APPLICATION OF GERMINATION INHIBITORS IN ORGANIC SOLVENTS TO CONIFER SEEDS

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ABSTRACT

Black spruce (*Picea mariana* [Mill.] B.S.P.) seeds were immersed in dichloromethane and acetone for 20 hours at 20°C with no ill effects on germination, but shorter immersion periods and lower temperatures were necessary to avoid affecting jack pine (*Pinus banksiana* Lamb.) germination. Dichloromethane did not appear to penetrate to the megagametophyte of black spruce seed. Abscisic acid, applied in these solvents, inhibited the germination of black spruce seeds effectively without reducing viability. This inhibition was partially reversed by cold stratification and by overwintering, but not by leaching. The germination of jack pine seeds was also inhibited by abscisic acid applied in acetone or in water, but to a lesser degree than with black spruce seeds. Stirring the solution during jack pine seed immersion increased the response to abscisic acid. Coumarin had no effect on the germination of either species.

RÉSUMÉ

Des graines d'épinette noire (*Picea mariana* [Mill.] B.S.P.) ont été immergées dans du dichlorométhane et de l'acétone durant 20 heures à 20°C, sans effet nuisible sur leur germination; il a cependant fallu des périodes d'immersion plus courtes et des températures plus basses pour qu'il en soit de même pour les graines de pin gris (*Pinus banksiana* Lamb.). Il ne semble pas que le macrogamétophyte de la graine de l'épinette noire ait absorbé de dichlorométhane. L'acide abscissique dilué dans ces solvants a, par contre, empêché la germination des graines de l'épinette noire sans toutefois en diminuer la viabilité. L'inhibition a été partiellement levée par stratification froide et hibernation, mais non par lixiviation. L'acide abscissique dans l'acétone ou dans l'eau a également eu un effet inhibiteur sur la germination des graines de pin gris, et, à un degré moindre, sur celle des graines d'épinette noire. L'agitation de la solution au moment de l'immersion des graines de pin gris a accru la réaction à l'acide abscissique. La coumarine n'a eu aucun effet sur la germination des deux essences.

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Note: The exclusion of certain manufactured products does not necessarily imply disapproval nor does the mention of other products imply endorsement by the Canadian Forestry Service.

INTRODUCTION

The purpose of this study was to determine whether the germination inhibitors abscisic acid and coumarin could be applied to black spruce *(Picea mariana* [Mill.] B.S.P.) and jack pine *(Pinus banksiana* Lamb.) seed with organic solvents to delay germination. Such a result would have field applicability if the germination of seeds sown in mid- to late summer could be delayed until the following spring. Sowing untreated seed in mid- to late summer presents the risk of immature plants being affected by fall frosts. Development of a successful treatment would allow the use of labor and seeding equipment over a much greater part of the year than is now possible.

The technique of introducing chemicals into seeds by means of organic solvents has received considerable attention, especially in species of agricultural importance. Many growth regulators have been effectively applied to seeds by means of this technique (Khan et al. 1973, Tao et al. 1974, Palevitch and Thomas 1974, Braun and Khan 1976, Braun et al. 1976, Rao et al. 1976, Heydecker and Joshua 1977, Phaneendranath and Funk 1978, Hegarty and Ross 1979, Nelson and Sharples 1980). There have been some differences in reports of how deeply solvents penetrate the seed (Meyer and Mayer 1971, 1973, Anderson 1973, Triplett and Haber 1973, Tao and Khan 1974, Brewer and Wilson 1975, Halloin 1977). It is possible that there are differences in solvent penetration among species, or that even superficial or shallow deposition of chemicals is sufficient to produce effects.

Dichloromethane and acetone are the organic solvents that have been used successfully. These generally have little effect on seeds, although germination may increase or decrease, depending on species (Brewer and Wilson 1975, Nelson and Sharples 1980). Reductions in O_2 uptake (Meyer and Mayer 1971) and CO_2 evolution (Brewer and Wilson 1975) have been reported. Other solvents have been tested, but these affected germination adversely (Meyer and Mayer 1971). If appreciable amounts of water are present in the solvent, germination may also be reduced (Triplett and Haber 1973).

In the previously cited studies seeds were usually immersed in acetone or dichloromethane for periods from 30 min to 24 hr. Following immersion, solvents were removed by evaporation or by vacuum desiccation.

