POLYEMBRYONY AND BREEDING BEHAVIOR OF POLYHAPLOID TWINS IN ALFALFA

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INTRODUCTION

Cultivated alfalfa is a 32 chromosome plant of unknown origin. Due to the existence of 16 chromosome species in nature, it is thought that the cultivated plant is a tetraploid, with a genome number of n = 8. Under this assumption the problem becomes one of origin, i.e. is it an allotetraploid or an autotetraploid. Cytologists, in general, favor the latter theory while geneticists have provided evidence for both ideas. It is thought that both Medicago falcata L. and Medicago sativa L. have played an important part in its background although other Medicago species have also been suggested.

The problem of allotetraploid or autotetraploid becomes of interest to the plant breeder when the difference in genetic ratios of the two are considered. The allotetraploid plant would be expected to give disomic ratios. Thus, for a single factor showing complete dominance, the expected F_2 ratio for an allotetraploid plant would be 3:1. The autotetraploid counterpart of this ratio would depend on the distance of the factor from the centromere, i.e. chromosome vs chromatid segregation. The result in the former case would give a 35:1 genetic ratio while the latter would give approximately 21:1. All three expected ratios are based on the assumption that there is complete dominance, random segregation and no double reduction.

The ratios become more complicated if two or more factors are considered. If two unlinked dominant genes are involved, an allotetraploid plant would give a 9:3:3:1 ratio. This corresponds

to a 1,225:35:35:1 ratio of an autotetraploid. If the plant breeder should wish to obtain a homozygous recessive plant, he obviously must grow a much larger population with an autotetraploid species.

Haldane (20) estimated that, considering three independently inherited factors, a diploid selfed for five generations (F_6) would be 82.9 percent homozygous for the triple dominant. In an autotetraploid only 4.71 percent of the triple dominant would be homozygous. He stated further, "Thus the probability of establishing a pure line in a self-fertile (auto)tetraploid is very small. In a self-sterile (auto)tetraploid or a higher polyploid, it is negligible." Therefore a fertile haploid plant of a tetraploid species (referred to as polyhaploid) may be a boon to the plant breeder. It was hoped that this study would yield such a plant since polyembryonic seeds are a better source of haploid plants than monogerm seeds.

The present study of polyembryony was inititated to: (1) learn more about the reproduction processes of alfalfa by studying the mode of origin of twin plants, (2) obtain a polyhaploid plant that might be utilized in a breeding program, and (3) gain information about the evolution of alfalfa through the study of polyhaploid plants.

REVIEW OF LITERATURE

Polyembryony

In 1940, Webber (52) reviewed the literature on polyembryony.

He stated that Leeuwenhoek found orange seeds containing two embryos in 1719. This was the first report on polyembryony. Little was done on the subject, other than reporting its occurrence, until 1874 when Strasburger (Webber, 52) wrote of finding multiple embryos in several genera. Primary interest at that time was the mode of origin of plural embryos. Theories were advanced to explain the manner in which they were formed and the cause behind their formation. In 1901, when Ernst (Webber, 52) summarized the literature, it became apparent that polyembryony was a common occurrence in seed plants. At that time research workers' attention turned to studying the seedlings and less emphasis was placed on their formation.

In alfalfa, twinning was first reported by Southworth (45) in 1914. Martin and Watt (34) were the next to find a polyembryonic seed in 1934. Neither report involved much more than a statement of its occurrence.

Frequency. Polyembryony has been reported in a great number of families, genera and species in both the angiosperms and the gymnosperms. The frequency of occurrence varies with the species and with the variety or subspecies. Cameron (8) reported a frequency of 0.04 to 0.25 percent in Nicotiana with monosomic cultures having higher averages than species, species hybrids and varietal hybrids. Johnstone (23) found 14 polyembryonic Pinus seeds among 8,464 germinated. Nielsen (39) in Bromus inermis found twins occurring one in 550 germinated seeds, with some plants producing as high as eight percent. Marshall (33)

stated he found 29 plural embryo among 26,000 tomato seeds. Morgan and Rappleye (35, 37) reported the frequency in Capsicum frutescens varied from 0.04 to 0.65 percent. They found 1,619 multiple embryos occurring in 300,683 seeds. The three varieties investigated showed a significant difference in frequency. Randal and Rick (42) tested 36 lots of Asparagus officinales seed and found the frequency of polyembryos ranged from 0.13 to 3.54 percent with a mean of 0.95 percent. Johnson (22) found all Eucenia hookeri seeds contained from 2 to 22 embryos, with 6, 10 and 15 being the most frequent. In alfalfa, Greenshields (16) reported from one in 1,000 to one in 7,000 germinated seeds, dependent on the variety or strain. He found a correlation between frequency of occurrence and percent germination of the seed. However, he stated that the difference might be due to variation among the varieties and not directly related to the degree of viability.

Most multiple embryos are twins, however other numbers have been reported. Cameron (8) found 120 sets of twins and 16 triplets in Nicotiana. Randal and Rick (42) in Asparagus officinales found that of 405 multiple seedlings, 97 percent were twins and the rest were triplets. Morgan and Rappleye (35) reported 291 sets of twins, three triplets and one quartet in Capsicum (37). Combined data of Greenshields (16) and Lesins (30) showed 174 sets of alfalfa twins and two triplets.

Methods of Increasing Frequency. Morgan and Rappleye (36) in studying effects of irradiation of pollen in maize and lily

found an increase in the number of polyembryos formed. The resulting seedlings were both unattached and conjoined but all were diploid-diploid twins.

Haccius (19) induced twinning in Eranthis hiemalis by immersing immature seeds in chemicals for a period of time. Frequency was increased from 0.03 percent (normal) to three to eight percent following treatment. All resulting embryos had the hypocotyls joined. The chemicals used were as follows: 2,4-dichlorophenoxyacetic acid, 2,4,5-trichlorophenoxyacetic acid and alpha naphthalene acetic acid, all having similar effects.

Kivi (25) increased the occurrence of polyembryony in cereals by the use of chloroform and ether.

Types. Webber (52) grouped the types of multiple embryos as to mode of origin under four headings. These are as follows:

Sporophytic polyembryony - one of the embryos arises by division and growth of a nucellar or integument cell. The cell starts dividing and pushes its way into the embryo sac where it may replace or compete with another embryo. This has been reported by Bacchi (4) in Citrus, by Arndt (2) in Mangifera indica, by Cameron (8) in Nicotiana, by Nielson (38) in Poa pratensis and by Woodsworth (54) in Alnus.

Cleavage polyembryony - the result of division of the zygote or young embryo into two or more units, each capable of forming an embryo. This has been reported by Buchholz (7) in Sequoia, by Pope (41) in barley, and by Skovsted, (Webber, 52) in Trifolium,

both members having a chromosome fragment, and in Medicago, both plants having an extra chromosome.

Simple polyembryony - the formation of multi-eggs from a single megaspore which unite with sperms that have been produced from one or more microspores. This type has been reported frequently in gymnosperms (Webber, 52), but since neither the formation of a plurality of eggs nor the liberation of multiple sperms in the embryo sac is characteristic of angiosperms, it is doubtful whether simple polyembryony occurs within this group. However, Woodsworth (54) reported a case in Alnus in which a synergid or antipodal may have been fertilized by a sperm resulting in a diploid embryo.

Euploid polyembryony - includes the cases not proven to be any of the other three types. Under this heading, Webber (52) includes multiple embryos that result in haploids as well as aneuploids since "Slight variations from exact multiples of a haploid probably have little significance in multiple embryo formation." Webber (52) listed the expected twins as to chromosome number as follows: haploid-diploid; haploid-haploid; haploid-triploid; diploid-diploid; diploid-triploid; diploid-tetraploid and triploid-triploid. Euploid polyembryony has been reported by Cameron (8) in Nicotiana and by Guttenberg et al (18) in Allium. Cooper (12) found in his study of seven Lilium species that one percent of the ovules contained synergids that were stimulated to divide. Morgan and Rappleye (37) found two functional embryo sacs in Capsicum. Nielson (38) reported plural

embryos in Poa pratensis were caused by two macrospore mother cells but occasionally they were due to two members of the egg apparatus, one arising apomictically. Cooper (11) in a cytological study of Medicago found usually one, occasionally two and sometimes three macrospore tetrads in a single ovule. Study of the female gametophyte revealed the presence of one embryo sac and sometimes two in an ovule.

Greenshields (16), in alfalfa, classed multiple embryos under three general headings, as follows: equal, unequal and conjoined. This classification was based on the appearance of the radicle upon emergence from the seed coat. The latter type was subdivided into six sub-classes as follows:

1. 4-cotyledons fused at the base of the cotyledons with the radicles free, each pair of cotyledons having a growing point.
2. 4-cotyledons fused from the tips of the radicles to the base of the cotyledons, having two growing points.
3. 4-cotyledons on what appears to be a single radicle, having a growing point.
4. 3-cotyledons on a single radicle, having two growing points.
5. 3-cotyledons on a single radicle, having a single growing point.
6. 2-cotyledons on a single radicle, having two growing points.

Haploid Plants

The practical use of a haploid plant was quickly noted when Blakeslee and Bergner, (Cooke, 10), in 1923, recognized and reported the first haploid plant in Datura. In 1924, Blakeslee

and Belling (5) in further investigations of Datura haploids outlined a method for creating and utilizing a homozygous plant by doubling the chromosome number of a haploid plant. Cooke (10) reported the first practical application of this theory in the creation of a pure line by doubling the chromosome number of a haploid tomato. Since the advent of colchicine this idea has become more and more practical.

In 1933, Kappert (Webber, 52), in Linum and Ramiah, Parthasarthi and Ramanerjam (Webber, 52), in Oryza, independently reported twin plants, one with haploid and one with diploid chromosome number.

