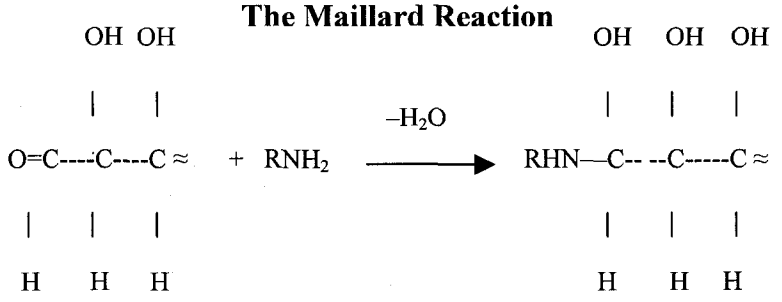
Alpha-galactosidase enzyme activity is induced by gibberellic acid and red light treatments in dry lettuce seeds before the end of germination (Leung and Bewley, 1983). Alpha-galactosidase enzyme activity is also increased by exogenous ethylene treatment in ACC-synthase antisense transgenic tomato fruits (Sozzi et al., 1998).

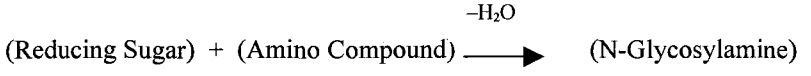
Alpha-galactosidase enzyme activity occurs throughout development of the endosperm in date palm as a result of endosperm cell wall mannan hydrolysis (DeMason et al., 1992). Alpha-galactosidase enzyme has been purified from coconut endosperms (Mujer et al., 1984; Balasubramaniam and Mathew, 1986) and high activity of α-galactosidase has been detected in the coconut endosperms due to hydrolysis of galactomannans and other stored carbohydrates (Mujer et al., 1984). Alpha-galactosidase is synthesized in carob endosperm to mobilize storage galactomannan from endosperm cell walls (Kontos and Spyropoulos, 1995) and enzyme activity is controlled by the seed coat in the growing carob seed endosperm and during seed maturation (Kontos and Spyropoulos, 1996). Enzyme activity is also found in soybean seeds due to α - galactosidase-hemagglutinin protein (Del Campillo and Shannon, 1982). The presence of α-galactosidase in both imbibed and dry endosperms in lettuce seeds is associated with the release of galactose from endosperm cell walls due to hydrolysis and mobilization of the sugars (Leung and Bewley, 1983).

Starch, the main carbon reserve in potato tubers, is important both as an industrial product and as a food source. Soluble sugar (sucrose, glucose, and fructose) content is related to tuber quality especially because high reducing sugar (glucose and fructose)

content lowers processed potato quality (Burton et al., 1992). The Maillard reaction, a product of reducing sugars and amino acids, causes unwanted potato brown coloring during potato high temperature processing and decreases nutritional level with nonenzymatic reaction in vivo (Van Es and Harmans, 1986; Burton et al., 1992).

The Maillard Reaction





Storage temperature and potato cultivar characteristics are the main determinants of soluble sugar content during storage of potatoes (Van Es and Harmans, 1986; Cottrell et al., 1993). Low temperature effects on reducing sugar content have been studied intensively. Low temperature (below 10°C) induces enzymes that enhance reducing sugar content (Burton, 1969; Van Es and Harmans, 1986; Richardson et al., 1990; Harris, 1992; Cottrell et al., 1993) and sucrose content (Van Es and Harmans, 1986) and is called low temperature sweetening.

It has been determined that starch breakdown is not a precondition for dormancy release in potato, since starch hydrolysis enzyme levels did not change just before dormancy release (Biemelt, 2000). Coleman and King (1984) did not find a relationship between reducing sugar content and potato dormancy.

Phenolic compounds are secondary plant metabolites commonly distributed in plants (Friedman M, 1997; Sellappan et al., 2002). They are mainly present in fruits and vegetables presumed to be related to defense mechanisms against predators and herbivores (Friedman M, 1997) and contribute to color, taste and flavor (Lea et al., 1978).

The quantity of total phenolics in potato tubers is not high (Malberg and Theander, 1984). The main phenolic constituent detected in potato tubers is chlorogenic acid (90 %) (Malberg and Theander, 1984; Friedman, 1997) and its concentration in tubers was found to be location dependent (Amberger and Schaller, 1975). The level of phenolic compounds in purple-flesh potatoes was induced by wounding, and not related to ethylene, temperature, and methyl jasmonate (Reyes and Cisneros-Zevallos, 2003). Three months cold storage (5-7 °C) increased the amount of phenolic compounds in potato cultivars (Peshin, 2000). It was also found that phenolic compound levels were significantly different between potato cultivars (Al-Saikhan et al., 1995; Peshin, 2000).

According to Korableva et al., (1973) as cited in Cvikrova et al., (1994), the content of phenolic compounds changed during potato tuber dormancy and after dormancy release, caffeic acid and scopoletin levels were reduced in meristematic tissues of potato tubers. Cvikrova et al., (1994) reported that free phenolic acid levels were higher during potato tuber dormancy and reached maximum values at the end of potato tuber dormancy followed by a decline after dormancy release in potato tubers. The level of conjugated phenolic acids (esters and glycosides) increased during potato dormancy break (Cvikrova et al., 1994).

The growth inhibitor level of phenolic batatasins was reported to be maximum at the beginning of dormancy, then slowly declined during dormancy in tubers of *Dioscorea*

sp (Ireland and Passam, 1984). According to Codignola et al., (1982) as cited in Cvikrova et al., (1994), in buds of *Betula alba,* phenolics compounds slowly declined. In tuberose (*Polianthes tuberose* L.) bulbs, the level of acidic and bound phenolic compounds differed during the dormancy periods and their role was not related to dormancy, but to physiological changes in this tuberose plant (Nagar, 1995). It was determined that total phenolic compounds were maximum at the inception of dormancy, but quickly declined with dormancy release in the basal buds and sprout growth of birch (Kauppi *et al.,* 1991). Codignola et al., (1988) found that qualitative phenol compound level did not change appreciably during dormancy in bud scales of *Fagus sylvatica* L. A correlation was not determined between bud dormancy and total phenolics content in this study.