Application of abscisic acid in aqueous solution to seeds or embryos of many species has inhibited germination (Sondheimer and Galson 1966, Khan 1967, Walbot et al. 1975, Poggi-Pellegrin and Bulard 1976, McDonald and Khan 1977, Schopfer et al. 1979, Aldasoro et al. 1981, Liptay and Schopfer 1983, Zagorski and Lewak 1983). Effective concentrations range from about 10^{-5} M to 10^{-3} M (saturation). Abscisic acid has also been effectively applied with acetone as a solvent (Khan et al. 1973, McDonald and Khan 1977, Phaneendranath and Funk 1978). Khan et al. (1973) found that the abscisic acid applied to seeds could be removed by washing with water or acetone.

Coumarin applied to seeds in solution with water, acetone or dichloromethane also has inhibited germination (Khan 1967, Berrie et al. 1968, Meyer and Mayer 1971, Anderson 1973, Tao and Khan 1974). The inhibitory effect of coumarin can be largely reversed by washing or leaching with water or dichloromethane (Meyer and Mayer 1971, Anderson 1973).

METHODS AND RESULTS

Materials and General Methods

High-quality seedlots of black spruce (collected in 1967 from Ontario seed zone 3200) and jack pine (collected in 1979 from Ontario seed zone 3400) were used.

Racemic abscisic acid was obtained from Calbiochem-Behring Corp., San Diego. Coumarin was obtained from Nutritional Biochemicals, Montreal. Dichloromethane and acetone of reagent grade were used.

Seeds were vacuum dried after immersion in organic solvent by pouring off the solvent and drying the seeds under vacuum in a desiccator for 30 min.

Seeds were germinated in covered plastic petri dishes on blotter paper over saturated Kimpack at 20°C and under low-intensity incandescent light. A seed was considered germinated when the radicle exceeded 2 mm in length. Germination was recorded daily for the first 28 days, and at less frequent intervals thereafter.

For each treatment, four replicates of 50 or 100 seeds each were used.

In general, results were expressed in germination value, peak day (Czabator 1962) and germination capacity (Bonner 1984), on the basis of a 28-day germination period. With some treatments, when it was clear that germination was not completed by day 28, germination results were expressed as the percentage germination that had occurred in a given time period.

Specific methods for individual experiments are outlined along with the results.

Effects of treatment on these variables were examined by means of one-way or two-way analysis of variance with completely randomized designs. Percentages were transformed to arcsin (proportion)$^{.5}$ for statistical analyses. Means were compared by Duncan's new multiple range test (Steel and Torrie 1960). All statistical tests of significance were made at the 5% level.

Effect of Organic Solvents on Black Spruce Seed Germination

To test for the effects of immersion on germination, black spruce seeds were immersed in acetone and in dichloromethane at 10°C and 20°C for 20 hr. No significant differences in germination value, peak day, or germination were observed among untreated seed and seed immersed in acetone or dichloromethane at either 10°C or 20°C for 20 hr (Table 1). Seedlings grown from seeds immersed in these solvents were normal in appearance and development.

Table 1. Effect of organic solvents on black spruce seed germination.

Treatment		Germination value	Peak day	Germination capacity (%)
Control		50.07	6.75	99.50
Acetone	10°C	48.03	6.25	98.50
Acetone	20°C	51.92	6.00	98.25
Dichloromethane	10°C	50.42	6.25	99.50
Dichloromethane	20°C	51.64	6.25	98.75

To investigate the depth of solvent penetration in black spruce seeds, shallow incisions, exposing the megagametophyte, were made with a scalpel in the seedcoats in two lots of 30 black spruce seeds. One lot was immersed in dichloromethane at 20°C for 20 hr and the other was not. Both lots were vacuum dried before being germinated. The germination capacity of immersed seeds was 0.0%, and of unimmersed seeds, 43.3%.

Effect of Germination Inhibitors Dissolved in Organic Solvents on Black Spruce Seed Germination

To test for the effects of inhibitors on black spruce seed germination, coumarin and abscisic acid were dissolved in dichloromethane and acetone at concentrations of 0, 1, 10, 100, 250, and 500 ppm (w/w). Seeds were immersed in these solutions for 20 hr at 20°C and then vacuum dried and germinated.

Over the range of concentrations of coumarin used, no effects were observed on germination value, peak day or germination capacity (Table 2). Solvent type had no effect on peak day or germination capacity, but did have a significant effect on germination value. Dichloromethane immersion resulted in higher germination values than did immersion in acetone.

