UNIVERSIDADE FEDERAL DE SANTA MARIA
CENTRO DE CIÊNCIAS RURAIS
PROGRAMA DE PÓS-GRADUAÇÃO EM AGRONOMIA

VERNALIZAÇÃO E APLICAÇÃO DE ÁCIDO GIBERÉLICO NA GERMINAÇÃO E DESENVOLVIMENTO DE *Penstemon digitalis* Nutt. cv HUSKER RED

TESE DE DOUTORADO

Anderson Machado de Mello

Santa Maria, RS, Brasil

2008

VERNALIZAÇÃO E APLICAÇÃO DE ÁCIDO GIBERÉLICO NA GERMINAÇÃO E DESENVOLVIMENTO DE *Penstemon digitalis* Nutt. cv HUSKER RED

por

Anderson Machado de Mello

Tese apresentada ao Curso de Doutorado do Programa de Pós-Graduação em Agronomia, Área de concentração em Produção Vegetal, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do grau de
Doutor em Agronomia

Orientador: Prof. Nereu Augusto Streck

Santa Maria, RS, Brasil

2008

Mello, Anderson Machado de, 1976-
M527v

Vernalização e aplicação de ácido giberélico na germinação e desenvolvimento de *Penstemon digitalis* Nutt. cv Husker Red / por Anderson Machado de Mello ; orientador Nereu Augusto Streck. - Santa Maria, 2009.
129 f. ; il.

Tese (doutorado) – Universidade Federal de Santa Maria, Centro de Ciências Rurais, Programa de Pós-Graduação em Agronomia, RS, 2009.

1. Agronomia 2. Quebra de dormência 3. Baixa temperatura 4. Produção de flores 5. Flor de corte I. Streck, Nereu Adroaldo, orient. II. Título

CDU: 635.912

Ficha catalográfica elaborada por
Luiz Marchiotti Fernandes – CRB 10/1160
Biblioteca Setorial do Centro de Ciências Rurais/UFSM

Endereço: Rua Gilberto Binatto, n. 606, Bairro Medianeira, Santa Maria, RS, 97060310
Fone (0xx)55 32219865; End. Eletr: andersonmellode@yahoo.com

Universidade Federal de Santa Maria
Centro de Ciências Rurais
Programa de Pós-Graduação em Agronomia

A Comissão Examinadora, abaixo assinada,
aprova a Tese de Doutorado

VERNALIZAÇÃO E APLICAÇÃO DE ÁCIDO GIBERÉLICO NA GERMINAÇÃO E DESENVOLVIMENTO DE P*enstemon digitalis* Nutt. cv HUSKER RED

Elaborada por
Anderson Machado de Mello

como requisito parcial para obtenção do grau de
Doutor em Agronomia

COMISSÃO EXAMINADORA:

_____
Nereu Augusto Streck, Ph.D.
(Presidente/Orientador)

_____
Ellen T. Paparozzi, Ph.D. (UNL**)**

_____
Luis Antonio de Avila, Ph.D. (UFSM**)**

_____
Dilson Antônio Bisognin, Ph.D. (UFSM**)**

_____
Erin Blankenship, Ph.D. (UNL**)**

Santa Maria, 16 de dezembro de 2008.

A minha família,
especialmente meus pais
Pedro Pereira de Mello e
Clelia Machado de Mello
e a todas as pessoas
que sempre estiveram ao meu lado.

Dedico...

AGRADECIMENTOS

À Universidade Federal de Santa Maria e ao Programa de Pós-Graduação em Agronomia, bem como a Universidade de Lincoln NE - EUA, pela oportunidade da realização do Doutorado através do Programa de Doutorado com Estágio no Exterior (PDEE) financiado pela Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

À CAPES pela concessão da bolsa de Doutorado.

Ao meu orientador professor Nereu Augusto Streck e aos meus coorientadores, professores Rogério Antônio Belle, Ellen T. Pararozzi, e Sidinei José Lopez, um agradecimento especial pelos conhecimentos transmitidos, pela amizade e dedicação. Aos colegas e amigos do Departamento de Fitotecnia da Universidade Federal de Santa Maria e da Universidade de Lincoln NE – EUA. Aos amigos Tom Fowler, Roob Crouch e Steven Smith pela amizade, apoio e incentivo nos EUA. Ao meu amigo e "roommate" Marcelo de Lima pela amizade e companheirismo. Aos meus pais Pedro Pereira de Mello, Clelia Machado de Mello e minhas irmãs, por todo o apoio. Enfim, a todos que de alguma forma contribuíram para a realização desta tese direta ou indiretamente e que não foram mencionados o **meu sincero agradecimento.**

“I wandered lonely as a cloud That floats on high o'er vales and hills, When all at once I saw a crowd, A host, of golden daffodils; Beside the lake, beneath the trees, Fluttering and dancing in the breeze Continuous as the stars that shine And twinkle on the milky way, They stretched in never-ending line Along the margin of a bay: Ten thousand saw I at a glance, Tossing their heads in sprightly dance”.

William Wordsworth,

"I Wandered Lonely as a Cloud," 1804.

RESUMO

Tese de Doutorado
Programa de Pós-Graduação em Agronomia,
Área de concentração em Produção Vegetal
Universidade Federal de Santa Maria, RS, Brasil

VERNALIZAÇÃO E APLICAÇÃO DE ÁCIDO GIBERÉLICO NA GERMINAÇÃO E DESENVOLVIMENTO DE *Penstemon digitalis* cv HUSKER RED

AUTOR: Anderson Machado de Mello
ORIENTADOR: Nereu Augusto Streck
Local e data da defesa: Santa Maria, RS, Brasil, 16 de dezembro de 2008.

Penstemon digitalis cv Husker Red tem flores exuberantes que possuem potencial para serem cultivadas e utilizadas tanto como flores de jardim como de corte. Foram conduzidos quatro experimentos, sendo o primeiro com o objetivo de determinar se tratamento de plantas de *Penstemon digitalis* cv. Husker Red com ácido (AG_3) e diferentes períodos de vernalização produziriam plantas de penstemon com alta qualidade para flor de corte no mínimo intervalo de tempo possível, e de maneira que possa ser repetida. As concentrações de AG_3 foram: 0, 100, 250 e 500 mg L^{-1} ppm e os períodos de vernalização foram: 0, 3, 6, 9 e 12 semanas de frio à 5°C. O experimento foi conduzido em casa de vegetação com plantas de *Penstemon digitalis* cv. Husker Red. A interação entre doses de AG_3 e diferentes períodos de vernalização não foi significativa. As variáveis número de dias para a emissão de botões florais, número de dias para as plantas florescerem, e número de dias para o florescimento a partir da emissão dos botões florais decresceram após a aplicação de AG_3, de acordo com os níveis de AG_3 e de acordo com os períodos de vernalização. As variáveis altura da planta, número de flores por planta e tempo de permanência das flores nas hastes não foram afetadas pelas aplicações de AG_3 nem pelos diferentes períodos de vernalização. Os outros três experimentos foram conduzidos como o objetivo de determinar se o tratamento de sementes de com AG_3 aumentaria a germinação de sementes de *Penstemon digitalis* cv. Husker Red. As concentrações de AG_3 utilizadas foram: 0, 10, 50, 100, 200 e 500 mg L^{-1} (segundo experimento) 0, 500, 1000 e 1500 (terceiro experimento) e 0, 500 e 1000 (quarto experimento). O segundo e o terceiro experimento foram conduzidos em câmara de crescimento enquanto que o quarto experimento foi conduzido em câmara de crescimento e casa de vegetação com sementes cobertas e não cobertas por substrato. Em todos os experimentos, o ácido giberélico aumentou a porcentagem e a taxa de germinação das sementes, sendo a dose de 1000 ppm de AG_3 o melhor tratamento. No quarto experimento, a porcentagem bem como a taxa de germinação foram mais elevadas, nas sementes que foram mantidas em câmara de crescimento, provavelmente devido a temperatura constante e a ausência de luz. Na casa de vegetação, a porcentagem de sementes que germinaram e a taxa de germinação, foram similares, não importando se as sementes foram cobertas ou não e também similares para as doses de 500 e 1000 mg L^{-1} de AG_3.

Palavras-chave: quebra de dormência, baixa temperatura, produção de flores, flor de corte.

ABSTRACT

Doctoral Thesis
Programa de Pós-Graduação em Agronomia,
Área de concentração em Produção Vegetal
Universidade Federal de Santa Maria, RS, Brasil

VERNALIZATION AND GIBBERELLIC ACID IN THE GERMINATION AND DEVELOPMENT OF *Penstemon digitalis cv* HUSKER RED

AUTHOR: Anderson Machado de Mello
ADVISOR: Nereu Augusto Streck
Location and date of presentation: Santa Maria, RS, Brazil, December 16th, 2008.

Penstemon digitalis cv Husker Red has showy flowers that have the potential to be cultivated as garden flowers as well as cut flowers. Two studies were conducted in Lincoln – NE, USA. The first study was carried out to determine if treating plants of *Penstemon digitalis* cv. Husker Red with gibberellic acid (GA_3) and vernalization (cold) periods would produce the best quality cut flowers of penstemon in the shortest possible time, in a repeatable manner. The GA_3 concentrations were: 0, 100, 250 and 500 mg L^{-1} and the vernalization periods were 0, 3, 6, 9 and 12 weeks of cold at 5°C. The experiments were conducted in a greenhouse with plants of *Penstemon digitalis* cv. Husker Red. There were no significant interactions between GA doses and vernalization periods. The variables: number of days to buds, number of days of plants to flowering and number of days to flowering since buds decreased after GA applications, according to the GA levels and according to the vernalization periods. The variables: plant height, number of flowers per plant and time that flowers lasted on the plants were not affected by GA_3 applications neither by different vernalization periods. The second study was conducted to determine if soaking seed with gibberellic acid (GA_3) would increase germination of *Penstemon digitalis* cv. Husker Red seed. The GA_3 concentrations were: 0, 10, 50, 100, 200 and 500 mg L^{-1} (first experiment) 0, 500, 1000 and 1500 (second experiment) and 0, 500 and 1000 (third experiment). The first and second experiments were conducted in a growth chamber while the third experiment was conducted in a growth chamber and greenhouse with seeds either covered or not covered in mix. In all experiments, gibberellic acid increased the percentage and rate of seed germination. The 1000 mg L^{-1} GA_3 was the best treatment. Germination percentage and rate were the highest for seeds grown inside of the growth chamber probably due to the consistency of temperatures and darkness. In the greenhouse, the percentage of seeds that germinated and the rate of germination were similar regardless of whether or not the seeds were covered or uncovered and whether they received either the 500 or 1000 mg L^{-1} GA_3 treatment.

Key words: breaking dormancy, low temperature, flower production, cut flowers.

LISTA DE TABELAS

CAPITULO III

TABELA 1 – The actual percentage of seed germination after 15 days and the estimate of the number of days it would take to 50% seed germination when seeds of *Penstemon digitalis* 'Husker Red' were soaked for 24 hours in varying concentrations of GA_3. All pair-wise comparisons between treatments were significantly different from each other at Pr <0.01 81

TABELA 2 – The actual percentage of seed germination after 15 days and the estimate of the number of days it would take for 50% seed germination when seeds of *Penstemon digitalis* 'Husker Red' were soaked for 24 hours in varying concentrations of GA_3. All pair-wise comparisons between treatments were significantly different from each other at Pr <0.01 82

TABELA 3 - The actual percentage of seed germination after 15 days and the estimate of the number of days it would take for 50% seed germination when seeds of *Penstemon digitalis* 'Husker Red' were soaked for 24 hours in varying concentrations of GA, and then sown in soilless mix. Seeds were sown either on top of the mix (uncovered) or covered lightly with mix and placed either in a growth chamber or the greenhouse. Unless indicated by an *, all pair–wise comparisons between treatments were significantly different from each other at Pr<0.01 83

LISTA DE FIGURAS

CAPÍTULO II

FIGURA 1- Effect of GA_3 (0, 100, 250 or 500 mg L^{-1}) sprayed on plants of *P. digitalis* cv. Husker Red on: (a) number of days to buds; (b) number of days to flowering; (c) number of days to flowering since buds; (d) plant height (cm); (e) number of flowers per plant and (f) time that flowers lasted in the plants 65

FIGURA 2 - Effect of weeks of cold (0, 3, 6, 9 or 12) at 5°C on plants of *P. digitalis* cv. Husker Red on: (a) number of days to buds; (b) number of days to flowering; (c) number of days to flowering since buds; (d) plant height (cm); (e) number of flowers per plant and (f) time that flowers lasted in the plants 66

CAPÍTULO III

FIGURA 1 - Percentage of *Penstemon digitalis* cv. 'Husker Red' seeds that germinated each day after soaking for 24 hrs in 0, 10, 50, 100, 200 or 500 $mg \cdot L^{-1}$ gibberellic acid (GA_3). Seeds were sown on moistened filter paper in Petri dishes and germinated in a dark growth chamber at 21-24°C 84

FIGURA 2 - Percentage of *Penstemon digitalis* cv. 'Husker Red' seeds that germinated each day after soaking for 24 hrs in 0, 500, 1000 or 1500 $mg \cdot L^{-1}$ gibberellic acid (GA_3). Seeds were sown on moistened filter paper in Petri dishes and germinated in a dark growth chamber at 21-24°C 85

LISTA DE APÊNDICES

CAPÍTULO III

APÊNDICE A – SAS output ANOVA with LSMEANS and regressions 99
APÊNDICE A – SAS algorithm

SUMÁRIO

1 INTRODUÇÃO..... 13
2 CHAPTER I - LITERARURE REVIEW..... 14
2.1 Cut flowers in Brazil..... 14
2.2 Penstemon spp. as cut flower..... 15
2.3 Germination..... 16
2.4 Seed germination..... 24
2.5 Plant Vernalization..... 25
2.6 Growth and temperature..... 26
2.7 Light..... 26
2.8 Gibberellic acid (GA)..... 27
2.9 Size of containers for greenhouse production 31
2.10 The medium..... 32
2.11 pH..... 32
2.12 Nutrition..... 33
2.13 Moisture..... 34
2.14 Preharvest..... 34
2.15 Postharvest..... 35
2.16. Diseases..... 36
3. References..... 37

3 CHAPTER II - *Penstemon digitalis cv* Husker Red: vernalization and gibberellic acid application..... 52

Abstract..... 53
Introduction..... 54
Material and Methods..... 56
Results and Discussion..... 58
Literature cited..... 61

4 CHAPTER III - Gibberellic Acid Promotes Seed Germination in *Penstemon digitalis* cv. Husker Red..... 67
Abstract..... 69
Introduction..... 70
Material and Methods..... 71
Results and Discussion..... 73
Literature cited..... 77
5 CONCLUSÕES GERAIS..... 86
6 SUGESTÕES PARA TRABALHOS FUTUROS..... 87
7 REFERÊNCIAS..... 88

1 INTRODUÇÃO

Nos últimos anos, *Penstemon* tem se tornado uma planta de jardim muito popular, devido a muitas (aproximadamente 280) espécies. Estas plantas crescem e toleram uma grande amplitude de temperaturas e também possuem uma ampla variedade de cores e formas de flores.

O interesse neste estudo é o *Penstemon digitalis* cv Husker Red, devido as suas flores exuberantes que possuem potencial para serem cultivadas e utilizadas tanto como flores de jardim como de corte. Estaquia é o método preferido de propagação de penstemon Husker Red, entretando para certos locais e regiões onde estacas não estão prontamente disponíveis, mudas podem ser produzidas e selecionas para a produção de plantas matrizes.

Testes foram conduzidos na Universidade de Lincoln Nebraska (UNL) para avaliar espécies de penstemon para serem utilizadas como flores de corte, sugerindo que algumas variedades possuem longa vida pós-colheita, durando cerca de (13 dias) somente em água sem a ocorrência de abscisão de flores, exemplos (*P. digitalis* e *P. confertus*) (Way James, 1988). Devido ao fato de que *Penstemon digitalis* possui um potencial tanto para ser cultivado como flor de jardim bem como flor de corte, esta planta poderia ser introduzida no Brasil com o objetivo de aumentar a variedade de flores de corte e de jardim no mercado.

Entretanto, plantas de *Penstemon digitalis* Husker Red necessitam de tratamento de frio, 2-7°C (35-45°F) por nove a 15 semanas para florescer. Na falta de tratamento de frio, o florescimento depende da intensidade de luz (Clough et al., 2000). Sementes de muitas espécies de *Penstemon* também possuem algum tipo de dormência, o qual limita a germinação. Requerimentos individuais para germinação variam amplamente entre e dentro de espécies (Kitchen and Meyer 1991; Meyer *et al.*, 1995). Os objetivos desta tese foram (i) estudar e avaliar o efeito do GA_3 e diferentes períodos de vernalização na qualidade de plantas de *Penstemon digitalis* cultivar Husker Red e (ii) determinar o efeito da concentração de ácido giberélico sobre a germinação desse genótipo.

2 CHAPTER I LITERARURE REVIEW

Penstemon digitalis cv Husker Red: vernalization and gibberellic acid application

In order to successfully grow a plant for cut flower production, one must be concerned with all aspects of seed germination, plant growth, and cultural procedures. Thus, this literature review focuses on the many factors involved in this process.

This thesis focuses on quantifying, elucidating and fine tuning the growing procedure in order to produce the best quality of cut flowers of penstemon in the shortest possible time.

2.1 Cut flowers in Brazil

The production of cut flowers and ornamental plants in Brazil occurs mainly in the state of Sao Paulo (FLORABRASILIS, 2002). This state is responsible for 70% of the national production (FNP consultoria & comercio, 2001). According to IBRAFLOR (2001) the total area of cut flowers production in Brazil in 1999 was around 4,850 ha.

However, production is increasing in the other states, such as in Rio Grande do Sul, due to the improvement in the market chain, the diversification of species and varieties, and the diffusion of new technologies of production (Tanio and Simoes, 2005).

The per capita consumption of cut flowers in Brazil is between 4 to 7 U.S. dollars. This rate of consumption appears modest when compared with Argentina's per capita consumption of 25 U.S. dollars. Switzerland, Germany, Sweden, and Denmark have a consumption rate of 100 U.S. dollars per capita. The Rio Grande do Sul consumes approximately 25 U.S. dollars per capita, which is close to the Argentina consumption, thus, differentiating it from the others Brazilian states. The greater consumption in Rio Grande do Sul State compared with other Brazilian States can be attributed to cultural characteristics of this State where people are more used to buy and use cut flowers for different purpose (SEBRAE, 1999).

During the last few years, Brazil has been searching for new markets such as Holland and the USA (IBRAFLOR, 2001). The U.S. is still a significant cut flower producer. However, since 1995 the production and number of U.S. cut flower growers is declining while cut flowers imports are growing especially from South and Central America (APDA, 1996).

Brazilian producers and exporters are still learning how to best satisfy the demands of the U.S. market. For example, many Brazilian cut flower products do not adequately meet the high standards for quality and variety necessary to successfully compete in the U.S. markets. An increased emphasis on trade associations between Brazil and the U.S. cut flowers sectors could be advantageous to both sides (Marques, 2002).

2.2 *Penstemon* spp. as cut flower

Over the past few years, *Penstemon* spp. have become increasingly popular garden flowers in the United States. Their flowers are now available in a wide range of colors, and a large number of varieties, some of which could be used as cut

flowers, (e.g. *Penstemon digitalis*). Tests have been carried out at University of Nebraska-Lincoln (UNL) to evaluate penstemon species as cut flowers, suggesting that some varieties have a markedly superior cut flower life, lasting (13 days) in plain water with no flower abscission, (e.g. example *P. digitalis* and *P. confertus*) (Way and James, 1988). Because *Penstemon digitalis* has potential as both garden and cut flower, this plant could be introduced in Brazil with the objective to increase the variety of cut flowers and garden plants on the market.

2.3 Germination

2.3.1 Dormancy

Dormancy can be defined as a measure of the state of readiness of the enclosed embryo to resume growth (Raghavan, 2000; Fenner, 2000). According to Baskin and Baskin, (1998) dormancy can be classified in: Physiological dormancy (due to physiological inhibiting mechanisms of the embryo), morphological dormancy (due to morphological characteristics of the embryo), morphophysiological dormancy (Physiological + morphological), Physical dormancy (due to the impermeability of the seed), Physical plus physiological dormancy, chemical dormancy (due to the presence of inhibitors in the pericarp) and mechanical dormancy (due to the presence of a hard endocarp).

Various methods can be applied to break dormancy and the choice of the right method depends on previous knowledge about the kind of dormancy that the seed has (Raghavan, 2000; Bradbeer, 1988). These methods can include single approaches such as scarification, leaching, exposure to light or dark, storage at low

or high temperature and exposure to chemicals (Raghavan, 2000) or by the exposure of two or more approaches (Bradbeer, 1988) for different kind of dormancies.

According to Atwater (1980) members of Scrophulariaceae family have endospermic seeds with axillary miniature embryos (endosperm occupies half or less of the seed and surrounds the embryo), are small, with thin and fragile coats. Seeds of many species of *Penstemon* are dormant, but seeds requirements vary widely, both among and within species (Kitchen and Meyer, 1991; Meyer et. al., 1995).

