

# REVIEW & INTERPRETATION

## Intraspecific Gene Flow in Bread Wheat as Affected by Reproductive Biology and Pollination Ecology of Wheat Flowers

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### ABSTRACT

Gene flow is transfer of genetic information between populations of the same or different species. Gene flow in bread wheat (*Triticum aestivum* L.) occurs via pollen or seed dispersal. Under normal conditions, gene flow between wheat cultivars is negligible due to selfing of flowers. Factors such as reproductive biology and environmental conditions at vegetative and reproductive stages bring variation in the extent of gene flow. Cultivars with open florets during pollination may have increased gene flow. High or low humidity or temperature during anthesis may decrease gene flow. Although pollen movement has been observed 1000 m from the source, substantial outcrossing beyond 30 m was not observed. Rigorous and systematic gene flow studies in wheat are absent from the scientific literature. Gene flow data could establish an appropriate isolation distance between cultivars and genetically modified (GM) plants or hybrids.

WITH THE DEVELOPMENT OF genetically modified (GM) crops questions have been raised about the potential for pollen mediated transfer of transgenes from GM crops to non-GM crops (Rieger et al., 2002) or to wild relatives (Goodman and Newell, 1985). So far, the majority of research on the potential environmental consequences of transgenes has dealt with transfer from a crop to wild relative (Ellstrand, 2001). However, gene transfer from GM to non-GM cultivars of the same crop may occur more frequently than to wild relatives because they are sexually compatible and may be grown in proximity to one another in a cropping season.

Bread wheat is the most widely grown and consumed food crop (Braun et al., 1998) in the world in which GM cultivars are in the process of development. It is likely that, when developed, many of the GM wheat cultivars will be grown next to non-GM wheats. Thus an understanding of pollen mediated gene flow between existing wheat cultivars would be useful in the development of crop management practices, as desired to maintain crop purity within acceptable levels for both GM and non-GM cultivars.

Gene flow is the movement of gametes, zygotes (seeds), individuals, or groups of individuals from one place to another and their subsequent incorporation in the gene pool of the new locality (Slatkin, 1987). Gene flow is a natural biological process and in plants it primarily occurs via pollen or seed dispersal (Levin and Kerster, 1974). In very few cases, it occurs via vegetative dispersal

of propagules (Golenberg, 1987). In flowering plants, pollen dispersal is the main mode of gene flow (Levin and Kerster, 1974).

These gene dispersal mechanisms have practical implications for crop husbandry and evolutionary consequences for wild populations (Anderson, 1949; Ellstrand et al., 1999). The relative importance of gene flow to population genetic structure depends on the distance between donor and recipient populations (Manasse, 1992), population size (Farris and Mitton, 1984), how long the process has been in effect (Klinger and Ellstrand, 1999), and whether the new gene(s) confers any fitness advantage to the recipient population (Arnold and Hodges, 1995; Arriola and Ellstrand, 1997). Populations isolated well beyond the pollen dispersal distance, or an insignificant level of gene flow without any fitness advantage may not have any noticeable effect on the recipient population. Furthermore, the success of gene flow depends on plant mating system (Govindaraju, 1988a, b), pollination mode (Govindaraju, 1988a; Hamrick et al., 1979), and flower structure (Ellstrand and Elam, 1993). For example, self-pollination and cleistogamous flowers (closed flower structure) severely reduce gene flow between populations, while cross-pollination and chasmogamous flowers (open flower structure) may facilitate significant gene flow between populations. Finally, the ultimate survival and maintenance of donor genes in the recipient population depends on the fitness consequences of foreign genes to the recipient population.

Outcrossing or cross-pollination is a type of mating in plants in which a male gamete of one individual fertilizes a female gamete of another individual. The term outcrossing generally refers to mating within a species. The term is often used to describe the movement of a gene *out* of the initial plant type, and has been used synonymously with gene flow (Gleaves, 1973; Handel, 1983). Mechanistically, outcrossing and gene flow are two different events. While outcrossing is a prerequisite for gene flow to occur, outcrossing per se does not effect gene flow. For example, there are many species whose mode of reproduction is primarily through cross-pollination, a mechanism by which genetic variation is distributed across populations. Gene flow is more typically associated with introgression. In the case of crop plants, the term gene flow is also used to describe possible movement of transgenes into traditional cultivars as well as gene flow into wild or weedy sister species. Gene flow by

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**Abbreviations:** CFIA, Canadian Food Inspection Agency; CIMMYT, International Maize and Wheat Improvement Center; GM, genetically modified.

definition includes movement of gametes, seeds, individuals or group of individuals (Slatkin, 1987); therefore, in a strict sense, outcrossing rates alone may not represent the total amount of gene flow experienced by a population (Govindaraju, 1988b).

While wheat is assumed to be predominantly a self-pollinated crop (Percival, 1921), it is recognized that low levels of outcrossing (hereafter referred as gene flow) can occur in this species (Allan, 1980). With the development and commercialization of new wheat cultivars, in particular those that have been genetically modified, there has been great interest in information about the distance and amount of gene flow that may occur in wheat. This information is useful for managing gene flow in wheat breeding, identity preservation, or other applications. The research record of cross-pollination and gene flow in bread wheat is sparse, unconnected, and often very narrowly focused. Moreover, much of this literature dates to the early part of the 20th century and is unknown to the current English-speaking research community. Even among a few published systematic studies, the main focus of the investigators was to quantify the outcrossing potential of wheat flowers. These outcrossing estimates reveal only a single episode of gene transfer between populations, but nothing about the integration of transferred genes in the recipient populations. In the absence of gene flow data in wheat, these outcrossing data may be helpful in speculating on a potential gene flow effect. As gene flow is considered, it is important to understand the process in the context of a biological system, especially the reproductive ecology of the wheat plant when dealing with pollen-mediated gene flow in wheat. There are two main advantages in this approach. First, the study of reproductive ecology highlights the genetic differences among cultivars related to flowering biology of the wheat plant and how these differences may influence gene flow. Second, as a prerequisite for pollen-mediated gene flow, pollen has to physically travel out of the donor plant to the recipient plant. The environment influences this pollen movement; therefore, knowledge of how environmental variation affects gene flow is essential to understand the dynamics of gene flow in wheat.

In this paper, we discuss gene flow in bread wheat (i.e., within or among bread wheat cultivars; not from wheat to wheat relatives) with respect to the reproductive biology and reproductive ecology of wheat. More specifically, we present (i) key reproductive features of bread wheat, (ii) how the environment influences the reproductive biology of bread wheat, (iii) how factors in (i) and (ii) affect gene flow in bread wheat, and (iv) the existing literature on outcrossing in bread wheat. We restrict our discussion only to pollen-mediated gene flow. However, we recognize that in wheat, gene flow by way of kernel dispersal or grain mixing may also bring about considerable genetic change in a recipient population.