Both concentration of abscisic acid and solvent type had significant effects on germination value, peak day and week 4 germination in the abscisic acid trial (Table 3). The interaction of these factors was significant as well. Germination value and week 4 germination decreased, and peak day increased, with increasing abscisic acid concentration. At high concentrations of abscisic acid, germination values and week 4 germination were lower, and peak day was higher, with dichloromethane than with acetone.

Figure 1 displays the long-term course of germination of seeds treated with 100, 250, and 500 ppm abscisic acid. Germination of all treatments at week 37 was 97% or greater.

Table 2. Effect of coumarin dissolved in acetone and DCM[a] on black spruce seed germination.

Treatment (solvent - coumarin concentration)	Germination value	Peak day	Germination capacity (%)
DCM - 0 ppm	53.10	6.00	97.50
Acetone - 0 ppm	48.79	6.25	98.50
DCM - 1 ppm	53.63	6.00	99.00
Acetone - 1 ppm	53.30	6.00	99.00
DCM - 10 ppm	51.00	6.00	98.50
Acetone - 10 ppm	51.62	6.00	98.00
DCM - 100 ppm	55.09	6.00	99.50
Acetone - 100 ppm	53.27	5.75	99.00
DCM - 250 ppm	54.20	6.00	99.50
Acetone - 250 ppm	52.40	5.75	97.50
DCM - 500 ppm	55.65	6.00	100.00
Acetone - 500 ppm	52.16	6.00	99.00

