ecious inbreds, support this conclusion. The effective range of concentrations is between 250 and 1500 ppm of potassium gibberellate, but the particular spraying regime to be used would depend on the objective of the experiment and the variety employed.

The increase in female tendency after GA treatments is often associated with: (i) increase in internode elongation, (ii) decrease in leaf size, (iii) suppression of shoot development at some lateral leaf axils, and (iv) delay in flowering.

It is essential to emphasize the importance of time in plant development at which the GA treatment is applied. If the application of GA is limited to a period prior to the differentiation of the first flower primordia, a prolonged delay in flowering will occur, and, during a long delay, the effect of GA upon sex is nullified. If this aspect of timing is overlooked, one may arrive at an erroneous conclusion concerning GA as a sex regulator, particularly in treating late-maturing varieties.

The most significant fact emerging from these results, in light of other reports, is that GA treatments of cucumber and castor bean plants provoke diametrically opposite changes in sex expression. Perhaps it is not a mere coincidence that the two species exhibit also opposite sex responses to photoperiod variation. In cucumbers, short days increase and long days decrease female tendency (*7*). In castor beans, the reverse seems to be true (*1*). Thus, in the two species involved, the effect of GA upon sex appears to simulate the action of long days (*2*).

OVED SHIFRISS
Department of Horticulture, Rutgers University, New Brunswick, New Jersey

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Identity of a Fungus Causing Blue Stain in Balsam Fir

Abstract. Blue-stained balsam fir wood [*Abies balsamea* (L.) Mill.] consistently yielded cultures of a nonsporulating fungus which were found to be the same as those obtained from ascospores of *Amphisphaeria thujina* (Peck) Sacc. collected from dead branches of this host. This blue stain appears to be identical with a previously reported but hitherto unidentified stain of conifers. The association of *Amphisphaeria* with stain in living trees does not appear to have been recognized before.

During the past 25 yr several investigators have reported the occurrence of a blue stain in the heartwood of balsam fir, but were unable to identify the causal organism. Kaufert (*1*), in 1935, described a blue stain occurring in the heartwood of overmature balsam fir in the Lake states. The fungus isolated from this stain produced "luxuriant grey-blue" mycelium in culture, but was not identified. A few years later, Crowell (*2*) described a blue stain of heartwood of white spruce and balsam fir from Shelter Bay, Quebec. He reported that the "bluestain was unusual in that almost the entire heartwood was deeply stained while the sapwood was free from attack," adding that "stained wood showed many short blackish lines on the radial surface, but the lines were not necessarily associated with wood rays, as invariably occurs in other stained wood." When blocks of wood were kept in a moist chamber for 5 wk, dark, olivaceous, cottony hyphae grew out of the stained wood but formed no spores. Interest in the blue-staining fungus was revived in 1942 when Christensen and Kaufert (*3*) confirmed that the fungus mentioned earlier by Kaufert was the same as that described by Crowell. They consistently isolated the same fungus from a blue stain of heartwood of northern white cedar (*Thuja occidentalis* L.) in Minnesota. Numerous isolations had yielded, in almost every case, a nonsporulating and very slow-growing fungus with dark hyphae, which was concluded to be the organism chiefly or solely responsible for the stain in the wood.

Although the presence of blue stain with heart rot in conifers has been recognized in Quebec for several years, only recently has its prevalence in branch stubs of balsam fir been observed. Blue stain was observed in over half of 648 living and dead branches that were sampled at Duchesnay, Quebec, in 1960, and a characteristic fungus was consistently isolated from the stained wood. The stain occurred as streaks at the bases of the branches, frequently extending for distances of 1 to 2 in. into the buried portion of the branch. There was no evidence that the discoloration had spread from the branches into the surrounding healthy wood, although the stain fungus was isolated several times from what appeared to be normal heartwood, as well as from areas of typical blue-stained wood adjacent to heart rot. Blue stain was not observed in branches which had been dead for less than 10 yr. The characteristics of the stained wood agreed both macroscopically and microscopically with the descriptions and illustrations of blue stain that have appeared in the earlier reports. The infected wood, both in radial and tangential sections, displayed numerous dark lines running at right angles to the wood elements, often continuing the characteristic pattern through adjacent areas of decayed wood. Sections of stained wood examined microscopically revealed long strands of thick, dark hyphae which were constricted where they passed through the walls of tracheids and rays.

On malt agar slants, the fungus produces abundant, sterile, dark grey-olivaceous aerial mycelium. The colonies have abrupt clear-cut margins, the reverse of the culture being black. At room temperature the growth is slow; it covers about two-thirds of the slant in 3 wk and usually ceases entirely before reaching the end of the tube.

The isolates from the blue stain were identified by comparing them with cultures made from fruiting structures found on dead branches of balsam fir. Dead branches, in various stages of deterioration, from stands where the blue stain occurred were critically examined for fruiting-bodies of fungi. From one of these fungi, seven cultures (representing three separate collections) were obtained that appeared to be identical with the cultures from the stained wood. This fungus was recognized as belonging to the genus *Amphisphaeria*.

In the literature, a number of species of *Amphisphaeria* are described which appear to be similar to the fungus collected on fir at Duchesnay, but the description of *A. thujina* (Peck) Sacc. agrees most closely in respect to the size of the perithecia and of the ascospores. The original collection of *A.*

thujina was made from dead branches of *Thuja occidentalis* in New York about 1875, and was deposited in the New York State Museum. Positive identification of this ascomycete was made by comparing the type collection (*4*) with the Duchesnay material.

