Optimization of Germination Requirement and Seed Production of Wild-type *Vaccinium myrtillus*

M. Ciordia, M. De Lucas, V. Mateos, L. Rodríguez, J.C. García and J. Majada
Servicio Regional de Investigación y Desarrollo Agroalimentario (SERIDA)
Apdo 13 - 33.300 Villaviciosa, Asturias
Spain

Keywords: bilberry, gibberellins, imbibition time, temperature

Abstract

Principado de Asturias develops programs and strategies to recover and conserve its natural resources. For the successful implementation of development goals the policy framework attaches significant importance to the natural resources available in the region, including fauna and indigenous flora recovery. The capercaillie (*Tetrao urogallus cantabricus*) from Northern Spain, an endangered bird that inhabits coniferous forests with an understory of *Vaccinium myrtillus*, is the smallest of those living in Europe and is a relic from the last glacial period. Seed dormancy is common among temperate zone bird-dispersed plants. Moreover, germination of seeds collected during and after the fruiting season might vary due to development of dormancy. Several studies conducted recently have shown a relationship between the presence of *V. myrtillus* and *T. urogallus* survival. Therefore we developed a seed extraction system for bilberry (*V. myrtillus*), as well as an efficient system for producing around 200.000 wild plants. Subsequently, these will be planted in strategic wild areas where there is reason to believe that the capercaillie survives. This work studied the influence of GA_3, imbibition time and temperature on bilberry production. The results indicate differences between germination rates that vary from 50% to 91% in the most efficient treatments. Besides, there were clear differences in germination among seeds that came from different provenances. Seeds from Somiedo Park germinated faster than those from Redes Park.

INTRODUCTION

Principado de Asturias develops programs and strategies to recover and conserve its natural resources. The policy framework is named "Plan de Ordenación de los Recursos Naturales de Asturias" PORNA (Management Plan of Natural Resources from Asturias) and it proposes, for first time, an environmental biodiversity analysis, as well as the design of a protected spaces network. Previous studies have indicated that the survival of some animal species threatened with extinction is correlated with the persistence or decline and distribution of some plant species.

PORNA has priority over some species like capercaillie (*Tetrao urogallus cantabricus*). Last year in Asturias the "Conservation Plan for the habitat of the *T. urogallus"* was approved (Order 36/2003, May 14th). The habitat of this bird has been closely related to the presence of bilberries (*V. myrtillus*) both in Somiedo and Redes Natural Parks. The diet of the capercaillie is mainly based on bilberries (buds, leaves and fruits) during spring and summer. Currently, there are about 1800 pair of capercaillies, in Spain, living only in the Pyrenean and in the Cantabrian areas. In the Cantabrian area there are now about 520 pairs and the population has been declining for the last ten years, with a decrease of around 40% during the last fifteen years (Purroy, 1999) in the Iberian Peninsula.

Bilberry seedling establishment is rarely observed in many locations, including parts of northern Europe, (Ritchie, 1956). Seedlings of *Vaccinium* sp. are also rare in North America (P. Stickney, pers. comm. 1990). Seedling establishment of dwarf bilberry appears variable, and the initial development of seedlings is very slow (Ritchie, 1956).

Seed dormancy is common among temperate zone bird-dispersed plants. However,

Proc. VIIIth IS on Vaccinium Culture
Eds.: L. Lopes da Fonseca et al.
Acta Hort. 715, ISHS 2006

germination of seeds collected early and late in the season might vary due to development of dormancy**.** The literature regarding pretreatments for highbush blueberry is not conclusive. However, cold requirements among various species appear to be species-specific. Although seeds of many species will germinate if sown immediately after they are extracted from fresh fruit, a dry cold treatment from 3 to 5 °C for about 90 days may increase germination or become necessary if seeds are allowed to dry (Ballington, 1998), and gibberellic acid treatment has been shown to promote germination.

The main objective of this work was to define the most important factors in obtaining *Vaccinium myrtillus* seed germination, as well as the factors that accelerate plant production in order to increase the establishment of seedlings in Somiedo and Redes Parks.

