Barriers to outcrossing in alfalfa seed production

. . . flowering alfalfa . . . field size . . . bare ground

Studies of the amount of outcrossing in alfalfa seed fields, as affected by flowering alfalfa, distance, and size of plots, at Davis in 1965 and 1966, indicated that field size probably contributed more toward minimizing contamination than the other factors. However, some evidence indicated that flowering alfalfa, and distance, also reduced the amount of outcrossing. These studies were made in Ranger alfalfa and a white-flowered type.

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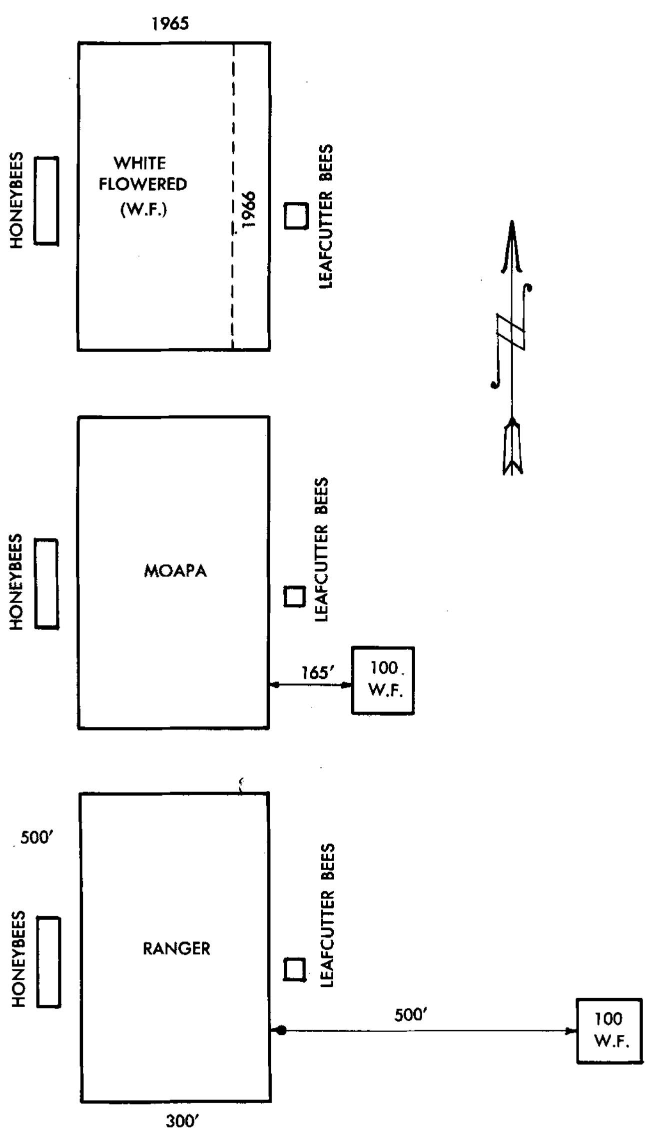
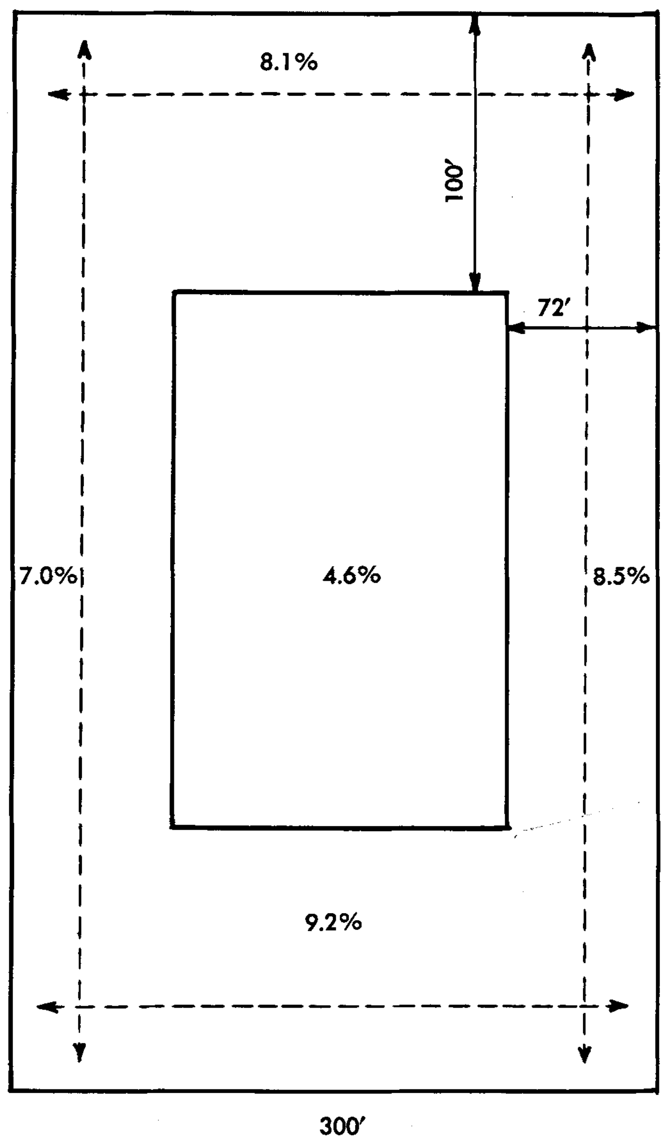
CERTIFIED ALFALFA SEED has been grown in California for over 20 years without serious difficulty in maintaining genetic purity of varieties. The effectiveness of current isolation requirements is largely responsible for this accomplishment. Varieties differing in winter hardiness must have 500 ft separation and those with similar hardiness characteristics, 165 ft. The isolation strip may be planted to other crops, left as bare ground, or seeded with the same alfalfa variety and seed class which may be either cut for hay or harvested as uncertified seed.

