A STUDY OF SEED-SETTING IN STRAWBERRY CLOVER,

Trifolium fragiferum, Linn.

By

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TABLE OF CONTENTS.

TABLE OF CONTENTS (Cont.)

LIST OF TABLES

LIST OF PHOTOGRAPHS

INTRODUCTION

Strawberry clover is already well-established on many of the wetter areas of New Zealand. In particular it is making a valuable contribution to the productivity of the once unstable and unproductive coastal regions of both the North Island and South Island.

It is felt, however, that this clover could be used to even greater advantage in this country if more seed of a high producing strain could be made available to agriculture, economically.

One of the major difficulties, hindering the development of Strawberry clover has been the low seed yields harvested, and for this reason the species has often been termed a "shy-seeder" (Ullmann,Kassel 1941, Gorman 1953). It is of interest to note that with bred strains under Australian conditions, high seed-yields have been obtained (Tiver 1954).

Trifolium fragiferum is considered by some authorities to be a self-fertile species (Ullmann-Kassel 1941, Williams 1931, Tiver 1954). However, the presence of incompatibility, which is known to occur throughout the genus Trifolium, should not be discounted and is a possible reason for the low seed-setting recorded in this species. In homostyled species, incompatibility is controlled by the single gene 'S', which has a large number of alleles (Sears 1937). These alleles act in a way which prevents pollen tubes which carry any one of them, from growing down the style of, and affecting fertilization in, any plant carrying the same allele. In incompatible pollination, pollen-tube growth is prohibited, but in compatible pollination, tube growth is normal.

The method of improving a crop may be determined only after its normal mode of pollination and fertilization are understood. In homozygous self-fertilizing plants, the simple, pure-line method may be used. In basically cross-fertilizing species, a more complex system of breeding must be adopted to maintain good growth vigour and fertility.

The aim of the present work was to obtain basic data on some of the factors which cause low seed-setting in this species, and in particular the degree of self- and cross-fertility that exists.

Factors other than incompatibility are known to affect seed yields in pasture legumes and these are discussed in a separate section below.

BACKGROUND.

1. Description of the Species.

Trifolium fragiferum Linn. is a perennial clover of haploid chromosome number, 8. (Ullmann-Kassel 1941).

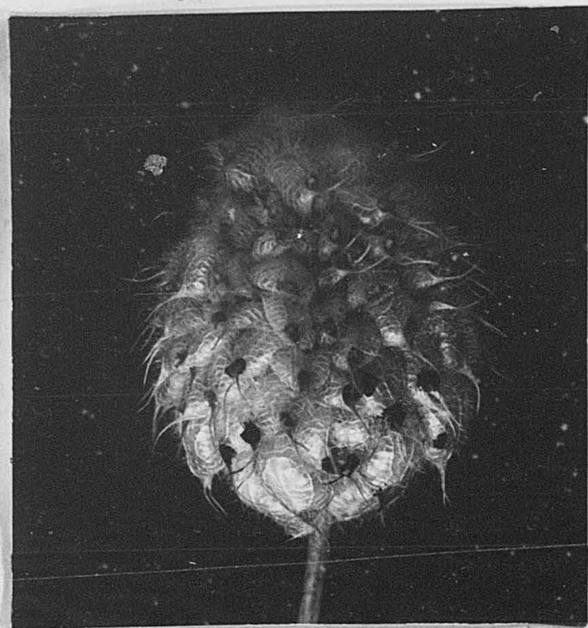
Vegetatively, the species resembles Trifolium repens, (see photograph 1) but Strawberry clover has a coarser leaf veination and has more oval-shaped leaflets. The leaflets of strawberry clover may be 0.5 cm. to 2.0 cm. long, by 0.4 cm. to 1.4 cm. wide.

Photograph 1, showing T. fragiferum and T. repens in flower.

The rounded flowerheads of T. fragiferum are 1.0 cm. to 1.5 cm. long. These heads often have pink colouration. The florets are on short stems and from 5.0 mm. to 7.0 mm. long.

After fertilization the calyces expand and form a papery bladder around the withering petals and style (see photograph 2). These expanded calyces allow the seedhead to float upon water and under natural conditions this may assist in seed dispersal.

In the present work it was found that only the calyces on florets which had set seed became enlarged to any extent (see photographs 4, 5 and 6, pages 24, 25). Expansion of the calyces was usually obvious a day or two after pollination, and this was used to detect which florets had set seed. Atwood (1940 p. 995) noted a similar response after fertilization in white clover.



Photograph 2. Seedhead from Clone No. 34 showing the expanded calyces on fertilized ovaries on the lower part of the head. The florets on the upper portion were not tripped and did not set seed.

The pods may contain one or two shiny, oval-shaped seeds of yellow or brown colouration. The length of a seed may be 1.25 mm to 2.0 mm, the width 1.2 mm to 1.5 mm and the diameter 0.95 mm to 1.0 mm.

The seeds of T. fragiferum, like those of other species of the family Leguminoseae are hard when ripe. However, if the heads fall into water when the seeds are mature but not fully ripened, (Hyde 1950) they will germinate and grow immediately. Hyde found the same phenomenon occurred in mature but unripened white clover seed.

2. Distribution of the Species.

According to Ullmann-Kassel (1941) the species probably originated in Southern Europe. It spread from coastal regions of the Black Sea over most of Europe, with the exception of the mountain regions of northern Scandinavia.

Strawberry clover is now well established in Asia Minor, North Africa, East Africa, Malta, the Canary Islands and the island of Madeira.

The species was taken by ship from Europe to the United States, Argentine, Australia and New Zealand.

3. **Habitat.**

A remarkable feature of strawberry clover is its wide range of habitat. It will thrive on heavy or light soils, on rich fertile loams or waterlogged, swampy land. Plants of the species are known to tolerate high salt concentration. Ahi and Powers (1938) found the degree of tolerance was related to temperature, moisture content of the soil and type, quantity and dispersion of the salts present.

The species will also survive in soils of high alkalinity, (Tiver 1954, Ullmann 1941) and withstands long periods of inundation. Ullmann describes the species as being frost resistant and winter hardy.

In Europe it may be found growing at all altitudes from sea level to 4,000 feet. In New Zealand too, this clover may be found in high country as well as lowlands.

4. **Place in Agriculture.**

Under most farming conditions, white clover is the more vigorous of the two clovers and will usually become dominant if the two are sown in the same sward. However, under certain environmental conditions and in difficult soil types, strawberry clover will thrive where white clover will not. It is in these conditions that strawberry clover becomes of importance, either as a pioneer plant or the dominant legume in permanent pasture.

Tiver (1954) reports the increasing use of this species in irrigated pastures in Australia.

REVIEW OF THE LITERATURE ON TRIFOLIUM FRAGIFERUM.

Most of the data published on this species are of an ecological nature.