2.3.2 Stratification

2.3.2.1 Low temperatures

The exposure to low-temperature can be described as chilling, cold after ripening or stratification (Bewley an Black, 1982). Low temperatures can range from 0° to 1°C (Bradbeer, 1988), 1° to 10°C (Bewley and Black, 1985) and 1.5° to 15°C (Totterdell and Roberts, 1979). The time of exposure to cold temperatures can generally vary from 60 to 90 days for woody species to a just few days for herbaceous species (Bewley and Black, 1985).

For snapdragon (*Antirrhinum majus)*, it is recommended to chill seeds for several days before sowing to improve germination (Nau, 1993). In the genus *Penstemon*, dormancy can be removed by subjecting the seed to low temperatures around 7°C (45°F) for weeks or months depending on the species (Nold, 1999). In addition, seeds usually benefit from chilling treatment at 4°C for at least four weeks (Way and James, 1988). *Penstemon* spp. were successfully germinated when seeds were cold-moist stratified or scarified (American Penstemon Society, 1981). *Penstemon parryi* seeds treated for 10 days in darkness and light at 15°C had the

highest total germination rate when compared with 5 days at the same light and temperature conditions (Raeber and Lee, 1991). Allen and Meyer (1990) working with seeds from three *Penstemon* species (*Penstemon strictus* Benth, *Penstemon palmeri* Gray and *Pensemon eatonii* Gray), subjected to various cold stratification and incubation temperature treatments obtained maximum germination rate after 4 weeks at 15ºC. Seeds of *Penstemon* 'Prairie snow' when stratified for 6 to 10 weeks at 0º to 3ºC had the best germination rates (Lindgren, 1990). Lindgren and Schaaf (2004) studying the effect of seed age together with cold stratification periods (0, 2, 4, 6, 8, and 10 weeks at ± 3ºC) on emergence of seven *Penstemon* species observed that seed stratification significantly increased emergence.

Penstemons plant populations exposed to severe winters (minimum average temperature of 15-20ºF) produced seeds with longer chilling requirements. Whereas plant populations exposed to mild winters (average minima being 30º to 42º F) produced seeds with shorter chilling requirements. Populations from mid-elevation habitats produced seeds with intermediate chilling requirements (Meyer and Kitchen, 1994).

2.3.2.2 Exposure to chemicals

Many chemicals can be used to break dormancy (Bewley and Black, 1982, 1985; Alderson 1987; Bradbeer, 1988; Raghavan, 2000). According to Bewley and Black, (1982; 1985) the chemical products can belong to the following groups: respiratory inhibitors, sulfhydryl compounds, oxidants, nitrogenous compounds, and growth regulators.

The growth regulator gibberellin (gibberellic acid GA_3, GA_4 and GA_7) can break the dormancy in numerous species of seeds (Bewley and Black, 1982; 1985),

including P*enstemon* (Atwater, 1980). Immature seeds in comparison with other parts of plants have relatively more gibberellins, demonstrating that GA(s) in seeds results from biosynthesis and not from transport (Salisbury and Ross, 1992). The use of GA to break seed dormancy has been applied in many species suggesting that ABA/GA ratio may be critical for seed dormancy (Bewley, 1997b). The effect of gibberellins on seed germination is to enhance cell elongation permitting the radical to push through the endosperm and seed coat that restricts its growth (Salisbury and Ross, 1992). But also, according to Taiz and Zeiger (2006), gibberellins are responsible for the activation of vegetative growth of the embryo where in this case embryonic GA is released triggering the weakening of seed-covering layers that surrounds the embryo is responsible for the stimulation and mobilization of foods and mineral elements in seed storage cells, as well as increasing and stimulating the extensibility of cell walls.

The weakening of the micropylar endosperm looks to be fundamental for seed germination and it occurs when GA acts on the cell walls through hydrolysis (Bewley, 1997a; Yamaguchi and Kamiya, 2002). According to Nonogaky (2000) the job of GA in germination is more “repressing a repressor” than inducing a factor or de-repressing a factor promoting germination. Dill and Sun (2001) and Peng and Harberd (2002) suggest that DELLA proteins like RGA (repressor of *ga1-3*) and GAI (gibberellin insensitive) may act as integrators of environmental and endogenous cues to regulate seed germination where RGL2 and the other DELLA-type proteins are transcription factors that inhibit GA responses and, thus, are considered to be negative regulators of GA signal transduction.

There are a variety of studies showing the positive effects of gibberellic acid on seed germination in many species. Kornegay and Doubrava (2006) working with seeds of *Verbena bonariensis* treated with GA_3 (125, 250, 500 and 1000 mg L^{-1}) at

24- 25ºC, demonstrated an increase in germination rate from 9 to 52% when compared with the control (0 mg L^{-1}). Gibberellic acid at 0.29 mM for 24h enhanced the germination of seeds of *Sesleria varia* (Castiglioni, et al. 2004). Seeds of *Dianthus glacialis* treated with GA_3 (0 to 0.72 mM) for 48h had higher germination at concentration of 0.72 mM. However this was not statistically different when compared with the control (Colombo et al., 2004). According to Meerow (2004) GA_3 presoaks has positive effects on germination rate of palm seeds, but GA_3 is not recomended because of it causes excessive elongation of the seedling. GA_3 soaks (0, 0.1, 0.2, 0.3 and 0.4 mM) for 12h improved the germination of corn and soybean seeds (Wang et al., 1996). Fontenot (2003) found an enhancement of germination of seashore paspalum 'Seaspray' seeds after 48h of soaking in 50µm of GA_3. Presoaking of *Sesamum indicum* seeds for 12h in GA_3 (100 or 500 mg L^{-1}) improved germination at 15 ºC (Kyauk et al., 1995). According to these authors the GA_3 may have diminished the adverse effects of low temperature (15ºC) on germination. Seeds of *Trichocereus terscheckii* had the germination improved when seeds received (0, 500 and 1000 mg L^{-1}) of GA_3 (Ortega-Bae and Rojas-Aréchig 2007). Seeds of *Ferula gummosa* and *Teucrium polium* had the germination percentage enhanced when soaked for 72h in GA_3 (1000, 1500, 2500 mg L^{-1}) for *Ferula gummosa* and (500, 100 and 1500 mg L^{-1}) for *Teucrium polium* (Nadjafi et al 2005). GA_3 pre-treatments of non-stratified black mulberry seeds significantly improved germination, being the highest germination percentage (60–67%) at 1000 and 2000 mg/l (Koyuncu, 2005). GA_3 (1500 mg L^{-1}) applied in true potato seeds increased percentage, rate and uniformity of emergence and dry weight of seedlings (Lerna and Tenorio, 2004). Puppala and Fowler (2003) using 100 mg GA_3 L^{-1} for 4 h stimulated the germination of *Lesquerella fendleri*

seeds. Soaking of 250 µM of GA_3 for 24h, improved the germination of *Podophyllum hexandrum* (Nadeem et al., 2000).

Gibberellic acid soak (100 mg L^{-1}) for 40 min applied in Marama bean (*Tylosema esculentum)* enhanced seed germination but just after 10h of water immersion being the positive GA_3 action attributed to a previous seed coat disruption (Travlos et al., 2007). Soak of *Eriostemon australasius* seeds in 0.5% of gibberellic acid for 20 hours after soaking in concentrated sulphuric acid for 20 min stimulated germination (Lidbetter et al. 2003). Rogis et al. (2004) demonstrated an improvement of seed germination as well as an increase in germination speed for Eastern gamagrass (*Tripsacum dactyloides* L.) after soaking with GA_3 (1mM) for 24 h combined with three weeks or less of stratification at 4°C. Application of 1000 mg L^{-1} of GA to scarified seeds (±4°C) of three species of Pseudopanax enhanced the speed of germination (Bannister and Bridgman, 1991). Exogenous application of 400 mg L^{-1} GA_3 to scarified seeds of Cyclocarya *paliurus* significantly enhanced seed germination (Fang et al. 2006). Application of GA_3 (250 mg L^{-1}) after 1 or 3 weeks of moist-chilling at ± 1°C promoted the germination of loquat *Eriobotrya japonica* (EL-DENGAWY El-Refaey, 2005). Çetinbaş and Koyuncu (2006) working with *Prunus avium* L. (mazzard cherry) seeds treated with exogenous GA_3 had 500 mg L^{-1} as the best dose in breaking dormancy in seeds with and without coat. Norden et al. (2005) soaking seabeach amaranth (*Amaranthus pumilus*) seeds for 24 hours in K-GA_3 prior to exposing them to an 8/16-hr thermoperiod of 30/20C without light increased linearly total germination percentages as the concentration of K-GA_3 increased from 0 to 1000 mg L^{-1}. Gibberellic acid (1000 mg L^{-1} + sand) stimulated germination of *Galeopsis speciosa* (Karlsson et al., 2006). Ruchala (2002) working with seeds of *Asarum canadense* stratified at 20°C for 0, 1, 2, or 3 months and then soaked in

gibberellic acid (0, 200, 400, or 600 mg L^{-1}) for 24 hours before being sowed in germination mix and placed in the greenhouse and with seeds that received the same stratification and GA treatments but placed in a cold storage facility 4℃ for 2 months did not get any germination.

Application of GA_3 (0.1, 0.3 and 0.5 mg/L) for 8h in seeds of *Echinacea angustifolia* after being prechilling for 18 days, and elimination of seed coat layers had stimulatory effects on the seed germination (Chuanren et al., 2004). Soaking of GA_3 1000 mg L^{-1} for 24 hours of scarified seeds of *Lupinus diffusus* Nutt. (sky-blue lupine) did not improve germination (Dehgan et al., 2003).

According to Yamaguchi and Kamiya (2002), GA can mimic the effect of red light. Evensen and Loy (1978) working with seeds of a monogenic recessive dwarf inbred lines of watermelon showed that GA_3 increased the rate of germination while light decreased the rate. The authors suggested that this result was due to a lipid degradation promoted by GA_3 in dark-incubated seeds, thus, providing a mechanism for generation of solutes during seed germination. Application of GA_3 (50 mg L^{-1}) incubated at alternating temperatures (20/30℃) for 16/8h in light did not enhance germination of *Echinacea angustifolia* (Macchia et al., 2001).

Ruchala (2002) soaking seeds of wintergreen *(Gautheria procumbens)* with (0, 200, 400, 600, 800, or 1000 mg L^{-1}) of GA_3 for 24 hours and placing seeds in petri dishes and them in the greenhouse under three different light regimes (long photoperiod of 18 hours, regular daylight and dark) had significantly increased germination percentages in all treatments due to GA, but no significant interaction between GA and light was observed, being the best germination percentage obtained with 1000 mg L^{-1} of GA_3 under ambient light conditions. This author

suggested that the low rate of germination in the dark was due to a lack of oxygen as the petri dishes were covered with plastic bags to create dark conditions.

Rouhi (2006) found that applied GA (0, 125, 250 and 500 mg L^{-1}) in seeds of *Prunus scoparia*, *a* native plant from Iran, did not significantly increase germination percentages after 10 days in dark at 7°C. However germination percenteages increased significantly after 10 days in dark at 22°C with the highest seed germination percentage obtained at a dose of 125 mg L^{-1} GA.

Kitchen and Meyer (1991), working with 50, 150, 250 and 500 mg $L^{-1}$of GA_3, recommend treatments with gibberellic acid to remove seed dormancy or shorten the chilling requirement for many (but not all) species of P*enstemon*, and recommended the use of gibberellins together with other treatments such as stratification or scarification, in order to increase the effects of GA in dormancy-breaking.

Raeber and Lee (1991), using 6 doses of gibberellic acid GA_3 (0, 10, 50, 100, 200, 500 mg L^{-1}) for 24h in 250ml of distilled water in *Penstemon parryi* seeds, obtained an increase in seed germination when compared with the control.

2.3.3. Scarification

Scarification is denoted for the name of the mechanical or chemical mechanism used to cause damage to the seed coat in order to break dormancy (Bradbeer, 1988). According to Alderson (1987), the physical dormancy of the seed coats of some species can be broken by the exposure of the seeds to concentrated sulphuric acid. Acid scarification with concentrated sulphuric acid is one of the most used treatments to softer/damage hard seed coats with treatments time varying from a few seconds to several hours. (Ellis, 1985).

Seed of *L. texensis* Hook. (Texas bluebonnet) scarified with sulfuric acid 36 N for 30 to 60 min improved seedling emergence (Davis et al*.,* 1991). Acid scarification of *L.havardii* S. Wats (Big Bend bluebonnet) with sulfuric acid 36 N for 120 min resulted in nearly 100% germination (Mackay et al., 1995). Seeds of *Lupinus diffuses* scarified in concentrated sulfuric acid 18 M for 90 min followed by immersion in water for 24 hr resulted in the best emergence of viable seed (Dehgan et al., 2003). It is suggested for rose seeds to soak in concentrated sulfuric acid for one hour (Blundell and Jackson, 1971). Flessner (1988) found that scarified seeds of *Penstemon haydenii* with sulfuric acid 18 M for 15, 30, 45, and 60 minutes to enhance germination.

2.4 Seed germination

According to Alderson (1987) most of the published literature on seed germination is dedicated to the problems of woody and agronomic species.

After dormancy has been broken, some important environmental factors such as light, temperature, shade, oxygen, and carbon dioxide will regulate the germination of seeds (Bewley and Black, 1982). For snapdragon, the optimum temperature germination is around 21°-24°C (70° to 75°F), and seeds germinate in 7 to 14 days in full light (Nau, 1993).

The germination requirements of *Penstemon* seeds vary widely, both among and within species (Kitchen and Meyer 1991; Meyer et al., 1995). Seeds from environments with protracted periods of snow cover and extended cold winters germinate slower than seeds from environments, which have snow cover of a shorter duration along with warmer winter temperatures (Meyer and Kitchen, 1994). Moreover, seeds from some types of penstemons including most mountain species

from the Rockies and certain hybrids require a germination temperature around 4℃ (40℉). However, Mexican species such as *P. hartwegii* and *P. gentianoides* and a few species, typified by *P. whippleanus* and *P. pinifolus* require a temperature of 21℃ (70℉) for germination (Way and James, 1988). Seeds of *P. barbatus* var. *Coccineus* will also germinate in seven to 10 days at 21℃ (70F) (Nau, 1996).

It has been reported that light is necessary for the germination of some species of *Penstemon*, such as *P. digitalis* (Deno, 1991). Lindgren and Schaaf (2004), however, studying the influence of seed stratification and seed age on emergence of *Penstemon* suggested that *P. digitalis* seeds emergence could be enhanced with stratification even without light.

2.5 Vernalization

Plants of *Penstemon digitalis* Husker Red require cold treatment at 2-7℃ (35-45℉) for nine to 15 weeks to flower. Without cold treatment, flowering depends on light intensity (Clough et al., 2000). Clements et al. (2002) found that *Penstemon tenuifiorus and P. hirsutus* also have the same vernalization requirements for flowering such that plants require exposure to ≥350 hours of vernalizing temperatures to flower (which temperatures).

Studies of the vernalization requirements for plants of *Penstemon pinifolius* and *Penstemon palmeri* that were stored in a cooler at 5℃ for 0, 3, 6, 9, 12, or 15 weeks under 9-hour photoperiod produced the following results. The effect of cold treatments on flowerering of either species was not significant. Final average internode length and final height were affected by the duration of the chilling. The chilling of *Penstemon palmeri* plants for periods of 12 or 15 weeks resulted in increased height and longer average internode length. In addition, chilling plants of

Penstemon pinifolius for 12 weeks resulted in longer average internode length. For both *Penstemon pinifolius* and *P. palmeri* plants, the effect of the chilling effect on height varied (Croft, 2003).

2.6 Growth and temperature

Penstemon barbatus plants can be grown at 16°C (60°F) nights from just after germination until they start to root. After this period they can be moved to a cold frame and grow at 10°C (50°F) until ready to sell (Nau, 1996).

On the other hand, *P. digitalis* cv. Husker Red requires two weeks at 18-21°C (65-70°F) for rooting cuttings and 3 to 4 weeks 18-21°C (65-70°F) for growth in to plugs (Clough et al., 2000).

Penstemon haydenii, an endangered plant species (blowout penstemon), requires temperatures between 20 and 30°C. The best growth of this species was obtained at 25 to 30°C daytime and 25 to 30°C nighttime in a greenhouse experiment (Lamphere, 1999).

2.7 Light

Not all species of *Penstemon* require exposure to full sun during the growth and development period, since some of them flourish in partial shade (Way and James, 1988). Plants of *Penstemon digitalis* Husker Red however, grow best in full sun. Furthermore, plants of this species, without cold treatment are dependent on light intensity for flowering. This is probably due to the existence of an alternative pathway to the cold treatment in response to the environmental factors.

Increased light intensity can also influence flower number of *Penstemon digitalis* cv. Husker Red. For this species, the use of high-pressure sodium (HPS) lamps is better than incandescent (INC) lamps due to the intensity of light and wave length (Clough et al., 2000). *Penstemon digitalis* Husker Red also needs full sun to keep the beautiful purple leaf color (Armitage, 1989).

The effect of shade on shoot dry mass and growth of two closely related species, *Penstemon tenuiflorus* and *Penstemon hirsutus,* were compared in a greenhouse study. Eighty to 100% of 15 replicate plants of *Penstemon tenuiflorus* and 60 to 93% of 15 plants of *Penstemon hirsutus* survived in the three light levels under which they were grown, i.e., no shade, intermediate shade, and heavy shade. Vernalized plants of *Penstemon tenuiflorus* and *Penstemon hirsutus* flowered similarly well under either long or short day photoperiods. Therefore, these two species are considered day-neutral species (Clements et al., 2002).

2.8 Gibberellic acid (GA)

Although many studies have been conducted on ending dormancy by cold temperature and GA treatment, little or no information is available on the effects of dormancy-breaking chemicals on Penstemons.

Flowering of many vernalization-responsive plants often requires long days (LD). This restricts their reproduction to spring and summer seasons. For simplicity, many studies have focused either on vernalization or daylength response. Thus, with the LD and cold responsive dicot Arabidopsis *(Arabidopsis thaliana)*, cultivars and lines have been chosen that respond strongly to vernalization, and they have generally been grown under LD to avoid any affect of daylength (Mouradov et al., 2002).

This vernalization response, at least for Arabidopsis, involves gene groups such as FLC, FRI, as well as a number of other flowering time genes (Sung and Amasino, 2004). Gibberellic acid (GA), vernalization, and photoperiod refer to the different pathways involved in floral development and induction (Soltis et al., 2002).

In many long day and cold-requiring plants, the application of GA promotes flowering in place of inductive photoperiods or cold treatment. By contrast, the flowering of most short-day plants is not affected by GA treatment (Michaels and Amasino, 1999; Zeevaart, 1983). Additionally, gibberellins (GAs) are involved in cold-induced stem elongation (King and Evans, 2003).

Evidence that GA has a role in vernalization comes from the discovery that exogenously applied GA induces flowering in biennial *Hyoscyamus niger* in the absence of cold treatment (Lang, 1986). Subsequent experiments showed that many cold-requiring species can be induced to flower in the absence of thermo-induction by the application of GA (Zeevaart, 1983). Additionally, in *Thlaspi arvense*, GA metabolism in shoot tips is altered by thermoinduction (0-10℃) (Hazebroek *et a*, 1993).

In Arabidopsis, applied GAs are effective in reducing the time to flowering in early- and late-flowering ecotypes as well as late-flowering mutants (Bagnall, 1992; Chandler and Dean, 1994). GA application led to earlier flowering in ecotype mutant *fca* under long days and *Landsberg erecta* under short days (both vernalization-sensitive mutants of *Arabidopsis thaliana*). The response was saturated across the range of GA dosage. Flowering was promoted by all of the GA treatments, relative to the control plants. There were no significant differences in the effectiveness of promotion by the different isomers GA_3, GA_5 and GA_9 (Bagnall, 1992). In Arabidopsis, GA may not have a direct role in the vernalization response, but may be

required for an alternate pathway that promotes flowering under non-inductive photoperiods (Michaels and Amasino, 1999).

The flower inducing ability of GA on *Rudbeckia* sp. has been reported for *R. hirta* and *R. bicolor* (Tanimoto and Harada, 1985). They found that GA induced remarkable bolting and flowering. Additionally, GA_3 treatments have been shown to increase the number of blooms in *Aglaonema* breeding stock (Henny, 2003).

Gibberellic acid is known to stimulate elongation of plant cells resulting in taller and larger leaves without inhibiting development. Researchers found that GA_{4+7} may enhance the long day effect on the apical meristem of *Rudbeckia*, but axillary meristems may remain unaffected. Additionally, GA application, significantly decreased days to terminal inflorescence anthesis (Harkess and Lyons, 1994).