According to our sources, changes in α-galactosidase activity, soluble sugar content, and total phenolics level in dormant and non-dormant potato minitubers have not been studied. Thus, the purpose of this study was to investigate the relationship of these metabolites to potato minituber bud dormancy and their emergence from dormancy in five short and long-dormancy cultivars.

4.3 Material and Methods

4.3.1 Plant materials and treatments for alpha-galactosidase studies

Two potato (*Solanum tuberosum* L.) minituber cultivars, Silverton Russet (short dormancy) and Russet Norkotah-selection3 (RNK-S3) (long dormancy) were obtained from San Luis Valley Research Center (SLVRC) in January 2002 and stored 79 days at

5±1 °C for Silverton Russet and 51 days for RNK-S3 under dark conditions. Treatments were 250 ppm Florel, 1000 ppm Florel, 2.28 10^{-6} M ProGibb, 10^{-4} M Absisic acid (ABA), Pro-florel (2.28 10^{-6} M ProGibb and 250 ppm Florel), 30 ppm (±) Catechin, 4-hour direct current (DC) electromagnetic field and control. The minitubers were soaked in these solutions and aerated with an aquarium pump for 24-hour in the dark at 15°C. Treated minitubers were stored for 0 and 4 weeks in the dark at 15°C.

The analysis of variance (ANOVA) and Least Significant Differences Method of Multiple Comparisons (SAS) were used for statistical analysis. Microsoft Excel software was used to create graphs.

4.3.1.1 Preparation of PNPG (P-Nitrophenyl α -D-Galactopyranoside) assay

Colorimetric tubes with corks were autoclaved on liquid cycle, containing 0.8% agar solution. The tubes and solutions were heated for 20 minutes at 121°C on liquid cycle. The agar was cooled to at least 60°C and a solution of 2.5 mM PNPG was added. The solution was then stirred, and 3 ml pipetted into each colorimetric tube. Each tube was recorked and the tubes were stored at 4°C in the dark.

4.3.1.2 Alpha-galactosidase assay

Cylindrical samples were taken from the bud sections of minitubers with a 5 mm diameter cork borer. Each cylinder was cut to 5 mm length, weighed, and placed basal side in contact with agar in a *para*-nitrophenol-α-galactopypyranoide (PNPG) tube and incubated in the dark at 20°C. A ''Spectronic 20D+, Spectronic Instrument'' spectrophotometer was used to obtain absorbance at 400nm for each tube. These

readings were taken every 24 hours for seven days. Alpha-galactosidase enzyme activity is based on the hydrolysis of PNPG to *para*-nitrophenol (PNP), catalyzed by alpha-galactosidase enzyme to PNP and absorbance is read at 400nm.

4.3.2 Plant materials and treatments for soluble sugar studies

Two potato minituber cultivars, Silverton Russet and RNK-S3 were obtained from San Luis Valley Research Center (SLVRC) in January 2002 and stored 75 days at 5±1°C for Silverton Russet and 46 days for RNK-S3 under dark conditions. Treatments applied were 250 ppm Florel, 2.28 10^{-6} M ProGibb, Pro-florel (2.28 10^{-6} M ProGibb and 250 ppm Florel), ABA and Control. For the treatments, minitubers were soaked in these solutions and aerated with an aquarium plump for 24-hours in the dark at 15°C. After treatments some minitubers were stored one week and some for four weeks at 15°C to examine potential differences between storage periods.

The analysis of variance (ANOVA) and Least Significant Differences Method of Multiple Comparisons (SAS) were used for statistical analysis. Microsoft Excel software was used to create graphs.

4.3.2.1 Soluble carbohydrate extraction and analysis

After each storage period, treated minitubers were sliced into two pieces and freeze dried for seven days. Minitubers were then ground into a fine powder and kept at – 20 °C for sugar analysis. Samples were derivitized using trimethylchlorosilane according to Sweeley et al., (1963).

The internal standard (IS) was a 1.0 mg/ml mixture of glucopyranoside. Fructose, glucose, sucrose and a mixture of these sugars (1.0 mg/ml) were used as sugar standards. Each time 9 samples were derivitized since the drying blocks have only 9 openings. IS (50 µl) was added into each of nine falcon tubes using a 100 µl pipette. Sugar standards, 25 µl of fructose, glucose, sucrose, and mixture of these sugars, were added to individual vials. The vials were dried using the blowing tubes under the laminar hood.

Between 0.8 and 1.5 mg samples were weighed and added to the vials, except standards. Then, 400 µl pyridine, 80 µl hexamethyldisilizane, and 40µl trimethylchlorosilane were added to each tube. Tubes were secured tightly with Teflon coated lids. The vials were set on the heating blocks at 80 °C for 20 minutes. The vials were removed from the heating blocks and dried under the air tubes about 40 minutes until the contents of the vial are visually dry. Then, 500 µl hexane was added to the vials. Another set of vials was marked with corresponding numbers to the first set. Pasteur pipettes were used to transfer the liquid from the old vials to the new set. These vials were dried under the air tubes. Finally, 200µl hexane was added to each vial and secured tightly with the Teflon coated caps for injection into a HP 5890 Series II gas chromatography (GC).

Samples were injected with a syringe needle (10µl Gastight#1801 by Hamilton Co.) from injection port to capillary column (J& W Scientific DB-1). Oven temperature was set at 180 °C and helium was used as a carrier gas. Samples were detected by a flame ionization detector. Soluble carbohydrates were quantified from peak area calculations and a 3-sugar standard using glucopyranoside as the internal standard.

4.3.3 Plant materials and treatments for total phenolics studies

Five minituber cultivars, Silverton Russet, Sangre-S14, Desiree, RNK, and RNK-S3 were obtained from San Luis Valley Research Center (SLVRC) at the end of January 2003 and stored for 75 days at 5±1°C for Silverton Russet, 71 days for Sangre-S14, and 67 days for Desiree, RNK and RNK-S3 under dark conditions. Treatments were 250 ppm Florel, Pro-florel (2.28 10^{-6} M ProGibb and 250 ppm Florel), 5 mM 2,2′-azobis- (2-amidinopropane) hydrochloride (AAPH) and Control. For the Florel and Pro-florel treatments, minitubers were soaked in these solutions and aerated with an aquarium plump for 24-hour in the dark at 15°C. Some minitubers were soaked in AAPH, a free radical producer, for 5 minutes. Some minitubers were stored to break dormancy and some were sliced into two pieces and freeze dried when they were dormant (just after treatments) and non-dormant (stored at 15 ±1 °C till they broke dormancy). After that they were ground and kept at – 20°C for total phenolic analysis.

