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CHANGES IN THE FATTY ACIDS AND WATER STATUS OF PHASEOLUS VULGARIS LEAVES AT TEMPERATURES INDUCING CHILL-HARDINESS

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Summary The primary cause of chilling-injury to Phaseolus vulgaris L. leaves on transfer from 25°C to 5°C, is leaf dehydration due to the rapid opening of the stomata at a time when the permeability of the roots to water is low. This rapid water loss and leaf injury at 5°C, can be prevented either by spraying the leaves with ABA or drought-hardening the plants at 25°C before chilling. The primary factor inducing hardening against chilling-injury in P. vulgaris leaves is a water stress and not low temperature per se. Phase transitions in the membrane lipids at 5°C are not considered to be important in the development of chilling-injury. Increases in the degree of unsaturation of the fatty-acids during chill-hardening at 12°C cannot be related to increased chill-hardiness and the lowering of the transition temperature as no increase in unsaturation occurred during drought-hardening at 25°C, a treatment which was as effective as chill-hardening in preventing chilling-injury at 5°C.

INTRODUCTION

Water loss and electrolyte leakage from the leaves of Phaseolus vulgaris during chilling at 5°C has been attributed to an increase in membrane permeability due to phase changes in the membrane lipids of the leaf cell plasmalemma (1,2). Therefore, increases in the degree of unsaturation of the fatty-acids associated with the phospholipids during the chill-hardening of P. vulgaris at 12°C were considered to prevent chilling-injury by lowering the temperature of the phase transitions to below 5°C (3). The present paper examines the protection against chilling-injury which is afforded by 4 days drought-hardening at 25°C. First, this paper compares the changes in the degree of unsaturation of the phospholipids during chill- and drought-hardening. Second, the cause of rapid water loss and leaf injury at 5°C and the mechanism of drought-hardening were investigated by following the changes in stomatal opening and the permeability of the roots to water at low temperature.

METHODS AND MATERIALS

Growth and hardening conditions

Seeds of Phaseolus vulgaris L. cv. Canadian Wonder were sown in 7 cm pots containing John Innes potting compost No. 2 and germinated in a growth cabinet at 25 + 2°C and 85 + 5% RH for 9 days under an 18 h light regime of 70 Wm^{-2} with a dark period from 23.00 to 05.00 hours. Plants of P. vulgaris were either chill-hardened at 12°C, 85 ± 5% RH for 4 days or drought-hardened at 25°C, 40% RH over a 4-day period. Drought-hardening was achieved by withholding water from the roots until the leaves wilted. Injury to severely wilted leaves was prevented by lightly watering the roots so that leaf turgor was regained. Over the 4-day period most of the plants wilted twice. Three hours before chilling at 5°C the plants were watered copiously so that the leaves regained almost complete turgor by the start of chilling. Drought-hardening was as effective as chill-hardening and prevented chilling-injury for up to 9 days on subsequent transfer to 5°C (4).

Lipid extraction, separation and fatty acid analysis

The first two leaves of P. vulgaris were used for the analysis which were performed as described previously (3).

Stomatal opening

The width of the stomatal aperture was measured by replication of the leaf surface using the silicon rubber monomer method (5).

Root permeability

The tops of the plants were removed just below the first node and 5-ml pipettes graduated in 0.05 ml were attached to the stumps by tygon tubing and sealed with a mixture of anhydrous lanolin and beeswax. The pipettes were attached to a vacuum pump by rubber tubing and a pressure gradient of 50 ± 3 cm Hg was maintained from the exterior to the interior of the root system. This pressure gradient was maintained for 4 h after which the amount of water absorbed by the roots was determined.

Abscisic acid (ABA)

The effectiveness of ABA in causing stomatal closure and in preventing chilling-injury to the leaves of Phaseolus vulgaris was investigated by spraying the leaves with a 10^{-4}M solution before transferring the plant to 5°C. Synthetic mixed isomers of ABA were supplied by the Sigma Chemical Co. Ltd. The ABA solutions were made up using a small quantity of dimethylsulphoxide as an initial solvent (0.5% of final volume), the required volume then being made up with distilled water. The leaves were sprayed twice using a Shandon chromatography spray gun until the entire leaf surface was wet. The first application was made 24 h before the start of chilling and the second application 1 h before the start of chilling. The controls were sprayed with 0.5% dimethylsulphoxide in water and this was found to have no effect on stomatal aperture. Leaf injury was assessed as the percentage of the leaf area showing necrosis 2 days after return to 25°C.