Guo et al. (2004), studying the effect of gibberellic acid (50 and 100 mg L^{-1}) in cauliflower plants at the 8-9 leaf stage, noted that GA_3 promoted elongation of the inflorescence stalk in vernalized plants (10ºC for 3, 4, 5 and 6 weeks) as compared to partially vernalized plants (15 or 20 ºC for 2, 3, 4 or 6 weeks) under a 10 h photoperiod.

Plants of 'Coreless' carrots were foliar sprayed with 50, 100, and 200 mg L^{-1} GA_3, three weeks after transplanting. On average, 84% of carrots flowered. Half of the plants were treated again after one week. While the second GA_3 treatment advanced flowering by at least 2 weeks but this only increased flowering to 88% (Bandara and Tanino, 1995).

Paroussi (2002) growing three cultivars of strawberry, applied with GA_3 (50 or 200 mg L^{-1}) under short (10 h) and long (16 h) photoperiods. These treatments reduced the time needed for inflorescence emergence, accelerated flowering and increased the number of flower buds and open flowers. However, GA_3 at 200 mg L^{-1},

combined with the long photoperiod increased the percentage of aborted flowers and malformed fruits. This resulted in a significant decrease in total marketable yield.

Gaillardia x grandiflora cultivars 'Dazzler' and 'Goblin' were induced to flower under long days with applications of GA_{4+7}. This gibberellic acid apparently substituted for long days and promoted flowering under short days in the same amount of time required by untreated, photoperiodically induced plants (Evans and Lyons, 1988).

In *Delphinium* sp. seedlings, the application of 100 mg L^{-1} GA_3 did not promote flowering, but promoted bolting of already differentiated tissues by causing rapid enlargement and stimulating leaf expansion of plants growth under chilled conditions (± 5ºC) (Ogasawara, 2001). Additionally, applications of gibberellic acid (GA) have been shown to break bud dormancy on various azalea cultivars (Joiner et al., 1983). Black et al. (1990) working with dormant-budded 'Gloria' azalea plants that had received cold for four weeks (± 0.5ºC) plus $GA_{4,7}$ at 500 mg.L^{-1} at 3, 10 and 17 days after removal from the cooler demonstrated that plants flowered sooner than did plants grown for six weeks of cold with no GA applications. Postharvest longevity and open flower diameter increased and days to harvest decreased.

In cut chrysanthemum 'Viking', the effect of gibberellic acid at four concentrations (0, 100, 200 and 300 mg L^{-1}) was evaluated five times (second, fourth, eighth and tenth week after planting). The tallest plants were produced with applications of 200 and 300 mg L^{-1} at the second and fourth weeks of growth. In addition, the GA_3 200 mg L^1 regardless of the time of application resulted in flowering (Schmidt, 2003).

Tamari et al. (1998), working with two cultivars of impatiens 'Tempo Pink' and 'Aruba' that were sprayed with ethrel alone or in combination with 25 mg L^{-1} GA,

observed that both cultivars produced taller plants than the ethrel-treated only plants. The same results were found for the length of harvested cuttings. In addition, in all treatments, gibberellin had no effect on the number of flowers, the number of cuttings per plant or the extent or quality of rooting of the cuttings.

Three-year-old plants of *Rhododendron pulchrum* were sprayed with 500 mg L^{-1} GA_3. Two applications were effective in breaking flower bud dormancy and caused the flower buds to show color 10 days earlier and induced anthesis 9 days earlier than the control (Chang, 2000). Dahanayake and Galwey (1999) noticed that the response of Spring rape treated with 0, 10 and 40 µg µ L^{-1} of GA_3 were similar to those for long days (LD) and low-temperature treatments, suggesting that the effect of photoperiod and the vernalization response are probably mediated through gibberellins.

2.9 Size of containers for greenhouse production

For *Penstemon* haydenni*,* the plant growth response to use of large and small tubes was examined for greenhouse production. Results indicated growth varied with tube size and was always linear relative to increasing volume. As a result, container size affected final plant height, root and shoot dry weight, and root-to-shoot ratio for length, but did not affect number of nodes or root-to-shoot ratio dry weight. Taller plants with longer root systems and greater root and shoot dry weights were produced in the largest tubes used (115 and 164 mL volume). Smaller plants were produced in the two smallest tubes (49 and 6 mL) volume compared to larger tubes (Lamphere, 1999).

2.10 The medium

The medium should be sterile and should provide adequate aeration and moisture. *Penstemon* spp. have been successfully grown in various soilless mixes, including washed river sand and vermiculite (1:1 v/v); vermiculite, perlite and peat moss (2:2:1, v/v/v) and vermiculite and perlite (3:2, v/v). For *Penstemon digitalis* cv. Husker Red, plants performed considerably better when grown in a porous soilless mix of sphagnum peat moss, perlite, vermiculite, and bark (Clough et al., 2000).

2.11 pH

Many species have the ability to grow well over a wide pH range, from neutral to highly alkaline (Way and James, 1988). Soils for most penstemons should be on the alkaline side of neutral (Nold, 1999). The tolerance of European Hybrids to at least moderate alkalinity has been shown extensively (Way and James, 1988). However, *Penstemon digitalis* can be cultivated in a pH range of 5.5 to 7.0 (USDA NRCS, 2006). Thus, the pH recommended is between 5.8 and 6.2 (Clough et al., 2000).

*S*eeds of *Penstemon haydenii were* sown in three treatments of pH levels (5, 7, and 9). The pH levels of 7 or 9 resulted in vigorous plants that were similar in appearance and growth; however, seedling emergence was greater in the pH 9 treatment. Plants grown at pH 9 had no more incidence of leaf tip burn than did plants in the pH 7 and both treatments had much less tip burn than plants in the pH 5 treatment group (Lamphere, 1999).

2.12 Nutrition

In general, it is safe to say that soils with relatively low fertility can adequately support the growth certain penstemon species (Way and James, 1988). Seedlings of penstemon should be fertilized with NO_3-N. It is recommended fertilizing seedlings with a low concentration of nutrients solution every 10 days (American Penstemon Society, 1981).

The effect of nitrogen and phosphorus fertilization (0, 75 and 150 mg L^{-1}) of seedlings of *Penstemon haydenii* on growth rate and axillary shoot production was examined in greenhouse studies using plants with two pairs of true leaves. Before cutting (done to stimulate growth and number of axillary breaks or shoots), nitrogen and phosphorus fertilization interacted to increase growth rate. After harvesting, fertilization temporarily stimulated the growth rate of harvested plants only. However, cutting did not affect axillary shoot production but plants fertilized with both nutrients produced more axillary shoots than plants fertilized with one nutrient or not fertilized. Therefore, nitrogen and phosphorus fertilization may be used to produce vigorous transplants in the greenhouse, and cutting fertilized plants just prior to transplanting outside is recomended. If soil moisture levels are below normal, fertilizing with 3-10-3 N-P-K at 150 mg L^{-1} of phosphorus, 45 mg L^{-1} of nitrogen, and 45 mg L^{-1} of potassium + H_2O is recommended. When using soilless mixes, fertilization should be started immediately, being careful not to allow build up of salt concentrations (Flessner, 1988).

In research using *Penstemon digitalis* cv. Husker Red, plants were fertilized at every irrigation using 125 mg L^{-1} N, 12 mg L^{-1} P, and 125 mg L^{-1} K, This was sufficient for good plant growth and development (Clough et al., 2000).

2.13 Moisture

Penstemons prefer well-drained soil. (Thomas, 1990). Like many perennials, penstemons require adequate moisture, but do not tolerate wet roots for long periods. (Hill, 1988; Nold, 1999).

All species of *Penstemon* are relatively drought tolerant and in fact, the biggest limitation to many of the hybrids doing well in the United States is the amount of winter moisture in many regions. Except for a few Eastern U.S. species, plants grow best in sunny, dry locations (Armitage, 1989).

Although drought tolerance is one of the merits of penstemons as garden plants, irrigation can be distinctly beneficial during long dry periods. This is particularly true for the European Hybrids and seed strains, and is consistent with their derivation from Mexican species. In this instance, the amount needed is similar to summer rainfall in their native habitat (Way and James, 1988).

Penstemon haydenii requires abundant soil moisture for seed germination (Flessner, 1988). *Penstemon tenuiflorus* is a bit more tolerant to drought than *Penstemon hirsutus.* Therefore, *Penstemon hirsutus* has the ability to grow better on a greater variety of substrate types than does *Penstemon tenuiflorus* (Clements et al., 2002). *Penstemon digitalis* Husker Red prefers low moisture (Clough et al., 2000).

2.14 Preharvest

For optimal floral stalk longevity, it is recommended to water plants the night before and then cut the stems early in the morning. Cut stems should be placed in

water for a few hours in a cool place. Cut penstemon last longer if floral preservatives are used, such as Universal Flower Food in particular (Way and James, 1988).

2.15 Postharvest

Pre-treatment of snapdragon and *Penstemon* flowers with 1-MCP (1-methylcyclopropene) at concentrations up to 20 nl L^{-1} for 6 hours increased flower longevity 4.5 and 6.0 days respectively. The treatment was applied at 20℃ (Serek et al., 1995).

Carnation and penstemon flowers treated with STS (silver thiosulfate complex) lasted longer than controls or those held in solutions containing either aminooxyacetic acid (AOA) or aminoetoxyvinyl glycine (AVG). Neither the solutions containing AOA nor AVG analogs offered improved vase life over the controls. STS greatly reduced or completely inhibited the abscission of flowers for both carnations and penstemons. The anti-ethylene solution containing the AVG analog only provided some abscission protection when ethylene levels were <0.005 mg L^{-1} for penstemon, and actually stimulated abscission in delphiniums (Staby et al., 1993). *Penstemon digitalis* cv. Husker Red should be shipped before the first flower opens. The stems will remain in flower for about three to four weeks (Clough et al., 2000).

Tests have been carried out at the University of Nebraska to evaluate *Penstemon* species as cut flowers, and the results were: *P. digitalis* and *P. procerus* lasted 13 days; *P. confertus, P. wilcoxii, P*. 'Scharf Hybrid' and *P. Buckley* lasted 9 days; *P. barbatus* lasted 7 days; *P. oklahomensis* and *P. grandiflorus* lasted 6 days and *P. pinifolius* only 5 days. All were placed in water to which a proprietary preservative had been added (Way and James, 1988).

2.16. Diseases

The most common diseases in penstemon are:

Die Back – SYMPTOMS: Loss of a sector of their stem structure; leaves turn brown and the affected part of the plant has a scorched appearance.

Grey mold - Is primarily a disease of concern at the propagation stage – SYMPTOMS: Cuttings wilting or dying back to compost level are often the first indication of infection. If left the stems and leaves become enveloped in a grey fur like fungus.

Leaf spot – SYMPTOMS: Spots are grey in color, vary in diameter from 3 to 13mm and in cases of severe infection may coalesce to form large irregularly shaped dead areas or completely kill the leaf.

Powdery mildew – SYMPTOMS: Whitish powdery coating over stems and leaves (Way and James, 1988). In several cases, **Phytophthora** was found on *Penstemon digitalis Husker Red* as well as P*. campanulatos,* causing stem blackening and eventual plant death (Clough et al., 2000).

Rust and root rot - SYMPTOMS: Yellowing, stunting and wilting of the above-ground portions of the plant. Plants often have a noticeable brown to black lesion at the soil line, since the fungi attack and girdle the plant. Under wet, humid conditions, a gray or off-white webby growth can be seen on the infected plants (Hill, 1988).

3 References

Allen P.S and Meyer S.E. 1990. Temperature requirements for seed germination of three *Penstemon* species. HortScience 25(2):191-193.

American Penstemon Society. 1981. American penstemon society manual for beginners. Dayton, Ohio, American Penstemon Society: 29p.

APDA (Agricultural and Processed Food Products Export Development Authority).1996. Word Trade in Floriculture (http://apeda.com/html/flori1.html).

Armitage, A.M. 1989. Herbaceous Perennial Plants. Varsity Press, Athens, Ga.

Atwater, B.R. 1980. Germination, dormancy and morphology of the seeds of herbaceous ornamental plants. Seed Science and Technology 8: 523-573.

Bagnall, D.J. 1992. Control of flowering in *Arabidopsis thaliana* by light, vernalisation and gibberellins. Aust. J. Plant Physiol. 19:401– 409.

Bandara, P.M.S. and Tanino, K.K. 1995. Effect of gibberellic acid (GA_3) on seedstalk development and flowering in carrot (*DAUCUS CAROTA* L. VAR. *SATIVA* DC). Acta Hort. 394:251-262.

Bannister, P. and Bridgman, J. 1991. Responses of seeds of three species of *Pseudopanax* to low temperature stratification, removal of fruit flesh, and application of gibberellic acid. New Zealand Journal of Botany. 29: 213-216.

Baskin, C. C. and Baskin, J. M. 1998. Seeds Ecology, Biogeography, and Evolution of Dormancy and Germination. Lexington, Kentucky. Academic Press. P.27-42.

Better homes and Gardens, 1978. Meredith Corp. Des Moines: Iowa.

Bewley, J.D. (1997a). Breaking down the walls – a role for endo-b-mannanase in release from seed dormancy? Trends in Plant Science. 2: 464–469.

Bewley, J.D. (1997b). Seed germination and dormancy. Plant Cell 9: 1055–1066.

Bewley, J.D. and Black, M. 1982. Physiology and Biochemistry of Seeds in Relation to Germination. 2. Viability, Dormancy and Environmental Control. Berlin, N. Y. Springer-Verlag. p.60-198.

Bewley, J.D. and Black, M. 1985. Physiology of Development and Germination. New York, N.Y. Plenum Press. P.175-236.

Black, L.A., Nell, T.A. and J.E. Barrett. 1990. Dormancy-breaking Method Effects on Azalea Longevity. HortScience 25(7):810.

Blundell, J.B. and Jackson, G.A.D. 1971. Rose seed germination in relation to stock production. Rose Annual. London, p 129-135.

Bradbeer, J. W. Seed Dormancy and Germination. 1988. Leicester Place, London. Chapman and Hall. P.38-80.

Castiglioni, A., Colombo, A. and Tosca, A. 2004. Evaluation of germination capacity and priming techniques in *Selsleria varia*, a plant for restoration of calcareous grasslands. Abstracts, 27th ISTA Congress. Seed Symposium Budapest, Hungary May 17th– 19th. Page 122.

Çetinbaş, M. and Koyuncu, F. 2006. Improving germination of *Prunus avium* L. seedsby gibberellic acid, potassium nitrate and thiourea. Hort. Sci. (Prague), *33*, (3): 119–123.

Chandler, J. and C. Dean, 1994. Factors influencing the vernalization response and w flowering time of late-flowering mutants of *Arabidopsis thaliana* (L.) Heynh. J. Exp. Bot. 45:1279–1288.

Chang, Y.S. and H.F. Sung. 2000. Effects of gibberellic acid and dormancy-breakingchemicals on flower development of *Rhododendron pulchrum* Sweet and R. *scabrum* Don. Scientia Hort. 83:331-337.

Chien, C., Kuo-Huang, L. and L. Tsan Piao. 1998. Changes in ultrastructure and abscisic acid level, and response to applied gibberellins in *Taxus mairei* seeds treated with warm and cold stratification. Annals of Botany 81:41–47.

Chuanren, D., Bochu, W., Wanqian, L., Jing, C., Jie, L., Huan, Z. 2004. Effect of chemical and physical factors to improve the germination rate of *Echinacea angustifolia* seeds. Colloids and Surfaces B: Biointerfaces 37: 101–105.

Clementes, R.K., Baskin, J.M. and C.C. Baskin. 2002. The comparative biology of the two closely-related species *Penstemon tenuiflorus Pennell* and *P hirsutus* (L.) Willd. (Scrophulariaceae, Section Graciles): III. Ecological life cycle, growth characteristics, and flowering requirements. Castanea 67:161-176.

Clementes, R.K., Baskin, J.M. and C.C. Baskin. 2002. The comparative biology of the two closely-related species *Penstemon tenuiflorus Pennell* and *P hirsutus* (L.) Willd. (Scrophulariaceae, Section Graciles): IV.Efects of shade, drought, and soil type on survival and growth. Castanea 64:299-309.

Clough, Emily, Cameron, Arthur, Heins, Royal and Will Carlson. 2000. Forcing perennials - crop-by-crop – *Penstemon digitalis* Husker Red. *Greenhouse Grower* 18(3):30-40.

Colombo, A., Castiglioni, A., Tosca, A. and Bonomi, C. 2004. Evaluation of germination capacity in *Dianthus glacialis*, an endangered alpine species. Abstracts, 27th ISTA Congress. Seed Symposium Budapest, Hungary May 17th– 19th. Page 69.

Croft, A.A. 2003. The production of native and adaptative plants for the intermountain west using the pot–in-pot nursery production system. Master of Science Thesis, Logan, Utah: Utah State University, 85p.

Dahanayake, R.S. and W.N. Galwey. 1999. Effects of Interactions between Low-temperatures, Gibberillin (GA_3) and Photoperiod on Flowering and Stem Height of Spring Rape (*Brassica napus* var. *annua*). Annals of Botany 84:321-327.

Davis, T. D., George, S.W., A. Upadhaya, J. M. Parsons. 1991. Improvement of seedling emergence of *Lupinus texensis* following seed scarification treatments. Journal of Environmental Horticulture 9:17-21.

Dehgan, B., Norcini G. J, S. M. Kabat, H. E. Perez. 2003. Effect of Seed Scarification and Gibberellic Acid Treatment on Seedling Emergence of Sky-Blue Lupine (*Lupinus diffusus)*. Journal of Environmental Horticulture 21(2):64-67.

Deno, N.C. 1991. *Penstemon Germination*. Bul. Amer. Penstemon Soc. 50-1:3-6

Dill, A. and Sun, T.P. 2001. Synergistic derepression of gibberellin signaling by removing RGA and GAI function in Arabidopsis thaliana. Genetics. 159: 777–785.

Douglas, E.S. et al. 2002. Missing links: the genetic architecture of flower and floral diversification TRENDS in Plant Science 7(1):22-31.

EL-DENGAWY El-Refaey F. A. 2005. Promotion of seed germination and subsequent seedling growth of loquat (*Eriobotrya japonica*, Lindl) by moist-chilling and GA3 applications. Scientia Horticulturae 105: 331–342.

Ellis, R. H. Hong, T. D., E. H. Roberts. 1985. Handbook of seed technology for genebanks. Rome. International Board for Plant Genetic Resources.

Evans and Lyons. 1988. Photoperiodic and Gibberellin-induced Growth and Flowering Responses of *Gaillardia x grandiflora.* HortScience 23(3): 584-586.

Fang, S., Wang, J. Wei, Z. and Zhu, Z. 2006. Methods to break seed dormancy in *Cyclocarya paliurus* (Batal) Iljinskaja. Scientia Horticulturae 110: 305–309.

Fenner, M. 2000. Seeds the Ecology of Regeneration in Plant Communities. (2nd ed.) Wallingford, UK. CABI Publishing. P.183-214.

Flessner, T.R. 1988. Propagation, establishment, and ecological characteristics of *Penstemon haydenii* S. Wats. Master of Science Thesis. Lincoln, NE: University of Nebraska-Lincoln, 192 p.

Florabrasilis. 2002. Relatorio do diagnostico da producao de flores e plantas ornametais brasileira (compact disc). Campinas: IBRAFLOR.

FNP Consultoria & Comercio. Agrianual 2001: Anuario da Agricultura Brasileira, Sao Paulo, 2001- p.337-348: Flores.

Fontenot, D. P. 2003. Evaluating Seashore Paspalum seed germination and enhancement, erosion abatement and potential use as a vegetative landfarm cap. Master of Science thesis. Louisiana State University, The School of Plant, Environmental and Soil Sciences. 76p.

Guo, D.P., Zheng, S.J., Zeng, G.W. and G.A. Shah. 2004. The interaction of plant regulators and vernalization on the growth and flowering of cauliflower (*Brassica oleracea* var. *botrytis)*. Plant Growth Regulation 43:163-171.

Harkess R. and R. Lyons. 1994. Gibberellin and cytokinin-induced growth and flowering responses in *Rudbeckia hirta* L. HortScience 29: 141-142.

Hazebroek, J.P., Metzger, J.D. and E.R. Mansager. 1993. Thermo inductive regulation of gibberellin metabolism in *Thlaspi arvense* L. Plant Physiol. 102:547–552.

Henny, R.J. 2003. Abundant Flower and Seed Produced from Aglaonema Breeding Stock Following Gibberellic Acid (GA_3) Treatment. Foliage Digest v6n. Suge H. 1970. Changes of endogenous gibberellins in Vernalized radish plants. Plant Cell Physiol 11:729–735.

IBRAFLOR. 2001. Brasil: Mostra tua flora**.** Informativo, 7(23):4.

Joiner, J.N. O. Washington, C.R. Johnson, and T. A. NELL. 1982. Effect of exogenous growth regulator on flowering and cytokinin levels in azaleas. Scientia Hort. 18:143-151.

Karlsson, L. M., Ericsson J. A. L. and Milberg, P. 2006. Seed dormancy and germination in the summer annual *Galeopsis speciosa*. Weed Research. 46: 353-361.