W. Ulmann - Kassel (1941a) reviewed the literature up to the time of his writing, under the following headings, ecology, nomenclature, origin, distribution and botany of the species. In his second review article Ullmann - Kassel (1941b) discusses cultivation, seed production, varietal differences, breeding, and the use of the species in agriculture. Ullmann concluded that the species was a poor seed producer and that this was its main disadvantage.

However, Tiver (1954) reported that under conditions found in South Australia, strawberry clover was a prolific seed producing plant and that yields of up to 280 lb per acre were obtained. Although no experimental evidence is given, Tiver states - "The flowers of strawberry clover are self-fertile, hence cross-pollination is not necessary as is the case with red clover and white clover. Honey bees visit the flowers and it is considered that they assist in the movement of pollen to the stigma and may therefore be important in increasing seed yields."

Williams (1931) in an experiment to determine the mode of fertilisation of lesser known pasture legumes, tested eight plants of T. fragiferum grown from seed collected in Canterbury, New Zealand. Four of these plants were isolated in a glasshouse and yielded 119 seeds from 17 heads. Another four plants were grown in the field. Two of these had their flowers protected from insects and 7 heads produced 246 seeds. The two remaining plants were left unprotected and from 13 heads, 230 seeds were harvested. Although no claims were made as to the exact degree of fertility, from the above, Williams concluded T. fragiferum was a spontaneously self-fertile species.

REVIEW OF LITERATURE RELATING TO SELF- AND CROSS-FERTILITY IN OTHER PASTURE LEGUMES.

To derive some indication as to the degree of self- and cross-fertility which might be expected in T. fragiferum it is of interest to review work done with related species.

Williams (1931), in his study of the lesser known species of Trifolium, concluded that all the voluntarily self-fertilizing species in his investigation were annuals with the exception of Trifolium fragiferum.

With T. repens, Williams found the species highly self-sterile in the absence of pollinating agents. However, when pollinated artificially there was a range of self-fertility, some individuals being highly self-fertile. He concluded that the species was more self-fertile than T. pratense. Atwood (1941) found individuals homozygous for self-compatability in white clover.

Reviewing the literature on Trifolium pratense, Williams (1925) stated that there was a wide diversity of opinion as to the amount of self-fertility in red clover and he concluded that although the evidence was strongly in favour of the view that red clover was not capable of affecting spontaneous self-fertilization, the evidence was too contradictory to be conclusive.

With Medicago sativa, Kirk and White (1933) in Canada found several autogamous plants which set seed fully without tripping. Pollination occurred in the early bud stage making the plants obligate self-fertilizers. Dwyer (1931) reports work to support this. Jenkin (1925) stated that cross-pollination is the natural mode of reproduction in M. sativa though self-fertilization may take place to a considerable extent; Williams (1931) using a wide range of material came to the same conclusion as Jenkin. However, it is generally agreed that tripping does improve seed setting in M. sativa, and that this may occur spontaneously under certain conditions (Dwyer 1932). Also many workers agree that a far greater amount of seed is set after crossing than after self-fertilization (Cooper & Brink 1940). Ufer (1933) sums up - "It is probable that the biology of the lucerne flower is ecologically governed, and with the distribution of this plant over varied geographical regions, a segregation into self-fertilizing and cross-fertilized types has taken place."

The work of Kirk and Stevenson (1931) supported by Ufer (1931) indicates that populations of *Melilotus* species may be mixtures of self-fertilizing and cross-fertilizing individuals. Similarly Silow (1931) found self-fertile and self-incompatible plants in his sample of *Lotus corniculatus*.

Stebbins (1957) put forward the hypothesis that regularly self-fertilizing types of plants are derived from cross-fertilizing ancestors. He quotes the autogamous species *Trifolium subterraneum* with its papillionate flower as an example. In Stebbins' opinion, facultative self-fertilizing species are intermediate between self-incompatible ones and those which are regularly self-fertilized. He concludes that plants resort to self-pollination when conditions are unfavourable to crossing or after long distance dispersal. Stebbins gives examples from the genera *Bromus*, *Hordeum* and *Secale* and the family *Plumbaginaceae*, where at the centre of greatest morphological diversity, the self-incompatible, cross-fertilizing species occur, while at the peripheral regions of distribution, self-fertilizing species occur. He believes that in cases of isolation, self-fertilization is favoured by natural selection and each successful biotype maintains itself as a pure-line.

Summarizing the above, the evidence strongly supports the hypothesis that in certain widely-dispersed, perennial, herbaceous legumes, there may be a tendency for basically cross-fertilizing species to form self-fertilizing or facultatively cross-fertilizing populations.

In these autogamous individuals two barriers to self-fertilization appear to be overcome. The first is the necessary deposition of pollen on the stigma accomplished by (a) spontaneous tripping (Engelbert 1931, Dwyer 1932) or (b) by changes in flower morphology (Kirk and Stevenson 1931). The second is incompatibility, overcome by the dominance of the self-fertility allele (Williams 1931, Silow 1931, Rinke and Johnson 1941, Atwood 1941) or some other mechanism, as yet, not described.

A REVIEW OF FACTORS AFFECTING SEED YIELD IN PASTURE LEGUMES.

Factors influencing seed-production in herbaceous legumes are many and varied, both as regards time, and mode of action. Each factor may act directly or indirectly upon the plant or crop and may affect a single aspect of seed development or may exert its influence upon many of the stages between flower initiation and the formation of ripe seed.

Seed yield is determined by the plant's genotype, environmental conditions, or interaction between the two.

The importance of each factor is governed by the number of individuals affected, the result varying from a small reduction in seed yield to complete failure of the seed crop.

Management of the crop is an important aspect of good seed yields but will not be discussed here.

The headings below are very broad and there is often interrelationship between the factors discussed.

1. CLIMATE.

Many workers have stressed the importance of weather conditions in relation to high seed yields (Martin 1914 and 1915, Williams 1931, Engelbert 1931, Dwyer 1931) and they are considered to be the main cause of annual fluctuations in crop yields.

The overall seasonal conditions have an effect upon the growth and reproduction of the plants but each aspect of the climate may exert its own influence upon the various stages of reproduction.

(a) Moisture. Martin (1915) found with red clover that there was a critical set of moisture conditions required at the stigma before pollen germinated. Engelbert (1931) considered that lucerne pollen viability was adversely affected by excess moisture at the stigma and by high atmospheric humidity. She found that rainfall, soil moisture, thickness of the stand and temperature were related to this moisture balance. She also maintained that insufficient moisture caused ovule and seed abortion. Martin (1914) believed excess moisture also favoured ovule abortion.

In lucerne, different varieties are known to have different optimum moisture requirements for pollen germination (Hector), and strains with the most water resistant pollen produce most seed in wet years (Engelbert).

(b) Temperature. According to Engelbert and Dwyer high temperatures induce automatic tripping and pollination in lucerne.

Sears (1937) concluded that for each species there was an optimum temperature for pollen-tube growth and Martin (1914) showed that pollen-tubes grew faster and fertilized the ovule in shorter time in hot weather than at low temperatures.

