5 Physiology of Growth in Specific Organs: Flowers, Fruit, and Seeds

In this chapter we will investigate the physiology of some specific growth characteristics of flowers, fruit, and seeds. As in the previous chapter, we will examine some of the mechanisms different plant organs have for growing and prospering as stationary organisms in a dynamic environment.

Flowers

Flowers are extremely important to life on earth. Much of our food is derived from flowers, whether from flowers in their earliest stages of development (such as artichokes, *Cynara cardunculus*), fruit (traditional fruits or vegetables that are botanically fruits), or the final product of their growth, seeds. In addition, flowers provide immeasurable aesthetic value making our lives richer and more colorful. From the bud to the seed, flower growth and development is a carefully orchestrated physiological phenomenon, never ceasing to amaze even the oldest veteran of horticulture.

Plant age and the flowering response – juvenility

All species have a genetically programmed minimal stage of development they must reach before they begin the flowering process. This minimal stage varies considerably among species. We can, however, make some general observations that are applicable to most plants. When a plant is capable of flowering given the appropriate environmental conditions, we say that the plant is 'competent' or 'ripe to flower'. We often measure the 'ripeness to flower' in time or plant stage, with plant stage being more accurate. The usual measure of a plant's stage of development is the number of nodes or leaves it has produced. This measure of competency to flower removes any variability caused by different growth rates due to different growing conditions which exist when measuring competency with time.

When considering perennial plants, we often refer to the age a plant must be before it will flower. Some perennial species will flower in their first year of growth (some conifers) while others require from several years to many decades of vegetative growth before they will flower (Lawson and Poethig, 1995).

Biennial plants require two growing seasons to flower. The first growing season is for vegetative growth, the second is for flowering. The two growing seasons are usually separated by a season of endo-dormancy which must be removed by exposure to cold temperatures (0–5°C). In many biennials, flower bud induction and initiation occur the first season while differentiation and development occur during the dormant and second growing season. In other biennials, all stages of flowering occur in the second growing season, but will only take place after exposure to the previously mentioned cold temperatures.

Most annuals reach a ripeness to flower after a predetermined number of nodes (or leaves) have been produced. Once the adult stage has been reached, the plant will produce a genetically programmed number of flowers. Some species produce a certain number of flowers then die, while others continue to flower until adverse environmental conditions prevent flowering. These differences are important in determining the potential yield of different cultivars, whether for cut flowers, fruit, or seed. With most crops there is a fine balance between vegetative growth and the crop load it can support. Cultivars which produce fewer flowers per plant often produce larger fruit due to lack of competition for photosynthates. In species which tend to produce copious numbers of flowers per plant, flowers or young fruit are often removed from the plant to encourage the production of larger fruit.

Besides the inability to flower, juvenile plants may differ from their adult counterparts in morphology and ease of vegetative propagation (Martin-Trillo and Martinez-Zapater, 2002).

Juvenile plants are often more easily vegetatively propagated than adult plants as juvenile stems often form adventitious roots more readily than adult stems. Adult leaves of English ivy (*Hedera helix*) are shaped differently from their juvenile counterparts and juvenile green bean (*Phaseolus vulgaris*) plants have simple leaves while the adults have compound leaves.

The flowering process

Many plant scientists tend to refer to flowering as a general process. The process as a whole is probably one of the most studied physiological processes in horticulture. It is extremely complex, intricately controlled by genetics and environment. Light and temperature are the two most important environmental factors controlling flowering.

As a process, flowering can be broken down into four major steps: (i) induction; (ii) initiation; (iii) differentiation; and (iv) development. Each step has its own set of responses to light and temperature (Fig. 5.1) (Durner and Poling, 1987). Many scientists fail to distinguish the stages when discussing flowering, which has led to much confusion in the scientific literature. When viewed as a four-step process, flowering is not as complicated as it first seems, even though its control is still quite complex.

Induction

Induction is that process which normally occurs in the leaf as a plant perceives the signal to switch from a vegetative state to a flowering state. This change from vegetative to flowering ultimately occurs at the meristem, but the signal (most often light or temperature) is detected by the leaves. In some plants, including strawberry (*Fragaria* spp.) floral induction can be macroscopically measured by monitoring leaf production (Durner and Poling, 1985). Induction is often monitored to determine effects of different variables on the flowering process.

The number of leaves a plant produces over time is monitored and an average leaf production rate is estimated. Leaf production is fairly constant until there is a sudden marked temporary increase in the rate of leaf production which coincides with the inductive trigger. By being able to determine when induction occurs, we are able to study the factors that influence it more easily.

Once the signal to switch from vegetative to floral growth has been detected, the signal must be transferred to the site of flower production, a meristem. The signal is transferred via the flowering hormone, florigen. More often than not, it is the apical meristem which changes from vegetative to floral, however, axillary meristems may also switch. Once the signal is received by the meristem, the next stage of flowering, initiation, begins.

Initiation

Floral initiation is associated with the observable morphological changes which occur at a meristem as the meristem transitions from leaf to floral production (Durner and Poling, 1985). Actually the change is merely a change in the direction of development as flowers are simply modified leaves. Initiation can be detected by observing changes in morphology of the meristem at the microscopic level. In general, a vegetative meristem is rather pointed and narrow. Once the transition has occurred, the meristem becomes rather flat and broad. The difference is rather easy to observe in most species. Once initiation has been detected at the meristem level, the next stage, differentiation, begins.

Differentiation

There is a fine line between initiation and differentiation. Differentiation is the process in which the various flower parts are formed after initiation, but before macroscopic production has commenced (Durner and Poling, 1985). Differentiation can be monitored via dissection of plant apices under a dissecting scope at magnification from ×10 to ×20. In many biennials and perennials, differentiation takes place over an extended period. In annuals it occurs quickly.

Specific genes have been identified which control: (i) what flower parts are produced; (ii) how many are produced within each flower; and (iii) where they are located (Smyth, 2001; Buzgo *et al*., 2004; Rijpkemaa *et al*., 2010). These genes are probably regulated by environmental cues.

Development

Development refers to the macroscopic production of flowers that are visible without magnification (Durner and Poling, 1985). It is monitored by visual counts of numbers of flowers per plant, inflorescences, etc. Monitoring development closely

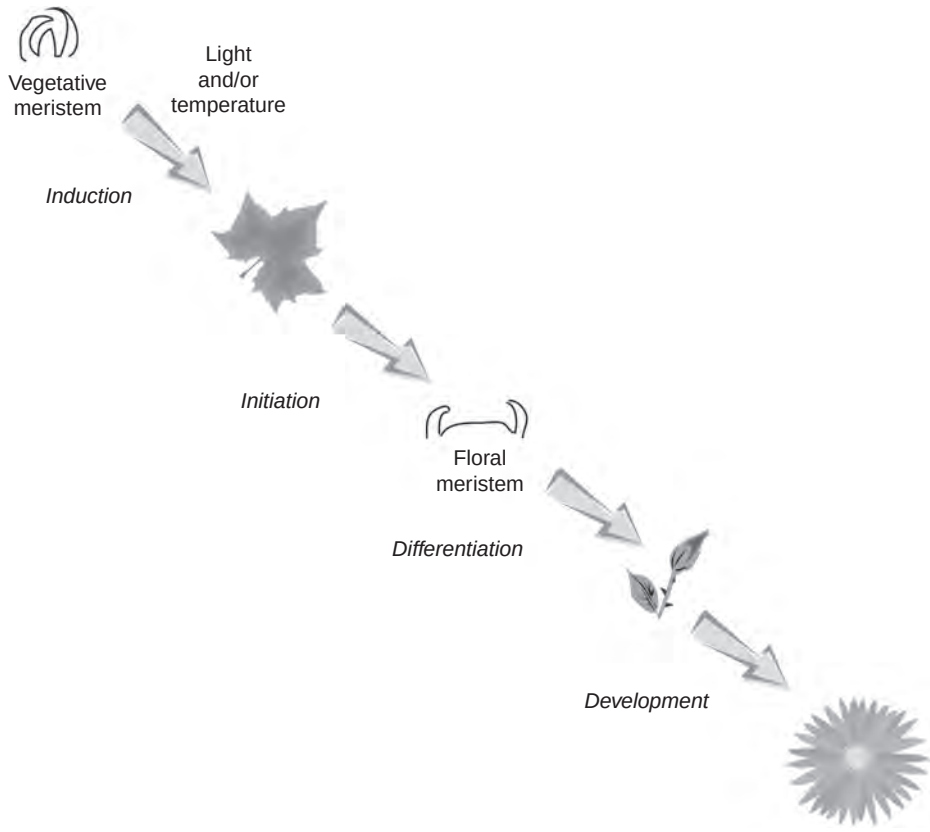

Fig. 5.1. The four stages of flowering. (Leaf, arrow, bud, and flower symbols courtesy of the Integration and Application Network, University of Maryland Center for Environmental Science, ian.umces.edu/symbols/.)

allows careful study of the factors that influence the fruiting potential of many plants. Note the term 'fruiting potential' is just that, potential. Many other steps are involved in the production of fruits and seeds after the production of flowers.

Reproductive mechanisms

Much of our horticultural production relies on adequate sexual reproduction in plants. It is therefore important to understand the underlying basic biology of sexual reproduction as well as the physiology behind the time of bloom. The reference for the technical details that follow is Evert and Eichorn (2006).

The alternation of generations

The life cycle of flowering plants consists of two separate generations, sporophyte and gametophyte, which alternate back and forth as plants sexually reproduce (Fig. 5.2). The generation most people recognize is the sporophyte generation, the more highly visible of the two. The sporophyte generation is, as the name suggests, the generation that produces spores. The gametophyte generation is the generation that produces the gametes involved in sexual reproduction, the egg and sperm cells. The gametophyte generation is small and actually takes place in flowers of a sporophyte plant. We'll look at each generation in detail.

SPOROPHYTES The sporophyte generation is what we see when we see a 'normal' plant. It is diploid, meaning it has two sets of chromosomes. The smallest sporophyte plant is the single-celled diploid zygote that results from the fertilization of an egg with one of the sperm cells from a pollen grain. Its cells undergo mitosis resulting in growth.

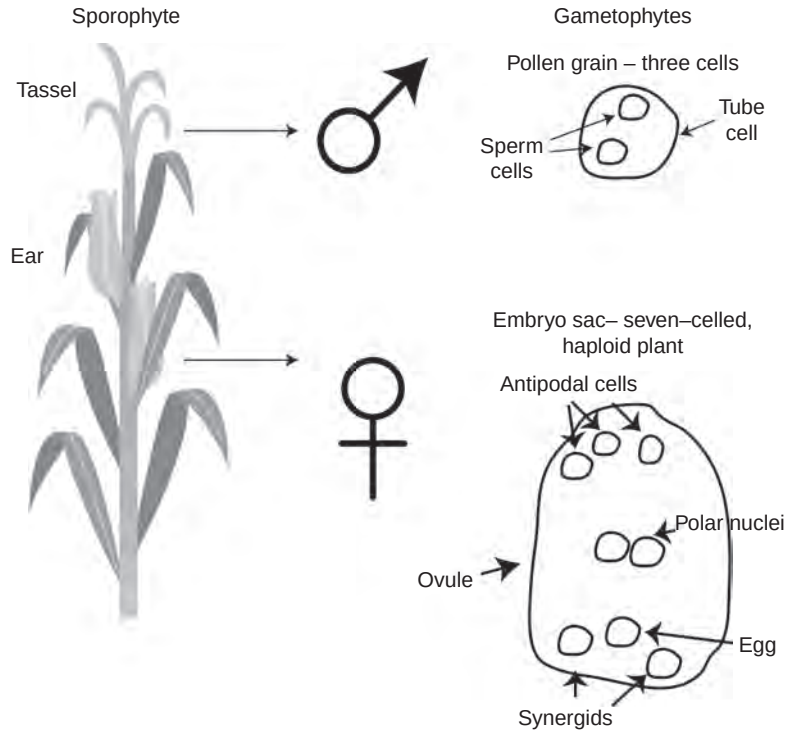

Fig. 5.2. The alternation of generations. (Corn and gender symbols courtesy of the Integration and Application Network, University of Maryland Center for Environmental Science, ian.umces.edu/symbols/.)