## Bread Wheat

In the USA, wheat is second only to maize in terms of area planted and grain production. Winter or spring

wheats are the two major classes of wheat grown in the USA (Briggle and Curtis, 1987). Winter wheat is more extensively grown than spring wheat and is favored anywhere that fall-sown seedlings survive the mild winter months. Spring wheat is grown in colder, drier areas that are unfavorable for winter wheat. In North America, the main winter wheat regions include the central and southern plains, and western states of the USA, and the main spring wheat regions include the northern plains of the USA and Canadian prairies (USDA-National Agricultural Statistics Service, 2000).

Wheat plants develop through distinct vegetative and reproductive growth stages. The vegetative stage begins within two weeks of sowing when the coleoptile emerges from the soil. The first leaf soon emerges through a vent at the tip of the coleoptile, with other leaves emerging in succession (Percival, 1921; Peterson, 1965). The shoot is a short rhizome bearing several axillary leafy culms (tillers) that may each grow to over a meter in height. After the first few leaves have appeared, tillers grow from buds at the nodes of the stem, at or below the soil surface (Lersten, 1987). Following tillering, the wheat plant enters a period of rapid growth known as stem extension. In this phase, both the stem and leaf-sheath elongate rapidly. The uppermost leaf is called the *flag leaf* (Peterson, 1965).

The vegetative growth stages are followed by a period of reproductive growth. In the following section, we describe the various components of the reproduction structure in wheat. Subsequently, we discuss how each of these components influences gene flow in wheat.

## Reproductive Stage

The top internode within the sheath of the flag leaf bears the spike and is the last internode to lengthen. As the spike grows in size, it inflates the sheath of the flag leaf, which is referred to as the *boot*. The emergence of the spike from the boot is called *heading*. The head of the primary tiller usually emerges first, followed in turn by heads of secondary tillers in the order of their origin (Peterson, 1965). Spikelet initiation occurs in the head of the primary tiller, followed by those in the secondary tillers, again in the order of their origin (Stern and Kirby, 1979). The spikelets are alternatively arranged along the rachis. One or more of the upper spikelets is usually imperfect with either or both male and female organs non-functional (Percival, 1921). The basal spikelets are often entirely sterile (De Vries, 1971).

## Inflorescence Description and Variation

The inflorescence of wheat is a spike with a main axis (rachis) bearing spikelets separated by short internodes. Each spikelet (Fig. 1a) has two bract-like empty glumes that enclose two to nine florets that are arranged alternately on a subaxis or rachilla. The two outer parts of each floret (Fig. 1) are a lemma and a thin-walled two-keeled palea. The lemma may have an awn or awnlet or may be awnless. The lemma and palea enclose the sex organs (Percival, 1921).

The majority of wheat flowers in a spike are hermaph-

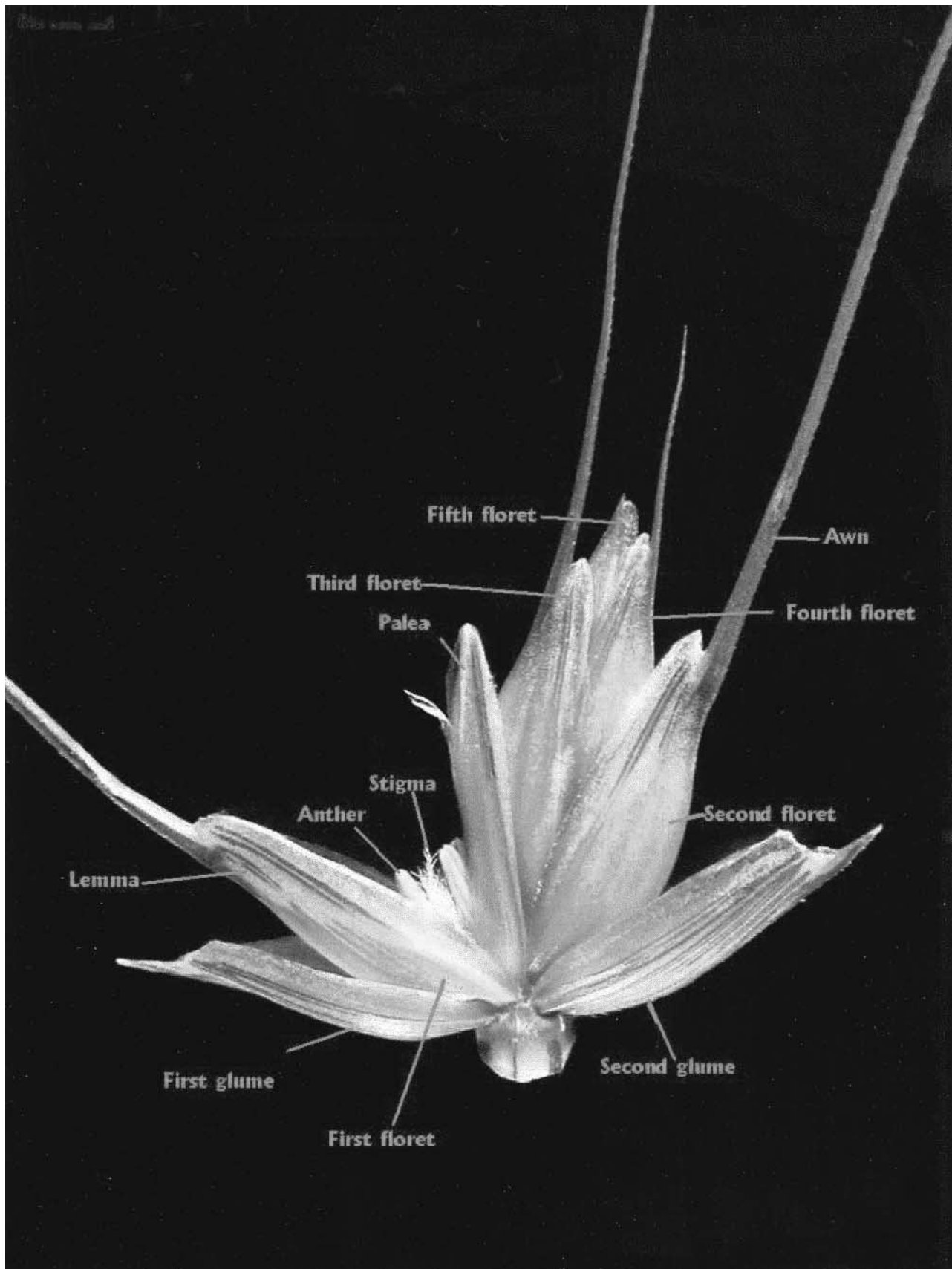


Fig. 1. Spikelet of wheat with five florets. The first floret on the left is open, showing the three anthers and a portion of the feathery stigma. [Courtesy: Research & Extension, Dep. of Agronomy, Kansas State Univ., Manhattan, KS; Available at: [http://www.oznet.ksu.edu/pr\\_aawf/May/may\\_4.htm](http://www.oznet.ksu.edu/pr_aawf/May/may_4.htm) (verified 19 Sept. 2002).]

roditic, but a few are unisexual. The unisexual flowers occupy the top and bottom portions of the spike (Percival, 1921; De Vries, 1971) and become potential sites for cross-pollination. The percentage of seeds set in a spikelet decreases from the lowest to the highest position or in the order of flowering (Leighty and Sando, 1924). If a nearby wheat field acts as a pollen source, and if the pollen grains are within a dispersal distance, then opportunity exists for gene flow between the two wheat cultivars, especially via male-sterile flowers.

