obtained. The growth pattern of the viruses is unlike that of cytomegalo-viruses (4).

The agents survive a temperature of 60°C for 40 min. Infectivity titers for embryonic human lung cultures vary from $10^{-8.5}$ to $10^{-5.5}$. Cross protection tests with convalescent sera indicated a serologic relationship between 13 of the 14 isolates. The only exception was the virus which grew in the monkey renal cultures. Acute sera from four of the hospitalized patients failed to neutralize homologous viral isolates, whereas sera from 1-yr convalescents neutralized 100 to 1000 TCID₅₀ of virus at serum dilutions of 1/25 and 1/50. Sera obtained from two adults who had recovered from infectious hepatitis 1 yr previously neutralized all the viruses except the monkey kidney isolate. The neutralization index with the latter sera was as great as that of convalescent sera from hospitalized cases (5).

ELDON V. DAVIS Communicable Disease Center, U.S. Public Health Service, Phoenix, Arizona

References and Notes

- E. V. Davis, Federation Proc. 19, 386 (1960).
 J. R. Paul and J. L. Melnick, in Diagnostic Procedures for Virus and Rickettsial Diseases (Am. Public Health Assoc., New York, ed. 2, 1956).
- 3. Immune sera were obtained from the National Foundation through the courtesy of H. A. Wenner.
- H. Weller, personal communication.
 Efforts to further characterize these isolates
- and studies on their epidemiology are being advanced.
- 13 March 1961

Gibberellin as Sex Regulator

in Ricinus communis

Abstract. Spraying monoecious inbreds with gibberellin A_3 can markedly increase their female tendency. The effect of this substance upon sex appears to simulate the action of long days.

The castor bean, *Ricinus communis* L., is a monoecious, potentially everflowering perennial. The main stem, which blooms first, and all subsequent shoots, are terminated by racemes, and each raceme differentiates both staminate and pistillate flowers. The percentage of pistillate flowers in the racemes of individual plants is taken as a measure of their female tendency. This character is subject to extreme genetic and nongenetic variations.

Table 1. Effect of spraying seedlings with gibberellin upon the female tendency of a monoecious inbred of the castor bean. Figures in parentheses refer to the total number of flowers. The spraying regimes are given in the text.

| Freatment | Individual plants employed | Pistillate flowers (%) in sequential racemes | | | |
|-----------|----------------------------------|--|-----------|-----------|-----------|
| | | First | Second | Third | Fourth |
| - | | Spr | aved | | |
| Α | 108-1 | 86.5(37) | 100.0(41) | 92.7(55) | 34.7(101) |
| Α | 108-2 | 87.9(83) | 100.0(85) | 73.9(85) | 73.9(130) |
| В | 108-3 | 66.0(97) | 97.6(42) | 44.7(132) | 40.8(49) |
| В | 108-4 | 74.6(63) | 88.7(53) | 59.4(69) | 36.3(113) |
| С | 108-5 | 57.9(107) | 73.8(84) | 38.6(132) | 21.1(109) |
| С | 108-6 | 61.2(67) | 93.5(46) | 38.0(108) | 26.2(130) |
| | | Uns | praved | | , |
| | 108-7 | 27.4(72) | 15.8(101) | 12.9(109) | 29.4(167) |
| | 108-8 | 32.8(67) | 24.2(62) | 14.7(75) | 14.9(134) |
| | 108-9 | 31.7(83) | 19.7(71) | 18.4(76) | 25.6(78) |
| | 108-10 | 25.7(105) | 24.3(66) | 15.8(54) | 25.8(159) |
| | 108-11 | 32.1(84) | 18.2(88) | 13.2(91) | 27.1(151) |
| | 108-12 | 27.3(77) | 25.9(81) | 17.4(115) | 24.5(188) |

In connection with a study of the relationship between female tendency and the incidence of female mutants in monoecious inbreds (1, 2), a search was made for substances which might regulate sex expression in these inbreds.