Kitchen, S.G. and Meyer S.E. 1991. Seed germination of Intermountain penstemons as influenced by stratification and GA_3 treatments. Journal of Environmental Horticulture 9: 51-56.

Koornneef, M., Bentsink, L. and Hilhorst, H. 2002. Seed dormancy and germination. Current Opinion in Plant Biology. 5:33–36.

Kornegay, J. and Doubrava, N. 2006. Friends of the JC Raulston Arboretum Newsletter. NC State University. VOL. 10, NO. 1.

Koyuncu, F. 2005. Breaking seed dormancy in Black Mulberry (*MORUS NIGRA* L.) by cold stratification and exogenous application of gibberellic acid. Acta Biologica Cracoviensia Series Botanica. 47/2: 23–26.

Kyauk, H., Hopper, N. W. and Brigham, R. D. 1995. Effects of temperature and presoaking on germination, root length and shoot length of Seasame (*Sesamum ndicum* L.) Environmental and Experimental Botany. 35, (3): 345-351.

Lamas, A.M. 1999. *Unidade Produtora de Flores de Corte*. Série Perfil de Projetos. Vitória: Sebrae, 38p.

Lamphere, J.A. 1999. Propagation and establishment of *Penstemon haydenii* S. Wats. Master of Science Thesis. Lincoln, NE: University of Nebraska-Lincoln, 212 p.

Lang, A. 1986. *Hyoscyamus niger*. In: Halevy AH, editor. CRC Handbook of Flowering. Vol V. Boca Raton, FL: CRC Press. P. 144–186.

Lerna, A. and Tenorio, J. 2004. Effects of pre-sowing treatments on emergence charecteristcs of twelve true potato seed (TPS) genotypes. Abstracts, 27th ISTA Congress. Seed Symposium Budapest, Hungary May 17th– 19th. Page 128.

Lewis, H. and N. Hill. 1988. Successful Perennial Gardening: A Practical Guide. Pownal, Vt. : Storey Communications.

Lidbetter, J., Slater, T., Cain, P., Bankier, M. and Vujovic, S. 2003. Grafting *Eriostemon australasius*. A report for the Rural Industries Research and Development Corporation. Publication No 02/140.

Lindgren, D. T. 'Prairie Snow' *Penstemon*. HortScience 25(4):489-1990.

Lindgren, D.T. and D.M. Schaaf. 2004. Influence of Seed Stratification and seed age on emergence of penstemon. HortScience 39(2):1385-1386.

Macchia, M., Angeline, L.G. and Ceccarini, L. 2001. Methods to overcome seed dormancy in *Echinacea angustifolia* DC.Scientia Horticulturae. 89:317-324.

Mackay, W. A., Davis, T. D., and D. Sankhla. 1995. Influence of scarification and

Marques, R.W.C. 2002. Avaliação da sazonalidade do mercado de flores e plantas ornamentais no estado de São Paulo. Dissertação de mestrado, ESALQ/USP. Piracicaba (Evaluation of the seasonality of the flowers and ornamental plants market in the state of Sao Paulo. Masters Thesis, ESALQ-University of Sao Paulo. Piracicaba).

Meerow, A. W. 2004. Palm Seed Germination. Environmental Horticulture Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences. University of Florida. BUL274. 11p.

Meyer, S.E. and Kitchen, S.G. 1994. Habitat-correlated variation in seed germination response to chilling in *penstemon* section glabri (Scrophulariaceae). American midland naturalist, 132(2):349-365.

Meyer, S.E. Kitchen, S.G. and S.L, Carlson. 1995. Seed germination timing patterns in Intermountain *Penstemon* (Scrophulariaceae). American Journal of Botany 82: 377-389.

Michaels, S. D. and Amasino, R.M. 1999. The gibberellic acid biosynthesis mutant *ga1–3* of *Arabidopsis thaliana* is responsive to vernalization. Developmental Genetics 25:194–198.

Mouradov, A., Cremer, F. and G. Coupland. 2002. Control of flowering time. Interacting pathways as a basis for diversity. Plant Cell (Suppl) 14:S111–S130.

Nadeem, M., Palni, L. M. S., Purohit, A. N., Pandey, H. and Nandi, S. K. 2000. Propagation and conservation of *Podophyllum hexandrum* Royle: an important medicinal herb. Biological Conservation. 92: 121-129.

Nadjafi, F., Bannayan, M., Tabrizi, L. and Rastgoo, M. 2005. Seed germination and dormancy breaking techniques for *Ferula gummosa* and *Teucrium polium*. Journal of Arid Environments 64: 542–547.

Nau, J. 1993. Ball Culture Guide. The Encyclopedia of Seed Germination (2nd ed.). Batavia, IL. Ball Publishing.

Nau, J. 1996. Ball Perennial Manual Propagation and Production. Batavia, IL. Ball Publishing.

Nold, R. 1999. Penstemons. Portland Oregon, USA. Timber Press. 259p.

Nonogaki, H., Gee, O.H. and Bradford, K.J. 2000. A germination-specific endo-b-mannanase gene is expressed in the micropylar endosperm cap of tomato seeds. Plant Physiology. 123: 1235–1245.

Norden, D. S., Blazich, F. A., Warren, S. L. and Nash, D. L. 2005. Seed Germination of Seabeach Amaranth (*Amaranthus pumilus*) in Response to Temperature, Light, and Gibberellin A3 Treatments. SNA Research Conference. 50: 411-413.

Ogasawara, N., Hiramasu, T., Ishiyana K.,. Fushimi H., Suzuki H. and H. Takagi. 2001. Effects of gibberellic acid and temperature on growth and root carbohydrates of *Delphinium* seedlings. Plant Growth Regulation 33:181-187.

Ortega-Bae, P. and Rojas-Aréchig, M. 2007. Seed germination of *Trichocereus terscheckii*(Cactaceae): Light, temperature and gibberellic acid effects. Journal of Arid Environments 69: 169–176.

Peng, J. and Harberd, N.P. 2002. The role of GA-mediated signalling in the control of seed germination. Current Opinion in Plant Biology. 5: 376–381.

Raghavan, V. 2000. Developmental Biology of Flowering Plants. New York, N.Y. Springer-Verlag. Inc. p. 292-303.

Reaber, A. C. and Lee, C. W. 1991. Gibberellic acid, temperature, light, stratification, and salinity affect germination of *Penstemon parry* seed. HortScience 26(12):1535-1537.

Rogis, C., Gibson, L. R., Knapp, A. D. and Horton, R. 2004. Enhancing Germination of Eastern Gamagrass Seed with Stratification and Gibberellic Acid. Crop Science. 44: 549-552.

Rosner, L.S., Harrington, J.T. Dreesen, D.R. and L. Murray. 2002. Gibberellic acid effect of and standard seed treatments on mountain snowberry germination. Native Plants Journal 3(2):165-162.

Rouhi, V. 2006. Seed germination of *Prunus scoparia* (Spach) C.K. Schneider and drought stress evaluation based on ecophysiological parameters and growth characteristics for three contrasting almond species (*P. dulcis* (Miller) D. Webb, *P. lycioides* (Spach) C.K. Schneider and *P. scoparia* (Spach) C.K. Schneider). PhD thesis. Ghent University, Belgium. 237p.

Ruchala, S. L. 2002. Propagation of several native ornamental plants. Master of Science in Horticulture thesis. The University of Maine, USA. 127p.

Salisbury, Frank B. & Ross, Cleon W. 1992. Plant physiology, 4th, Belmont, California: Wadsworth Publishing.

Schmidt, M. C. et al. 2003. Ácido giberélico (GA_3) no crisântemo (*Dendrathema grandiflora* Tzvelev.) de corte 'viking': cultivo verão/outono. Cienc. Rural 33(2):267-274.

Serek, M. Sisler, E.C., and M.S. Reid. 1995. Effect of 1-MCP on the vase life and ethylene response of cut flowers. Plant Growth Regulation 16:93-97.

Staby, G. L., R. M. Basel, M. S. Reid, and L. L. Dodge. 1993. Efficacies of commercial antiethylene products for fresh cut flowers. HortTechnology 3:199-202.

Stubbendieck, J. and W. G. McCully, 1976. Effect of temperature and photoperiod on germination and survival of sand bluestem. J. of Range Mgnt. 29:206-208

Sung, S. and R.M. Amasino. 2004 Vernalization and epigenetics: how plants remember winter. Curr Opin Plant Biol 7: 4–10.

Taiz, L., and Zeiger, E. 2006. Plant Physiology (Sinauer Associates, Inc) 4th edition.

Tamari, G., Pappa, L., Zered, T., and A. Borochov. 1998. Effects of ethrel and gibberellin on impatiens plants. Scientia Horticulturae 76:29-35.

Tanimoto and Harada, 1985. *Rudbeckia,* In: A. Halevy (ed.) CRC Handbook of Flowering Vol. IV. CRC Press, Boca Raton, Fla. p. 239-242.

Tanino, S.D. and C. S. Simoes. 2005. Estufos Realizados. GELOG-UFSC, 2005.temperature treatments on seed germination of *Lupinus havardii*. Seed Sci. Technol. 23:815-821.

Thomas, G. S. 1990. Perennial garden plants or the modern florilegium. Saga Press, Portland. Thomsen-Stork, M. 1995. Neue Taglilien aus den USA. Gartenpraxis 21(12):8-13.

Travlos, I. S., Economou, G., Karamanos, A. I. 2007. Germination and emergence of the hard seed coated Tylosema esculentum (Burch) A. Schreib in response to different pre-sowing seed treatments. Journal of Arid Environments 68: 501–507.

USDA NRCS. 2006. The PLANTS database (http://plants.usda.gov/plants). Baton Rouge, LA: National Plant Data Center.

Wang, Q., Zhang, F. and Smith, D. L. 1996. Application of GA_3 and kinetin to improve corn and soybean seedling emergence at low temperature. Environmental and Experimental Botany. (36),4: 377-383.

Way, D. and P. James. 1998 In: The Gardener's Guide to Growing Penstemons. Portland Oregon, USA. 160p.

Yamaguchi, S. and Kamiya, Y. 2002. Gibberellins and lightstimulated seed germination. Journal of Plant Growth Regulation 20: 369–376.

Zeevaart, J.A.D. 1983. Gibberellins and flowering. In: Crozier A, (ed). The Biochemistry and Physiology of Gibberellins. Vol 2. New York: Praeger. p 333–374.

3 CHAPTER II

Penstemon digitalis cv Husker Red: Vernalization and Gibberellic Acid Application

Penstemon digitalis **cv Husker Red: Vernalization and Gibberellic Acid Application**

Abstract

A study was conducted to determine if treating plants of *Penstemon digitalis* cv. Husker Red with gibberellic acid (GA_3) and vernalization periods would produce the best quality cut flowers of penstemon in the shortest possible time, in a repeatable manner. The GA_3 concentrations were: 0, 100, 250 and 500 mg L^{-1} and the vernalization periods were 0, 3, 6, 9 and 12 weeks of cold at 5°C. The experiment was conducted in a greenhouse with plants of *Penstemon digitalis* cv. Husker Red. There was no significant interaction between GA doses and vernalization periods. The variables: number of days to buds, number of days of plants to flowering and number of days to flowering since buds decreased after GA applications, according to the GA levels and according to the vernalization periods. The variables plant height, number of flowers per plant and time that flowers lasted on the plants were not affected by GA_3 applications or different vernalization periods.

Key words: breaking dormancy, low temperature, flower production, cut flowers.

Introduction

Penstemons are native to North America, from Guatemala to Canada, growing in different environments, from wetlands to deserts and alpine conditions (Clements et al., 2002). Penstemon flowers are now available in a wide range of colors, and a large number of varieties, some of which could be used as cut flowers such as *Penstemon digitalis*. This species has become increasingly popular in the United States over the past few years. An important genotype is *Penstemon digitalis* cv Husker Red with its tall showy flower spikes and purple leaves, it has potential as both a garden and cut flower.

Plants of *Penstemon digitalis* Husker Red require cold treatment at 2-7℃ (35-45℉) for nine to 15 weeks to flower. Without a cold treatment, flowering depends on light intensity (Clough et al., 2000). Clements et al. (2002) found that *Penstemon tenuifiorus and P. hirsutus* also have the same vernalization requirements for flowering such that plants require exposure to ≥ 350 hours of vernalizing temperatures to flower.

In many long-day and cold-requiring plants, the application of GA promotes flowering in place of inductive photoperiods or cold treatment. On the other hand, flowering of most short-day plants is not affected by GA treatment (Zeevaart, 1983; Michaels and Amasino,1999). The hypothesis that GA is involved in vernalization relies on the fact that exogenously applied GA induces flowering in biennial *Hyoscyamus niger* in the absence of cold treatment (Lang, 1986). Other experiments showed that many cold-requiring species can be induced to flower in the absence of cold by the application of GA (Zeevaart, 1983). The flower inducing ability of GA on *Rudbeckia* has been reported for *R. hirta* and *R. bicolor* inducing remarkable bolting

and flowering (Tanimoto and Harada, 1985). GA_3 treatments have been shown to increase the number of blooms in *Aglaonema* breeding stock (Henny, 2003).

Gaillardia x grandiflora cultivars 'Dazzler' and 'Goblin' were induced to flower under long days with applications of GA_{4+7}. Gibberellic acid apparently substituted for long days and promoted flowering under short days in the same amount of time required by untreated, photoperiodically induced plants (Evans and Lyons, 1988).

In *Delphinium* seedlings, the application of 100 mg L^{-1} GA_3 did not promote flowering, but promoted bolting of already-differentiated tissues by causing rapid enlargement and stimulating leaf expansion of plants grown under chilled conditions (± 5ºC) (Ogasawara, 2001). Furthermore, applications of gibberellic acid (GA) have been shown to break bud dormancy on various azalea cultivars (Joiner et al., 1983). Black et al. (1990) working with dormant-budded 'Gloria' azalea plants that had received cold for four weeks (± 0.5ºC) plus $GA_{4,7}$ at 500 mg L^{-1} at 3, 10 and 17 days after removal from the cooler, demonstrated that plants flowered sooner than did plants grown for 6 weeks of cold with no GA applications. Postharvest longevity and open flower diameter increased and days to harvest decreased. In cut chrysanthemum 'Viking', the effect of gibberellic acid at four concentrations (0, 100, 200 and 300 mg L^{-1}) was evaluated five times (2, 4, 8 and 10 weeks after planting). The tallest plants were produced with applications of 200 and 300 mg L^{-1} at 2 and 4 weeks after planting. In addition, the GA_3 200 mg L^1 treatment resulted in flowering regardless of the time of application (Schmidt, 2003). Tamari et al. (1998), worked with two cultivars of impatiens 'Tempo Pink' and 'Aruba': These plants were sprayed with ethrel alone or in combination with 25 mg L^{-1} GA. It was observed that both cultivars produced taller plants than the ethrel-treated only plants. Similar results (Tamari et al., 1998) were found for the length of harvested cuttings. In addition, in all

treatments, gibberellin had no effect on the number of flowers, the number of cuttings per plant or the extent or quality of rooting of the cuttings. Three-year-old pot-grown plants of *Rhododendron pulchrum* were sprayed with 500 mg L^{-1} GA_3. Two applications were effective in breaking flower bud dormancy and caused the flower buds to show color 10 days earlier and induced anthesis 9 days earlier than the control (Chang, 2000). Although many studies have been conducted on ending dormancy by cold temperature and GA treatment, little information is available on the effects of dormancy-breaking chemicals on penstemons. The purpose of this study was to evaluate the effect of GA and different vernalization periods on the quality of plants of *Penstemon digitalis* cultivar Husker Red, elucidating and fine tuning the growing procedure in order to produce the best quality cut flowers of penstemon in the shortest possible time, in a repeatable manner.

Material and Methods

The experiment was conducted in the Horticulture Greenhouse Research Complex at the University of Nebraska - Lincoln (UNL), USA, from February 23 to July 26, 2006. Plants of *Penstemon digitalis* cultivar Husker Red were grown in a flat and the treatments were the combinations of five weeks of vernalization (0, 3, 6, 9 and 12) at 5°C and four levels of gibberellic acid GA_3 (0, 100, 250 and 500 mg L^{-1}).

The cold treatments were accomplished by the use of a cold room with controlled temperature set at 5°C with high-pressure sodium lamps (HPS) to provide sun light. A time clock turned the lights on at 7:00 AM and off at 4 PM (9 hours of photoperiod + SD). After each cold treatment, plants were potted in 6 inch plastic

azalea pots using a potting mixture of 2 vermiculite: 2 perlite: 1 peat moss by volume plus dolomite to obtain a pH between 5.8 - 6.2. Plants then received either 0, 100, 250 or 500 mg L^{-1} of GA. GA solutions were prepared by mixing GA_3 in deionized/distilled water. Plants were sprayed with 0.5ml of water or GA_3 solution at the end of each cold treatment and before placing in the greenhouse. Before GA_3 application, the mix of each pot was covered with a plastic sheet to protect the mix and permit the absorption of the product just by the leaves. Applications were made between 8:00 AM and 9:00 AM with spray bottles where each plant received one spray of solution from four directions for a total of four sprays of 5.5 ml of solution per plant. The same amount of deionized/distilled water was sprayed on control plants.

After GA_3 application, the plastic was removed and plants were placed inside the greenhouses in a randomized block complete design with four replications. Natural light was supplemented for with high-pressure sodium lamps (HPS) to give a 16 hour photoperiod such that lights were on at 6:00 AM and off at 10 PM. Plants were fertilized with 125 mg L^{-1} of N from Peters Potash Special from 25 -5 -30 at each watering (200ml per pot).

Data taken included light measurements (total light and photosynthetic radiation PPFD), days to visible buds, days to flowering, number of days to flowering since buds, plant height (length of the inflorescence - cm), number of flowers per plant and time that flowers lasted in the plants and soil temperature.

The measures of light inside of the greenhouse were made once a week at noon (12:00) using a digital light meter (**Phytotronics, Inc**. model FCM-10M+) to measure the total amount of light. To measure the photosynthetic radiation a basic quantum meter (**Spectrum Technologies, Inc.** model BQM-SUN) was used. Both light measurements were made above the top of the plants.

Days to buds set were counted from the days that plants came out of the cooler until visible bud. Days to flowering were counted from the day that plants came out of the cooler until petals began to open and show their specific color (anthesis). Plant height was measured from top of the potting mix to the shoot apex, blooms, or flower tip. Soil temperature was recorded twice a day.

In order to control white flies and thrips, (Orthene) - O,S-Dimethyl acetylphosphoramidothioate was used during the experiment and to control powdery mildew, kocide 101 Cupric hydroxide was used.

The experimental design was complete randomized block with two factors (GA and cold). There were four replications and the experimental unit was 1 pot with 1 plant per pot. Statistical analyses were conducted using analysis of variance (ANOVA) implemented in SAS (SAS Institute 2002). Interaction GA X cold was tested and regression analyses were performed accordingly using orthogonal polynomials.

Results and Discussion

ANOVA indicated that the interaction GA and cold periods was not significant for the six variables (Apendix numero…). These results are maybe due to the fact that in this experiment the light intensity effect was not taken into account during the time when plants were growing in the greenhouse. The flowering of most plant species is initiated by different environmental factors such as day length, temperature, light quality and nutrient availability (Samach and Wigge, 2005).

The observed number of days to buds decreased with GA levels (Fig.1. a). However the linear regression analysis was not significant. A trend of decreasing in

the observed number of days to buds with the increase of cold time was confirmed by the linear regression (Fig.2.a). This result agrees with Joiner et al. (1982) that affirm that GAs are used to promote bud break for many cut flowers. Oka (2001) reported similar results with vernalized and non-vernalized plants of *Eustoma grandiflorum* when the same GA_3 concentration was used. A possible explanation for these results could be that both GA and cold temperature somehow supplied one or more of those specific environment requirement conditions that accelerate flowering.

The application of GA decreased the number of days of plants to flowering when compared with the control treatment, but the linear regression analysis was not significant (Fig.1.b). The vernalization treatments also decreased the number of days to flowering, confirmed by the linear regression (Fig. 2.b). Dole and Wilkins (1999) state that gibberellins are most commonly used to induce flower development. GA_3 was as well effective on bud growth rate and flowering of *R. pulchrum*, causing the flower buds to show color 10 days earlier and inducing anthesis nine days earlier than the control (Chang and Sung, 2000). Cold temperature is an important environmental signal for stem elongation from a vegetative rosette, and subsequent flowering of many winter annuals and biennials plants. However, Croft (2003) working with *Penstemon pinifolius* and *Penstemon palmeri* held in a cooler at 5°C for 0, 3, 6, 9, 12, or 15 weeks treatments had no significant effect on flowering for either species. On the other hand, the number of days to flowering since buds did not show the same response to GA levels, and the linear regression analysis was not significant, with the longest period obtained with the highest GA level, 500 mg L^{-1} (Fig. 1.c). The vernalization treatments decreased the number of days to flowering since buds with the increase of vernalization time, with a quadratic response (Fig. 2.c).