MATERIALS AND METHODS

Plant material. Berries from *V. myrtillus* were hand collected from two provenances located in the Cantabrian Asturian Mountains (Somiedo and Redes Parks) during the fruiting season (July and August), and were immediately refrigerated at 4ºC.

Seed extraction was based on a modification of the protocol described by Galletta and Ballington (1996), which consists of three main stages: maceration of the fruits, dilution of the mixture obtained and a decantation. In our trial, several decantations were made in order to obtain, as sediment, as many viable seeds as possible (previous assays showed that floating seeds had much-reduced viability). Number of fruits per kilogram and number of seeds per berry were recorded. Three replicates with 10, 20 50, 80 and 100 seeds were randomly selected and weighed in order to establish a regression between weight and seed number.

Germination tests were carried out in controlled environment chambers at 25 °C using cool-white fluorescent tubes (16 h, photosynthetic photon flux of 90 µmol m^{-2} s^{-1} at the germination surface). Seeds for different experiments were placed in clear-plastic boxes (600 x 650 x 60 mm) on cellulose paper (Fanoia 1516/400) moistened with water through an absorbent wick except as indicated, then covered with 80 mm diameter Petri dishes to maintain the relative humidity close to 100 %.

Experimental design. Three treatments were compared: seed origin, with two levels (Redes and Somiedo); temperature of the imbibition water, with three levels (4ºC, 25ºC and a control without imbibition) and gibberellin concentration, with four levels (0.1, 0.5, 1.0 mg l^{-1} and a control without GA_3). A factorial (2 x 3 x 4) randomized block design with three replications per treatment was established. Each experimental unit contained 10 mg of seeds, ranging from 45 to 50 seeds.

Seeds were sowed and gibberellins were dissolved in the irrigation water. Percentages of germination were calculated, and germination rates were obtained. For the purpose of this study, germination was defined as being complete when the radicle emerged from the seed. Germinated seeds were counted and removed every 24 h until germination ceased.

Bilberry production. After selection of the best germination treatment, two production procedures were used: **1.** In late summer, 40.000 bilberry seeds, previously water imbibed at 25ºC for 24 h, were put into seedbeds containing a mixture of peat:vermiculite 80:20 v v. Irrigation with water and GA_3 0.5 mg. l^{-1} was applied during four weeks. After germination, bilberries were grown in a greenhouse with controlled culture conditions and were moved outdoors in spring 2004. **2.** Early spring bilberry seeds (around 150.000 seeds), previously pelleted in Oxypil® (Germain's Technology Group), were automatically sowed into rigid containers using a Conic-system sower, PRO-110 model.

RESULTS AND DISCUSSION

Dwarf bilberry contains an average of 3350 to 2970 fruits per kilogram (Somiedo and Redes Parks, respectively). Seed production generally begins when the plants are three years old (Pakonen et al., 1988) and is subject to considerable annual variation. Our data showed higher numbers of seeds per fruit (41.2 and 50.6 in Somiedo and Redes Parks respectively) compared to previous reports, where fruit contained an average of 18 to 20 viable seeds per berry (Ritchie, 1956; Vander Kloet, 1983). Seeds weigh an average of 25 mg per 100 seeds (Vander Kloet, 1983). Asturian bilberry seeds are smaller. Model x= (y-0.2333)/0.23; y:weight of seed sample in mg, x: number of seeds in the sample, fitting with r^2=0.95.

Although debated, it appears that seeds of some *Vaccinium* species do not require any pre-treatment for satisfactory germination. Devlin and Karczmarczyk (1975) and Devlin and others (1976) demonstrated that cranberry seeds would germinate after 30 days of storage at room temperature if light requirements were fulfilled during germination. Aalders and Hall (1979) reported that seeds of lowbush blueberry will germinate readily if extracted from fresh fruit and sown immediately.

