Table 1. Analysis of variance that includes a column indicating which mean square was used in each F test to indicate which contrasts were considered random or fixed.

Source	Degrees of freedom	Mean square	Mean square used in F test	Significance level
Main effects				
Years	1	179.31	Year × loc. int.	not sig.
Locations	2	150.57	(Year × loc. int.) + (Gen. × loc. int.) − Residual	not sig.
Genotypes	3	20.07	Gen × loc. int.	<.05
Replications (in location-years)	18	2.12	Residual	<.01
Interactions				
Locations × years	2	133.65	Residual	.01
Locations × genotypes	6	2.43	Residual	.01
Genotypes × years	3	.26	Residual	not sig.
Residual*	60	.76		

* Includes Location × genotype × year interaction. Separately, the mean square for the interaction was .43 (d.f. = 6) and the residual was .80 (d.f. = 54).

Table 2. Mean yield of cultivars and isogenic lines.

Genotype	Mean yield kg/ha
'Red Kidney'	2531.4a[z]
Red isogenic line	2182.7b
Dark red isogenic line	2130.4bc
'California Dark Red Kidney'	2049.0c

[z] Mean separation by Duncan's multiple range test at the 5% level.

analysis, and 2) the 2 isogenic lines are combined and treated as a third genetic background. This can be justified on the *a priori* argument that the selfing process is expected to develop a single genetic background except for the R/r locus. Since the *RR* and *rr* isogenic lines are not significantly different (their means differ by only .22 kg. per ha. and the difference required for significance is .50 kg. per ha.), it appears that the R/r locus has no effect on yield. If this argument is accepted the interpretation is that there are 2 genetic backgrounds 'Red Kidney', 'California Dark Red Kidney', a third, intermediate background that was derived from the first 2; the 3 lines with different genetic backgrounds produce different yields, and the R/r alleles have no effect on yield. Therefore, it should be possible to

Table 3. Estimates of variance components for seed yield with year × location × genotype interaction pooled with residual to estimate error component. Genotype × year components was <0.

	Variances of parameters for:						
	Years	Locs.	Gens.	Reps.	Yr. × loc.	Gen. × loc.	Error
Estimated components of variance	0.951	0.476	0.735	0.340	8.306	0.209	0.762

develop dark red kidney cultivars that yield as well as light red kidney cultivars.

The estimated components of variance showed an overwhelming contribution of the year-location interaction to the overall pattern of variation (Table 3). This suggests that many years and locations need to be sampled to evaluate yield and, since the year component is twice as large as the location component, more years than locations should be used. However, genotypes did not interact with years but did interact with locations, suggesting that more locations than years are required for the evaluation of genotypes.

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Outcrossing in Common Bean *Phaseolus vulgaris* L.[1]

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Abstract: Twelve common bean cultivars were utilized to obtain estimates of outcrossing at Davis in 1971 and 1972. The maximum and minimum rates of outcrossing were estimated to be 0.007 and zero. Thus, outcrossing blocks cannot be used successfully in environments like that at Davis in 1971 and 1972, and little or no isolation is needed to obtain selfed seed.

A limited number of estimates of outcrossing rates in the common bean have been made by counting colored-seeded phenotypes that appeared among white-seeded phenotypes in the generation after white-seeded and colored-seeded phenotypes were planted in mixed populations. Emerson (2), Barrons (1) and Mackie and Smith (5) reported estimates of outcrossing of 10%, 8.26% and 0.63%, respec-

tively. Harding and Tucker (3, 4) found that frequency of outcrossing was also extremely variable in lima bean *Phaseolus lunatus* L. and that the propensity to outcross was associated with the genotype of the individuals and that erroneous conclusions can result from a failure to take male gametophytic selection into account. This paper reports the results of a study where 12 cultivars were used to estimate outcrossing in common bean.