Isolation restrictions among states dif-

FIELD DIAGRAM OF THREE ALFALFA VARIETIES USED IN EVALUATING EFFECTIVENESS OF ISOLATION BARRIERS



EFFECT OF FLOWERING ALFALFA AS A BARRIER TO OUTCROSSING IN A WHITE-FLOWERED SEED FIELD IN 1965



fer somewhat in the ways isolation strips are managed, but most regulations follow the minimum width requirement of 165 ft set forth by the Association of Official Seed Certifying Agencies. California alfalfa seed fields generally are much larger than fields in most other states. In 1967, for example, 69% of the alfalfa fields certified in this state exceeded 50 acres in size and this group accounted for over 92% of the total alfalfa acres certified. Less than 10% of the fields were smaller than 25 acres.

Isolation strips

From time to time, the need for wide isolation strips has been challenged—especially when large fields are involved. Consider a certified seed grower with a 160-acre alfalfa seed field which is square and lies adjacent to a different variety but one that is similar in hardiness characteristics. An isolation strip 165 ft wide along one side, and 2,640 ft long, would amount to 10 acres. If the field has a good yield of clean seed per acre, the seed from this isolation strip could be of more value to the grower if a portion of it was marketable as certified rather than common seed.

The wide isolation strips pose not only an economic question but also raise the question of contamination. How effective is this border in minimizing outcrossing? This study stressed the latter aspect in an effort to determine what constitutes an adequate isolation barrier in relation to outcrossing. Its objective was to test the effectiveness of flowering alfalfa, relative field size and bare ground as barriers against outcrossing in alfalfa seed fields.

Test plots

A field trial consisting of three large blocks of alfalfa, each 300 by 500 ft, and two small plots, each 30 by 30 ft was begun in 1965 (see diagram). One field was planted to Ranger, a winter-dormant or hardy variety. Another was planted with Moapa, a non-hardy variety, and the third with a white-flowered variety. The latter was started with rooted cuttings. This field was reduced to a block 60 by 500 ft for the 1966 season.

The two small plots each included 100 plants of white-flowered alfalfa spaced equidistantly at 3-ft intervals. One of these blocks was placed 500 ft from the Ranger field, and the other 165 ft from the Moapa field.

Three strong hives of honeybees per acre were centrally located along the west side of each 3-acre field, and one domicile of leafcutter bees was placed similarly on the opposite side. Honeybees were therefore 800 and 665 ft, respectively, from small blocks of white-flowered alfalfa.

Each of the three large fields was segmented into plots four rows (12 ft) wide and 16 ft long. All samples within each plot were taken from the middle two rows. Seed pods were harvested at random from plants within these rows, but the 100-plant blocks were harvested on an individual plant basis.

Evaluation

The outcrossing of Moapa to Ranger was observed by (1) special planting to observe recovery in the field following cutback in late fall with observations made in late winter and early spring—plants which did not show good dormancy characteristics were scored as outcrosses—and (2) outcrossing between non-dormant and winter dormant types was made in the greenhouse using the stem-length test (a more precise estimate). With seed of pure Ranger used as a control, a representative sample of seed from harvested plots was planted in flats, thinned after one week, then transferred to a cold chamber for 16 hours daily. This cold treatment continued for four weeks, after which each seedling was measured from the cotyledons to the apical meristem. Seedlings taller than the tallest pure-seeded Ranger plants were classed as outcrosses and expressed as percent impure seed for each field plot.