In general, it has been reported that larger seeds germinated in higher percentages, although optimal size was clone specific. With our provenances, discarding the floating seeds in each decantation stage maximizes seed viability. Aalders and others (1980) demonstrated that seed size may be an indication of seed viability in clones of lowbush blueberry. Seeds that passed through a screen with openings of 600 µm germinated poorly (1 to 14%), whereas seeds retained on that screen germinated at higher percentages (5 to 74%).

Germination percentages observed in some of our treatments have been among the highest published to date. Comparing provenances and treatments, differences between germination rates varied from 50% to 91% in the most efficient treatment. Other published results showed averages from 35 to 46 percent following many types of pretreatment. Germination of seeds exposed to low temperatures (0 °C) for 3 weeks averaged from 41 to 64 percent (Ritchie, 1956). Good germination has been reported after seeds were exposed to 14 hours of light at 28 °C followed by 10 hours of darkness at 13 °C (Vander Kloet, 1983). Fresh seed germinated well under a similar regime, or when exposed to alternating periods at 22 °C and 5 °C (Vander Kloet, 1983). Heat treatments were found to produce some germination, although this was irregular in agreement with the data obtained by Mallik and Gimingham (1985).

The germination function for the different treatments has a classical sigmoidal shape, as described by Black (2000), with the slope depending on the treatment (Figures 1a to 1f), (Models $f=a/(1+\exp(-(x-x_o)/b))$ with R values indicating fit of the model ranging from 0,96 to 0,99). Clear differences between the two provenences of seeds exist at the beginning of germination; those from Somiedo germinate before than those from Redes (figures 1a, 1c, 1e vs 1b, 1d, 1f), and also show a greater slope.

Analysing the behaviour of seeds from Somiedo, we can conclude that water imbibition before sowing acelerated germination. Imbibition at 25ºC was better than imbibition at 4ºC. Imbibition with 25ºC water almost doubled germination percentages compared to the control (Fig. 1e vs 1a) after 15-20 days.

Exogenous application of gibberelins had different effects, positive or negative, depending on concentration. For example with seeds from Somiedo provenance, at the end of the germination period, the least and most effective treatments were 1 mg GA_3 l^{-1} giving a germination percentage of 58.9% and 0.5 mg GA_3 l^{-1} giving 84.4%. 0.1 mg GA_3 l^{-1} had medium results. In general, the use of GA_3 did not increase total germination, though it reduced the necessary hours of light, and in some instances, it overcame the light requirement and stimulated early and uniform germination (Ballington 1998; Ballington and others 1976; Devlin and Karczmarczyk 1975; Giba and others 1993; Smagula and others 1980). Gibberellic acid treatment enhanced germination in light as well as dark germination, with 1 mg GA_3 l^{-1} sufficient to overcome dark inhibition (Dweikat and Lyrene, 1988). In 4 weeks, 4% germination of nontreated seeds was reported, whereas 50% germination of seeds treated with 0.9 mg GA_3 l^{-1} was reported. In our case, concentrations higher than 0.5 mg l^{-1}decreased the beneficial effects of GA_3. On the contrary, Ballington et al. (1976) found that GA treatments did not influence the final seed germination percentage of the rabbiteye blueberry 'Tifblue'. Devlin and

Karczmarczyk (1977) found that cranberry seeds failed to germinate without light. However, seeds treated with 500 ppm GA showed 69% germination after 20 days in the dark following treatment. They also reported that, under low light conditions GA_3 stimulated early germination.

Evidence for field seed banking in dwarf bilberry appears contradictory. Some researchers have observed very few seeds in the soil despite high coverage of plants at the site and questioned whether seed banking is an important regenerative strategy in this species (Thompson and Grime, 1979). Martin (1979) stated that most Vacciniums are characterized by seeds of relatively short viability which are readily damaged by heat. However, other workers emphasize the importance of seed banking in dwarf bilberry (Eriksson, O. 1989; Granstrom, 1982; Vander Kloet, 1988). Moreover, it has been well established that seeds of various species of *Vaccinium* are photoblastic and require several daily hours of light for germination (Devlin and Karczmarczyk 1975, 1977; Giba and others 1993, 1995; Smagula and others 1980). Our results showed that, using treatments with GA_3, nursery seedbeds can maintain germination levels similar to the rate defined in the laboratory under light conditions (data not showed). Nevertheless, industrial seedling production in greenhouses has high hand-labour costs, and economic studies should be done to evaluate the convenience of using the automatic Conic-system sower PRO 110-model.