[a] Dichloromethane

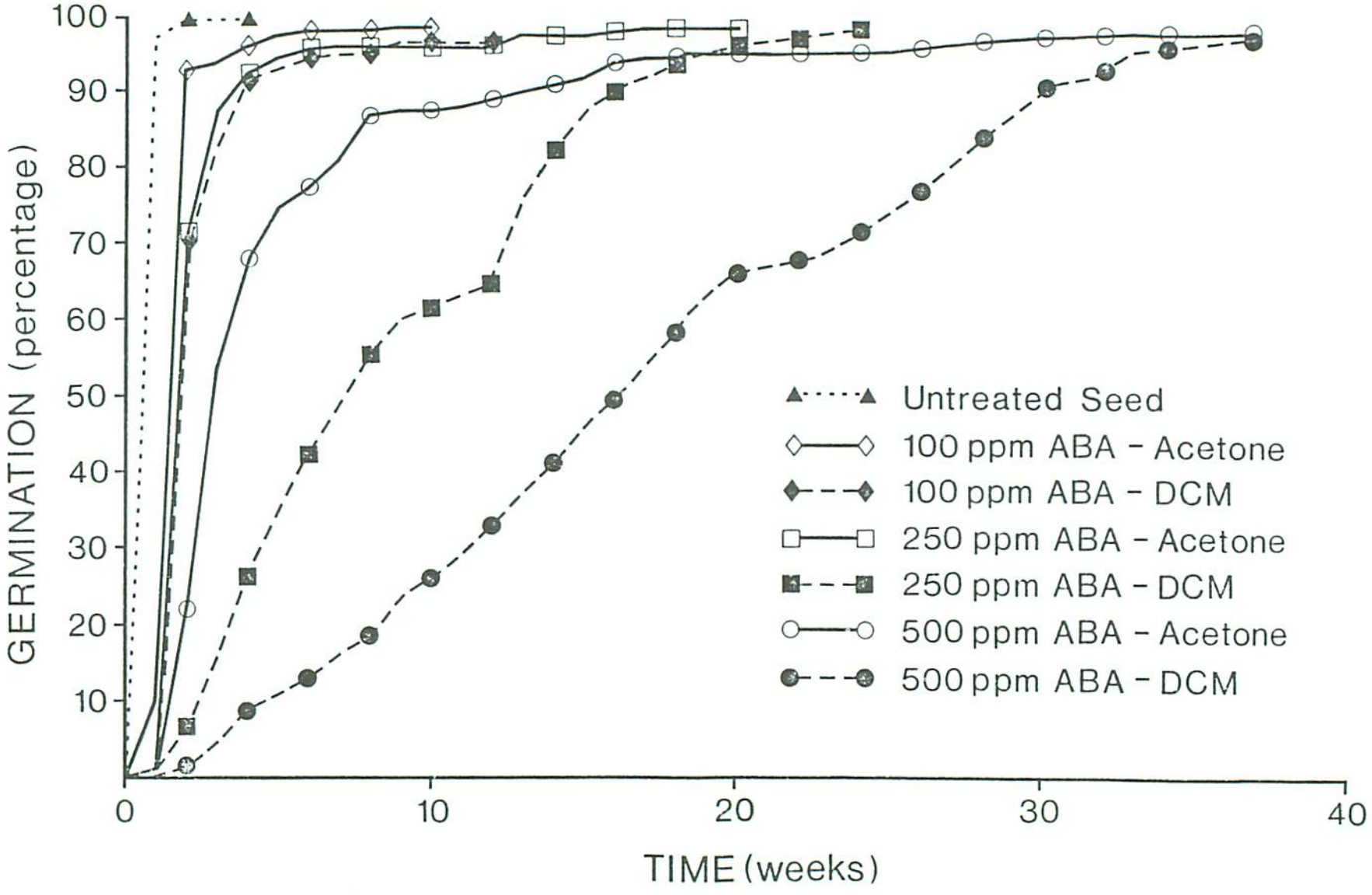

Figure 1. Germination of black spruce seed treated with abscisic acid.

Table 3. Effect of ABA[a] dissolved in organic solvents on black spruce seed germination.

Treatment (solvent - ABA concentration)	Germination value	Peak day	Week 4 germination (%)
DCM[b] - 0 ppm	54.83	6.00	98.50
Acetone - 0 ppm	49.59	6.00	98.50
DCM - 1 ppm	53.64	6.00	99.00
Acetone - 1 ppm	49.17	6.00	95.50
DCM - 10 ppm	52.21	6.25	99.00
Acetone - 10 ppm	47.85	6.00	98.00
DCM - 100 ppm	16.32	14.25	92.00
Acetone - 100 ppm	31.31	8.75	96.00
DCM - 250 ppm	0.95	25.00	26.00
Acetone - 250 ppm	17.05	13.50	92.50
DCM - 500 ppm	0.13	24.75	9.00
Acetone - 500 ppm	6.75	21.25	68.00

[a] Abscisic acid
[b] Dichloromethane

Infection of seeds by moulds and fungi often occurs during long germination periods, but in this trial few seeds were so afflicted, even during this exceptionally long germination period.

Effect of Leaching on Germination of Black Spruce Seed Treated with Abscisic Acid

An experiment was carried out to determine whether leaching would remove the effect of abscisic acid, as has been observed in other studies.

Black spruce seeds were immersed in abscisic acid-dichloromethane solutions of 500 and 1000 ppm for 20 hr at 20°C and then vacuum dried. The treated seeds were then placed in 20 ml of water or 20 ml of dichloromethane and immersed again for 20 hr, with each liquid poured off and replaced at 2, 4, 7, and 10 hr. The seeds were again vacuum dried.

Abscisic acid concentration had a significant effect on week 4 and week 10 germination, but the type of liquid used had a significant effect only on 10-week germination. The interaction was not significant in either case. Germination was lower with 1000 ppm abscisic acid than with 500 ppm, and at week 10 was also lower with water leaching than with dichloromethane leaching (Table 4).

Effect of Stratification on Germination of Black Spruce Seed Treated with Abscisic Acid

Stratification is routinely employed to overcome internal dormancy in seeds. To examine the effect of stratification on black spruce seeds treated with abscisic acid, which appear to be in a state similar to dormancy, seeds were immersed in abscisic acid-dichloromethane solutions of 500 and 1000 ppm for 20 hr at 20°C, vacuum dried, placed between two layers of moist Kimpack at 2°C for 3 or 6 weeks, and then germinated.

Table 4. Effect of leaching on germination of seed treated with ABA[a].

Treatment	Week 4 germination (%)	Week 10 germination (%)
500 ppm ABA, leached in H_2O	6.5	29.5
1000 ppm ABA, leached in H_2O	3.5	19.0
500 ppm ABA, leached in DCM[b]	11.5	51.0
1000 ppm ABA, leached in DCM	3.0	29.5

[a] Abscisic acid
[b] Dichloromethane

Period of stratification had no effect on week 4 germination, but concentration of abscisic acid did. Week 4 germination was lower for 1000 ppm than for 500 ppm (Table 5).

Effect of Outdoor Overwintering on Germination of Black Spruce Seed Treated with Abscisic Acid

In practical application, seed treated with abscisic acid could be sown in late summer to germinate the following spring after overwintering on the ground. The effects of overwintering on black spruce seeds treated with abscisic acid were tested by overwintering them outdoors in 1982 and 1983. Seeds were placed in abscisic acid-dichloromethane solutions of 500 and 1000 ppm for 20 hr at 20°C in October 1982 and October 1983. In each case the seeds were vacuum dried.