The analysis of variance (ANOVA) and Least Significant Differences Method of Multiple Comparisons (SAS) were used for statistical analysis. Microsoft Excel software was used to create graphs.

4.3.3.1 Extraction and determination of total phenolics

Total phenolic content of potato minitubers was determined according to the Folin-Ciocalteu colorimetric method as described by Spanos and Wrolstad (1990) with modifications (Lister and Wilson 2001). Gallic acid solution was used to prepare a standard curve for estimation of total phenolic content as gallic acid equivalents. Gallic acid solution (25 mg gallic acid + 25ml 80 % acetone) was made up daily. A standard

curve was prepared from gallic acid concentrations of 0, 5, 10, 20, 40, 60, and 100 µl and H_2O. Minitubers were freeze-dried and ground. A 600 mg sample was transferred to 15 ml centrifuge tubes, with 10 ml 80 % acetone. The sample was rotated for 2 hours and 10 minutes and centrifuged 10 min at 3800 rpm at 4 °C. One ml supernatant from each sample was transferred to 1.5 ml eppendorf tube to vacufuge the extracts for 3.00 hours at 45°C in dark. Extracts were stored at -20 °C for total phenolic content measurement. One ml of 80% acetone was added to each sample extract. The samples were transferred to a 36 °C water bath for 10 minutes and vortexed 10 seconds. 50 µl sample solutions were transferred to three (10ml-test) tubes and 450 µl H2O and 2.5 ml of 10 % folin and 2.0 ml 7.5 % Na_2CO_3 solution added in a sequence to each tube. The tubes were vortexed 10 sec and incubated at 45 °C in water bath for 15 min. Samples were cooled at room temperature and absorbance was taken as soon as possible at 765 nm at 25 °C.

4.4 **Results**

4.4.1 Alpha-galactosidase activity

In Silverton Russet and RNK-S3 cultivars, alpha-galactosidase enzyme activity was higher in 0-week stored minitubers than 4-week stored minitubers for all treatments except the 4-hour direct current (DC) electromagnetic field treatment (Table. 4.1, 4.2). Alpha-galactosidase activity was significantly higher (3 to 4 fold) in both cultivars treated with 4-hour direct current (DC) electromagnetic field stored 4 weeks compared to the other treatments (Fig. 4.2, 4.4, Table. 4.1, 4.2). Pro-florel and ProGibb applications did not significantly alter alpha galactosidase activity in RNK-S3 and Silverton Russet minitubers after 0 and 4 weeks storage periods (Table. 4.1, 4.2).

4.4.2 Soluble sugar studies

Three soluble sugars (fructose, glucose and sucrose) levels were compared in potato minitubers, stored 1 week and 4 weeks after treatment. All three sugars were higher after one week storage in RNK-S3 minitubers (long dormancy cultivar) than 4 weeks storage in RNK-S3 minitubers for all treatments, with the exception of sucrose in the control and 250 ppm florel applications (Fig. 4.6, 4.7, 4.8, Table. 4.4, 4.5). However, application of Pro-florel significantly increased the levels of fructose (ranging 10 to 15 μM/gdw) and glucose glucose (ranging 20 to 355 μM/gdw) after one week storage of RNK-S3 minitubers (Fig. 4.6, 4.7). However, Pro-florel treatment did not increase sucrose level significantly in RNK-S3 minitubers (Fig. 4.8, Table. 4.6).

In Silverton Russet (short dormancy cultivar), there was no significant difference between treatment effects on soluble sugar levels, although glucose and sucrose levels were higher after one week than after four weeks storage (Fig. 4.7, 4.8).

4.4.3 Total phenolics studies

None of the treatments significantly increased total phenolic level with any of the cultivars except dormant minitubers of the short dormancy cultivar (Silverton Russet) and non-dormant minitubers of the long dormancy cultivar (RNK-S3) (Fig 4.9, 4.10, 4.11).

4.5 **Discussion**

Alpha-Galactosidase enzyme activity increased gradually with time due to daily accumulation of PNP. Alpha-galactosidase enzyme activity was not significantly

induced by Pro-florel and ProGibb treatments that were found to be very effective for minituber bud dormancy release (Fig. 3.1, 3.2, 3.3). However, alpha-galactosidase enzyme activity was increased by exogenous ethylene and gibberellic acid treatments in transgenic tomatoes and dry lettuce seeds (Sozzi et al., 1998; Leung and Bewley, 1981). Alpha-galactosidase activity was significantly induced in both cultivars treated with 4-hour direct current (DC) electromagnetic field stored 4 weeks (Fig. 4.2, 4.4, Table. 4.1, 4.2). However, studies with direct current (DC) electromagnetic field revealed that this treatment did not significantly shorten minituber bud dormancy. So, from these results, it can be concluded that potato tubers have very low amount of raffinose type oligosaccharides to use as energy during potato minituber dormancy release and not related to dormancy release.

Application of Pro-florel significantly increased the levels of fructose and glucose in 1 week stored dormant RNK-S3 minitubers (Fig. 4.6, 4.7). However, Pro-florel treatment did not significantly increase sucrose level in RNK-S3 minitubers (Fig. 4.8). Pro-florel treatment has been found as a dormancy release agent in minitubers. It can be concluded minitubers use starch (not raffinose family oligosaccharides) as an energy source during dormancy release.

Although Cvikrova et al., (1994) found that free phenolic acids and conjugated phenolic acids (esters and glycosides) increased during potato dormancy break, our studies were different. In the case of total phenolics during minituber dormancy, we did not find a correlation between dormant and non-dormant minituber TP levels with treatments. Pro-florel application significantly increased TP level in dormant long dormancy cultivars more than non-dormant long dormancy cultivars. Treatment and

cultivar effects on TP levels varied, but it can be concluded that there was no relationship between TP level and potato minituber dormancy release.