Wheat spikes differ noticeably in shape, size, and density. Although in the majority of cultivars the distribution of spikelets along the rachis is fairly uniform, great variation is found among wheats in respect of the number of spikelets per unit length of the rachis. In some, the spikelets are crowded together, the lengths of the internodes between each being very short and hidden from view [e.g., club wheat, *T. aestivum* ssp. *compactum* (Host) Mackey]; in others, they are widely separated and the rachis is visible between adjacent spikelets (lax-headed wheat). In a few cultivars, the spikelets are especially crowded at the tip of the rachis and arranged in an open manner toward the base (Percival, 1921).

The compactness of the spikelets in the spike influences the width of the bract-separation angle (Johnson et al., 1967), in the sense that the lemma and palea of lax-headed wheats reach a wider angle of separation than those of dense-headed ones (Tsunewaki, 1969). Therefore, it may be possible that lax-headed wheats might be more amenable to gene flow effects than dense-headed wheats. Hucl (1996), while studying 10 Canadian spring wheat cultivars, noticed that the cultivar Oslo, which has open spikelets, produced one of the highest outcrossing rates, whereas cultivars CDC Makwa and Columbus, which have denser spikes, had the lowest outcrossing rates.

### Variation in the Time of Anthesis

During the time of flowering or pollination, flowers open (or remain closed as in the case of cleistogamous flowers) and the three anthers open releasing pollen (anthesis). Flowering starts several days after the wheat spike emerges. The process normally begins midway up the spike and proceeds upwards and downwards with opposite spikelets on the rachis usually undergoing synchronized flowering. The first floret of a spikelet flowers first and is followed by the secondary, tertiary, and so forth. The successive florets often open on successive days (Percival, 1921). Flowering continues throughout the day, with 2 to 6 d required for a spike to finish blooming (Percival, 1921; Leighty and Sando, 1924). After the first round of blooming, unfertilized florets open again, exposing the stigma for another 2 to 3 d with the ovaries remaining receptive for an additional 2 to 3 d (Hoshikawa, 1960). Likewise, in male-sterile plants the second round of flowering starts 36 h after the first round and the florets remain opened for another 5 d (Molnár-Láng et al., 1980).

There are apparent genotypic differences for flowering among wheat cultivars as a result of deliberate

selection by plant breeders. In wheat cultivars, the percentage of extruding anthers in the florets of a spikelet ranged from 25 to 80% (Joppa et al., 1968; D'Souza, 1970). This wide range of estimates for flower opening was partly attributed to the inclusion of a diverse selection of flowering types (e.g., small openings between the glumes, short duration of opening, cleistogamous and chasmogamous flowers) (De Vries, 1971). In an evolutionary sense, floret opening in a cleistogamous flower is not surprising in view of the close relationship of wheat to allogamous genera within the *Triticum-Aegilops* complex (Cook, 1913; Stebbins, 1957). Moreover, bread wheat itself is a hybrid species originated spontaneously via cross-pollination between tetraploid and diploid wheats (Kihara, 1944; McFadden and Sears, 1946). Such outcrossing events between related species are still ongoing within the *Triticum-Aegilops* complex in nature (van Slageren, 1994).

Glume opening, the extrusion of anthers, and the duration of opening are significant factors affecting cross-pollination and potential gene flow. Open flowering is very important for cross-pollination to occur. Zukov (1969) found that  $r = 0.93$  in hexaploid wheats between the percentage of open florets and the percentage of extruded anthers. When the angle of separation between the glumes was too narrow, as in some higher-level florets or in dense-headed wheats, anther extrusion was substantially reduced (Obermayer, 1916; Percival, 1921). These findings may have implications for gene flow. Cultivars with a large number of open florets before anther dehiscence and/or with a longer duration of flower opening might be prone to greater outcrossing than cultivars with a short anthesis period or with a large proportion of cleistogamous flowers. Even in opened, unpollinated flowers, gene flow is possible if adjacent plants are in synchronized flowering. However, variation in the degree of flower opening and pollen shedding influence gene flow. Finally, genotype and environmental (e.g., dry weather) interaction for flower opening likely results in a continuum of gene-flow effects among wheat cultivars.

The most important factor that influences cross-pollination and potential gene flow during anthesis is the extent to which the flower opens. As the extent of flower opening increases, the flower becomes a potential pollen donor to nearby flowers, and at the same time the donor flower itself becomes a site for pollen reception. It was shown in 10 Canadian spring wheats that cultivars with high outcrossing rates tended to have a greater degree of floret opening at anthesis (Hucl, 1996). Stigma size, style length, stigma exertion, stigma receptivity, anther size, filament size, and pollen grain number are important floral characters that influence gene flow in wheat. Significant cultivar differences for these traits exist in wheat (Cahn, 1925; Kherde et al., 1967; De Vries, 1974).

Observation of chasmogamous and cleistogamous florets in a spikelet shows a distinct spatial arrangement. For example, the first two basal florets of a spikelet generally flower chasmogamously and the rest cleistogamously (Obermayer, 1916; Rajki, 1962). The spatial distribution of florets within a spikelet appears to have arisen as a consequence of branch elongation along the rachilla. The first two florets, on average, have a 30° to 60°

angle separation between them, while the florets located higher on the rachilla are closely packed and have less space to open fully (Früwirth, 1905). Moreover, chasmogamous flowering is predominantly observed in the upper part of the spike and wheat cultivars differ in the frequency of chasmogamous flowers (Bohác and Miklová, 1962).

### Variation in Stigma Receptivity

The pistil consists of an ovary with two short styles and a branched feathery stigma (Fig. 1). Stigmas are receptive for 2 (Khan et al., 1973) to 13 d after anthesis, and are most receptive for the first 2 to 5 d (Rajki and Rajki, 1966; De Vries, 1971). The wide range in stigma receptivity has been attributed to the influence of weather conditions during flowering—in dry and warm weather, all stages of the flowering process were shorter than in rainy weather (Rajki, 1961). Stigma receptivity decreases gradually from the moment of flower opening, and is optimal at moderate temperature and humidity (De Vries, 1971).

