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Methods to Enable the Coexistence of Diverse Cotton Production Systems

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Upland cotton *(Gossypium hirsutum)* and Pima cotton *(G. barbadense)* are the two types of cotton produced commercially in California. In acreage as well as crop value, over the past 5 years cotton has typically ranked in the top three in agronomic field crops grown in California. During that period, plantings of upland cotton in California have ranged from about 400,000 to over 650,000 acres (160,000 to 260,000 ha), while Pima plantings have ranged from about 140,000 to over 250,000 acres (56,000 to 101,000 ha).

Does cross-pollination occur in cotton?

Both upland and Pima cotton are variously referred to as "largely self-pollinated" or "partially cross-pollinated." These descriptions acknowledge that these types of cotton are mostly self-pollinated but some cross-pollination can occur, albeit at relatively low incidence rates, through activity of pollinating insects or by wind dispersion. The pollen of both wild and cultivated *Gossypium* species is large in size and among the heaviest among angiosperms, the group of plants that produces flowers, fruit, and seeds. Individual flowers of Pima and upland types are open only for part of a single day, typically opening in the morning, changing color, and withering late in the same day. The pollen of cultivated *Gossypium* species has been described as being sticky and having pronounced spines, with a marked tendency for groups of pollen grains to clump together. In combination with the location of pollen-bearing organs, or anthers, within the flowers, these pollen characteristics greatly reduce the opportunity for cotton pollen to be easily windborne. The duration of pollen viability has also been found to be affected by environmental conditions as well as some characteristics of pollinator species (Richards et al. 2005). In that study, most cotton pollen carried on mouthparts of moths was nonviable within 8 hours after removal from flowers as compared with 16 hours for cotton pollen mechanically removed from flowers.

Insect-mediated cross-pollination has been shown to increase in incidence when higher populations of suitable insect pollinators are present in the fields. Numerous types of bees, including *Melissodes* species, honey bees *(Apis mellifera),* and bumblebees (*Bombus* spp.) have been mentioned in multiple studies as the primary pollinators of upland varieties (Moffet et al. 1980, 1978; McGregor et al. 1976). Several wild bee species have been described as nectar collectors that carry pollen stuck on their bodies as they move from flower to flower, acting as pollinators, and this results in outcrossing (Vaisseire et al. 1984). By comparison, honey bees have been described as secondary pollinators that do not collect and carry pollen for significant distances in part because they clean pollen from their bodies when it accumulates (Waller et al. 1985; Waller 1972).

McGregor (1976) discussed several studies that demonstrated that the number of viable seed per cotton boll could be consistently increased with the introduction of higher populations of bees suitable as pollinators of cotton. Later studies where hybrids were produced verified that insect pollinators have significant potential for improving pollination frequencies and increasing pollen transfer across short distances (Waller et al. 1985; Vaisseire et al. 1984). Limited data available for Pima cotton and field observations support a similar contention that self-pollination is also predominant as in upland cottons, with lower potential for wind and insect-mediated pollen transfer as seen with upland cotton.

What is the frequency of cross-pollination?

In recent years, multiple studies have been conducted to investigate pollen transfer from cotton (some incorporating genetically transformed traits) with the goal of quantifying pollen transfer over various distances. Studies have been done across a range of environmental and production conditions, with an array of insect pollinator populations, which may in part explain the range of results seen in these field evaluations. In a study done in Greece (Xanthopoulos and Kechagia 2002), glandless and red leaf morphological markers in cotton were used as indicators to determine the percentage of natural outcrossing. With the glandless trait, the percentage dropped from an average of 2.17% in adjacent rows to 1.42% in rows 2 meters (6.5 ft) apart, to near zero at 10 meters (33 ft) distance. In the red leaf evaluation in that same study, natural crossing dropped from 3.85% in adjacent rows to 0.31% at 10 meters. A similar percent of outcrossing was obtained in studies done with upland cotton in Turkey (Sen et al. 2004).