In 1982, the seeds were placed in a fabric screen pouch on a bed of peat-vermiculite. Flats containing the pouches and peat-vermiculite were covered with one layer of cheesecloth and were placed outdoors at Sault Ste. Marie, Ontario on 22 October 1982. The first snow fell in mid-November, but snow cover did not become continuous until early December. The flats became completely snow free in early March. In late March seeds were removed from the flats for germination testing.

In 1983, the seeds were put in fabric screen pouches placed on the surface of mineral soil contained in plastic trays. These trays were placed outside at Sault Ste. Marie in late October, 1983. Snow cover became continuous at the end of November. The trays became completely snow free at the end of March, and the seeds

Table 5. Effect of stratification on germination of black spruce seed treated with abscisic acid.

Treatment	Week 4 germination (%)
500 ppm, stratified 3 weeks	38.5
1000 ppm, stratified 3 weeks	17.0
500 ppm, stratified 6 weeks	41.0
1000 ppm, stratified 6 weeks	15.5

were removed on 9 April 1984 for germination testing.

Week 4 germination of black spruce seeds still decreased with abscisic acid concentration following overwintering (Table 6). Germination of untreated seeds was also reduced by overwintering in 1983-1984.

Effect of Germination Inhibitors Dissolved in Organic Solvents on Jack Pine Seed Germination

To test for the effects of inhibitors on jack pine seed germination, seeds were immersed in solutions of abscisic acid and coumarin in acetone and dichloromethane for 2 hr at 10°C,

Table 6. Effect of overwintering on week 4 germination of black spruce seed treated with ABA[a].

Treatment	Overwintered 1982-1983 (%)	Overwintered 1983-1984 (%)
untreated	99.00	73.00
100 ppm ABA	97.50	95.50
250 ppm ABA	89.75	73.50
500 ppm ABA	45.50	52.00
1000 ppm ABA	37.75	23.00

[a] Abscisic acid

Effect of Organic Solvents on Jack Seed Pine Seed Germination

To determine suitable immersion periods and temperatures for jack pine seeds in acetone and dichloromethane, a series of immersion trials was carried out. After immersion, seeds were vacuum dried and germinated.

Results of these trials are summarized in Table 7. In general, adverse effects on germination value, peak day and germination capacity increased with immersion period and temperature. Dichloromethane was more injurious to germination than was acetone. Short immersion periods in acetone increased germination values.

Attempts to reduce the effects of solvents on jack pine seeds by reducing seed moisture content before immersion, and by placing a desiccant (Na_2SO_4) in the solution to remove H_2O, were unsuccessful.

a combination of immersion temperature and period that was not detrimental to jack pine germination. Concentrations were 0, 1, 10, 100, 250, and 500 ppm (w/w) for each solvent-inhibitor combination. The seeds were vacuum dried following immersion, and then germinated.

Coumarin concentration had no significant effect on germination value, peak day, or germination capacity. The kind of solvent had no effect on germination value or peak day, but seeds immersed in acetone had significantly greater germination capacity than seeds immersed in dichloromethane (96.00% versus 94.00%). Interactions were not significant.

Similar analysis of the germination value, peak day, and germination capacity of seeds treated with abscisic acid showed that abscisic acid concentration significantly affected peak day, but not germination value or

Table 7. Effect of organic solvents on jack pine seed germination.

Treatment[a]	Germination value[b]	Peak day	Germination capacity (%)
ACE 2 C 2 hr	52.70a	6.00a	95.50abc
ACE 2 C 1 hr	52.04a	6.00a	95.50abcd
ACE 2 C 4 hr	51.69a	6.00a	97.00abc
ACE 10 C 1 hr	51.04a	6.00a	98.00abc
ACE 2 C 10 hr	49.23ab	6.00a	94.00bcd
DCM 10 C 1 hr	48.64ab	6.00a	95.50abc
ACE 10 C 2 hr	45.66bc	6.50ab	98.00a
ACE -4 C 10 hr	43.36cd	6.00a	94.00bcd
DCM 10 C 2 hr	42.84cd	6.25ab	95.00abcd
ACE 2 C 6 hr	40.35de	7.00ab	97.50ab
ACE 10 C 10 hr	40.30de	6.50ab	94.50abcd
ACE 10 C 5 hr	38.55de	6.50ab	93.00cde
ACE -4 C 20 hr	36.41ef	6.50ab	89.50de
DCM 10 C 5 hr	32.46f	6.75ab	87.50ef
ACE 2 C 20 hr	27.90g	7.25abc	81.50fg
DCM -4 C 10 hr	23.92gh	7.25abc	78.00g
ACE 2 C 20 hr	21.63h	8.25bcde	75.50g
ACE 10 C 20 hr	8.48i	9.25cde	51.25h
DCM 10 C 10 hr	6.51ij	8.00abcd	43.50h
DCM 2 C 20 hr	3.43jk	9.50de	32.00i
DCM -4 C 20 hr	2.00jk	8.00abcd	25.50ij
DCM 10 C 20 hr	0.97k	10.50e	19.00j
ACE 20 C 20 hr	0.05k	10.50e	4.50k
DCM 20 C 20 hr	0.04k	11.25e	3.50k
untreated	44.75	6.00	95.75

