Artificial Hybridization and Self-Pollination

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The wide diversity of floral structure and development among crop species has led to an array of methods for artificial hybridization and self-pollination. Despite this diversity, some principles common to all methods must be followed to be consistently successful. The purpose of this chapter is to summarize these general principles. Examples and illustrations are taken from other chapters that describe methods for individual crops. The reader is referred to those chapters for additional detail and references.

I. SELECTION OF PARENTS

Selection of parental material is the first step in a plant breeding program. The choice depends on the objective of the program and the relative merits of available germplasm (Chapter 4). It also may depend on the feasibility of hybridization when genotypes from different species or genera are involved (Chapter 7).

When the choice of parents has been made, hybridization may be facilitated by carefully determining the genotype to be used as either female or male. Male-sterile genotypes automatically would be used as females. The choice is not so obvious in many other situations; nevertheless, proper choice of the female can determine success of the cross. In sweet potato, reciprocal crosses routinely are made between clones, and the relative rates of pollen tube development are measured in the two parents. Compatible sweet potato crosses are those in which pollen grains adhere to the stigma, and pollen tubes penetrate the style. Selection of the female also can be important for interspecific and intergeneric hybridization, for which the breeder must consider chromosome number, seed size, and other characteristics of the parents (Chapter 7).

Copyright © 1980 American Society of Agronomy-Crop Science Society of America, 677 S. Segoe Road, Madison, WI 53711. Hybridization of Crop Plants.
A common technique in the choice of female parents is the use of genetic markers to identify accidental self-pollinations. Genetic markers preferably are qualitative characters controlled by one or a few genes. The parent chosen as female would have the recessive allele, and the male would have the dominant allele. Seeds or plants from self-pollination would have the recessive character of the female, and hybrids would have the dominant character of the male.

Some genetic markers can be used to differentiate selfed and hybrid seed on the female plant. Waxy endosperm of sorghum is controlled by a single recessive allele and normal endosperm by the dominant allele. In a cross between a female parent with waxy endosperm and a male parent with normal endosperm, waxy seed would result from self-pollination and normal seed from hybridization. Seed markers frequently are used for genetic studies or for determining the amount of outcrossing in a species. They seldom are used as genetic markers in a conventional breeding program because usually only one form of the character is desired and present in breeding lines. Shrunked endosperm, a genetic marker of corn, is an undesirable character in commercial hybrids; therefore, genotypes commonly used by corn breeders have only the dominant allele for normal endosperm.

Genetic markers of seedlings or mature plants are used routinely in some species. To use a seedling or mature plant marker, seeds are harvested from the female parent with the recessive allele. The seeds are sown and the plants examined for the recessive character of selfed plants or the dominant character of hybrid plants. Hypocotyl color of sugarbeet, awnedness of wheat, and pubescence color of soybean are examples of commonly used markers.

Selection of the female parent determines the cytoplasm present in progeny from the cross; therefore, many commercial cultivars may have the same cytoplasm if the same genetic marker is consistently used. Dwarfness of rice recently has become a desirable agronomic character. The dwarf cultivar ‘Cina’ was used as female in crosses with conventional tall cultivars because dwarfness is recessive. Continued use of dwarfness as a genetic marker has caused many cultivars to have the same cytoplasm from Cina. Lack of cytoplasmic diversity may have undesirable consequences for improvement of the species or susceptibility to plant pests.

II. PREPARATION OF THE FEMALE PARENT

A. Coordination with Time of Pollination

Preparation of the female parent must coincide with availability of pollen from the male. Synchronization of flowering may require use of special techniques at planting or during the growing season, particularly when early and late flowering parents are to be mated (Chapter 1 and 4).

The timing of female preparation during the day depends on the time of pollen availability and the need for immediate pollination of the female after it is prepared. Pollen of lentil is available in the morning, and it is most efficient to emasculate the tiny flower and pollinate immediately while the flower is in hand and readily identified.
Preparation of the female during one period of the day and pollination during another may be required to minimize selfing, to obtain maximum success, or to efficiently spread the work load throughout the day. In northern India and Mexico, hybridization of chickpea is most successful with evening emasculation and morning pollination, even though immediate pollination is possible. Females of corn can be prepared any time of the day with equal success; however, the optimum time of pollen availability will vary with weather conditions. Corn breeders adjust their schedule to pollinate whenever pollen is most available and prepare females the remainder of the day.

A delay of 1 or more days between emasculation and pollination of perfect flowers can reduce the possibility of accidental self-pollination. Flowers of wheat, oat, and barley commonly are emasculated before the anthers are mature and are pollinated 2 or 3 days later when the stigmas become receptive.