Studies of pollen transfer distances were evaluated in herbicide-resistant genetically transformed upland cotton at multiple sites in California in recent years (Van Deynze et al. 2005). In these studies, seedling herbicide resistance bioassays and DNA tests were used to evaluate outcrossing frequencies identified as pollen-mediated gene flow. When insect pollinator activity was relatively high, gene flow resulting from pollen transfer was found not to be influenced by the direction of the nontransformed plants from the plots containing the transformed variety; frequencies declined from an average of approximately 7.65% at 0.3 meters (1 foot) to less than 1% beyond 9 meters (30 feet). When insect pollinator populations were low to near zero, the Van Deynze study reported that pollen-mediated gene flow was even lower, falling to less than 1% at a 1 meter (3.3 feet) from the genetically transformed plant source. Evaluations done in commercial cotton fields were a good match to experimental plots. When measurements were made at 1,625 meters (1 mile) from the genetically transformed plants, the measured pollen-mediated gene flow was detected at less than 0.04%. Multiple studies agree with the observations that increased outcrossing percentages occur with higher pollinator insect populations, and near-exponential reductions in this type of gene flow are observed with increasing distance from the source plants.

Other cotton species not commercially produced in the United States, such as *Gossypium tomentosum,* have been reported to have a greater incidence of insect-mediated pollination. *Gossypium tomentosum* was found to be successfully pollinated by various lepidopteran insects, including several types of moths (Fryxell 1979). In the *G. tomentosum* species of cotton, which is not of commercial interest in the United States, floral structures are typically not compatible with self-pollination, and significant frequencies of self-pollination do not occur. Insect pollinators therefore could greatly enhance pollination rates in *G. tomentosum.*

How far must cotton varieties be separated to ensure production of Foundation, Registered, and Certified seed?

Studies over the past decade in locations around the world have developed data that help draw a distinction between the adequacy of isolation standards for commercial cotton varietal maintenance and purity versus isolation that might be required to greatly lower the chances of any unwanted gene flow into nongenetically transformed seeds from genetically transformed varieties within the same production region. According to California Seed Certification Standards approved by the California Crop Improvement Association (http://ccia.ucdavis.edu/seed_cert/cotton.htm) and associated U.S. regulations stated in USDA 7 CFR Part 201, isolation distances required between "similar" cotton types of the same species for Foundation or Registered cotton seed production in California are 660 feet plus 20 feet of buffer rows if there is no intervening cotton field. "Widely different" cotton types require separation of 1,320 feet plus 20 feet of buffer rows for Foundation and Registered seed. Cotton varieties are considered "similar types" if they are of the same species, as long as they are not considered as having naturally colored fiber according to official U.S. cotton color grade standards. Cotton types are considered "widely different types" when they are of different species, such as Pima *(G. barbadense)* versus upland *(G. hirsutum)*. Distance requirements suggested between naturally colored cotton fields and other seed production fields are even greater, at 1 to 3 miles depending on crops grown in intervening fields.

Through many years of commercial seed production in California, there is general consensus from the California Crop Improvement Association (CCIA, see http://ccia.ucdavis.edu/) and seed companies that isolation requirements for certified seed production have been adequate for maintenance of a perception by users of varietal purity based on specific plant morphological and fiber quality characteristics evaluated for short-term or long-term changes. Although the exact basis for these isolation distances is not stated in the regulations or background materials, these distances appear to have resulted from field analyses of distances that reduce the incidence of identifiable morphological characteristics, which would be an indication of outcrossing. The approach used in assessing the adequacy of isolation distances has also considered that the combination of pollen characteristics (heavy, sticky, clumping) and studies of honey bee movement distances through cotton fields indicate that current isolation distances severely restrict pollen transfer and hence gene flow.

Are there examples where more stringent limits on gene transfer are desired?

An indication of the potential for outcrossing that is possible in closely located Pima and Acala fields (2,640 feet apart) is provided by observations by CCIA field inspectors. They determined that, with this separation distance, Acala-Pima hybrids were occasionally observed at frequencies close to 1 per 9,000 plants, which is close to the allowable limit of 1:10,000 plants in Foundation seed production. This type of observation of Pima-Acala hybrids is typically based on flower coloring characteristics and plant height. Since these simple visual markers represent only a few easily observable characteristics indicative of outcrossing, it could be argued that harder-to-evaluate additional changes due to outcrossing could also be occurring at similar or more frequent rates.

Outside of commercial cotton seed production, there are situations where the need for more stringent limits on pollen transfer are considered highly desirable, suggesting a need for production systems with greater limitations on potential gene transfer. The most prominent examples in cotton would be concerns of producers of seed for organic cotton production or of export seed to markets not accepting seed that contain genes introduced by genetic transformation. Due to the advent of sensitive DNA-based and protein-based testing methods, the capacity to track down seeds with some of these transformed traits has become feasible and cost-effective, increasing the likelihood that testing could be used to reject seed for some markets based on some measure of contamination by an undesired genetically transformed trait.