[a] ACE = Acetone; DCM = Dichloromethane

[b] Values in vertical columns followed by the same letter are not significantly different.

germination capacity. Despite this significant effect, no clear relationship between peak day and abscisic acid concentration was evident (Table 8). The kind of solvent used had no effect on peak day or germination capacity, but seeds immersed in acetone had significantly greater germination value than seeds immersed in dichloromethane (41.89 versus 37.79). Interactions were not significant.

Because of the poorly defined or nonexistent effects of inhibitors with a 2-hr immersion time, immersions of jack pine seed in coumarin/acetone and abscisic acid/acetone for 6 and 10 hr at 2°C were tested. Solution concentrations were 10, 100, 250, and 500 ppm. Seeds were vacuum dried after immersion and then germinated.

For seeds treated with coumarin, neither immersion time nor coumarin concentration had a significant effect on germination value, peak day or germination capacity.

Table 8. Effect of abscisic acid concentration on jack pine seed peak day (seeds immersed for 2 hr at 10°C).

Abscisic acid concentration	Solvent	
	Acetone (peak day)	Dichloromethane (peak day)
0 ppm	7.00	6.50
1	7.50	9.00
10	8.75	9.50
100	6.75	8.75
250	8.50	8.50
500	8.25	7.00

For seeds treated with abscisic acid, concentration had a significant effect on germination value and peak day, but not on germination capacity. Germination value decreased and peak day increased with increasing abscisic acid concentration (Table 9). Immersion time significantly affected germination value, but not peak day or germination capacity. Surprisingly, germination values were lower with 6-hr immersion than with 10-hr immersion (34.35 versus 37.64). No interactions were significant.

Effect of Stirring during Immersion on Germination of Jack Pine Seed Treated with Abscisic Acid

The most effective immersion treatment for jack pine, 10 hr in acetone at 2°C, was used, and a comparison was made between stirring and not stirring the seeds during immersion in abscisic acid solutions of 0 and 500 ppm. Stirring was done continuously with laboratory magnetic mixers.

Table 9. Effect of abscisic acid dissolved in acetone on jack pine seed germination (immersion temperature of 2°C).

Abscisic acid concentration	6-hr immersion		10-hr immersion	
	Germination value	Peak day	Germination value	Peak day
10 ppm	38.93	6.50	43.55	6.75
100 ppm	37.43	7.00	39.61	6.75
250 ppm	31.97	8.25	34.50	8.00
500 ppm	29.05	8.50	32.89	8.50

Both abscisic acid concentration and stirring contributed significantly to the variance of germination value, peak day, and week 4 germination, and the interaction of the two factors was significant for each variable as well. Especially in the stirred seed, germination was delayed by abscisic acid (Table 10).

The effects of abscisic acid dissolved in water were much more pronounced on black spruce than on jack pine germination.

DISCUSSION

Intact black spruce seeds were not adversely affected by immersion in acetone or dichloromethane for 20 hr at 20°C. These two solvents have potential for introducing many substances into black spruce seeds without the complication of simultaneous H_2O uptake. Dichloromethane, at least, was fatal when seed coats were incised, and this suggests that the seed coat is an effective barrier to solvent penetration. It is of interest to note that the incisions which just exposed the megagametophyte were of themselves injurious to seed germination.

Table 10. Effect of stirring abscisic acid dissolved in acetone on jack pine seed germination (10-hr immersion at 2°C).

Abscisic acid concentration	Not stirred			Stirred		
	Germination value	Peak day	Week 4 germination (%)	Germination value	Peak day	Week 4 germination (%)
0 ppm	38.09	7.25	94.00	39.80	7.00	92.00
500 ppm	32.39	8.25	95.00	9.50	13.00	68.00

Effects of Abscisic Acid Dissolved in Water on Jack Pine and Black Spruce Seed Germination

Jack pine and black spruce seeds were immersed in solutions of abscisic acid in water for 24 hr. Seeds were air dried for 30 min to 1 hr before germination. Concentrations of the abscisic acid solutions were 0 ppm and saturated (approximately 250 ppm). For jack pine the solutions were either stirred during immersion or not stirred.