Duration of stigma receptivity may influence the delay between emasculation and pollination. The duration is short for species like cowpea, in which receptivity occurs from the day before anthesis until noon of the day of anthesis. In contrast, pollinations of sugarbeet can be made immediately after emasculation or up to 12 days later.

B. Selection of Female Flowers

An important step in artificial hybridization or self-pollination is identification of female flowers at the optimum stage for preparation. Flowers that are too immature may be difficult to manipulate, and the percentage of successful pollinations may be reduced. Flowers that are too fully developed may be accidentally selfed or may be pollinated by an unselected male.

The optimum stage for the female flower commonly is determined by its outward appearance. Knowledge of the relationship between floral appearance and development of the sex organs improves the efficiency of selecting appropriate flowers. The broadbean has a perfect flower; therefore, a flower may serve as either a female or male for hybridization. A broadbean flower used as a female usually is emasculated when the petals are about twice the length of the calyx (Fig. 1). A broadbean flower used as male would be at a more advanced stage of development. In chickpea and flax, flower buds that have a small amount of the petals protruding beyond the calyx are selected for emasculation. Panicles of wild rice to be used as females are prepared during the boot stage.

Differences among genotypes and environments can alter the relationship between outward appearance and development of the sex organs; therefore, precisely determining the suitability of a flower for preparation often requires that the flower be opened. The female flowers of cassava, a monoecious species, are bagged the morning of the day they open (Fig. 2). A drop of nectar at the base of the corolla is the most certain way to tell that the female cassava flower will open shortly (Fig. 3). Florets of wheat appropriate for emasculation have light-green, well-developed anthers and feathery stigmas that extend about one-fourth the length of the florets. If
the anthers are yellow or cream-colored, mature pollen may be shed if the anthers are ruptured during emasculation.

Success of hybridization can be influenced by age of the flowers relative to others on the same inflorescence and among other inflorescences on the same plant. The first inflorescences of broadbean generally are considered the best choice, and success is more likely with flowers from the base and middle of the inflorescence than from the top part. A vigorous plant of lentil in the initial stages of flowering is best suited for hybridization. The flowers that first appear in a spike of jute have the highest percentage of successful pollinations.

C. Preparation Without Emasculation

Emasculation is not a universal requirement for artificial hybridization. A few self-pollinated species can be prepared and pollinated immediately without emasculation. Genotypes with male sterility, self-incompatibility, or protogyne, and monoecious and dioecious species do not require

Fig. 1—Broadbean inflorescence with flowers at various stages of development. The flower marked with the arrow is at the stage suitable for emasculation. The largest flower is at the stage in which pollen shed usually occurs (Reproduced by permission of D. A. Bond, D. A. Lawes, and M. H. Poulsen from Chapter 11).
Fig. 2—Branches of cassava with female flowers are covered with a cloth bag in the morning and pollinated later in the day (Reproduced by permission of K. Kawano from Chapter 13).

Fig. 3—A drop of nectar at the base of the corolla of a female cassava flower is the best indication that the flower will open shortly (Reproduced by permission of K. Kawano from Chapter 13).
emasculation.
When self-pollinated species with male-fertile flowers are not emasculated, the stigma must be receptive at least 1 day before anthers of the flower can shed pollen. In soybean, the calyx and corolla are removed, and the stigma is pollinated immediately. Lack of self-pollination in such cases suggests that when cross-pollination is unsuccessful, the flower dries up before its anthers can dehisce.

The most common procedure for preparing a female flower that will be hand pollinated without emasculation is to cover it to avoid accidental pollination. Female flowers of cassava are enclosed in cloth bags (Fig. 2). The female ear of corn is covered with a glassine bag before the silks emerge.

D. Emasculation

Several procedures of emasculation have been developed. Some of the procedures are used in many different species, and others are used in a restricted few.

1. Direct Anther Emasculation

Direct removal of anthers is the most widely used procedure for emasculation. Techniques and instruments vary among species and breeders, but they all have the common objective of manually removing the anthers.

A common first step in direct anther emasculation is removing underdeveloped or overdeveloped flowers from the inflorescence. This reduces competition for nutrients, eliminates accidental self-pollination of emasculated flowers, and prevents accidental harvest of selfed seed. In barley and other grass species, underdeveloped spikelets at the base and tip of the spike and unwanted florets in the remaining spikelets are removed (Fig. 4B). Usually only one flower of a peanut inflorescence is emasculated, and all immature buds and previously pollinated flowers are removed.