(c) Light. Herbaceous legumes flower in response to a definite photoperiod and set of temperature conditions. Wexelsen (1936) found there was an inherent variation in earliness of flowering and length of flowering period. It is possible that flowering is induced at a period unsuitable for high seed production or in extreme cases not at all. Care must be taken to ensure that the basic plants in a synthetic strain have similar peak flowering periods.

2. FACTORS WHICH MAY AFFECT THE HEALTH OF THE PLANTS.

Pathological conditions in the plant as a whole or of the reproductive organs, animal predators, nutrient deficiencies, old age or inherent lack of vigour will all adversely affect seed yields.

3. POLLINATION.

In autogamous plants any mechanism assisting pollen to the stigma will help increase seed-setting. In self-incompatible plants the deposition of foreign pollen on the stigma is essential to normal seed development.

(a) Flower Morphology. Hector describes the explosive mechanism of the legume flower which scatters pollen when triggered by insects.

Kirk and Stevenson (1931) found seven floral characteristics in *Melilotus alba* which aid in self-pollination without tripping.

Coffman, quoted by Hector, found that in lucerne, pollen dehiscence often took place in the early bud stage, the pollen being forced up the keel to the stigma. Fertilization could thus take place before tripping depending on the self-compatibility of the plant and the receptivity of the stigma. Also in white clover (Hector) and red clover (Martin 1913) the anthers may dehisce early, even in the bud stage. Williams (1931) obtained a slight increase in self-fertility by selfing unopened florets of red clover. Sears (1937) quotes workers who found viable seed could be obtained from self-incompatible plants, (these were not Leguminoseae), by self-pollination in the bud stage. This may be the result of apomixis or stimulated by pollen-tube growth or perhaps this is a method of ensuring some seed is set in self-incompatible plants in case cross-pollination does not occur. Perhaps only a small amount of seed, if any, is set after self-pollination in the bud, the bulk of the seed being set after cross-pollination.

Hector concluded that in lucerne in both highly self-compatible and incompatible plants, fertilization did not occur in untripped flowers despite the presence of pollen on the stigma. It was found that tripping lucerne flowers ruptured a fine membrane over the stigma and this allowed the pollen to germinate. Kirk and Stevenson (1931) confirmed this with Melilotus species where scarification of the stigma greatly increased seed setting. These workers suggest that under natural conditions insects may rupture this stigmatic membrane with their bodies. Other workers have noted that inhibition of pollen germination may be overcome by tripping, (Silow (1931) with Lotus uliginosus, Atwood (1931) with T. repens).

It would appear that tripping, either automatically or by insects, besides carrying pollen to the stigma, renders the stigma receptive to pollen germination by rupturing a covering membrane. This apparently is not necessary in highly autogamous plants where floral morphology aids pollen transfer to the stigma and incompatibility is not present. Pollination in the bud stage may be responsible for pseudo- self-fertility in some self-incompatible individuals (Sears 1937)

(b) Insect Pollinators. Basically the legume flower is adapted to insect cross-pollination. It is thought that the Leguminoseae and members of the Lepidoptera and Hymenoptera have evolved together.

The importance of insects in cross-pollination has been known since the time of Darwin.

Wild bee populations tend to be unstable and are not usually dependable for continuous high seed yields. There is no apparent relationship between date of flowering of a species, such as red clover, and the number of insects about (Todd and Vansell 1952) (F.A.O. Report 1953).

Akerberg (1952) maintained that attempts to increase wild bee populations had, on the whole, not been successful.

The problem of obtaining optimum useful insect populations on seed crops at peak flowering, has, in some instances, been solved by crowding honeybee hives onto the cropping area at this period (F.A.O. Report 1953).

4. INTERNAL FACTORS.

(a) Floral abnormalities. These are usually the result of recessive factors which become apparent after inbreeding and give rise to such characters as small untrippable floral envelopes, double styles or ovaries in each floret, and non-dehiscing anthers (Hector) (Engelbert 1931).

Only a few individuals in a population may be homozygous for these characters, and these plants, if self-incompatible, may be able to reproduce only in rare circumstances.

(b) Sterility. This may be defined as the partial or complete suppression of the reproductive organs and the failure of gamete formation.

The formation of non-viable pollen and ovules ~~are~~ is usually the result of chromosomal abnormalities or heritable factors (mutant recessives). However, environmental factors may also exert an influence upon gamete formation.

(c) Incompatibility. This is any hindrance to the normal fusion of gametic nuclei within a regular mating group, except when fusion is prevented by a defect of the nucleus itself. Incompatibility is always genetically determined but may be influenced in expression by environmental conditions. It is a physiological barrier between pollination and fertilization. Two plants may be entirely self-incompatible but reciprocally fertile, therefore there is no abnormality in development, merely a functional limitation.

East and Park (1917) first studied incompatibility in *Nicotiana* and concluded that it was inherited by definite combinations of transmissible factors. Prell in 1921 first put forward the oppositional factor hypothesis.

Sears (1937) and Lewis (1954) reviewed work done on incompatibility and gave classifications of the various forms.

Williams (1931) and Silow (1931) concluded that the number of alleles conditioning incompatibility in the legume species they studied was extensive.

East and Park found that in some incompatible plants they studied there was a slight but temporary increase in self-fertility late in the flowering season. This phenomenon they termed end-of-season pseudo- self-fertility. Lewis (1942) concluded that altering the temperature could induce self-fertilization in incompatible plants and Emerson (1938) found that incompatibility in Oenothera organensis could be overcome to some extent by placing the plants in the dark.

Correns in 1912 is believed to be the first to propose that incompatibility was due to the inhibitive action of the stylar tissue on the pollen. Martin in 1913 concluded that incompatibility in Trifolium pratense was due to slow pollen tube growth.

Silow (1931) (red clover) found no difference in pollen germination on the style after compatible and incompatible pollination. He observed that the majority of pollen tubes, both compatible and incompatible, grew only a short distance into the style. Only about 3 or 4 passed beyond this point of interference. Silow found no evidence of the anomalous tube growth reported in species of other families (Sears 1937).

The point of retardation of incompatible tubes was considerably beyond the point where the majority of pollen tubes ceased to grow, and at a point about half-way down the length of the style. Atwood (1941) (white clover) reported that inhibition of incompatible pollen tubes took place after they had grown through approximately three-quarters of the style length.

Pande (1954-55) supported the conclusions of Atwood and Silow, finding that the "interference zone" and the "incompatibility zone" were closer together in T. repens than in T. pratense. He observed that the main difference between the two clovers was the number of compatible tubes which grew beyond the interference zone, only a few (3 or 4) were found in red clover whereas in white clover a greater number grew beyond this point. Pande found in red clover that the ends of the pollen tubes in the "interference zone" were directed back up the style.

(d) Ovule abortion. Usually many more ovules are formed in the ovary of pasture legumes than there are seeds set per pod.

Martin (1914) and Engelbert (1931) concluded that environmental conditions influenced ovule abortion, but there was variation between plants in response to these.

