

MAINTAINING RAGWEED CULTURES

Willard W. Payne and Walter F. Kleinschmidt, Ann Arbor, Mich.

EXPERIMENTS being carried out on the nature of the ragweed plant and hay fever have made it necessary to grow many specimens in the greenhouse and garden. These ragweed cultures provide a continuous supply of material for botanical studies and implement physiologic research on growth and pollen production by the ragweeds. Specimens are also produced for studying the dissemination of ragweed pollen outside the natural ragweed season, and fresh pollen is made regularly available for immunologic and clinical studies.

Techniques for maintaining an adequate supply of viable ragweed seeds available throughout the year to the experimenter were worked out in the growing season and winter of 1959-1960. As research workers in allergy and related fields may be interested in growing ragweed plants for experimentation, our after-ripening technique is described later, along with methods of collecting and storing fruits, culturing plants, and collecting pollen.

COLLECTING RAGWEED FRUITS

Because of their adaptation for wind pollination rather than insect pollination, ragweeds have evolved a structure and appearance quite different from most other members of the composite family, the botanical family to which they belong, along with such familiar plants as chrysanthemums, sunflowers, and dandelions. Flower colors and forms that function to attract insects have been lost. Individual flowers are much reduced and the functions of pollen and fruit production have been relegated to different flowers on different parts of the plant. A normal ragweed plant, therefore, bears heads or flower clusters of two kinds: pollen-producing or male flowers are clustered in heads which are borne in spikes at the tips of the main stem and branches; seed-producing or female flowers are borne singly in modified heads located in the axils of leaves and bracts below the male flower spikes. Fig. 1, A-C, shows male and female heads and their usual arrangement on a branch of giant ragweed.

The seeds are produced in the fall. Each seed is enclosed in a dry fruit surrounded by the fused, hardened bracts (involucre) of the female head.

From the Department of Botany and Botanical Gardens, University of Michigan, Ann Arbor, Mich.

Publication No. 24 on Atmospheric Pollution by Aeroallergens under Research Grant No. E-1379 from the National Institute of Allergy and Infectious Diseases, U. S. Public Health Service.

Received for publication Feb. 16, 1961.

Typical fruits of the more abundant ragweed types are shown in Fig. 1, *D-H*. These are collected in September or October, after they have matured, by grasping the stem a few inches below the tip and stripping it of leaves, fruits, and spikes. After drying for a few days, the fruits are freed from the unwanted elements by rubbing all of the material between the hands. Light gloves are usually worn to protect the hands from the hard, sharp spines. By carefully pouring the mixture of fruits and vegetative fragments from one pan to

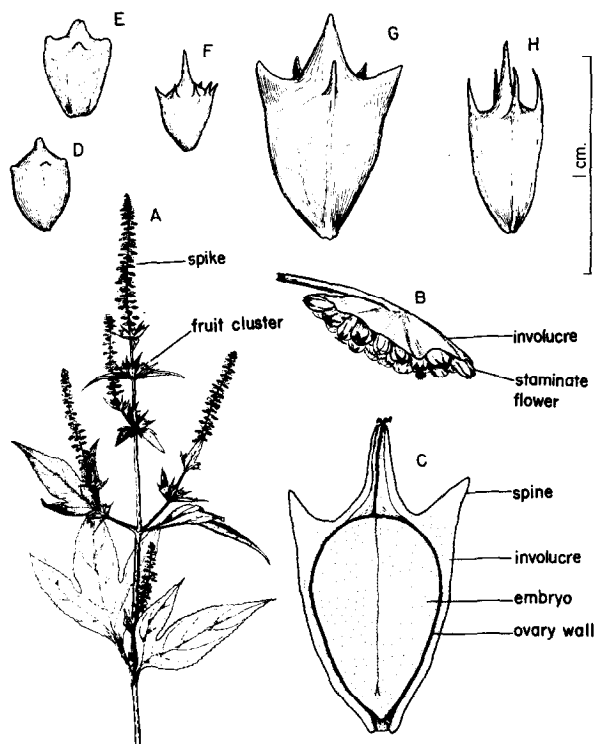


Fig. 1.—Ragweed inflorescence and fruit types. A, Flowering branch of giant ragweed *Ambrosia trifida*, showing the position of male and female heads. B, Enlargement of male head. C, Longitudinal section of a mature fruit. D, Fruit of eastern perennial ragweed, *A. coronopifolia* T. & G. E, Fruit of western perennial ragweed, *A. psilostachya* DC. F, Fruit of common ragweed, *A. artemisiifolia* L. G, Fruit of giant ragweed, *A. trifida* L. H, Fruit of southern ragweed, *A. bidentata* Michx.

another in front of a fan, the fruits are winnowed out. Lighter debris, consisting of pieces of leaves, male heads and stems, is blown away while the heavier fruits fall through the air stream. Empty fruits, those which develop with no seeds enclosed, are light and are blown away with the other debris.