The plant height was not affected by GA applications (Fig.1.d) and weeks of cold (Fig.2.d). Donnel (2005) had similar results concluding for both species of *Catharanthus* 'Pacifica Lilac' and *Celosia* 'Century Fire' that the final height and diameter were both not affected by GA concentration (100, 200 and 400 mg L^{-1}) applied one week before transplanting. However, Ben-Jaacov (2006) spraying flowering buds of *Protea* 'Pink Ice' with aqueous solution of 1000 mg L^{-1} of GA_3 four times per week obtained at harvesting time flowers 121 mm longer than the control (93 mm) long.In addition Croft (2003) reported an effect of vernalization treatments on plants of *Penstemon pinifolius* and *Penstemon palmeri* that were chilled for 12 or 15 weeks at 5℃ had an increment of final plant height.

The GA_3 levels did not affect the number of flowers per plant (Fig. 1.e). Regarding the vernalization periods control treatment was the one that had the highest number of flowers per plant (Fig.2.e). Tamari et al. (1998) show similar results working with applications of gibberellin (25 mg L^{-1}) applied in two cultivars of Impatiens (Impatiens balsamina L.) plants of Sultanii (cv.`Tempo Pink') and New-Guinea (cv. `Aruba'), with no significant effect on the number of flowers, on the number of cuttings per plant and on the extend and quality of the rooting.

The time that flowers lasted in the plants was almost the same for all GA levels, including the control (Fig. 1f) an did not vary with the vernalization treatments where the control was the one that hold the flowers in the plants longer than the other treatments (fig.2.f).

We expected in this study greater differences between control plants and plants treated with GA and cold as the effect of these two factors on the development of various species of plants has been reported in the literature (Zeevaart, 1983; Çetinbaş and Koyuncu 2006). A possible reason for the lack of or small response to

GA and cold in this study could be that plants used in this experiment were at a late vegetative developmental stage. Therefore, further research on *Penstemon digitalis* cultivar Husker Red with the same and different treatments and plants at different developmental stages is needed.

Literature cited

Ben-Jaacov, J. 2006. Gibberellic acid spray increased size and quality of *protea ´*pink ice´ flowers - a preliminary experiment. Acta Hort. (ISHS) 716:135-140.

Black, L.A., Nell, T.A. and J.E. Barrett. 1990. Dormancy-breaking Method Effects on Azalea Longevity. HortScience 25(7):810.

Chang, Y.S. and H.F. Sung. 2000. Effects of gibberellic acid and dormancy-breakingchemicals on flower development of *Rhododendron pulchrum* Sweet and R. *scabrum* Don. Scientia Hort. 83:331-337.

Clementes, R.K., Baskin, J.M. and C.C. Baskin. 2002. The comparative biology of the two closely-related species *Penstemon tenuiflorus Pennell* and *P hirsutus* (L.) Willd. (Scrophulariaceae, Section Graciles): IV.Efects of shade, drought, and soil type on survival and growth. Castanea 64:299-309.

Clough, Emily, Cameron, Arthur, Heins, Royal and Will Carlson. 2000. Forcing perennials - crop-by-crop – *Penstemon digitalis* Husker Red. *Greenhouse Grower* 18(3):30-40.

Croft, A. A. 2003. The production of native and adapted plants for the intermountain west using the pot-in-pot nursery production system.A thesis submitted in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Plant Science UTAH STATE UNIVERSITY Logan, Utah.

Dole, J.M. and H.F. Wilkins. 1999. Floriculture: Principles and species. Prentice–Hall, Inc., Upper Saddle River, N.J.

Donnel, A. A. 2005. Effect of plug flat on plant growth and prevention of post-transplanting stunting. Master of Science thesis. North Carolina State University. 92p.

Evans and Lyons. 1988. Photoperiodic and Gibberellin-induced Growth and Flowering Responses of *Gaillardia x grandiflora.* HortScience 23(3): 584-586.

Henny, R.J. 2003. Abundant Flower and Seed Produced from Aglaonema Breeding Stock Following Gibberellic Acid (GA_3) Treatment. Foliage Digest v6n. Suge H. 1970. Changes of endogenous gibberellins in Vernalized radish plants. Plant Cell Physiol 11:729–735.

Joiner, J.N. O. Washington, C.R. Johnson, and T. A. NELL. 1982. Effect of exogenous growth regulator on flowering and cytokinin levels in azaleas. Scientia Hort. 18:143-151.

Lang, A. 1986. *Hyoscyamus niger*. In: Halevy AH, editor. CRC Handbook of Flowering. Vol V. Boca Raton, FL: CRC Press. P. 144–186.

Michaels, S. D. and Amasino, R.M. 1999. The gibberellic acid biosynthesis mutant *ga1–3* of *Arabidopsis thaliana* is responsive to vernalization. Developmental Genetics 25:194–198.

Ogasawara, N., Hiramasu, T., Ishiyana K.,. Fushimi H., Suzuki H. and H. Takagi. 2001. Effects of gibberellic acid and temperature on growth and root carbohydrates of *Delphinium* seedlings. Plant Growth Regulation 33:181-187.

Oka , M; Tasaka, Y; Iwabuchi, M and Mino, M. 2001.Elevated sensitivity to gibberellin by vernalization in the vegetative rosette plants of *Eustoma grandiflorum* and *Arabidopsis thaliana.* Plant Science 160: 1237–1245.

Samach, A. and Wigge, P. 2005. Ambient temperature perception in plants. Current Opinion in Plant Biology. 8:483–486.

Schmidt, M. C. et al. 2003. Ácido giberélico (GA_3) no crisântemo (*Dendrathema grandiflora* Tzvelev.) de corte 'viking': cultivo verão/outono. Cienc. Rural 33(2):267-274.

Statistical Analysis System – SAS. 2002. Getting started with the SAS® Learning Edition. Cary, NC: SAS Institute, 81p.

Tamari, G., Pappa, L., Zered, T., and A. Borochov. 1998. Effects of ethrel and gibberellin on impatiens plants. Scientia Horticulturae 76:29-35.

Tanimoto and Harada, 1985. *Rudbeckia,* In: A. Halevy (ed.) CRC Handbook of Flowering Vol. IV. CRC Press, Boca Raton, Fla. p. 239-242.

Zeevaart, J.A.D. 1983. Gibberellins and flowering. In: Crozier A, (ed). The Biochemistry and Physiology of Gibberellins. Vol 2. New York: Praeger. p 333–374.

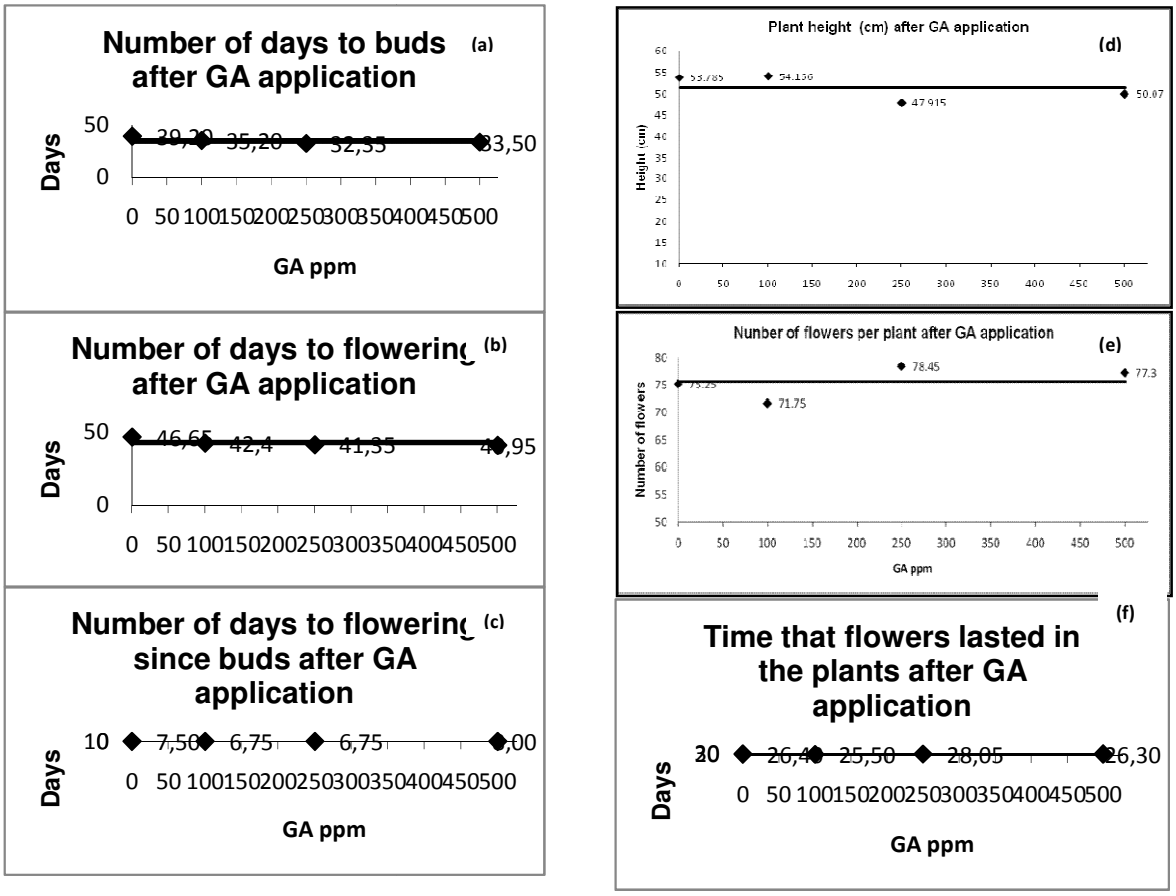

Fig.1. Effect of GA_3 (0, 100, 250 or 500 ppm) sprayed on plants of *P. digitalis* cv. 'Husker Red' on: (a) number of days to buds; (b) number of days to flowering; (c) number of days to flowering since buds; (d) plant height (cm); (e) number of flowers per plant and (f) time that flowers lasted in the plants.

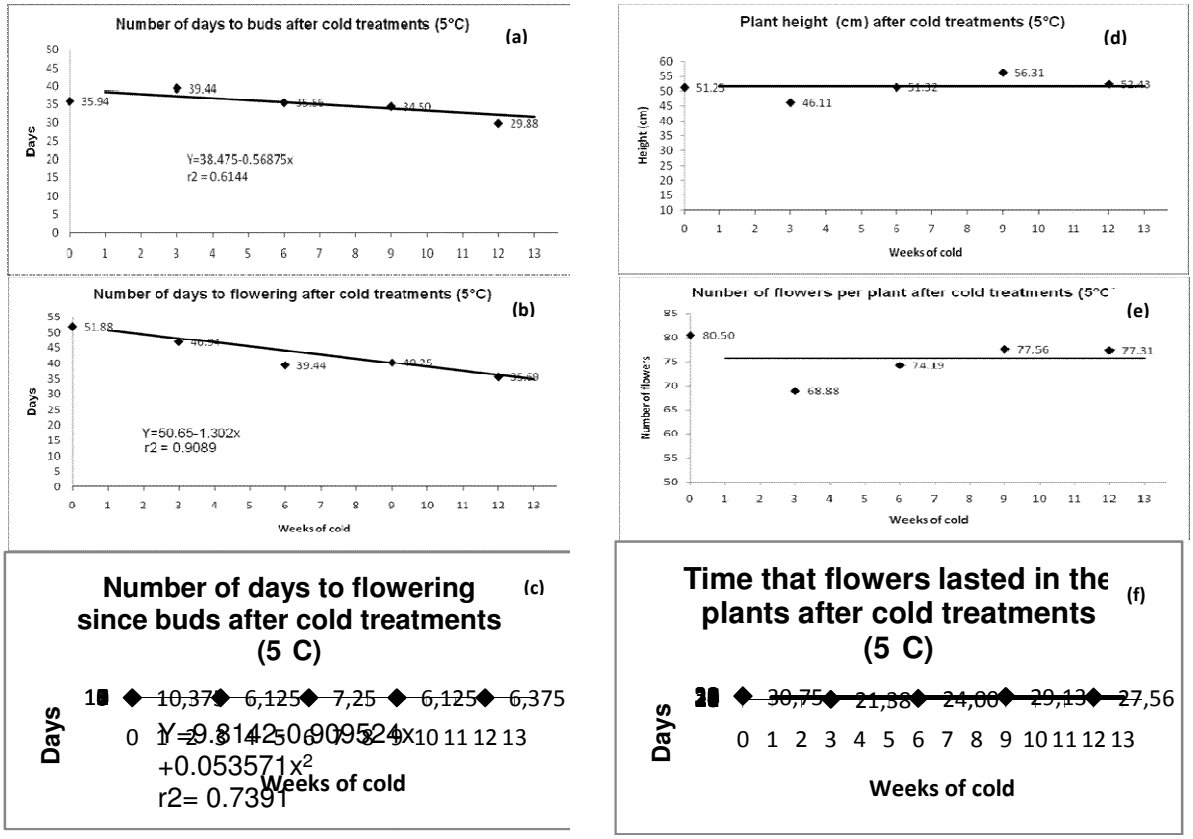

Fig.2. Effect of weeks of cold (0, 3, 6, 9 or 12) at 5℃ on plants of *P. digitalis* cv. 'Husker Red' on: (a) number of days to buds; (b) number of days to flowering; (c) number of days to flowering since buds; (d) plant height (cm); (e) number of flowers per plant and (f) time that flowers lasted in the plants.

4 CHAPTER III

TRABALHO ACEITO PARA PARA PUBLICAÇÃO:

Gibberellic Acid Promotes Seed Germination in *Penstemon digitalis* cv Husker Red

HortScience, **2008**

Gibberellic Acid Promotes Seed Germination in *Penstemon digitalis* cv. Husker Red

Anderson Machado de Mello

Programa de Pós-graduacao em Agronomia, Universidade Federal de Santa Maria, Santa Maria, Rio Grande do Sul State, 97105-900, Brazil.

Nereu Augusto Streck

Departamento de Fitotecnia, Universidade Federal de Santa Maria, Santa Maria, Rio Grande do Sul State, 97105-900, Brazil.

Erin E. Blankenship

Department of Statistics, University of Nebraska, Lincoln, NE 68583-0963

Ellen T. Paparozzi[1]

Department of Agronomy and Horticulture, University of Nebraska, Lincoln, NE 68583-0724

We wish to thank Dr. Dale Lindgren, for seed, advice on cultural procedures and critical review of this manuscript.

[1] To whom reprint requests should be addressed: Email address: etp1@unl.edu

Subject Category: Short communication

Gibberellic Acid Promotes Seed Germination in *Penstemon digitalis* cv. Husker Red

Additional Index Words: dormancy, seed soak, GA_3

Abstract.

Penstemon seed often shows an inconsistent or a low germination percentage. While most select cultivars are propagated by cuttings, for export to other countries seed is preferred. Three experiments were conducted to determine if soaking seed in gibberellic acid (GA_3) would increase seed germination of *Penstemon digitalis* cv. 'Husker Red'. GA_3 concentrations used were: 0, 10, 50, 100, 200 and 500 $mg \cdot L^{-1}$ (first experiment) 0, 500, 1000 and 1500 $mg \cdot L^{-1}$ (second experiment) and 0, 500 and 1000 $mg \cdot L^{-1}$ (third experiment). The first and second experiments were conducted in a growth chamber while the third experiment was conducted in both a growth chamber and greenhouse with seeds either covered or not covered by the mix. In all experiments, gibberellic acid increased the percentage and rate of seed germination. The 1000 $mg \cdot L^{-1}$ GA_3 was the best treatment. In the third experiment, percentage and rate of seed germination were the highest for seeds grown inside of the growth chamber probably due to the consistency of temperatures and darkness. In the greenhouse, the percentage of seeds that germinated and the rate of germination were similar whether or not the seeds were covered with mix and whether they received either the 500 or 1000 $mg \cdot L^{-1}$ GA_3 treatment.

Over the past few years, various penstemons (from approximately 280 species) have become increasingly popular as a garden flower. These plants grow and tolerate a wide range of temperatures and have a variety of flower types and colors. Of particular interest to us is *Penstemon digitalis* cv Husker Red, as its tall showy flower spikes have potential as a specialty cut flower. While cuttings are the preferred method of propagating 'Husker Red' penstemon, for locations and regions where cuttings are not readily available, seedlings could be screened and then selected for stock plant production. However, seeds of many *Penstemon* species possess a dormancy, which limits seed germination. As a result, individual germination requirements vary widely, both among and within species (Kitchen and Meyer 1991; Meyer et al., 1995). In perennial production classes at UNL, fresh penstemon seed sown without any soaking will take 4-5 weeks to germinate with only a maximum of 50% success rate (data unpublished).

Gibberellic acid (GA_3, GA_4 and GA_7) has been shown to break dormancy and increase germination in seeds of several genera (Bewley and Black, 1982; 1985), including certain penstemon (Atwater, 1980; Meyer, et al., 1995). Using Intermountain region penstemon species, Kitchen and Meyer (1991) working with five concentrations of GA_3 (0-500 ppm) in combination with a cold treatment (2^{o}C), found that seeds that were treated with gibberellic acid and stratified (to simulate natural conditions that a seed must endure before germination) had higher germination percentages. Raeber and Lee (1991) soaked seeds of *Penstemon parryi* in six concentrations of gibberellic acid GA_3 (0 to 500 ppm) for 24 h and found 87-97% seed germination. Neither low temperature stratification nor NaCl soaks were as effective as 500 ppm for GA_3 seeds of *P. parryi.* Mexican species such as *P. harwegii* and *P. gentianoides* as well as *P. barbatus* 'Coccineus' germinated, without stratification, at 21^{o}C (Way and James, 1988; Nau, 1996).

Given the desire to grow 'Husker Red' penstemon as a specialty cut flower in a country where cuttings/plugs are not available and difficult to impossible to send from the US and as seeds of 'Husker Red' are usually sown while fresh (when used for breeding and selection purposes) and often result in uneven seed germination (Lindgren, 2007) or are reported to take up to eight weeks for germination (Swayne, 2000), three experiments were conducted to determine if gibberellic acid soaks would promote seed germination in order to facilitate stock plant establishment.

Materials and Methods

Two seed germination experiments were conducted in a dark growth chamber in the Plant Science Building at the University of Nebraska, Lincoln USA (UNL). The first experiment was conducted from Apr. 13 to Apr. 27, 2007 and the second from Aug. 12 to Aug. 26, 2007. The third experiment was conducted from Sept. 23 to Oct. 8, 2007 in a growth chamber and the UNL double polyethylene greenhouses. Fresh seeds of *Penstemon digitalis* 'Husker Red' were obtained from Dr. Dale Lindgren's breeding program and were soaked for 24 hours in 50 ml of one of the following treatments: 0, 10, 50, 100, 200 and 500 (first experiment – used by Raber and Lee (1991) on *P. parryi)*, 0, 500, 1000 and 1500 (second experiment) and 0, 500 and 1000 (third experiment) $mg \cdot L^{-1}$ GA_3. All seed mixtures were gently swirled at the beginning, middle, and end of the 24 hours soak period in each experiment. Floating (empty) seeds were discarded. Seed mixtures were then filtered and rinsed under cold distilled water for 2 minutes.

In the first and second experiments, fifteen seeds were placed in a small petri dish (5 cm) with No. 2 Whatman filter paper which was saturated with 1 ml of distilled deionized (d/d)

water, initially and then watered as needed. There were 15 petri dishes per treatment placed randomly across four shelves in a dark growth chamber at 21˙3°C.

In the third experiment, seeds were soaked and then sown into six packs (128 cells per tray; each cell holds 80 ml of mix) such that each treatment had eight cells with one seed per cell. Seeds were sown by either placing them on top of the mix (uncovered) or lightly covered by the mix (1:1 peat vermiculite by volume). There were eight flats total with two blocks per flat. Six flats were placed in the greenhouse (24 -32°C D/21-24°C N) and two flats were placed in a dark growth chamber (21˙3°C). All flats were covered with a plastic dome and each cell was watered with 4 ml of distilled deionized (d/d) water initially and then as needed.

For experiments one and two, seeds were observed daily for germination and were considered germinated when the radical was visible. For experiment three, seeds were considered germinated when the cotyledons appeared.

The experimental design for the first and second experiment was a completely randomized design. The third experiment was a randomized complete block design with the covered and uncovered planting treatments in the greenhouse or in the growth chamber analyzed as 4 separate experiments. Thus, there were 12 replications for each - the uncovered and covered in the greenhouse and there were 4 replications for each - the uncovered and the covered in the growth chamber.

All experimental data was subjected to a Shapiro-Wilk test of normality to determine if the data needed to be transformed. Residuals versus predicted values were also plotted to assess the assumption of constant variance. As the variance was found to be constant and the seed count data were normally distributed, the data were not transformed. Statistical analyses were then conducted using analysis of variance (ANOVA) implemented in SAS PROC MIXED (SAS

Institute, 2006), identifying significant differences through the use of contrasts. LSMeans were expressed as percent germination.