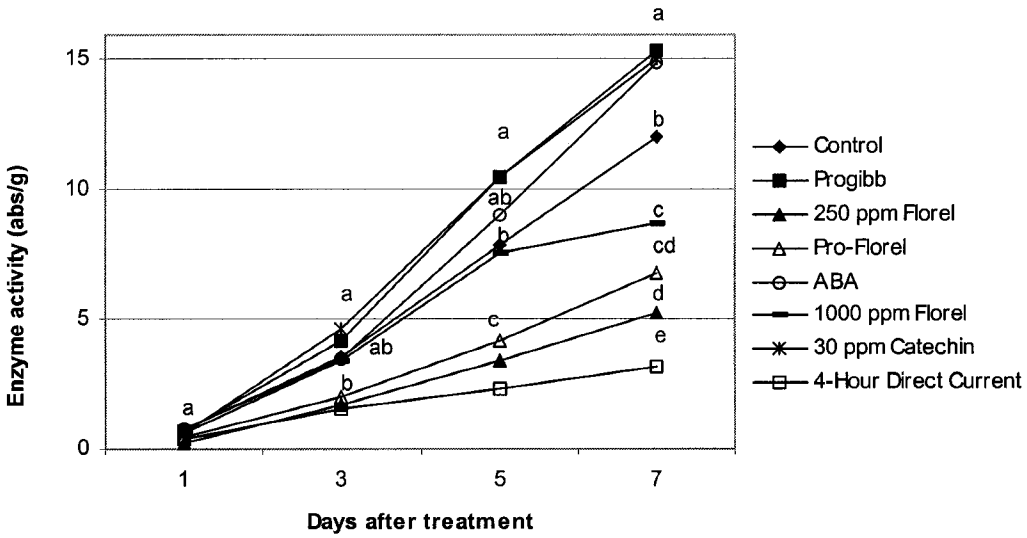


Fig. 4.1 Alpha-galactosidase activity in RNK-S3 minitubers for eight treatments after 0-week storage at 15°C. For each data point n=9. Treatments with the same letter are not significantly different at $p<0.05$ by LSD (SAS).

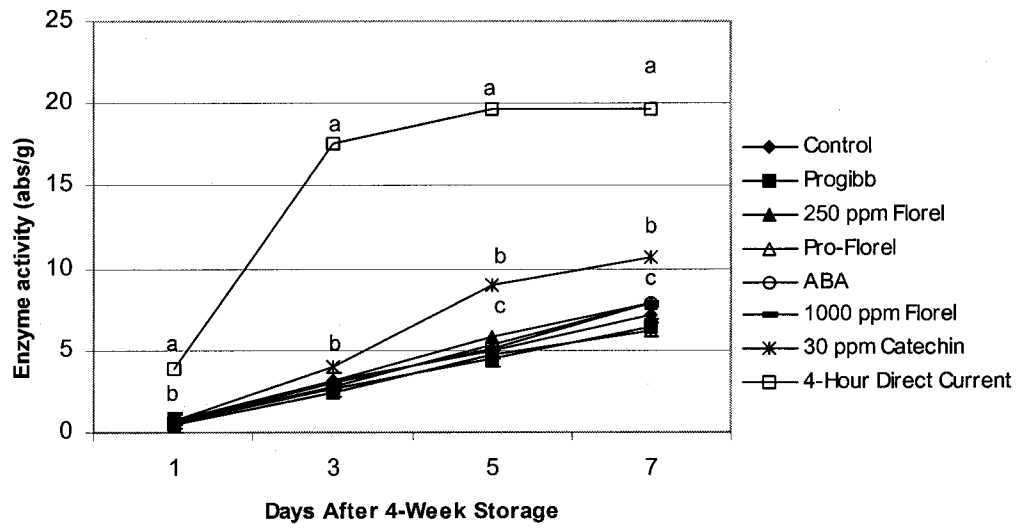


Fig. 4.2 Alpha-galactosidase activity in RNK-S3 minitubers for eight treatments after 4-week storage at 15°C. For each data point n=9. Treatments with the same letter are not significantly different at $p<0.05$ by LSD (SAS).

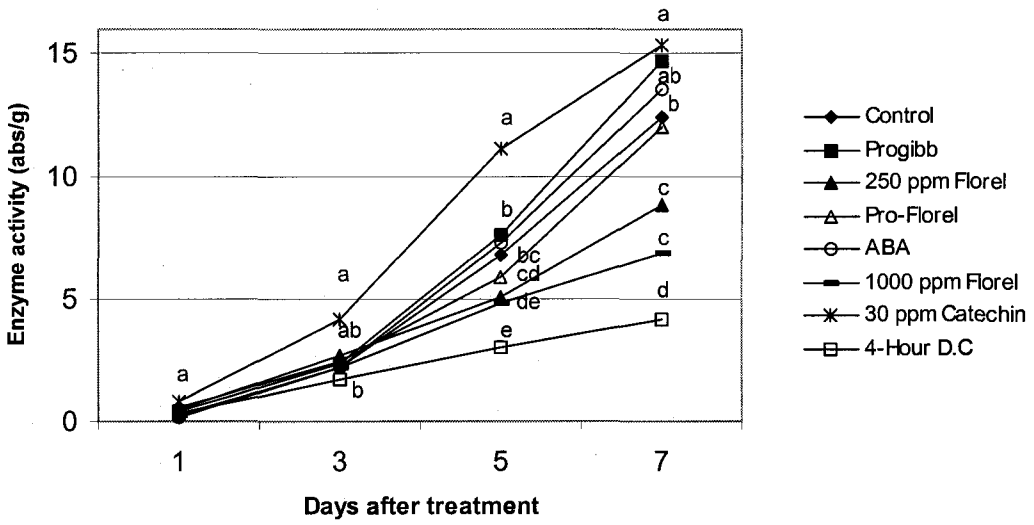


Fig. 4.3 Alpha-galactosidase activity in Silverton Russet minitubers for eight treatments after 0-week storage at 15°C. For each data point n=9. Treatments with the same letter are not significantly different at $p<0.05$ by LSD (SAS).