Ultimately this diploid sporophyte, which over time has acquired the multi-cellular appearance of a 'normal plant', will undergo meiosis in certain tissues (the anthers and ovules of flowers) to produce two different types of gametophyte plants, the male and female gametophytes.

GAMETOPHYTES Gametophytes are multi-cellular haploid organisms (plants) residing in the flowers of sporophyte plants. The male gametophyte is called the pollen grain and is derived from a microspore mother cell which is found in the anther of a flower. There are many microspore mother cells in any given anther. Each of these cells divides to form four haploid microspores. Each microspore develops into a pollen grain which consists of a large vegetative cell, called the tube cell, inside of which lives a smaller generative (germ) cell. The haploid germ cell divides by mitosis to produce two sperm cells. Thus the pollen grain is a three-celled haploid male gametophyte plant made up of two sperm cells living inside a tube cell.

The female gametophyte is a seven-celled haploid plant which develops from a megaspore. A diploid megaspore mother cell located in the ovule of a sporophyte plant undergoes meiosis to form four haploid megaspores, three of which disintegrate. The remaining haploid megaspore undergoes mitosis four times to form eight haploid cells. One of these cells is the egg cell, which if successfully fertilized will become part of the new sporophyte. The egg cell is flanked by two cells called synergids. These three cells (the egg plus two synergids) sit at the micropyle end of the ovule (this is the end of the ovule with space for the pollen tube to grow through during pollination). Three of the remaining five haploid cells sit at the antipodal end of the ovule (the end away from the micropyle) and they are called antipodal cells. The remaining two haploid cells fuse into a single cell with two polar nuclei and this cell sits in the middle of the ovule. Thus we've accounted for all eight haploid cells which formed from a single megaspore. They have now developed into a seven-celled haploid female gametophyte plant.

Upon pollination and subsequent fertilization (both will be covered shortly), the new sporophyte generation begins as a single-celled zygote which eventually grows into an embryo. The new sporophyte plant we see develops from this embryo. Pretty amazing, isn't it?

Controlling sexual reproduction

Sexual reproduction in plants is important for genetic diversity. There are a number of mechanisms which have evolved in plants that impart at least some degree of control on plant sexual reproduction. Many of these mechanisms ensure that self-pollination does not occur. Forcing pollination by another individual reduces inbreeding which could lead to homozygosity, which often leads to expression of undesirable traits or the lack of expression of desirable traits. Some mechanisms which encourage heterozygousity include: (i) dioeciousness; and (ii) asynchronous time of bloom on monoecious plants.

Plant types

Although many species (85%) of plants have flowers that are perfect (i.e. include both male and female gametophytes residing in the same flower), there are many species which employ a different approach to sexual reproduction (Yampolsky and Yampolsky, 1922). This mechanism is such that separate male and female flowers exist. If both male and female gametophytes are produced on the same plant but they reside in separate flowers, the species is monoecious. About 7% of plant species are monoecious (Dellaporta and Calderon-Urrea, 1993). Good examples of this are corn (*Zea mays*) (Fig. 5.3) and squash (*Cucurbita maxima*) (Fig. 5.4). When a species' male and female gametophytes reside on separate plants, that species is dioecious. About 6% of plant species are dioecious (Renner and Ricklefs, 1995). A popular example of this type of plant is holly (*Ilex opaca*). In dioecious species, male and female plants must be close enough together such that pollen is easily transferred from the male plant to the female plant if sexual reproduction is to be successful. If fruit are a nuisance, as is often the case in landscaping situations, plants should be screened to include only male plants to ensure that fruit won't be produced. Selecting all-female plants may not be effective, since there may be male plants of the same species on neighboring properties.

Time of bloom

Time of bloom of two different plants within the same species can be synchronous or asynchronous. Some cultivars, especially in fruit crops, are self-incompatible with respect to pollination, thus they

Fig. 5.3. A typical corn (*Zea mays*) plant with separate flowers: (a) male tassel, top; and (b) female ear, on stalk.

Fig. 5.4. The female flower of a typical winter squash (*Cucurbita* spp.) plant. Female flowers are easily identified due to the often prominent ovary subtending the corolla. Male flowers conspicuously lack the ovary.

require pollen from a different cultivar of the same species. Synchronous bloom among cultivars is important for effective pollination in these crops. Asynchronous time of bloom is an effective mechanism for ensuring that cross-pollination occurs to encourage heterozygousity.

This last macroscopic step in the flowering process is under both genetic and environmental control. Once flowering occurs, two more steps, often microscopic, occur: (i) pollination; and (ii) fertilization. We will first explore the control of bloom time then look at pollination and fertilization.

GENETIC CONTROL OF BLOOM – LOW-CHILL CUTIVARS Every species has an internally programmed time to bloom, thus the ultimate control is genetic. The appropriate environmental conditions must exist for the genetics to be expressed, thus environment is secondary, yet equally important. Many deciduous species require exposure to a period of relatively cold temperatures (0–5°C) between growing seasons before they will resume growth. This is called the chilling requirement. Once the chilling requirement has been fulfilled, plants begin growth when exposed to warm temperatures as measured via heat units. The physiology of chilling and heat units will be covered extensively in Chapter 9, this volume.

Some cultivars of peach (*Prunus persica*), apple (*Malus domestica*), and blueberries (*Vaccinium* spp.) have a greatly reduced chilling requirement when compared with other cultivars within their respective species. They are called 'low-chill' cultivars. These cultivars are often used for commercial production in regions which normally do not receive enough cold weather to fulfill the 'normal' chilling requirement. They should not be used in areas where significant chilling accumulation occurs, since their chilling requirement would be fulfilled early in the dormant season. Any exposure to warmer weather would then lead to premature heat-unit accumulation and spring-like growth which would probably be injured or killed with the return of harsh temperatures. The low-chill character is genetically controlled and has been quantified for many commercial cultivars.

GENETIC CONTROL OF BLOOM – LATE-BLOOMING CULTIVARS While the low-chill cultivars have bloom times regulated primarily by their exposure to cold temperatures, there is a group of apple cultivars which tend to bloom much later than the average cultivar due to a greater heat-unit requirement. The greater heat-unit requirement may be due to an absolute increase in the amount of heat needed for development or it could be a result of decreased sensitivity to lower temperatures. In the latter case, fewer heat units would accumulate at lower temperatures while in the former, more heat units must accumulate for bloom to occur.

ENVIRONMENTAL CONTROL OF BLOOM All species have an inherent genetic control of the time of bloom which may be modified by the environment. In general, species have a genetically controlled chilling and heat-unit requirement which is constant and quantifiable. Time of bloom can be modified by altering the chilling/heat required for bloom or by altering the chilling/heat accumulated. Accelerated bloom is usually not a desirable trait for most field-grown horticultural crops. Accelerated bloom may be desirable for out-of-season production in greenhouses or high tunnels. Most often our attempts to modify the time of bloom are attempts at delaying bloom to avoid frost or freezing injury to fruit blossoms. In certain regions of the world, frost or freezing injury may result in 100% crop loss. Methods that have been studied for delaying bloom and the physiology behind the plant response will be discussed in Chapter 9, this volume.

Pollination

While the general mechanisms for regulating sexual reproduction have been discussed, specific control

of the process lies in pollination and fertilization. Both processes are highly regulated at the physiological level.

MECHANISMS OF POLLINATION Sexual reproduction is a highly specialized process closely regulated anatomically and physiologically. Only similar genomes can cooperate to form a new individual via sexual reproduction. Various 'security measures' have evolved in plants to ensure only compatible gametes unite.

Successful pollination requires several steps. Initially, pollen must land on the stigma of a pistil. The transfer of pollen from the anther to the stigma is accomplished either via wind or insects. Once the pollen grain lands on the stigma, it must germinate, grow into the stigma then down through the style into the locule of the ovary towards the awaiting egg. Pollen tube growth through the style is accomplished with the weakening of stylar tissue by enzymes produced by both the pollen tube and the style. These enzymes are only produced when compatible pollen attempts to grow through the style. Once the pollen tube reaches the egg, the egg and one of the sperm cells from the pollen grain unite to form the zygote. The zygote eventually develops into the embryo.

Pollination is accomplished by either biotic or abiotic means. Approximately 10% of flowering plants are pollinated abiotically by either wind (anemophily) or water (hydrophily), with wind pollination accounting for 98% of abiotic pollination. Abiotically pollinated flowers are often inconspicuous and non-showy.

Nearly 90% of all flowering plants require a pollinator, that is, an organism that can move pollen from the anther to the stigma. The plant from which the pollinator takes the pollen is called the pollinizer. Most of the 200,000 or so pollinators are insects. Pollination by insects is called entomophily and is accomplished by organisms such as: (i) bees, wasps, and ants (*Hymenoptera*); (ii) beetles (*Coleoptera*); (iii) moths and butterflies (*Lepidoptera*); and (iv) flies (*Diptera*). Most plants pollinated by insects have colorful, strongly scented flowers.

Pollination by vertebrates is called zoophily, and is accomplished by birds and bats. Plants that are pollinated by bats or moths tend to have white petals with strongly scented flowers. Plants pollinated by birds usually have red petals and not much of a scent.

INTERACTIONS OF POLLEN AND STIGMA/INCOMPATIBILITY A pollen grain is a three-celled haploid male gametophyte plant consisting of two sperm cells living inside a tube cell. Pollination occurs when the haploid pollen grain lands on the diploid stigma surface. If the stigma is covered in mucus, it is called a wet stigma. If it is covered in cutin, it is called a dry stigma. The pollen grain can be compatible or incompatible with the stigmatic surface. Compatibility will result in pollen tube growth and potential fertilization of the egg cell. Pollen tube growth and subsequent fertilization will not occur if there is incompatibility.

Pollination and subsequent fertilization between genera is relatively rare. In general, pollen from different genera is not compatible primarily because of differences in chromosome number. However, there are some notable exceptions to this general rule. The showy ornamental plant × *Heucherella tiarelloides*, is an intergeneric hybrid of *Heuchera sanguinea* × *Tiarella cordifolia*. Many common orchids are intergeneric hybrids, as is the leyland cypress, × *Cupressocyparis leylandii*.

Within a genus, interspecific pollination and fertilization can occur resulting in interspecific hybrids. French–American hybrid grapes (*Vitis*) are well-known examples of this. Other examples include: (i) loganberry (*Rubus* × *loganobaccus*), a hybrid of blackberry (*Rubus ursinus*) × raspberry (*Rubus idaeus*); (ii) peppermint (*Mentha* × *piperita*), a hybrid of spearmint (*Mentha spicata*) × water mint (*Mentha aquatica*); (iii) tangelo (*Citrus* × *tangelo*), a hybrid of mandarin orange (*Citrus reticulata*) × pomelo (*Citrus maxima*) or grapefruit (*Citrus* × *paradisi*); (iv) triticale (× *Triticosecale*), a hybrid of wheat (*Triticum* spp.) × rye (*Secale cereale*); and (v) grapefruit (*Citrus* × *paradisi*), a hybrid of pomelo (*C. maxima*) × sweet orange (*Citrus sinensis*).

Within a species, crosses between cultivars may or may not occur readily due to one of several types of incompatibility. One type of incompatibility is called self-incompatibility. Nearly 60% of angiosperm species have some form of self-incompatibility (Hiscock and Tabah, 2003). In self-incompatible species, the pollen of a plant will not develop a pollen tube on the stigma of the same plant. Pollen from another plant of the same species will develop a pollen tube, as long as it does not carry the same allele for incompatibility as the stigma on which it lands. The self-incompatibility ensures cross-fertilization and is a barrier to inbreeding and the homozygosis it causes.

Gametophytic self-incompatibility is regulated by a single gene (SI) with multiple alleles (SI1, SI2, SI3, SI4 . . . S*i*n) and is the most common (Hiscock

and Tabah, 2003). The incompatibility reaction occurs whenever the pollen and the stigma have the same alleles. Remember that the pollen is haploid with one allele while the stigma is diploid with two alleles. Effective pollination and fertilization may only occur if the alleles of the pollen and stigma are different.

Sporophytic self-incompatibility occurs when some molecular component in the pollen is repulsed by the stigma which prevents effective germination of the pollen tube.

