later date if no action was taken in the first instance. Your lawyer will consider if you have legal grounds for taking action. If so, they will in the first instance contact the third party and ask that they "cease and desist" their apparent infringement of your trademark. Ideally the infringement will either cease absolutely or you reach some settlement acceptable to both parties. If this does not occur it may be necessary to take court action to obtain an injunction instructing the third party to stop infringement and, in some cases, to pay damages for their infringement.

CONCLUSION

By selectively and knowledgeably choosing your brand and protecting it by registering it as a trade mark, then by appropriately marketing your brand and enforcing any infringement of it, you will build a strong and long-lasting brand of great value.

ADDITIONAL READING

Intellectual Property Office of New Zealand <www.iponz.govt.nz>.

The Effect of Gibberellic Acid, Potassium Nitrate, and Cold Stratification on the Germination of Goldenseal (*Hydrastis canadensis*) Seed©

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A laboratory experiment was conducted to measure the effects of various factors on germinating goldenseal seed. Seeds were soaked in gibberellic acid (0.5 g·L^{-1}, GA_3) or potassium nitrate (2 g·L^{-1}, KNO_3) for 2, 6, 12, or 24 h, followed by periods of cold stratification at 4 °C (0, 2, 4, 8, or 12 weeks). GA_3 treatment accelerated the germination process with 61% of the seed germinating to the seed splitting stage in 47 days compared to 4% in the non-GA_3 treatments. Neither soak time nor osmotic conditioning with KNO_3 had any effect on germination. Cold temperature stratification at 4 °C had a negative effect on germination. Increasing the stratification time from 0 to 12 weeks induced secondary seed dormancy and reduced overall germination after 6 months incubation at 1 °C by 23% compared to fresh, untreated seed, which gave 80%–90% germination after 3 months. Germinating seed from all treatments was observed to be highly susceptible to disease under laboratory conditions suggesting this may also be a factor in the poor establishment of goldenseal crops in the field. The lack of seedling development following seed splitting suggests that the conditions, which favour the early germination process are different from those that promote seedling growth.

Keywords: Goldenseal, germination, gibberellic acid, stratification, Ranunculaceae, osmotic conditioning.

[1]Deceased

INTRODUCTION

Goldenseal (*Hydrastis canadensis* L., Ranunculaceae) is a highly valued North American medicinal herb which has traditionally been gathered from wild populations (Foster, 1993). To ensure its survival goldenseal was listed under the CITES Treaty (Convention on International Trade in Endangered Species) in 1997 (Bannerman, 1998). Research into the production of this species is now focused on developing sustainable cultivation methods (McGuffin, 1999).

Goldenseal can be propagated by division of the underground rhizome, root cuttings, and seed (Henkel and Klugh, 1908; Lloyd, 1912; van Fleet, 1914; Foster, 1993; Sturdivant and Blakley, 1999; Davis, 1999; Davis and McCoy, 2000) with propagation by seed considered to be the most difficult and unpredictable method (Sturdivant and Blakley, 1999; Davis, 1999; Davis and McCoy, 2000). As goldenseal seed is recalcitrant; seed for propagation is extracted from the ripe fruit pulp in summer and stored in moist sand until sowing (Henkel and Klugh, 1908; van Fleet, 1914; Hardacre, 1962; Davis and McCoy, 2000). Seed stored dry does not germinate (Deno, 1993). Hardacre (1962) successfully grew goldenseal from both autumn and spring sowings but generally found autumn sowing was more successful than spring sowing. Foster (1993) suggested goldenseal seed should be refrigerated for 3 months before sowing in spring, while Sturdivant and Blakley (1999) advocated leaving seed sown in trays outside until early spring to achieve winter chilling before bringing them into a greenhouse to germinate. Davis (1999) considered that the best seed germination was achieved by sowing fresh seed in the late summer-autumn period immediately after extraction. Experiments by Davis and McCoy (2000) found that where seed was stored in moist sand at 21 °C and sown in late autumn an average germination rate of 37% (range 25%–88%) was achieved the following spring. Seed, which was held at 21 °C for 30 days and subsequently at 4 °C, or held over the entire period at 4 °C, and planted the following spring, gave an average germination rate of 45% (range 30%–70%) but with the germination occurring two seasons later (Davis and McCoy, 2000).