The floral parts that cover the sex organs may be entirely or partly removed, or may be left intact. The petals of guar are completely removed to facilitate removal of the anthers. The lemma and palea of barley florets are clipped off just above the anthers (Fig. 4C). There is some evidence that clipping the floret of barley and wheat may slightly reduce seed set and seed weight, but it is much easier and faster than spreading the lemma and palea apart to remove the anthers. Clipping of the florets usually is not practiced if desiccation is a problem. Seed set of jute is poor if the calyx and corolla are removed during emasculation.

The anthers may be extracted during removal of the floral parts covering the sex organs. Sesame breeders routinely pull the corolla from the flower and simultaneously remove the stamens, which are attached to the corolla. The anthers and petals of cotton flowers can be removed with fingernails in one operation.
Fig. 4—Steps in the emasculation and pollination of barley. (A) Unwrapping spike from flag-leaf sheath, (B) removal of lateral florets, (C) clipping lemma and palea before removal of the anthers, (D) removal of the anthers, (E and F) pollination of the female spike by the swirl method (Reproduced by permission of T. M. Starling from Chapter 10).
Anthers commonly are removed with forceps that are appropriate for the size and structure of the flower. Some oat breeders prefer sharp, pointed forceps with curves ends, while others prefer straight ends. Sometimes they file the tip of the forceps to create a flat edge that is less likely to damage the glumes, lemma, and palea. Some barley breeders prefer forceps that can be easily closed to prevent fatigue of the fingers. They may grind the tips to a thin, square shape with a blunt point and use them as a cutting tool for removing the tip of the spike and other unwanted florets. Forceps for emasculation of field pea usually have a sharp point.

A spear-pointed needle, pencil, stick, or scalpel is used instead of a forceps by breeders of a few species. A needle is used to make an incision in the keel of the lentil flower, and the 10 anthers are teased out. The procedure is best suited to conditions of low relative humidity when it is desirable to keep the stigma from drying. A pencil or stick of similar size is preferred by some sorghum breeders to tease the anthers from the spikelet. A scalpel reduced to about half its normal blade width can be used to remove the anthers of potato flowers.

Special tools have been developed to aid emasculation of a few species. A Spinlab device is used for emasculation of cotton. It consists of a circular opening with a cutting surface for removing the petals and a smaller orifice for stripping away the anthers.

Removal of anthers by suction is preferred by some breeders. The basic components of a suction system are an electric aspirator with adjustable
vacuum pressure, a filter to prevent anthers from entering the aspirator, and a glass tube inserted in a rubber tube. The system used by the International Rice Research Institute permits several persons to use a single power source (Fig. 5). The glass tube is drawn to a fine point with an aperture appropriate for the size of the anthers. A 1-mm aperture is used for emasculation of alfalfa flowers. The amount of suction is adjusted to permit removal of the anthers without injuring the stigma. A suction of 500 mm Hg is used to emasculate rice flowers.

Fig. 6—Pipette placed in the floret of rice to remove the anthers by suction emasculation (Reproduced by permission of W. R. Coffman and R. M. Herrera from Chapter 36).
Flowers for suction emasculation are prepared in the same manner as those in which forceps are used. The glass tube is inserted into the flower and gently moved until all anthers are removed (Fig. 6).

No tools are required to emasculate a few species. Sainfoin, a forage legume, is emasculated by applying pressure to the base of the keel, which causes the staminal column to protrude. The stamens are removed by rubbing them between the thumb and forefinger.

2. Scissor Emasculation

Emasculation of rye, barley, and other grass species can be accomplished by cutting the young anthers, but not removing the fragments that remain. A spike is chosen about 1 week before any of its florets will have anthers with mature pollen, a stage several days earlier than for direct anther removal (Fig. 7). The anther tips are removed by cutting off the

Fig. 7—Scissor emasculation of rye. Left to right: Rye spike at the proper stage for emasculation, and emasculation of the spike (Reproduced by permission of D. D. Morey and R. D. Barnett from Chapter 37).
upper one-third of the floret with small, sharp scissors. Care must be taken to assure that all anthers are cut. The cut cannot be made too low because the stigma may be injured excessively. Parts of the anthers below the cut are not removed because they dry up and do not produce viable pollen. The florets are pollinated at the same stage as those prepared by direct anther removal.

Scissor emasculation is preferred by some breeders because it is faster than direct anther removal. The frequency of self-pollination seems to be slightly higher than with direct anther removal, and the use of genetic markers to identify hybrid plants is desirable. The stigmas of florets prepared by scissor emasculation are vulnerable to desiccation and must be properly covered until they are receptive to pollination.