Since that time it has been reported in numerous species that haploids do occur more frequently in multiple embryos than in mono-embryonic seeds. Silow and Stephens (43) summarized the studies on cotton and found that of 55 pairs of twins of Sea Island cotton, 51 pairs were haploid-diploid combination. All showed relatively little male fertility and low female fertility. Cameron (3) in Nicotiana found seven haploid plants from 136 polyembryonic seeds. Morgan and Rappleye (37) discovered in peppers that of 46 plural embryos seven were haploid. The combined data of Skovsted (Greenshields, 16), Greenshields (16) and Lerins (30) in alfalfa shows that of 192 polyembryonic seeds only one polyhaploid plant has been found.

Elliott and Wilsie (15) in Bromus inermis found a polyhaploid plant that was highly fertile under open-pollinated conditions.

On the other hand, Harland (21) in Gossypium, Lamm (26) in potato and Nielson (38) in Poa pratensis all have reported finding highly sterile haploid plants. Lesins (28) found a polyhaploid alfalfa plant that showed a very low percent of viable pollen, but showed some viable female gametes by setting seed when selfed or crossed.

The polyhaploid alfalfa found by Lesins (28) was one of a set of triplets from the variety Grimm. It was characterized by reduced top growth and erect growth habit. It showed two definite morphological abnormalities: (1) some of the florets had open keel petals exposing the staminal column, the latter being without the normal tension and (2) the polyhaploid exhibited necrosis at the tips of the older leaves. It proved to have 15 percent stainable pollen, with wide variation in size (from 22 to 65 u). The chromosome number of this plant was doubled and the pollen was again studied. The chromosome-doubled plant showed 30 percent stainable pollen, with less variation in size than before (28 to 53 u).

Lesins (30) obtained 23 selfed seeds from the polyhaploid plant, none of which proved to be 2n = 16. He made 732 crosses, using a diploid M. falcata as the female. These resulted in two seeds, neither of which germinated. Two seeds resulted from 251 reciprocal crosses, both of which proved to be 2n = 16 chromosome number. When crossed with tetraploids, a seed set of 86 per 100 flowers was obtained. The chromosome-doubled polyhaploid produced six seeds using a natural tetraploid as the female plant. The reciprocal cross produced 240 seeds from 100

flowers or three times more than before chromosome doubling.

Stanford and Clements (48) found a haploid M. sativa plant (16 chromosomes). It was completely self-sterile but when used as a female parent it showed 50 percent fertility when a tetraploid was used as the pollen parent. The chromosome number of the progeny (24, 31-33 and 39-40) showed both reduced and unreduced gametes were functional. When it was used as the female in crosses with diploid M. sativa and M. falcata less than five percent pod set was obtained. These crosses produced only 16 chromosome plants. Pollen production was low in the haploid and most of the grains were empty. The plant showed 43 percent of the cells had eight bivalents at meiosis with one or two occasional quadrivalents.

Origin of Alfalfa

The origin of cultivated alfalfa is as yet unknown. It is believed to be a tetraploid plant with 32 chromosomes but the question of allo- or auto-tetraploid remains unanswered.

In 1951, Atwood and Grun (3) reviewed the literature on the cytogenetics of alfalfa. Of the 36 separate genetic studies involving 25 distinct characters, where an interpretation had been suggested, only disomic ratios were proposed. They stated, however, that possibly consideration had not been given to tetrasomic ratios since at that time the tetrasomic theories were more recent. They also stated that there is a tendency to report only the simpler ratios for which there is a ready explanation. They concluded that while cytological studies favored

the autotetraploid theory, the genetic work indicated allotetraploid origin. Stanford (47) stated that genetic studies reviewed by Atwood and Grun (3) were inadequate to detect the difference between disomic and tetrasomic ratios.

Grun (17) reported an average of 0.62 quadrivalents per cell from studying 1,257 diakinesis cells from 12 plants. Plant averages ranged from 0.21 to 0.87 quadrivalents per cell, the difference being statistically significant. In some cells as many as four quadrivalents were found. Univalents were found in 28 percent of the cells.

Cleveland (Stanford and Cleveland, 49) found 1.7 quadrivalents per cell in his study of meiosis in alfalfa. This low frequency does not disprove autoploidy, since homozygous autoploids are known which have all chromosomes pairing as bivalents (31). Cleveland (Stanford and Cleveland, 49) found only 2.7 quadrivalents per cell in an induced autotetraploid of M. falcata.

Ledingham (27), studying meiosis in the tetraploid progeny resulting from a cross between tetraploid M. sativa (2n = 32) as the pollen parent and diploid M. falcata (2n = 16) as the female, concluded that the F_1 tetraploid resulted from fertilization of unreduced eggs in M. falcata by normal M. sativa male gametes. Cytological studies revealed normal pairing in the F_1 tetraploid. He concluded that the two sets of genomes of M. sativa and M. falcata must be closely homologous.

In his studies, Julen (24), crossed a colchicine induced octoploid, M. sativa, (2n = 64), with a tetraploid (2n = 32), producing a hexaploid (2n = 48) in which the first meiotic

metaphase showed 24 bivalents. He concluded that at least one of the M. sativa genomes must be pairing with a M. falcata genome, which suggested autoploidy in the original tetraploid.

Ledingham (27) found the diploid form of M. falcata did not readily cross with the tetraploid M. sativa. This was attributed to faulty development after fertilization, not because the two were incompatible. He obtained two triploids by using M. sativa as the female and the diploid M. falcata as the male, suggesting the reduced male gametes were functional in such a cross. When he used M. falcata as the female only tetraploids were obtained indicating the unreduced female gametes were functional. In the latter cross many ovaries developed though the ovules aborted. The tetraploid showed high fertility, with 16 bivalents formed at meiosis indicating complete homology of the genomes.

Twamley (50) also obtained a very fertile F_1 tetraploid from a diploid M. falcata and a white flowered tetraploid cross.

Bolton and Greenshields (6) reported on a diploid M. sativa which was highly self-sterile and highly cross-sterile when crossed to 32-chromosome forms of M. sativa and M. falcata, but was cross-fertile when crossed to 16-chromosome forms of M. falcata.

Oldemeyer and Brink (40) have shown that fertility in offspring was not reduced if the genome of diploid M. falcata was joined with the genomes of cultivated alfalfa.

Lesins (30) in his work on M. sativa and M. falcata crosses found no reduction in fertility in crosses of F_1 hybrids as compared to intraspecific crosses.

Atwood and Grun (3) concluded that Ledingham's (27) and Julen's (24) examples indicated that while M. sativa may be a hybrid it probably resulted from a cross involving closely related parents. Armstrong (1), in studying the cytology of a tetraploid resulting from a cross between a diploid M. falcata by a tetraploid M. sativa, stated that the two genomes in the tetraploid possess in common a considerable number of homologous sections or even whole chromosomes.

In 1942, Tysdal et al (51) cited data that Korohoda in 1933 had presented concerning F_2 segregation of plants for typical leaf shape of M. sativa and M. falcata. Korohoda (Atwood and Grun, 3) had interpreted the data on the basis of disomic ratios. Tysdal et al (51) suggested that the results more closely fit a tetrasomic ratio.

In 1951, Stanford (47) reported the first definite example of tetrasomic inheritance in alfalfa. He concluded that the inheritance of purple flower color in the population he was working with was controlled by a single factor inherited in a tetrasomic manner. In 1954, Stanford and Cleveland (49), studying two leaf characteristics (mottled and folded leaf) found each to be due to one factor inherited tetrasomically. Likewise Davis (13), in 1956, reported autoploid inheritance for one factor in his study of an elongated hypocotyl mutant.

Twamley (50) found that one of the two factors associated with purple pigment production in Medicago sativa appeared to follow a tetrasomic ratio only, whereas the second factor followed disomic ratios in some plants and tetrasomic ratios in others.

He concluded that the majority of the evidence favored the autotetraploid theory, but still enough evidence existed in favor of allotetraploidy that a definite conclusion could not be made.

Dudley and Wilsie (14), studying the inheritance of two characters, branched inflorescence and vestigal flowers in M. sativa, concluded that there were two complementary dominant genes in the normal plant, one inherited on a disomic level and the other tetrasomically inherited.

MATERIALS AND METHODS

One hundred and five polyembryonic seeds were selected from 15 alfalfa varieties. In addition, 13 polyembryonic seeds were found in the process of germinating seed from various crosses. Two plants showing two growing points were obtained from a flat of F_1 plants. The total number of polyembryonic seeds found in the crosses and in each variety, along with the number of complete sets grown to maturity and the origin of the seeds are listed in Table 1.

Germinated seeds of four varieties, (Buffalo, Vernal, Lahontan and Rambler) were counted and recorded to obtain an estimate of the frequency of polyembryony in a normal seed population of alfalfa. In addition, polyembryonic seeds were selected from germination tests of 10 varieties, (Atlantic, Buffalo, Du Puits, Ladak, Ranger, Rhizoma, Semipalatinsk, Sevelra, Vernal and Williamsburg). Seeds were germinated between blotters in accordance with the method adopted by the Association of Official Seed Analysts. The data on twin frequency were based on the total

Table 1. Number of polyembryonic seeds found in various alfalfa varieties and crosses.

Varieties and crosses	F.C. No.	Total No. of polyembryonic seeds	Number of surviving sets
Atlantic	32954	10	5
Buffalo	32984	12	6
Du Puits	24697	11	3
Ladak	32566	1	0
Lahontan	33087	5	4
Mississippi Polycross	---	1	0
Narragansett	32768	13	6
Rambler	33701	15	6
Ranger	24802	7	4
Rhizoma	24798	13	4
Sc Ma 531	32667	1	0
Semipalatinsk	24822	2	1
Sevelra	24660	1	0
Vernal	31983	12	8
Williamsburg	24803	1	1
Various crosses	---	15	9
		120	57

number of seeds actually germinated. Seeds showing two or more radicles were planted into pots. At the time the seedlings reached the third trifoliate leaf stage, they were taken from the pot and the soil was washed from around their roots. They were then carefully separated, repotted and labeled A or B, with the A plant always equal in size or larger than the B plant at at that particular stage of growth.

Fifty-seven complete sets of twins and one set of triplets were grown to maturity. These plants formed the basic material for this study.