ACKNOWLEDGEMENTS

Thanks to Ministerio de Medio Ambiente for finnancial assistance.

Literature Cited

Aalders, L.E., Hall, I.V. and Brydon, A.C. 1980. Seed production and germination in four lowbush blueberry clones. HortScience 15(5): 587-588.

Ballington, J.R., Galletta, G.J. and Pharr, D.M. 1976. Gibberellin effects on rabbiteye blueberry seed germination. HortScience 11(4): 410-411.

Black, M.J., Bradford, K.J. and Vázquez-Ramos, J. 2000. Seed Biology: Advances and Applications. CABi Publishing. 528 Pages. ISBN: 0851994040.

Dweikat, I.M. Lyrene, P.M. 1988. Adventitious shoot production from leaves of blueberry cultured in vitro. HortScience 23(3): 629.

Devlin, R.M. and Karczmarczyk, S.J. 1977. Influence of light and growth regulators on cranberry seed dormancy. Journal of Horticultural Science 52(2): 283-288.

Eriksson, O. 1989. Seedling dynamics and life histories in clonal plants. Oikos. 55: 231-238.

Galletta, G.J. and Ballington, J.R. 1996. Blueberries, cranberries, and lingonberries. In: Janick J, Moore JN, eds. Fruit breeding, Volume 2, Vine and small fruit crops. New York: John Wiley & Sons: 1B107.

Granstrom, A. 1982. Seed banks in five boreal forest stands originating between 1810 and 1963. Canadian Journal of Botany. 60:1815-1821.

LHBH [Liberty Hyde Bailey Hortorium]. 1976. Hortus third: a concise dictionary of plants cultivated in the United States and Canada. 3rd ed. New York: MacMillan. 1290 p.

Mallik, A.U. and Gimingham, C.H. 1985. Ecological effects of heather burning. II. Effects on seed germination and vegetative regeneration. Journal of Ecology. 73: 633-644.

Martin, A.E. 1979. Productivity and taxonomy of the *Vaccinium globulare*, V. membranaceum complex in western Montana. Missoula, MT: University of Montana. 136 p. Thesis.

Pakonen, T., Laine, K., Havas, P. and Saari, E. 1988. Effects of berry production and deblossoming on growth, carbohydrates and nitrogen compounds in Vaccinium myrtillus in north Finland. Acta Botanica Fennica. 136: 37-42.

Ritchie, J.C. 1955. A natural hybrid in Vaccinium I. The structure, performance, and chorology of the cross Vaccinium intermedium Ruthe. New Phytology. 54: 49-67.

Ritchie, J.C. 1956. Biological flora of the British Isles: *Vaccinium myrtillus* L. Journal of Ecology. 44(1): 290-298.
Smagula, J.M., Michaud, M. and Hepler, P.R. 1980. Light and gibberellic acid enhancement of lowbush blueberry seed germination. Journal of the American Society for Horticultural Science 105(6): 816-818.
Thompson, K. and Grime, J.P. 1979. Seasonal variation in the seed banks of herbaceous species in ten contrasting habitats. Journal of Ecology. 67: 893-921.
Vander Kloet, S.P. 1983. The taxonomy of Vaccinium and cyanococcus: a summation. Canadian Journal of Botany. 61 1: 256-266.
Vander Kloet, S.P. 1988. The genus Vaccinium in North America. Publication 1828. Ottawa: Research Branch, Agriculture Canada. 201 p.