Atwood (1940) found with white clover that the number of seeds set per flower in incompatible crosses could be related to the number of ovules produced, and that this character appeared to be inherited.

Cooper and Brink (1940) working with lucerne, found that the ovules nearer the style tended to be fertilized more often than those occupying positions further along the pod towards the stem. Pollen-tubes often failed to reach these basal ovules. They found that abortion of normal ovules was common in lucerne and that many ovules remain unfertilized even when pollen tubes were near the micropyles.

(e) Embryo Abortions. Engelbert (1931) considered that small abnormal seeds were the result of inadequate nutrient supply at the stage of rapid embryo growth immediately after fertilization (Hyde 1950). Williams (1931) however, believed these seeds were the result of apomictic development. Cooper and Brink (1940) showed that 34.4% of their inbred lucerne embryos and endosperms collapsed within 6 days after fertilization. However, after cross-pollination only 7.1% of the hybrid embryos collapsed. These workers concluded that the higher survival following crossing was the result of more active growth of the hybrid endosperm.

Small abnormal seeds may therefore be the result of a number of factors:- poor nutrition, apomixis, chromosal abnormalities, lethal factors, and lack of vigour of inbred endosperm and embryo.

MATERIALS AND METHOD.

As a starting point in the improvement of seed yield and agronomic merit of strawberry clover it was decided to study plants from regions of New Zealand in which the species was already well-established. It was hoped that the results would show why seed yields were relatively so low in this country and also be a guide as to the possible use of local ecotypes as a basis for breeding work.

Fifty-four plants were grown from seed collected from the various habitats listed in Table 1. Twelve plants were grown from the seed of two Australian commercial lines, "Palestine" and "O'Connors". The following table gives the locality from which the seed was collected and the experimental number allotted to the plants from these localities:-

Table I.

District or Strain and Habitat.	Latitude (approx).	Plant Numbers Allotted.
North Island.		
Dargaville, North Auckland (Low lying)	35° 55'	45, 46, 47
Aoroa, North Auckland (River flats)	35° 58'	63, 64, 65
Kopaurahi, Hauraki Plains (Peat Swamp)	37° 14'	57, 58, 59
Ngatea, Hauraki Plains (Peat Swamp)	37° 16'	60, 61, 62
Raglan, Auckland (Coastal)	37° 48'	4, 5, 6
Wairoa, Hawkes Bay (River flats)	39° 3'	1, 2, 3
Haumoana, Hawkes Bay (Coastal)	39° 38'	54, 55, 56
Hastings, Hawkes Bay (Lagoon area)	39° 39'	39, 40, 41
Flock House, Bulls, Wellington (Coastal)	40° 16'	22, 23, 24
Himatangi, Wellington (Coastal)	40° 23'	33, 34, 35
South Island.		
Nelson (Coastal swamp)	41° 15'	19, 20, 21
Blenheim (sown with Australian seed) (River flats)	41° 30'	51, 52, 53
Kaiapoi, Canterbury (Coastal)	43° 22'	42, 43, 44
Lake Ellesmere, Canterbury (Lake side)	43° 45'	25, 26, 27
" " " " "	" "	28, 29, 30
" " " " "	" "	7, 8, 9
Oamaru, North Otago (Limestone washings)	45° 5'	31, 32, 66
Omakau, Central Otago (sown with seed from (Ellesmere)	45° 5'	10, 11, 12
Australian Commercial Lines.		
O'Connor's strain		13, 14, 15
" "		16, 17, 18
Palestine strain		36, 37, 38
" "		48, 49, 50

As can be seen from the table, three plants from each locality were used, excepting Lake Ellesmere, from which came nine plants and six plants from each of the Australian commercial lines.

Data were required on (1) the degree of self- and cross-fertility to be expected (2) other inherent factors influencing low seed setting and (3) the importance of insects, especially bees, as pollinating agents of this species.

Five cuttings were taken from each of the plants, and planted in boxes on 24 October, 1957. Hereafter the plants will be referred to as clones and each plant of a clone, as a clonal propagule. When the clonal propagules were well rooted, they were transplanted into "six-inch" clay pots (19 November, 1957).

The potted clones were kept in the open until just prior to the time of flowering. At this stage they were transferred to an insect-proof glasshouse. Gamexane bombs were used from time to time to destroy possible insect contaminants.

The pots were spaced well apart in trays, filled to a depth of about 2" with water. Under these conditions from the beginning of January 1958 most of the plants grew vigorously. However, some of the clones showed poor growth and appeared to be inherently non-vigorous. These clones later flowered poorly and many proved to be spontaneously self-fertilizing.

The first florets opened during the first week of January and 23 of the clones had begun to flower by 7 January 1958. The first florets of the last plant to flower opened on 3 February 1958. The majority of clones continued to flower until mid-March. Selfing treatments were completed by the second week of February to avoid possible end-of-season pseudo- self-fertility.

The unit of study was the individual floret. To obtain some idea of possible relative seed-yielding capacity of each clone, counts were made of the number of seeds set in each ovary examined, the number of florets per raceme (at least ten heads were counted), and the total number of flower heads produced by the five clonal propagules of each clone.

As each flowerhead was formed and the lower florets were about to open, the head was allotted to one of the treatments described below. Each treatment was distinguished from another by differently coloured pieces of wool tied around the stems. Two pots containing clonal propagules of each clone were kept in the glasshouse and the flowerheads produced on these were used in the hand-crossing and selfing treatments. The remaining three pots of clonal propagules were used in the treatments involving bees.

A. Selfing and Crossing Treatments.

The sixty-six clones were to be self-pollinated, and cross-pollinated with pollen from two other clones in the group. Pollination was accomplished, 1) artificially by hand and, 2) by bumble bees, Bombus terrestris workers, in cages. Some of the clones, however could not be subjected to both selfing and crossing treatments because of indifferent flowering and/or self-fertilization early in floral development.

The weak plants and those which were obviously not going to produce many flowerheads were placed in one group to be selfed and crossed. There were thirty-four of these. The remaining thirty-two were studied in greater detail in the second group.

The reasons for using Bombus terrestris worker bees in this experiment were. 1) They are efficient pollinators of white clover (Hadfield and Calder 1934). 2) They are numerous and easy to catch and handle. 3) They will work and live a relatively long period in captivity.

The techniques used in selfing and crossing were adaptations of those used by Williams (1931) with red clover, white clover and lucerne.

The thirty-two clones studied in detail were subjected to the following treatments.

1. Selfing without being tripped.

These heads were left entirely alone, any seed set, being the result of self-pollination and fertilization or else apomixis.

2. Selfing with tripping.

(a) Rolling the head between the fingers and thumb. This was done on alternate days until the petals on the last florets to open had begun to wither.

After the heads of each plant had been treated in this way the fingers and thumb were dipped into 95% alcohol to destroy adhering pollen and thus avoid contamination (Silow, April 1931, p.234).

(b) Selfing by tripping, using a pointed plastic rod.

