Germination of cascara seed

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Use of potassium gibberellate, but not of thiourea, represents a practical alternative to artificial stratification when spring planting is desirable.

Cascara buckthorn *(Rhamnus purshiana DC.),* a deciduous shrub or small tree, has a natural range extending from British Columbia east to western Montana and south to central California (4). In addition to the well-known medicinal value of its bark, cascara provides protection for watersheds and food and cover for wildlife.

The limited information on germination and seedling production of cascara includes reports on embryo dormancy, a pregermination treatment of about 90 days of cold, moist stratification to break dormancy, fall or spring (after stratification) sowing in the nursery at 1-inch 12.54cm) depth and shading of seedbed areas, and a maximum germination by stratified seed of only 40 percent (4, 6). Clearly, much more information is needed if maximum seedling production is to be achieved in the nursery and in the field.

This study was designed to determine effects of different periods of cold, moist stratification, various concentrations of potassium gibberellate ($K\text{-}GA_3$) and thiourea solutions, and light on germination of dormant cascara seeds. Limited pot experiments were also conducted to evaluate seedling production after seed were sown in soil at two different depths.

Materials and Methods

The seeds.-Ripe fruit were gathered in mid-August 1972 from three wild, mature plants growing near Olympia, Washington. Fruit were macerated in water, and "full" seeds were cleaned of other material by repeated decantation. Seeds were then blotted to remove moisture, airdried for 2 to 3 days at room temperature to a moisture content of 7.8 percent, packed in a plastic bag, and stored at 5`C until used in tests.

Stratification. -Seeds, enclosed in nylon netting, were soaked in distilled water for 48 hours in the dark at room temperature. Seeds were then drained of excess water, placed in sealed plastic vials covered with heavy-duty aluminum foil to exclude light. and placed in a refrigerator at 2° to $5^{\circ}C$. Stratification periods (treatments) were 0, 28, 56, 81, 112, and 110 days: and times at which stratification was begun were scheduled so that all treatments were completed the same day.

Chemical treatments. - $K\text{-}GA_3$ and thiourea were tested in two separate experiments. Solutions used were: 0, 100, 250, and 500 p/m of K-GA3. and 0, 1, 2, and 3 percent of thiourea. Test solutions were freshly prepared with distilled water, and seeds were soaked in the appropriate solution for 48 hours in the dark and drained as with water in the stratification experiment. Treated seeds were not rinsed with water or allowed to dry before germination.

In all experiments, pregermination treatments were carried out in the dark to accentuate effects of light during germination.

Germination tests. - Following treatment (stratification, $K\text{-}GA_3$, or thiourea I, seeds were placed in lots of 50 in 9-cm plastic Petri dishes on one circle of Whatman No. 3 filter paper moistened with 7 ml of dis. tilled water; more water was added during tests as needed. In each of the three experiments, seeds of each treatment (six stratification periods and four $K\text{-}GA_3$ and four thiourea test solutions) were germinated simultaneously both in light and in darkness. Seeds tested for germination in the dark were placed in the dishes under a darkroom light with a green safelight filter and then wrapped in aluminum foil. There were six 50-seed replicates for each treatment, equally divided between germination tests in light and in darkness.

In each experiment, dishes were randomly transferred to an incubator programmed for alternating diurnal temperatures of 30±1°C for 10 hours and 20±1°C for 14 hours; preliminary experiments showed such regime superior to constantly maintained temperature. Cool-white fluorescent light of about 900 lux was available to seeds germinating in light during the higher temperature. Germinants were counted at weekly intervals for 4 weeks and, for dark germination, counts were made under the green safelight. Protrusion of radicle through the seedcoat was used as criterion for germination.

Germination percents were calculated. Final, cumulative germination data of each experiment were subjected to analysis of variance after arc-sine transformation, and means were compared according to Tukey's test (7).