The number of germinating seeds out of 15 for the experiment number one and out of 10 for the experiments number two and three was also modeled as a function of time using a logistic model implemented in SAS PROC NLMIXED (SAS Institute, 2006). From the fitted models, time until 50% germination (T50) was calculated, and differences between T50s for different levels of GA_3 were calculated.

Results and Discussion

Experiment 1. Gibberellic acid increased seed germination and the rate of germination as compared with the control (Fig.1, Table 1). The increase was directly proportional to the increase in GA_3 concentrations within the range used. All concentration of gibberellic acid reduced the number of days to 50% seed germination, but particularly 500 $mg \cdot L^{-1}$ (12 days), as compared with the control (23 days; water soak only) (Table 1). However, total germination percentages were not much higher than 50% of the seed sown.

Experiment 2. As in experiment one, all GA_3 treatments were effective for increasing seed germination when compared with the control (Fig. 2). Moreover, seeds from the control treatment (0 $mg \cdot L^{-1}$) did not start to germinate until five days after the beginning of the experiment while seeds from all other GA_3 treatments started to germinate after 48 hours. The highest percentage of seed germination occurred when seeds were soaked with 1000 $mg \cdot L^{-1}$ GA_3, (almost 100% germination after eight days). Again, all GA_3 treatments were effective for increasing the speed of seed germination by 7 to 13 days (Table 2). Seeds germinated quicker when they were soaked in either 1000 or 1500 $mg \cdot L^{-1}$ GA_3 (4 and 5) days.

Experiment 3. All levels of GA_3 were again effective for increasing seed germination when compared with the control treatment (Table 3). In all treatments the speed of germination and the number of seeds that germinated was higher for plants grown in the growth chamber compared to plants in the greenhouse (for figures see Mello, 2009).

All concentrations of GA_3, except the control, were effective for increasing the speed of germination when seeds were either not covered or covered with mix (Table 3). When seeds were not covered with mix, seeds soaked in either the 500 or 1000 $mg \cdot L^{-1}$ GA_3 germinated in half the time compared to the control. When seeds were covered with mix, seeds soaked in 1000 $mg \cdot L^{-1}$ GA_3 germinated more rapidly.

Seed flats placed in the greenhouse were slower to germinate than all the seeds that were grown in the dark growth chambers (Table 3). In the greenhouse, it did not matter whether the seeds were covered with mix or not, either GA_3 concentration caused seeds to germinate faster. There was no difference in speed of germination between the two concentrations of GA_3 .

In these experiments we have shown that 24 hr GA_3 soaks increase the number of seeds germinated and the speed of seed germination for 'Husker Red' penstemon. Similar increases in germination when seeds were soaked at similar concentrations as those used in this research have been shown to effectively increase seed germination for a wide variety of seeds such as *Verbena bonariensis* (Kornegay and Doubrava, 2006), *Sesamum indicum* (Kyauk et al.,1995), *Trichocereus terscheckii* (Ortega-Bae and Rojas-Arechig, 2007), *Sesleria varia* (Castiglioni, et al., 2004), *Sesamum indicum* (Kyauk et al., 1995), *Ferula gummosa* and *Teucrium polium* (Nadjafi et al., 2005), black mulberry (Koyuncu, 2005), *Cyclocarya paliurus* (Fang et al., 2006), *Prunus avium* (Çetinbaş and Koyuncu 2006), *Gautheria procumbens* (Ruchala 2002) and *Galeopsis speciosa* (Karlsson et al., 2006).

So the question arises as to whether *Penstemon digitalis* seeds possess a dormancy. According to Nold (1999) in the genus *Penstemon*, dormancy can be removed by subjecting the seeds to temperatures below about 7°C (45°F) for weeks or months depending on the species. Gibberellins (GA_3, GA_4 and GA_7) have been shown to break dormancy in numerous genera of seeds (Bewley and Black, 1982; 1985), including *Penstemon* (Atwater, 1980). Kitchen and Meyer (1991) working with 50, 150, 250 and 500 $mg \cdot L^{-1}$ GA_3 recommend treatments with gibberellic acid to remove seed dormancy or shorten the chilling requirement for many, but not all, species of *Penstemon,* and recommended the use of gibberellins together with other treatments such as stratification or scarification, in order to increase the effects of GA_3 in breaking dormancy. However, according to Atwater (1980) plants in the Scrophulariaceae family to which *Penstemon* belongs have seeds in which the endosperm surrounds the embryo and occupies up to half of the seed. In our data, there was a trend that, over experiments (and thus over time), the % germination increased for the same treatment, particularly the 0 $mg \cdot L^{-1}$ GA_3 treatment. One reason may be that these seeds were undergoing after-ripening. Another possibility could be an increasing permeability of the seed coat as penstemons have been shown to have thin, fragile seed coats (Atwater, 1980). However, 'Husker Red' seeds which were acid scarified for 15 to 60 minutes at 15 minute intervals did not show improved germination (Mello and Paparozzi, unpublished data). Thus, if there is a dormancy, it is probably a physiological one, endodormancy (Baskin and Baskin, 1998; Raghavan, 2000; Bradbeer, 1988), that could be released by a gibberellin soak.

Light can either stimulate or inhibit seed germination. As expected, seeds in the growth chamber that did not need to push through the growth medium germinated faster than covered seeds. In the third experiment our results showed that seeds flats placed in the greenhouse were slower to germinate than seeds that were grown in the completely dark growth chambers.

Evensen and Loy (1978) had some similar results working with seeds of a monogenic recessive dwarf inbred line of watermelon. They found that GA_3 increased the rate of germination, but light decreased the rate of seed germination. The authors suggested that this was due to lipid degradation promoted by GA_3, thus providing solutes necessary for seed germination. Application of GA_3 (500 $mg \cdot L^{-1}$) to seeds that were incubated at alternating temperatures (20/30°C for 16/8h) in light did not enhance germination of *Echinacea angustifolia* (Macchia et al., 2001). Thus, the consistent warm not hot temperatures in the growth chamber may have aided germination. For *Penstemon digitalis* cv Husker Red, we hypothesize that the effect of darkness, consistency of moisture and warm temperature together with GA_3 soaks helped seeds to overcome endodormancy.

In conclusion, soaking seeds of *Penstemon digitalis* cv. Husker Red in 1000 $mg \cdot L^{-1}$ GA_3 for 24h increases the rate and number of germinating seeds whether seeds are grown in Petri dishes in a dark, warm growth chamber or a soilless mix with a clear plastic dome over the flat in a warm greenhouse. Soaking seeds in either 500 or 1000 $mg \cdot L^{-1}$ GA_3 and then sowing them in a soilless mix either lightly covered or on the top of the mix in a flat covered with a plastic dome in the greenhouse will also increase the number and rate of germinating seeds. Over all experiments, seeds soaked in a high concentration of GA_3 consistently gave greater than 50% germination within 2 weeks.

Literature cited

Atwater, B.R. 1980. Germination, dormancy and morphology of the seeds of herbaceous ornamental plants. Seed Sci. and Technol. 8: 523-573.

Baskin, C. C. and J. M. Baskin. 1998. Seeds: Ecology, biogeography, and evolution of dormancy and germination. p. 27-42. Academic Press, New York, NY.

Bewley, J.D. and M. Black. 1982. Physiology and biochemistry of seeds in relation to germination. Volume 2. Viability, dormancy and environmental control. p. 60-198. Springer-Verlag Inc., New York, NY.

Bewley, J.D. and M. Black. 1985. Seeds: Physiology of development and germination. p.175-236. Plenum Press, New York, NY.

Bradbeer, J. W. 1988. Seed dormancy and germination. p. 38-80. Chapman and Hall, London., UK.

Castiglioni, A., A. Colombo, and A.Tosca. 2004. Evaluation of germination capacity and priming techniques in *Selsleria varia*, a plant for restoration of calcareous grasslands. 27th International Seed Testing Symposium, Budapest, Hungary May 17th– 19th. p.122. (abstr.).

Çetinbaş, M. and F. Koyuncu. 2006. Improving germination of *Prunus avium* L. seeds by gibberellic acid, potassium nitrate and thiourea. Hort. Sci. (Prague) 33 (3): 119–123.

Evensen, K. B. and J. B. Loy. 1978. Effects of gibberellic acid and gold light on germination, enzyme activities, and amino acid pool size in a dwarf strain of watermelon. Plant Physiol. 62:6-9.

Fang, S., J. Wang, Z. Wei, and Z. Zhu. 2006. Methods to break seed dormancy in *Cyclocarya paliurus* (Batal) Iljinskaja. Scientia Hort. 110: 305–309.

Karlsson, L. M., J. A. L. Ericsson, and P. Milberg. 2006. Seed dormancy and germination in the summer annual *Galeopsis speciosa*. Weed Res. 46: 353-361.

Kitchen, S.G. and S.E. Meyer. 1991. Seed germination of Intermountain penstemons as influenced by stratification and GA_3 treatments. J. Environ. Hort. 9: 51-56.

Kornegay, J. and N. Doubrava. 2006. Friends of the J.C. Raulston Arboretum Newsletter. Bul. 10, No. 1. North Carolina State University, Raleigh, NC.

Koyuncu, F. 2005. Breaking seed dormancy in black mulberry (*Morus nigra* L.) by cold stratification and exogenous application of gibberellic acid. Acta Biol Cracov Bot. 47(2): 23–26.

Kyauk, H., N. W. Hopper, and R. D. Brigham. 1995. Effects of temperature and presoaking on germination, root length and shoot length of sesame (*Sesamum indicum* L.) Environ. Exp. Bot. 35(3): 345-351.

Lindgren, D. 2007. Personal communication.

Macchia, M., L.G. Angeline, and L. Ceccarini. 2001. Methods to overcome seed dormancy in *Echinacea angustifolia* DC. Scientia Hort. 89:317-324.

Mello, A, M. 2009. Vernalization and gibberellic acid in the germination and development of *Penstemon digitalis* cv Husker Red. Federal University of Santa Maria, Brazil. PhD Diss.

Meyer, S.E. S.G. Kitchen, and S.L. Carlson. 1995. Seed germination timing patterns in Intermountain Penstemon (Scrophulariaceae). Am. J. Bot. 82: 377-389.

Nadjafi, F., M. Bannayan., L. Tabrizi, and M.Rastgoo. 2005. Seed germination and dormancy breaking techniques for *Ferula gummosa* and *Teucrium polium.* J. Arid Environ. 64: 542–547.

Nau, J. 1996. Ball perennial manual: Propagation and production. Ball Publishing Co., Batavia, Ill.

Nold, R. 1999. Penstemons. Timber Press, Portland, Ore.

Ortega-Bae, P. and M. Rojas-Aréchig. 2007. Seed germination of *Trichocereus terscheckii* (Cactaceae): Light, temperature and gibberellic acid effects. J. Arid Environ. 69: 169–176.

Raghavan, V. 2000.. Developmental biology of flowering plants. p. 292-303.Springer-Verlag Inc., New York, NY.

Raeber, A. C. and C. W. Lee. 1991. Gibberellic acid, temperature, light, stratification, and salinity affect germination of *Penstemon parryi* seed. HortScience 26(12):1535-1537.

Ruchala, S. L. 2002. Propagation of several native ornamental plants. University of Maine, Orono. MS Thesis.

SAS Institute. 2006. Statistical analysis system for mixed models. SAS Institute, Cary, NC.

Swayne. J. 2000. The penstemons. Seed germination methodology. tomclothier.hort.net/page08.html

Way, D. and P. James. 1998. The gardener's guide to growing penstemons. Timber Press. Portland, Ore.

Table 1. The actual percentage of seed germination after 15 days and the estimate of the number of days it would take to 50% seed germination when seeds of *Penstemon digitalis* 'Husker Red' were soaked for 24 hours in varying concentrations of GA_3. All pair-wise comparisons between treatments were significantly different from each other at Pr <0.01.

GA_3 $mg \cdot L^{-1}$	**Germination %**	**Estimate (days)**
0	8.63	22.83 ±0.84
10	21.18	18.11 ±0.34
50	18.43	19.6±0.50
100	26.47	17.00±0.30
200	33.33	15.82±0.23
500	54.31	12.10±0.13

Table 2. The actual percentage of seed germination after 15 days and the estimate of the number of days it would take for 50% seed germination when seeds of *Penstemon digitalis* 'Husker Red' were soaked for 24 hours in varying concentrations of GA_3. All pair-wise comparisons between treatments were significantly different from each other at Pr <0.01.

GA_3 $mg \cdot L^{-1}$	**Germination %**	**Estimate (days)**
0	24.00	17.94±0.82
500	71.00	10.19±0.25
1000	95.00	4.17±0.17
1500	88.00	5.43±0.21

Table 3. The actual percentage of seed germination after 15 days and the estimate of the number of days it would take for 50% seed germination when seeds of *Penstemon digitalis* 'Husker Red' were soaked for 24 hours in varying concentrations of GA, and then sown in soilless mix. Seeds were sown either on top of the mix (uncovered) or covered lightly with mix and placed either in a growth chamber or the greenhouse. Unless indicated by an *, all pair–wise comparisons between treatments were significantly different from each other at Pr<0.01.

GA_3	Greenhouse				Growth Chamber			
	Uncovered		Covered		Uncovered		Covered	
$mg \cdot L^{-1}$	Germination %	Estimate (days)	Germination %	Estimate (days)	Germination %	Estimate (days)	Germination %	Estimate (days)
0	50.00	15.68 ± 2.35	40.63*	17.94 ± 3.36	45.83	14.77 ± 0.40	21.88	26.68 ± 2.98
500	100.00	5.87 ± 0.43	46.88*	12.92 ± 1.03	56.25*	12.29 ± 0.45	45.83*	14.09 ± 0.38
1000	84.38	7.43 ± 0.38	75.00	7.06 ± 0.43	53.13*	12.74 ± 0.35	44.79*	13.85 ± 0.43

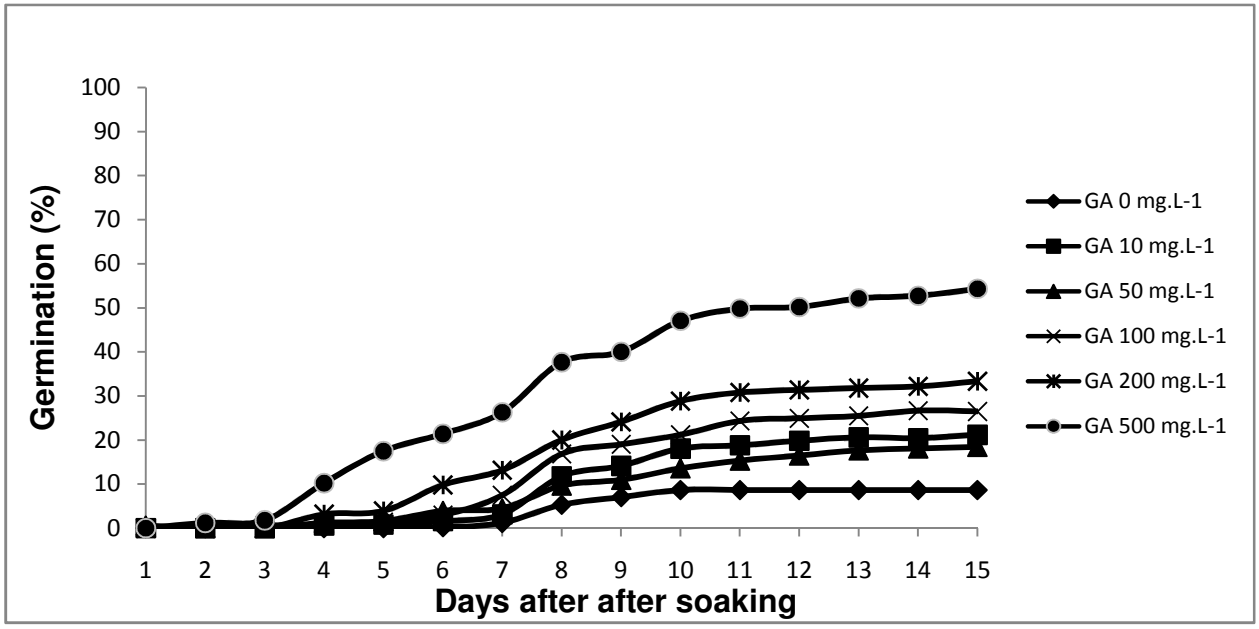

Figure 1. Percentage of *Penstemon digitalis* cv. 'Husker Red' seeds that germinated each day after soaking for 24 hrs in 0, 10, 50, 100, 200 or 500 $mg \cdot L^{-1}$ gibberellic acid (GA_3). Seeds were sown on moistened filter paper in Petri dishes and germinated in a dark growth chamber at 21-24°C.

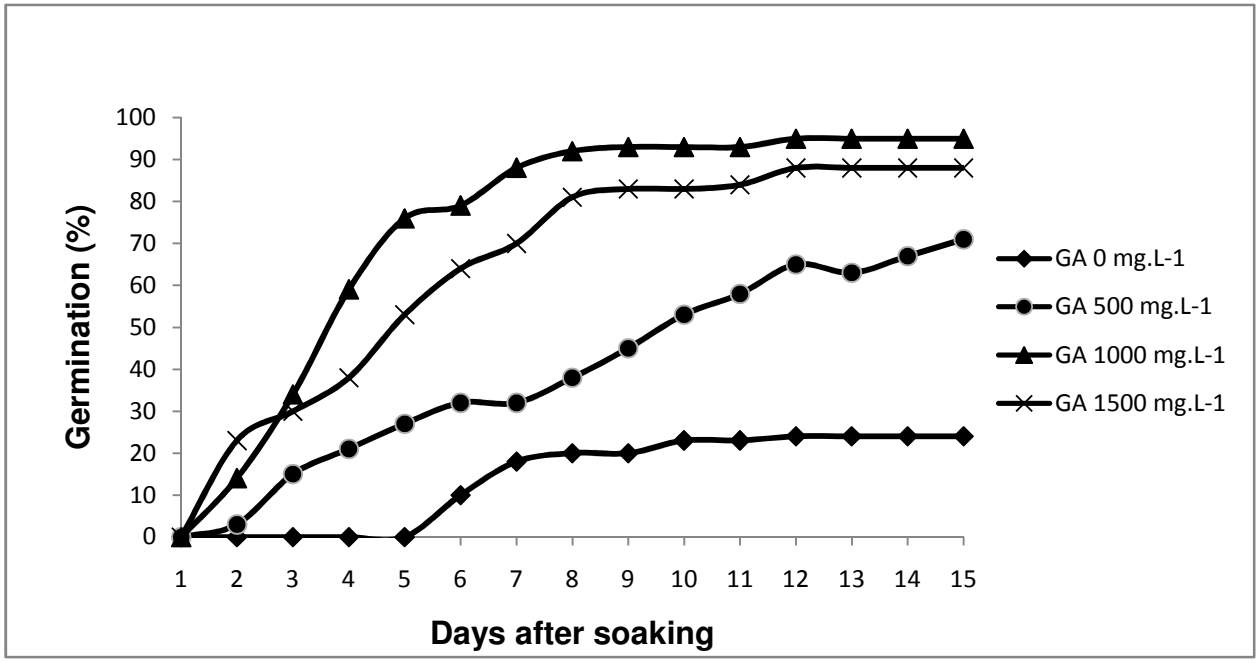

Figure 2. Percentage of *Penstemon digitalis* cv. 'Husker Red' seeds that germinated each day after soaking for 24 hrs in 0, 500, 1000 or 1500 $mg \cdot L^{-1}$ gibberellic acid (GA_3). Seeds were sown on moistened filter paper in Petri dishes and germinated in a dark growth chamber at 21-24°C.

5 CONCLUSÕES GERAIS

1. Não há interação entre doses de AG_3 e diferentes períodos de vernalização. As variáveis número de dias para a emissão de botões florais, número de dias para as plantas florescerem, e número de dias para o florescimento a partir da emissão dos botões florais decrescem após a aplicação de GA_3, de acordo com os níveis de AG_3 e de acordo com os períodos de vernalização. A altura da planta, número de flores por planta e tempo de permanência das flores nas hastes não são afetadas pelas aplicações de AG nem pelos diferentes períodos de vernalização.

2. O ácido giberélico aumenta a porcentagem e a taxa de germinação das sementes, sendo 1000 mg L^{-1} de AG_3 a melhor dose. A porcentagem bem como a taxa de germinação são mais elevadas nas sementes que são mantidas em câmara de crescimento, provavelmente devido a temperatura constante e a ausência de luz. Na casa de vegetação, a porcentagem de sementes que germinaram e a taxa de germinação, são similares, não importando se as sementes estão cobertas ou não e também similares para as doses de 500 e 1000 mg L^{-1} de AG_3.

6 SUGESTÕES PARA TRABALHOS FUTUROS

Neste estudo não foi possível determinar ou confirmar o requerimento necessário para vernalização em *Penstemon digitalis* cultivar Husker Red, devido a interferência de fatores ambientais sobre os tratamentos incicalmente planejados. Assim, pesquisas futuras se fazem necessárias com a utilização dos mesmos tratamentos bem como com tratamentos diferentes usados nesse estudo, utilizando-se plantas em diferentes estágios de desenvolvimento.