Abscisic acid significantly decreased the germination value and week 4 germination and increased the peak day in both jack pine and black spruce (Tables 11 and 12). In jack pine, stirring significantly decreased germination value and increased peak day. These effects were most pronounced at the saturated level of abscisic acid (significant interaction). Stirring also significantly reduced germination capacity, with no interaction.

Although coumarin applied to seeds of other species is an effective germination inhibitor (Khan 1967, Berrie et al. 1968, Meyer and Mayer 1971, Anderson 1973, Tao and Khan 1974), coumarin applied to black spruce seeds in organic solvents had no effect on germination. Abscisic acid had a very strong inhibiting effect on black spruce seed germination.

Table 11. Effect of stirring abscisic acid dissolved in water on jack pine seed germination.

Abscisic acid level	Unstirred			Stirred		
	Germination value	Peak day	Week 4 germination (%)	Germination value	Peak day	Week 4 germination (%)
0 ppm	62.39	5.0	97.0	56.09	5.0	95.5
saturated	39.70	7.0	93.0	20.32	11.0	88.5

Table 12. Effect of abscisic acid dissolved in water on black spruce seed germination.

Abscisic acid level	Germination value	Peak day	Week 4 germination (%)
0 ppm	65.42	5.00	99.0
saturated	2.40	2.75	41.0

Leaching did not appear to reduce the effect of abscisic acid on black spruce seed germination. At week 4, germination of seeds treated with 500 ppm abscisic acid in dichloromethane was similar for leached and unleached treatments. In other studies (Meyer and Mayer 1971, Anderson 1973, Khan et al. 1973) washing or leaching reversed the effects of growth regulators. This raises an interesting question about the site of activity of exogenous abscisic acid in black spruce seed. Although the solvent does not penetrate the seed coat, the ineffectiveness of leaching indicates that abscisic acid is not superficially held to the seed coat. Possibly abscisic acid is carried into the seed coat by the solvent, and is then carried farther into the seed by another transport mechanism.

Stratification and outdoor overwintering partially reversed the inhibiting effect of abscisic acid on black spruce germination. Week 4 germination of stratified seed or overwintered seed was at least several times that of seed which had not been treated in such a manner.

The effect of abscisic acid on black spruce seed appears similar to embryo dormancy in that the seed is in a viable state, but does not germinate in favorable conditions. There are dissimilarities, however, because seed treated with abscisic acid eventually germinates without a dormancy-breaking treatment, and because dormancy-breaking treatments only partially reverse the effect of abscisic acid.

Jack pine seed germination was affected adversely by solvent immersion treatments that did not affect black spruce seeds. Similar adverse effects of dichloromethane on seeds of oats *(Avena sativa* L.) and pigweed *(Amaranthus retroflexus* L.) have been noted (Brewer and Wilson 1975). Short immersion periods at low temperature in acetone, however, were not detrimental to jack pine seed germination and even appeared to speed germination slightly.

Under solvent immersion conditions that were not injurious to jack pine seeds, coumarin had no effect on germination, and abscisic acid had a significant effect, although it was much less pronounced than the effect of abscisic acid on black spruce seeds.

Delay of jack pine seed germination was also achieved by immersing seeds in an abscisic acid-water solution, but again the response was much less pronounced than with black spruce treated in a similar manner.

The delay of germination observed in jack pine when abscisic acid was used in either water or organic solvents was not great and appears unlikely to lead to any practical application. In contrast, extremely effective inhibition of black spruce seed germination was achieved with abscisic acid applied in organic solvents, and this may lead to practical applications.

LITERATURE CITED

Aldasoro, J.J., Matilla, A., and Nicolas, G. 1981. Effect of ABA, fusicoccin and thiourea on germination and K^+ and glucose uptake in chick-pea seeds at different temperatures. Physiol. Plant. 53:139-145.

Anderson, J.D. 1973. Dichloromethane and lettuce seed germination. Science 179:94-95.

Berrie, A.M.M., Parker, W., Knights, B.A., and Hendrie, M.R. 1968. Studies on lettuce seed germination-I. Coumarin-induced dormancy. Phytochemistry 7:567-573.

Bonner, F.T. 1984. Glossary of seed germination terms for tree seed workers. USDA For. Serv., Gen. Tech. Rep. SO-49.