RESULTS

Phospholipid changes during chill- and drought-hardening

Wilson and Crawford (3) considered that chilling-injury to the leaves of species such as P. vulgaris, Cucumis sativus and Gossypium hirsutum may be due to phase changes in the membrane lipids and that these phase changes were prevented during hardening at 12°C by an increase in the degree of unsaturation of the phospholipid fatty-acids (Table 1). The possibility that drought-hardening also resulted in an increase in the degree of unsaturation of the membrane phospholipids

Table 1. Changes in the percentage fatty-acid composition of phosphatidylcholine from leaves of Phaseolus vulgaris during chill-hardening at 12°C and drought-hardening at 25°C.

Fatty-acid*	Control	Chill-hardened at 12°C for 4 days	Drought-hardened at 25°C for 4 days
14:0	3.8	2.1	5.3
16:0	20.4	12.8	24.3
16:1	0.9	1.0	0.7
16:2	0.9	1.1	0.4
18:0	6.5	4.3	6.2
18:1	4.0	3.5	2.0
18:2	27.5	40.0	24.1
18:3	36.0	35.2	37.0
Total per cent unsaturated fatty-acid	69.3	80.8	64.2

* The numbers shown are the ratios of the number of carbon atoms to the number of double bonds in the molecule.

was investigated. Table 1 shows that during drought-hardening there is no increase in the degree of unsaturation of the fatty-acids associated with phosphatidyl choline, the main phospholipid of P. vulgaris leaves. Drought-hardening resulted in a decrease in the amount of unsaturated fatty-acid associated with the phospholipids, mainly due to a fall in the level of linoleic (18:2) acid (Table 1). Similarly, the degree of unsaturation of the fatty-acids associated with phosphatidyl ethanolamine, phosphatidyl inositol, phosphatidyl glycerol and phosphatidic acid also decreased during drought-hardening and no changes were detected in the fatty-acid compositions of the glycolipids, monogalactosyl diglyceride, digalactosyl, diglyceride and sulpholipid. Therefore, on the basis of the phase change theory of chilling-injury, we would expect the drought-hardened plants to be more chill-sensitive rather than less, since the transition temperature will be raised with a

decrease in unsaturation. This result led to a re-examination of the cause of water loss from the leaves at 5°C.

The effect of direct chilling in stomatal opening and root permeability

Dehydration of P. vulgaris leaves and subsequent injury and electrolyte leakage on transfer from 25°C to 5°C may be caused by stomatal opening at a time when the permeability of the roots to water is low. An examination of the changes in stomatal aperture on direct transfer from 25°C to 5°C, showed that the stomata reach their maximum width after approximately two hours from the start of chilling (Table 2).

It has long been known that low temperature decreases the permeability of the roots to water. At 5°C the permeability of P. vulgaris roots is very low being only 0.02 ml per hour (Table 2) which is approximately one tenth of the rate of water absorption at 25°C. Hence the low permeability of the roots to water prevents the replacement of that lost by evapo-transpiration from the leaf so that rapid leaf dehydration and injury occur at 5°C. The severity of chilling-injury therefore depends on a synergistic effect between stomatal opening and reduced permeability of the roots to water at 5°C.

Table 2. Changes in stomatal aperture and root permeability on chilling unhardened and hardened plants of Phaseolus vulgaris

Treatment	Root permeability (ml/h)	Observation
1. Direct chilling 25°C → 5°C	0.020	Stomata open 50% leaf injury after 1 day
2. Chill-hardened 25°C → 12°C → 5°C	0.042	Stomata closed No injury up to 9 days
3. Drought-hardened 25°C → 25°C(40% RH) → 5°C	0.010	Stomata closed No injury up to 9 days
4. ABA (10^{-4} M spray) 25°C → 5°C	0.018	Stomata closed Injury prevented for 2 days
5. High humidity 25°C → 12°C(100% RH) → 5°C	0.019	Stomata open 50% leaf injury after 1 day

The effects of chill- and drought-hardening on stomatal aperture and root permeability

Chill-hardening at 12°C conditions the stomata so that they close on transfer to 5°C thus preventing water loss. Similarly, drought-hardening over the 4 day period at 25°C also results in stomatal closure at the start of chilling and the stomata remain closed for the duration of the chilling treatment (Table 2). It is the closure of the stomata during chill and drought-hardening that is the most important factor in

the prevention of chilling-injury to Phaseolus vulgaris. Although the permeability of the roots to water during chill-hardening doubles from 0.02 to 0.04 ml per hour drought-hardening results in no increase in root permeability (Table 2), although it is just as effective as chill-hardening in preventing chilling-injury.