To determine the percentage of outcrossing between white-flowered alfalfa and either the Moapa or Ranger variety, a seedling hypocotyl color test was used. This greenhouse technique involved planting seed from each plot of white-flowered alfalfa. Seedlings having green hypocotyls resulted from a white × white cross; seedlings with reddish-colored hypocotyls were considered outcrosses resulting from a white × Ranger or Moapa cross.

The average amount of outcrossing in the 3-acre Ranger field, as determined by the greenhouse stem-length test, was 6.2 % in 1965 and 7.9% in 1966. These results were considerably higher than those based on a subjective score for recovery in winter and early spring in the field. The latter appears to be an unsatisfactory method for evaluating outcrossing at this location. Relative growth differences were minimized under field conditions making scoring difficult, which lends support to the greenhouse technique as a more realistic measure of the outcrossing that actually took place.

Relation of field size to percent outcrossing in white-flowered alfalfa for the 30-by-30 ft plots and the three-acre field in 1965, as compared with the reduced white-flowered field in 1966

Year	Size	Outcrossing
	Acres	%
1965	3.0	7.1
1966	0.6	10.4
1965	0.02*	23.3
1965	0.02†	31.6

* 800 feet from honeybees
† 665 feet from honeybees

In the large white-flowered field, 7.1% outcrossing occurred in 1965 as measured by the seedling hypocotyl color test. This is strikingly similar to results in the large Ranger field which, if averaged over two years, shows 7.05% outcrossing. Plots in the white-flowered field in 1966 averaged 10.4% outcrosses with at least part of the increase resulting from reduction of the size of the plot to one-fifth of its original dimensions.

For the small white-flowered plots, after a 90-day seed production period in 1965, the average amount of outcrossing per plant (500 ft from the nearest alfalfa) was 23.3%. In the block 165 ft from the Moapa field, 31.6% outcrossing per plant occurred in 1965, and 21.4% in 1966 over a similar crossing period. Cutback in the 500-ft block in 1966 was purposely delayed to provide only a 30-day pollination period and resultant outcrossing was only 6.9%.

Flowering alfalfa

The large amount of outcrossing found through these experiments probably was inflated because of the relatively small fields used and the continually saturated bee population. Nevertheless, some evidence that flowering alfalfa provides a partial barrier even under these conditions was noted in the large white-flowered field. Greenhouse results from the seedling hypocotyl test for each of the 600 small plots indicated that an area six plots wide along both sides, and both ends, averaged 8.2% outcrosses, whereas the 156 centrally located plots averaged 4.6%. The barrier of flowering alfalfa which was 72 ft on the east and west, and approximately 100 ft on the north and south, did provide some protection from contaminant pollen (see diagram).

Nearly one-fourth of the plots in this centrally located block showed no evidence of outcrossing, whereas 84% of the surrounding plots received contaminating pollen.

Contamination in the Ranger field was determined for six north-south cross sections covering the 500 ft length. Results were somewhat different than in the white-flowered field, in that centrally located plots averaged only slightly less in percent outcrosses as compared with border plots (based on the stem length test. It is doubtful that testing samples from each plot throughout the entire Ranger field would have appreciably changed this result. Flowering alfalfa might have been a more effective deterrent to outcrossing in larger fields than methods used in these experiments.

Field size and distance

Field size appeared to be a definite factor affecting outcrossing in these experiments, as both 30 by 30 ft blocks of white-flowered alfalfa exhibited at least three times more contamination than did the 3-acre white-flowered field (see table). This occurred even though honeybees were adjacent to the large plots and 665 and 800 ft from the small plots. Bare ground, or crops other than alfalfa, comprised the balance of the area between the large and small blocks.

An increased amount of contamination occurred in the white-flowered field in 1966—possibly because it was reduced (in size) from 3 acres to 0.6 acres.

Evidence that distance from bees and contaminant pollen also influences outcrossing was shown by comparing the two small plots of white-flowered alfalfa. In 1965 the plot nearest the bees and large alfalfa fields contained 31.6% outcrossing whereas the plot at a more distant location averaged 23.3%.

Wind effects

Although the effects of wind or endemic pollinators were not evaluated, the results in these experiments indicate that size and distance may be factors of great importance in larger alfalfa seed fields. Size probably contributes more toward minimizing contamination.

Some of these data indicate that flowering alfalfa can minimize outcrossing, but its contribution needs further substantiation. Sampling, followed by greenhouse-testing areas in larger fields (25, 50, and 100 acres), which lie at various distances from other alfalfa fields, could pinpoint the relative importance, along with field size and distance, as isolation barriers.

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OBLONGA

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Response of young olive plants to inoculations with strains of Verticillium albo-atrum. Top: Manzanillo after inoculation with severe (T-1) strain (left) and mild (SS-4) strain (right). Bottom: Oblonga after inoculation with severe (T-1) strain (left) and mild strain (right).