TYPES OF POLLINATION Plant systems include several different types of pollination. Self-pollination occurs when the pollen of a plant lands on its own stigma and germinates into a pollen tube. Cross-pollination occurs when pollen from one plant lands on the stigma of another (of the same species, of course). In addition to self- and cross-pollination, there are the controlled crosses of hybrid development. This type of pollination occurs when the pollen of a specific cultivar is used to pollinate the stigma of a second specific cultivar to produce seed of a hybrid cultivar. The process is usually labor intensive and also is the culmination of many years of work by one or more plant breeders. That is why hybrid seed is more expensive than open-pollinated cultivars. Successful pollination does not ensure fertilization with the seed and fruit development that normally follows. There are a number of other fates that may await the pollen tube.

STERILITY There is a difference between sterility and incompatibility. Sterile plants fail to produce spores or gametes or those that are produced are abnormal. In systems of incompatibility, the male and female gametes are normal and functional but will only produce a zygote with a compatible mate.

Sterility is a reproductive system in plants where plants fail to produce functional gametes. Male sterility is much more prevalent or at least more widely known than female sterility. Perhaps it is more prevalent due to the fact that the male sporophyte and gametophyte are less protected from the environment compared with their female counterparts and exposure to the elements may induce greater chances of sterility. It could also be that the condition is simply more widely recognized since it is easier to detect male sterility because of the much greater number of male gametophytes produced when compared with the number of female gametophytes produced. In addition, it is easy to assay male sterility with staining techniques. Detection of female sterility requires crossing.

MALE STERILITY Male sterility arises from spontaneous mutations in nuclear or cytoplasmic genes and appears in a number of different ways (Schnable and Wise, 1998; Budar and Pelletier, 2001). It can appear as: (i) an absence or malformation of the stamen; (ii) lack of male flowers; or (iii) a failure of flowers to produce anthers. If anthers are produced, microsporogenesis may be abnormal resulting in deformed or non-viable pollen. Pollen may form but fail to mature normally, thus preventing germination upon pollination. Finally, totally viable pollen may be produced but is not released due to a failure of anther dehiscence.

We will focus our discussion on male sterility since it is more prevalent than female sterility. Male sterility is classified as phenotypic or genotypic (Fig. 5.5).

PHENOTYPIC MALE STERILITY Phenotypic male sterility occurs as: (i) structural male sterility; (ii) sporogenous male sterility; or (iii) functional male sterility.

Structural male sterility occurs when there is some structural malfunction of the male sex organs. Sporogenous male sterility occurs with a malfunction of the anthers during pollen development such that very little pollen is formed or the pollen that is formed is defective. Functional male sterility occurs when functional pollen is produced by the anthers but some barrier to fertilization occurs. These barriers include: (i) lack of anther dehiscence and subsequent lack of pollination; (ii) pollen clumping such that it isn't released; and (iii) excessive pollen tube growth.

GENOTYPIC MALE STERILITY There are three types of genotypic male sterility: (i) genic; (ii) cytoplasmic; and (iii) genic-cytoplasmic (Budar and Pelletier, 2001; Fig. 5.5).

Genic male sterility involves nuclear genes and follows laws of Mendelian inheritance (Kaul, 1988; Chaudhury, 1993). In most cases it arises as a spontaneous mutation. This type of male sterility has been identified in about 175 species and is controlled by a single recessive gene in most of the species in which it occurs. Multi-gene control also occurs in some species.

Cytoplasmic male sterility is controlled by cytoplasmic rather than nuclear genes and follows non-Mendelian inheritance. This form of male sterility

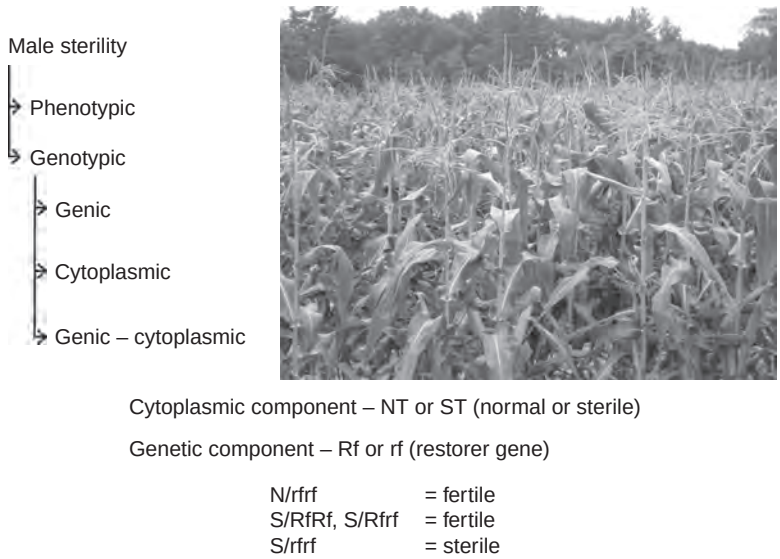

Fig. 5.5. Cytoplasmic and genetic control of male sterility.

is not very common, it is inherited maternally and there are normal (N) and sterile (S) cytoplasms (Kaul, 1988).

In cases of genic-cytoplasmic male sterility, both nuclear and cytoplasmic genes are involved (Schnable and Wise, 1998; Skibbe and Schnable, 2005). Normal and sterile cytoplasms combine with genes which restore fertility (Rf) which are distinct from genic male sterility genes. The Rf gene is required to restore fertility with sterile cytoplasms. The following combinations of cytoplasm and nuclear genes can occur:

- N/rfrf which is fertile.
- S/RfRf or S/Rfrf which are fertile.
- S/rfrf which is sterile.

Mutations of the restorer gene are frequent, thus the combination of normal cytoplasm with RfRf genes is best to maintain stable fertility. The best known system for this kind of male sterility is in corn (*Zea mays*) (Skibbe and Schnable, 2005). Several sterile cytoplasms occur in corn, among which T, S and C are the most well known. Since T cytoplasm was involved in the great corn blight epidemic of the 1970s in the USA, let's take a look at what happened a little more closely.

The T (T is for Texas) cytoplasm is very stable under many environmental conditions (Levings, 1990). It works by preventing anther exertion with pollen abortion. Unfortunately, plants with the T cytoplasm are highly susceptible to race T of southern corn leaf blight (*Cochliobolus heterostrophus* = *Bipolaris maydis*). This race of southern corn leaf blight is incredibly vigorous and virulent allowing it to spread rapidly through any population with the T cytoplasm.

But why would anyone use male sterility in crop production in the first place? Male sterility makes the production of hybrids very efficient since emasculation of the female parent is not needed. If the female parent has cytoplasmic male sterility, no viable pollen will be produced, thus there is no need to remove anthers to get a hybrid. Nearly all commercially produced corn cultivars are hybrids, thus male sterility is widely used in hybrid corn seed production.

In the 1960s there was widespread use of the T-cytopolasmic male sterility system in hybrid corn seed production, which meant that many of the hybrids used for field production had the T cytoplasm and were therefore extremely susceptible to race T of *C. heterostrophus*. Mitochondria in T cytoplasm are extremely sensitive to a toxin (β-polyketol also called T-toxin) produced by the fungus.

A gene in T cytoplasm (T-urf13) confers both male sterility and mitochondrial sensitivity to fungal toxins. This gene is only present in T cytoplasm

and encodes a large protein on the inner mitochondrial membrane. The fungal toxin interacts with this protein and causes abnormal plasma membrane permeability (Levings, 1990).

The urf13 gene causes male sterility by causing the tapetum, a layer of nutritive cells in the pollen sac, to degenerate during microsporogenesis. This disrupts pollen development leading to pollen abortion. The urf13 protein is only toxic in anther cells, even though the protein can be found throughout the plant. The protein may cause a minor reduction in plant vigor and yield, but for the most part, has little other effect on the plant.

Basically the gene causes mitochondrial dysfunction in anthers of corn. But why only in the anthers even though it's found all over the plant? Anthers have an exceptionally high energy requirement during pollen development. There are 40 times more mitochondria present in tapetum cells and 20 times more mitochondria in sporogenous cells during pollen development compared with other cell types. Any perturbation in energy production during pollen development could have lethal consequences. Tapetum cells are evidently extremely sensitive to slight changes in mitochondrial activity. When multiplied by 40, this severely impacts pollen production. Other cells are evidently not nearly as sensitive, thus there are no observed deleterious effects of the urf13 protein.

There are two genes, Rf1 and Rf2, involved in restoring fertility to T-cytoplasmically sterile corn plants. Sterility is eliminated in plants with a dominant set of these alleles even though they continue to produce the urf13 protein. Fertility is restored prior to mitosis in the anther, thus this type of fertility restoration is called sporophytic. Even though these genes restore fertility, they do not impart full resistance to corn blight. Only normal cytoplasm confers resistance.

The Rf1 gene is specific for restoring fertility. The Rf2 gene is important in the production of aldehyde dehrogenase, an enzyme needed to detoxify accumulated ethanol and acetaldehyde in cells. The highest levels of Rf2 protein are found in the tapetum. When mitochondrial activity is negatively affected by male sterile cytoplasm, Rf2 might scavenge the toxic ethanol and acetaldehyde produced by alternate energy production pathways in the cells of the tapetum induced by the dysfunctionality of the mitochondria. Thus even though the mitochondrial activity is adversely affected by the male sterile cytoplasm, the Rf2 gene restores pollen fertility by getting rid of toxic ethanol and acetaldehyde. If the Rf2 isn't present, the ethanol and acetaldehyde build to lethal levels in tapetum cells, thus there is no viable pollen.

Other cytoplasmic male sterility systems have been identified in green beans (*P. vulgaris*), sorghum (*Sorghum* spp.), beet (*Beta vulgaris*), carrot (*Daucus carota*), onion (*Allium cepa*), petunia (*Petunia* × *hybrida*), *Brassica napus*, rye (*Secale cereale*), sunflower (*Helianthus annuus*), and wheat (*Triticum* spp.).

DICHOGAMY Dichogamy is the separation in time of maturation of male and female flower parts such that self-pollination is avoided. Protandry occurs when the anthers mature before the pistils. Many species in the family *Compositae* and family *Leguminosae* exhibit protandry with pollen released from anthers before the stigma in the same flower is receptive. Corn (*Zea mays*) is a protandrous, monoecious, diclinous plant. (Diclinous species are those species which have separate male and female flowers on the same plant.) The tassel is the male flower while the ear is the female flower. The silk on an ear of corn are the styles of the pistil. Sweet corn is harvested as soon as the silks begin to whither and turn brown. At that point, the kernels have had just enough time to develop so that they are tender and sweet when harvested.

Protogyny occurs when the stigma becomes receptive before the anthers in the same flower shed their pollen. Many species of the family *Rosaceae* and family *Cruciferae* are protogynous.

Both protandry and protogyny may occur within a flower (intrafloral dichogamy) or among flowers in diclinous species (interfloral dichogamy). Sometimes the maturation of the two sexes are completely separated (complete dichogomy) while in other cases sexual maturity overlaps (incomplete dichogamy). Dichogamy is often considered an evolutionary mechanism for reducing the frequency of self-pollination and inbreeding. If this is the reason (if there is one) for dichogamy to exist, it is a redundant feature in many species, since many species that are dichogamous are also self-incompatible. Besides separation in time, pollen and stigmas can be separated in space. Herkogamy, the spatial separation of pollen and stigma, does not necessarily prevent self-pollination. All monoecious plants exhibit herkogamy.

EFFECTIVE POLLINATION PERIOD Whether or not pollination leads to fertilization also depends

on the effective pollination period. The effective pollination period is the length of time viable pollen has the chance of being deposited on a receptive stigma and germinating. The simplest estimate is length of ovule longevity minus the number of days it takes the pollen tube to grow down the style. Estimates have been made for many crops and varies from less than 24 h to as long as 1 week or more. Estimates of the effective pollination period must take into consideration: (i) stigmatic receptivity; (ii) pollen viability; (iii) pollen tube growth; and (iv) ovule longevity. Dichogamy adds further complexity to the situation, as there may be lags between anthesis, ovule maturation, stigma receptivity, and pollen tube growth.

TEMPERATURE EFFECTS ON POLLINATION Temperature affects pollination by influencing pollen dispersal, pollen growth, and pollinator activity. In general pollen will not germinate at low temperatures (below 4°C) and pollen tube growth is slow below 5°C.

Insect pollinators

Many economically important crops are pollinated by insect vectors (McGregor, 1976). Of all the insects capable of pollination, bees are the most important vectors for horticultural crops. While not strictly a physiological issue, pollination by insects is so crucial to the survival of many species, including our own, a discussion of bee biology and function is justified here.