Abscisic acid (ABA)

The necessity for stomatal closure in the prevention of chilling-injury to P. vulgaris was further demonstrated by spraying leaves of plants grown at 25°C with 10^{-4}M ABA. This caused stomatal closure at 25°C and at 5°C the stomata remained closed, the leaves did not wilt and injury was prevented for up to 2 days, by which time the effectiveness of ABA was wearing off (Table 2).

Chilling and chill-hardening in a saturated atmosphere

Chilling-injury to P. vulgaris leaves can be prevented for up to 9 days on direct transfer from 25°C to 5°C, by enclosing the plant inside a polythene bag - thus maintaining a saturated (100% RH) atmosphere around the leaves. Plants held in a saturated atmosphere at either 5°C or 12°C, for 4 days do not acclimatize to withstand chilling injury at 5°C, 85% RH and incur chilling-injury at approximately the same rate as plants transferred directly from 25°C to 5°C (Table 2).

DISCUSSION

The hardening mechanism

With the above results it is now possible to explain the mechanism by which plants transferred to 12°C harden against chilling-injury. During effective chill-hardening at 12°C the plant experiences a water stress, as shown by the temporary wilting of the leaves at the start of hardening. This water stress during chill-hardening is produced by the temporary opening of the stomata at 12°C (a temperature just above the chilling range) and the reduced permeability of the roots to water at 12°C. At this intermediate temperature of 12°C the stress is not severe enough to result in damage and the temporary wilting at 12°C vanishes after 12 hours. Similarly, during drought-hardening at 25°C the water stress is imposed simply by witholding water from the roots under conditions of high evapo-transpiration so that the leaves wilt. In contrast, a 4 day period in a water saturated atmosphere at either 12°C or 5°C does not result in any increase in chill-hardiness as the plant experiences no water stress. On removing the polythene bag from plants held at either 12°C or 5°C for 4 days and transfer to 5°C, 85% RH the stomata do not close and remain fully open with the result that rapid leaf dehydration causes over 50% leaf injury with 24 hours.

The relationship between chill- and drought-hardening has shown that an intermediate temperature of 12°C is not essential for hardening to occur (4). Therefore, a water stress and not low temperature per se is the primary factor inducing hardening against chilling-injury in P. vulgaris. It is considered that the water stress imposed during chill-hardening or drought-hardening prevents rapid water loss from the leaves on transfer to 5°C by modifying stomatal behaviour at low temperatures through an increase in the level of ABA present. Elevated ABA levels in the leaves of drought-hardened P. vulgaris have been reported (6). It is known that leaf dehydration often increases the resistance of plants to a wide variety of environmental factors (7) and it has been suggested that a common regulatory mechanism which facilitates cross

adaptation in plants involves the hormone ABA (8). To elucidate the mechanism of ABA action in relation to stress adaptation more research is needed on the effects of cold on ABA synthesis and on the effects of ABA on the lipid and protein composition of membranes and membrane permeability.

Recent research (9) has suggested that phase transitions in the membrane lipids can occur in both chill-resistant and chill-sensitive plants within the same low temperature ranges. The importance of methodology in detecting phase transitions by electron spin resonance and polarographic techniques has been stressed by Raison, Chapman and White (10) who maintain that transition temperatures for chill-resistant species occur at a lower temperature than for chill-sensitive species. However, the present research has demonstrated that in P. vulgaris phase changes in the membrane lipids and associated increases in membrane permeability and activation energies of membrane bound enzymes can only be of minor importance in the development of chilling-injury since injury can be prevented for up to 9 days simply by maintaining a saturated atmosphere around the leaves.