To test the ability of the fungus to produce blue stain, blocks of balsam fir were inoculated with cultures of *Amphisphaeria thujina* derived both from stained wood and single ascospores. After 2 mo, typical blue stain had developed in all of the test blocks. The fungus was reisolated from the artificially inoculated wood and identified as the species first isolated. These experiments prove that *A. thujina* is the causal agent of the blue stain in balsam fir (*5*).

DAVID E. ETHERIDGE
RENÉ POMERLEAU
Forest Entomology and Pathology Laboratory, Sillery, Quebec

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4. We are grateful to S. J. Smith, curator, New York State Museum, for his kindness in providing the type collection of *A. thujina*. A detailed description of *A. thujina*, including the results of comparative taxonomic studies with Peck's original material, is in preparation.
5. Further laboratory studies are in progress to determine the factors that may influence the establishment of blue stain in the living tree, and to ascertain its effect on subsequent development of decay organisms. This report is contribution No. 723, Forest Entomology and Pathology Branch, Department of Forestry, Ottawa, Canada.

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Freezing-Out, a Safe Technique for Concentration of Dilute Solutions

Abstract. The principle of freezing-out has been adapted for the quantitative concentration of small or large volumes of dilute solutions. Mechanical stirring is an essential part of the process. The method should prove of greatest value where volatile or heat-labile substances are to be concentrated and is applicable to solutions in certain organic solvents as well as to aqueous solutions.

The purpose of this report is to call attention to an exceedingly useful, but hitherto neglected, method for the laboratory concentration of dilute solutions. The method, which possesses advantages over most of those in use, is based on the principle of freezing-out. It is simple, relatively inexpensive, and is almost above reproach with regard to chemical or physical alteration of the substances being concentrated. The method is applicable to a very wide range of volumes, having been used in this laboratory on samples as small as 100 ml and as large as 300 liters. Preliminary work has shown that in addition to its use with aqueous solutions it is applicable also to solutions in organic solvents, such as benzene, which solidify at relatively high temperatures.

Although freezing-out is widely used industrially, it has not become a part of the analyst's armamentarium, and references to its use in the laboratory are rare. As described below, the method is similar to that used in the purification of benzoic acid by Schwab and Wichers (*1*). It differs from the previous applications by Haurowitz (*2*), Gibor (*3*), and Schildknecht and Mannl (*4*), in that mixing is an essential part of the process and part of the solution is left unfrozen. Thus materials which are adversely affected by freezing are not harmed, and substances which dissolve with difficulty are recovered while still in solution. In addition, the volumes which can be concentrated are much greater than in the previous applications.

Volumes of 1 liter or less are treated as follows. The solution is poured into either a glass or plastic bottle, leaving sufficient room for the expansion which accompanies freezing. The bottle is then attached to a mechanical shaker and placed in a freezing chamber. After several hours of gentle shaking, most of the solution will be frozen except for the central spherical or egg-shaped portion which contains the concentrated solutes. This portion is recovered by punching or melting a hole in the ice and pouring it out. The equipment used in this laboratory consists of a freezer cabinet of the type used to store ice cream, operating with a ⅓-hp motor at −30°C, and a Burrell Wrist Action shaker which rests on the wooden cover of the freezer. Arms with clamps attached extend down into the freezer from the shaker. In this way numerous samples of different volumes can be concentrated simultaneously. The results with this method have been excellent, with recoveries of greater than 99 percent after concentrations of as much as 20-fold in volume. However, in order to achieve such recoveries, it is essential to keep a close watch over the bottles so that they may be emptied when cloudy ice indicates that entrapment is occurring.

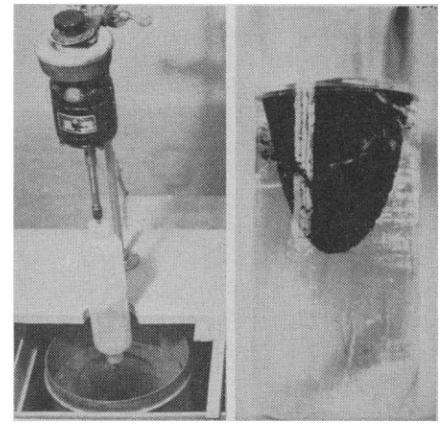

Fig. 1. (Left) A 5-gal container in the freezer chest with the polyethylene bottles on the stirrer. (Right) The block of ice removed from the container showing the concentrate in the core.

Large volumes are treated somewhat differently. The solutions are placed in cylindrical metal containers which are positioned in the freezing chest so that the open tops of the containers are just above the freezing level in the chest (Fig. 1). The solutions are stirred mechanically, and the propeller is raised in several steps as freezing proceeds. Ice forms from the sides inwards and from the bottom upwards, and the result is a smooth, sharply defined shallow conical core in which the solutes are concentrated into a small volume (Fig. 1).

The time required for concentration varies with the volume and the efficiency of operation. For example, with the equipment in use it has been possible to concentrate three 16-liter containers of water to a volume of 1 liter each in about 50 hr. On the other hand, when the solution was placed directly in the compartments of the cabinet instead of in containers, 300 liters were concentrated to 35 liters in the same length of time.

Several refinements are possible. To avoid losses due to splashing of the concentrate onto the top of the already formed ice, two 250-ml polyethylene bottles, bored through the cap and bottom, are slipped onto the stirring shaft (Fig. 1). Thus, as the stirrer is raised the liquid level falls. Recovery of the last traces of concentrate from the core is simplified by gently melting the surface ice with warm air, such as from