During the summer of 1957, 13 pairs of twins were set out in the field in a randomized complete block design with five replications. The remaining plants were transplanted to the field in the spring of 1958. Comparative morphological studies, budding dates and flower color notes were taken both years. Rate of recovery notes were recorded on the basis of one to nine, one being the fastest to recover and nine the slowest. Similarly, growth habits were listed with one being the most upright and nine the most prostrate. The recovery notes were analyzed by Wilcoxon's non-parametric method for paired comparisons (Snedecor, 1946). The same studies were made in the greenhouse on all 57 sets.

The amount of stainable pollen was determined by staining the pollen with IKI solution which was specific for starch. The formula used is as follows: one gram potassium iodide, one gram crystal iodine, 100 milliliters of absolute alcohol and sufficient lactophenol to increase the viscosity of the stain. The latter prolonged the preparation and allowed a thorough examination of the slide. It did not impair the stainability of the IKI.

A drop of IKI was placed on a clean slide and five florets from a single raceme were tripped into it. The material was then covered with a cover glass and two transverse counts, from left to right and from top to bottom, were made under the high dry lense of the microscope (9). The pollen grains were classed as either stained or aborted. If there were evidence of plasmolysis the grains were classed as aborted. The two classes were recorded separately by using two Veeder hand tally counters. Three

such slides from each plant were counted. With the exception of plant T39B which produced relatively few pollen grains, the total number counted ranged from 843 to 3,783. The results were transformed by the arcsin method (44) and then compared by use of the L.S.D. method of analysis.

Pollen diameter was ascertained at the same time the pollen were counted. Ten pollen grains from each plant were measured by using an ocular micrometer calibrated for the high dry lens. Distance measured was from the outer edge of the exine on one side to the germ pore on the opposite side. Only well stained pollen grains were measured.

Root tips obtained from newly rooted cuttings were used for cytological studies. The method employed was a modification of that described by Lesins (29). The rooted cuttings were carefully dug and the sand was washed from the newly formed roots. The cuttings were then placed in a vial of water and kept on ice for 12 to 14 hours at room temperature. The roots were then removed from the plant and killed and fixed in 1:3 acetic alcohol. After at least three hours the roots were transferred to 70 percent alcohol and stored at about four degrees centigrade. The roots to be studied were placed in a solution of 2.5 parts of three percent ferric ammonium sulfate stock solution and seven parts 95 percent alcohol. The roots were left in this mordant solution for at least three hours, after which the root tips were excised and placed in a few drops of acetocarmine for 10 minutes. The root tips were then transferred to a glass slide in a drop of acetocarmine. To this was added two drops of 45

percent acetic acid. The slide was heated with an alcohol lamp until the material was completely soft, at which time it was flattened with a glass rod. The material was covered with a coverslip, the slide reheated and pressure applied by tapping the coverslip with the wooden end of a dissecting needle. Fingernail polish was applied to the edges of the coverslip to prevent the slide from drying out before a thorough study could be made of it. Since the main interest was in the euploid series, the plants were classified in this respect only.

All pollinations were made in the greenhouse. Selfing was accomplished by tripping the florets with a toothpick. Any floret not tripped was removed with tweezers. Members of a set were inter-pollinated without emasculation by alternately tripping florets of the two plants on a toothpick. An attempt was made to self- and cross-pollinate at least 100 florets on each plant. In cases where a plant did not produce many flowers, preference was given to self-pollination.

The S_1 seed from each twin was germinated and the number containing polyembryos was recorded. An examination of the S_1 twin plants was made in an attempt to detect unusual characters the A and B plants might have had in common.

Photomicrographs were taken through a Bausch and Lomb microscope with a 10X ocular and a 90X oil immersion lens (Fig. 3 and 4, Plate 2) a 47.5X high dry lens (Fig. 2, Plate 2) and a 20X lens (Fig. 1, Plate 2). All figures were photographed with a 35mm. Exakta camera on panchromatic film with the exception of Fig. 1, Plate 1. This was taken by Mr. Floyd J. Hanna, Illustrations

photographer, Kansas State College.

EXPERIMENTAL RESULTS

Twins

Frequency. The percent of polyembryonic seeds occurring in various varieties is shown in Table 2. The results suggest a difference in frequency among the varieties. The highest monogerm to multiple embryo seed ratio was shown by Buffalo (1,330:1) while Rambler showed the lowest (514:1). The overall total indicated that polyembryos occurred about one in 800 germinated seeds in the population studied.

A striking difference was evident when frequency in the varieties was compared with that of the S_1 seeds of twin plants. The results show an increase of nearly 10 times that of the

Table 2. Frequency of polyembryony in various varieties and S_1 progeny from twin plants of alfalfa.

Variety or plant number	Total seeds germinated	Polyembryonic seeds found	Ratio	Percent
Rambler	11,811	23	514:1	0.195
Vernal	10,906	16	682:1	0.147
Lahontan	11,101	11	1,009:1	0.099
Buffalo	11,968	9	1,330:1	0.075
10 misc. varieties	25,956	31	837:1	0.119
Total	71,742	90	797:1	0.121
S_1 progeny from twins	10,260	127	81:1	1.238
T14A selfed	300	56	5.4:1	18.67
T14B selfed	242	37	6.5:1	15.29

studied varieties. An increase in the number of multiple embryos was evident in three sets of twins, T13, T14 and T104. T13 and T104 produced about one in 60 and one in 90 germinated seeds respectively. T14A and B exhibited a definite increase in the occurrence of plural embryos, A producing one in 6.4 seeds and B producing one in 7.5 seeds.

Morphological Characteristics. Size. A summary of the comparative length of the primary root shows that of 120 seeds, 43 displayed equal length, 46 were unequal, 16 were conjoined to varying degrees and 14 were not classified for this characteristic. Fifty-six sets of twins grew to maturity and were studied. Of these sets, 23 had primary roots of equal length, 17 possessed unequal roots, 11 were conjoined while six were unclassified. Table 3 shows the comparative size of the plants. Figure 1, Plate 1, illustrates the various types of polyembryonic seeds found. At the time the members of twin sets were separated 37 of the total 57 sets showed unequal height. Seventeen of the pairs were equal, one pair still conjoined and two were unclassified at this stage of growth. A summary of Table 3 shows that of the 23 sets having equal primary roots at emergence time only 13 were equal in height at separation. Fifteen of the 18 showing unequal root length showed unequal height. Eight of the 11 conjoined twins separated naturally, seven of which showed unequal height and one showed equal height. Only one set remained attached and had to be cut apart. Its height was unequal.

Table 3. Comparative size of the twin alfalfa plants at various stages of growth.

Code No.	Variety	Primary root length	Seedling size	Height at separation A	Height at separation B
T 2	Buffalo	Unequal	Unequal	---	
T 4	Buffalo	---	Unequal	---	
T 5	Buffalo	---	Unequal	---	
T 8	Buffalo	Unequal	Unequal	---	
T 9	Buffalo	Conjoined	Conjoined	---	
T 10	Williamsburg	---	Equal	---	
T 13	Semipalatinsk	Unequal	Equal	---	
T 14	Atlantic	Equal	Equal	---	
T 18	Buffalo	---	Unequal	---	
T 19	Atlantic	Equal	Unequal	---	
T 21	Atlantic	Equal	Equal	---	
T 23	Atlantic	Equal	Equal	---	
T 25	Atlantic	Equal	Equal	---	
T 29	Ranger	Conjoined	Unequal	8 1/4"	6 1/2"
T 36	Lahontan	Unequal	Unequal	10 3/4"	6 1/2"
T 38	Narragansett	Unequal	Unequal	9 "	6 1/2"
T 39	Lahontan	Conjoined	Unequal	13 3/4"	3 3/4"
T 40	Lahontan	Conjoined	Unequal	10 "	3/4"
T 41	Lahontan	Equal	Unequal	9 1/4"	6 "
T 42	Narragansett	Conjoined	Unequal	10 "	7 1/2"
T 44	Du Puits	Conjoined	Unequal	5 "	3/4"
T 45	Ranger	Equal	Equal	1 3/4"	1 1/2"
T 46	Rhizoma	Unequal	Unequal	5 "	3 3/4"
T 47	Rhizoma	Equal	Equal	6 3/4"	6 1/2"
T 50	Du Puits	Unequal	Unequal	5 3/4"	3 1/4"
T 54	Vernal	Equal	Unequal	7 1/2"	4 3/4"
T 55	Vernal	Unequal	Unequal	12 "	6 3/4"
T 62	Rambler	Equal	Unequal	2 3/4"	1 3/4"
T 64	Rambler	Equal	Unequal	9 3/4"	7 1/4"
T 67	Narragansett	Equal	Unequal	11 1/4"	6 1/4"
T 70	Narragansett	Conjoined	Unequal	2 1/2"	1 1/4"
T 71	Narragansett	Unequal	Unequal	2 "	1 "
T 72	Narragansett	Unequal	Equal	2 "	2 "
T 73	Rambler	Equal	Equal	2 "	1 3/4"
T 74	Vernal	Unequal	Unequal	11 1/4"	8 1/4"
T 75	Vernal	Equal	Unequal	5 1/2"	4 1/4"
T 77	Rhizoma	Equal	Equal	4 3/4"	4 1/4"

Table 3. (concl.).