In hybrid wheat (F1 or the first generation offspring obtained from crossing two genetically distinct parents), a prolonged stigma receptivity is considered essential because exact synchronization of male anthesis with flowering in the female is often difficult to accomplish in seed production blocks (Virmani and Edwards, 1983). Duration of stigma receptivity has generally been measured by examining seed set on male-sterile lines upon pollination at different times after the onset of stigma receptivity. Most studies suggest that environmental effects are of greater significance than genetic effects (Rajki and Rajki, 1966; De Vries, 1971; Khan et al., 1973). With open pollination under moderate conditions, De Vries (1971) obtained the highest percentage seed set on the third and fourth day after the start of flowering; by the seventh day, practically no more seed was set. The author suggested that the highest seed set on the third and fourth day might be because of a wider angle of opening of the lemma and palea and/or a higher stigma receptivity on those days than on the first day after the start of flowering.

Ten square millimeters represents the average area a wheat stigma would intercept as pollen disperses through the air (Bitzer and Patterson, 1967). Thus, the amount of gene flow may be increased by a larger stigmatic surface and longer style exertion. If the angle of separation between the lemma and palea is not wide, it may be important for cross-fertilization that both branches of the stigma protrude from the floret during flowering. Under normal development, researchers have never observed stigmas exerting outside the glumes (Früwirth, 1905; Obermayer, 1916; Wilson, 1968). Under special circumstance, such as under delayed pollination, the stigmas of some cultivars tend to grow beyond the lips of the lemma and palea, affording excellent opportunity for gene flow (De Vries, 1974).

### Environmental Effects on Anthesis and Seed Set

Wheat florets have two lodicules at the base of the ovary, which function to open the floret at anthesis. The

lodicules are dependent on adequate moisture and cool temperature for proper functioning. When flowers have reached flowering stage, the exact time and rate of flowering is strongly influenced by meteorological conditions such as variation of temperature, light, and humidity throughout the day (Leighty and Sando, 1924). Some flowers open in <1 min and others require 3 min or more. Flowering has been observed at temperatures ranging from 13 to 26°C and from 0430 to 1900 h, yet a majority of the flowers (86%) reach their peak opening between 0900 and 1130 h on a sunny day. Subsequently, a second flush of flower opening occurs around 1500 to 1700 h (Virmani and Edwards, 1983).

Both genotype and environment appear to influence the number of extruding anthers (Früwirth, 1905). In a multiyear investigation involving different genotypes and variable environments, Rajki (1962) observed the percentage of extruded anthers varied from 61 to 93%; in the driest year, the fewest anthers were extruded. An environmental influence on anthesis and seed set was also supported by Livers (1964), who obtained the lowest seed set on open-pollinated, male-sterile plants in the most droughted sites due to the poor exertion of anthers in self-fertile pollinator plants. Furthermore, low temperatures and rain were shown to increase the number of cleistogamous flowers (Früwirth, 1905). Anther dehiscence is also influenced by weather conditions. In warm weather and at high atmospheric and soil humidity, anthers dehiscence faster with as few as 45 to 50% fertile pollen grains produced as compared with >65% fertile grains found in dehisced anthers under normal weather conditions (De Vries, 1971).

Robertson et al. (1968) reported that high relative humidity and moderate temperatures appeared important for high seed set. Stockman et al. (1983) showed that light enhancement or shading during an 8-d period of floret development in a controlled environment altered the number of fertile florets at anthesis and the number of kernels formed per spikelet. Compared with a control treatment, a 15% additional light period increased the number of fertile florets and the number of kernels per spike by 37 and 22%, respectively, whereas 70% shading compared with the control decreased the fertile florets and kernel number per spike by 43 and 51%, respectively. Within a spike, the rate of response to assimilate change decreased from the basal to apical spikelets (Stockman et al., 1983). In other words, the base of the spike is normally the sink of lowest priority in the spike, suffering the most with unexpected shortage of assimilates and gaining the most from unexpected abundance. Increase in kernel number prompted by assimilate enhancement was attributed to greater floret survival. The assimilate supply to a developing spikelet appeared to be important for its development and may be potentially limiting, especially at high seeding rates (Scott et al., 1975; Mohapatra et al., 1982).

Elimination of plant stress during flowering and the early stages of seed development will diminish the success of gene flow, and will favor self-pollination. Wheat is primarily self-fertilized and, under favorable environmental conditions, anther dehiscence takes place within

the floret. Under stressed conditions, a large proportion of pollen is shed into the air. It is estimated that probably one-third of the pollen falls within its own flower, and the remaining pollen is scattered about and may fall upon the stigmas of other flowers (Leighty and Sando, 1924). Environmental stress that results in a large proportion of extruded anthers would likely represent an ideal situation for gene flow to nearby wheat fields. Cross-pollination apparently is sometimes effected in this way. Under certain environmental conditions, wheat flowers may be generally cleistogamous. When conditions are unfavorable for opening of the glumes (e.g., high moisture, low stress), anthers of wheat flowers shed their pollen and effect self-fertilization without being extruded, or they may be extruded only at the tips. These latter conditions effectively reduce gene flow between wheat cultivars.

### Pollen Production

There is a positive association between anther size and the quantity of pollen produced per anther (Cahn, 1925; Beri and Anand, 1971). Pollen production in predominantly self-pollinating wheat plants is nearly one-tenth (450 000 pollen grains) of rye (4 200 000 pollen grains), a near outcrossing relative (Pohl, 1937; D'Souza, 1970). Furthermore, within wheat cultivars, large variation is reported for the number of pollen grains produced per anther. For instance, in bread wheat the range of estimates for pollen production per anther is 856 to 1380 grains among four wheat cultivars in North Dakota (Cahn, 1925), 1282 to 1629 grains among 12 winter wheat cultivars in Kansas (Khan, 1967), 2687 to 3867 grains among eight hard-red spring wheat cultivars in Montana and North Dakota (Joppa et al., 1968), 581 to 2153 grains among 22 wheat cultivars in Punjab, India (Beri and Anand, 1971), and 2236 to 3022 grains among four wheat cultivars in Köln-Vogelsang, Germany (D'Souza, 1970). Reduced sunlight during pollen development (microsporogenesis) in the anther decreases pollen grain production and thereby may result in male sterility in florets (Fesenko, 1963).

The amount of pollen produced in predominantly self-pollinating wheat plants is substantially lower compared with open-pollinated crops like rye and maize. Together with the cleistogamous nature of some flowers, reduced pollen production is responsible for low levels of gene flow between wheat cultivars under normal growing conditions. Although this is the case in general, the potential still exists for gene flow to occur as 30 to 80% of the pollen is shed outside the flower (D'Souza, 1970; Beri and Anand, 1971). Such a high amount of pollen shed suggests that the presumption of *predominant self-pollination* of the wheat plant may be less than accurate. More likely, the literature has established this point for a narrow base of cultivars with which plant breeders are familiar in studies that themselves are narrowly focused. If the environmental conditions are conducive and the isolation distance is not great enough between cultivars, gene flow could occur if pollen from one cultivar could pollinate unfertilized flowers of another nearby cultivar.