Figures

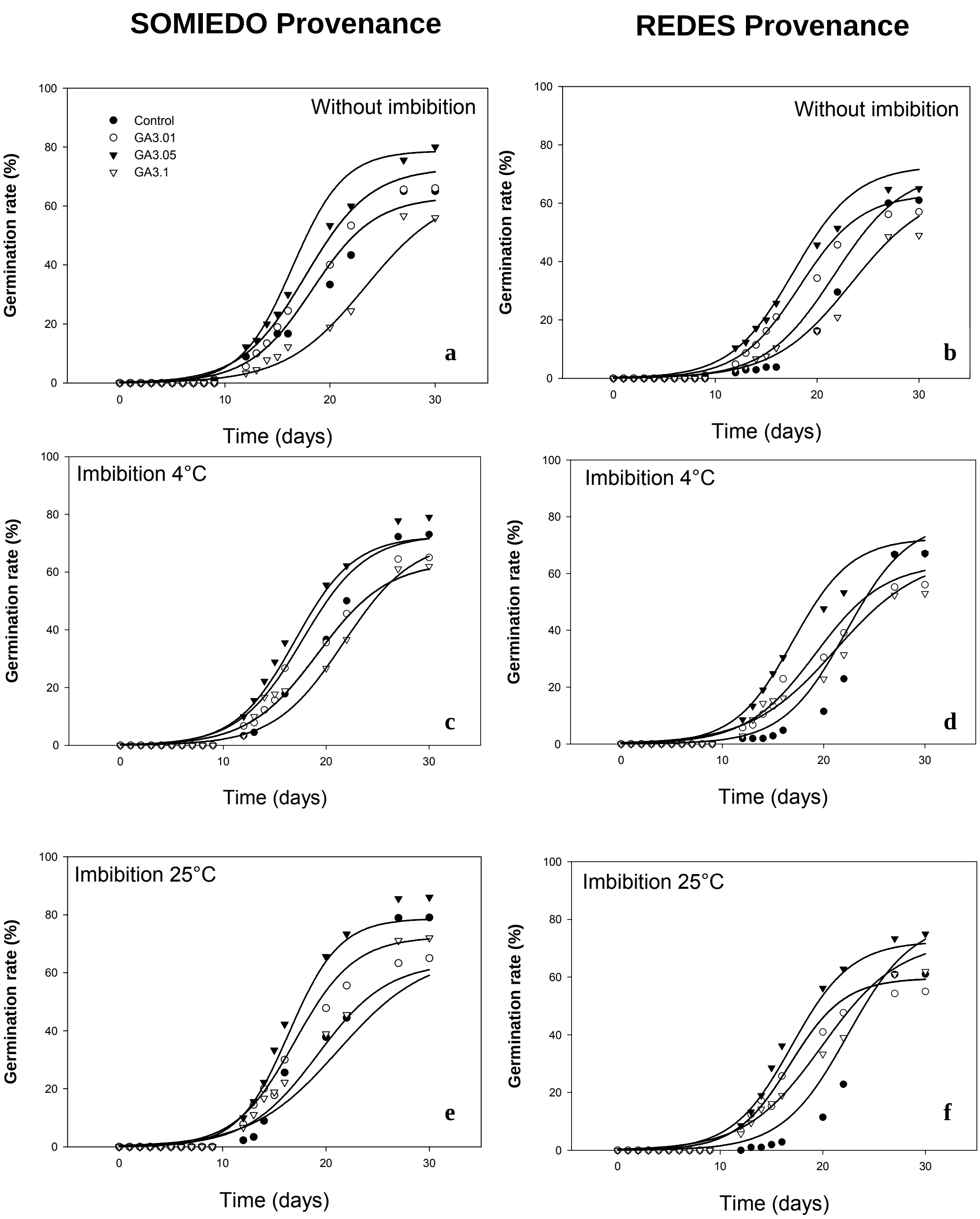

Fig. 1. Germination of *Vaccinium myrtillus* affected by seed origin (provenances Somiedo and Redes), temperature of the imbibition water, with three levels (4ºC, 25ºC and a control without imbibition) and gibberelins concentrations, with four levels (0.1, 0.5, 1.0 mg l^{-1} and a control without GA_3.