Code No.	Variety	Primary root length	Seedling size	Weight at separation A	B
T 79	Ranger	Unequal	Unequal	2 1/4"	3/4"
T 80	Vernal	Equal	Equal	1 1/4"	1 1/4"
T 81	Vernal	Equal	Equal	10 1/2"	9 1/2"
T 86	Rhizoma	Conjoined	---	---	
T 87	Vernal	Equal	Unequal	6 1/4"	5 "
T 89	Rambler	Unequal	Unequal	10 1/4"	6 3/4"
T 93	50-1216 Sc 25342	---	Unequal	8 1/2"	10 1/2"
T 94	Sc 25342 50-1216	---	Unequal	6 1/4"	3 1/4"
T104	Ranger	Unequal	Unequal	8 3/4"	7 "
T105	Rambler	Conjoined	Equal	9 1/2"	9 1/2"
T106	30-1106 Sc 25352	Equal	Equal	7 3/4"	7 3/4"
T107	Du Puits	Conjoined	---	---	
T110	30-1106 Sc 25365	Unequal	Unequal	7 1/2"	3 1/4"
T111	Rambler	Conjoined	Unequal	1 3/4"	1 1/4"
T112	30-1106 Sc 25390	Equal	Unequal	8 1/4"	5 1/4"
T116	30-1151 C-32	Equal	Equal	5 3/4"	5 1/2"
T117	30-1106 Sc 25410	Unequal	Unequal	4 1/4"	2 1/2"
T118	30-1176 Sc 25352	Equal	Unequal	2 "	3/4"
T119	30-1151 Sc 25352	Equal	Equal	2 1/2"	2 1/4"
TT 1	Vernal	Unequal	Unequal	---	

EXPLANATION OF PLATE I

Fig. 1. Twin seedlings from polyembryonic seeds in alfalfa. The various types shown are from left to right:

Top row: separate and equal, conjoined primary root (cotyledons enclosed in seed coat), separate and unequal, separate and equal, conjoined primary root.

Bottom row: separate and unequal, conjoined hypocotyls and separate and unequal primary roots, separate and equal, separate and unequal, separate and equal.

PLATE I

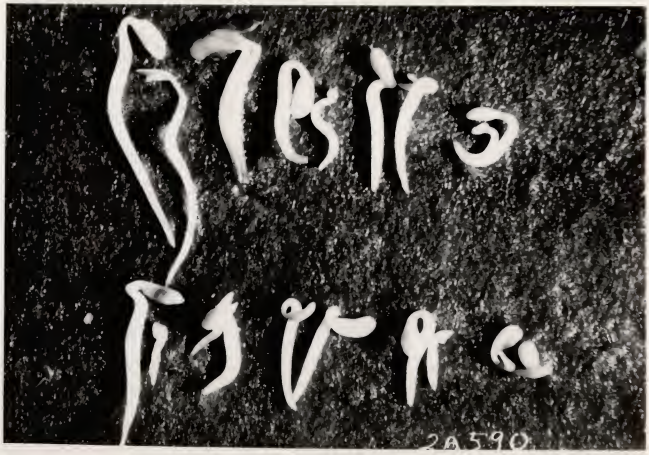

Fig. 1

All size differences had disappeared following flowering and cutting back. The regrowth was equal within twin pairs with two exceptions, T39 and T40. In these two sets the B plants consistently showed reduced growth.

Cotyledons. Plants T39B, T42A and T64B all exhibited three cotyledons while their twins T39A, T42B and T64A had the normal two cotyledons.

Plant T54B differed from its twin in the appearance of the cotyledons. In contrast to the normal green color of the A plant, T54B displayed a dark green color on the adaxial side of the leaves and a reddish-purple color on the abaxial side. The cotyledons appeared wilted and never were fully open. The plant remained in this condition for a month before it formed green uni- and trifoliate leaves.

Plants T62B and T118A each had one cotyledon while their twins T62A and T118B had two.

Unifoliate Leaf. Plants T39B, T40B and T47B did not form unifoliate leaves. All three remained at the primary cotyledonary stage of growth longer than normal, then formed trifoliate leaves. T39A, T40A and T47A formed unifoliate leaves five to eight days after the seedlings emerged above the soil.

Plant 89B exhibited an unusual type of unifoliate blade attachment. The normal blade attachment as displayed by T39A was with the petiole joined to the base of the blade, which appears as a natural extension of the petiole at nearly the same angle pointing away from the base of the stem. T89B had

the petiole attached to the base of the blade, but the blade was lying at a 180 degree angle to the normal blade, pointing toward the main stem.

Leaf Shape and Serrations. Plants T39B and T40B exhibited much narrower leaves than their twins.

Plants T14A and B had no distinct serration on the leaf edges of either plant.

Plants T23A and B displayed deeper than normal serration in their leaves. The A plant, however, consistently had the deeper serration of the two.

Insect Resistance. Plants T25A and B, T47A and B, T77A and B and T118A and B all displayed relative resistance to pea aphids in the field. Both members of the other sets that were studied showed susceptibility.

Insect Susceptibility. Plants T39A and B and T40A and B were susceptible to spotted alfalfa aphids.

Disease Susceptibility. Plants T23A and B showed susceptibility to black stem disease in the field.

Fumigation Susceptibility. Plants T46A and B and T79A and B all showed relative susceptibility to fumigation in the greenhouse. T46A and B which were the more susceptible of the two sets, showed severe burning of the leaves following fumigation.

Flower Abnormalities. Plants T18A and B displayed abnormal flowers on both plants. The keel petals of some florets were

open which exposed the staminal column, the latter thus being without the characteristic tension of normal flowers.

Foliage. Plants T111A and B in the field exhibited a yellowish cast to the foliage and their leaves were curled in a convex manner.

Plants T2A and B displayed a yellowish color of the foliage. Both were characterized by reduced growth.

Conjoined Twins. Plants T9A and B were conjoined directly below the cotyledons. The fused pair had three cotyledons and two primary roots which were equal and separate below the union. This was the only fused set of twins that remained attached when the pairs were separated.

Plants T29A and B proved to have conjoined primary roots that were slightly unequal in length. Two cotyledons were present for each growing point. The plants were still attached after 15 days but separated naturally a few days later.

Plants T39A and B were completely united below the cotyledons with one primary root slightly longer than the other.

Plants T40A and B resembled T39A and B except the roots were of equal length.

Plants T42A and B were fused below the cotyledons and had primary roots of equal length.

T44A and B had equal primary roots which were fused at the cotyledons only. The two plants each had a single cotyledon. The plants were still fused 50 days following planting but had separated naturally by 75 days.

Plants T45A and B proved to have a thin thread of tissue about one-half inch long connecting the two hypocotyls together. The thread remained attached to both plants for over 20 days but finally became detached from the B plant.

Plants T70A and B were fused the entire length of the hypocotyl and primary root.

Plant T86 had the hypocotyl fused with two separate primary roots. It had two cotyledons, one unifoliate leaf and one growing point.

Plants T93A and B displayed one root, four cotyledons and two growing points. The root had no apparent line of division. These plants were obtained from a flat when two growing points became apparent.

Plants T94A and B were like T93 with the exception that they had only three cotyledons. They also were selected from a flat on the basis of two growing points.

Plants T105A and B had conjoined primary roots and separate hypocotyls.

Plants T107A and B showed conjoining at the cotyledons only, having two separate primary roots and three cotyledons. One root died but both shoots survived.

Plants T111A and B were fused below the cotyledons. The primary roots were equal in length but showed a difference in diameter, one being twice as large as the other. The B plant emerged above the soil several days after its twin.

Recovery. The recovery of the plants following cutting

Table 4. Recovery rates, growth habits and budding dates of twin alfalfa plants.

Code No.	Recovery[1]		Growth habit[2]	Budding date[3]	
	Green house	Field		Green house	Field
T 2A	8.0	---	---	---	---
T 2B	8.5	---	---		
T 4A	5.0	4.0	4.0	5.0	0.5
T 4B	4.0	5.5	3.5		
T 5A	1.5	5.0	5.0	3.2	3.0
T 5B	4.0	3.0	4.0		
T 8A	1.5	5.5	4.5	3.5	6.0
T 8B	2.0	7.0	3.0		
T 9A	6.0	4.5	4.5	5.8	2.5
T 9B	3.5	3.5	4.5		
T 10A	2.5	3.0	2.0	2.0	0.0
T 10B	3.0	3.0	2.0		
T 13A	8.5	7.5	6.5	0.0	0.5
T 13B	6.5	7.5	6.5		
T 14A	5.5	---	---	4.0	---
T 14B	3.5	---	---		
T 18A	3.5	7.0	3.0	9.3	---
T 18B	1.0	7.0	3.0		
T 19A	6.0	4.0	4.5	0.4	0.0
T 19B	5.0	5.0	6.0		
T 21A	7.0	5.5	2.5	2.5	0.5
T 21B	6.0	5.0	2.5		
T 23A	3.5	---	---	0.5	---
T 23B	4.5	---	---		
T 25A	5.0	5.0	6.5	0.0	0.0
T 25B	4.5	4.5	6.5		
T 29A	4.3	7.0	3.5	4.5	1.5
T 29B	5.0	5.5	3.5		
T 36A	4.3	4.0	1.5	0.0	6.5
T 36B	5.0	4.0	1.5		
T 38A	5.0	5.5	3.5	2.3	0.5
T 38B	6.3	5.5	4.0		
T 39A	3.7	1.0	2.0	0.0	4.5
T 39B	7.7	7.0	1.0		
T 40A	3.7	5.0	2.0	11.0	---
T 40B	8.5	---	---		
T 41A	4.0	6.5	3.5	4.0	1.0
T 41B	5.0	7.5	3.5		
T 42A	4.0	6.5	8.0	2.0	0.0
T 42B	6.3	7.0	8.0		

Table 4. (Cont.).

Code No.	Recovery[1]		Growth habit[2]	Budding date[3]	
	Green house	Field		Green house	Field
T 44A	3.7	2.0	2.5	2.5	0.5
T 44B	3.0	3.5	2.5		
T 45A	7.3	6.5	2.5	0.0	1.0
T 45B	6.0	7.0	2.5		
T 46A	5.7	5.0	8.0	2.0	0.0
T 46B	5.6	5.0	8.0		
T 47A	4.7	5.0	8.0	1.5	1.5
T 47B	6.7	6.0	8.0		
T 50A	3.3	3.5	1.5	7.0	1.0
T 50B	5.0	3.5	1.5		
T 54A	5.7	6.0	3.0	2.0	3.0
T 54B	5.0	5.5	3.0		
T 55A	4.3	5.0	4.5	0.0	0.5
T 55B	4.3	5.0	4.5		
T 62A	7.0	7.5	4.0	---	---
T 62B	7.3	7.0	3.5		
T 64A	6.3	8.0	7.5	3.0	0.0
T 64B	7.3	8.5	7.5		
T 67A	5.3	7.5	4.0	0.0	3.0
T 67B	6.0	7.0	4.0		
T 70A	8.0	6.5	4.5	8.0	2.5
T 70B	7.0	7.0	4.0		
T 71A	8.0	6.0	9.0	5.2	0.5
T 71B	7.0	6.0	9.0		
T 72A	4.7	5.0	6.5	4.5	2.0
T 72B	4.3	5.0	6.5		
T 73A	5.3	4.0	3.5	1.0	1.5
T 73B	5.3	4.5	3.5		
T 74A	6.0	4.0	7.0	3.0	0.0
T 74B	4.7	4.0	7.0		
T 75A	6.3	3.5	4.5	4.0	0.5
T 75B	7.3	2.5	4.5		
T 77A	6.7	3.0	6.0	5.0	0.0
T 77B	6.0	3.0	6.5		
T 79A	3.7	3.5	4.5	1.5	0.5
T 79B	5.0	4.0	4.5		
T 80A	3.7	7.5	3.0	2.5	4.5
T 80B	6.3	8.0	3.0		
T 81A	3.3	4.0	5.0	1.3	1.0
T 81B	5.0	6.0	5.0		

Table 4. (Concl.).