As each floret was tripped, it was marked on the standard with a small spot of "indian-ink", this clearly showed which of the florets had been treated (see photographs 2, 4, 6). After the heads of any clone had been so treated, the rod was dipped in alcohol. Any florets thought to be past the receptive stage were carefully removed with forceps.

(c) Selfing with bees.

The bees used in the experiment were caught in the field in test-tubes and washed free of pollen with cotton-wool and water.

One clonal propagule of each of the thirty-two clones was placed out-of-doors, inside a fine-mesh, wire cage 24" by 18" by 27" high, with a glass top. Two bees were kept in the cages at all times during the flowering period. As the bees died they were replaced by others. Although the bees were fed on a syrup of sugar and water, each bee had to be replaced approximately every three days. Some survived a week or more.

Before being placed within the cages any unreceptive florets were removed.

Trays of water in the bottom of the cages ensured adequate watering.

3. Crossing (Chain System).

Each of the thirty-two clones was crossed separately with two others in the group. There was no conscious selection of which clones were to be crossed. The main problem being to obtain two clonal propagules for each cross to be made, with approximately the same number of heads about to mature on each.

(a) With Bees.

The procedure was the same as that described for selfing except that two pots, each containing one plant of the cross, were placed inside the cage. Again two B. terrestris workers were used.

(b) By Hand.

Attempts to emasculate the florets before crossing, were unsuccessful, mainly because pollen was usually shed at a very early stage of floral development. Efforts to remove the petals of the buds damaged the florets too much. Kirk's suction pump method could not be used successfully.

The technique for hand crossing was as follows. A pointed plastic rod was inserted between the standards and keels of three florets on the head of one of the plants, after which pollen could usually be seen on the rod. The rod was then inserted into a similar number of florets on the second plant. The rod was then returned to the florets of the first plant. A mixture of pollen from the two plants was thus deposited on the stigmas of each plant of the cross. The amount of self-pollination to be expected could be judged from the results of the selfing treatments and the efficiency of the method could be found by comparison of seed-setting after the same cross had been made with bees.

As each group of three florets had been pollinated a small spot of "indian ink" was deposited on the standards (See photographs 2,4). This indicated clearly which florets had been pollinated. These dots were made by pressing against the standard, the tip of a capillary tube fitted to an eye-dropper and filled with ink. The marks could be seen clearly on the withered standard, months after seed had been set.

To test the technique, one half of the florets on some heads were pollinated and the other half not treated.

Only receptive florets were treated, and as each head in the cross was completed, a label stating the date of crossing, and the number of the pollen parent, was tied around the stem.

The 34 clones not subjected to the treatments described above, were, where possible, (a) self-pollinated by rolling (b) crossed with two other plants with bees (c) left untreated to determine spontaneous self-fertilization. The method of crossing these clones was to place all three clonal propagules in the cage with two bees. i.e. a polycross.

<u>Seed Counting.</u> After all the treatments had been completed,

and the seed-heads had ripened, the heads were harvested and placed in labelled packets for examination later.

Each pod on each head was examined separately and the number of viable seeds, abnormal seeds and two-seeded pods were recorded.

B. Ancillary Studies.

1. Pollen Viability.

Pollen grains of *T. fragiferum* were found to germinate and grow readily in 15% sucrose solution at 25°C. Small and shrunken grains did not germinate.

Pollen viability counts were made as follows. Three receptive florets were taken at random from two flowerheads. All the anthers were gently pressed into a drop of dilute alcohol on a microscope slide. The anthers were agitated to release the grains which were then spread over the slide. The slide was warmed, evaporating the alcohol and a drop of molten gelatine containing basic fuschin was spread over the grains. A cover slip was then gently applied (this technique is used at Grasslands Division, Department of Scientific and Industrial Research, Palmerston North).

Only the large rounded grains were counted as viable (See photographs 7 and 8).

2. Pollen Tube Growth.

An attempt was made to determine the region of inhibition or retardation of pollen tubes after incompatible pollination, (selfing). The place of inhibition has been described in other species of *Trifolium* by Silow (1931 p.228) Atwood (1941) and Pande (1954-55).

The techniques adopted were those described in The Microtomist's Vademecum and by Silow (1931), Darlington and La Cour, Esser (1955) and Dionne and Spicer (1958).

Dionne and Spicer reported that of a number of standard techniques tried, none proved satisfactory for their material.

The writer used various fixitives, hydrolysing agents and stains, without successfully tracing pollen-tube growth beyond the first ¼ of the style length. Perhaps at this point gross inhibition occurred and only a few tubes grew beyond this, as workers found in related species.

3. Insect trials.

These are discussed in details on page 38.

RESULTS.

Detailed results for the individual clones are set out in the tables below and in the appendices.

There was a wide variation in the relative ability of the clones to set seed. This was first observed in the number of flowers produced (See table II, and histogram). The range of total heads produced from the five clonal propagules of a clone was 0 - 382.

As a general observation the more vigorously growing clones produced more heads than the weaker ones.

The histogram below illustrates the fact that the majority of clones used in the experiment tended to produce a small number of flowerheads. (30 of the clones having less than 50 heads (or ten heads per plant)).