AFTER-RIPENING*

Ragweed fruits are dormant when they are collected from the plant. This dormancy is broken by the process termed after-ripening. After-ripening

*Limited quantities of fruits which have been after-ripened in the manner described will be available during the growing seasons of 1961 and 1962. Fruits of the following species can be obtained by addressing inquiries to the senior author: Low or common ragweed, *Ambrosia artemisiifolia* L.; giant ragweed, *A. trifida* L.; eastern perennial ragweed, *A. coronopifolia* T. & G.; southern ragweed, *A. bidentata* Michx.

is accomplished by allowing the embryos in the seeds to undergo a period of rest during which the conditions required for germination are not present. In northern latitudes it is done by submitting them to a period of low temperatures while supplying them with sufficient water and oxygen. Under natural conditions, the occurrence of winter accomplishes after-ripening. The artificial technique given below is designed to simulate natural environmental conditions as closely as possible.

1. The fruits are placed in cloth bags. Inexpensive cotton broadcloth or similar material made from a blend of cotton and synthetic fibers is used. Old or worn material tends to decompose during the winter treatment and is not satisfactory. The size of the bags is varied to suit the number of fruits to be treated, but the largest bags we use are approximately eight by twelve inches. Plastic bags or jars do not permit development of the proper moisture and oxygen conditions and their use often results in loss of the seed through death of the embryos or failure to break dormancy. If several kinds of fruits are gathered, necessary data is written on the bags with a laundry pen of the ball-point type. Such a pen does not catch in the material as the ordinary quill-type laundry marker does.

Care is taken not to put too many fruits in one bag. Respiration of crowded seeds may raise the temperature in the bag and inhibit after-ripening or promote germination in the bag. Crowding also restricts the oxygen supply by keeping the fruits sodden.

2. The fruits are soaked in lukewarm water for an hour. Since after-ripening is successful and uniform only if the embryos are saturated with water,¹ this is a very necessary step.

3. Approximately an inch of washed sand is placed in a wooden flat and the bags are spread on the sand. The layer of fruits is distributed evenly in each bag and the bags are then covered with another inch of washed sand. It is important that clean sand be used, as the movement of water and gases may be restricted if the sand contains too much clay or humus. At our Botanical Gardens a commercially available type known as "fine, washed, plastering sand" is used, but any clean sand is satisfactory.

4. The flats are sprinkled well with water and placed outside where they will not be disturbed. Since after-ripening requires approximately three months, this should be done by late November or early December. If done too early, however, mice or other rodents may be attracted to these handy caches of food and may eat or carry away most of the fruits. If rodents are abundant the flats can be protected by covering them with screens. The flats should not be covered with boards or other impermeable materials which will shield them from snow or frost. A shallow, open cold-frame is ideal.

5. The flats are brought inside in the early spring while the seeds and sand are still frozen. At our location this is late February or early March. Seeds may be germinated immediately or stored for future use. The after-ripened fruits are stored in two ways: (a) The frozen fruits are left in their after-ripening bags and placed in a freezer. Seeds stored in this way in our laboratories have remained viable for nearly a year. When plants are needed, a

quantity of fruits is removed from the bag, thawed, and sowed immediately. (b) The fruits are removed from the bag, spread in a cool place and air-dried as quickly as possible. Two days is usually adequate. Dried fruits are stored in loosely covered jars which allow circulation of oxygen between the air of the jar and the outer atmosphere. Seeds stored thus give us approximately 70 per cent germination ten months from the date of drying. Although no marked decrease in viability had been noted at the time this article was prepared, the seeds were expected to return eventually to a dormant condition. For this reason it is recommended that a new supply of fruits be grown and after-ripened annually to maintain a reliable source of viable seeds.