Code No.	Recovery[1]		Growth habit[2]	Budding date[3]	
	Green house	Field		Green house	Field
T 86A	3.0	4.0	7.0	2.0	0.0
T 86B	3.0	4.0	7.0	0.0	1.0
T 86C	3.0	4.5	7.0		
T 87A	6.3	7.0	4.0	---	7.5
T 87B	6.0	7.0	4.0		
T 89A	5.7	7.0	6.0	3.0	0.0
T 89B	4.3	5.5	6.0		
T 93A	8.0	7.0	4.5	3.0	2.0
T 93B	1.0	6.5	5.5		
T104A	3.3	7.0	3.5	6.3	1.5
T104B	6.7	7.0	2.5		
T105A	7.0	1.5	6.5	2.0	3.0
T105B	7.0	3.0	6.5		
T106A	4.3	9.0	3.0	---	3.5
T106B	4.7	8.5	2.5		
T107A	6.3	1.5	2.0	---	2.0
T107B	7.7	3.5	2.0		
T110A	5.7	4.0	3.5	4.0	2.0
T110B	5.7	3.5	3.5		
T111A	6.5	3.0	4.5	---	0.5
T111B	7.0	3.0	4.5		
T112A	7.3	3.5	2.5	---	1.0
T112B	6.7	3.5	2.5		
T116A	5.3	4.5	7.0	3.0	1.0
T116B	7.0	5.0	6.0		
T117A	7.3	8.0	5.5	6.0	4.5
T117B	7.0	8.0	4.0		
T118A	5.7	7.5	6.5	0.5	1.5
T118B	6.3	7.5	6.5		
T119A	4.7	4.0	6.0	5.0	3.0
T119B	6.0	3.5	5.5		
TT 1A	4.7	5.5	6.0	0.0	0.0
TT 1B	5.0	6.0	6.0	0.0	0.0
TT 1C	4.0	4.5	6.0		

1. Recovery: 1=fastest; 9=slowest.
2. Growth habit: 1=upright; 9=slowest.
3. Budding: Days difference between A and B and between B and C.

Table 5. Recovery rates and budding dates of twin alfalfa plants transplanted to the field in the spring of 1957.

Code No.	Rate of recovery[1]	Date of budding[2]
T 2A	9.0	
T 2B	9.0	---
T 4A	2.0	
T 4B	2.0	0.9
T 5A	2.6	
T 5B	3.0	2.0
T 8A	4.0*	
T 8B	5.1	4.0
T 9A	5.1	
T 9B	4.9	0.0
T10A	1.0	
T10B	1.0	0.0
T13A	8.0	
T13B	8.6	---
T14A	5.1	
T14B	4.9	0.6
T18A	4.5	
T18B	4.3	0.2
T19A	4.2	
T19B	4.7	0.9
T21A	3.3	
T21B	3.2	3.2
T23A	3.0	
T23B	3.3	1.0
T25A	7.3	
T25B	7.0	1.0

1. Recovery: 1=fastest; 9=slowest.
2. Budding: Days difference between A and B.
* Significant at .05 level.

back in the field is shown in Tables 4 and 5. The results of the 13 sets of twins transplanted in the field in 1957 were analyzed, (Table 5). T8A and B was the only set that showed a significant difference in recovery. This difference was also apparent in the

greenhouse and in the field in 1950.

Environmental conditions such as light, temperature and moisture relationships proved to be a major factor in the greenhouse. Considerable variation was noted in recovery that was not apparent when the same plants were taken to the field. For example, the A plant of set T5 showed faster recovery in the greenhouse (1.5 vs 4.0). This was reversed in the field with T5B displaying the faster recovery (5.0 vs 3.0). No difference was noted in the two plants when randomized and replicated.

T39A and T40A showed faster growth than their twins in the greenhouse as did T39A in the field.

T81A, T89B and T107A showed consistently faster recovery than their respective twins in both the greenhouse and the field.

Twin sets T25, T46, T55 and T86 all showed comparative equal recovery both in the greenhouse and the field.

Flower Color. In all cases both members of a set of twins and the triplet set displayed equal flower color.

Growth Habit. All sets of twins showed equal or nearly equal growth habits (Table 4). The twin pairs were quite easy to distinguish in the field because of the close resemblance within sets as compared to between sets of the same variety.

Miscellaneous Characters. Foliage color, stipule appearance, leaf characteristics and texture of the plant were also studied. Only T39 and T40 showed any difference within sets. These will be discussed later.

Reproductive Characteristics. Budding Date. The dates of budding of the twin plants are shown in Tables 4 and 5. The figure given is the average days difference in budding between A and B.

The results indicate that T8A, T41B, T89B, T104A, T110B and T116A showed a consistently shorter period from cutting back to budding than their respective twins.

Twin sets T10, T19, T23, T25 and T45 proved to have nearly equal budding dates for both members.

T39A and B and T40A and B, though not indicated in Table 4 produced flower buds at nearly the same time in numerous tests.

Self- and Inter-fertility. The results of the self- and intra-pollination of twin sets are shown in Tables 6 and 7. Four sets of twins (T23, T39, T75 and T117) exhibited over 20 percent variation in self-fertility between members of a set. The greatest difference was 75 percent between T39A and B. In contrast, 18 sets of twins displayed less than five percent difference.

Only two sets of twins (T8 and T39) showed over 20 percent variation between members on the basis of inter-fertility of members of a set. In contrast, 15 sets displayed less than five percent difference.

If the members of a twin set were identical, no appreciable increase in fertility (above self-fertility) would be expected if the plants were inter-crossed. On the other hand, an increase might be possible if the twin plants differed in their genetic make-up. In 10 twin sets both members displayed from 10 to 27

Table 6. Self-fertility in twin plants of alfalfa.

Code No.	No. of florets tripped	No. of pods per floret	No. of seeds per floret	No. of seeds per pod
T 2A	55	0.11	0.04	0.40
T 2B	102	0.01	0.00	0.00
T 4A	165	0.46	0.74	1.60
T 4B	170	0.59	1.29	2.23
T 5A	121	0.35	0.45	1.31
T 5B	132	0.15	0.17	1.15
T 8A	116	0.29	0.10	0.35
T 8B	124	0.03	0.02	0.75
T 9A	779	0.54	1.48	2.72
T 9B	139	0.68	2.18	3.19
T 10A	143	0.59	1.28	2.18
T 10B	162	0.75	1.89	2.51
T 13A	130	0.64	1.61	2.52
T 13B	139	0.46	0.75	1.62
T 14A	148	0.22	0.19	0.88
T 14B	146	0.13	0.09	0.74
T 18A	107	0.02	0.02	1.00
T 18B	104	0.00	0.00	0.00
T 19A	148	0.63	1.16	1.84
T 19B	102	0.53	1.05	1.98
T 21A	171	0.24	0.25	1.10
T 21B	148	0.20	0.33	1.58
T 23A	211	0.40	0.82	2.26
T 23B	214	0.64	1.46	2.26
T 25A	102	0.66	1.70	2.58
T 25B	105	0.62	1.72	2.74
T 29A	128	0.37	0.38	1.04
T 29B	195	0.44	0.54	1.21
T 36A	104	0.20	0.27	1.33
T 36B	106	0.39	0.66	1.71
T 38A	96	0.27	0.47	1.73
T 38B	145	0.32	0.65	2.00
T 39A	119	0.75	1.02	1.34
T 39B	51	0.00	0.00	0.00
T 40A	156	0.09	0.15	1.71
T 40B	281	0.00	0.00	0.00
T 41A	75	0.41	0.69	1.68
T 41B	125	0.28	0.38	1.33

Table 6. (Cont.).

Code No.	No. of florets tripped	No. of pods per floret	No. of seeds per floret	No. of seeds per pod
T 42A	132	0.44	0.98	2.22
T 42B	101	0.53	0.92	1.72
T 44A	114	0.33	0.71	2.13
T 44B	108	0.43	0.97	2.23
T 45A	111	0.40	0.54	1.36
T 45B	99	0.23	0.25	1.09
T 46A	102	0.03	0.03	1.00
T 46B	101	0.14	0.18	1.28
T 47A	109	0.03	0.03	1.00
T 47B	109	0.04	0.04	1.00
T 50A	102	0.27	0.45	1.64
T 50B	131	0.04	0.52	1.31
T 54A	128	0.14	0.18	1.28
T 54B	108	0.10	0.65	0.64
T 55A	100	0.62	1.49	2.40
T 55B	101	0.66	1.62	2.45
T 62A	68	0.12	0.15	1.25
T 62B	5	0.00	0.00	0.00
T 64A	109	0.09	0.10	1.10
T 64B	93	0.19	0.20	1.06
T 67A	109	0.27	0.32	1.21
T 67B	92	0.16	0.26	1.60
T 70A	107	0.14	0.09	0.67
T 70B	122	0.18	0.16	0.86
T 71A	126	0.28	0.35	1.26
T 71B	108	0.31	0.28	0.88
T 72A	104	0.64	1.38	2.15
T 72B	112	0.60	1.30	2.16
T 73A	110	0.54	0.82	1.52
T 73B	108	0.47	0.77	1.63
T 74A	106	0.43	0.77	1.78
T 74B	161	0.58	1.14	1.98
T 75A	104	0.60	1.04	1.74
T 75B	108	0.33	0.71	1.83
T 77A	102	0.64	1.32	2.08
T 77B	65	0.47	0.95	2.00
T 79A	140	0.79	2.14	2.69
T 79B	121	0.69	1.48	2.13

Table 4. (concl.).