### Pollen Viability

Under optimal field conditions (20°C, 60% relative humidity), wheat pollen retains viability for ≈0.5 h (D'Souza, 1970; De Vries, 1971). Wheat is predominantly self-pollinated, with pollen viability attributed to this characteristic. All the viable pollen grains that have access to the stigma surface germinate as soon as they come in contact with the stigma (Chandra and Bhatnagar, 1974). Because the entire length of the stigma is receptive to pollen grains in wheat, the location of pollen landing generally does not affect pollen germination. It was observed that the primary and secondary florets supply the most viable pollen (Ovcinnikov, 1952).

Temperature and relative humidity influence the duration of pollen viability, as extreme cold or hot temperatures are unfavorable for pollination and fertilization. Cool temperatures reduce the duration of pollen shed and high temperatures shorten both the duration of pollen shed and pollen viability (D'Souza, 1970; Major, 1980). However, for artificially stored pollen grains, cool temperatures and relatively high humidity levels prolong viability (De Vries, 1971). D'Souza (1970) tested the viability of stored pollen at combinations of three temperature regimes (5, 20, and 30°C) and three relative humidity levels (40, 60, and 95%) combinations and found 5°C and 60% relative humidity was the best combination for extending pollen viability. Stored pollen grains at 5°C and 60% relative humidity resulted in 65, 43, 28, 15, 10, and 2% seed set after 15, 30, 45, 60, 90, and 120 min of storage time, respectively.

According to Dowding (1987), matured anthers of grasses do not hold pollen for more than a few hours. Anthers appear unable to retain ripe pollen and therefore dehisce within the floret if it remains closed in wet conditions. Pollen grains quickly become desiccated after their release from the anthers (Heslop-Harrison, 1979); therefore, pollen of wheat generally is viable for only a very short time. Highest values for percentage seed set are obtained when pollination is performed with fresh, yellow pollen from newly dehisced anthers (Rajki, 1962). The time period that pollen remains viable would also influence the potential for long-distance gene flow. In addition, weather conditions play an important role. Humid weather makes the pollen heavy, so pollen may not disperse far from the parent plant, whereas dry weather causes desiccation and loss of viability, reducing the chance of effective gene flow. However, humid weather with light winds might favor gene flow (Dowding, 1987).

### Pollen Movement

There are very few studies in the literature regarding pollen dispersal in bread wheat, and it is important to emphasize that physical movement of pollen does not necessarily result in gene flow. Wheat pollen is relatively heavy compared with other grass pollen (Lelley, 1966). In wind-pollinated species, anthesis may be phased to concentrate pollen release to coincide with fine weather conditions (Frankel and Galun, 1977; Dowding, 1987). Wheat, evidently, has not been subjected to selection for this character and may be unable to adjust pollen

release. The pollen load in the air at a given time is a function of the amount of pollen produced per anther, the amount of anther extrusion, and the number of anthers per unit area (Joppa et al., 1968; also see Virmani and Edwards, 1983, for a detailed discussion). Joppa et al. (1968) investigated the relative pollen-shedding ability of 11 hard red spring wheats. Percentage of anther extrusion had the largest direct effect on pollen shedding. The expected pollen load in the air per 10 mm<sup>2</sup> was compared with the actual number obtained on slides situated within field plots. The calculated and observed numbers of pollen grains were of the same order of magnitude, with  $r = 0.91$ . Their results indicated that the relative pollen shedding capacity of a cultivar could be predicted from knowledge of the number of pollen grains per anther, the percentage of anther extrusion, and the number of fertile florets per plot.

Wheat pollen grains per se typically travel very short distances (Lelley, 1966). This is evident from hybrid wheat research where male-sterile plants had low seed set when pollinator plants were >3 m from them (Suneson and Cox, 1964; Jensen, 1968; De Vries, 1971). Jensen (1968) showed that in pollinator wheat lines, 90% of the pollen remained within 6 m of its source, and very few pollen grains traveled as far as 60 m. However, this long distance pollen movement did not proportionately increase gene flow, as gene flow decreased rapidly after 1.5 m. Long distance pollen movement was also reported by several researchers: wheat pollen grains were detected from a source population at a distance of 20 to 24 m (Suneson and Cox, 1964; De Vries, 1971), 50 to 60 m (D'Souza, 1970; Khan et al., 1973), or even at 1000 m (Virmani and Edwards, 1983). For example, Khan et al. (1973) measured the distance pollen moved from a pollinator (B line) using a pollen trap method for a 3-yr period from 1967 to 1969 at Newton, KS. Collection stations were placed at 0- and 3-m distances from the pollen source, and thereafter collection stations were placed every 6 m up to 60 m. The average number of pollen grains collected hourly from 0700 to 1700 h on glass rods ranged from 72 to 153 grains at the 0- to 3-m distance, and 33 to 43 grains at 48 m. A small number of pollen grains were also detected at 60 m from the pollen source. However, beyond 3 m the amount of collected pollen decreased rapidly. A similar study was also performed at Pioneer Hi-Bred International, Inc., Kansas, where researchers conducted pollen trap studies using glass slides and tested the trapped pollen grains for their viability. They observed viable wheat pollen as far as 1000 m from a very large pollen source (Virmani and Edwards, 1983). Bitzer and Patterson (1967) reported that above-normal temperature and the absence of rain during pollination favored pollen dispersal but shortened the pollination period. They also noted that seed set on hand-emasculated female flowers was directly related to wind-borne pollen load and female plants differed significantly in seed set.

Wheat pollen is relatively heavy compared with other grasses and travels  $\approx 1$  m from the pollen source in still air (Lelley, 1966). This may be one reason why so little seed is set on male-sterile plants at some distance from

a pollinator (Kihara and Tsunewaki, 1964; Holland and Roberts, 1966; Rajki and Rajki, 1966; Bitzer and Patterson, 1967; Tsunewaki, 1969; De Vries, 1974; Miller and Lucken, 1976). The number of pollen grains that arrive on the stigma under open pollination is important since gene flow in wheat is directly related to the wind-borne pollen load (Bitzer and Patterson, 1967). If the pollen source is not large enough, then substantial gene flow may not occur between wheat cultivars that are separated beyond 6 m because the majority (90%) of the pollen grains disperse within 6 m of its source (Jensen, 1968). However, with large pollen sources, pollen grains have been detected even at 1000 m (Virmani and Edwards, 1983). Therefore, without a large number of comparable pollen dispersal studies in wheat, it would be difficult to make an association between pollen movement and gene flow. Although pollen grain dispersal up to 60 m and beyond has been reported, their role in outcrossing is still questionable as experiments to test the seed set by foreign pollen at that distance did not support long distance pollen dispersal followed by fertilization (Pickett, 1993).