Germination of genera within the family Ranunculaceae is often enhanced by pre-chilling, exposure to light, gibberellic acid (GA_3), and osmotic conditioning with potassium nitrate (KNO_3) (Ellis et al., 1985). Recent examples in the literature show that low temperature and GA_3 promoted the germination of *Cimicifuga nanchuanensis* (Fu et al., 1998) and *Thalictrum aquilegiifolium* (Sim et al., 1996), and KNO_3 enhanced the germination of *Ranunculus sceleratus* (Shim et al., 1998). Foster (1993) has previously recommended prechilling goldenseal before sowing but no information could be found on the use of GA_3 or KNO_3 to aid the germination of goldenseal seed. The success of these exogenous chemical treatments in enhancing the germination of other Ranunculaceae genera raised the question of whether the germination of goldenseal could also be improved to give a consistently high rate. To investigate this, goldenseal seed was treated with GA_3 or KNO_3 and stratified at 4 °C before being placed under constant warm temperature incubation to measure the effects of the treatments on germination.

MATERIALS AND METHODS

Ripe berries were collected in mid summer (February in New Zealand) from 5-year-old goldenseal plants grown outdoors under shade cloth in the Waikato, New Zealand (latitude 37° 50′ S, longitude 175° 18′ E). The berries were mashed in a sieve and the

seed was separated from the pulp under running water similar to method described by Davis and McCoy (2000). The extracted seed was surface sterilised by soaking it in a 0.3% sodium hypochlorite solution for 5 min and then air dried and weighed.

A factorial experiment combing 2 putative-dormancy-breaking agents (0.5 $\cdot L^{-1}$ GA_3 or 2 $g \cdot L^{-1}$ KNO_3) × 4 soak times (2, 6, 12, 24 h) × 5 moist-seed stratification treatments at 4 °C (0, 2, 4, 8, 12 weeks) with the control treatments consisting of two replicates of the seed stratification treatments (0, 2, 4, 8, 12 weeks) after soaking in water was laid down on 13 March 1996 with one replicate. This design gave a total of 50 treatment units with each unit having ten seeds placed on moist filter paper in a covered Petri dish and kept constantly moist for the duration of the trial. Following the stratification treatments at 4 °C the Petri dishes were placed in a fluorescent lit incubation cabinet at 14 °C. Seed germination, indicated by the splitting of the hard seed coat, was recorded weekly for the next 264 days (180 days incubation after the longest stratification treatment). The germination counts recorded after 47, 89, 131, and 180 days incubation were used to analyse the results. After 180 days, all ungerminated seeds were dissected and assessed for seed viability by soaking them in a 1.0% tetrazolium solution for 6 h (Hartmann and Kester, 1975).

Analysis of variance was carried out on the seed germination counts after 47, 89, 131, and 180 days in 14 °C incubation using the inbuilt replication of the factorial design to assess the main effects and two-factor interactions. The precision of the main effect was further enhanced by combining treatments which did not differ statistically from each other.

RESULTS

The fresh seed extracted from the berries had a mean seed weight of 1.33 g/100 seed (SD = 0.042) and after soaking for 24 h in either GA_3 or KNO_3 solutions had a seed weight of 1.50 g per 100 seeds (SD = 0.092). There was no effect on germination from varying the seed soaking time or soaking the seed in KNO_3. This data was subsequently pooled with the control treatments to increase the precision of the GA_3 and the stratification effects.