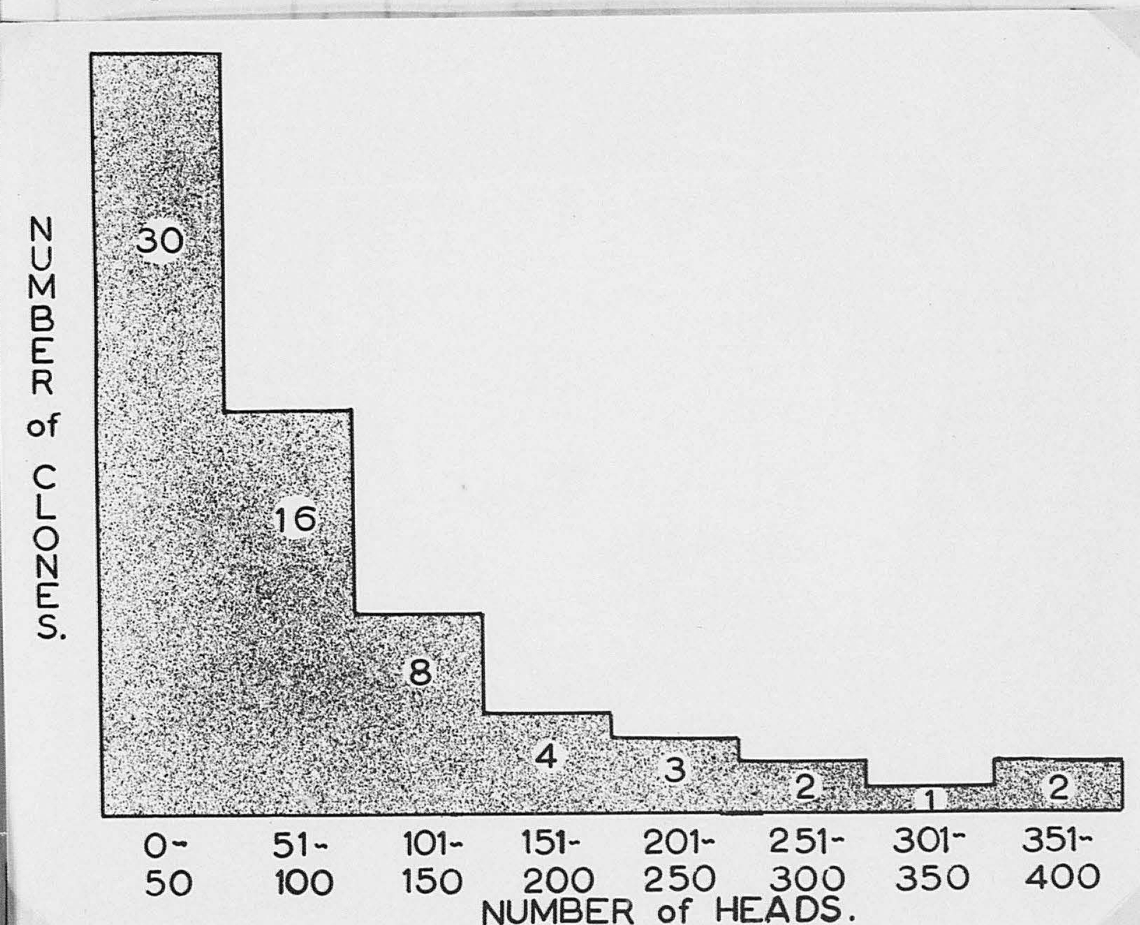


TABLE II.

District or strain	Plant number	Heads Counted	Average number of florets per head and S.E.	Locality Mean Counts and S.E.	Total number of heads produced
Dargaville	45	10	40.6 ± 4.9		45
	46	10	58.3 ± 6.0	47.3 ± 5.4	50
	47	10	44.2 ± 7.8		57
Aoroa	63	10	54.0 ± 7.8		79
	64	10	52.0 ± 8.1	55.1 ± 2.2	114
	65	10	59.3 ± 11.2		91
Kopurahi	57	7	59.6 ± 12.3		46
	58	10	57.8 ± 10.7	60.3 ± 2.0	34
	59	10	63.2 ± 8.3		104
Ngatea	60	10	47.7 ± 6.5		127
	61	10	56.5 ± 7.7	52.9 ± 2.5	198
	62	10	48.1 ± 4.6		160
Raglan	4	10	67.5 ± 11.3		65
	5	10	65.3 ± 7.2	61.5 ± 4.9	34
	6	10	51.8 ± 6.1		89
Wairoa	1	10	56.3 ± 7.5		98
	2	10	55.0 ± 4.9	53.0 ± 2.7	370
	3	10	47.7 ± 5.9		120
Haumoana	54	10	52.4 ± 7.9		82
	55	5	80.4 ± 3.5	66.3 ± 9.8	62
	56	10	80.0 ± 1.4		44
Hastings	39	10	72.6 ± 13.2		175
	40	10	80.3 ± 21.7	69.0 ± 7.7	342
	41	10	54.2 ± 7.8		214
Flock House	22	10	47.8 ± 8.2		80
	23	10	65.5 ± 12.0	52.6 ± 7.6	68
	24	6	39.2 ± 5.9		19
Himatangi	33	10	46.1 ± 7.5		66
	34	10	65.1 ± 8.3	53.9 ± 5.7	112
	35	10	50.6 ± 4.7		382
Nelson	19	10	59.5 ± 11.2		78
	20	6	46.0 ± 6.1	52.6 ± 4.1	15
	21	10	49.3 ± 5.9		18
Blenheim	51	10	45.3 ± 10.7		84
	52	10	64.6 ± 6.4	55.4 ± 5.6	26
	53	10	56.2 ± 10.7		205
Kaiapoi	42	5	54.0 ± 3.7		23
	43	6	54.7 ± 2.2	52.4 ± 1.6	11
	44	8	49.6 ± 5.9		36
Lake Ellesmere	25	6	47.5 ± 3.6		20
	26	10	47.5 ± 4.5	49.9 ± 2.2	39
	27	10	53.8 ± 7.1		248
" "	28	10	44.1 ± 6.5		88
	29	10	46.2 ± 7.4	45.8 ± 1.1	53
	30	5	48.4 ± 6.6		13
" "	7	Non-flowering			0
	8	5	47.2 ± 6.2	47.9 ± 2.4	38
	9	5	48.6 ± 9.0		11

TABLE II (contd.)

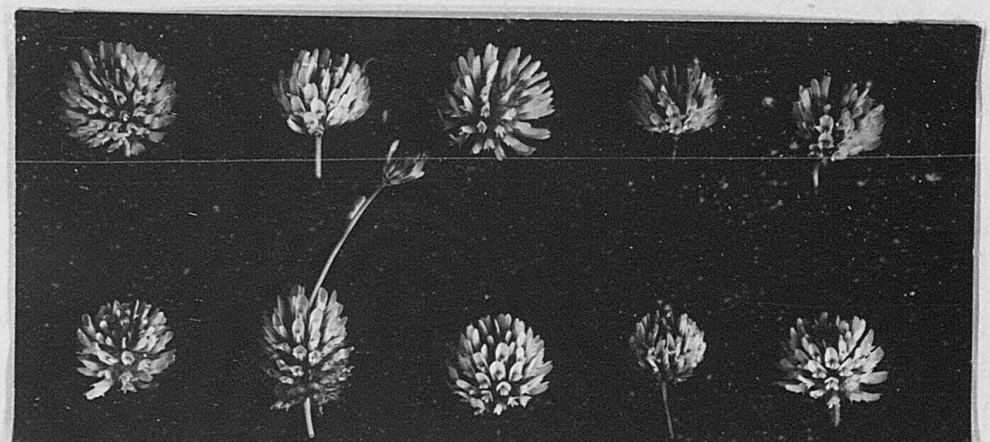
District or strain	Plant number	Heads Counted	Average number of florets per head and S.E.	Locality Mean Counts and S.E.	Total number of heads produced
Nth Otago	31	10	50.6 ± 6.1		268
	32	6	55.5 ± 5.8	54.9 ± 3.0	36
	66	7	60.4 ± 8.8		38
Omakau	10	5	63.6 ± 3.8		12
	11	5	55.6 ± 9.3	67.0 ± 5.6	12
	12	10	74.4 ± 10.7		41
O'Connors	13	2	63.5 -		2
	14	10	44.4 ± 6.6	46.0 ± 2.5	267
	15	10	46.5 ± 5.4		40
	16	10	47.3 ± 2.6		52
	17	10	38.1 ± 5.2		132
	18	7	51.9 ± 5.7		34
Palestine	36	4	43.8 ± 4.8		4
	37	6	47.0 ± 4.7		28
	38	10	56.4 ± 6.0	60.5 ± 6.7	142
	48	10	48.7 - 9.1		105
	49	10	66.7 = 9.3		39
	50	10	85.0 = 5.4		190

There was variation in the number of florets formed per head, both within any one plant and between clones of a given locality (See table II and photograph 3). In the majority of cases there were significant differences in floret number per head between the clones representing any one locality. Counting 4 or 5 heads would give sufficient accuracy to detect locality differences in floret number, but at least 12 clones would be required from each locality to detect significantly locality differences.