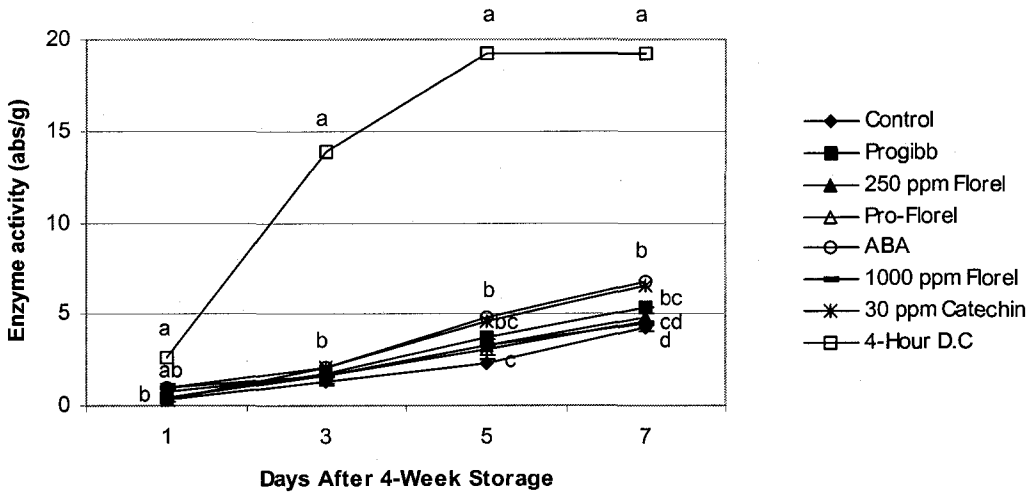


Fig. 4.4 Alpha-galactosidase activity in Silverton Russet minitubers for eight treatments after 0-week storage at 15°C. For each data point n=9. Treatments with the same letter are not significantly different at $p<0.05$ by LSD (SAS).

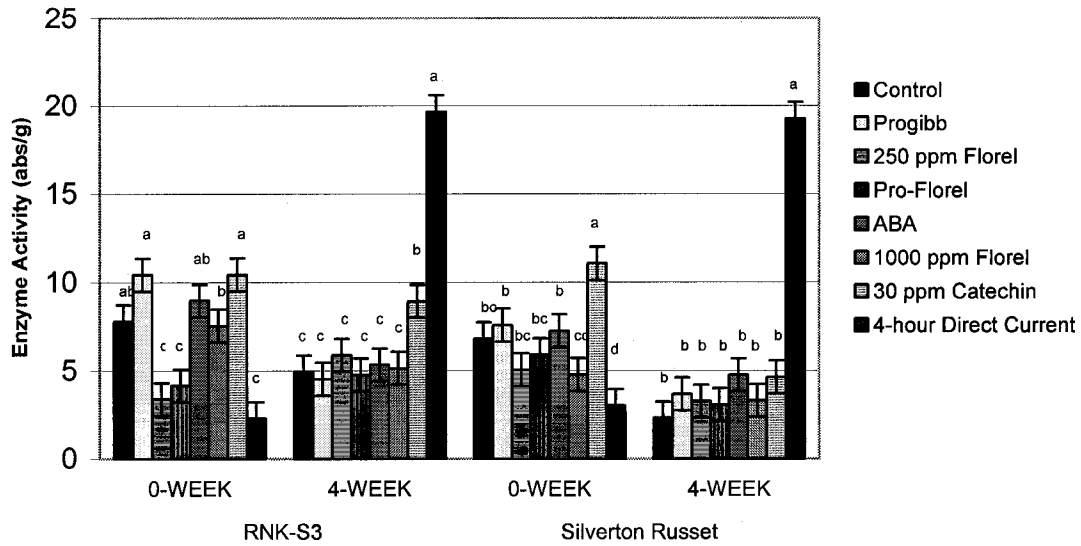


Fig. 4.5 Alpha-Galactosidase activity in Silverton Russet and RNK-S3 minitubers, on the 5th day after treatments, stored 0-week and four-week at 15°C. For each data point n=9. Treatments with the same letter are not significantly different at $p<0.05$ by LSD (SAS).

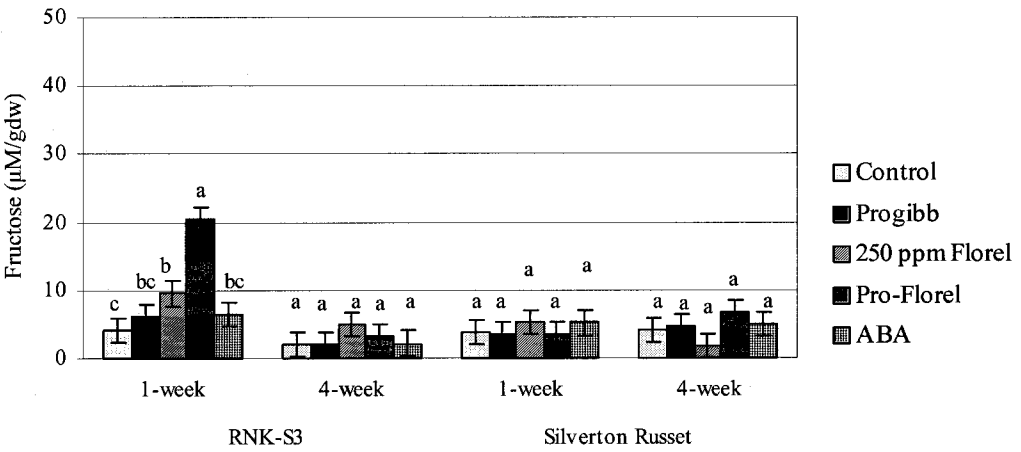


Fig. 4.6 Fructose levels (µM/gdw) in Silverton Russet and RNK-S3 minitubers from 5 treatments, stored one and four weeks at 15°C. For each data point n=3. Treatments with the same letter are not significantly different at $p<0.05$ by LSD (SAS) within each storage period. Error bars indicate mean ± SEM.