Seed treated with GA_3 when incubated at 14 °C began germinating after 19 days with 61% germinating after 47 days and 82% after 180 days. By comparison untreated seed or seed treated with KNO_3 had 4% seed germinated after 47 days and 58% by 180 days (Fig. 1). Seed given a short stratification (0, 2, or 4 weeks) germinated more quickly than those given a long stratification (8 or 12 weeks) with the use of GA_3 both speeding up the germination process and overcoming the stratification effect (Fig. 2). Seed soaked in GA_3 gave similar germination counts in all stratification treatments following 47 days incubation, but the subsequent germination was 20% less in the longer stratification treatment. The short stratification treatments without GA_3 had a similar but slower pattern of germination than the seed with GA_3 treatments with germination continuing over a longer period and ending with a lower final germination (Fig. 2). The longer stratification treatment without GA_3 showed an entirely different pattern of germination with low germination in the first 87 days and germination continuing over the entire 180 days (Fig. 2).

Regression analysis of the stratification treatments showed a significant linear effect on seed germination as stratification time increased (Fig. 3). Stratification of goldenseal seed for 12 weeks, reduced the overall germination by 23% from 81% to 58% compared to no stratification, with the effect being more pronounced for the

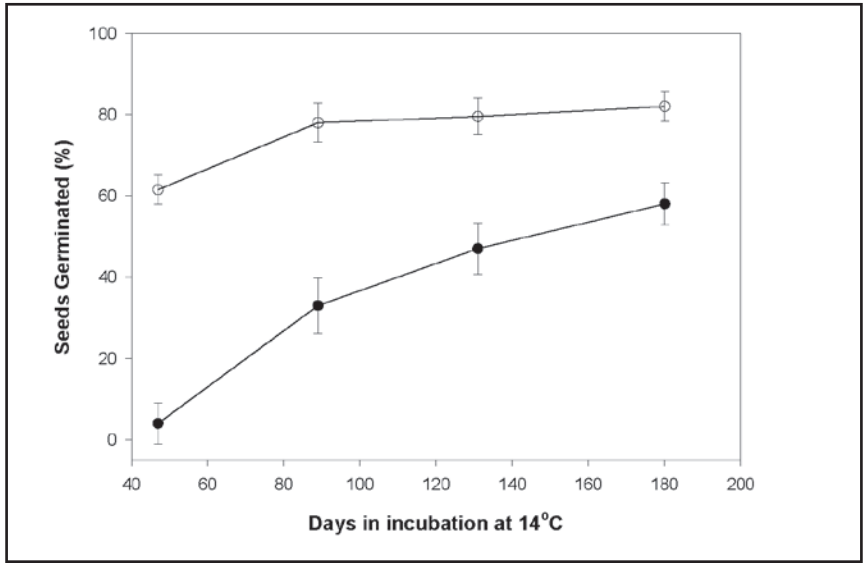

Figure 1. The main effect of soaking seed with (O) and without (●) GA_3 on germination when incubated at 14 °C.

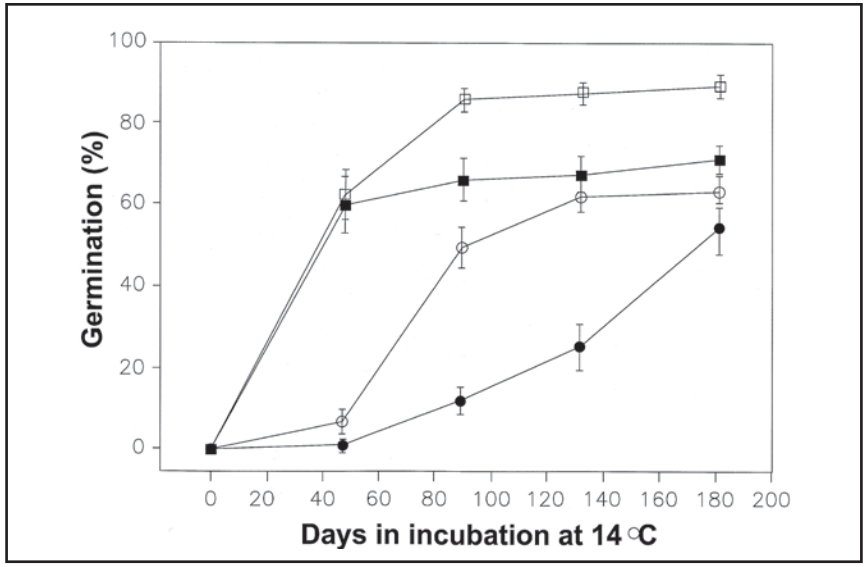

Figure 2. The interaction between a short stratification period (0, 2 or 4 weeks) with (□) and without (O) GA_3 and a long stratification period (8 or 12 weeks) with (■) and without (●) GA_3 on seed germination. Error bars indicate the standard error of the treatment means.