Previous investigations demonstrated that in monoecious inbreds of cucumbers, *Cucumis sativus* L., certain treatments with 1-naphthalene acetic acid (NAA) increase (3) and certain treatments with gibberellic acid or gibberellin (GA) decrease (4) female tendency. It is also known that staminate flowers can be induced by GA in some genetic females of cucumbers (5, 6). These findings prompted us to test NAA and GA as possible sex regulators in the castor bean.

Various spraying regimes with NAA at concentrations ranging from 10 to 100 parts per million (ppm) did not affect female tendency. On the other hand, spraying with GA markedly increased female tendency (Table 1). In the experiment with GA, I used an early-flowering monoecious inbred No. 108 which was self-reproduced for 11 generations. This inbred was originated from a selection made in the variety Gamadon (1). The seed was germinated at 30°C on 8 August 1960, and 12 uniform plants were grown in 12-in. pots, in soil, under greenhouse conditions, without supplementary light, at day temperatures of 24° to 27°C and night temperatures of 18° to 21°C (thermostatically controlled). The sprayed and unsprayed plants were randomized.

The sprays used were aqueous solutions of Merck's Gibrel, a potassium salt of gibberellin A_3 . Each treated plant was sprayed three times—at the two-leaf, three-leaf, and four-leaf stages of growth, successively. In treatment A, the concentrations of the gibberellin solutions employed were 1500 ppm for the first spraying, 1500 ppm for the second, and 500 ppm for the third. In treatment B, the corresponding concentrations were 1500, 1000, and 500 ppm; and in treatment C, they were 1500, 500, and 125 ppm (Table 1). The spraying began on 26 August and the first racemes appeared on 8 September. In both sprayed and unsprayed plants the number of leaves to the first raceme on the main stem ranged from six to eight and averaged seven.

By selective pruning of side shoots it was possible to obtain four sequential racemes on each plant, and these racemes were developmentally comparable in all plants.

As is shown in Table 1, the first three sequential racemes on each of the six sprayed plants were greatly affected by the GA treatments. One should also note the trend in the differences between treatments A, B, and C.

In the unsprayed plants (Table 1) there is a drop in female tendency from the first to the third raceme in each individual, and this drop is followed by a recovery in the fourth raceme. The racemes of the sprayed plants do not exhibit the same developmental change, presumably because of the cumulative effect of the three GA treatments at seedling stages. Cyclic changes in sex tendency during development are not uncommon in some untreated monoecious species of plants (6).

It is evident that GA is an effective substance for increasing female tendency in the castor bean. Other GA experiments with inbred No. 108, as well as with several distinctly different monoecious 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

References and Notes

- 1. O. Shifriss, Genetics 41, 265 (1956); J. Genet.,
- O. Shifriss, Genetics 41, 265 (1956); J. Genet., in press.
 Project No. 489, Ricinus, 1960-61 University Research Fund; paper of the journal series, New Jersey Agricultural Experiment Station, Rutgers University, New Brunswick. I wish to thank Ming-Yu Li and William L. George for their help in this study.
 F. Laibach and F. J. Kribben, Ber. deut. botan. ges. 62, 53 (1949); J. Heslop-Harrison, Biol. Revs. Cambridge Phil. Soc. 32, 38 (1957).
 T. Yabuta and T. Hayashi, J. Agr. Hort. 13, 21 (1938) [This work, written in Japanese, was reviewed by F. H. Stodola, "Source book on gibberellin, 1828-1957," U.S. Dept. Agr. ARS (1958)]; S. H. Wittwer and M. J. Bukovac, Econ. Botany 12, 213 (1958).
 E. Galun, Phyton (Buenos Aires) 13, 1 (1959); C. E. Peterson and L. D. Anhder, Science 131, 1673 (1960); O. Shifriss and G. A. Taylor, unpublished data.
 O. Shifriss, J. Heredity, in press.
 J. P. Nitsch, E. B. Kurtz, Jr., J. L. Liverman, F. W. Went, Am. J. Botany 39, 32 (1952).
 February 1961 in press.

- 16 February 1961

2062

Identity of a Fungus Causing Blue Stain in Balsam Fir

Abstract. Blue-stained balsam fir wood [Abies balsamea (L.) Mill.] consistently vielded 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 bluestaining 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, Que-

bec, 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.