3. Thermal Inactivation of Pollen

Viable pollen can be destroyed at temperatures that do not injure the pistil. This principle is used for hot water emasculation of some grass species. Temperatures of 46 to 47°C for 3 min or 48°C for 1 min kill the pollen of smooth bromegrass. Sorghum pollen is killed when exposed to about 47 to 48°C for 10 min. Pollen of rice is inactivated by soaking the panicles for 5 min at 43°C.

An inflorescence is chosen that has just begun to flower and the open florets are removed. The inflorescence is immersed in hot water or is exposed to moist, heated air of precisely the correct temperature. The water usually is contained in a vacuum bottle (Thermos) of adequate size to accept the inflorescence. It is difficult in the field, however, to bend a sorghum plant sufficiently to place the panicle in a bottle. The panicle is enclosed in a sleeve of rubber or plastic tied tightly around the peduncle and open at the top. Water of the proper temperature is poured into the top of the sleeve.

Panicles are allowed to dry before they are enclosed in a bag to await pollination. Florets of rice are pollinated 30 to 60 min after hot water treatment, and any unopened florets are removed.

The principle of thermal inactivation of pollen has been used to achieve temporary male sterility by regulation of greenhouse temperatures. Bluegrass plants become male-sterile, but retain female fertility when maintained at 29°C day and 20°C night temperatures for 11 to 19 days. Low temperatures of 0 to 5°C can be used to achieve temporary male sterility of timothy.

4. Alcohol Emasculation

Sterilization of hybridization equipment is routinely accomplished by dipping the instruments into alcohol. Exposing flowers of forage legumes to alcohol for a brief period will inactivate pollen within their anthers.

Alcohol emasculation of alfalfa is accomplished by immersing the whole raceme in a beaker of 57% ethanol for 10 sec and then washing it in water for a few seconds. Exposure of red clover to concentrations of
ethanol as low as 66.5% for 10 to 20 sec, followed by a water rinse, prevents self-pollination of self-compatible genotypes.

Species differ in susceptibility of the stigma to injury by exposure to alcohol. A lower percentage of alfalfa flowers set seed when exposed to alcohol than do flowers emasculated by suction. In contrast, concentrations of ethanol up to 88.5% have no effect on the pistil of red clover.

5. Dehiscence Control

High humidity delays anther dehiscence and can be used to facilitate or eliminate emasulation, primarily of grass species. Flowers of some grass species open at the time of pollen shed. To reduce the labor required for direct anther removal from immature buds, the florets are held under high humidity during floral opening so that the anthers do not dehisce. Anthers are removed from open flowers much more easily than from immature ones. In sorghum, open florets are pollinated without anther removal.

A panicle can be kept in high humidity for several days until the desired number of florets have opened and been pollinated. Compared with direct anther removal from immature florets, this method increases the number of florets on a plant that can be pollinated.

High humidity can be achieved by 1) covering the panicles with a paper bag under very humid field conditions, 2) covering panicles with a polyethylene bag, 3) using a humidifier in a greenhouse or growth chamber, or 4) spraying the greenhouse floor with water when natural humidity is high. Temperatures may become excessive under a polyethylene bag; therefore, the bag sometimes is put on the plant in the evening and removed after hand pollination the following morning.

Dehiscence control is considered effective when many seeds are desired and some selfs are not objectionable.

E. Prevention of Contamination

An important consideration during preparation of the female is to prevent undesired pollen from reaching the stigma. One of the most important means of avoiding contamination is to select female flowers at the proper stage of development. Use of flowers that are excessively mature increases the risk of accidental self-pollination. When emasculation is used, inspection of flowers for complete anther removal and accidental anther dehiscence can minimize self-pollination. A magnifier may be necessary to adequately examine flowers that are extremely small (Fig. 8).

Undesired pollen can be present on plant parts, on hybridization equipment, and on the hands of the breeder. When changing parents, the most common practice for disinfection is to rinse the instruments used for emasculation and pollination in alcohol or water. A similar rinse is needed when anthers accidentally dehisce during emasculation. Some breeders
prefer to wash their hands when changing parents, particularly during pollination.

Rinsing the flowers is not a common practice for disinfection; however, it is used in a few situations. Sorghum panicles are rinsed in water before emasculation under greenhouse conditions in which free pollen can remain viable for extended periods. A similar rinse is seldom used in the field, where free pollen loses its viability quickly. The stigma of cotton sometimes is disinfected with 30% alcohol to destroy pollen grains when an anther is accidentally crushed during emasculation.