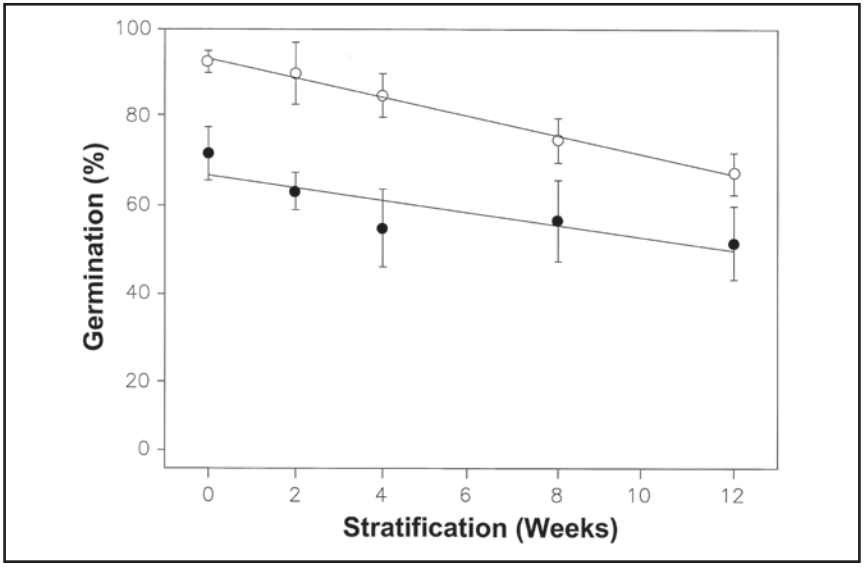

Figure 3. Effect of stratification on the germination of seed soaked with (O) and without (●) GA_3 after 180 days incubation at 14°C. The regression equations are y = 93.32 - 2.18 strat week, (R^2 = 0.99) for the GA_3 treatment and y = 68.07 - 1.55 strat week, (R^2 = 0.67) for the KNO_3 and water treatment.

GA_3-treated seed (93% to 68%) than for seed treated with KNO_3 or water (67% to 49%) (Fig. 3).

At the end of the experiment 13% of the KNO_3 and water-treated seed, and 2% of the GA_3-treated seed, that had not germinated were viable but dormant when tested with tetrazolium. There were also more dormant seed left ungerminated in the 12-week stratification treatment (28%) than in the unstratified seed (7%) in the KNO_3 and water treatment. All treatments had a final germination potential of 80%–100% when the alive but dormant seed numbers were combined with the seed germination numbers.

DISCUSSION

The propagation of goldenseal from seed is known to be difficult and unpredictable (Sturdivant and Blakley, 1999; Davis, 1999; Davis and McCoy, 2000). It is well known that goldenseal seed needs to be stored moist to maintain viability (Henkel and Klugh, 1908; van Fleet, 1914; Hardacre, 1962; Foster, 1993; Davis and McCoy, 2000) and, the increased seed dormancy after cold storage is one explanation for the variable and prolonged germination of spring-sown crops compared to autumn-seeded crops found by Hardacre (1962). More recent results have found that seed stored under warm (21 °C) rather than cold conditions has been beneficial to germination (Davis, 1999; Davis and McCoy, 2000). From our studies and those of Davis (1999), the most reliable germination of goldenseal seed is from fresh seed sown immediately. Treating seed with GA_3 had a significant positive impact on the rate of germination and in addition it gave the lowest number of ungerminated seeds

at the end of the experiment indicating it was effective in breaking seed dormancy as well as accelerating the germination. There was no benefit from prolonging the seed soaking in GA_3 beyond 2 h although soaking for 24 h is recommended for most species (Hartmann and Kester, 1975).