Code No.	No. of florets tripped	No. of pods per floret	No. of seeds per floret	No. of seeds per pod
T 80A	105	0.20	0.23	1.14
T 80B	156	0.22	0.22	1.00
T 81A	147	0.53	0.88	1.67
T 81B	100	0.42	0.79	1.88
T 86A	154	0.08	2.66	3.30
T 86B	118	0.77	2.30	2.99
T 86C	119	0.74	2.00	2.70
T 87A	107	0.13	0.12	0.93
T 87B	112	0.16	0.22	1.39
T 89A	158	0.22	0.30	1.38
T 89B	263	0.27	0.40	1.46
T 93A	102	0.75	2.34	3.10
T 93B	97	0.70	1.82	2.60
T104A	174	0.72	1.40	1.79
T104B	127	0.67	1.46	2.18
T105A	108	0.18	0.21	1.15
T105B	111	0.23	0.27	1.15
T106A	106	0.11	0.11	1.00
T106B	106	0.12	0.15	1.23
T107A	103	0.72	1.86	2.59
T107B	46	0.74	1.72	2.32
T110A	123	0.54	0.84	1.54
T110B	103	0.58	1.04	1.78
T111A	107	0.39	0.68	1.74
T111B	68	0.31	0.50	1.62
T112A	101	0.08	0.06	0.75
T112B	101	0.09	0.09	1.00
T116A	108	0.68	1.07	1.66
T116B	178	0.48	0.89	1.85
T117A	104	0.26	0.35	1.33
T117B	120	0.48	0.71	1.49
T118A	108	0.75	2.28	3.01
T118B	99	0.68	1.54	2.27
T119A	127	0.35	0.52	1.50
T119B	105	0.34	0.33	0.97
TT 1A	76	0.05	0.09	1.75
TT 1B	117	0.07	0.08	1.25
TT 1C	103	0.10	0.09	0.89

Table 7. Inter-fertility of members of twin sets in alfalfa.

Cross	Total florets crossed	No. of pods per floret	No. of seeds per floret	No. of seeds per pod
T 2A x T 2B	34	0.00	0.00	0.00
T 2B x T 2A	11	0.00	0.00	0.00
T 4A x T 4B	132	0.49	1.12	2.28
T 4B x T 4A	134	0.36	0.78	2.12
T 5A x T 5B	60	0.37	0.30	0.82
T 5B x T 5A	57	0.19	0.28	1.45
T 8A x T 8B	101	0.44	0.76	1.75
T 8B x T 8A	104	0.21	0.17	0.82
T 9A x T 9B	94	0.53	1.52	2.86
T 9B x T 9A	108	0.61	1.77	2.89
T 10A x T 10B	110	0.52	1.44	2.79
T 10B x T 10A	105	0.65	2.00	3.09
T 13A x T 13B	25	0.88	1.92	2.18
T 13B x T 13A	20	0.35	0.08	2.28
T 14A x T 14B	113	0.19	0.31	1.67
T 14B x T 14A	101	0.23	0.30	1.30
T 18A x T 18B	27	0.00	0.00	0.00
T 18B x T 18A	19	0.00	0.00	0.00
T 19A x T 19B	122	0.75	1.81	2.51
T 19B x T 19A	122	0.88	2.25	2.57
T 21A x T 21B	116	0.25	0.34	1.34
T 21B x T 21A	116	0.27	0.29	1.10
T 23A x T 23B	104	0.67	2.14	3.18
T 23B x T 23A	129	0.83	2.10	2.53
T 25A x T 25B	53	0.21	0.57	2.73
T 25B x T 25A	46	0.50	1.28	2.56
T 29A x T 29B	59	0.52	0.54	1.03
T 29B x T 29A	56	0.43	0.52	1.21
T 38A x T 38B	122	0.43	0.74	1.70
T 38B x T 38A	122	0.43	0.77	1.77
T 39A x T 39B	103	0.80	1.81	2.27
T 39B x T 39A	51	0.00	0.00	0.00
T 40A x T 40B	67	0.07	0.09	1.20
T 40B x T 40A	129	0.00	0.00	0.00
T 42A x T 42B	119	0.48	1.01	2.10
T 42B x T 42A	115	0.63	1.58	2.49
T 44A x T 44B	112	0.51	1.07	2.10
T 44B x T 44A	98	0.49	1.10	2.25

Table 7. (Cont.).

Cross	Total florets crossed	No. of pods per floret	No. of seeds per floret	No. of seeds per pod
T 45A x T 45B	45	0.31	0.60	1.93
T 45B x T 45A	44	0.18	0.27	1.50
T 46A x T 46B	108	0.09	0.09	1.00
T 46B x T 46A	108	0.12	0.15	1.21
T 47A x T 47B	107	0.01	0.01	1.00
T 47B x T 47A	101	0.06	0.06	1.00
T 50A x T 50B	107	0.45	0.87	1.94
T 50B x T 50A	110	0.54	1.08	2.02
T 54A x T 54B	133	0.11	0.14	1.27
T 54B x T 54A	109	0.05	0.05	1.00
T 55A x T 55B	56	0.70	1.96	2.82
T 55B x T 55A	57	0.72	1.61	2.24
T 67A x T 67B	64	0.11	0.17	1.57
T 67B x T 67A	69	0.14	0.17	1.20
T 70A x T 70B	55	0.29	0.24	0.81
T 70B x T 70A	64	0.26	0.26	1.00
T 71A x T 71B	102	0.35	0.56	1.58
T 71B x T 71A	106	0.42	0.63	1.52
T 72A x T 72B	121	0.76	1.99	2.62
T 72B x T 72A	128	0.70	1.58	2.24
T 73A x T 73B	98	0.63	0.96	1.52
T 73B x T 73A	110	0.65	0.91	1.40
T 75A x T 75B	52	0.57	1.27	2.23
T 75B x T 75A	52	0.73	1.60	2.18
T 79A x T 79B	127	0.79	3.03	2.39
T 79B x T 79A	116	0.72	1.88	2.63
T 80A x T 80B	110	0.14	0.15	1.06
T 80B x T 80A	121	0.17	0.26	1.48
T 81A x T 81B	86	0.59	1.13	1.90
T 81B x T 81A	109	0.45	0.90	2.00
T 86A x T 86B	103	0.74	2.18	2.96
T 86B x T 86A	110	0.79	2.50	3.16
T 86A x T 86C	109	0.85	2.61	3.05
T 86C x T 86A	110	0.68	2.38	3.49
T 86B x T 86C	161	0.65	2.14	3.28
T 86C x T 86B	156	0.71	2.40	3.37
T 87A x T 87B	65	0.23	0.29	1.27
T 87B x T 87A	60	0.27	0.13	1.12

Table 7. (Concl.).

Cross	Total florets crossed	No. of pods per floret	No. of seeds per floret	No. of seeds per pod
T 89A x T 89B	110	0.29	0.42	1.44
T 89B x T 89A	105	0.40	0.64	1.60
T 93A x T 93B	37	0.65	1.57	2.42
T 93B x T 93A	37	0.76	2.35	3.11
T104A x T104B	137	0.77	1.53	1.98
T104B x T104A	102	0.74	1.67	2.24
T106A x T106B	100	0.22	0.25	1.14
T106B x T106A	91	0.11	0.09	0.80
T116A x T116B	105	0.77	1.61	2.09
T116B x T116A	119	0.75	1.82	2.44
T118A x T118B	42	0.88	2.29	2.59
T118B x T118A	44	0.73	1.89	2.59
T119A x T119B	104	0.38	0.49	1.28
T119B x T119A	103	0.37	0.61	1.66
TT 1A x TT 1B	115	0.17	0.27	1.55
TT 1B x TT 1A	121	0.14	0.19	1.35
TT 1A x TT 1C	118	0.19	0.24	1.26
TT 1C x TT 1A	105	0.20	0.31	1.57
TT 1B x TT 1C	62	0.27	0.39	1.41
TT 1C x TT 1B	57	0.24	0.33	1.36

percent increase in fertility above self-fertility.

Greenhouse conditions brought about considerable variation in fertility. The percent of pod set was affected especially by the moisture relationship. Also, due to the low number of flowers produced, not all pollinated florets were at the same stage of growth.

Pollen Stainability. The results of pollen staining with IKI are recorded in Table 8. The highest average percent of stainable pollen found in a plant was 96.11 percent in T4A. Three other plants, T93A, 107A and 107B, showed greater than

Table 8. Percent of stainable pollen, pollen diameter and chromosome counts in twin plants of alfalfa.

Code No.	Percent stainable pollen: Original data	Percent stainable pollen: Transformed data*	Pollen diameter	Chromosome count
T 2A	82.62	65.35	38.21	--
T 2B	84.35	66.74	39.22	32
T 4A	96.11	78.61	41.65	32
T 4B	93.02	74.66	40.59	--
T 5A	88.42	70.09	40.19	32
T 5B	90.36	71.95	41.32	--
T 8A	59.25	50.30	39.43	32
T 8B	84.00	66.55	41.25	32
T 9A	83.78	66.27	41.84	--
T 9B	78.27	62.24	40.49	32
T 10A	82.84	65.50	40.26	32
T 10B	84.75	67.05	39.60	32
T 13A	84.37	66.74	39.30	32
T 13B	80.82	64.01	39.60	32
T 14A	60.99	51.35	39.60	32
T 14B	61.66	51.77	40.07	32
T 18A	74.28	59.54	39.68	--
T 18B	69.74	56.60	39.44	--
T 19A	90.96	72.54	41.25	--
T 19B	91.34	72.84	39.43	32
T 21A	46.60	43.05	40.85	32
T 21B	51.04	45.57	40.99	32
T 23A	73.73	59.15	39.76	32
T 23B	77.57	61.75	38.49	32
T 25A	83.25	65.80	40.66	32
T 25B	84.18	66.58	38.68	32
T 29A	49.41	44.66	41.08	32
T 29B	53.09	46.78	40.33	32
T 36A	92.54	74.11	40.30	--
T 36B	94.33	76.19	39.84	--
T 38A	56.55	48.79	41.25	32
T 38B	56.54	48.73	39.93	32
T 39A	74.04	59.34	38.50	32
T 39B	3.55	10.94	-- --	16
T 40A	72.25	58.18	39.67	32
T 40B	1.47	7.04	-- --	16
T 41A	89.62	71.19	39.37	32
T 41B	90.63	72.15	40.19	32
T 42A	81.60	66.89	38.21	32
T 42B	80.20	63.58	37.95	--

Table 8. (Cont.).