Female flowers of some species must be covered to prevent undesired pollinations, even if emasculation is not necessary. Types of covering include cloth bags (Fig. 2), soda straws (Fig. 9), paper bags (Fig. 10), dialysis tubing, and tissue paper. Covering emasculated flowers prevents desiccation, as well as excluding pollen.

The need for covering the female flowers is not universal among species and may vary among environments. Flowers of tomato, potato, chickpea, and peanut are not contaminated if left uncovered. Heads of safflower are covered with a paper bag in the field, but not in a greenhouse that is free of insects.

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Fig. 8—Use of magnifying glasses to prepare an inflorescence of red clover for pollination (Reproduced by permission of N. L. Taylor from Chapter 16).
F. Identification of the Female Flower

Identification of the female flower may be necessary to locate it for pollination. The identification may be simply the paper bag protecting the flower. Stamping on the bag the date on which the flower was covered aids in determining when the flower is ready for pollination.

A tag may be hung on the plant to record the date of preparation and other necessary information. The same tag may be used at pollination to record additional information (Fig. 11). Tags of different colors may be used to permit quick identification of flowers prepared on different days. Wood stakes or plastic flags may be used to identify plants on which female flowers have been prepared, particularly when the prepared flowers are obvious.

III. POLLINATION

A. Selection of Male Flowers

Pollen collection is most efficient when anther development can be related to outward appearance of the flower or inflorescence. A spike of barley with suitable pollen generally is near emergence from the flag leaf.
sheath. Pollen of soybean generally is obtained from an open flower with a fresh appearance (Fig. 12).

The relationship between anther development and flower appearance may differ among genotypes within a species, and among locations, days, and time of the day. Availability of pollen can be determined precisely by stimulating the anthers to dehisce. This may involve striking or stroking the inflorescence and watching for pollen, or removing an anther from the flower with a forceps and brushing it on the thumbnail to check for pollen shed.

B. Pollen Collection

Collection of pollen is simplest when it is used immediately for pollination; therefore, preparation of the female usually is timed to correspond with natural pollen shed (Section IIA). Immediate use of pollen assures...

Fig. 10—Bag placed over the inflorescence of sesame to protect it from foreign pollen (Reproduced by permission of D. M. Yermanos from Chapter 39).
maximum viability, minimizes identification of the male flower, and minimizes the time required for a pollination. Pollen may be removed from the male flower without removing it from the plant, or flowers may be collected and carried to the female. Tomato pollen can be collected by stripping the anther cone with a flattened or spearpoint needle, and the pollen-laden needle is used to pollinate the stigma of the female parent. Flowers of pigeonpea are removed from the plant, placed on moist filter paper in a covered petri dish, and used for pollination throughout the day.

Pollen carried by wind or insects can contaminate the desired pollen source. To prevent contamination, male flowers may be prevented from opening or covered with a bag. Flowers of sweet potato that are to be used as pollen sources are prevented from opening by placing a paper clip or piece of soda straw over the corolla the day before they open (Fig. 13).

Covering male flowers to prevent contamination usually coincides with pollen collection. Free pollen of grass species loses its viability in a few hours; therefore, male flowers of crops like wild rice, corn, and sorghum

Fig. 11—Wings and standard of cowpea returned to their original position after pollination and secured with tape to prevent desiccation. The tag can be used to identify emasculated female flowers and to record parentage of the cross and other information after pollination (Reproduced by permission of H. T. Blackhurst and J. C. Miller, Jr. from Chapter 21).
are bagged 12 to 24 hours before they are used for pollination. During the period, foreign pollen grains on the flowers lose their viability, additional foreign pollen is excluded, and pollen is collected for use the next day. Before the bag is removed, the male inflorescence is bent horizontally, and the bag is struck sharply to cause additional anther dehiscence.

Pollen of some species is collected from detached branches or flowers. Lateral branches of hop, a dioecious species, are cut from male plants the day before anthers will shed pollen. The branches are taken to the laboratory and placed in water in a glass beaker or test tube that is taped to the inside of a Plexiglas cylinder (Fig. 14). The anthers shed their pollen onto wax paper below the cylinder, from which it is collected and used for pollination. Flowers of tobacco are collected about 24 hours before they are to be used for pollination, placed in a 50-cc beaker containing 10 cc of water, and stored in an insect-free room at room temperature and ambient relative humidity. Although the flowers generally are used within 1 day, they are useful for up to 10 days, and seed has been obtained with 3-week-old flowers if stigmas were heavily covered with pollen.