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EFFECTS OF ENVIRONMENTAL AND GROWTH REGULATOR TREATMENTS ON THE FLOWERING OF MATURE SITKA SPRUCE

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Summary Keeping small grafted plants of Sitka spruce (Picea sitchensis (Bong.) Carr.) at high temperatures in a polythene house for several months preceeding the differentiation of reproductive buds, led to a 7-fold increase in numbers of male strobili and a 10-fold increase in female strobili, suggesting that low temperatures can limit flowering under natural conditions in the United Kingdom. A gibberellin $A_{4|7}$ mixture applied under field conditions through cuts in the branch strongly promoted flowering and this was further enhanced by combined treatment with benzyladenine. Naphthalene acetic acid reduced the ratio of female to male strobili. Gibberellins also promoted flowering on trees under polythene or glass when applied directly to buds, gibberellin A_9 being most active, but gibberellin A_4 alone giving no response. The promotive effect of gibberellin was reversed by either Phosphon D or abscisic acid. Gibberellin $A_{4|7}$ applied to mature trees in the forest enhanced flowering, whilst gibberellic acid was inactive.

INTRODUCTION

The ability to induce flowering of forest trees enables breeding programmes to be quicker and more efficient and seed supplies to be improved (1,2).

Sitka spruce (Picea sitchensis (Bong.) Carr.) is economically the most important species used in British forestry, comprising more than 40% of the plants produced by the Forestry Commission in the last decade. However, Sitka spruce does not flower readily in the U.K.; little is observed before trees are 25-30 years old. Even on sexually mature trees good cone crops are irregular and unpredictable, making breeding difficult.

Puritch (2) refers to several correlations between high temperatures in summer and plentiful flowering of forest trees in the subsequent year, but very few studies have assessed the effect of artificially altering the environment around the tree. A study of this type was carried out at the Forestry Commission Northern Research Station near Edinburgh (3) and the results are reviewed below.

Growth regulator treatments also may affect the flowering of conifers. Gibberellin applications can induce flowering in certain groups of herbaceous plants (4); these findings were first extended among conifers to the Taxodeaceae and Cupressaceae (5,6), but spruces and other members of the Pinaceae, which include most of the important forest trees, appeared to be relatively unresponsive (2,7). More recently, promotion of flowering in sexually mature plants following gibberellin treatment has been noted in several Pinaceae species (8); statistically significant promotion of strobilus numbers has been reported for Douglas fir (9) and for Sitka spruce (10).

The experiments on sexually mature Sitka spruce reviewed below suggest that low temperatures may inhibit flowering in the field and that hormone treatments can remove this inhibition.

MATERIALS AND METHODS

Detailed accounts of materials and methods may be found elsewhere (3,10), but essential features are described below.

Small potted plants comprising mature scions grafted 5 years previously to young seedling rootstocks were used for experiments in a polythene house at the Forestry Commission Northern Research Station near Edinburgh, plants grafted 10 years previously and grown in 'orchard' conditions in a 'Tree Bank' were used at Wauchope near Hawick in Scotland.

Hormone treatments were applied in pure ethanol in July, August and September 1975 on 12 occasions in the polythene house or on two occasions at Wauchope Tree Bank. In the polythene house gibberellins were applied at the same molarity in each treatment. Solutions were applied directly to buds (in the polythene house) or beneath freshly cut flaps of bark on the appropriate branches (at Wauchope). Bark removal treatments ('ringing') were made by excising two half rings of bark just proximal to the site of hormone application. Care was taken to ensure that the first-order branches used were comparable between treatments. One experiment (at Long Ashton) was carried out in a greenhouse using similar materials and methods to those used in polythene house experiments.

A further experiment was carried out on mature trees at Brendon Forest, Somerset. In early June a brace and bit were used to drill a hole in the upper surface of the branch and the tip of a hypodermic syringe containing 0.5 ml of hormone solution was firmly implanted. In late July the syringe was removed, recharged with 0.5 ml of solution, and re-inserted into the branch. Treatments were allocated at random to upper branches on the tree.

The total numbers of male and female strobili were counted in 1976 for all branches in hormone experiments and subjected to statistical analysis.

The effect of keeping grafted spruce in a polythene house was tested using 19 clones of grafted plants prepared in the same way as for the polythene house experiments described above. Plants were kept inside for 3.5 months in the summer of 1973 and for the entire spring and summer periods in 1974 and 1975. The number of replicates was reduced in 1976 and treated plants remained inside for all or part of the summer period.

RESULTS

Retaining grafted plants in a polythene house gave greatly increased male and female flowering in four successive years (Table 1). Mean daily temperature maxima were 37.8°C inside and 21.5°C outside the polythene house during the period between April and September 1976 (Table 2), so there were large temperature differences during the period before differentiation of reproductive buds. Average minima, on the other hand, were similar around the two groups of plants. Large temperature differences were also observed in previous years.

