This type of experimentation provides a rapid method of carrying on certain phases of breeding work with this long-lived group of plants since the results of each year's work are available at the end of the current season. The kernel of other nut-producing species may also be affected by genetic constitution of the pollen, since wide variations in nut size and flavor are noticeable in several species.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

CHLORAZOL FAST PINK BKS AS AN ANTI-COAGULANT

CLOTTING in systems recording blood pressure of animals is a frequent source of annoyance in kymograph experiments. The inexpensive methods of inhibiting clotting are, generally speaking, inffective, while heparin, which is effective, is so expensive that its use is limited.

An effective and inexpensive anti-coagulant would be eminently desirable in any laboratory in which an appreciable amount of work is done which involves the direct recording of blood pressure. We were therefore interested in the properties of an anti-coagulant dye which was suggested by a former member of this department.[1] We have used it with such success, and we have been requested for information concerning its properties, use and preparation from so many sources that it was thought desirable to point out again that such a substance is available, and to record a simple method of purification of the crude product.

In 1932 Huggett and Silman[2] pointed out the anti-coagulant properties of Chlorazol Sky Blue FF (Chicago Blue). They found that the dye acted by inhibiting the clotting effect of calcium and thrombokinase on the fibrogen-prothrombase complex. The dye raised blood pressure slightly and had little or no effect on the gas-carrying power of the blood or the buffering action of the plasma. Later Huggett and Rowe[3] reported that many other azo dyes possessed anti-coagulant properties. Of a group which were examined Chlorazol Fast Pink BKS (Color Index 353) was found the most efficient; even more effective than heparin.

The crude dye was obtained from the General Dyestuff Corporation of New York City (trade name: Fastusol Pink BBA). Inasmuch as the crude dye is toxic it must be separated from salts and other impurities. A simple method of purification was sought. It was found that the dye was precipitated from an aqueous solution by alcohol, and a method of separation from impurities was based on this observation.

The crude dye is dissolved in about 15 parts of water and filtered. To the filtrate an equal volume of 95 per cent. alcohol is added, producing almost complete precipitation of the dye. The mixture is filtered and the precipitate saved. The filtrate should be colored only slightly by unprecipitated dye. The precipitate is washed with 70 per cent. alcohol, dried over steam and ground into a coarse powder.

This method of extraction yields about 20 per cent. of purified dye from the crude commercial product. A so-called commercially pure dye prepared by the General Dyestuff Corporation, which is too toxic as such, yields about 50 per cent. of purified dye by the same method. A dye supplied by the Calco Chemical Company (trade name: Calcomine Fast Pink 2BL) appears to be identical with Fastusol.

The purified dye is used in a 5 per cent. solution. It is relatively non-toxic. As much as 1.0 gm per kg produces only a slight increase in blood pressure, with some slowing of the heart and no effect on respiration. We have found that a single intravenous dose of 100 mg per kg (2.0 cc per kg of the solution) prevents clotting for many hours, and that specimens of blood from animals so treated do not clot in the test-tube for twelve hours or more. For most kymograph experiments 50 or 75 mg per kg suffice, but the 100 mg dose is more certain. These doses stain the animal and its urine, and cause bleeding and oozing from recently ruptured capillaries. It is best, therefore, to complete all operative procedures and to obtain complete hemostasis before the dye is injected. The dye should be injected immediately after cannulation.

A method of using the dye which does not involve intravenous injection has also been found satisfactory. Small amounts of the dye (0.5 cc portions) are introduced at about 30-minute intervals into the pressure system of the recording manometer just above the junction of the cannula and the rubber tubing. A fine needle (No. 25 or No. 26) is used. Some of the dye should go into the blood vessel connected with the cannula.

With the use of the dye other anti-coagulating agents in the pressure system are not necessary. We have discarded saturated sodium sulfate and magnesium sulfate and use only physiological saline in the pressure system.

Several hundred experiments of a variety of types have been conducted in this laboratory without any

[1] Dr. David Robert Climenko.
[2] A. StG. Huggett and H. Silman, *Jour. Physiol.*, 74: Proc. 9P, 1932.
[3] A. StG. Huggett and F. M. Rowe, *ibid.*, 78: Proc. 25P, 1933.

clotting or interference with the course of the experiment. Clotting has been prevented even in experiments in which agents which are known to produce intravascular clotting were used. The dye has proved very valuable in experiments conducted by students.

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EFFECTIVE CONTROL OF CULTURE MITES BY MECHANICAL EXCLUSION

INVESTIGATORS working with fungi *in vitro* are unhappily familiar with the common mycophagous mites, which invade their test-tubes and cause many inconveniences by destroying pure cultures or by contaminating them with other fungi or bacteria. Control of these pests is claimed to have been effected by the use of various volatile chemicals which purport to kill the mites and their eggs. Since plant and other materials harboring these mites are brought almost daily into most mycological laboratories reinfestations readily occur necessitating repeated use of these chemicals, most of which are detrimental to fungous growth and noxious to the person using them. The method of control which was developed and is now being used in this laboratory is based on the positive exclusion of the mites from test-tube cultures by mechanical means. The materials to be used are: (a) 10 per cent. gelatin in water to which has been added 2 per cent. $CuSO_4$ to prevent fungous and bacterial growth, (b) 1 book of L.L.F. cigarette papers and (c) a heavy blotter. About 25 cc of the melted gelatin is poured into a petri dish and allowed to solidify. The cigarette papers are taken from the cover, the small dab of glue that holds the sheets together is cut off, the bundle of sheets is cut in halves, placed in a small preparation dish and sterilized in the dry oven. This treatment with dry heat tends to make the papers separate more readily. The ordinary laboratory procedure now follows: The cotton plug is removed, the tube is seeded and flamed, but instead of replacing the plug the tube is inverted and the hot rim is pressed gently against the surface of the solidified gelatin, thus becoming coated with a thin film of melted gelatin. By touching the gelatin-coated rim to the cigarette papers in the preparation dish the top sheet is neatly picked up and removed and then made to adhere more firmly by pressing it against the resilient surface of the blotter. The tube is now placed upright in a rack with other tubes similarly prepared and so arranged that the corners of the projecting pieces of paper touch. By igniting at a single point, the projecting paper on all the tubes will burn off, leaving neat, circular, paper seals that effectively keep out all faunal and floral contaminants. When sub-cultures are to be made the seal is readily burned off by flaming, and after the transfer is completed the process of sealing is repeated as before. For cultures in liquid media the gelatin is melted and painted on the rim of the tube with a small brush and the paper then placed on top by means of forceps. If so desired, the cotton plug may be retained but should of course be shoved well below the rim of the tube before sealing. We have tested several brands of cigarette papers, many other kinds of paper, several grades of Cellophane and other materials. All Cellophanes and treated papers such as waxed papers greatly depressed growth of the fungi and of all the others tested only the one brand of cigarette papers made a perfect seal and burned without leaving an undesirable black residue. The efficacy of the method was tested by placing together in the same drawer sealed cultures, unsealed, cotton-stoppered cultures and unsealed cultures heavily infested with mites. After a period of months only the sealed cultures remained free of mites. This new method of mite control has the advantage over older methods of being effective, nontoxic to fungi, inexpensive and easily applied. An illustrated account of this and other mite-control methods will be published elsewhere.

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