Special tools have been developed for collection of bulk pollen from some species. A mechanical vibrator is used to collect pollen of tomato and potato (Fig. 15). Vibration of the flower causes the anthers to shed their pollen into a receptacle. A person can use the vibrator to collect enough pollen in 30 min to use for about 1 day of pollination. More elaborate vibrators are available for collection of tomato pollen for hybrid seed production. A small vacuum pump and a spore collector are used to collect pollen of some grass species.

Fig. 12—Soybean flower at the stage when pollen shed usually occurs (Reproduced from Chapter 42).
C. Pollen Storage

Maintenance of viable pollen may be necessary when male and female flowers are not available at the same time. Procedures for pollen storage are well understood for some species, but not for others.

Pollen of some species remains viable for extended periods without special treatment. Pollen of castor will remain viable several days after the flower is picked, and flowers have been sent through the mail when delivery could be made in 3 days or less. Tomato pollen retains good viability under dry room conditions for several weeks.

Low temperature is a common requirement for storage of pollen. Pollen of pearl millet collected in glassine bags and sealed in glass jars at 4°C will retain its viability for over a week, but its viability approaches zero after

Fig. 13—Male flower of sweet potato closed with a soda straw or paper clip to prevent insects from contaminating the flower before it is used for pollination (Reproduced by permission of A. Jones from Chapter 46).
only 3 days at 27°C. Pollen of potato can be stored for a month at 2.5°C or a year at −24°C.

Low relative humidity is considered important for pollen storage of some species. Potato pollen stored in a desiccator has longer viability than pollen stored at higher humidity. Pollen of sugarbeet stored in closed vials in a desiccator for 5 years at 0 to −10°C has been used to achieve normal seed set.

High relative humidity is preferred for pollen storage of some species of Gramineae. Storage of rye pollen requires relative humidity of 80 to 100%.

D. Pollen Application

1. Direct Pollen Application

The most common method of hand pollination is the direct application of pollen to the stigma using one of several different techniques and instruments. The anthers of tobacco are brushed onto the stigma without any

Fig. 14—A detached branch of hop containing male flowers from which pollen will be collected in the laboratory (Reproduced by permission of A. Haunold from Chapter 27).
Fig. 15—Collection of bulk pollen of potato with a battery-powered vibrator (Reproduced by permission of R. L. Plaisted from Chapter 34).

Fig. 16—Application of pollen to the stigma of a tobacco flower (Reproduced by permission of E. A. Wernsman and D. F. Matzinger from Chapter 47).
special instruments (Fig. 16). A pollen-laden stigma of common bean is removed from the male flower with forceps and brushed onto the stigma of the female (Fig. 17). The anther column of soybean is removed by forceps from the male flower and brushed against the stigma. Pollen of sunflower is applied with a piece of cotton or an artist’s bush, or the heads of the two parents may be rubbed together. The alfalfa flower is tipped and its stigma strikes pollen on the tip of a piece of cardboard (Fig. 18). Pollen collected in a bag from the tassel of corn is poured on the silk.

Direct pollen application is the only practical way to pollinate some species. For species in which indirect pollination is possible, the direct method may be used only when pollen is limited.

2. Indirect Pollen Application

It is possible in some species to pollinate without placing pollen by hand onto the stigma of each flower. Indirect pollen application involves dissemination of enough pollen close to the female flower that some will
land on the stigma. Direct pollination of hop, a dioecious species, requires that the bag covering female flowers be removed and pollen brushed against the stigmas. A more convenient procedure of indirect pollination involves inserting a hypodermic syringe into the bag and injecting pollen. The hole is covered with tape or glue, and the bag is agitated to disperse the pollen.

The swirl method of pollination, also referred to as the go-go or twirl method, is used for wheat, triticale, and similar species when the pollen source is plentiful. For this technique, a male inflorescence is chosen that is shedding pollen, and the upper one-third of each floret is removed to facilitate pollen dispersal. The top of the bag protecting the emasculated flowers is cut off, the male inflorescence is inserted and rotated vigorously to stimulate anther dehiscence, then the bag is resealed (Fig. 19). Some breeders prefer to remove the bag protecting the female, use one hand to enclose the female and male inflorescence, and roll the male around the female (Figs. 4E and F). Other breeders prefer to remove the bag protecting the female, insert the female in a paper cone, and shake the male inside it.