Materials and Methods

Six populations were formed by mixing equal portions of seed of 2

cultivars. The populations were planted at Davis in 1971 and 1972 and contained approximately 700 plants of each cultivar. Populations were planted in 10 rows, 12.8 m long, on 76.2 cm centers. Two populations were mixtures of indeterminant growth habit cultivars 'Great Northern Nebraska 1' plus 'Sutter Pink' and 'Pinto U. I. 111' plus 'Big Bend'; 2 populations were mixtures of 2 determinant growth habit cultivars, 'Contender' plus 'Royal Red' and 'White Kidney' plus 'Red Kidney'; 2 populations were mixtures of an indeterminant growth habit cultivar and a determinant growth habit cultivar 'French's Horticulture' plus 'California Dark Red Kidney' and 'Romano' plus 'Charlevoix'. In the 2 years that the populations were grown, 1 determinant population, 1 indeterminant and 1 mixed determinant and indeterminant were planted in early May and again in early June. Each population was bulk harvested and seeds were separated by using their distinctive seed coat colors and patterns. A random sample of 1200 seeds of each cultivar was planted the following year and all progeny were scored for selfs and outcrosses. Cultivars in each population were selected so that reciprocal crosses could be recognized in the subsequent generation and on coincidence of flowering time. Bees visited all populations during flowering time; however, no definitive observations were made on their effect on outcrossing. The stand establishment of the progeny of every cultivar was generally more than 80%; therefore, approximately 1000 plants of each cultivar in each population were scored for selfing or outcrossing.

Results

Among the 20,000 progeny scored from all populations grown in 1971 and 1972 (Table 1), not one outcross was observed. The estimate of outcrossing for *P. vulgaris* is, therefore, zero. Mackie and Smith's (5) estimate of outcrossing was 0.63% for California-grown beans.

The zero estimate is recognized as the minimum value for outcrossing. The maximum value for outcrossing that is statistically compatible with the data can be derived in the following manner. The parameters, following Harding and Tucker (3), are α, the frequency of cross-fertilization; $1-\alpha$, the frequency of self-fertilization; T, the observed frequency of outcrosses in a progeny; $1-T$, the observed frequency of selfs and intra-cultivar (homogeneous) outcrosses in a progeny; and q, the frequency of the cultivar being tested in its population. The probability of observing N consecutive selfs or homogeneous outcrosses which could not be identified, *i.e.* no observable outcrosses, in a progeny of size N is $(1-T)^N$. The probability of observing at least one outcross, P, is $P = 1-(1-T)^N$, Equation [1]. The value for T, consistent with observing 1 or more outcrosses with a confidence of 95%, is obtained by setting P at .95

and solving for T. The ln $(1-T)$ can be solved from $\ln(1-T) = [\ln(1-P)]/N$, Equation [2] gives $\ln(1-T) = -.00299573$. Taking the antilog, T is found to be .00299124. This is the value of T for observations of one or more outcrosses and since none were observed, the true value of T must be less than .00299124. This value can be taken, therefore, as the absolute maximum rate of observable outcrossing that is consistent with each progeny estimate. But the true outcrossing rate, α, is higher because some outcrossing can occur among members of the cultivar being sampled. The rate of homogeneous outcrosses is expected to be αq. Thus, the rate of observable outcrosses T is expected to be $\alpha(1-q)$ or, $T = \alpha(1-q)$, and $\alpha = T/(1-q)$, Equation [3]. The maximum value for α could be obtained by solving [3] but that would ignore sampling variations in the estimation of q. Values for q were obtained from samples of seed taken from the populations. The size of these samples, K, ranged from 132 to 379. To obtain an estimate of maximum α, the minimum value of $(1-q)$ consistent with the data is needed. Assuming a normal distribution for $(1-q)$, its minimum value at the .95 level of certainty is $(1-q)-2\sigma q$. The maximum rate of outcrossing is, therefore, given by:

$$\hat{\alpha}_{max.} = \frac{T_{max.}}{(1-q)_{min.}} \text{ which for N}$$

$$= 1000 \text{ is, } \hat{\alpha}_{max.} = \frac{.00299124}{1-q-2[q(1-q)/K]^{\frac{1}{2}}},$$

Equation [4] where K is the sample size for the estimate of q. Estimates of the maximum outcrossing rate using [4] are presented in Table 1. Outcrossing rates for these individual populations are about .006 or .007 at an absolute maximum. If the samples are all combined (N = 20,000), an average $(1-q)$ is set at .5, and no error in 1-q is assumed, the maximum outcrossing rate is 3.0×10^{-4}.