Two hormone application experiments were performed inside a polythene house (Table 3). The results show that $GA_{4}{}_{7}$ applied directly to the buds increased male and female flowering significantly both alone and in combination with GA_3, but kinetin and GA_4 alone did not affect flowering. Phosphon D (PHOS) reversed the effect

TABLE 1

Effect on flowering of keeping grafted plants in a polythene house

Year of assessment	Polythene house			Control		
	Mean number of strobili per plant		Number of plants flowering/ total number	Mean number of strobili per plant		Number of plants flowering/ total number
	Male	Female		Male	Female	
1974	1.5	0.8	36/171	0.1	0	4/57
1975	16.2	7.2	153/171	0.1	0.2	4/57
1976	4.0	0.1	63/162	1.1	0	9/54
1977	15.3	3.5	22/27	3.8	1.0	4/9

TABLE 2

Mean daily maximum and minimum air temperature (°C) in 1976

	May		June		July		August	
	Min	Max	Min	Max	Min	Max	Min	Max
In the polythene house	7.9	34.5	11.6	39.3	13.1	39.3	11.0	38.2
Around controls (outside)	6.1	16.8	9.7	22.3	10.0	23.1	9.3	23.7

of gibberellins and a similar, but less marked, reversal was observed with abscisic acid (ABA).

Results from a field experiment using large, grafted plants at Wauchope Tree Bank are presented in Table 4. Gibberellins applied under flaps of bark again promoted flowering and simultaneous application of benzyladenine (BA) further enhanced the response. Naphthalene acetic acid (NAA) reduced the number of female and increased the number of male strobili when applied with gibberellins and a similar effect was produced when NAA was combined with both gibberellins and BA.

In a further experiment on small, grafted plants in a glasshouse at Long Ashton GA_3, GA_5 and $GA_{4/7}$ gave negligible flowering, whilst GA_9 gave a significant increase above controls (means <0.1 and 0.44 strobili per branch respectively). The generally low level of flowering may have been caused by relatively good ventilation and low temperatures in this experiment.

At Brendon Forest flowering was increased by gibberellin $A_{4/7}$ alone and in combination with BA, but gibberellic acid was inactive (Table 5).

DISCUSSION

Keeping grafted plants in a polythene house produced striking increases in male and female flowering above controls outside the house. The large differences in average day temperatures observed between the two locations are believed to have caused the increases in flowering although other causes have not been excluded.

Inadequate water supply ('water stress') can promote the flowering of beech (11) and of some other trees (2). However, in the present experiments sufficient water was supplied to keep the mean weight of pots containing the plants inside the house approximately equal to that of the pots containing the control plants outside. Other factors, such as relative humidity, light quality and light quantity were considered unlikely to have caused the observed effect.

Brondbo (12) observed a relatively small increase in male flowering of Norway spruce by covering grafted plants with polythene tents for periods of two weeks; the stronger flowering of Sitka spruce in the present study may have been due to the longer period of treatment.

TABLE 3

Effect on flowering of hormone treatments applied to plants in a polythene house for (i) ♂ + ♀ flowering clones, (ii) ♂ flowering clones and (iii) ♀ flowering clones*

Experiment	Treatment	(i) mean numbers of ♂ + ♀ strobili per branch	(ii) Mean numbers of ♂ strobili per branch	(iii) Mean numbers of ♀ strobili per branch
(A)	$GA_{4}|_{7}$	2.07a	1.89a	1.71a
	$GA_{4}|_{7}$	2.07a	2.22a	1.29a
	GA_{4}	0.43b	0.22b	0.57ab
	Control	0.29b	0.11b	0.43b
	KN	0.27b	0.11b	0 b
(B)	$GA_{4}|_{7}$ + GA_{3}	2.50a	1.06a	2.33a
	$GA_{4}|_{7}$ + GA_{3} + ABA	1.61a	1.19a	0.83b
	$GA_{4}|_{7}$ + GA_{3} + PHOS	0.83b	0.69ab	0.33b
	Control	0.22c	0.25b	0 b
	PHOS	0 c	0 b	0 b

* A mean followed by a particular letter or group of letters is significantly different at the 5% level from means not followed by the same letter or any of the letters in the group.