The approach method is a form of indirect pollen application used in grass species. Female inflorescences are prepared in the same manner as for direct pollen application. One or more male inflorescences are chosen that have just begun to shed pollen, and a portion of each floret may be removed to expose the anthers. One or more females are positioned slightly below a male inflorescence, and both are enclosed in a bag (Fig. 20). Pollen from the male drops onto the female below to effect pollination. Some breeders strike the bag sharply each day to stimulate pollen shed.

Fig. 18—Tripping an alfalfa flower with a folded piece of cardboard (Reproduced by permission of D. K. Barnes from Chapter 9).
Detached male inflorescences can be used for the approach method (Fig. 20). The detached male inflorescence is placed above the female flowers, and the cut stem is placed in a container of water that has been tied to a pole or taped to the stem of the female plant. Detached inflorescences usually will supply pollen for several days if provided with adequate water.

The approach method has the advantage of providing pollen over an extended period of time and usually results in a higher percentage of seed set than direct pollen application. The method is considered more rapid than direct pollination, but slower than the swirl method.

Mutual pollination is a method of indirect application used to hybridize genotypes with a high degree of self-incompatibility, such as forage grasses. Inflorescences of similar maturity from each genotype are placed together at the same height in a bag or isolated enclosure. The bag or stalks are agitated several times a day to stimulate pollen dispersal. Seed from the mutual pollination may be bulked when only two genotypes are used, or kept separate when several male and female parents are in the same enclosure.

Fig. 19—Pollination of an emasculated spike of triticale by the swirl method (Reproduced by permission of E. N. Larter and J. P. Gustafson from Chapter 49).
E. Prevention of Contamination

Cleaning pollination equipment when changing male parents is essential for avoiding contamination with foreign pollen. This includes fingers and hands when they are in contact with the pollen, such as for the swirl method of pollination (Fig. 4E and F). Ethanol commonly is used to clean equipment. Water also is used, and some persons prefer to place forceps or brushes in their mouth to clean them.

Covering the female after emasculation prevents contamination and minimizes desiccation of the pistil. The covering is removed for pollination and usually is replaced immediately (Figs. 9 and 10). The bag used to collect pollen also may be used to cover the female. The plant parts that enclose the pistil may be opened for pollination, then returned to their original position. The flower also may be held closed to minimize desiccation (Fig. 11).

![A spike of wheat from the male parent is positioned above the female parent for the approach method of pollination. The detached male spike is placed in a reservoir of water that is taped to the stem of the female parent (Courtesy of R. E. Allan, USDA-SEA, Washington State Univ., Pullman, WA 99164).](image)
Bagging the female flower after pollination can reduce seed set of some species. Pod set of pigeonpea is reduced under glassine bags.

Foreign pollen carried by wind or insects can contaminate the source of pollen. Such contamination can be avoided by covering the male flower for the time necessary for the foreign pollen to lose its viability (Section IIIB).

F. Identification of a Pollinated Flower

Pollinated flowers usually are identified by a tag or by the bag used to cover the female. Information recorded for identification may include the parentage of the cross, date of pollination, and number and position of pollinated flowers (Fig. 11). When parents are planted in pairs and only one cross is possible, parentage may be recorded at the time of seed harvest, thereby reducing the time spent recording information during pollination. Pollinated flowers may be marked only with string or plastic ties that are color-coded or stamped to identify different male parents. Such a practice is used for large-scale hybridization of tomato. It also is used for pigeonpea flowers, which are too small to be identified with a tag.

Identification of peanut flowers is unique because the seed develops underground. The portion of the flower that penetrates the soil must be marked with foil or wire, so that it can be identified at harvest.

New buds may develop on an inflorescence after hand pollination is complete and seed set by self- or cross-pollination. Such flowers can be confused with hand-pollinated ones, particularly when the identification tag is placed below the inflorescence and not on an individual flower. Removal of part of the calyx, glume, lemma, or other floral covering can differentiate hand pollinated from naturally pollinated flowers. Systematic removal of new flowers may be necessary to eliminate errors at harvest.

IV. SELF-POLLINATION

Natural self-pollination occurs in species with perfect flowers that are self-compatible and do not open until pollen shed has taken place. A small percentage of cross-pollination occurs in almost all species, but the amount often is so low that manual exclusion of foreign pollen is not practiced. Oat, wheat, and lentil are a few of the species that do not require any outside interference to effect self-pollination.