#### **Outcrossing Studies in Traditional Wheat Cultivars and Male-Sterile Lines**

Floral structure, anthesis, and anther dehiscence patterns in wheat make wheat largely a self-pollinating crop. Although wheat has a perfect flower, conducive to self-pollination, various cross-pollination percentages have been reported for this plant throughout the last two centuries (Shirreff, 1873; Nilsson-Ehle, 1915; Hayes, 1918; Meister, 1921; Leighty and Taylor, 1927; Garber and Quesenberry, 1923). Seed set of as much as 75% on male-sterile wheats is reported to be a common occurrence (Virmani and Edwards, 1983). That means there is indirect evidence to document gene flow for wheat.

Thomas Andrew Knight, the most distinguished 18th century horticulturist from England stated, "I readily obtained as many [new] varieties as I wished by merely sowing the different kinds together: for the structure of the blossom of this plant freely admits the ingress of adventitious farin [pollen grains], and is thence very liable to sport in varieties, some of those obtained were excellent, others very bad, and none of them permanent" (Shirreff, 1873). Shirreff (1873) observed formation of natural hybrids between wheat cultivars in both experimental pedigree cultures and in open fields. Many of the sports discovered among wheats in all parts of the world have proved to be natural hybrids or their segregants. Since spontaneous hybridization occurs among widely different races and cultivars when grown in small plots, it may be assumed that it takes place also in the field between plants of the same crop, and it is possible that the greater vigor and yield of some wheats may be due to the fact that they cross more frequently with other wheats than the less prolific types (Shirreff, 1873).

Shirreff (1873) also reported that natural hybridization is seldom observed for some wheat populations. He noted: "If cultivars growing contiguous are always instrumental in fecundating one another, my experimen-

tal plots must have long since become a heterogeneous mass, when between one and two hundred [plots] have been grown within a foot of each other for nearly fourteen years." In the early part of the 20th century, scientists in the USA (Hayes, 1918; Garber and Quesenberry, 1923; Leighty and Taylor, 1927), and around the globe reported natural outcrossing in wheat {e.g., Howard in India, Körnicke in Germany, Gordon and Vilmorin in France, Nilsson-Ehle and Kajanus in Sweden [see Percival (1921) for a detailed description]}. From the preceding discussion, it is clear that outcrossing frequently occurs in nature between wheat cultivars, even though the magnitude of outcrossing was not clearly estimated in those studies. Also, the higher presumed out-crossing rates in some of these earlier reports might be due to the cultivation of landraces as cultivars. The presence of several genotypes in such landrace populations allows for both the formation of hybrid genotypes or differential expression of genotypes in different environments that could be mistaken as hybrid individuals. Present-day wheat cultivars are genetically uniform; therefore, the higher outcrossing rates reported in some of the earlier investigations may not hold true for many of the present-day wheat cultivars.

Recent reports quantify average cross-pollination levels of  $\approx 3$  to 4% among wheat cultivars in adjacent rows (Allan, 1980), although much higher levels have been detected for some cultivars. Some cultivars have a higher tendency to undergo outcrossing than others (Leighty and Taylor, 1927; Bitzer and Patterson, 1967; Griffin, 1987; Hucl, 1996). Leighty and Taylor (1927) found varying amounts of natural cross-pollination in common winter wheat across a 10-yr period. They observed that some cultivars were more likely to outcross than others under certain environmental conditions and stated that 34% natural outcrossing occurred in a line of 'Fulcaster' in 1917. They also found that 'Nebraska No. 28' and 'Kan-red' were cross-fertilized annually. Harrington (1944) reported that several seed increases of 'Triumph' breeder's seed had to be discarded because of outcrosses to a red-glumed cultivar, Concho. Generally, high seed set was obtained on emasculated heads in close association with pollen-shedding wheat (Wilson and Ross, 1961). For example, in 10 Canadian spring wheat cultivars, Hucl (1996) observed outcrossing rates ranging from 0.00 to 6.70% in adjacent rows (Table 1). Harrington (1932)

observed outcrossing at Saskatoon across a 5-yr period and reported levels ranging from 0 to 2.16% for four hard red spring wheat cultivars. Although, while estimating outcrossing rates in wheat cultivars, the majority of the investigators used phenotypic markers to identify hybrid individuals, it is possible that in some of these studies there could be an overestimation of outcrossing rates due to seed contamination or off-type plants. Nevertheless, the overall results of outcrossing-experiments show that while self-fertilization may be considered the rule, examples of formation of intervarietal hybrids among wheat cultivars are more frequent than is generally assumed (Percival, 1921); or, on the other hand, the perception that wheat is a predominantly self-pollinating plant may not be quite accurate.

A serious purity problem was noticed in Kansas with winter wheat cultivar TAM 105 and with an experimental winter semidwarf KS75210 wheat, which was attributed to gene flow effect (Martin, 1990). Accordingly, increased gene flow has not been shown to be associated with breeding origins or with specific plant traits such as semidwarf stature. During 1982 through 1984 crop years, Martin examined 11 hard red winter wheat cultivars and one experimental line for outcrossing rates at Hays, KS. The awnless pollinator and awned female parents, including the semidwarf wheat, were isolated 30 cm from each other. The outcrossing rate ranged from 0.1 to 5.6% for individual cultivars in a given year. The semidwarf experimental line, KS 75210, had the highest 3-yr average outcrossing rate of 3.1%, whereas cultivar Centurk 78 had the lowest average outcrossing rate of 0.3% (Martin, 1990). In New Zealand, Griffin (1987) estimated outcrossing rates in 10 wheat cultivars planted 0.15 m from a pollen source using purple grain color as the marker. These cultivars included five spring wheats derived from the International Maize and Wheat Improvement Center (CIMMYT) germplasm and five locally bred wheats with no CIMMYT germplasm backgrounds. The outcrossing rates ranged from 0.14 to 3.95% across the 10 cultivars without any significant difference between the two groups. At 20-cm distance, the average outcrossing rate for 11 spring wheat cultivars across 2 yr (1992 and 1993) at Saskatoon, SK, Canada, was 0.88%. Between the 2 yr, the average outcrossing rates for the 11 cultivars ranged from 0.22 ('Katepaw') to 4.64% ('Oslo') (Hucl, 1996).