Discussion

Estimates of outcrossing in the lima bean and in common bean have varied from less than 1% to greater than 10%. Occasional high rates of outcrossing have received much attention because of the genetic variability generated and the contamination of pure seed lots. In lima bean, plant breeders have made extensive use of natural outcrossing blocks to utilize the low levels of outcrossing to produce hybrid seeds. Outcrossing rates have occasionally been reported that are quite low, .63% in common bean (5), and .53% for Population III, Irvine, 1962 in lima beans (3). Our results suggest that, occasionally, outcrossing rates can go more than 2 orders of magnitude lower than that. Where outcrossing rates are as low as 10^{-3} or 10^{-4}, natural crossing blocks

Table 1. Estimates of maximum and minimum outcrossing rates for 10 populations of common bean grown in 1971 and 1972.

Population	Year	Female Parent	Frequency (1-q)	K	Outcrossing Rate Minimum	Outcrossing Rate Maximum
Romano + Charlevoix	1971	Romano	.43	325	0	.00798
		Charlevoix	.57	325	0	.00581
	1972	Romano	.31	132	0	.01303
		Charlevoix	.69	132	0	.00491
Pinto U. I. Ill + Big Bend	1971	Pinto	.49	235	0	.00704
		Big Bend	.51	235	0	.00673
		Pinto	.54	379	0	.00612
		Big Bend	.46	379	0	.00732
Sutter Pink + Great Northern Nebraska 1	1971	Sutter Pink	.40	2.5	0	.00898
		G. North.	.60	2.5	0	.00561
	1972	Sutter Pink	.46	277	0	.00748
		G. North.	.54	277	0	.00623
Contender + Royal Red	1971	Contender	.54	186	0	.00641
		Royal Red	.46	186	0	.00773
	1972	Contender	.48	286	0	.00711
		Royal Red	.52	286	0	.00649
Red Kidney + White Kidney	1972	Red Kidney	.51	243	0	.00671
		White Kidney	.49	243	0	.00702
French's Horticultural + California Dark Red Kidney	1972	French's Hort.	.54	345	0	.00615
		Dk. Red Kid.	.46	345	0	.00736

cannot be used to produce hybrids and problems of seed contamination via cross pollination are nil. Future studies on outcrossing in common bean should focus on insect activity related to pollination, variation in temperature and humidity during pollinating and rate of male sterility.

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Hormonal Control of Flower Bud Dormancy in Sour Cherry (*Prunus cerasus* L.). I. Identification of Abscisic Acid[1]

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Abstract. We demonstrated the presence of inhibitory substances in both the free and base-hydrolyzable fractions of methanol extracts of sour cherry buds. One of these, occurring in both fractions, we identified as abscisic acid (ABA) by combined gas-liquid chromatography-mass spectrometry (GC-MS). The base hydrolyzable form of ABA was assumed to be the 1'-glucose ester.

Hemberg (8, 9) first related endogenous inhibitors to bud dormancy, finding higher levels of inhibitory substances in *Fraxinus* bud scales in Oct., when buds on forced cuttings were unable to grow, than in Feb. when rest had been broken. A similar inhibitory zone was later termed the β-inhibitor complex (2). Abscisic acid (ABA) was isolated in crystalline form from birch and sycamore leaves as the main inhibitor of this zone (6, 7, 12, 17), and has been identified as a component of the β-inhibitor complex from willow sap (11) and tung bud scales (20). It has been tentatively identified by thin-layer chromatography (TLC) and bioassay in buds of apple (14) and peach (4, 5), characterized by gas-liquid chromatography (GLC) in apple buds (19) and olive leaves (1), and positively identified in coffee flower buds by GC-MS (3). A water soluble form of ABA, the 1'-glucose ester, was characterized in apple buds by GLC (16).

The purpose of this study was to determine if ABA, in both the free and bound forms, was present in flower buds of sour cherry.