Seedling production tests.- Chemically treated (500 p/m K-GA$_3$ and 3-percent thiourea soaks as indicated above) and untreated seeds were used in three four-pot tests. In each test, seed were sown in two 1-gallon (3.8-liter) pots at a depth of $^1/_8$ inch (0.3 cm) and in the other two at 1 inch (2.54 cm). Pots contained a soil-vermiculite-peat moss mixture and held 100 seeds each. After they were watered, pots of treated seeds were placed immediately in the incubator used above. Remaining pots were transferred to the incubator after 16 weeks of natural stratification outdoors. In each test, emerged seedlings were counted at weekly intervals for 6 weeks after emergence began.

Results

Germination

Effects of stratification duration on germination are shown in table 1. Without stratification, germination was less than 20 percent in light and only 3 percent in the dark. Stratification was effective in promoting germination. In the dark, however, germination remained very low 15 percent or less) for up to 84 days of stratification, then significantly increased to 55 percent after 112 days of chilling, and reached a maximum of 88 percent after 110 days. In contrast, seeds exposed to light after stratification always germinated faster and more completely. Thus, germination in light amounted to 23 percent after 28 days of stratification, increased steadily with length of stratification period, and reached 92 percent after seeds had been chilled for 112 days.

Table 2 summarizes effects of K-GA3, and thiourea soaks on germination of unstratifled, dormant cascara seeds. Data indicate that effectiveness of these compounds as substitutes for stratification varied with the chemical u-ed and its concentration and, more importantly, with light conditions during germination. Thus, for K-GA:; solutions of equal concentration, germination in light was faster and higher than germination in darkness. Maximum germination of 87 percent was obtained in light after treatment with 500 p/m K-GA :;. Similarly, thiourea promoted faster and more complete germination in light than in darkness and was also most effective at the highest concentration of 3 percent, producing maximum germination of 88 percent.

Highest total germination for the most effective stratification 1"92 percent), K-GA3 (87 percent), and thiourea 188 percent) treatments was essentially the same. Speed of germination, however, varied considerably among treatments. Thus, at midpoint of the germination tests (1 1 days), percents of total germination achieved were 96, 57, and 5 for stratification. K-GA$_3$, and thiourea, respectively.

Germinants from K-GA:, treatments appeared as healthy and normal as those resulting from stratified seeds. The thiourea germinants, on the other hand, were relatively weak, and many radicles had brownish tips. Similar phytotoxic effects after treatment by thiourea have been reported with other species (8).

Seedling Production

Average number of seedlings produced varied among treatments and by depth of sowing. Thus, at $^1/8$ inch (0.3 cm), seedling production from thiourea treatment 154 percent) was significantly (P = 0.05) less than from K-GA:; (87 percent) or stratification (80 percent) treatments. Similar differences among treatments were also evident at 1 inch (2.54 cm) where percents of seedlings produced were: thiourea = 33, K-GA., = 60, and stratification = 40. Fewer seedlings, therefore, were consistently produced when seeds were sown deeper in the soil. In addition, the greater sowing depth always delayed seedling emergence by an average of 2 days and produced spindly plants. There were

Table 1.—Cumulative germination percents of cascara seed in light and in darkness after stratification in the dark [1]

Stratification period (days)	Days in germination test: 7	14	21	28
	In the light			
0	0	0	5	19 d
28	0	0	14	23 d
56	0	6	16	29 d
84	4	22	52	72 b
112	80	88	91	92 a
140	88	90	90	90 a
	In the dark			
0	0	0	0	3 e
28	0	0	0	2 e
56	0	0	0	2 e
84	0	0	4	5 e
112	48	52	53	55 c
140	72	84	86	88 a

[1] Percents are averages of three 50-seed replicates. In the last column, means followed by the same letter do not differ significantly (P = 0.05) by Tukey's test.

no differences in appearance among seedlings resulting from the various seed treatments when seeds were planted at the [1]/8-inch (0.3 cm) depth.