Hucl and Matus-Cadiz (2001) examined gene flow rates

**Table 1. Estimation of outcrossing rate in winter and spring wheat cultivars.†**

Pollinator distance	Seed set (range)	Comments	Reference
m	%		
0.3	0.79 (0–2.16)	The experiment involved five female parents and six pollinators and was conducted across a 5-yr period.	Harrington (1932)
7.8	0.12 (NA‡)		
0.3	1.2 (0.1–5.6)	The experiment involved 12 female parents and a mixture of 24 F <sub>4</sub> pollinator lines and was conducted across a 3-yr period.	Martin (1990)
0.2	0.89 (0–6.7)	The experiment involved 11 female parents and two pollinator lines in three different planting dates and was conducted across a 2-yr period.	Hucl (1996)
0	0.77 (0–3.23)	The experiment involved four female parents and one pollinator line and was conducted across a 2-yr period. In both years, the experiment was repeated in four wind directions.	Hucl and Matus-Cadiz (2001)
27	0.004 (0–0.09)		

† We included outcrossing studies in bread wheat which specifically tested cross-pollination with respect to distance or where it was possible to infer gene flow and distance association from existing data. In all the above experiments, phenotypic markers were employed to quantify outcrossing rates.

‡ NA = not available; Range values are not provided in the original reference.

of four wheat cultivars. Percentage gene flow was determined for distances from 30 cm to 33 m from the pollen source. Gene flow rate decreased with greater distance from the pollen source, and was dependent upon wind direction and wheat genotype. Maximum gene flow rates at 30 cm (adjacent rows) were 3.8, 2.6, 0.4, and 0.2% for the four cultivars. At 27 m, gene flow was recorded in 2 of 32 samples (2 yr  $\times$  4 cultivars  $\times$  4 directions).

Spontaneous cross-fertilization in wheat, unlike male-sterile wheat, appears to be associated with variations in the components of floral biology. For example, differences in stigma receptivity (Lacedena, 1966) and pollinating capacity (Joppa et al., 1968), which facilitate outcrossing, are known in wheat. The presence of these features in wheat suggests that modern wheat cultivars still retain some of the reproductive features of the progenitor species favoring cross-pollination. Wild emmer wheats in Palestine possess a relatively high cross-pollination potential (Cook, 1913), so also Mexican wheats that tend toward cross-pollination (De Vries, 1971). Cultivation of wheat outside its center of origin may have caused a tendency toward selection of self-pollinated forms (Stebbins, 1957; Wilson, 1968).

### Gene Flow Studies in Male-Sterile Lines

Male-sterile wheat flowers produce fertile ovules and sterile pollen, and the proportion of seed set on male-sterile wheat plants can be a rough estimate of outcrossing potential in bread wheat. Generally, outcrossing esti-

mates are expected to be higher on male-sterile female parents because these lines have been developed for both vegetative and reproductive traits that enhance outcrossing potential. Spontaneous male-sterile mutants rarely occur in nature, hence most of the existing male-sterile wheat lines are created by humans. Additionally, foreign pollen is devoid of any competition from self-pollen on the stigma of a male-sterile floret. Therefore, it may be erroneous to compare gene flow data of cultivars with that of male-sterile lines. Nevertheless, male-sterile lines facilitate to quantify upper limits of gene flow for crop plants in the absence of self-pollination.

Provided that an adequate pollen load is present in the field, and that there is correct synchronization of flowering between male and female parents, seed set on male-sterile plants is also influenced by the distance pollen can travel and remain viable (Virmani and Edwards, 1983). In wheat, a number of studies on the effect of pollinator distance on seed set have been conducted (Table 2). Male-sterile spikes flowering during the optimum pollen-shedding period, had an average of 71% seed set when the distances between male and female lines were between 0.75 and 2.25 m in Fort Hays, KS (Wilson and Ross, 1962). The authors concluded that high cross-pollination at short distance is possible in wheat and pollen may not be a limiting factor in seed set when the distance is small between cultivars.

In Wichita, KS, Johnson et al. (1967) observed a linear relation between seed set percentage and distance of

**Table 2. Estimation of outcrossing rate in male-sterile bread wheat.†**

Pollinator distance	Seed set (range)	Comments	Reference		
m	%				
0.75	73 (20–76)	The experiment involved one male-sterile line and one pollinator line; Percentage seed set was calculated on the two lowest florets of spikelets.	Wilson and Ross (1962)		
1.5	70 (35–90)				
2.25	70 (41–79)				
1.5	31 (6–88)				
4.6	20 (2–67)				
7.6	14 (2–45)	Common wheat cultivars with hand-emasculated spike; The experiment was repeated in two wind directions and in each direction three female parents and one pollinator line were used; The experiment was repeated twice in a 2-yr period at two pollinator densities (high and low); The data presented here are for the low density pollinator experiment where percentage seed set was determined for both wind directions in both years.	Bitzer and Patterson (1967)		
0.3	58 (NA‡)				
3.0	54				
6.0	41				
12.0	33				
18.0	26				
24.0	16				
28.5	15				
0.2	25 (12–37)			The experiment involved one male-sterile line and four isogenic pollinator lines.	Tsunewaki (1969)
0.4	26 (11–43)				
0.6	31 (17–45)	The experiment involved four male-sterile lines and two pollinator lines and was conducted at two locations in a 2-yr period; The data presented here are from the Guelph location for the year 1967 with the male-sterile line 48 and the pollinator cultivar 'Genesee' where percentage seed was determined for all distances studied.	Stoskopf and Rai (1972)		
0.8	14 (6–31)				
0.2	60 (NA)				
1.5	17				
3	8				
4.5	3				
6	1.4				
7.6	0.7				
9	0.4				
10.7	0.3				
0	14 (NA)	The experiment was repeated in four wind directions, and in each direction one male-sterile line and one pollinator line were used; The experiment was repeated thrice in a four-year period; The data presented here are for the years 1968 and 1969, where percentage seed set at various distances was determined for both years.	Khan et al. (1973)		
3	11				
6	9				
12	9				
24	5				
48	6				

† We included outcrossing studies in male-sterile bread wheats which specifically tested cross-pollination with respect to distance or where it was possible to infer gene flow and distance association from existing data. In all the above experiments, phenotypic markers were employed to quantify outcrossing rates.

‡ NA = not available; Range values are not provided in the original references.

female plants from a pollen source in a winter wheat cultivar. The seed set percentages at a distance of 0.3, 3.0, 6.0, 12.0, 18.0, 24.0, and 28.5 m were 58, 54, 41, 33, 26, 16, and 15%, respectively. This conclusion was supported by other similar studies conducted in the USA (Table 2) (Bitzer and Patterson, 1967; Khan et al., 1973), Japan (Tsunewaki, 1969), and Canada (Stoskopf and Rai, 1972). Weather conditions at blooming time, day and night temperatures, light, moisture, and wind direction and speed have a large influence on gene flow in male-sterile wheat lines (Khan et al., 1973).