Materials and Methods

Extraction. Fifty g of dormant cherry, cv. Montmorency, buds were collected on March 28, 1970 from an orchard at East Lansing, frozen on dry ice and held at 18°C until analyzed.

The buds were homogenized for 6 min in absolute methanol, transferred to an Erlenmeyer flask, methanol added to give a final vol of 125 ml, and shaken for 18 hr at room temp. The homogenate was filtered and the filtrate centrifuged at 25,000 × *g* for 30 min. The supernatant was evaporated in a flash evaporator at 40°C and resuspended in 100 ml distilled water.

The extract was adjusted to pH 7.3 with water saturated with $NaHCO_3$ and washed 3 times with ethyl acetate (EtAc). The $NaHCO_3$ phase was adjusted to pH 3.0 with H_2SO_4 and partitioned 3 times with 25 ml EtAc to obtain the acidic or "free" fraction. The water phase was adjusted to pH 11.0 with NaOH and heated for 1 hr at 60°C, then adjusted to 3.0 with H_2SO_4 and partitioned 3 times with 25 ml EtAc to obtain the base hydrolyzable or "bound" fraction.

One-half of each fraction (25 g equivalents) was evaporated on glass wool and placed on top of a 19 mm i.d. silica gel column (8 g Mallinkrodt 100 mesh silicic acid equilibrated with 4.5 ml 0.5M formic acid) and eluted with a gradient consisting of 160 ml hexane

and 120 ml EtAc, both solvents having been redistilled and saturated with 0.5M formic acid (15). Fifty-two 5-ml fractions were collected and 0.5 ml of each fraction was evaporated and analyzed by the wheat coleoptile bioassay.

Wheat coleoptile bioassay. 'Ionia' wheat seeds were soaked for 3 hr in water with aeration, sown on vermiculite moistened with one-half volume of water, and grown for 3 days in the dark at 25°C. A 4-mm section was removed 3 mm behind the tip and floated on distilled water approximately 3 hr until used. Each fraction to be analyzed was resuspended in 0.3 ml phosphate-citrate buffer (1.794 g K_2HPO_4 + 1.019 g citric acid/liter H_2O), pH 5.0, containing 2% sucrose. Five coleoptiles were placed in each tube and the tubes rotated in the dark for 20 hr in a clinostat at 25°C, after which the segments were measured utilizing a photographic enlarger. The difference between the increase in length of control and test segments, divided by the increased length of the control equaled percent inhibition.

Gas-liquid chromatography (GLC). The extract remaining in each fraction in the zone of inhibition (fractions 22–25) and the remaining one-half of the acidic and bound fractions were methylated with diazomethane (18), evaporated to dryness, resuspended in EtAc, and injected into a Packard 7300 Gas-Liquid Chromatograph equipped with a H flame ionization detector, and 2 mm i.d. × 1.83 m columns containing either 2% QF-1 on Chromosorb W 80/100 mesh, 2% OV-1 on Chromosorb W 100/120 mesh or 3% SE-30 on Supelcoport 60/80 mesh. The carrier was N at a flow rate of 40 ml/min at 40 psi. Inlet, column, and detector temperatures were 250°C, 180°C and 250°C, respectively.

Mass spectrometry. Combined gas-liquid chromatography-mass spectrometry (GC-MS) was performed utilizing a LKB-9000 Combined Gas-Liquid Chromatograph-Mass Spectrometer, interfaced with a Digital Electronic Corporation PDP 8/I computer. The column was 2 mm i.d. × 1.22 m containing either 3% SE-30 on Supelcoport 60/80 mesh or 2% DC-200 (12,500 cstk.) on Gas-Chrom Q 80/100 mesh. The carrier gas was He at a flow rate of 30 ml/min. Temperatures of column, inlet flash heater, source, and molecular separator were 180°C, 230°C, 220°C and 230°C, respectively, and entrance and exit slit widths were 0.08 and 0.3 mm, respectively. The ion source was operated at 70.0 eV.

Results and Discussion

Following column chromatography, 5 major zones of inhibition were evident (fractions 7–9, 15–18, 21–27, 37–40 and 48–51, labeled

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