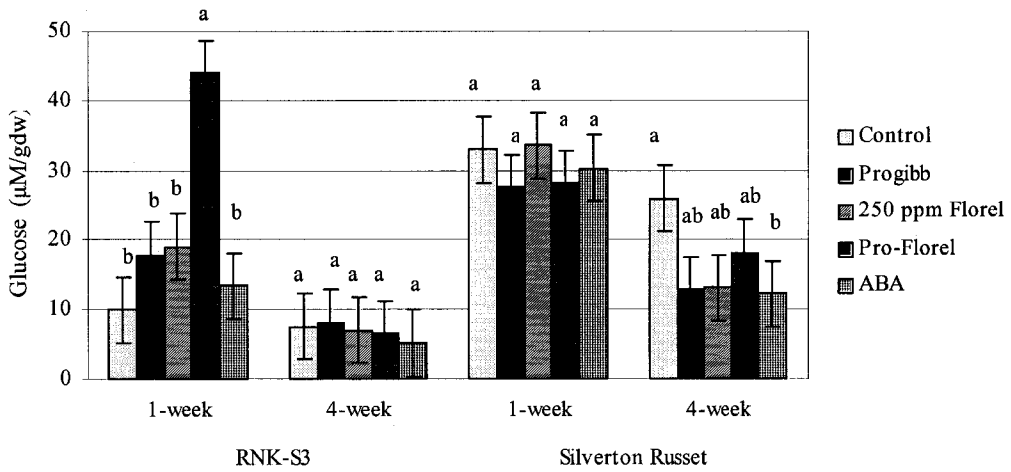


Fig. 4.7 Glucose levels (µM/gdw) in Silverton Russet and RNK-S3 minitubers from 5 treatments, stored one and four weeks at 15°C. For each data point n=3. Treatments with the same letter are not significantly different at $p<0.05$ by LSD (SAS) within each storage period. Error bars indicate ± SEM.

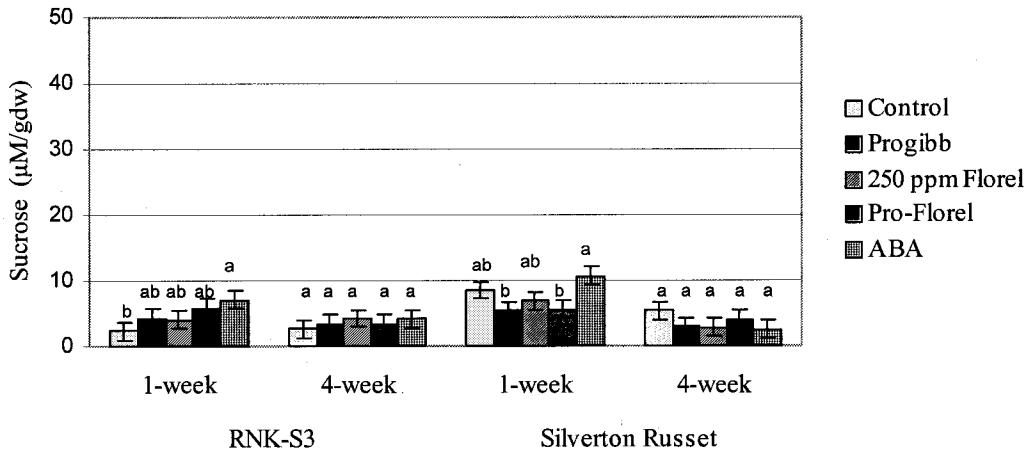


Fig. 4.8 Sucrose level (µM/gdw) in Silverton Russet and RNK-S3 minitubers from 5 treatments, stored one and four weeks at 15°C. For each data point n=3. Treatments with the same letter are not significantly different at $p<0.05$ by LSD (SAS) within each storage period. Error bars indicate mean ± SEM.

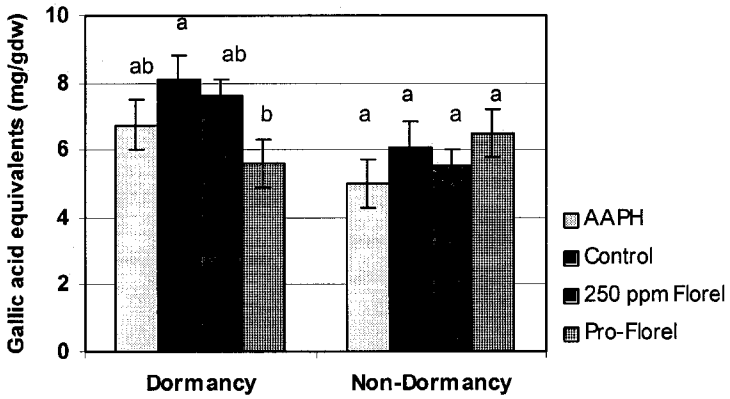


Fig. 4.9 Total phenolics level (mg/gdw) in the short dormancy cultivar (Silverton Russet) from four treatments. For each data point n=3. Treatments with the same letter are not significantly different at $p<0.05$ by LSD (SAS) within dormant and non-dormant minitubers. Error bars indicate means ± SEM.

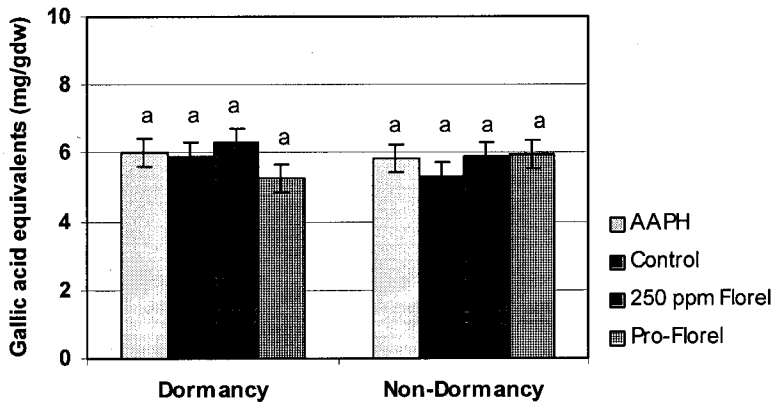


Fig. 4.10 Total phenolics level (mg/gdw) in the intermediate dormancy cultivar (Desiree, RNK, Sangre-S14) from four treatments. For each data point n=3. Treatments with the same letter are not significantly different at $p<0.05$ by LSD (SAS) within dormant and non-dormant minitubers. Error bars indicate ± SEM.