BEE BIOLOGY AND POLLINATION There are over 19,000 species of bees worldwide! Bees are members of one of seven families in the superfamily *Apoidea*, order *Hymenoptera*, class *Insecta* in the animal phylum *Arthropoda* (Winston, 1987). All bees other than the domesticated honeybee (*Apis mellifera* L.) are considered wild. Though bees are incredibly important for the pollination of many worldwide crops, few species have been utilized for such purposes. Besides honeybees, only five other genera have been domesticated for their assistance in pollinating mankind's crops. These include: (i) leafcutter bees (*Megachile pacifica*); (ii) alkali bees (*Nomia melanderi*); (iii) Osmia bees (*Osmia* spp.); and (iv) bumblebees (*Bombus* spp.) (Mader *et al*., 2010).

Bees feed their young pollen and nectar, thus they forage for food on flowers. While doing so they transfer pollen from anther to stigma and from flower to flower and *voila*, pollination!

Contrary to popular belief, many bees do not sting. In those that do, the sting is accomplished by modified ovipositors in females or exposed genitalia in males. Besides the obvious, you can determine the sex by looking at antennae. Male bees have antennae with 13 segments while female antennae have 12 segments.

Bees are solitary, gregarious or social. Solitary bees are characterized by females who construct one single-celled nest at a time. The female bee deposits an egg then seals the cell, all with no help from any other bee. Gregarious bees are solitary individuals but they tend to nest close to each other. Social bees live together in hives and work cooperatively with each other to maintain their community. The main life activity of bees is foraging for nectar and pollen. Most bees forage for pollen and nectar from as little as a few hundred feet to as much as 25 km from their nest depending on species.

HONEYBEES The bee most common to horticulturists is the European honeybee, *Apis mellifera* L. There are a number of varieties of European honeybees that have been bred for honey production, disease resistance, pollination efficiency, and temperament. Extensive cross-breeding can occur and only queens from commercial breeders should be used when reinvigorating a hive.

Some of the more prevalent varieties or stocks of honeybees include the German, Italian, Carniolan, Caucasian, Buckfast, and the Russian bee.

The German or black bee (*Apis mellifera mellifera* L.) is a hardy, aggressive honeybee which has been all but wiped out in North America by disease.

The Italian bee (*Apis mellifera ligustica*) is favored by beekeepers for their excellent honey production and long brood life. They tend to consume surplus honey if it is not removed from the hive in a timely manner and they also tend to steal honey from neighboring, weaker hives. They are also less defensive and prone to diseases than the German race. The Buckfast bee, which is a descendant of the Italian race, was developed by a monk to thrive in the cold, wet conditions of the British Isles.

The Carniolan bee (*Apis mellifera carnica*), also from middle Europe, is favored for its rapid colony growth early in the spring. They are docile, build very nice wax combs and do not rob other hives of honey. They do have a tendency to swarm.

The Caucasian bee (*Apis mellifera caucasica*), from Eastern Europe, are prized for their long tongues that can reach nectar other honeybees can't. They are docile, but very slow to get started in the spring. They also tend to use excessive propolis (a sticky substance) when building their hives, making the hives hard to manage.

The Russian bee, originated from Russia near the Sea of Japan. It is resistant to the varroa mite which has devastated honeybee hives around the world. They have good, clean hives and are also resistant to the tracheal mite. While most bees have queens only during hive replacement or swarming, Russian bees have queen bee cells present in their hives at all times. Cross-contamination with other strains greatly reduces their resistance to varroa mites.

Some other stocks include: (i) the Minnesota Hygienic stock, known for its hive-cleaning character; (ii) the SMR (Suppression of Mite Reproduction) stock, which is a collection of mite-resistant stocks used in breeding projects; and (iii) the Cordovan stock, a very light yellow Italian bee.

Many commercial hybrid stocks have been generated by bee breeders, including the Midnight, the Starline, the Double Hybrid and the Smart strains. The Midnight strain is a cross of the Caucasian and Carniolan stocks which was developed to decrease the excessive propolis use of the Caucasians and reducing the swarming tendency of the Carniolans while maintaining the docility of both. The Starline was developed for its exceptional honey production. The Double Hybrid is a Midnight and Starline cross. The Smart strains are crosses of previously mentioned stocks with the SMR line.

In order to understand how honeybees do what they do best, make honey and pollinate, you must understand their life cycle and social nature. There are three levels of bees in the honeybee social scene: (i) the queen; (ii) the drones; and (iii) the workers. The queen lays eggs and she regulates whether or not the egg that is laid is fertilized or not. Fertilized eggs become females while non-fertilized eggs become drones. Females are workers unless as larvae they were fed copious amounts of royal jelly by workers, when they would then be virgin queens.

A queen can lay as many as 1000 eggs/day in hexagonal cells of the honeycomb, one egg per cell. Eggs hatch into larvae in 3 days and are fed royal jelly for 2 days. Workers must eat tremendous amounts of pollen and nectar to produce enough royal jelly to feed the developing larvae. On the third day of development, larvae destined to be workers are fed honey, pollen, and water while those destined to be queens are fed royal jelly for their entire larval life. Larvae molt five times to become pupae. During the larval stage, each larva is fed nearly 10,000 times and their weight increases 1500-fold. Development time in the larval stage varies with their adult destiny:

- Queens are larvae for 5.5 days.
- Workers are larvae for 6 days.
- Drones are larvae for 6.5 days.

Once they become pupae, the cells are capped by the workers. The length of pupation varies from 7.5 days for queens, 14.5 days for drones and 12 days for workers. Thus the time from egg laying to emergence as an adult is approximately 15 days for the queen, 24 days for the drones, and 21 days for workers.

An active honeybee colony typically consists of 50,000–60,000 workers, 500–1000 drones and one queen who typically lives for 4 or 5 years. Workers collect pollen and nectar from flowers sometimes from as far as 4.8 km away from the hive. Each worker typically makes ten trips a day. Pollen is fed to larvae or stored in cells of the hive. The nectar is regurgitated along with an enzyme (invertase) into honeycomb cells where it is converted to honey and evaporated. Wax produced as small flakes is chewed and reshaped to form the honeycomb by workers. While the workers are busy constructing the hive and gathering nectar and pollen, the colony continues to grow as the queen continues to lay eggs and workers feed the brood. A worker normally lives for only 1–2 months.

After emerging from their pupal stage, worker bees are cared for by older worker bees for 4 days. After 4 days, adults work around the hive for about 21 days. During that time they may clean the hive, build more honeycombs, feed larvae, process honey or cool the hive by fanning. After about 21 days, workers switch to pollen and nectar gathering, which they do for about 20 days before they leave the hive to die.

Collected pollen is brought back to the hive and fed to larvae or stored for later use. Nectar is regurgitated to younger 'hive workers' where it is processed in their digestive system and regurgitated into storage cells where it ripens for 5 days. After 5 days it is honey and the cell is capped for storage. It takes the nectar from about 5 million flowers to make 1 pint (473 ml) of honey.

If a new queen is needed due to death or expansion of the colony, a special wax cell is built around seven or eight fertilized eggs. The eggs and subsequent larvae are fed royal jelly for their entire larval life which causes them to become fertile females (queens) rather than workers. The first queen to emerge from her pupal case kills all of her sister queens and sometimes her mother as well. Within 2 weeks of her emergence, the new queen will fly off and mate with up to 10 drones. Drones who mate die immediately. The queen will store their sperm in a special organ called a spermatheca. The sperm remains viable for up to 4 years and she will never mate again. When she returns to the hive, she will lay up to 1500 eggs/day. In 2–4 years, she will have used up all the stored sperm and begin laying unfertilized eggs which will become drones. Workers will raise one or two new queens from the last of the fertilized eggs. Commercially, beekeepers often replace the queen each year to maximize hive productivity.

If the hive has become too large, the queen will leave the colony with some worker bees to find a new home in the often maligned process of swarming. Swarming usually occurs in the spring. The initial departure of the queen with about 60% of the workers is called a prime swarm. One to five afterswarms with virgin females may occur. In the process of swarming, colonies reproduce, one original hive becoming two or more new hives.

Commercial beekeepers normally do not want their colonies to swarm. They therefore control swarming by removing brood honeycombs from the hive to make sure the colony doesn't get too big. The removed combs can be sold or used to create new hives.

As the growing season ends and winter approaches in the temperate zone, honeybees begin preparing to overwinter. Drones that did not mate are expelled from the hive and die. The remaining queen and workers do not hibernate, they cluster.

As air temperatures drop below about 14°C, honeybees cling to each other on combs inside the hive forming a cluster inside the hive (Owens, 1967). This cluster keeps the temperature inside the hive warm regardless of the outside temperature. The cluster moves within the hive to reach honey as needed. Some bees die during the cold season. In warmer climates, colonies may not cluster but rather, continue to forage and make honey while the queen lays eggs.

Once the queen resumes egg laying towards the end of the winter, the colony must keep the temperature of the cluster between 34°C and 37°C. If the temperature dips below this range, the brood will die.

COLONY COLLAPSE DISORDER In 2006, an alarming number of honeybee hives in the USA began to die off for no apparent reason. Oddly enough, no dead bees are ever found in or near the hive, and a queen and the brood are still alive. This mysterious problem was given the name 'colony collapse disorder' (vanEngelsdorp *et al*., 2009).

The number of managed honeybee hives in the USA has dropped from 5 million in the 1940s to 2.5 million presently. To gain perspective, consider that the almond crop in California alone requires the pollination efforts of 1.3 million colonies!

No single factor has been identified as being the causal agent for this disorder. There are three major theories being investigated by researchers. One theory is that there has been a build up in the level of pesticides in bee colonies that has reached a fatal level in many hives. A second theory is that a new parasite or pathogen is to blame. One possible organism being studied is a microbe called *Nosema* which lives in the bee's gut. The third theory is that viruses may be the cause of the problem. The most likely cause is a combination of stressors that have weakened colonies which has led to the collapse. Stress tends to negatively affect a bees immune system and the general social structure of the colony. Before the colony collapse disorder appeared, honeybee colonies were already under stress from varroa and tracheal mites. The varroa mite is a blood-feeding parasite that transmits viruses. The tracheal mite itself causes little negative consequences for a bee hive, but it may exacerbate weakness in already weakened colonies.

It is interesting that a similar phenomenon of disappearing hives was documented in the 1880s, 1920s, and the 1960s. Whether or not it was the same ailment is not known.

AFRICANIZED HONEYBEES Despite the often dramatic presentations concerning "Africanized honeybees" by the media, this group of honeybees for the most part have not and do not cause permanent injury to the general population (Delaplane, 2006). Certainly attacks which have resulted in death to

humans and livestock have occurred, hence the name "killer bees" but these attacks are rare.

The European honeybee is not well suited for life in the tropics. In an attempt to improve honey production in the tropical environment of South America, honeybees were imported from Africa to Brazil in 1956 by researchers. These bees did well in the tropics and began to hybridize with colonies of European honeybees. The "Africanized" honeybee was born. The bees slowly spread north through Central America and into North America by the 1990s.

Africanized honeybees are extremely defensive and large groups of them have been known to attack humans and livestock with very little provocation. They have also been known to take over European honeybee hives and kill the queen.

A major difference between Africanized and European bees is their permanency. European honeybees gather large amounts of honey and are well adapted for overwintering in a permanent location. Africanized honeybees do not gather large quantities of honey and respond to food shortages by migrating or swarming.

Generally Africanized honeybees do not overwinter in cold temperate climates beyond 34° latitude. The region between 32° and 34° latitude is considered the hybrid zone for African and European honeybees. Below 32° latitude, African honeybees thrive.

The venom of an Africanized bee sting is less toxic than that of a European honeybee sting! It's the number of stings inflicted during an attack that matters. When aggravated to the point of attack, the average European honeybee colony will inflict a dozen or so stings to the victim compared to hundreds of stings from a disturbed Africanized honeybee colony. The average person can tolerate 15–25 stings without medical attention. However, those with known or possible allergies should seek medical attention immediately regardless of the number of stings sustained. Anyone stung by more than 25 bees should seek attention as responses to the bee venom may be delayed in some individuals.