Code No.	Percent stainable pollen: Original data	Transformed data*	Pollen diameter	Chromosome count
T 44A	82.29	65.12	38.75	32
T 44B	82.87	65.57	40.14	32
T 45A	91.38	72.95	39.10	--
T 45B	90.67	72.24	39.76	--
T 46A	55.11	47.93	40.42	32
T 46B	56.56	48.79	40.33	--
T 47A	91.08	72.64	43.90	32
T 47B	92.62	74.21	42.30	32
T 50A	81.44	64.45	39.00	--
T 50B	83.94	66.34	38.64	--
T 54A	80.95	64.16	39.76	--
T 54B	83.05	65.65	39.96	--
T 55A	89.33	70.91	40.00	--
T 55B	83.55	66.11	38.84	--
T 62A	51.41	45.80	37.98	--
T 62B	51.36	45.80	38.38	--
T 64A	38.35	38.29	38.12	--
T 64B	44.32	41.73	38.44	--
T 67A	60.89	51.30	39.86	--
T 67B	62.33	52.12	40.99	--
T 70A	38.84	38.53	40.92	--
T 70B	37.04	37.47	40.19	--
T 71A	49.04	44.43	41.42	--
T 71B	44.19	44.54	40.99	--
T 72A	71.92	57.99	40.66	32
T 72B	71.18	57.54	40.52	--
T 73A	84.20	66.58	42.90	--
T 73B	86.81	68.70	40.61	--
T 74A	79.71	63.22	40.67	--
T 74B	78.22	62.17	39.67	--
T 75A	77.24	61.48	41.63	--
T 75B	80.12	63.51	40.76	--
T 77A	53.28	46.79	43.65	--
T 77B	57.67	49.43	40.86	--
T 79A	83.28	65.88	41.25	--
T 79B	67.43	55.18	40.85	--
T 80A	41.44	40.05	40.26	--
T 80B	47.12	43.34	42.08	--
T 81A	83.65	66.11	39.93	32
T 81B	75.20	60.13	39.37	--

Table 8. (Concl.).

Code No.	Percent stainable pollen		Pollen diameter	Chromosome count
	Original data	Transformed data*		
T 86A	76.92	61.27	41.74	--
T 86B	75.32	60.53	42.17	--
T 86C	78.46	61.00	40.66	--
T 87A	76.83	61.21	42.08	--
T 87B	74.73	59.80	40.26	32
T 89A	42.09	40.46	43.92	--
T 89B	36.29	37.05	51.89	--
T 93A	95.92	78.32	37.88	--
T 93B	94.11	75.94	38.28	--
T104A	54.87	47.81	40.19	--
T104B	53.95	47.29	40.92	--
T105A	64.12	53.19	37.29	32
T105B	72.85	58.56	37.55	32
T106A	61.84	51.83	39.81	--
T106B	62.61	52.30	38.13	--
T107A	95.65	77.89	40.55	--
T107B	95.05	77.08	40.36	--
T110A	69.86	56.73	39.60	--
T110B	61.63	51.71	38.53	--
T111A	31.11	38.12	37.20	32
T111B	32.23	34.57	39.20	--
T112A	57.12	49.08	39.62	32
T112B	58.26	49.78	40.14	--
T116A	85.93	67.94	39.93	32
T116B	82.61	65.35	39.93	--
T117A	29.71	33.02	37.45	--
T117B	29.28	32.77	37.57	--
T118A	81.47	64.52	39.20	--
T118B	78.67	62.51	38.35	--
T119A	46.27	42.88	40.67	--
T119B	46.33	42.88	40.67	--
TT 1A	41.21	39.93	39.76	32
TT 1B	37.57	37.92	41.25	32
TT 1C	38.08	38.12	40.76	32
H. sativa	86.45	-- --	34.97	--
H. vastula	95.07	-- --	34.10	--
L.S.D. .05 between members of a set.		4.38		

* Data transformed by the arcsin method (44).

95 percent. The lowest amount was in plants T39B and T40B which had 3.55 and 1.47 percent respectively. T40B produced considerably more pollen grains than did T39B (2,319 vs 197 on the basis of three slides).

The greatest variability between members of a set was displayed by the T39 and T40 twins, (Fig. 1 and 2, Plate 2). T39A and T40A showed 70.49 and 70.78 percent respectively more stainable pollen than the B plant of the sets. The least variation was in T38A and B, T62A and B and T119A and B all showing less than 0.06 percent difference between the A and B plant.

Eight sets of twins proved to be significantly different by the L.S.D. method of analysis. These sets were T8, T39, T40, T55, T79, T81, T105 and T110. T5 and T9 approached significance.

Plants T117A and B displayed the lowest percent of stainable pollen for both members of a set (A = 29.71 and B = 29.28).

Sets T14, T74 and T112 exhibited sticky pollen in both the A and B plants.

Plants T47A and B showed a majority of pollen grains with four germ pores instead of the usual three.

Pollen Diameter. Pollen diameters are listed in Table 8. Four plants (T73A, T77A, T89A and T111B) displayed pollen grains which were two microns larger than the pollen grains of their twins. The smallest pollen grain found in a twin plant was 37.20 u in T111A. Four sets of twins (T42, T62, T93 and T105) displayed small pollen in both members. T89A exhibited the largest pollen grains of any single plant (43.92 u), while T47A

EXPLANATION OF PLATE II

Fig. 1. Plant T40A showing 72.25 percent stainable pollen. Approximately 200X

Fig. 2. Plant T40B showing 1.47 percent stainable pollen. Approximately 475X

Fig. 3. Plant T40A, a somatic cell showing 32 chromosomes. Approximately 900X

Fig. 4. Plant T39B, a somatic cell showing 16 chromosomes. Approximately 900X

PLATE II

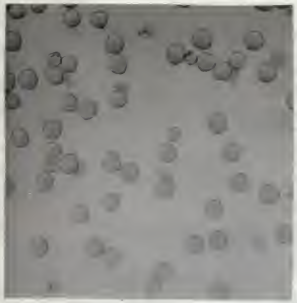

Fig. 1

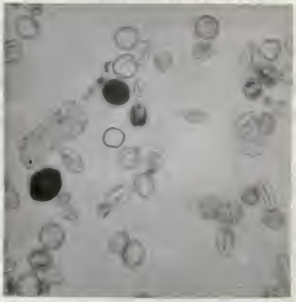

Fig. 2

Fig. 3

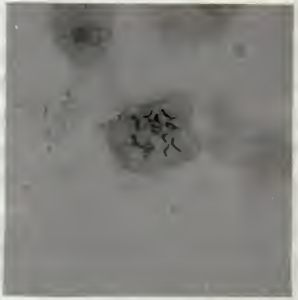

Fig. 4

and B had the largest grains of any twin set.

Plants T21A and B exhibited a wide variation in size of pollen grains.

Chromosome Counts. The results of the chromosome counts are listed in Table 8. The figures listed represent the approximate number of chromosomes in terms of euploid, since no attempt was made to determine if a plant was an aneuploid. Only two plants proved to be polyhaploids (2n = 16), T39B and T40B, both from the variety Lahontan, (Fig. 4, Plate 2). The remainder of the studied plants were 32 chromosomes.

Progeny Study. The polyembryonic seeds found in germination of the S_1 seeds from the twin plants were planted and the seedlings were studied. Various abnormal characteristics were noted.

Both T14A and B produced a high percent of polyembryonic seeds, 18.67 and 15.29 percent respectively (Table 2). Neither plant displayed serration on the edge of the leaf. The S_1 generation for both plants showed faint or no serration of the leaf. Similarly both T14A and B produced progeny having bifoliate leaves instead of the unifoliate leaf produced on normal plants.

Plants T13A and B both produced progeny which exhibited a funnel-shaped blade on the unifoliate leaf. They each produced three sets of twins from 197 and 103 germinated seeds respectively. This would suggest both plants were able to produce more multiple embryos than a normal seed population.

Plants T104A and B also displayed higher than normal production of polyembryos. T104A showed three from 273 seeds and

its twin showed two from 179 seeds.

The twin plants T116A and B produced progeny exhibiting an elongated epicotyl.

All the progeny of the T23A and B (300 and 261 plants respectively) displayed deeper than normal serration, similar to the parent plants.

Triplets

One set of triplets was found in the 120 polyembryonic seeds germinated. The three plants were separate and showed extreme differences in length of the primary root. Two plants had equal roots while the third was nearly twice as long as the other two. The seed was planted in sterile sand and complete nutrient solution was supplied to keep the plants alive. The largest plant (A) formed only one cotyledon but had a growing point. The plant tagged C was an extremely small plant and showed very slow growth. The B plant was intermediate in size between the A and C plant. They retained this size relationship until they were cut back. At that time A was 12 inches, B was 8 inches and C was 3 inches in height. All three plants displayed equal regrowth.

The triplets proved to be equal for recovery rate, budding date, growth habit, flower color, pollen stainability and chromosome number. All three plants exhibited between 5.4 to 9.7 percent self-fertility. Intra-triplet fertilization varied from 14.0 to 27.4 percent. This would suggest that the members of the triplet set were not identical.