Osmotic conditioning with 2 $g \cdot L^{-1}$ KNO_3 for 2 to 24 h had no effect on germination of goldenseal seed but it is unknown whether longer soak times of 2 to 21 days as recommended by Khan (1992) would be more effective.

Our experiment successfully germinated goldenseal seed to the seed cracking stage but the subsequent development of seedlings was very slow. Once the seed had cracked there was a high mortality from a number of diseases identified as *Alternaria alternata, Fusarium oxysporum, F. proliferatum, F. sacchari*, and *Mucor* species. In spite of the disease deprivation the slowness of the seedling growth suggests that seedling development in goldenseal is either hampered by a secondary dormancy or else the environmental requirements for seedling development are markedly different than those for the germination process. This aspect of growing goldenseal from seed is the subject of further research.

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LITERATURE CITED

Bannerman, J.E. 1998. Goldenseal in world trade: Pressures and potentials. HerbalGram 41:51–52.

Davis, J.M. 1999. Forest production of goldenseal. Agroforestry Notes, USDA Forest Service, July 1999.

Davis, J.M. and **J. McCoy.** 2000. Commercial goldenseal cultivation. North Carolina State University Horticulture Information Leaflet 131.

Deno, N.C. 1993. Seed germination theory and practice. N.C. Deno, private publication, 139 Lenor Drive, State College, Pennsylvania.

Ellis, R.H., T.D. Hong, and **E.H. Roberts.** 1985. Handbook of seed technology for genebanks, Volume II. Compendium of specific germination information and test recommendations. Handbooks for Genebanks No 3, International Board for Plant Genetic Resources, Rome, Italy.

Foster, S. 1993. Herbal renaissance: Growing, using and understanding herbs in the modern world. Gibbs-Smith, Salt Lake City, Utah.

Fu, J., W. Qi, Z. Gu, and **R. You.** 1998. Seed dormancy and germination in *Cimicifuga nanchuanensis*. Acta Botanica Sinica. 40(4):303–308.

Hardacre, J.V. 1962. The wildcrafters goldenseal manual, Laurence Barcus, Rockville, Indiana, USA:1–11.

Hartmann, H. T. and **D.A. Kester.** 1975. Plant propagation principles and practices. Prentice-Hall, Inc., Englewood Cliffs, New Jersey.

Henkel, A. and **G.F. Klugh.** 1908. The cultivation and handling of goldenseal. U.S. Dept. Agric., Bureau of Plant Industry Circular No. 6.

Khan, A.A. 1992: Preplant physiological seed conditioning. Hort. Review 132, 131–181.

Lloyd, J.U. 1912. The cultivation of *Hydrastis.* J. Amer. Pharmaceutical Assoc. 1:5–12.

McGuffin, M. 1999. AHPA Goldenseal survey measures increased agricultural production. HerbalGram 46:66–67.

Shim, S.J., S.G. Lee, and **B.H. Kang.** 1998. Effects of several chemicals and burial of seeds into the soil on dormancy breaking of weed species. Korean J. Weed Sci. 18(4):295–303.

Sim, Y.G., Y.Y. Han, I.K. Song, T.Y. Kwon, J.S. Jung, J.T. Yoon, and **B.S. Choi.** 1996. Influence of GA_3 and chilling treatment on seed germination in several native plants. Rural Development Admin. J. Agric. Sci. (Horticulture) 38(1):700–704.

Sturdivant, L. and **T. Blakley.** 1999. The Bootstrap Guide to medicinal herbs in the garden, field and marketplace. San Juan Naturals, Friday Harbor, USA.

van Fleet, W. 1914. Goldenseal under cultivation. U.S. Dept Agric. Farmers Bull. 613.