Banning beekeeping in your municipality is not a good idea and is not a defense against Africanized honeybees. By removing European honeybees from a region, you are actually making it easier for the nomadic Africanized bee to move in. Local beekeepers should be encouraged since they can maintain strong, healthy European colonies that would dilute any possible contamination by Africanized honeybees.

ALTERNATIVES TO HONEYBEES An excellent reference for studying alternatives to honeybee pollinators is *Managing Alternative Pollinators: a Handbook for Beekeepers, Growers, and Conservationists* (Mader *et al.*, 2010), which is available for download at www.sare.org or www.nraes.org.

BUMBLEBEES Bumblebees (*Bombus* spp.) have become increasingly popular as pollinators. There are more than 250 species of bumblebees worldwide. They are social and relatively docile. Mated females overwinter in solitary hibernation and emerge in the spring. They immediately seek a nesting site, and build a wax cell filled with pollen and nectar and lay several eggs.

As with the honeybee, a queen bumblebee mates and stores sperm in her spermatheca, located in the queen's vagina. Most bumblebee queens mate only once. When she lays eggs, she decides whether or not to fertilize an egg. Fertilized eggs develop into female workers and unfertilized eggs develop into males. Most of the eggs she lays are fertilized and become female workers. In order for a fertilized egg to become a queen, it must be fed copious amounts of pollen, and this usually only occurs towards the end of the colony life cycle.

Once the workers emerge, the queen's sole purpose is to lay eggs. The workers construct the hive and fill cells with pollen and nectar. Bumblebees collect nectar, but only enough to last for several days of inclement weather. They do not transform nectar into honey. Towards the end of the summer, female larvae are fed enough to produce queens and the reigning queen lays unfertilized eggs to develop into males. The only function of male bumblebees it to mate with the queens. After mating, the males and worker females die. The mated queens go into solitary hibernation only to start the cycle all over again the following spring.

Commercially available hives of *Bombus impatiens* generally have around 50–300 bees, which include a queen, workers, and a brood which consists of pupae, eggs and larvae. Sugar solutions are often supplied with the hive when hives are used for pollination of nectarless crops such as tomato. Different hive sizes are available depending on the size of the crop to be pollinated.

Bumblebees are not as easy as honeybees to manage, but they are extremely efficient as pollinators. They have long tongues to gather nectar from flowers whose anatomy makes nectar gathering by honeybees

difficult. In anatomically difficult flowers with long petal tubes, bees with short tongues unable to reach the nectar will often bite a hole in the base of the flower to reach the nectar. This bypasses pollination by the bee altogether.

Temperature greatly affects pollinator activity. Honeybees are not very active below 10°C, while bumblebees are active down to 7°C. While this is a very small difference, it can be important for crops that bloom when it is cool (e.g. raspberries, *Rubus* spp.) or that release pollen early in the day when it tends to be cooler (e.g. squash, *Cucurbita* spp., and cucumbers, *Cucumis sativus*).

Some flowers release pollen through pores in the anthers. In order for the pollen to be released, the anther must be vigorously shaken. A bumblebee can pollinate by grabbing an anther with its mandibles and shaking vigorously to release pollen. There is an audible buzz that emanates from the bee's throat, thus the term 'buzz pollination' was adopted for this process. Crops that benefit from buzz pollination of bumblebees include tomatoes (*Solanum lycopersicum*), peppers (*Capsicum annuum*) and blueberries (*Vaccinium* spp.). Bumblebees are also more docile than honeybees, thus they are good for greenhouse use.

Carpenter bees (*Xylocopa* spp.) are often confused with bumblebees. They look like large black bumblebees without pollen baskets on their hind legs. They are solitary and have not been of much use in pollination enhancement since they tend to cut holes in the base of corollas to access nectar, thus they don't distribute pollen very effectively.

OSMIA BEES Osmia bees (often called mason bees, blue orchard bees, and hornfaced bee) are solitary bees that are extremely important in crop pollination. While current interest in the mason bee began to increase sharply in the 1990s, mason bees (*Osmia cornifrons*) have been used for apple (*Malus domestica*) pollination since the 1940s. While there are dozens of species in the genus *Osmia*, only three species (*Osmia lignaria, Osmia californica*, and *Osmia cornifrons*) are currently commercially available for pollination.

Osmia bees are solitary, there is no hive or colony and each bee is an independent entity. The female constructs her nest, gathers pollen and lays eggs on her own with no help from other bees. Even though they are independent, many Osmia bees are gregarious, meaning that they build their nests next to other Osmia bee nests. This makes it easier to commercially raise Osmia bees as large numbers of nests can be cultivated in a single structure.

The females are the pollinators; males may gather a little nectar from flowers, but they really only live to mate. Once they mate, they die. Males are smaller than females and both are generally metallic blue, black or green in color. Females have pollen-collecting hairs on their abdomen as well as a pair of horn-like appendages on their faces. Males do not have facial horns, but often have a tuft of white hair in the center of their face. Even though they have a stinger, females rarely sting and if they do, the sting is not very painful.

Most Osmia bees are univoltine, meaning that there is only one generation per year. All *Osmia* species are only active in the spring, with the exception of *O. californica*. Adults emerge from eggs laid the previous spring and are active for only a few weeks. During this time they mate, build their nests, lay eggs and die. Larvae develop into adults over the summer and lie dormant over the winter. Since they are only active early in the spring, they are effective pollinators for a limited, yet important, number of crops. They are extremely important in fruit crop pollination.

Managed Osmia bees build nests in tunnels lined with cardboard in holes drilled in wooden blocks. The tubes are lined with a type of cement (hence the name mason bees) made up of mud gathered from nearby areas by females as they prepare to lay their eggs. The females construct one cell at a time, fill it with pollen and nectar, lay an egg, then cap the cell with more mud. Each nest is finished with one empty cell with a thick end wall. The empty cell provides extra protection from predators and wasps who might try to lay eggs in the nest. Each female normally lives for about 1 month and builds one to six tunnels, each with five to 15 eggs in each.

There are two options to choose from when considering using mason bees as supplemental pollinators. Mason bees can be 'trap nested' from wild populations or purchased from commercial sources. Trap nesting ensures that the bees are those native to your habitat. Purchased bees may or may not be native to your area.

ALKALI BEES (*NOMIA MELANDERI *COCKERELL) Alkali bees are an effective pollinator of alfalfa in the western USA. The alkali bee is a gregarious bee almost as large as a honeybee which nests in the soil. They are black with copper-green stripes across

the abdomen and the males have much larger antennae than the females. They generally build up to 100,000 nests over a 23 m^2 area. Their nests, each with 15–20 cells, are generally 1 cm wide and extend 7–13 cm vertically into the soil. Each cell is oval and slightly larger than the main nesting tunnel and is about 1 cm long. It is lined with soil followed by a waterproof liquid produced by the bee. A pollen ball made up of eight to ten bee loads of pollen mixed with nectar is placed in each cell.

Adults emerge from their cell after spending 10 months as a dormant, fully grown larva from late June to late July. Males emerge several days before the females. A female lays 15–20 eggs over the course of her active, 1 month-long life as an adult. Males mate with the female during the first day of hive construction and each male mates with multiple females. On average each female lays one egg/day.

The primary food source for alkali bees is alfalfa. Females seek nectar and pollen while males seek nectar only. Most flowers are pollinated by female bees as pollen is generally only released when females are searching for pollen.

Artificially prepared nesting sites or beds can be developed in regions where the alkali bee is not normally found and the reader is advised to examine the book by McGregor (1976) if interested. Construction of alkali bee beds may be more cost effective than honeybee hive rental. However, the utility of alkali bees for pollination is somewhat limiting as: (i) beds can only be used in areas with limited rainfall during bloom; (ii) beds must be constructed where the crop is to be grown; and (iii) they must be constructed months before the crop is planted. In addition, an alkali bee bed can be lost to pests with little or no warning.

ALFALFA LEAFCUTTER BEES (MEGACHILE PACIFICA PANZER) The alfalfa leafcutter bee gets its name from the fact that it lines its above-ground nests, that are hollow tubes or tiny holes, with circular sections of alfalfa (*Medicago sativa*) leaves, and on occasion, petals of ornamental flowers.

The alfalfa leafcutter bee is a gregarious solitary bee slightly larger than a housefly. The female begins constructing nests in hollow tunnels in which she barely fits, as soon as she emerges in the spring. Each cell in the nest is lined with leaf sections cut from alfalfa, then filled with pollen and nectar. Pollen and nectar are gathered from mostly alfalfa, clovers (*Melilotus* spp., *Trifolium repens* L.), and mint (*Mentha* spp.). The female lays her egg, caps off the cell with leaf sections, and begins construction of the next cell in the nest. This process continues for about 2 months as long as pollen and nectar are available. The average female mates only once and lays about 30–40 eggs, of which two or three of the resultant adults will be male. The male generally seeks nectar only and is inefficient in pollinating crops. The female seeks pollen and nectar and may visit up to 15 flowers/min, thus she is a very effective pollinator.

Development from egg to adult is extremely temperature dependent. It takes 15 days for eggs to hatch at 16°C and 2 or 3 days at 35°C. Development through four instars to the pre-pupal stage takes 11 and 35 days from egg hatch at 16°C and 35°C, respectively. Many larvae can be lost during the first two instars if the temperature is above 26°C or too cold for feeding. If larvae die during their development, an empty, pollen-filled celled called a 'pollen ball' is all that remains.

In cooler climates, development stops at the pre-pupal stage and the bee will overwinter in a dormant state. As temperatures warm in the spring, the pre-pupae molt into pupae. After about 1 week, adults emerge. In warmer climates, development may continue past the pre-pupal stage without dormancy and a second generation of bees may emerge. One major problem with second-generation bees is a high incidence of chalkbrood mortality, a disease of leafcutter bees. When second-generation bees emerge from the nest, they chew through chalkbrood contamination from the previous generation and may become covered with spores in the process. The second-generation adults then return to the nest and contaminate any new nests they build.

Males emerge before females, and once females emerge, mating begins. Females mate once while males may mate several times. Sperm is stored in a spermatotheca and the female will begin to lay eggs a 1 or 2 days after mating.

Alfalfa flowers are like most other legume flowers in that they have a unique design which is important in pollination strategies. A legume flower normally has one large 'normal' petal with two 'wing' petals, one on each side of the 'normal' petal. Two other petals on the bottom of the flower are fused together to form the 'keel' petal which encloses the stamen under tension. When the fused keel petals are slightly separated by a pollen-seeking insect, the stamen is released rapidly, smacking the

'normal' petal. Once the stamen has been released from the keel, it does not return to its former position, but remains exposed. The flower is then said to have been tripped. During the tripping process, the insect is dusted with pollen. Insects are often hit on the head during tripping. Leafcutter bees are much less annoyed by the process compared with honeybees, as honeybees will seek other sources of pollen and nectar rather than get smacked on the head.

Alfalfa leafcutter bee nesting is easily encouraged using commercially available nesting boards. Larvae-filled nesting boards are stored in a cool, dry place between –1°C and 4°C. They are removed from storage the following spring as pollinators are desired.

STINGLESS WILD BEES Two important genera of stingless social bees are *Melipona* and *Trigona*. These bees are particularly important in crop pollination in Mexico, Central and South America, Africa, Southern Asia, and Australia. Besides their importance in pollination, they also produce honey and wax.

Even though these bees are stingless, some do have mandibles strong enough to inflict a mild bite and some emit a substance from their mouth that is irritating to the skin. In general, these bees do not injure humans working with or around them.

The honey produced by stingless bees is quite variable in quality. The wax secreted by stingless bees is mixed with propolis, thereby causing the wax to be black. It is used for waterproofing and ink.

These social bees have queens who are attended to by worker bees. Each nest has a single egg-laying queen and up to 50 virgins.

COMMERCIALLY AVAILABLE POLLINATORS Many horticultural crops benefit from the introduction of extra pollinators into the production field due to a lack of natural pollinators. While most of the pollinators available for either rental or purchase are expensive, the benefit of their use is quickly realized by increased yield.

There are many factors which determine how many pollinators you might need for a specific crop at a specific location. These factors include: (i) crop; (ii) size of planting; (iii) time of year; (iv) wild pollinator populations; and (v) what commercial pollinator you plan to use. As a very general rule of thumb, you need at least two to five honeybee hives, ten bumblebee hives, 50,000 leafcutter bees or 620 female mason bees per hectare.

