XENIA IN THE CHESTNUT

THE number of species of plants of horticultural importance in which xenia[1] is known to occur is surprisingly small. Since Focke in 1881 used the word xenia with reference to the immediate effect of pollen on the endosperm of *Zea mays* L., this plant has become the classical example of the process. Few references are found in the literature in regard to the type of xenia dealt with in chestnuts (*Castanea* spp.) namely, a measurable effect of the pollen on the embryo of the seed. Blaringhem[2] reported but gave no data on a case of xenia in the chestnut in which time of ripening and size of nut were affected by the kind of pollen used. However, his observations were based upon a small number of hybrid nuts. It should be stated that metaxenia, or the immediate effect of pollen on the maternal or sporophytic tissues of the plant, apparently is of much wider occurrence than xenia due to the complete absorption of the endosperm in most seeds.

In the chestnut the pistillate catkin produces from one to several "burrs," and each of these from one to five flowers, usually three, which if pollinated develop into nuts. Each nut develops from a single ovary. Normally one ovule develops in each ovary to form the seed of the nut, the kernel or fleshy edible portion of the seed being composed of the thickened cotyledons of the embryo. An extensive endosperm is formed soon after fertilization, but the growing embryo quickly absorbs this and "filling" of the nut consists largely of a thickening and increase in size of the cotyledons. Hence the nut of the chestnut is both a fruit and a seed, and the edible portion is the embryo.

The immediate effect of pollen on the seed of the chestnut is expressed through an increase or decrease in the size of the nut, depending upon the kind of pollen used. Since the nut is filled entirely with tissue of the embryo any differential size effect caused by different pollens on the same tree would be a result of the action of the pollen on extent of growth of the embryo. For the past two seasons a tree of the Japanese chestnut, *Castanea crenata* Sieb. and Zucc., has shown this differential effect, producing nuts of distinctly different size as a result of using two kinds of pollen. The tree normally produces large nuts typical of certain varieties of Japanese chestnut. On this tree when pollen from a variety of Chinese chestnut, *C. mollissima* Bl., bearing small nuts is used the mean weight of nuts produced is 18.77 grams. However, where pollen from a variety of Japanese chestnut bearing large nuts is used the mean weight of nuts is increased to 27.12 grams. A difference between the means of eight grams per nut has been observed both seasons, 1937 and 1938, as a result of the two kinds of pollen applied to the stigmas of this tree. With a variance of difference between the means of 1.828 grams this difference is highly significant. Analysis of data from this and other crosses will be presented elsewhere.

During the season of 1938 extensive crosses were made between varieties of three species of chestnut, *C. crenata, C. mollissima* and the European chestnut, *C. sativa* Mill. Many varieties upon which several pollens were used failed to show significant size differences between the nuts harvested. In all three species, however, certain varieties showed a variation in nut size that is clearly indicative of pollen effect on this character. The fact that this effect is not uniformly obtained in all the crosses between varieties or species suggests that nut size in the chestnut may be affected by genetic factors which are inherited from the pollen parent. Since the chestnut is considered to be largely cross-pollinated, the varieties within a species are probably heterozygous to a high degree. On this basis a genetic effect such as herein described could not be interpreted with certainty in all the varieties worked with due to a lack of knowledge of the genetic constitution of the trees. Increase in size of seed when two species are crossed is attributed by certain authors to heterosis or hybrid vigor. This interpretation could be given to instances in the chestnut in which the size of the nut produced by a tree is increased beyond that of either parent by using pollen from a tree bearing large nuts. Since the size of nuts produced by a tree bearing large nuts may be reduced by the use of the proper pollen, the writers consider that variation in nut size in certain chestnut crosses is due to the immediate effect of the pollen on the development of the embryo. It has long been a common observation that certain trees of the American chestnut, *C. dentata* Borkh., produced nuts with split shells, and this may possibly be explained by over-development of the kernel as a result of the action of the pollen of certain varieties.

The results of pollination experiments in the chestnut are at the present time entirely preliminary in nature. The practical implications of the problems involved, however, are many. One of the most significant of these is the fact that the quality of the kernel, as well as the size of nut, may possibly be affected by the variety of pollen used. If this is the case it should be possible to obtain greater uniformity of size and quality in chestnuts by planting the proper combination of varieties for pollination purposes. Chestnuts with split shells are attacked readily by molds and spoil quickly. Since the pollen of certain varieties determines the size of the nuts produced by other varieties, proper pollinators become important in the production of nuts free from split shells.

[1] The generally accepted definition of the term "xenia" is used here, namely, "the immediate effect of pollen on the embryo or endosperm of the seed."

[2] M. L. Blaringhem, *Bull. Soc. Bot. de France*, 66: 354–356, November 14, 1919 (1920).

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.