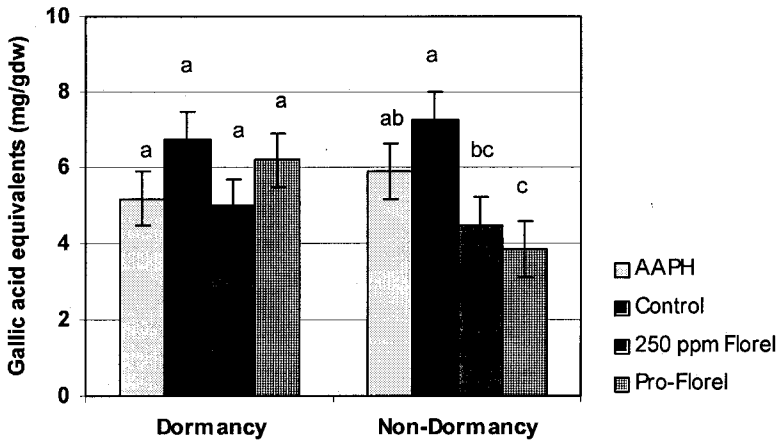


Fig. 4.11 Total phenolics level (mg/gdw) in the long dormancy cultivar (RNK-S3) from four treatments. For each data point n=3. Treatments with the same letter are not significantly different at $p<0.05$ by LSD (SAS) within dormant and non-dormant minitubers. Error bars indicate ± SEM.

Table 4.1 Alpha-Galactosidase activity in RNK-3 for eight treatments after 0-week and four -week storage at 15 °C

	0-WEEK				4-WEEK			
Treatment	1-Day	3-Day	5-Day	7-Day	1-Day	3-Day	5-Day	7-Day
Control	0.76 ^{AW}	3.55 ^{ABX}	7.80 ^{BY}	11.98 ^{BZ}	0.72 ^{BW}	3.16 ^{BX}	4.94 ^{CX}	7.15 ^{CY}
2.28 10^{-6} M ProGibb	0.68 ^{AVU}	4.13 ^{AW}	10.43 ^{AY}	15.32 ^{AZ}	0.55 ^{BU}	2.68 ^{BWV}	4.53 ^{CXW}	6.38 ^{CX}
250 ppm Florel	0.24 ^{AV}	1.72 ^{BWV}	3.38 ^{CXW}	5.21 ^{DYX}	0.59 ^{BV}	3.19 ^{BXW}	5.88 ^{CY}	7.92 ^{CZ}
Pro-florel	0.49 ^{AW}	1.98 ^{BW}	4.16 ^{CYX}	6.74 ^{CDZ}	0.48 ^{BW}	2.43 ^{BXW}	4.77 ^{CZY}	6.13 ^{CZY}
ABA	0.74 ^{AV}	3.49 ^{ABXW}	8.96 ^{ABY}	14.83 ^{AZ}	0.59 ^{BV}	2.81 ^{BW}	5.34 ^{CX}	7.94 ^{CY}
1000 ppm Florel	0.63 ^{AW}	3.38 ^{ABYX}	7.54 ^{BZ}	8.71 ^{CZ}	0.59 ^{BW}	3.00 ^{BX}	5.15 ^{CY}	7.94 ^{CZ}
30 ppm Catechin	0.65 ^{AW}	4.59 ^{AX}	10.44 ^{AY}	14.99 ^{AZ}	0.77 ^{BW}	4.05 ^{BX}	8.94 ^{BY}	10.70 ^{BY}
4-Hour D.C	0.41 ^{AV}	1.51 ^{BWV}	2.29 ^{CXWV}	3.16 ^{EXW}	3.86 ^{AX}	17.64 ^{AY}	19.67 ^{AZ}	19.67 ^{AZ}

Differences determined using LSD. $^{A\rightarrow Z}$ Means followed by the same letter within columns are not significantly different. $^{Z\rightarrow A}$ Means followed by the same letter within rows are not significantly different.

Table 4.2 Alpha-Galactosidase activity in Silverton Russet for eight **treatments after 0-week and four-week storage at 15 °C**

	0-WEEK				4-WEEK			
Treatment	1-Day	3-Day	5-Day	7-Day	1-Day	3-Day	5-Day	7-Day
Control	0.26 ^{AV}	2.24 ^{ABXWV}	6.81 ^{BCY}	12.39 ^{BZ}	0.30 ^{BWV}	1.35 ^{BWV}	2.31 ^{CXW}	4.21 ^{DX}
2.28 10^{-6} M ProGibb	0.37 ^{AU}	2.39 ^{ABWV}	7.58 ^{BY}	14.72 ^{AZ}	0.45 ^{BWU}	1.77 ^{BWVU}	3.68 ^{BCXW}	5.38 ^{BCDX}
250 ppm Florel	0.50 ^{AW}	2.66 ^{ABX}	5.06 ^{CDY}	8.84 ^{CZ}	0.48 ^{BW}	1.63 ^{BXW}	3.27 ^{BCYX}	4.84 ^{BCDY}
Pro-florel	0.60 ^{AV}	2.45 ^{ABWV}	5.90 ^{BCDY}	12.03 ^{BZ}	0.72 ^{ABV}	1.69 ^{BWV}	3.07 ^{BCXW}	4.57 ^{CDYX}
ABA	0.14 ^{AV}	2.20 ^{ABW}	7.25 ^{BY}	13.52 ^{ABZ}	0.95 ^{ABWV}	2.04 ^{BWV}	4.77 ^{BX}	6.78 ^{BYX}
1000 ppm Florel	0.27 ^{AW}	2.19 ^{ABXW}	4.78 ^{DEY}	6.87 ^{CZ}	0.95 ^{ABW}	1.61 ^{BXW}	3.31 ^{BCYX}	4.53 ^{CDY}
30 ppm Catechin	0.84 ^{AV}	4.17 ^{AW}	11.07 ^{AY}	15.36 ^{AZ}	0.34 ^{BV}	2.09 ^{BV}	4.64 ^{BXW}	6.51 ^{BCX}
4-Hour D.C	0.29 ^{AV}	1.71 ^{BWV}	3.02 ^{EXW}	4.13 ^{DX}	2.59 ^{AXW}	13.90 ^{AY}	19.28 ^{AZ}	19.28 ^{AZ}

Differences determined using LSD. $^{A\rightarrow Z}$ Means followed by the same letter within columns are not significantly different. $^{Z\rightarrow A}$ Means followed by the same letter within rows are not significantly different.