HONEYBEES Probably the most widely used accessory pollinator is the honeybee. Hives are available for rental, and demand is usually high. Thus it is important to set up rental agreements early to ensure that you will be able to get the hives you need. Make sure that you only sign a rental and liability agreement that you are comfortable with and seek professional guidance if you are unsure about the agreement.

There are a number of factors which should be considered when entering a hive rental agreement. All parties involved in the transaction must be clearly identified. This includes the grower, the beekeeper, and the transportation specialist. The crop which will be pollinated and its location should also be included as well as a glossary of terms which may not be familiar to all parties. The number and size (or strength) of hives, the rental price, and the length of the rental must be included, as well as any transportation or maintenance fees. An explicit listing of pesticide practices utilized by the grower is often useful in preventing any miscommunications regarding appropriate exposure of hives to pest management techniques. This list should include pest management practices utilized prior to the hive rental. Make sure that both parties agree on removal of hives after the pollination season, so that they don't interfere with ongoing farm practices, especially pesticide application. Liability for injuries caused by bees should be agreed upon. This is especially true if visitors will be on farm during the pollination season.

BUMBLEBEES Bumblebee (*Bombus terrestris* and *Bombus impatiens*) hives are purchased rather than rented (Fig. 5.6). A colony with a queen along with workers and a brood (pupae, eggs, and larvae) are supplied in a plastic and cardboard hive along with a sugar solution for supplemental feeding of the hive. Different hive types are available with different pollination capacities. Bumblebees are most often used in protected culture of fruits and vegetables. They are not as aggressive as honeybees and a hive of 50 or so bumblebees can do the work of 20,000 honeybees.

It is important that the hive be adequately shaded in the greenhouse. Bumblebees work best at a temperature range of 10–30°C. Air vents must be screened to prevent the escape of bees from the

Fig. 5.6. A commercial bumblebee hive often used for pollinating greenhouse crops such as raspberries (*Rubus* spp.) or strawberries (*Fragaria* × *ananassa*).

greenhouse. The hive should be located as far away from carbon dioxide supplementation as possible.

Since bumblebees rely on UV light for navigation, it is important to manage lighting conditions of the greenhouse with the bees in mind. Their hive should be about 2 m off the floor with the hive entrance facing east. If daylengths are extended with artificial lighting, extend the light period before sunrise, not after sunset. Before sunrise the bees will be in the hive and turning lights on will stimulate them to pollinate. If you extend the light before sunset, bees are disoriented when the lights are shut off and are unable to return to the hive.

Great care must be given to the use of any pest management schemes employed in the greenhouse. It is often necessary to remove the hive(s) from the greenhouse when pesticides are used. Most commercial hives have two entrances. One allows bees to fly in and out of the hive while the other only allows flight into the hive. To remove the hive from the greenhouse, simply close the two-way entrance. After about 1 h, all bees will have returned to the hive. It can then be removed from the greenhouse until it is safe for the bees to return to flight. Make sure the hive is protected from ants, since ants are highly attracted to the sugar solution feeding the bees. Larger hives are available for field or high tunnel use.

GREENBOTTLE FLIES (LUCILIA CAESAR) The greenbottle fly is available for pollination enhancement that is often needed in the production of certain seed crops. Sometimes both male and female lines in field hybridization produce little pollen or nectar, and are thus not attractive to "normal" pollinators. Such crops include cauliflower (*Brassica oleracea* Botrytis Group), cabbage (*Brassica oleracea* Capitata Group), rapeseed (*Brassica napus*), lettuce (*Lactuca sativa*), endive (*Cichorium endivia*), radicchio (*Cichorium intybus*), carrot (*Daucus carota*), onion (*Allium cepa*), leek (*Allium ampeloprasum* var. *porrum*) and asparagus (*Asparagus officinalis*). The adults visit flowers searching for nectar and in the process distribute pollen on their bodies. In order to be effective pollinators, their bodies must come into contact with anthers when searching for nectar. This is totally dependent on flower structure. The pollen is carried to the next flower they visit.

Greenbottles are purchased as pupae mixed with sawdust. Approximately 1 kg of this mixture is evenly dispersed over 100 m^2 area once a week during pollination. It should be dispersed under the shade of the canopy in the morning or evening and protected from mice. The pupae–sawdust mixture has a shelf life of only 1 week and should be stored at 6°C in the dark. Flies emerge in about 3–4 days at 24°C.

ORCHARD MASON BEES (OSMIA SPP.)/ALFALFA LEAFCUTTER BEES Many commercial sources of Osmia bees and alfalfa leafcutter bees exist. Bees may be purchased in a number of forms. Which you choose is up to you but it is imperative that you become educated in handling the dormant bees before embarking on their use. For specific information see the before-mentioned reference (Mader *et al.*, 2010) on alternative pollinators, as a discussion of their handling is beyond the scope of this text.

Fertilization

The final phase of floral development occurs with fertilization. Fertilization ultimately results in the formation of a seed or seeds which then influence the growth and development of the fruit. Fruit growth and development are really extensions of floral development; however, we separate the two for clarity.

DOUBLE FERTILIZATION Fertilization in most plants is a two component event called double fertilization. In one phase of fertilization, one of the sperm cells from the pollen grain unites with the egg cell to form a single-celled zygote. The zygote undergoes mitosis and transforms into a multi-celled embryo. The second sperm cell from the pollen grain fuses with the polar nuclei (remember it's a diploid entity

of the female gametophyte) to form the triploid endosperm. The endosperm is parenchymatic tissue with the primary function of food storage. In some seeds it is absorbed into large fleshy seed leaves (e.g. green beans, *P. vulgaris*) while in other seeds, it remains separate as a starchy entity (corn, *Zea mays*). Together the embryo and the endosperm form the bulk of a seed. The seed or seeds formed from the fertilization of a flower's egg(s) have a profound influence on the development of the fruit.

Fruit

There is often confusion about whether certain horticultural commodities are a fruit or a vegetable. It depends on whether you are speaking botanically or from a horticultural perspective. Botanically, a fruit is a ripened ovary along with any harvested accessory tissues. The horticulturist would probably refine that definition to limit fruits to a commodity used for dessert or treats compared with those employed in the main meal. For example, a green bean is a fruit to a botanist while a horticulturalist would place it in the vegetable category. Unfortunately, sometimes the classification is for political reasons, such as the United States Department of Agriculture (USDA) classification of the tomato as a vegetable so that ketchup served with French fried potatoes can be considered a vegetable in school lunch programs.

Fruit set

The first stage of fruit growth and development is called fruit set. Fruit set describes a visible stage of development where it is apparent on inspection of the remnants of the flower that a harvestable fruit is beginning to develop. It is a point in development where a physiological decision is made to either abort the embryo and ovary or to go ahead with fruit development. Fruit set is most often quantified as a percentage of flowers present at bloom which appear to be developing into a harvestable fruit. Most fruit and vegetable crops require both pollination and fertilization for fruit set and growth.

Seedless fruit/parthenocarpy

Seedless fruit production is known as parthenocarpy. There are three different forms of parthenocarpy: (i) vegetative parthenocarpy; (ii) stimulative parthenocarpy; and (iii) stenospermocarpy.

Vegetative parthenocarpy

Fruit set without pollination or fertilization is called vegetative parthenocarpy. Seedless cucumber (*C. sativus*), pineapple (*Ananas comosus*), 'Satsuma' mandarin (*Citrus reticulata*), 'Washington' navel orange (*Citrus sinensis*), some sycomore figs (*Ficus sycomorus*) and dessert banana (*Musa acuminata*) are examples of fruits that form by vegetative parthenocarpy.

Stimulative parthenocarpy

Fruit set with pollination but without fertilization is called stimulative parthenocarpy. Seedless clementines (*Citrus reticulata*), eggplant (*Solanum melongena*) and 'Black Corinth' grape (*Vitus vinifera*) are examples of fruit formed via stimulative parthenocarpy. Stimulative parthenocarpy can also be achieved via stimulation with plant growth regulators or other stimuli, such as wasps as is the case in some figs (*F. sycomorus*).

Stenospermocarpy

Fruit production when pollination and fertilization are quickly followed by embryo abortion is called stenospermocarpy. Seedless grapes (*Vitis* spp.) such as 'Thompson Seedless' (*Vitis vinifera* 'Thompson Seedless') result from stenospermocarpy. Fruits without seeds tend to be smaller than those with seeds. This is because seeds are a rich source of the plant hormone, gibberellic acid (GA). Many seedless grape cultivars are sprayed with GA after bloom to increase size. (GA is also used during bloom to reduce the number of berries set in each grape cluster.)

In some horticultural crops, parthenocarpy is controlled through controlled crosses. This is the case with seedless watermelon (*Citrullus lanatus* var. *lanatus*). This is a good example of controlled stimulative parthenocarpy, since only pollination is required for fruit growth and development. The pollinated cultivar does not produce viable eggs, thus fertilization is impossible (Fig. 5.7). The pollinated cultivar is obtained by crossing an inbred tetraploid female with an inbred diploid male parent to produce triploid seed. (The reciprocal cross of diploid female parent with tetraploid male parent does not produce seed.) Tetraploid plants produce only 5–10% the amount of open-pollinated cultivars, thus triploid seed is very expensive. Plants from triploid seeds (33 chromosomes) are sterile

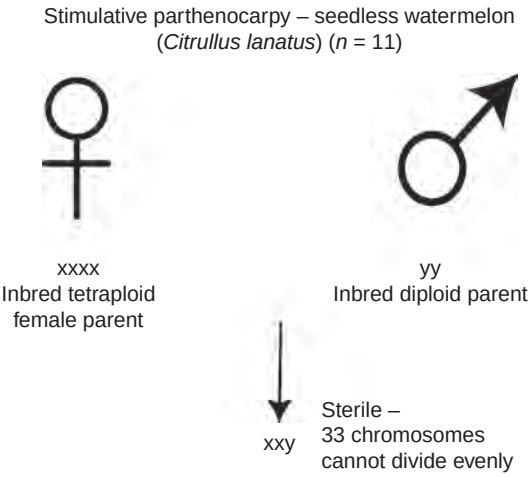

Fig. 5.7. Genetic control of seedlessness in watermelon (*Citrullus lanatus*). (Gender symbols courtesy of the Integration and Application Network, University of Maryland Center for Environmental Science, ian.umces.edu/symbols/.)

since their chromosomes cannot be divided equally during meiosis. However, parthenocarpic fruit will develop if a diploid pollinizer is planted in with the triploid cultivar.

The fruit-set signal

Unless the species is vegetatively parthenocarpic, the most important factor determining the amount of fruit set is pollination. The act of pollination sends a signal to the ovary to continue development. Growing pollen produces GA which enhances auxin production in the style and ovary. GA is the primary signal which induces the secondary signal, auxin. The flower abscisses if this gibberellin signal is not present. In some species, pollination can be replaced with an application of GA, and GA or auxin can also be effective in enhancing fruit set in some species in years when pollination is poor.

Seed set

While pollination is all that is needed for fruit growth to continue in some species, in most species fertilization must occur for the fruit to continue developing. In these crops seeds are needed for fruit growth. Assuming effective pollination occurs, temperature will probably determine whether or not fertilization takes place. The pollen tube must grow down the style quickly enough so that the sperm cells can effect fertilization before the ovule dies. The temperature that is best for pollen growth varies with species.

Seed set occurs once fertilization of the egg has taken place and the chance of embryo abortion has diminished. While one might argue that seed set and fertilization are one and the same, the chance of embryo abortion immediately after fertilization distinguishes the two events. Seed set is characterized by a sharp increase in the production of gibberellins, auxins and cytokinins, each playing an important role in further fruit and seed growth.

Fruit drop/fruit thinning

In all cases, once fruit growth begins, adequate photosynthates must be translocated to the developing fruit. If there are too many fruit developing on a plant, the available photosynthate will often be translocated to the larger, stronger fruit, resulting in the abortion of the embryo and abscission of the weaker fruit from the plant. In tree-fruit production, this is called 'June drop'. One of the tasks of a fruit grower is to prevent excessive fruit set while at the same time promoting sufficient set for a full crop.