7 REFERÊNCIAS

ALLEN, P.S.; MEYER, S.E. Temperature requirements for seed germination of three *Penstemon* species. **HortScience**, v. 25, n. 2, p. 191-193, 1990.

American Penstemon Society. American penstemon society manual for beginners. Dayton, Ohio, American Penstemon Society: 29 p. 1981.

APDA (Agricultural and Processed Food Products Export Development Authority).1996. Word Trade in Floriculture (http://apeda.com/html/flori1.html).

ARMITAGE, A.M. **Herbaceous Perennial Plants**. Athens, Ga: Varsity Press,1989.136p.

ATWATER, B.R. Germination, dormancy and morphology of the seeds of herbaceous ornamental plants. **Seed Science and Technology**, v. 8, p. 523-573, 1980.

BAGNALL, D.J. Control of flowering in *Arabidopsis thaliana* by light, vernalization and gibberellins. Aust. J. **Plant Physiol**, v. 19, p. 401– 409, 1992.

BANDARA, P.M.S. AND TANINO, K.K. Effect of gibberellic acid (GA_3) on seedstalk development and flowering in carrot (*DAUCUS CAROTA* L. VAR. *SATIVA* DC). **Acta Hort**, v. 394 p. 251-262, 1995.

BANNISTER, P. AND BRIDGMAN, J. Responses of seeds of three species of *Pseudopanax* to low temperature stratification, removal of fruit flesh, and application of gibberellic acid. **New Zealand Journal of Botany**, v. 29, p. 213-216, 1991.

BASKIN, C. C.; BASKIN, J. M. **Seeds Ecology, Biogeography, and Evolution of Dormancy and Germination.** Lexington, Kentucky: Academic Press,1998. p. 27-42.

BEN-JAACOV, J. Gibberellic acid spray increased size and quality of *protea ´*pink ice´ flowers - a preliminary experiment. **Acta Hort. (ISHS)**, v. 716, p. 135-140, 2006.

Better homes and Gardens. Des Moines, Iowa: Meredith Corp, 1978.45 p.
BEWLEY, J.D. Breaking down the walls – a role for endo-b-mannanase in release from seed dormancy. **Trends in Plant Science**, v. 2, p. 464–469, (1997a).

BEWLEY, J.D. Seed germination and dormancy. **Plant Cell**, v. 9, p. 1055–1066, (1997b).

BEWLEY, J.D. AND BLACK, M. Physiology and Biochemistry of Seeds in Relation to Germination. **2. Viability, Dormancy and Environmental Control**. Berlin, N. Y. Springer-Verlag, 1982. p. 60-198.

BEWLEY, J.D.; BLACK, M. **Physiology of Development and Germination**. New York, N.Y.: Plenum Press, 1985. p. 175-236.

BEWLEY, J.D.; M. BLACK. **Physiology and Biochemistry of Seeds in Relation to Germination. 2. Viability, Dormancy and Environmental Control.** Berlin, N. Y.: Springer-Verlag,1982. p.60-198.

BLACK, L.A., NELL, T.A. AND J.E. BARRETT. Dormancy-breaking Method Effects on Azalea Longevity. **HortScience,** v. 25, n. 7, p. 810, 1990.

BLUNDELL, J.B. AND JACKSON, G.A.D. Rose seed germination in relation to stock production. **Rose Annual**. London. p. 129-135, 1971.

BRADBEER, J. W. **Seed Dormancy and Germination.** Leicester Place, London: Chapman and Hall, 1988. p. 38-80.

CASTIGLIONI, A., COLOMBO, A. AND TOSCA, A. Evaluation of germination capacity and priming techniques in *Selsleria varia*, a plant for restoration of calcareous grasslands. Abstracts, **27th ISTA Congress**. **Seed Symposium** Budapest, Hungary May 17th– 19th. P. 122, 2004.

ÇETINBAŞ, M. AND F. KOYUNCU. Improving germination of *Prunus avium* L. seedsby gibberellic acid, potassium nitrate and thiourea. **Hort. Sci**, (Prague), v. 33, n. 3, p. 119–123, 2006.

CHANDLER, J. AND C. DEAN, Factors influencing the vernalization response and flowering time of late-flowering mutants of *Arabidopsis thaliana* (L.) Heynh. **J. Exp. Bot**, v. 45, p. 1279–1288, 1994.

CHANG, Y.S. AND H.F. SUNG. Effects of gibberellic acid and dormancy-breakingchemicals on flower development of *Rhododendron pulchrum* Sweet and R. *scabrum* Don. **Scientia Hort**, v. 83, p. 331-337, 2000.

CHIEN, C., KUO-HUANG, L. AND L. TSAN PIAO. Changes in ultrastructure and abscisic acid level, and response to applied gibberellins in *Taxus mairei* seeds treated with warm and cold stratification. **Annals of Botany**, v. 81, p. 41–47, 1998.

CHUANREN, D., BOCHU, W., WANQIAN, L., JING, C., JIE, L., HUAN, Z. Effect of chemical and physical factors to improve the germination rate of *Echinacea angustifolia* seeds. **Colloids and Surfaces B: B iointerfaces**, v. 37, p. 101–105, 2004.

CLEMENTES, R.K., BASKIN, J.M. AND C.C. BASKIN. The comparative biology of the two closely-related species *Penstemon tenuiflorus Pennell* and *P hirsutus* (L.) Willd. (Scrophulariaceae, Section Graciles): III. Ecological life cycle, growth characteristics, and flowering requirements. **Castanea**, v. 67, p. 161-176, 2002.

CLEMENTES, R.K., BASKIN, J.M. AND C.C. BASKIN. The comparative biology of the two closely-related species *Penstemon tenuiflorus Pennell* and *P hirsutus* (L.) Willd. (Scrophulariaceae, Section Graciles): IV.Efects of shade, drought, and soil type on survival and growth. **Castanea**, v. 64, p. 299-309, 2002.

CLOUGH, EMILY, CAMERON, ARTHUR, HEINS, ROYAL AND WILL CARLSON. Forcing perennials - crop-by-crop – *Penstemon digitalis* Husker Red. **Greenhouse Grower**, v.18, n. 3, p.30-40, 2000.

COLOMBO, A., CASTIGLIONI, A., TOSCA, A. AND BONOMI, C. Evaluation of germination capacity in *Dianthus glacialis*, an endangered alpine species. Abstracts, 27th ISTA Congress. **Seed Symposium Budapest**, Hungary May 17th– 19th. Page 69. 2004.

CROFT, A.A. The production of native and adaptative plants for the intermountain west using the pot–in-pot nursery production system. **Master of Science Thesis**, Logan, Utah: Utah State University, 85p. 2003.

DAHANAYAKE, R.S. AND W.N. GALWEY. Effects of Interactions between Low-temperatures, Gibberillin (GA_3) and Photoperiod on Flowering and Stem Height of Spring Rape (*Brassica napus* var. *annua*). **Annals of Botany**, 84:321-327. 1999.

DAVIS, T. D., GEORGE, S.W., A. UPADHAYA, J. M. PARSONS. Improvement of seedling emergence of *Lupinus texensis* following seed scarification treatments. **Journal of Environmental Horticulture**, v. 9, p. 17-21, 1991.

DEHGAN, B., NORCINI G. J, S. M. KABAT, H. E. PEREZ. Effect of Seed Scarification and Gibberellic Acid Treatment on Seedling Emergence of Sky-Blue Lupine (*Lupinus diffusus)*. **Journal of Environmental Horticulture,** v. 21, n. 2, p. 64-67, 2003.

DENO, N.C. *Penstemon Germination.* **Bul. Amer. Penstemon Soc**., v. 50, n. 1, p. 3-6, 1991.

DILL, A. AND SUN, T.P. Synergistic derepression of gibberellin signaling by removing RGA and GAI function in Arabidopsis thaliana. **Genetic**,. v.159, p. 777–785, 2001.

DOLE, J.M.; H.F. WILKINS. **Floriculture: Principles and species**. Upper Saddle River, N.J.: 1999. Prentice–Hall, Inc. 236p.

DONNEL, A. A. Effect of plug flat on plant growth and prevention of post-transplanting stunting. **Master of Science thesis**. North Carolina State University. 92p. (2005).
DOUGLAS, E.S. et al. Missing links: the genetic architecture of flower and floral diversification. **TRENDS in Plant Science**, v. 7, n.1, p. 22-31, 2002.

EL-DENGAWY EL-REFAEY F. A. Promotion of seed germination and subsequent seedling growth of loquat (*Eriobotrya japonica*, Lindl) by moist-chilling and GA3 applications. **Scientia Horticulturae**, v. 105, p. 331–342, 2005.

ELLIS, R. H. HONG, T. D., E. H. ROBERTS. **Handbook of seed technology for genebanks**. Rome. International Board for Plant Genetic Resources. 1985.
EVANS AND LYONS. Photoperiodic and Gibberellin-induced Growth and Flowering Responses of *Gaillardia x grandiflora.* **HortScience**, v. 23, n. 3, p. 584-586, 1988.

EVENSEN, K. B. AND J. B. LOY. Effects of Gibbereilic Acid and Gold Light on germination, Enzyme Activities, and Amino Acid Pool Size in a Dwarf Strain of Watermelon. **Plant Physiol**, v. 62, p. 6-9, 1978.

FANG, S., J. WANG, Z. WEI, AND Z. ZHU. Methods to break seed dormancy in *Cyclocarya paliurus* (Batal) Iljinskaja. **Scientia Horticulturae**, v. 110, p. 305–309, 2006.

FENNER, M. **Seeds the Ecology of Regeneration in Plant Communities. (2nd ed.)** Wallingford, UK: 2000. CABI Publishing. p.183-214.

FLESSNER, T.R. Propagation, establishment, and ecological characteristics of *Penstemon haydenii* S. Wats. **Master of Science Thesis**. Lincoln, NE: University of Nebraska-Lincoln, 192 p. 1988.

Florabrasilis. Relatorio do diagnostico da producao de flores e plantas ornametais brasileira (compact disc). Campinas: IBRAFLOR. 2002.

FLORES FNP Consultoria & Comercio. Agrianual: **Anuario da Agricultura Brasileira**, Sao Paulo, p.337-348, 2001.

FONTENOT, D. P. Evaluating Seashore Paspalum seed germination and enhancement, erosion abatement and potential use as a vegetative landfarm cap. **Master of Science thesis**. Louisiana State University, The School of Plant, Environmental and Soil Sciences. 76p. 2003.

Guo, D.P., Zheng, S.J., Zeng, G.W. and G.A. Shah. The interaction of plant regulators and vernalization on the growth and flowering of cauliflower (*Brassica oleracea* var. *botrytis)*. **Plant Growth Regulation**, v. 43, p.163-171, 2004.

HARKESS R. AND R. LYONS. Gibberellin and cytokinin-induced growth and flowering responses in *Rudbeckia hirta* L. **HortScience,** v. 29, p. 141-142, 1994.

HAZEBROEK, J.P., METZGER, J.D. AND E.R. MANSAGER. Thermo inductive regulation of gibberellin metabolism in *Thlaspi arvense* L. **Plant Physiol**, v. 102, p. 547–55, 1993.

HENNY, R.J. Abundant Flower and Seed Produced from Aglaonema Breeding Stock Following Gibberellic Acid (GA_3) Treatment. Foliage Digest v6n. Suge H. 1970. Changes of endogenous gibberellins in Vernalized radish plants. **Plant Cell Physiology**, v. 11, p. 729–735, 2003.

IBRAFLOR. Brasil: Mostra tua flora**. Informativo**, 7(23):4. 2001.

JOINER, J.N. O. WASHINGTON, C.R. JOHNSON, AND T. A. NELL. Effect of exogenous growth regulator on flowering and cytokinin levels in azaleas. **Scientia Hort,** v. 18, p. 143-151, 1982.

KARLSSON, L. M., ERICSSON J. A. L. AND MILBERG, P. Seed dormancy and germination in the summer annual *Galeopsis speciosa*. **Weed Research**, v. 46, p. 353-361, 2006.

KITCHEN, S.G. AND MEYER S.E. Seed germination of Intermountain penstemons as influenced by stratification and GA_3 treatments. **Journal of Environmental Horticulture**, v. 9, p. 51-56, 1991.

KOORNNEEF, M., BENTSINK, L. AND HILHORST, H. Seed dormancy and germination. **Current Opinion in Plant Biology**, v. 5, p. 33–36, 2002.

KORNEGAY, J. AND N. DOUBRAVA. **Friends of the JC Raulston Arboretum Newsletter**, NC State University. Bul. 10, NO. 1. 2006.

KOYUNCU, F. Breaking seed dormancy in Black Mulberry (*MORUS NIGRA* L.) by cold stratification and exogenous application of gibberellic acid. **Acta Biologica Cracoviensia Series Botanica**, v. 47, n. 2, p. 23–26, 2005.

KYAUK, H., N. W. HOPPER, AND R. D. BRIGHAM. Effects of temperature and presoaking on germination, root length and shoot length of Seasame (*Sesamum ndicum* L.) **Environmental and Experimental Botany**, v. 35, n.3, p. 345-351, 1995.
LAMAS, A.M. **Unidade Produtora de Flores de Corte**. Série Perfil de Projetos. Vitória: Sebrae, 38p. 1999.

LAMPHERE, J.A. Propagation and establishment of *Penstemon haydenii* S. Wats. **Master of Science Thesis**. Lincoln, NE: University of Nebraska-Lincoln, 212 p. 1999.

LANG, A. ***Hyoscyamus niger*** **CRC Handbook of Flowering**. Vol V. Boca Raton, FL: 1986. CRC Press. p.144–186.

LERNA, A. AND TENORIO, J. Effects of pre-sowing treatments on emergence charecteristcs of twelve true potato seed (TPS) genotypes. **Abstracts, 27th ISTA Congress. Seed Symposium Budapest**, Hungary May 17th– 19th. Page 128. 2004.

LEWIS, H.; N. HILL. **Successful Perennial Gardening: A Practical Guide.** Pownal, Vt. : 1988. Storey Communications. 176p.

LIDBETTER, J., SLATER, T., CAIN, P., BANKIER, M. AND VUJOVIC, S. Grafting *Eriostemon australasius*. **A report for the Rural Industries Research and Development Corporation**. Publication No 02/140. 2003.

LINDGREN, D.. **Personal communication**, 2007.

LINDGREN, D. T. 'Prairie Snow' *Penstemon*. **HortScience**, v, 25. n. 4, p. 489-1990.

LINDGREN, D.T. AND D.M. SCHAAF. Influence of Seed Stratification and seed age on emergence of penstemon. **HortScience**, v. 39, n. 2, p.1385-1386, 2004.

MACCHIA, M., ANGELINE, L.G. AND CECCARINI, L. Methods to overcome seed dormancy in *Echinacea angustifolia* DC.**Scientia Horticulturae**, v. 89, p. 317-324, 2001.

MACCHIA, M., L.G. ANGELINE, AND L. CECCARINI. Methods to overcome seed dormancy in *Echinacea angustifolia* DC. **Scientia Horticulturae**, v. 89:317-3242001.

MARQUES, R.W.C. Avaliação da sazonalidade do mercado de flores e plantas ornamentais no estado de São Paulo. Dissertação de mestrado, ESALQ/USP. Piracicaba (Evaluation of the seasonality of the flowers and ornamental plants market in the state of Sao Paulo. **Masters Thesis**, ESALQ-University of Sao Paulo. Piracicaba). 2002.

MEEROW, A. W. Palm Seed Germination. **Environmental Horticulture Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences**. University of Florida. BUL, 274. 11p. 2004.

MEYER, S.E. AND KITCHEN, S.G. Habitat-correlated variation in seed germination response to chilling in *penstemon* section glabri (Scrophulariaceae). **American midland naturalist**, v. 132, n. 2, p. 349-365, 1994.

MEYER, S.E. KITCHEN, S.G. AND S.L, CARLSON. Seed germination timing patterns in Intermountain *Penstemon* (Scrophulariaceae). **American Journal of Botany**, v. 82, p. 377-389, 1995.

MICHAELS, S. D. AND AMASINO, R.M. The gibberellic acid biosynthesis mutant *ga1–3* of *Arabidopsis thaliana* is responsive to vernalization. **Developmental Genetics**, v. 25, p. 194–198, 1999.

MOURADOV, A., CREMER, F. AND G. COUPLAND. Control of flowering time. Interacting pathways as a basis for diversity. **Plant Cell** (Suppl), v.14, p.111–130, 2002.

NADEEM, M., PALNI, L. M. S., PUROHIT, A. N., PANDEY, H. AND NANDI, S. K. Propagation and conservation of *Podophyllum hexandrum* Royle: an important medicinal herb. **Biological Conservation**, v. 92, p. 121-129, 2000.

NADJAFI, F., BANNAYAN, M., TABRIZI, L. AND RASTGOO, M. Seed germination and dormancy breaking techniques for *Ferula gummosa* and *Teucrium polium*. **Journal of Arid Environments**, v.64, p. 542–547, 2005.

NADJAFI, F., M. BANNAYAN., L. TABRIZI, AND M.RASTGOO. Seed germination and dormancy breaking techniques for *Ferula gummosa* and *Teucrium polium*. **Journal of Arid Environments**, v. 64, p. 542–547, 2005.

NAU, J. **Ball Culture Guide**. The Encyclopedia of Seed Germination (2nd ed.). Batavia, IL.: 1993. Ball Publishing.456p.

NAU, J. **Ball perennial Manual Propagation and Production**. Co. Batavia, IL.: 1996. Ball Publishing. 493p

NOLD, R. **Penstemons.** Portland Oregon, USA.: 1999. Timber Press. 259p.

NONOGAKI, H., GEE, O.H. AND BRADFORD, K.J. A germination-specific endo-b-mannanase gene is expressed in the micropylar endosperm cap of tomato seeds. **Plant Physiology**, v.123, p. 1235–1245, 2000.

NORDEN, D. S., BLAZICH, F. A., WARREN, S. L. AND NASH, D. L. Seed Germination of Seabeach Amaranth (*Amaranthus pumilus*) in Response to Temperature, Light, and Gibberellin A3 Treatments. **SNA Research Conference**, v. 50, p. 411-413, 2005.

OGASAWARA, N., HIRAMASU, T., ISHIYANA K.,. FUSHIMI H., SUZUKI H. AND H. TAKAGI. Effects of gibberellic acid and temperature on growth and root carbohydrates of *Delphinium* seedlings. **Plant Growth Regulation**, v. 33, p.181-187, 2001.

OKA , M; TASAKA, Y; IWABUCHI, M AND MINO, M. Elevated sensitivity to gibberellin by vernalization in the vegetative rosette plants of *Eustoma grandiflorum* and *Arabidopsis thaliana.* **Plant Science**, v. 160, p. 1237–1245, 2001.

ORTEGA-BAE, P. AND M. ROJAS-ARÉCHIG. Seed germination of *Trichocereus terscheckii*(Cactaceae): Light, temperature and gibberellic acid effects. J**ournal of Arid Environments**, v. 69, p. 169–176, 2007.

PENG, J. AND HARBERD, N.P. The role of GA-mediated signalling in the control of seed germination. **Current Opinion in Plant Biology**, v.5, p. 376–381, 2002.

RAGHAVAN, V. **Developmental Biology of Flowering Plants**. New York, N.Y. Springer-Verlag. Inc. p. 292-303, 2000.

REABER, A. C. AND C. W. LEE. Gibberellic acid, temperature, light, stratification, and salinity affect germination of *Penstemon parryi* seed. **HortScience**, v. 26, n. 12, p. 1535-1537, 1991.

REABER, A. C. AND LEE, C. W. Gibberellic acid, temperature, light, stratification, and salinity affect germination of *Penstemon parry* seed. **HortScience**, v. 26, n. 12, p.1535-1537, 1991.

ROGIS, C., GIBSON, L. R., KNAPP, A. D. AND HORTON, R. Enhancing Germination of Eastern Gamagrass Seed with Stratification and Gibberellic Acid. **Crop Science**, v. 44, p. 549-552, 2004.

ROSNER, L.S., HARRINGTON, J.T. DREESEN, D.R. AND L. MURRAY. Gibberellic acid effect of and standard seed treatments on mountain snowberry germination. Native **Plants Journal**, v. 3, n. 2, p. 165-162, 2002.

ROUHI, V. Seed germination of *Prunus scoparia* (Spach) C.K. Schneider and drought stress evaluation based on ecophysiological parameters and growth characteristics for three contrasting almond species (*P. dulcis* (Miller) D. Webb, *P. lycioides* (Spach) C.K. Schneider and *P. scoparia* (Spach) C.K. Schneider). **PhD thesis**. Ghent University, Belgium. 237p. 2006.

RUCHALA, S. L. Propagation of several native ornamental plants. **Master of Science in Horticulture thesis**. The University of Maine, USA. 127p. 2002.

SALISBURY, FRANK B.; ROSS, CLEON W. **Plant physiology**, 4th. Belmont, California: 1992. Wadsworth Publishing.634p.