Some species with perfect flowers must be protected to assure complete self-pollination. Sesame often is described as a self-pollinated species, but cross-pollination up to 65% can occur. Inflorescences of sesame are placed in bags to exclude insects (Fig. 10). Bagging also is used to self forage grasses that are wind pollinated. Selfing of cotton and sweet potato involves closing the corolla with a paper clip, wire, straw, or similar device to exclude insects (Fig. 21). Shaking the enclosed flowers aids pollen dispersal and increases seed set.
Self-pollination of some insect-pollinated legumes requires tripping of the flowers. Four ways in which flowers can be tripped are: inserting a toothpick in the throat of the flower, inserting a toothpick that has the tip covered with emery paper to rupture the stigmatic surface, using a folded cardboard that retains pollen into which the stigma falls (Fig. 18), and gently rolling or squeezing the racemes between the fingers. Rolling the racemes is the most efficient method for alfalfa, but care must be taken to clean hands between pollinations of genotypes. Self-pollination of red clover is most effective by inserting the closed inflorescence in a chamber at 40 C, removing it when the flowers are open, and tripping with a needle or toothpick. Self-pollination of insect-pollinated species also can be accomplished by placing plants in cages with the appropriate insect.

Some insect-pollinated species must be self-pollinated by manually transporting pollen from the anther to the stigma of the same flower. Potato flowers can be selfed by removing pollen from an anther with a scalpel and applying it to the stigma. When large quantities of selfed seed are needed, a vibrator can be used to collect pollen for application to stigmas of the clone (Fig. 15). Seed set of sunflower is increased considerably when heads are brushed with a piece of cotton.

Selfing of monoecious species usually involves the same procedures for female preparation and pollination used for hybridization. The only difference is that pollen for self-pollination is obtained from the same plant on which the female flower occurs. For species like wild rice, which have pistillate and staminate flowers on the same inflorescence, selfing is accomplished by bagging the inflorescence before the pistillate flowers open, and assuring that pollen is dispersed over the stigmas during anther dehiscence.

Fig. 21—Three methods of selfing Upland cotton. Left to right: paper clip attached to the end of the corolla; corolla wrapped with copper wire; and cardboard cylinder over the flower (Reproduced by permission of J. A. Lee from Chapter 20).
V. SEED DEVELOPMENT

A. Determination of Successful Pollinations

A visual indication of successful fertilization and seed development is desirable soon after pollination, so that additional pollinations can be made if necessary before flowering ceases. Positive identification of success may be possible in less than a day or may not be possible for a week or more after pollination. Fertilization of sunflower can be determined the morning after pollination by observing withering and receding of the stigmas. Success of oat pollination can be determined 2 to 3 days after pollination by noting a wilted stigma and an enlarged ovary. Success in tobacco can be seen within a week of pollination because unfertilized flowers drop from the inflorescence, while fertilized ones have enlarged seed capsules.

Abortion of developing seed can cause difficulty in determining the success of pollination. Flowers of the common bean that have not been fertilized usually drop 1 to 2 days after pollination. Some pods, however, reach up to 7 cm in length and remain on the plant, but contain only aborted seeds. Broadbean pods frequently abort until they are about 3.5 cm long. Enlargement of the potato ovary can be observed a week after pollination and usually signifies seed development, but the ovary may enlarge without fertilization and later abort.

B. Percentage of Success

Large differences exist among species for the average percentage of successful pollination expected under favorable conditions. Less than 10% of buckwheat flowers set seed after hybridization. Success approaches 100% for pearl millet and sesame. The number of seed obtained from successful pollination of a flower ranges from one in species like sesame and barley to several hundred in tobacco.

Variability in success is affected by environment, health of the plant, the parents involved, and skill of the breeder (Chapter 1). The percentage of success in field pea exceeds 80% when pollinations are made on cool, sunny days with vigorous plants in the early stages of blooming. Only 20% success may be obtained, however, when using older plants under hot, dry, windy conditions. Crosses of field pea involving genotypes with small or fragile flowers also have a low percentage of success.

Skill of the breeder can affect the percentage of successful crosses. Careful preparation of a female flower at the proper stage and adequate pollination with viable pollen can require much practice in some species. The technique used to hold a flower with the fingers can influence the speed, ease, and success of hybridization.

Hormones have been used to a limited extent to improve seed set. Application of 0.005% p-chlorophenoxyacetic acid in lanolin on the pedicel or calyx of pollinated tomato flowers may double the percentage of fruit set under unfavorable environmental conditions, when partially sterile parents are used, or for interspecific crosses. A drop of a 100 ppm gibberellic acid solution applied at the base of the anther column of cotton at emasculation promotes fruit set of genotypes that are difficult to hybridize.