Although several gene flow experiments were conducted on male-sterile lines, most of the studies were conducted in small plots that have little practical value for drawing inferences about gene flow for commercial hybrid seed production plots (Virmani and Edwards, 1983) and even similar studies produced contrasting results (Wilson and Ross, 1962; Kihara and Tsunewaki, 1964) because of differences in the population density of male-sterile and fertile lines. The findings of the majority of above-mentioned gene flow studies in both normal and male-sterile wheat plants suffer from experimental artifacts; either unnatural crop stands or lack of follow up studies, to name a few. Moreover, gene flow studies, if conducted on emasculated female plants (male sterile), might seriously undermine the validity of the results. However, the majority of the gene flow studies in wheat (Tables 1 and 2) show that gene flow effect varies widely between experiments for a similar set of isolation distances and is likely dependent upon the environment and biological factors discussed previously.

#### **Gene Flow and Isolation Distance Practices for U.S. and Canadian Seed Producers**

According to the USDA, for nonhybrid wheats, seed multiplication plots should be isolated from other wheat fields by a distance adequate to prevent mechanical mixture (USDA-National Agricultural Statistics Service, 2000). No particular isolation distance is mentioned as a guideline between Foundation, Registered, or Certified seed plots and wheat fields. However, for  $F_1$  hybrid wheats, the USDA has the following guidelines: (i) foundation and registered seed fields should be separated from all other wheat fields by 198 m, and (ii) certified seed fields should be separated from all other wheat fields by 99 m. Recently, the Kansas Research and Extension Service (Shroyer, 2000) suggested the following isolation distances between newly released hard white winter wheat and red wheat: (i) white wheat in foundation seed fields should be 15 m or more, (ii) white wheat in registered seed fields should be 9 m or more, and (iii) white wheat in certified seed fields should be 6 m or more. Although the Canadian Food Inspection Agency (CFIA) suggests a 3- to 10-m isolation distance for various pedigree seed classes of wheat (Anonymous, 1994; CFIA, 2000), based on their recent outcrossing estimation study, Hucl and Matus-Cadiz (2001) suggested a minimum isolation distance of 30 m for the production of pedigreed seed of cultivars suspected to exhibit higher-than-normal levels of outcrossing.

#### **Future Production Practices with Transgenic Crops**

The Food and Agriculture Organization (FAO) wheat seed production guidelines require the seed field to be isolated from all other fields of wheat or crop species with similar seed size by a distance adequate to prevent mechanical mixing, or to be separated by a physical barrier like a fence (Anonymous, 1993). Natural hybridization involves successful mating in nature between individuals from two populations, or groups of populations, which are distinguishable on the basis of one or more heritable characters (Harrison, 1990). Between transgenic and nontransgenic cultivars there always will be at least one heritable trait difference. The major issue to be addressed between transgenic and nontransgenic cultivars is how to maintain cultivar purity when they are grown some distance from one another, and what should be the appropriate isolation distance to minimize mixing through gene flow. Since gene flow in wheat will occur, thresholds of acceptable levels must be established. Currently, the number of systematic studies on gene flow in wheat is too small to make any valid inference about the isolation distance between adjacent cultivars (Table 1). But there is enough evidence to show that cross-pollination regularly occurs in wheat and the reproductive biology of wheat is favorable to facilitate varying degrees of gene flow in a variety of situations (e.g., high temperature, drought). In addition, the results so far discussed demonstrate that gene flow in wheat must be addressed appropriately, especially when diverse cultivars are grown adjacent to one another. Factors such as low pollen viability (Hucl, 1996) or severe moisture stress during flowering (Briggs et al., 1999) that enhance outcrossing rates in wheat should be considered. Moreover, at any particular distance from a pollinator, cross-pollination rate is substantially higher on a male-sterile line than on a male-fertile female parent (Table 1). Therefore, isolation distances should be developed separately for the two types of female parents.

The impact of gene flow depends on its magnitude. Gene flow among natural plant populations is idiosyncratic, varying among species, populations, individuals, and even years. Even when compatible species with similar flowering phenologies grow in spatial proximity, levels of gene flow may vary. When gene flow and selection work in concert, gene flow will accelerate the increasing frequency of favorable alleles in a recipient (sink) population (Ellstrand et al., 1999).

The U.S. and Canadian seed certification guidelines suggest an isolation distance of 10 m for wheat cultivars and  $\approx 17$  m for hybrid seed production. One empirical report on gene flow in wheat recorded pollen movement at a distance of 20 to 64 m, although the quantity of pollen that traveled was  $<10\%$  (D'Souza, 1970; De Vries, 1971). From the limited information available, it seems that effective gene flow is nearly absent beyond 3 to 6 m from the pollen source (Suneson and Cox 1964; Jensen, 1968; De Vries, 1971) and the extreme pollen dispersal distances of 50 to 1000 m (Jensen, 1968; D'Souza, 1970; Khan et al., 1973; Virmani and Edwards, 1983) resulting in significant outcrossing have not been sup-

ported with gene flow studies, as none of the experiments on seed set with isolation distance supported the above claims (Pickett, 1993). It seems, therefore, that most of the available information on seed set may have only limited usefulness for commercial projects (De Vries, 1974). In terms of the evolutionary potential of gene flow, even a small pollen load can change the genetic structure of a recipient population in a few generations (Hedrick, 1995). For instance, if the transgene confers any fitness advantage to the recipient population, frequency dependent selection gradually increases the percentage of the transgene in the recipient population. Gene flow of >1% is considered biologically important and, because of this, phrases such as *gene flow drops off rapidly with distance* are misleading (Ellstrand et al., 1999).

At the same time, workable guidelines on isolation distance have to be worked out for *real world situations*. The most important criterion to be considered when making decisions on isolation distance is "... balancing risk of pollen movement from nearby crops with the cost of spatial separation" (Pickett, 1993). Presently, a more complicated picture of pollen dispersal and gene flow is emerging, which suggests a high degree of temporal (Gaines and Bertness, 1993) and spatial (Ellstrand and Marshall, 1985) variation in the pollen dispersal process, which was also evident in the case of bread wheat cultivars. This makes it hard to provide any generalization about isolation distance in bread wheat. Rather, it seems practical to develop a set of isolation distances based on acceptable levels (thresholds) of trait presence in cultivars and the amount of gene flow risk involved. There is a need for research in wheat gene flow studies to see if the greater distances recorded for pollen movement by Jensen (1968), D'Souza (1970), Khan et al. (1973), and Virmani and Edwards (1983) are repeatable, and to see if this pollen movement results in substantial gene flow effect that is considered biologically important and meaningful in a recipient population.

For any crop that sheds pollen, including wheat, a guarantee of zero gene flow is not possible. However, based on sound experimental evidence on wheat, gene-flow management practices, such as isolation distance between GM and non-GM wheat cultivars, can be implemented that will result in levels of gene flow that can be kept below specified thresholds. Thus, the critical factor in gene-flow management of transgenic wheat will be dependent upon the establishment of an appropriate threshold or acceptable level.

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