SAMACH, A. AND WIGGE, P. Ambient temperature perception in plants. **Current Opinion in Plant Biology**, v. 8, p. 483–486, 2005.

SCHMIDT, M. C. et al. Ácido giberélico (GA_3) no crisântemo (*Dendrathema grandiflora* Tzvelev.) de corte 'viking': cultivo verão/outono. **Cienc. Rural**, v. 33, n. 2, p. 267-274. 2003.

SEREK, M. SISLER, E.C., AND M.S. REID. Effect of 1-MCP on the vase life and ethylene response of cut flowers. **Plant Growth Regulation**, v. 16, p. 93-97, 1995.

STABY, G. L., R. M. BASEL, M. S. REID, AND L. L. DODGE. Efficacies of commercial antiethylene products for fresh cut flowers. **HortTechnology**, v. 3, p. 199-202, 1993.

Statistical Analysis System – SAS. Getting started with the SAS® Learning Edition. Cary, NC: 2002. SAS Institute, 81p.

STUBBENDIECK, J. AND W. G. MCCULLY, Effect of temperature and photoperiod on germination and survival of sand bluestem. **J. of Range Mgnt**, v. 29, p. 206-208, 1976.

SUNG, S. AND R.M. AMASINO. Vernalization and epigenetics: how plants remember winter. **Curr Opin Plant Biol**, v. 7, p. 4–10, 2004.

TAIZ, L., AND ZEIGER, E. 2006. **Plant Physiology** (Sinauer Associates, Inc) 4th edition.

TAMARI, G., PAPPA, L., ZERED, T., AND A. BOROCHOV. Effects of ethrel and gibberellin on impatiens plants. **Scientia Horticulturae**, v. 76, p. 29-35, 1998.

TANIMOTO HARADA, ***Rudbeckia.*** **CRC Handbook of Flowering.** Vol. IV., Boca Raton, Fla. A.: 1985. Halevy CRC Press p. 239-242.

TANINO, S.D. AND C. S. SIMOES. Estufos Realizados. GELOG-UFSC. temperature treatments on seed germination of *Lupinus havardii*. **Seed Sci. Technol**, v. 23, p. 815-821. 2005.

THOMAS, G. S. **Perennial garden plants or the modern florilegium**., Portland: Saga Press, 1990. 87p.

THOMSEN-STORK, M. Neue Taglilien aus den USA. **Gartenpraxis**, v. 21, n. 12, p. 8-13, 1995.

TRAVLOS, I. S., ECONOMOU, G., KARAMANOS, A. I. Germination and emergence of the hard seed coated Tylosema esculentum (Burch) A. Schreib in response to different pre-sowing seed treatments. **Journal of Arid Environments**, v. 68, p. 501–507, 2007.

USDA NRCS. The PLANTS database (http://plants.usda.gov/plants). Baton Rouge, LA: **National Plant Data Center**. 2006.

WANG, Q., ZHANG, F. AND SMITH, D. L. Application of GA_3 and kinetin to improve corn and soybean seedling emergence at low temperature. Environmental and **Experimental Botany**, v. 36, n. 4, p. 377-383, 1996.

WAY, D.; P. JAMES. In: **The Gardener's Guide to Growing Penstemons**. Portland Oregon, USA.1998.160p.

YAMAGUCHI, S. AND KAMIYA, Y. Gibberellins and lightstimulated seed germination. **Journal of Plant Growth Regulation**, v. 20, p. 369–376, 2002.

ZEEVAART, J.A.D. The Biochemistry and Physiology of Gibberellins. Vol 2. In: **Gibberellins and flowering**. New York: 1983. Praeger Crozier A, p. 333–374.

APÊNDICE A – SAS output ANOVA with LSMEANS and regressions

****

The SAS System 1
19:04 Wednesday, December 15, 2008

Obs	Bl	A	B	V1	V2	V3	V4	V5	V6	A2	A3
1	1	0	0	50	48	9	51.60	74	23	0	0
2	1	0	100	43	55	12	54.50	76	32	0	0
3	1	0	250	40	50	9	48.40	86	39	0	0
4	1	0	500	31	50	18	46.20	72	34	0	0
5	1	3	0	54	61	7	54.00	80	25	9	27
6	1	3	100	54	63	0	54.60	64	23	9	27
7	1	3	250	46	54	8	59.00	92	27	9	27
8	1	3	500	50	61	9	53.30	87	28	9	27
9	1	6	0	36	40	9	53.40	76	18	36	216
10	1	6	100	36	40	9	50.00	54	21	36	216
11	1	6	250	36	33	9	48.00	68	25	36	216
12	1	6	500	36	40	9	53.30	96	21	36	216
13	1	9	0	34	43	9	48.50	76	28	81	729
14	1	9	100	34	39	5	55.00	82	33	81	729
15	1	9	250	28	39	4	45.50	75	35	81	729
16	1	9	500	34	39	5	53.00	81	28	81	729
17	1	12	0	29	35	5	47.30	85	31	144	1728
18	1	12	100	28	40	6	42.00	71	34	144	1728
19	1	12	250	32	36	6	52.50	89	22	144	1728
20	1	12	500	32	39	6	50.00	78	27	144	1728
21	2	0	0	37	48	11	56.40	82	30	0	0
22	2	0	100	39	50	11	51.50	71	28	0	0
23	2	0	250	39	50	11	62.00	80	29	0	0
24	2	0	500	43	54	11	48.30	76	34	0	0
25	2	3	0	46	47	8	52.50	84	27	9	27
26	2	3	100	39	49	10	54.60	72	23	9	27
27	2	3	250	39	46	7	56.30	96	33	9	27
28	2	3	500	0	0	0	0.00	0	0	9	27
29	2	6	0	43	49	6	51.70	78	28	36	216
30	2	6	100	36	33	10	54.52	108	26	36	216
31	2	6	250	40	46	6	49.20	92	27	36	216
32	2	6	500	36	42	6	56.00	59	17	36	216
33	2	9	0	36	43	7	51.00	54	26	81	729
34	2	9	100	34	39	5	58.00	67	29	81	729
35	2	9	250	34	39	5	50.00	86	29	81	729
36	2	9	500	34	39	5	50.00	92	25	81	729
37	2	12	0	33	37	6	55.00	76	27	144	1728
38	2	12	100	29	38	6	46.00	80	30	144	1728
39	2	12	250	32	36	7	53.00	79	26	144	1728
40	2	12	500	28	36	6	47.00	73	33	144	1728
41	3	0	0	43	48	5	62.20	108	32	0	0
42	3	0	100	42	50	8	51.30	79	33	0	0
43	3	0	250	40	50	10	51.60	77	35	0	0
44	3	0	500	31	44	13	64.50	90	30	0	0
45	3	3	0	46	52	6	52.50	77	24	9	27
46	3	3	100	42	49	7	42.00	54	20	9	27
47	3	3	250	39	46	7	54.30	78	26	9	27
48	3	3	500	39	47	8	52.00	75	23	9	27
49	3	6	0	42	50	8	48.40	73	22	36	216
50	3	6	100	0	0	0	56.00	0	0	36	216

****

The SAS System 2
19:04 Wednesday, December 15, 2008

Obs	Bl	A	B	V1	V2	V3	V4	V5	V6	A2	A3
51	3	6	250	36	41	8	51.3	93	30	36	216
52	3	6	500	42	43	7	44.0	66	28	36	216
53	3	9	0	38	43	9	50.0	63	33	81	729
54	3	9	100	34	39	5	60.0	74	24	81	729
55	3	9	250	36	41	11	58.0	88	34	81	729
56	3	9	500	34	39	5	49.0	83	32	81	729
57	3	12	0	31	35	7	60.5	48	31	144	1728

58	3	12	100	31	34	7	57.5	78	28	144	1728
59	3	12	250	28	34	8	49.5	84	31	144	1728
60	3	12	500	28	34	6	51.5	96	29	144	1728
61	4	0	0	31	71	10	48.0	85	23	0	0
62	4	0	100	35	50	15	69.0	81	27	0	0
63	4	0	250	0	68	0	0.0	62	29	0	0
64	4	0	500	31	44	13	54.5	89	34	0	0
65	4	3	0	48	61	8	45.2	80	20	9	27
66	4	3	100	50	63	0	51.0	82	18	9	27
67	4	3	250	0	0	0	0.0	0	0	9	27
68	4	3	500	39	52	13	56.5	81	25	9	27
69	4	6	0	42	44	7	55.0	56	32	36	216
70	4	6	100	36	44	8	50.1	92	25	36	216
71	4	6	250	36	43	7	52.7	96	31	36	216
72	4	6	500	36	43	7	47.5	80	33	36	216
73	4	9	0	36	43	7	70.5	73	27	81	729
74	4	9	100	34	39	5	71.5	86	32	81	729
75	4	9	250	38	41	6	62.5	67	30	81	729
76	4	9	500	34	39	5	68.5	94	21	81	729
77	4	12	0	29	35	6	62.0	77	21	144	1728
78	4	12	100	28	34	6	54.2	64	24	144	1728
79	4	12	250	28	34	6	54.5	81	23	144	1728
80	4	12	500	32	34	8	56.3	78	24	144	1728

****

The SAS System 3
19:04 Wednesday, December 15, 2008

The ANOVA Procedure

Class Level Information

Class	Levels	Values
Bl	4	1 2 3 4
A	5	0 3 6 9 12
B	4	0 100 250 500
V1	19	0 28 29 31 32 33 34 35 36 37 38 39 40 42 43 46 48 50 54
V2	25	0 33 34 35 36 37 38 39 40 41 42 43 44 46 47 48 49 50 52 54 55 61 63 68 71
V3	13	0 4 5 6 7 8 9 10 11 12 13 15 18
V4	54	0 42 44 45.2 45.5 46 46.2 47 47.3 47.5 48 48.3 48.4 48.5 49 49.2 49.5 50 50.1 51 51.3 51.5 51.6 51.7 52 52.5 52.7 53 53.3 53.4 54 54.2 54.3 54.5 54.52 54.6 55 56 56.3 56.4 56.5 57.5 58 59 60 60.5 62 62.2 62.5 64.5 68.5 69 70.5 71.5
V5	36	0 48 54 56 59 62 63 64 66 67 68 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 92 93 94 96 108
V6	20	0 17 18 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 39

Number of Observations Read 80
Number of Observations Used 80

****

The SAS System 4
19:04 Wednesday, December 15, 2008

The ANOVA Procedure

Dependent Variable: V1

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F

Model	22	2881.175000	130.962500	1.42	0.1465
Error	57	5267.512500	92.412500		
Corrected Total	79	8148.687500			

R-Square	Coeff Var	Root MSE	V1 Mean
0.353575	27.41716	9.613142	35.06250

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Bl	3	361.237500	120.412500	1.30	0.2823
A	4	758.125000	189.531250	2.05	0.0993
B	3	538.737500	179.579167	1.94	0.1329
A*B	12	1223.075000	101.922917	1.10	0.3758

****

The ANOVA Procedure

Dependent Variable: V2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	22	4961.07500	225.50341	2.05	0.0155
Error	57	6261.81250	109.85636		
Corrected Total	79	11222.88750			

R-Square	Coeff Var	Root MSE	V2 Mean
0.442050	24.46744	10.48124	42.83750

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Bl	3	283.437500	94.479167	0.86	0.4672
A	4	2685.825000	671.456250	6.11	0.0004
B	3	410.037500	136.679167	1.24	0.3022
A*B	12	1581.775000	131.814583	1.20	0.3057

****

The ANOVA Procedure

Dependent Variable: V3

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	22	366.3000000	16.6500000	2.02	0.0178
Error	57	470.7000000	8.2578947		
Corrected Total	79	837.0000000			

R-Square	Coeff Var	Root MSE	V3 Mean
0.437634	39.63662	2.873655	7.250000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Bl	3	7.3000000	2.4333333	0.29	0.8291
A	4	209.0000000	52.2500000	6.33	0.0003
B	3	22.5000000	7.5000000	0.91	0.4428
A*B	12	127.5000000	10.6250000	1.29	0.2517

****

The SAS System 7
19:04 Wednesday, December 15, 2008

The ANOVA Procedure

Dependent Variable: V4

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	22	2187.04216	99.41101	0.64	0.8762
Error	57	8866.28776	155.54891		
Corrected Total	79	11053.32992			

R-Square	Coeff Var	Root MSE	V4 Mean
0.197863	24.22486	12.47192	51.48400

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Bl	3	106.4490400	35.4830133	0.23	0.8764
A	4	850.1529200	212.5382300	1.37	0.2570
B	3	544.4976400	181.4992133	1.17	0.3304
A*B	12	685.9425600	57.1618800	0.37	0.9697

****

The SAS System 8
19:04 Wednesday, December 15, 2008

The ANOVA Procedure

Dependent Variable: V5

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	22	5029.67500	228.62159	0.56	0.9339
Error	57	23365.51250	409.92127		
Corrected Total	79	28395.18750			

R-Square	Coeff Var	Root MSE	V5 Mean
0.177131	26.75014	20.24651	75.68750

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Bl	3	169.237500	56.412500	0.14	0.9371
A	4	1247.625000	311.906250	0.76	0.5551
B	3	518.537500	172.845833	0.42	0.7382
A*B	12	3094.275000	257.856250	0.63	0.8087

****

The SAS System 9
19:04 Wednesday, December 15, 2008

The ANOVA Procedure

Dependent Variable: V6

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	22	1487.675000	67.621591	1.61	0.0756
Error	57	2388.012500	41.894956		
Corrected Total	79	3875.687500			

R-Square	Coeff Var	Root MSE	V6 Mean
0.383848	24.36755	6.472631	26.56250

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Bl	3	88.2375000	29.4125000	0.70	0.5547
A	4	937.2500000	234.3125000	5.59	0.0007
B	3	68.7375000	22.9125000	0.55	0.6522
A*B	12	393.4500000	32.7875000	0.78	0.6657

****

The GLM Procedure

Class Level Information

Class	Levels	Values
Bl	4	1 2 3 4
A	5	0 3 6 9 12
B	4	0 100 250 500

Number of Observations Read	80
Number of Observations Used	80

****

The GLM Procedure

Dependent Variable: V2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	22	4961.07500	225.50341	2.05	0.0155
Error	57	6261.81250	109.85636		
Corrected Total	79	11222.88750			

R-Square	Coeff Var	Root MSE	V2 Mean
0.442050	24.46744	10.48124	42.83750

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Bl	3	283.437500	94.479167	0.86	0.4672

A	4	2685.825000	671.456250	6.11	0.0004
B	3	410.037500	136.679167	1.24	0.3022
A*B	12	1581.775000	131.814583	1.20	0.3057

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Bl	3	283.437500	94.479167	0.86	0.4672
A	4	2685.825000	671.456250	6.11	0.0004
B	3	410.037500	136.679167	1.24	0.3022
A*B	12	1581.775000	131.814583	1.20	0.3057

****

The GLM Procedure
Least Squares Means

A	V2 LSMEAN	LSMEAN Number
0	51.8750000	1
3	46.9375000	2
6	39.4375000	3
9	40.2500000	4
12	35.6875000	5

Least Squares Means for effect A
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: V2

i/j	1	2	3	4	5
1		0.1880	0.0014	0.0027	<.0001
2	0.1880		0.0477	0.0764	0.0036
3	0.0014	0.0477		0.8272	0.3158
4	0.0027	0.0764	0.8272		0.2233
5	<.0001	0.0036	0.3158	0.2233	

NOTE: To ensure overall protection level, only probabilities associated with
pre-planned comparisons should be used.

****

The GLM Procedure

Class Level Information

Class	Levels	Values
Bl	4	1 2 3 4
A	5	0 3 6 9 12
B	4	0 100 250 500

Number of Observations Read	80
Number of Observations Used	80

****

The GLM Procedure

Dependent Variable: V3

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	22	366.3000000	16.6500000	2.02	0.0178
Error	57	470.7000000	8.2578947		
Corrected Total	79	837.0000000			

R-Square	Coeff Var	Root MSE	V3 Mean
0.437634	39.63662	2.873655	7.250000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Bl	3	7.3000000	2.4333333	0.29	0.8291
A	4	209.0000000	52.2500000	6.33	0.0003
B	3	22.5000000	7.5000000	0.91	0.4428
A*B	12	127.5000000	10.6250000	1.29	0.2517

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Bl	3	7.3000000	2.4333333	0.29	0.8291
A	4	209.0000000	52.2500000	6.33	0.0003
B	3	22.5000000	7.5000000	0.91	0.4428
A*B	12	127.5000000	10.6250000	1.29	0.2517

****

The GLM Procedure
Least Squares Means

A	V3 LSMEAN	LSMEAN Number
0	10.3750000	1
3	6.1250000	2
6	7.2500000	3
9	6.1250000	4
12	6.3750000	5

Least Squares Means for effect A
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: V3

i/j	1	2	3	4	5
1		0.0001	0.0032	0.0001	0.0002
2	0.0001		0.2728	1.0000	0.8065
3	0.0032	0.2728		0.2728	0.3927
4	0.0001	1.0000	0.2728		0.8065
5	0.0002	0.8065	0.3927	0.8065	

NOTE: To ensure overall protection level, only probabilities associated with
pre-planned comparisons should be used.

****

The GLM Procedure

Class Level Information

Class	Levels	Values
Bl	4	1 2 3 4
A	5	0 3 6 9 12
B	4	0 100 250 500

Number of Observations Read 80
Number of Observations Used 80

****

The GLM Procedure

Dependent Variable: V6

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	22	1487.675000	67.621591	1.61	0.0756
Error	57	2388.012500	41.894956		
Corrected Total	79	3875.687500			

R-Square	Coeff Var	Root MSE	V6 Mean
0.383848	24.36755	6.472631	26.56250

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Bl	3	88.2375000	29.4125000	0.70	0.5547
A	4	937.2500000	234.3125000	5.59	0.0007
B	3	68.7375000	22.9125000	0.55	0.6522
A*B	12	393.4500000	32.7875000	0.78	0.6657

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Bl	3	88.2375000	29.4125000	0.70	0.5547
A	4	937.2500000	234.3125000	5.59	0.0007
B	3	68.7375000	22.9125000	0.55	0.6522
A*B	12	393.4500000	32.7875000	0.78	0.6657

****

The GLM Procedure
Least Squares Means

A	V6 LSMEAN	LSMEAN Number
0	30.7500000	1
3	21.3750000	2
6	24.0000000	3
9	29.1250000	4
12	27.5625000	5

Least Squares Means for effect A
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: V6

i/j	1	2	3	4	5
1		0.0001	0.0046	0.4805	0.1691
2	0.0001		0.2561	0.0013	0.0090
3	0.0046	0.2561		0.0290	0.1251
4	0.4805	0.0013	0.0290		0.4975
5	0.1691	0.0090	0.1251	0.4975	

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

****

The REG Procedure
Model: MODEL1
Dependent Variable: V2

Number of Observations Read	80
Number of Observations Used	80

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	2535.26786	1267.63393	11.24	<.0001
Error	77	8687.61964	112.82623		
Corrected Total	79	11223			

Root MSE	10.62197	R-Square	0.2259
Dependent Mean	42.83750	Adj R-Sq	0.2058
Coeff Var	24.79596		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	51.94464	2.49915	20.78	<.0001
A	1	-2.16518	0.98681	-2.19	0.0312
A2	1	0.07192	0.07886	0.91	0.3646

****

The REG Procedure
Model: MODEL1
Dependent Variable: V2

Number of Observations Read	80
Number of Observations Used	80

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	2441.40625	2441.40625	21.69	<.0001
Error	78	8781.48125	112.58309		
Corrected Total	79	11223			

Root MSE	10.61052	R-Square	0.2175
Dependent Mean	42.83750	Adj R-Sq	0.2075
Coeff Var	24.76923		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	50.65000	2.05472	24.65	<.0001
A	1	-1.30208	0.27961	-4.66	<.0001

****

The REG Procedure
Model: MODEL1
Dependent Variable: V3

Number of Observations Read	80
Number of Observations Used	80

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	180.07143	60.02381	6.94	0.0003
Error	76	656.92857	8.64380		
Corrected Total	79	837.00000			

Root MSE	2.94003	R-Square	0.2151
Dependent Mean	7.25000	Adj R-Sq	0.1842
Coeff Var	40.55219		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	10.21429	0.72974	14.00	<.0001
A	1	-1.86508	0.61879	-3.01	0.0035
A2	1	0.27579	0.13096	2.11	0.0385
A3	1	-0.01235	0.00717	-1.72	0.0893

****

The REG Procedure
Model: MODEL1
Dependent Variable: V6

Number of Observations Read	80
Number of Observations Used	80

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	937.22768	312.40923	8.08	<.0001
Error	76	2938.45982	38.66395		
Corrected Total	79	3875.68750			

Root MSE	6.21803	R-Square	0.2418
Dependent Mean	26.56250	Adj R-Sq	0.2119
Coeff Var	23.40907		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	30.74554	1.54336	19.92	<.0001
A	1	-6.14459	1.30872	-4.70	<.0001
A2	1	1.18204	0.27697	4.27	<.0001
A3	1	-0.05768	0.01517	-3.80	0.0003