Whilst differences in the maximum temperatures are large, differences between minimum (night) temperatures were relatively small in the present study. These observations suggest that the day temperatures, here represented by the maxima, are especially important in controlling flowering of Sitka spruce.

The results suggest that outdoor temperatures during the day may inhibit the flowering of Sitka spruce under field conditions in the U.K. Clearly, it is impractical artificially to raise air temperatures around large numbers of big grafted plants in the field, so the practical interest of this work concerns the possibility of modifying the temperature-induced limitation of flowering by applying growth regulators.

TABLE 4

Effect on flowering of hormone treatments at Wauchope for (i) ♂ +♀ flowering clones, (ii) ♂ flowering clones and (iii) ♀ flowering clones*

Treatment	(i) Mean numbers of ♂ +♀ strobili per branch	(ii) Mean numbers of ♂ strobili per branch	(iii) Mean numbers of ♀ strobili per branch
$GA_{4}|_{7}$ + GA_3 + BA + NAA ('ringed')	24.91a	22.78a	7.67a
$GA_{4}|_{7}$ + GA_3 + BA	17.00ab	14.11b	6.67a
$GA_{4}|_{7}$ + GA_3 + BA + NAA	14.27bc	12.22b	5.22a
$GA_{4}|_{7}$ + GA_3 + NAA	10.73bc	10.00b	3.11b
$GA_{4}|_{7}$ + GA_3	8.36c	3.45c	6.78a
$GA_{4}|_{7}$	8.00c	4.11c	5.67a
Controls	1.70d	0.59d	1.48b

* Letters indicate statistical significance as in Table 3.

TABLE 5

Effect on flowering of hormone treatments applied to fifteen 50-years-old trees at Brendon Forest*

Hormone (concentration in mg/ml)	Mean number of ♂ strobili per branch	Mean number of ♀ strobili per branch
$GA_{4}|_{7}$ (2.0) + BA (0.02)	73.1ab	9.2a
$GA_{4}|_{7}$ (2.0)	108.9a	5.9ab
$GA_{4}|_{7}$ (1.0)	58.4ab	2.0b
Control	48.6b	1.1b
GA_3 (2.0)	48.6b	2.7ab
GA_3 (1.0)	44.4b	0.9b

* Letters indicate statistical significance as in Table 3.

The physiological implications of these growth regulator experiments are also of interest. Exogenous gibberellins applied to the plant must have raised internal levels, at least temporarily, and this may have been the cause of the increased flowering observed. This explanation is supported by the fact that gibberellins are naturally-occurring in Sitka spruce (13,14) and by the finding that Phosphon D, which is known to inhibit gibberellin biosynthesis in higher plants, can reverse the promotion of flowering by gibberellins.

It has been suggested that the number of hydroxyl groups on the basic ring system of the molecule may determine the activity of a gibberellin in promoting flowering of the Pinaceae, those with fewer such groups having greater activity (15). In agreement with this concept, GA_9, which has no such hydroxyl groups, had the greatest activity of the gibberellins tested. On the other hand, GA_4 was inactive but a $GA_{4}|_{7}$ mixture gave strong promotion of flowering; since GA_4 and GA_7 both have a single hydroxyl group on the basic ring system, some other explanation must be found for this difference in activity.

Cytokinins occur naturally in Sitka spruce (16) and applying benzyladenine (BA)

enhanced the promotion of flowering in response to gibberellins (Table 4), suggesting that cytokinins may also be involved in the natural control of flowering in this species. However, in these experiments BA could have acted by improving gibberellin uptake or gibberellin translocation, since the hormones were supplied through cuts in the branch.

Applying ABA directly to buds reversed the promotion of flowering by gibberellins in Sitka spruce: furthermore ABA has been identified in extracts of several different Pinaceae species (7). These findings suggest an antagonistic role for ABA in the natural control of flowering in Sitka spruce.

Bark removal ('ringing') further increased the flowering induced by hormone applications to Sitka spruce, confirming the results of Ross and Pharis (9) for Douglas fir. This effect may have been caused by accumulation of strobilus-promoting substances in the branch, by preventing strobilus-inhibiting substances from entering the branch, or by a combination of these possibilities.

The results at Brendon Forest show that promotion of flowering can be obtained by gibberellin $A_{4|7}$ applications to ungrafted Sitka spruce under natural conditions but that gibberellic acid has no such effect. The fact that only gibberellic acid was used in previous attempts to induce flowering by gibberellins in spruce may explain the former lack of success.

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