Can outcrossing of commercial cotton occur with other cotton varieties?

Cotton can cross-pollinate between numerous compatible species, such as wild and cultivated *Gossypium hirsutum* and *G. barbadense*, and domestic cotton varieties are recognized as genetically compatible with *G. tomentosum*. Gene flow by normal sexual transmission, however, requires parental plants within the same geographical area to have coincident flowering periods, a method for pollen transfer, and potential for production of fertile seed that remains viable under the prevailing environmental conditions. When it comes to the potential for successful transfer of pollen between uncultivated wild *Gossypium* species and commercial *Gossypium* varieties, these necessary conditions very rarely exist with wild or native species that are near commercial cotton production areas, greatly limiting the potential for gene flow to native or wild *Gossypium* species. In Australia, extensive field surveys identified wild *Gossypium* species in addition to commercially grown *G. hirsutum* or *G. barbadense* varieties (Brubaker and Brown 2001). They stated that cross-pollination mediated via an insect pollen vector is the most likely means of gene flow. However, they also stated that geographical isolation and separation of wild cotton populations from commercial production areas greatly reduce the likelihood of this outcrossing, serving as an effective natural barrier to cross-pollination. If these essential combinations of conditions occur, however, the potential does exist for introgression of traits, such as the glyphosate tolerance (Roundup Ready) trait, into some compatible wild *Gossypium* plants as well as into cultivated species, such as other *G. hirsutum* varieties. It should be recognized, however, that in the example of the glyphosate tolerance trait, no competitive advantage would be conferred to that progeny in the absence of glyphosate applications, and there would be no selective advantage to retaining that trait in the population.

Organic cotton production acreage, where the presence of a genetically transformed trait would be undesirable, has been quite limited in California and other U.S. cotton production regions. Limited adoption of organic practices to date has been due to a number of factors, including weed and insect control, harvest preparation difficulties, production costs, and limited identification of markets willing to pay a profitable price. During the past 15 years, acreage certified for organic cotton production in California has varied from a high of about 800 acres (324 ha) in the late 1990s to a low of about 80 to 100 acres (32 to 40 ha) in recent years (OTA 2005). This represents less than 0.1% of total California cotton acreage during the last decade. With relatively limited acreage of organic cotton and more predictable locations associated with "certified organic" field designations, larger isolation distances of conventional or genetically transformed cotton production could significantly reduce the opportunity for pollen movement and introduction of genetically transformed traits into organic cotton fields.

What practices are used to reduce contamination frequencies of cotton seed produced in California?

The practices described above to reduce the potential for undesired outcrossing can be considered in improving genetic purity of cotton seed produced in California. In addition, seed producers also need to maintain practices that increase assurance that seed sources are not mixed during planting, harvest, and cleaning operations. Every seed company, in cooperation with their growers, has procedures in place to reduce chances for contamination during each step of seed production. Seed handling standards have been established by the Association of Official Seed Certifying Agencies (see ASOCA 2003) that form the basis for these operations, and individual seed companies also generally develop additional in-house procedures to reduce opportunities for contamination, including pre- and postharvest cleaning and inspections of harvesters, module makers used in harvest, transport vehicles, bins used for storage, and ginning facilities. Fields are rogued, off-types and weeds removed, and the fields are inspected multiple times by both company and industry staff. The CCIA is involved in monitoring the production of Foundation, Registered, and Certified seed, providing third-party inspections of seed fields to assess compliance with quality standards related to isolation and potential contamination from other crops, weeds, or disease.

PERSPECTIVE

Pollen and flower characteristics, relatively short pollen viability times, and lack of wild or weedy cotton species in geographical proximity to commercial cotton production all serve to greatly limit chances of outcrossing among any *Gossypium* species growing in California. Field studies of pollen transfer distances and outcrossing percentages in California and elsewhere have shown that nearly no pollen moves aerial distances greater than a few meters via wind movement. However, these same studies have shown that in the presence of insects serving as pollen vectors, very small amounts of pollen-mediated gene flow (less than 0.04%) were detectable at distances up to 1,600 meters (1 mile) from the tested pollen sources. These studies together indicate that current large required isolation distances between different cotton types and varieties for Certified, Foundation, and Registered seed production provide a large measure of isolation to limit potential gene flow.

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