Discussion and Conclusions

As with the seeds of many woody plants, freshly harvested cascara seeds exhibited very low germination and were considered dormant at fruit maturity and dispersal. Dormancy was completely broken by stratification, and light promoted higher germination until seeds had been stratified for 140 days when influence of light was completely eliminated. This response is similar to that of other species with dormant seeds and light-requiring germination where dormancy is typically broken by a period of stratification during which germination becomes decreasingly sensitive to light (2, 9). In some of these species, the light requirement can he fulfilled during stratification (5).

Stratification-germination data show a requirement of about .1 months for full stratification followed by total germination of approximately 90 percent. These results are not consistent with available information indicating maximum germination of 414 percent after a recommended 3-month stratification period (4, 6). This discrepancy is probably due to differences in viability and dormancy of seeds tested and methods used for stratification and germination.

Dormant cascara seeds responded favorably to chemical treatments. Thus, application of 500 p/m K-GA:, or 3 percent thiourea broke seed dormancy and replaced the chilling requirement when light was available during germination. Although similar germination responses to these chemicals have been reported with other species (1), it is conceivable that concentrations or soak times other than those tested would effect complete germination also in the dark (3).

Results from the pot experiments show that the 1-inch (2.54)cm sowing depth now recommended (4) is much too deep for maximum production of seedlings. The results. also, support germination data. Thus. K-GA3, proved to be as effective as stratification, indicating that amount of light necessary to trigger germination is low and, hence, not critical when K-GA_3-treated seeds are planted in soil. On the other hand, the much lower seedling production with thiourea reflects phytotoxic effects on the radicles observed during germination tests.

Treatment with K-GA3, but not with thiourea, represents a practical alternative to artificial stratification for breaking cascara's dormancy when spring sowing in the nursery or in the field is desirable. Moreover, the treatment should prove useful for quick evaluation of germination potential of different seed lots.

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Table 2.—Cumulative germination percents of cascara seed in light and in darkness after treatments with potassium gibberellate (K-GA_3) and thiourea [1]

Chemical treatment	Days in germination test: 7	14	21	28
		EXPERIMENT I		
K-GA_3 (p/m)		In the light		
0	0	0	3	14 e
100	2	12	24	36 c
250	2	16	37	56 b
500	4	50	75	87 a
		In the dark		
0	0	0	2	5 f
100	2	2	7	9 ef
250	0	6	16	25 d
500	2	10	21	31 cd
		EXPERIMENT II		
Thiourea (percent)		In the light		
0	0	0	3	12 cd
1	0	0	4	18 c
2	0	0	24	36 b
3	0	4	60	88 a
		In the dark		
0	0	0	0	1 e
1	0	0	0	2 e
2	0	0	0	8 d
3	0	4	8	8 d

[1] Percents are averages of three 50-seed replicates. In the last column, within each of the two experiments, means followed by the same letter(s) do not differ significantly ($P = 0.05$) by Tukey's test.

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Results and Discussion

Class 1 seeds germinated 66 percent compared to less than 16 percent for the other classes (table 1). The poor germination of Class 2 seeds was not expected because the seeds appear to be essentially nor*mal,* both on *X-rays* and when the seeds are cut. The low germination for seed classes other than Class I suggests that when seed physiology studies are contemplated, only seeds in Class 1 should be used. The data also suggest that cutting tests to determine percent filled seed in low-quality seed lots such as these may be in error *by* as much as 100 percent or more. Also included in table 1 are the percentages of seed in each seed class in the original sample, based on X-ray analysis.

Table 1.—Percent germination anddistribution for white ash seed quality classes

Class	Percent Germination	Percent of Total Seed in Sample [1]
1	66.0	10.8 (range = 8–12)
2	15.8	17.4 (17–18)
3	11.6	32.2 (30–36)
4	2.6	12.3 (10–16)
5	0.0	5.1 (4–6)
6	7.5	6.7 (6–7)
7	0.0	15.5 (14–16)
		100.0

[1] Average of three replicate 250-seed samples.

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Questions that remain unanswered are:

1. What is the optimum duration of the stratification treatment?
2. Are there other treatments that will produce even better germinatinn?

As a means of partially answering question number 1, seed will be stratified starting in November 1975 for July 1976 sowing.

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