CULTURE OF PLANTS FOR POLLEN PRODUCTION

Three to four months before mature plants or fresh pollen are needed, a quantity of fruits is planted in a pot filled with light garden soil. The fruits are pressed firmly into the surface of the soil and covered with a thin layer of dry sand. The pot is then watered generously and placed in a cool greenhouse until germination occurs. The pot is covered with a pane of glass to prevent drying of the soil before the seedlings have developed.

The seedlings are potted off into small pots (two- or three-inch) as soon as they have emerged. If delayed too long the risk of loss of the seedlings through the activities of damping-off fungi is increased. After approximately a month the plants are potted in five- or six-inch pots.

Under a regime of normal day lengths, that is, the comparatively long days of spring and summer, the plants reach the pollen-producing stage in approximately three months. As each male head contains numerous flowers (Fig. 1, *B*) which mature in a gradual succession, the period of pollen production of a given head may extend over three or four weeks. A spike will produce the greatest quantity of pollen daily approximately a week to a week and one half after the initial flowering. Since spikes of the lower branches mature later than the terminal spikes, the period of pollen production for a given plant may be a month or more. Best results are obtained over prolonged periods by staggering plantings of fruits a week apart so that only the best producers need be used during the period for which pollen is needed.

The short days of winter cause the plants to mature when young and produce but few male heads or none. This phenomenon has been investigated by Jones^{2, 3} and has been amply demonstrated in our greenhouses. Nearly normal plants can be grown if incandescent or fluorescent lights are used to extend the day length to twelve hours. In general, however, satisfactory pollen production is difficult to obtain in November, December, and January.

COLLECTING THE POLLEN

Pollen can be gathered in a number of ways. As the pollen is shed shortly after dawn, it must be collected by eight or nine o'clock in the morning. If many plants are grown, large quantities of pollen can be collected with a vacuum pump to which is attached a Buchner-type funnel with a fritted disc.

The funnel is run up and down the side of the spike while the spike is gently agitated. A few days' observation of the producing plants will reveal the best time for this operation. A somewhat more efficient but more painstaking method is to tap the spikes over waxed paper, allowing the pollen to fall onto the paper. The most efficient technique for gathering pollen is to allow the spikes to shed their pollen on paper after they have been removed from the plant.⁴ For this last technique a quantity of spikes is cut the evening before the pollen is needed. The spikes are arranged on clean paper and covered to protect them from air currents. The flowers will discharge the following morning and the spikes are then lightly tapped against the paper to dislodge clinging grains and discarded. This method is of special value if greenhouse facilities are not available and the plants must be grown out of doors where morning breezes blow the pollen from the plants before it can be collected. The technique has the disadvantage of limiting the effective period of pollen production of a given spike.

If not used immediately pollen is dehydrated and stored in an air-tight chamber containing calcium chloride.

SUMMARY

A practical method is described for maintaining ragweed cultures. Fruits are stripped from mature wild or cultivated plants in the fall, winnowed, and placed in appropriately labeled cloth bags. These are soaked for an hour in warm water, placed on clean sand in wooden flats and covered with another inch of sand. The flats are placed out of doors in late November or December where they will be undisturbed but exposed to normal climatic conditions. In the spring the flats are brought inside before the ground has thawed. If not planted immediately, the fruits are stored frozen in their bags or are dried and placed in loosely covered jars. Seeds are planted in light loam. Seedlings are potted as soon as possible and repotted as required by the growth of the plants. Pollen is collected with a vacuum pump, by tapping the spikes on waxed paper at the time of discharge, or by allowing covered, cut spikes to shed their pollen on clean paper.

REFERENCES

1. Davis, W. E.: Primary Dormancy, After-Ripening and the Development of Secondary Dormancy in Embryos of *Ambrosia trifida*, Am. J. Bot. 17: 58-76, 1930.
2. Jones, K. L.: Studies on *Ambrosia*. II. Effect of Certain Environmental Factors on Floral Development of *A. elatior*, Bot. Gaz. 98: 296-306, 1936.
3. Jones, K. L.: Studies on *Ambrosia*. IV. Effects of Short Photoperiod and Temperature on Sex Expression. Am. J. Bot. 34: 371-377, 1947.
4. Kelley, J. W.: Methods of Collecting and Preserving Pollen for Use in the Treatment of Hay Fever, U. S. Dept. Agric. Circ. 46, 1928.