Polyhaploid Plants

T39B. This plant (16 chromosome, Fig. 4, Plate 2) was fused to its twin at the primary root stage, but later they separated naturally. It consistently exhibited reduced growth as compared to its tetraploid twin. The distinguishing characteristics of this plant were its upright growth, stiff stems and narrow leaves of near normal length. The flowers, though reduced in size were apparently normal. The flowering date of T39B was approximately equal to the A plant. In comparison to their relative heights, the polyhaploid recovered as rapidly from cutting back as its twin. At flowering time in the field it was seven and one-half inches tall as compared to 14 inches for A.

The polyhaploid displayed 3.6 percent stainable pollen on the basis of 197 pollen grains from 45 florets. The low amount of pollen produced and the low percent of stainable pollen made it very difficult to get a measurement of the pollen size. Therefore, a comparison between T39B and diploid M. sativa or M. caetula has not been made on the basis of pollen size.

T39B proved to be self-sterile (Table 9) as might be expected on the basis of low percent of stainable pollen. It did not set seed when used as the female in crosses with the diploid species, M. sativa, M. falcata and M. caetula, and with the other polyhaploid T40B. This suggests that few reduced female gametes are functional. When its tetraploid twin was used as the pollen parent, T39B showed some fertility, setting nine pods from 251

Table 9. Self- and cross-fertility of polyhaploid alfalfa twins.

Code number	Number of florets	Pods set	Seeds set	Percent pods set
T39B selfed	51	0	0	0.00
T39B x T39A	251	9	11	3.58
T39B x M. sativa (16 chromosome)	45	0	0	0.00
T39B x M. falcata (16 chromosome)	60	1	1	1.67
T39B x M. caetula (16 chromosome)	77	0	0	0.00
T39B x T40B	45	0	0	0.00
T40B selfed	295	0	0	0.00
T40B x T40A	159	0	0	0.00
T40B x M. sativa (16 chromosome)	133	0	0	0.00
T40B x M. falcata (16 chromosome)	37	0	0	0.00
T40B x M. caetula (16 chromosome)	9	0	0	0.00
T40B x T39B	31	0	0	0.00

florets pollinated. This suggests that the unreduced female gametes were functional. Chromosome counts of the seven plants from the nine seeds have not been ascertained as yet.

The twin set, T39, was from the variety Lahontan, which shows relative resistance to the spotted alfalfa aphid. Both A and B plants proved to be susceptible to the insect.

T39B was transplanted to the field in the spring of 1958. There it has shown excellent growth, though short, and appears to be able to maintain itself well in the field.

T40B. This plant was very similar to T39B in most respects. It was fused to T40A at the start, but separated naturally. Its'

growth habits were nearly identical to T39B although it was not as vigorous. T4OB was much more difficult to maintain in the greenhouse. No clones were available for field studies.

T4OB showed less fertility in crosses than the other polyhaploid plant (Table 9). Due to the low number of stainable pollen grains (Fig. 2, Plate 2) again no comparison can be made with M. sativa and M. caetula on pollen size.

Though from the variety Lahontan, T4OA and B were susceptible to the spotted alfalfa aphid.

DISCUSSION

The results indicate that more than one process is involved in the formation of polyembryonic seeds in alfalfa. Twin sets T39 and T4O probably have originated by euploid polyembryony. The tetraploid A plant in each set might have been the result of normal fertilization, while the B plant (2n = 16) may have developed parthenogenetically from an antipodal, a synergid or an egg cell in a second embryo sac. Cooper (11) reported that both the antipodals and synergids remained until after the pollen tube had entered the embryo sac. Thus, these cells would be present to start division if a stimulus was supplied by the pollen tube.

Seventeen sets of twins (other than T39 and T4O) and the triplet set exhibited a distinct difference between members for at least one characteristic. Therefore, they could not be considered the result of zygotic cleavage but may have arisen in one of three ways: (1) they could have been brought about by

fertilization of two cells in a single embryo sac. These cells could have been the egg cell and a synergid or antipodal. In such a case the plants would have the same material characters but would differ in the paternal characters. This would assume that two pollen tubes were able to penetrate a single embryo sac. It is not known if this is possible (39), (2) two embryo sacs may develop within a single ovule. This would result in two or more embryos in the same seed which could differ both in male and female characters and (3) one embryo might arise by sporophytic budding of a nucellar or integument cell. In this case one embryo would be a hybrid between the male and the female while the second embryo would show maternal characters only.

The conjoined twins probably arose from the same embryo sac and hence would be the result of zygotic cleavage, fertilization of a synergid or antipodal or apomictic development of some cell. Twin sets T39 and T40 have already been discussed under the latter possibility. T105A and B, which proved to be significantly different on the basis of stainable pollen, probably were the result of fertilization of a second cell in the egg apparatus. No definite conclusion may be drawn concerning the remaining six conjoined sets.

The other 30 twin pairs may have arisen by zygotic cleavage or any of the other mention ways with the exception of parthenogenesis of a reduced cell.

Greenshields (16) concluded from his study of 55 sets that twinning in alfalfa was generally the result of cleavage of a normal zygote or else normal fertilization occurring in two

embryo sacs. Cooper (11) substantiated this idea when he reported as many as three macrospore tetrads and sometimes two embryo sacs in an ovule. In the multiple embryo studies of alfalfa, the only chromosome number reported have been 32 or its aneuploid, with the exception of Lesins' (30) polyhaploid.

During the seedling stage of growth, 36 sets of twins and the triplet set showed differences in height between members. In all cases, with the exception of T39 and T40, the members of each set showed equal height at maturity. This indicated that the height difference of the seedling plants was not a genetic difference. Perhaps the best explanation is that it was due to the relative position of the embryo to the endosperm. The embryo with the larger food reserve would be the faster growing plant.

The average frequency of polyembryos in the varieties studied was about one in 800 germinated seeds. However, this is probably the minimum rate of occurrence. Only seeds showing two or more primary roots at germination time were called polyembryonic. Therefore, any conjoined set displaying only one undivided radicle would not be included in the count. However it is questionable if a plant with one radicle and one growing point could be considered a twin on the basis of its having three or four cotyledons such as Greenshields (16) stated.

Polyembryony appears to be genetically controlled in many cases. Kappert (Webber, 52) concluded from his study of Linum that it was a recessive character probably conditioned by a series of multiple factors. Greenshields (16) suggested that the occurrence of polyembryos in alfalfa maybe similar. This

study corroborates his theory. The frequency of multiple embryos in S_1 seed from twin plants proved to be 10 times greater than that of the studied seed population. Plants T14A and [illegible] both produced a high percent of twin plants (18.7 and 15.3 respectively) suggesting that some factor other than chance is involved.

The polyhaploids found in this study resembled the plant reported by Lesins (28) and the haploid found by Stanford and Clements (43) both in breeding behavior and pollen abortion. Lesins (27) concluded from his study that alfalfa is an autotetraploid which has undergone a great many cryptic structural changes. He stated, "The 8-chromosome set has become extremely weak under the tetraploid condition as none of the male and few female gametes were found to be functional." If the polyhaploids had been fertile it might be assumed that alfalfa were an autotetraploid plant. However, the finding of two more sterile polyhaploids does not prove conclusively that it is an allotetraploid.

SUMMARY

Fifty-six sets of alfalfa twins and one set of triplets were studied for various morphological and reproductive characteristics. Nineteen sets of twins and the triplet set proved to have members which differed in at least one studied character. The remaining 37 sets appeared to be identical.

The results of testing 12 alfalfa varieties showed that polyembryony occurred about once in 800 germinated seeds. One in 81 S_1 seeds from twin plants proved to be a multiple embryo.

One set of twins (T1L4A and B) showed a frequency of one in 6.4 and one in 7.5 S_1 seeds respectively.

Two polyhaploid plants (2n = 16) were found among the twin sets. Each of these plants was fused to its tetraploid twin at germination. They later separated naturally. Each twin set proved to be equal for flower color, growth habit and date of budding. They differed in recovery rate, percent of stainable pollen and self- and cross-fertility.

The polyhaploids (T3OB and T4OA) showed reduced growth, stiff upright stems and narrow leaves. Both plants exhibited less than four percent stainable pollen. Each proved to be highly self-sterile and highly cross-sterile when crossed with diploid (2n = 16) M. sativa, M. falcata and M. raetula and the other polyhaploid. T3OB showed some female fertility when crossed with its tetraploid counterpart.

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POLYEMBRYONY AND BREEDING BEHAVIOR OF POLYHAPLOID TWINS IN ALFALFA

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Fifty-six sets of twins and one triplet set selected from 15 alfalfa varieties and crosses were studied in an attempt to determine more about the reproductive process and evolution of alfalfa. The polyembryonic seeds were determined by germinating seed between blotters and selecting the seeds showing two or more primary roots.

The frequency of twinning proved to be about one in 800 germinated seeds in the alfalfa varieties studied. S_1 seeds from the twin plants displayed nearly 10 times greater rate of occurrence. Twin set T14A and B produced one multiple embryo in 6.4 and 7.5 germinated seeds respectively.

The plants were studied in the greenhouse and the field to detect intra-set differences. Six sets differed in number of cotyledons; four in appearance of the unifoliate leaf; one showed difference in leaf serration; three sets displayed different recovery rates; eight differed in percent stainable pollen; one in percent self- and intra-set fertility and two in euploid chromosome number.

All sets were found to be identical for flower color and growth habit.

Two polyhaploid plants (2n = 16) were found among the twin sets. Each of these plants was fused to its tetraploid twin at germination. They later separated naturally. The twin sets proved to be equal for flower color, growth habit and date of budding. They differed in recovery rate, percent of stainable pollen and self- and cross-fertility.

The polyhaploids (T39B and T40B) showed reduced growth,

stiff upright stems and narrow leaves. Both plants exhibited less than four percent stainable pollen. Each proved to highly self-sterile and highly cross-sterile when crossed with diploid (2n = 16) M. sativa, M. falcata and M. gaetula and the other polyhaploid. T39B showed some female fertility when crossed with its tetraploid counterpart.