Fruit growth

After fruit and/or seed set, fruit growth results from both cell division and expansion. In most crops, an initial period of rapid cell division occurs in all parts of the fruit and is dependent on growth regulators produced by the developing seeds. Fruit usually do not appear to grow very much during this stage even though the number of cells in it is increasing dramatically. Cell division in many crops seems to be regulated by hormones, particularly gibberellins, produced by the developing seeds. Final fruit size is often highly correlated with the number of seeds developing in the fruit. When one or more seeds in a multi-seeded fruit abort, lopsided and misshapen fruit result. Parthenocarpic fruits don't have seeds and how their growth is regulated is not well understood.

Fruit gradually shift from cell division into a period of cell expansion. Some tissues within the fruit continue division while other tissues begin cell expansion. In general the tissues that make up

the bulk of the mature fruit undergo greater cell expansion, while the other tissues are likely to continue cell division for any increase in size. By far, cell expansion accounts for the greatest increase in fruit size.

Fruit ripening

An in-depth look at maturity and ripening is presented in Chapter 15, this volume. We briefly address both here for completeness. Fruit are considered mature if they will ripen once removed from the parent plant. Fruit ripening is the process of becoming edible after maturity has been reached. Crops can be harvested either: (i) mature but not ripe; or (ii) mature ripe. Strawberries will not continue to ripen once harvested, thus they are harvested mature ripe. Pears are not edible when harvested and must ripen before being consumed.

In general ripening coincides with embryo maturation. We often limit our discussion of ripening to the fleshy fruited commodities. Fleshy fruit are either climacteric or non-climacteric, a quality very important in postharvest considerations. Climacteric fruits have a rapid concomitant increase in respiration and ethylene production. This increase occurs just prior to ripening and is quickly followed by fruit senescence. Climacteric fruit include tomatoes (*S. lycopersicum*), bananas (*Musa* spp.) and peaches (*P. persica*). Non-climacteric fruit such as grapes (*Vitis* spp.) and strawberries (*Fragaria* × *ananassa*) do not exhibit this phenomenon. Ethylene is the hormone regulating ripening and senescence, but exposure to ethylene does not automatically induce ripening. A fruit must be 'ready to ripen' before ethylene can exert its effect. At that point they are called mature green. Ethylene production by plant tissues is autocatalytic, meaning that ethylene induces ethylene production.

Many changes to fruit tissues occur during ripening and they will be discussed in detail in Chapter 15, this volume. Generally speaking, color changes, sugars increase, acids decrease and tissue softening takes place. Ultimately fruit quality will deteriorate as the fruit begins to senesce after ripening.

Seeds

A seed, consisting of an embryo, the endosperm and a testa (seed coat) develops from an ovule in an ovary. The embryo is the newly developing plant inside the seed. In order to truly appreciate how marvelous its development is, one must step back and review the situation existing inside the ovary just prior to fertilization. The multi-cellular embryo sac resides inside the ovary and has a polar organization from one end to the other. The two ends of the embryo sac are the micropylar end and the chalazal end. The egg cell and its synergid cells are close to the micropylar end while the antipodal cells are close to the chalazal end. A central cell with two nuclei is located about midway between the two ends. The pollen tube grows through the micropyle opening and one of the sperm cells traveling down the pollen tube fertilizes the egg cell to form a one-celled diploid zygote. The other sperm cell combines with the two polar nuclei of the central cell to form the triploid endosperm. The endosperm serves as a nutrient reservoir for the embryo and may become a separate entity or it may be absorbed into the embryo during seed development.

Embryo development – morphogenesis

Embryo development is often divided into three overlapping stages: (i) morphogenesis; (ii) maturation; and (iii) desiccation (Evert and Eichorn, 2006). During morphogenesis, the polarity first observed in the embryo sac is preserved in the typical plant body which consists of a basal root apex, a distal shoot apex and connecting tissue between the two (de Smet *et al*., 2010). The polar nature of development in the embryo seems to be controlled maternally and determined in the embryo sac. However, since somatic cells of many plants can be forced to undergo embryogenesis, other regulators of polarity must exist.

The single-celled zygote undergoes an uneven transverse (divides along the equator of the cell) cell division resulting in a smaller apical cell which ultimately forms the embryo and a larger basal cell which develops into the suspensor and hypophysis. The suspensor connects the embryo with maternal tissue while the hypophysis gives rise to the root cortex and cap.

An embryo's polarity defines its axis of development. Precisely regulated cell division leads to further development in a series of events that partition the embryo into three distinct zones: (i) an apical zone which includes the cotyledons, the shoot apex and the upper part of the hypocotyl; (ii) a mid-zone which includes most of the hypocotyl; and (iii) a basal zone which includes the root apex. These zones

have been observed in many species during embryogenesis. The regulation of the development of these zones has been shown to be under precise genetic control in studies with *Arabidopsis* mutants and auxin appears to be the primary phytohormone involved in embryo developmental patterns (de Smet *et al*., 2010).

There may be a role for cytokinins for primary root development (Müller and Sheen, 2008) and brassinosteroids may take part in embryo development (Chandler *et al*., 2009; Scacchi *et al*., 2009). Gibberellins and abscisic acid (ABA) are mainly involved in the later stages of embryo development, particularly ABA maintenance of dormancy and gibberellin promotion of germination (Holdsworth *et al*., 2008).

The shoot, cotyledons and hypocotyl are all derived from the apical cell which formed after the first division of the fertilized egg. Root development is a little more complicated. The top cell of the suspensor, which was derived from the basal cell after the first division of the fertilized egg, forms the hypophysis which develops into the root cap, root cap initials and ground meristem initials. The remainder of the root apex develops from the apical cell.

The first two embryonic organ systems to develop are the cotyledon and the axis, which form in response to polar auxin transport. Their formation begins in the globular stage of embryo development but they are not visible until the heart stage. We know that differentiation begins in the globular stage since embryos treated with auxin transport inhibitors in this stage, or mutants deficient in auxin transport develop deformed cotyledons. Separate cotyledons do not form, but rather, a ring of cotyledon-like tissue develops as a ring around the apex. Another important step in embryo development is the development of specific tissues within the organs, specifically the protoderm, the ground tissue and the procambium. These tissues become apparent in the transition from the globular to the heart stage.

The embryo eventually moves into a stage where lipid, protein and carbohydrate reserves are developed in all cells. These reserves are especially important in the cotyledons of species which do not develop distinct food reserves in an endosperm. These reserves serve as a nutrient source for developing seedlings until the seedling can become photosynthetic. The embryo finally enters a period of desiccation.

Endosperm development

The endosperm results from the union of the polar nuclei with a sperm cell from the pollen grain (Evert and Eichorn, 2006; Dumas and Rogowsky, 2008). In some species it remains a separate entity from the embryo, but in others it is absorbed by the cotyledons. The endosperm has two very important roles in seed development. One is to be a source of nutrition for the developing embryo and the other is to at least partially direct development of the seed (Berger *et al*., 2006).

In angiosperms there are two types of endosperm: (i) nuclear; and (ii) cellular. The cellular type of endosperm is limited to the lower or basal angiosperms. Its development is similar to that of the embryo, which suggests that it may have evolved as some sort of simple auxiliary embryo.

The nuclear type of endosperm is the most common type of endosperm in angiosperms. Nuclear endosperms have a series of nuclear divisions followed by polar migration of the nuclei forming sequestered units or domains, much like in the developing embryo. The cell-like structure of the nuclear endosperm is achieved with the formation of alveoli, open-ended tube-like structures. Alveoli are found only in nuclear-type endosperm and in the female gametophyte of gymnosperms. Gymnosperms do not have endosperm, but rather the female gametophyte enlarges considerably while storing nutrients during embryo development.

Though endosperms vary considerably among angiosperm species, they all have common attributes. These include: (i) a mother–offspring interface; (ii) an embryo–endosperm interface; (iii) an epidermis; and in some (iv) a separate storage tissue.

The endosperm is designed to transfer nutrients from the mother plant to the developing embryo. In both monocots and eudicots (most but not all dicots) most nutrients, mainly sucrose, is absorbed by the embryo through the mother–offspring interface by means of specialized haustoria, or food-absorbing outgrowths, in the chalazal area of the ovule (the end away from the micropyle). This area of the endosperm is often called the chalazal endosperm. The area right next to the embryo is called the micropylar endosperm. It is important for regulating development of the embryo by regulating sugar uptake. It also protects the embryo with anti-fungal proteins.

Endosperms accumulate certain compounds as food reserves for the embryo. Starch is the main

storage compound in monocots and it is stored in a mass of enlarged cells called the starchy endosperm. Most other angiosperms store lipids and proteins in their cotyledons rather than a large separate region as in the monocots.

All endosperm possess an epidermis called the aleurone layer. It contains proteins which prevent precocious germination and promote desiccation tolerance. Enzymes are released from the aleurone layer during imbibition that mobilize stored nutrients for use by the rapidly growing embryo. The aleurone layer is present even in species where most of the endosperm is absorbed by the embryo, indicating a role in seed maturation and germination.

The endosperm is triploid, two sets of chromosomes are maternal and one is paternal. In order for normal development to occur, this two-to-one balance must be maintained. This suggests that a large portion of endosperm development is maternally controlled. When the balance is switched via novel breeding and molecular techniques, endosperm development is abnormal.

Seed coat development

The testa originates from the integuments of the ovule and serve a protective function surrounding the embryo/endosperm complex. The seed coat often has adaptations for improved dispersal such as hook-like hairs or glue-like substances that allow seeds to become attached to animals for deposition away from the parent plant.

Maturation and entrance into dormancy

As the embryo reaches a stage of maturation, most cell division has been completed. Further growth is through cell expansion and an embryo may grow 100-fold during that time. During the cell expansion period a large accumulation of storage compounds (starches, lipids, and proteins) occurs. Many of these storage compounds are harvested by humans for food. From a seed's perspective, storage compounds are an important energy source for germination.

Another important physiological process that occurs during seed maturation is the development of desiccation tolerance by the seed, the embryo in particular. There is a group of proteins called deyhdrins which develop as the seed matures. These proteins seem to sequester ions to prevent crystallization during desiccation. Dehydrins may also form a protective layer surrounding various membranes in embryonic cells.

Most seeds enter a period of dormancy after maturation which can range from several days to years. In addition, some seeds require exposure to cool moist conditions before they will germinate. This exposure is called stratification, and will be discussed in Chapter 9, this volume. Seeds of some species do not go through a maturation stage per se but rather may germinate immediately upon release from the fruit. This type of seed is called viviparous.

Seed dormancy

Definitions of dormancy are difficult to develop because dormancy can only be measured by the absence of germination. Even so, seed dormancy has been organized into five major categories: (i) morphological; (ii) physical; (iii) physiological; (iv) morphophysiological; and (v) combinational dormancy (Baskin and Baskin, 2004). It is also often characterized as primary or secondary dormancy. Generally speaking, a seed is dormant if it cannot germinate in a specified amount of time under any combination of physical environmental factors that are otherwise favorable for its germination once it is non-dormant (Baskin and Baskin, 2004).

Morphological dormancy

Morphological dormancy is characterized by an incompletely developed embryo at seed harvest. Once the embryo grows to maturity it will readily germinate. Celery (*Apium graveolens*) is an example of seed with morphological dormancy.

Physical dormancy

Physical dormancy is caused by some characteristic of the seed which prevents it from imbibing water and germinating under normally favorable conditions. Inability to imbibe water may be caused by a layer of waterproof cells in the seed coat. Imbibition can only take place once this barrier is broken down by fluctuating temperatures, repeated freezing and thawing, fire or passage through an animal's digestive tract.

Another characteristic which prevents water imbibition is the presence of a specialized structure at the hilum, the area where seed was attached to the ovary via a placenta. One such structure is

called a lens and occurs in legumes in the family *Fabaceae*. Only when cells in the lens are disrupted can the seed imbibe water. Another structure called the chalazal plug or cap control water imbibition in some species in the *Bixaceae, Cistaceae*, and *Malvaceae*.

Physiological dormancy

Physiological dormancy is the most widespread type of seed dormancy and is often subdivided into three further levels: (i) deep; (ii) intermediate; and (iii) non-deep. In order to determine the type of physiological dormancy of seeds in a particular species, embryos are excised from the seeds and placed under conditions known to favor germination in that species. Seeds with deep physiological dormancy require 3–4 months of warm (>15°C) or cold (0–10°C) stratification in order for their embryos to germinate and treatment with GA does not hasten germination. Some examples include *Acer platanoides* and *Leptecophylla tameiameiae*.