Table 4.3 Alpha-Galactosidase activity in RNK-3 & Silverton on the 5th day after treatment, stored 0-week and four -week at 15 °C

	RNK-3		Silverton Russet	
Treatment	0-WEEK	4-WEEK	0-WEEK	4-WEEK
Control	7.80 ^{ABZ}	4.94 ^{CYX}	6.81 ^{BCZY}	2.31 ^{BX}
2.28 10^{-6} M ProGibb	10.43 ^{AZ}	4.53 ^{CX}	7.58 ^{BY}	3.68 ^{BX}
250 ppm Florel	3.38 ^{CZ}	5.88 ^{CZ}	5.06 ^{BCDZ}	3.27 ^{BZ}
Pro-florel	4.16 ^{CZY}	4.77 ^{CZY}	5.90 ^{BCZ}	3.07 ^{BY}
10^{-4} M ABA	8.96 ^{ABZ}	5.34 ^{CY}	7.25 ^{BCZY}	4.76 ^{BY}
1000 ppm Florel	7.54 ^{BZ}	5.15 ^{CZY}	4.78 ^{CDY}	3.31 ^{BY}
30 ppm Catechin	10.44 ^{AZY}	8.94 ^{BY}	11.07 ^{AZ}	4.64 ^{BX}
4-hour Direct Current	2.29 ^{CY}	19.67 ^{AZ}	3.02 ^{DY}	19.28 ^{AZ}

Differences determined using LSD. $^{A\rightarrow Z}$Means followed by the same letter within columns are not significantly different. $^{Z\rightarrow A}$Means followed by the same letter within rows are not significantly different.

Table 4.4 Fructose levels (µM/gdw) in Silverton Russet and RNK-S3 minitubers cultivars from five treatments, stored one and four weeks at 15 °C

	RNK-3		Silverton Russet	
Treatment	1-WEEK	4-WEEK	1-WEEK	4-WEEK
Control	4.09 ^{CZ}	2.05 ^{AZ}	3.87 ^{AZ}	4.12 ^{AZ}
2.28 10^{-6} M ProGibb	6.05 ^{BCZ}	2.10 ^{AZ}	3.37 ^{AZ}	4.67 ^{AZ}
250 ppm Florel	9.51 ^{BZ}	4.92 ^{AZY}	5.34 ^{AZY}	1.79 ^{AY}
Pro-florel	20.48 ^{AZ}	3.24 ^{AY}	3.55 ^{AY}	6.80 ^{AY}
ABA	6.41 ^{BCZ}	2.15 ^{AZ}	5.16 ^{AZ}	4.96 ^{AZ}

Differences determined using LSD. $^{A\rightarrow Z}$Means followed by the same letter within columns are not significantly different. $^{Z\rightarrow A}$Means followed by the same letter within rows are not significantly different.

Table 4.5 Glucose levels (µM/gdw) in Silverton Russet and RNK-S3 minitubers cultivars from five treatments, stored 1 and 4 weeks at 15 °C

	RNK-3		Silverton Russet	
Treatment	1-WEEK	4-WEEK	1-WEEK	4-WEEK
Control	9.93 ^{BY}	7.48 ^{AY}	32.92 ^{AZ}	25.88 ^{AZ}
Pro-florel ProGibb	17.82 ^{BZY}	8.11 ^{AY}	27.52 ^{AZ}	12.89 ^{ABY}
250 ppm Florel	19.06 ^{BY}	6.92 ^{AY}	33.54 ^{AZ}	13.19 ^{ABY}
Pro-florel	43.88 ^{AZ}	6,51 ^{AX}	28.02 ^{AY}	18.10 ^{ABYX}
ABA	13.46 ^{BY}	5.17 ^{AY}	30.24 ^{AZ}	12.22 ^{BY}

Differences determined using LSD. $^{A\rightarrow Z}$ Means followed by the same letter within columns are not significantly different. $^{Z\rightarrow A}$ Means followed by the same letter within rows are not significantly different.

Table 4.6 Sucrose levels (µM/gdw) in Silverton Russet and RNK-S3 minitubers cultivars from five treatments, stored one and four weeks at 15 °C

	RNK-3		Silverton Russet	
Treatment	1-WEEK	4-WEEK	1-WEEK	4-WEEK
Control	2.36 ^{BY}	2.66 ^{AY}	8.49 ^{ABZ}	5.44 ^{AZY}
ProGibb	4.28 ^{ABZ}	3.47 ^{AZ}	5.35 ^{BZ}	2.88 ^{AZ}
250 ppm Florel	4.03 ^{ABZY}	4.24 ^{AZY}	6.93 ^{ABZ}	2.75 ^{AY}
Pro-florel	5.89 ^{ABZ}	3.38 ^{AZ}	5.52 ^{BZ}	3.97 ^{AZ}
ABA	7.05 ^{AZY}	4.12 ^{AYX}	10.64 ^{AZ}	2.44 ^{AX}

Differences determined using LSD. $^{A\rightarrow Z}$ Means followed by the same letter within columns are not significantly different. $^{Z\rightarrow A}$ Means followed by the same letter within rows are not significantly different.

Table 4.7 Total phenolics levels (mg/gdw) in dormant and non-dormant minituber cultivars of short dormancy cultivar (Silverton Russet), intermediate dormancy cultivars (Desiree, Sangre-S14, RNK), and long dormancy cultivar (RNK-S3) from four treatments

	DORMANT			NON-DORMANT		
Treatment	Short	Intermediate	Long	Short	Intermediate	Long
AAPH	6.8 ^{ABZ}	6.0 ^{AZ}	5.2 ^{AZ}	5.0 ^{AZ}	5.9 ^{AZ}	5.9 ^{ABZ}
Control	8.1 ^{AZ}	5.9 ^{AYX}	6.7 ^{AZYX}	6.1 ^{AZYX}	5.3 ^{AX}	7.3 ^{AZY}
250 ppm Florel	7.6 ^{ABZ}	6.3 ^{AZY}	5.0 ^{AYX}	5.5 ^{AYX}	5.9 ^{AZYX}	4.5 ^{BCX}
Pro-florel	5.6 ^{BZY}	5.3 ^{AZY}	6.2 ^{AZ}	6.5 ^{AZ}	6.0 ^{AZ}	3.8 ^{CY}

Differences determined using LSD. $^{A\rightarrow Z}$ Means followed by the same letter within columns are not significantly different. $^{Z\rightarrow A}$ Means followed by the same letter within rows are not significantly different.

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