Photograph 3 showing the variation in floret size, form, and number per head, of clones used in the experiment.

Palestine | O'Connor's

No.50 No.48 No.2 No.17 No. 14



No. 39 Twin styles | No. 40 Multiple head | No. 53 | No. 63 | No. 58

The photographs below show the expansion of the calyces after seeds have been set. Clone No. 19 has set seed without being tripped while the other two clones required cross-pollination before an appreciable amount of seed was set.

Photograph 4.

SEEDHEADS of Clone No.50 (self-incompatible).

CROSSED with No.38	ROLLED	UNTRIPPED	HAND-TRIPPED	CROSSED with No.22
72 Florets	90 Florets	97 Florets	75 Florets	104 Florets
74 Seeds	0 Seed	0 Seed	6 Seeds	45 Seeds

Photograph 5.

SEEDHEADS of Clone No. 19 (Spontaneously self-fertile)

UNTRIPPED	UNTRIPPED	CROSSED with No. 39	CROSSED with No. 39
66 Florets	61 Florets	38 Florets	48 Florets
37 Seeds	45 Seeds	46 Seeds	46 Seeds

Photograph 6.

Clone No. 61 (Self Incompatible)

CROSSED with No.53	UNTRIPPED	UNTRIPPED	CROSSED with No.53
46 Frorets	43 Florets	53 Florets	50 Florets
21 Seeds	0 Seed	0 Seed	35 Seeds

SELF-FERTILITY.

It was found soon after the plants had begun to flower and were self-pollinated, that some set seed readily, spontaneously, whereas others would not set seed even after artificial self-pollination.

~~The photographs on page 24 show the expansion of the calyces after seed has been set. Clone No. 19 has set seed, without being tripped. The other two clones require cross-pollination for normal seed setting.~~

The clones could thus be grouped into (1) those which were self-fertile and (2) those which were self-incompatible.

Insufficient data were obtained to determine the degree of self-fertility of 7 of the clones but of the remaining 59, 44 could be considered as self-incompatible and 15 as self-fertile, i.e. approximately one in four.

1. Self-Incompatible Clones.

The following 22 clones set no seed after selfing treatments.

Clones No.

3	15	66	39	48	62
4	23	33	45	49	63
13	25	34	46	57	
14	31	37	47	60	

All of these, with the exception of Clone No. 13, which produced only two heads and was not treated, did set seed after outcrossing and therefore must be considered as self-incompatible.

Some of the clones did, however, set a few seeds after selfing treatments, but were obviously self-incompatible. Williams (1931) found the same phenomenon in red clover where 19 of the plants he studied set from 1 to 7 seeds per 100 florets after selfing. He termed this "pseudo- self-fertility", which is not used in the same sense as originally defined by East and Park (1917). Clones studied here that come within this category were:-

Clones No.

2	17	35	50	54	61
5	28	38	51	56	64
6	29	40	52	58	65
16	32	41	53	59	

The range of seed set by these "pseudo- self-fertile" plants was 0.25 ± 0.25 to 6.0 ± 1.5 seeds per 100 florets pollinated.

The cause of this seed-setting in self-incompatible plants is not clear. Possible reasons may be, a) accidental cross-pollination, b) some abnormality by which incompatibility becomes partially ineffective, c) the most probable cause may be apomoxis, which according to Darlington (1957) is more common than is generally realized, as it is seldom apparent.

2. <u>Self-Fertile Clones</u>.

The following clones could be considered as highly self-fertile:-

<u>Clones No.</u>

1	10	18	21	27
8	11	19	22	42
9	12	20	26	44

All of these with the exception of Nos. 1, 12, 18, 19, 22 and 27, were spontaneously self-fertilized in the bud stage, as judged by the withering petals and expansion of the calyces.

Of the spontaneously self-fertilizing clones which were artificially tripped i.e. Nos. 12, 18, 19, 20, 26, 27, there was an increase in seed-setting after tripping in No. 12 (significant at the 5% level of p) and Nos. 18, 19 and 27 (significant at the 0.1% level). In Clones No. 20 and 26 there was no significant difference between spontaneous self-pollination and artificial self-pollination.

It would appear, that in some spontaneously self-fertilizing clones tripping increased seed setting.

Clone No. 1 set only 1.3 ± 0.65 seeds per 100 florets, spontaneously, but set 50.4 ± 3.0 when artificially tripped (difference highly significant). This clone is apparently self-compatible but is unable to set appreciable amounts of seed unless the florets are tripped. This also applies to Clone No. 18 where 4.2 ± 1.24 seeds per 100 florets counted were set spontaneously, yet 28.0 ± 4.5 were set after being tripped (* *). These two clones apparently needed to be tripped to deposit pollen on the stigma or perhaps rupture a stigmatic membrane before self-fertilization could take place effectively.

Clone No. 22 set 11.9 ± 2.1% seed spontaneously and 7.8 ± 1.7% after rolling (difference N.S.) and no seed at all after selfing with bees. Pollen counts of this clone revealed that very little viable pollen was produced. A few viable grains found on the slides must have represented sufficient pollen to affect the self-fertilization found. If this clone had produced more viable pollen it might have proved to be highly self-fertile.
Engelbert observed that bees tended to avoid plants with sterile anthers, which could therefore not reproduce unless cross-pollinated. This may in part explain why no seed was set in this clone after selfing with bees.

3. <u>Comparison of Selfing Methods</u>.

As most of the clones proved to be self-incompatible and many of the self-fertile ones set seed spontaneously, the data on the different selfing techniques are not very complete. The results are summarized in the table below for the four self-compatible clones from which data were obtained.

Table III. Comparison of Selfing Treatments

Clone No. 1

Treatment	Florets	Seeds	No. seed/100 flts.	Difference	
Rolled	258	130	50.4	N.S:	**
Hand tripped	32	20	62.5		
Bees	308	111	36.0		

Clone No. 12

Treatment	Florets	Seeds	No. seed/100 flts.	Difference
Rolled	294	203	69.1	N.S:
Hand tripped	228	163	71.5	
Bees	No data			

Clone No. 19

Treatment	Florets	Seeds	No. seed/100 flts.	Difference
Rolled	366	220	60.1	N.S:
Hand tripped	No data			
Bees	176	102	58.0	

Clone No. 27

Treatment	Florets	Seeds	No. seed/100 flts.	Difference	
Rolled	263	126	47.9	N.S:	**
Hand tripped	250	126	50.4		
Bees	258	64	24.8		

The two methods of artificial tripping, rolling with the fingers and tripping with a rod, gave similar results. Seed-setting with bees as the pollinating agent, however, gave a significantly lower result than artificial tripping in two cases out of three. This may have been due to differences in the environmental conditions existing between the glasshouse and the bee-cages which were out-of-doors. During periods of wet weather the bees refused to work but florets continued to open and die off, unpollinated. Wet conditions might also be expected to affect pollen viability. Moreover, single plants in the cages may have been unattractive to the bees.