Braun, J.W., and Khan, A.A. 1976. Alleviation of salinity and high temperature stress by plant growth regulators permeated into lettuce seeds via acetone. J. Am. Soc. Hort. Sci. 101:716-721.

Braun, J.W., Rao, V.S., and Khan, A.A. 1976. Release of lettuce seed thermodormancy by plant growth regulators applied in organic solvent. HortScience 11:29-30.

Brewer, P.E., and Wilson, R.E. 1975. Dichloromethane: variability in penetration and resulting effects on seed germination and CO_2 evolution. Bot. Gaz. 136:216-218.

Czabator, F. 1962. Germination value: an index combining speed and completeness of pine seed germination. For. Sci. 8:386-396.

Halloin, J.M. 1977. Effects on cottonseed of immersion in acetone or methylene chloride. Crop Sci. 17:867-869.

Hegarty, T.W., and Ross, H.A. 1979. Effects of light and growth regulators on germination and radicle growth of lettuce seeds held under high temperature stress and water stress. New Phytol. 82:49-57.

Heydecker, W., and Joshua, A. 1977. Alleviation of thermodormancy of lettuce *(Lactuca sativa* L.) seeds. J. Hort. Sci. 52:87-98.

Khan, A.A. 1967. Antagonism between cytokinins and germination inhibitors. Nature 216:166-167.

Khan, A.A., Tao, K.L., and Roe, C.H. 1973. Application of chemicals in organic solvents to dry seeds. Plant Physiol. 52:79-81.

Liptay, A., and Schopfer, P. 1983. Effect of water stress, seed coat restraint, and abscisic acid upon different germination capabilities of two tomato lines at low temperature. Plant Physiol. 73: 935-938.

McDonald, M.B. Jr., and Khan, A.A. 1977. Factors determining germination of Indian rice grass seeds. Agron. J. 69:558-563.

Meyer, H., and Mayer, A.M. 1971. Permeation of dry seeds with chemicals: use of dichloromethane. Science 171:583-584.

Meyer, H., and Mayer, A.M. 1973. Dichloromethane and lettuce seed germination. Science 171:96.

Nelson, J.M., and Sharples, G.C. 1980. Effect of growth regulators on germination of a cucumber and other cucurbit seeds at suboptimal temperatures. HortScience 15:253-254.

Palevitch, D., and Thomas, T.H. 1974. Thermodormancy release of celery seed by giberellins, 6-benzylaminopurine, and ethephon applied in organic solvent to dry seeds. J. Exp. Bot. 25:981-986.

Phaneendranath, B.R., and Funk, C.R. 1978. Germination stimulation of Kentucky bluegrass seed permeated with plant growth regulators dissolved in acetone. Crop Sci. 18:1037-1039.

Poggi-Pellegrin, M.C., and Bulard, C. 1976. Interactions between abscisic acid, giberellins and cytokinins in Grand Rapids lettuce seed germination. Physiol. Plant. 36:40-46.

Rao, V.S., Braun, J.W., and Khan, A.A. 1976. Promotive effects of organic solvents and kinetin on dark germination of lettuce seeds. Plant Physiol. 57:446-449.

Schopfer, P., Bajracharya, D., and Plachy, C. 1979. Control of seed germination by abscisic acid. Plant Physiol. 64:822-827.

Sondheimer, E., and Galson, C.E. 1966. Effects of abscisin II and other plant growth substances on germination of seeds with stratification requirements. Plant Physiol. 41:1397-1398.

Steel, R.G.D., and Torrie, J.H. 1960. Principles and procedures of statistics. McGraw-Hill, New York. 481 p.

Tao, K.-L., and Khan, A.A. 1974. Penetration of dry seeds with chemicals applied in acetone. Plant Physiol. 54:956-958.

Tao, K.-L., McDonald, M.B., and Khan, A.A. 1974. Synergistic and additive effects of kinetin and ethrel on the release of seed dormancy. Life Sci. 15:1925-1933.

Triplett, L.L., and Haber, A.H. 1973. Dichloromethane and lettuce seed germination. Science 179:95-96.

Walbot, V., Clutter, M., and Sussex, I. 1975. Effects of abscisic acid on growth, RNA metabolism, and respiration in germinating bean axes. Plant Physiol. 56:570-574.

Zagorski, S., and Lewak, S. 1983. Interactions between hydrogen cyanide, giberellin, abscisic acid and red light in germination of lettuce seeds. Physiol. Plant. 59:95-98.