Intermediate physiological dormancy is characterized by a requirement for 2–3 months of cold stratification. Dry storage shortens the stratification period in some species and treatment with GA promotes germination in some species. An example is *Acer pseudoplatanus*.

Most species exhibit non-deep dormancy. Treatment with GA breaks the dormancy as can scarification, dry storage, or cold or warm stratification. Non-deep physiological dormancy is divided into five levels numbered one through to five, and most species are in type one or two. The five types differ in seed germination temperature requirements.

Morphophysiological dormancy

Seeds that exhibit morphophysiological dormancy have an underdeveloped embryo *and* some other physiological element to their dormancy, which usually requires some form of stratification. An example of a species with this form of dormancy is ash (*Fraxinus excelsior*).

Physical with physiological dormancy

Seeds with a hard seed coat and a non-deep physiological requirement to end dormancy are put into this category (e.g. *Geranium* spp.).

Requirements for breaking dormancy

Before sowing seeds of any horticultural crop, seed dormancy of that species should be well understood if success is expected. Usually specific requirements involve light, water, temperature or scarification treatment(s) to improve germination. Specific requirements are easily found in plant propagation texts, seed packets or with a quick search of the Internet.

Germination

Germination is a highly regulated process which occurs only when environmental conditions are favorable for growth of the species in question (Finch-Savage and Leubner-Metzger, 2006; Nonogaki, *et al*., 2010). Germination encompasses those events occurring from water imbibition through to radicle emergence (Bewley, 1997; Nonogaki, *et al*., 2010). It is interesting that almost all cellular and metabolic events which occur between imbibition and radicle emergence in non-dormant seeds occur in dormant seeds, yet the radicle fails to emerge from dormant seeds.

Imbibition

Water imbibition is the first step of germination (Nonogaki, *et al*., 2010). Water uptake by seeds is triphasic. There is a rapid initial uptake of water (phase I) followed by a plateau phase (phase II). A third phase (phase III) involves the rapid uptake of water with elongation of the embryonic axis which results in radicle emergence and the completion of germination. Dormant seeds can progress through phases I and II, however, dormant seeds do not enter phase III.

Structural changes

Water uptake in phase I immediately leads to structural changes in membranes. They go from a gel phase that was induced during the maturation and drying stages of the seed development to a 'normal' hydrated liquid-crystal state (Crowe, *et al*., 1989). During the transition from gel to liquid-crystal, extensive leakage of solutes and low molecular weight substances into the surrounding imbibition fluid occurs (Copeland and McDonald, 1985). Membrane repair to correct the leakage problem occurs during the hydration process, but the mechanism of repair is unknown.

Metabolic changes

There is a rapid increase in metabolic activity on imbibition (Nonogaki, *et al*., 2010). The structures and enzymes needed for cellular metabolism are present but are not necessarily functional within the dry seed. Part of the amazing physiology of a seed is the fact that it survives the intense dehydration associated with desiccation at maturity. Full metabolic status and functioning occurs within several hours after the initiation of imbibition.

One of the first metabolic changes observed during germination is a rapid increase in respiration which is detected within minutes. The rate of oxygen uptake and carbon dioxide release slowly declines after the initial surge. Radicle emergence from the seed is accompanied by another respiratory burst. Though mitochondria are poorly differentiated due to desiccation during seed development, there are enough enzymes present to produce enough ATP for several hours after imbibition (Hourmant and Pradet, 1981). Two distinct patterns of mitochondrial development occur during germination, depending on the type of food reserves in the seed. Starch-storing seeds normally repair and activate pre-existing mitochondria while oil-storing seeds typically produce new mitochondria (Morohashi, 1986). In both cases, the glycolytic and oxidative pentose phosphate pathways both resume during phase I, and the Kreb's cycle is activated.

All cellular parts needed for protein synthesis are present in mature dry embryos except for polysomes (Nonogaki, *et al*., 2010). Immediately upon imbibition, singular ribosomes gather into polysomes and start synthesizing protein. New ribosomes are also made. Initially, mRNA that is already present is used for protein synthesis, but with time, new transcripts are utilized for the protein code. The new mRNAs transcribed as germination proceeds encode proteins needed for normal growth and maintenance of the developing seedling, rather than those needed for germination. Even though many references in the literature speak of specific mRNAs responsible for food-storage mobilizing enzymes needed for germination, this remobilization actually occurs post-germination.

Radicle extension and the completion of germination

Radicle extension through the tissues surrounding the embryo signals the end of germination and the beginning of seedling growth (Nonogaki, *et al*., 2010). This extension growth may or may not be accompanied by cell division. There are two distinct phases of DNA synthesis in radicle cells after imbibition. The first phase is soon after imbibition and involves the repair of DNA damaged during the desiccation associated with maturation and subsequent imbibition. There is also synthesis of mitochondrial DNA at this stage. The second phase is DNA synthesis associated with post-germinative cell division.

Radicle extension is driven by turgor pressure and requires flexibility in cell walls of the root axis between the cap and the hypocotyl. How does turgor increase in these cells to cause radicle elongation and emergence? Osmotic potential of radicle cells somehow becomes more negative due to accumulation of solutes with concomitant uptake of water, but the mechanism for such changes is not yet known.

The radicle must go through tissues surrounding the embryo to emerge from the seed (Nonogaki, *et al*., 2007). In some species the tissue yields easily to the pressure exerted by the radical, while in other species there is considerable resistance. The resistance declines during germination due to the activity of cell-wall loosening enzymes (hydrolases).

Metabolism of dormancy maintenance and termination

Some seeds may lose their dormancy while dry and their metabolism is very low. Many, however, lose their dormant status only after imbibition. Imbibed dormant seeds have a high level of metabolic activity and they are receptive to external stimuli (light, temperature, chemical treatment) that remove dormancy and/or induce germination. The primary event in release from dormancy is the reception of the dormancy-breaking signal by the embryo followed by metabolic and hormonal changes which result in the emergence of the radicle.

One of the most well-known primary signal receptors is phytochrome. P_r is transformed to P_{fr} by red light. P_{fr} is the active form, but what this active form of phytochrome causes within the seed that leads to germination is not known.

Primary versus secondary dormancy

Freshly harvested mature seeds are in a state of primary dormancy that was induced by ABA while

the seeds were still on the mother plant (Finch-Savage and Leubner-Metzger, 2006). Secondary dormancy is often associated with unfavorable environmental conditions after primary dormancy has been removed that do not favor germination.

Seed coat and endosperm control of germination

In some seeds, the endosperm and seed coat covering the embryo may present a mechanical constraint that must be overcome by growth of the embryo and thus cause a form of dormancy called coat dormancy.

In non-endospermic seeds and in *Arabidopsis* (which has one-cell layer of endosperm), the seed coat alone is responsible for coat dormancy. Completion of germination in seeds with coat dormancy requires embryo growth to overcome the mechanical constraint as well as a decrease in resistance of the seed coat. In endospermic seeds both the testa and the endosperm layers have to be considered in evaluating coat dormancy.

Endosperm rupture is the germination-limiting process in seeds of members of the *Asteraceae* (lettuce), *Solanaceae* (tomato and tobacco), and *Rubiaceae* (coffee). In these species, weakening of the endosperm surrounding the radicle tip is required for radicle protrusion.

Hormonal regulation of seed dormancy

One of the most intriguing questions in horticultural physiology cannot be easily answered. How does an embryo emerge from a seed to complete germination, and how is emergence blocked so that seeds can be maintained in the dormant state? The seed is the beginning of the next independent generation of any plant species and dormancy is the intrinsic block to its germination into a new seedling.

We can't figure out what controls dormancy because we don't really know the defining events of dormancy. Most seed populations don't complete the process of dormancy release in a synchronous fashion, thus there is large variability in a population of seeds. Additionally, the events required for dormancy release may occur in relatively few cells in the embryonic axis, thus making it difficult to observe the real changes occurring. Since the seed is an entire package of embryo, endosperm and testa, dormancy should be studied on a whole-seed basis.

Is dormancy a lack of some key cellular event or an imposed event that must be negated before germination can be completed? Is the release from dormancy mediated through a common signal transduction chain that coordinates diverse cellular responses? It has been suggested that there are related or common receptors for dormancy-breaking agents within the plasma membrane of the responsive embryonic cells. When triggered, these receptors then initiate a signal transduction cascade, perhaps involving synthesis of or sensitization to germination-promoting substances, that leads to the completion of germination.

Probably the most widely studied aspect of dormancy regulation is regulation via plant hormones. In this section we will review the singular and interactive effects of the known plant hormones on the release of seeds from dormancy.

ABA

There is a tremendous amount of circumstantial evidence that ABA regulates the onset and maintenance of seed dormancy (Kucera *et al*., 2005). There is an incredible lack of understanding how it does so and no receptor for ABA has been identified (Finkelstein *et al*., 2002).

When seeds are dispersed from their parent, they are in a state of primary dormancy which is induced by ABA produced by the embryo during their development in the fruit. The level of ABA is low during the initial stages of seed development, it increases substantially during mid-development, and then declines during maturation and desiccation. Dormancy is only initiated when the embryo itself produces ABA as ABA produced by maternal tissue does not induce dormancy (Karssen *et al*., 1983; Groot and Karssen, 1992; Koornneef and Karssen, 1994; Hilhorst, 1995; Frey *et al*., 1999; Nambara and Marion-Poll, 2003).

Precocious seed germination and vivipary (germination before release from the parent) are usually associated with a deficiency of ABA or deficiency in sensitivity to ABA (Kucera *et al*., 2005). After-ripening is a period of dry storage at room temperature of freshly harvested mature seeds. It is a common process for releasing dormancy. ABA content and seed sensitivity to ABA declines and sensitivity to gibberellins increases as after-ripening proceeds (Kucera *et al*., 2005). ABA inhibits phase III water uptake, endosperm rupture, further embryo extension and seedling growth after radicle emergence (Kucera *et al*., 2005).

GA

While ABA induces dormancy during seed maturation, gibberellins play a key role in release from dormancy and promotion of germination. Even though there is considerable gibberellin biosynthesis in developing seeds, gibberellins seem to be involved with embryo and fruit growth rather than dormancy.

A high gibberellin:ABA ratio favors vivipary, adding support to the idea that gibberellins promote germination. Two key functions for gibberellins during germination have been suggested: (i) promoting embryo growth; and (ii) weakening tissues surrounding the radicle, thereby enhancing emergence.

Some seeds such as lettuce (*L. sativa*) are photodormant and require exposure to red light for germination. Exposure to red light transforms phytochrome from the red form to the physiologically active far-red form, inducing gibberellin production, thereby promoting germination.

Dormancy release followed by germination is the result of a balance between many promoting and inhibiting factors which target both the testa and the embryo. The main promoter is gibberellin while the main inhibitor is ABA. It seems that the gibberellin requirement for dormancy release and germination depends on the amount of ABA produced in the developing seeds which sets the level of dormancy as well as the amount of ABA produced upon imbibition which maintains the dormancy already set. It appears that gibberellin levels are always adequate for germination but can only lead to it if ABA synthesis is inhibited. The transition through dormancy to germination is accompanied by decreased sensitivity to ABA and increased sensitivity to gibberellins as well as a reduction in synthesis of ABA.

Ethylene

Ethylene production increases with germination of many seeds and ethylene production is often higher in non-dormant compared with dormant seeds. Even though ethylene seems to promote dormancy release and germination, no clear role in either process has been established (Kucera *et al*., 2005).

Brassinosteroids

Brassinosteroids in general, promote germination by acting in conjunction with gibberellins to promote cell elongation and counteract the inhibitory effects of ABA. Brassinosteroids may also stimulate gibberellin biosynthesis.

Cytokinins

Cytokinins are present in developing seeds, predominantly in the endosperm, and are known to break seed dormancy in many species by enhancing ethylene production. Cell division, especially during root elongation, is also promoted by cytokinins.

Auxins

Auxins play a major role in embryogenesis as previously discussed. During germination, auxin regulates catalase activity which is important in getting rid of toxic metabolites.

Summary

The effects of hormones on seed dormancy and subsequent germination has been studied extensively. Even so, all that work can be summarized into a very brief space regarding what we really know. ABA induces and maintains dormancy, and for a prolonged effect, it must come from the embryo. Gibberellins promote release from dormancy and germination by counteracting the inhibitory effects of ABA. The roles that brassinosteroids, ethylene, auxins and cytokinins play are still being explored.