There is apparently no relationship between any of the selfing methods and the occurrence of pseudo- self-compatibility.

Summary of Results of Self-Pollination.

It may be concluded, that of the clones used here, under the conditions described, some proved to be highly self-fertile, of these most had set seed before the florets had opened. Some of the self-compatible plants set more seed after being tripped than they did after spontaneous self-pollination.

Of the self-incompatible plants, which were in the majority, some set no seed after self-pollination while others showed varying degrees of "pseudo- self-fertility". The explanation of this is not clear.

4. Results of Seed-setting after Cross-Pollination.

Although approximately three out of every four of the clones proved to be self-incompatible, in the crossing experiments where each of the thirty-two clones was out-crossed to two others in the group, there was no evidence of any two plants being cross-incompatible. It may be assumed from this that the number of alleles conditioning incompatibility in this species may be very large. This agrees with the findings of workers with related species (Williams 1931, Sears 1937).

Detection of two plants with one incompatibility allele in common would not be possible from the results obtained here, as 50% compatible pollen in the volume of pollen usually applied to the stigma would be expected to give normal seed-setting. If a larger number of crosses had been made or related plants had been crossed, cross-incompatibility would undoubtedly have been found.

Of the fifty clones which were both artificially self-pollinated and cross-pollinated, in every case there was an increase in seed number set per hundred florets treated, after cross-pollination. In most cases the difference was highly significant. This was to be expected as 44 of these clones were self-incompatible, but even the highly autogamous plants so treated gave an increased seed-set after cross-pollination.

Table IV summarizes the reciprocal of the out-crosses done

TABLE IV.

SEED-SET, per 100 FLORETS, AFTER CROSSING BY TWO METHODS.

(Pollen parent shown in brackets above figures).

Female Parent Number.	With Bees.		By Hand.	
	Cross 'A'.	Cross 'B'.	Cross 'A'.	Cross 'B'.
1	(38) 52.4 ± 3.3	(4) 54.2 ± 3.3	(38) 84.6 ± 2.4	(4) 87.5 ± 2.6
2	(17) 52.9 ± 3.2	(62) 47.8 ± 3.0	(17) 52.8 ± 3.6	(62) 50.6 ± 2.7
3	(12) 20.0 ± 2.6	(23) 37.6 ± 3.1	(12) 36.4 ± 4.2	(23) 34.3 ± 3.1
4	(1) 19.7 ± 2.0	(58) 13.9 ± 2.1	(1) 12.0 ± 2.3	(58) 11.8 ± 1.9
6	(19) 29.7 ± 2.6	(14) 43.1 ± 2.5	(19) 37.6 ± 3.4	(14) 43.7 ± 2.5
12	(3) 52.8 ± 2.6	(63) 87.8 ± 1.7	(3) 74.5 ± 3.2	(63) 77.3 ± 2.4
14	(6) 67.9 ± 3.1	(50) 75.7 ± 2.8	(6) 74.3 ± 3.2	(50) 75.7 ± 3.2
17	(2) 16.5 ± 2.7	(40) 34.2 ± 3.5	(2) 35.4 ± 3.9	(40) 51.3 ± 3.2
19	(39) 88.0 ± 1.9	(6) 87.7 ± 1.9	(39) 75.5 ± 2.7	(6) 80.1 ± 2.7
22	(34) 58.9 ± 3.1	(61) 41.8 ± 3.1	(34) 60.5 ± 2.8	(61) 52.8 ± 2.7
23	(3) 55.0 ± 2.6	(59) 52.8 ± 2.5	(3) 45.1 ± 2.8	(59) 52.2 ± 2.8
27	(31) 69.1 ± 3.0	(33) 47.8 ± 3.3	(31) 82.2 ± 2.7	(33) 82.5 ± 2.8
28	(64) 38.9 ± 3.3	(29) 21.2 ± 2.8	(64) 55.0 ± 3.6	(29) 22.8 ± 4.7
29	(28) 31.6 ± 3.1	(59) 25.1 ± 3.0	(28) 40.2 ± 5.6	(59) 43.9 ± 5.0
31	(40) 72.7 ± 2.4	(27) 65.2 ± 2.3	(40) 45.5 ± 3.0	(27) 62.7 ± 2.9
33	(27) 51.8 ± 3.1	(41) 52.4 ± 3.5	(27) 47.7 ± 3.8	(41) 52.8 ± 3.5
34	(35) 36.2 ± 2.6	(22) 0	(35) 29.0 ± 2.4	(22) 3.0 ± 0.9
35	(34) 57.6 ± 2.8	(65) 25.2 ± 2.7	(34) 55.8 ± 2.8	(65) 53.8 ± 3.3
38	(50) 54.3 ± 3.1	(1) 57.3 ± 3.0	(50) 52.7 ± 3.2	(1) 67.0 ± 3.4
39	(53) 31.6 ± 2.8	(19) 5.7 ± 1.2	(53) 38.2 ± 3.3	(19) 26.1 ± 2.8

TABLE IV (contd.).

Female Parent Number.	With Bees.		By Hand.	
	Cross 'A'.	Cross 'B'.	Cross 'A'.	Cross 'B'.
40	(31) 54.3 ± 3.2	(17) 48.2 ± 3.2	(31) 53.2 ± 3.1	(17) 71.5 ± 2.3
41	(60) 57.7 ± 3.0	(33) 49.8 ± 3.2	(60) 43.3 ± 3.4	(33) 66.0 ± 3.4
50	(38) 67.6 ± 2.2	(14) 60.4 ± 2.4	(38) 80.3 ± 2.0	(14) 115.8 ± 2.6
53	(61) 30.5 ± 2.4	(39) 22.6 ± 2.3	(61) 35.1 ± 2.6	(39) 17.6 ± 2.4
58	(63) 5.9 ± 1.4	(4) 9.4 ± 1.7	(63) 10.2 ± 1.7	(4) 11.7 ± 2.3
59	(29) 6.6 ± 1.4	(23) 13.6 ± 1.3	(29) 37.9 ± 4.0	(23) 35.8 ± 2.6
60	(41) 38.8 ± 2.9	(64) 33.6 ± 3.1	(41) 48.0 ± 5.0	(64) 36.3 ± 6.5
61	(22) 0.4 ± 0.4	(53) 50.4 ± 3.0	(22) 3.03 ± 1.3	(53) 54.0 ± 3.0
62	(2) 15.7 ± 2.4	(65) 31.3 ± 2.8	(2) 72.2 ± 3.5	(65) 97.9 ± 0.9
63	(58) 39.5 ± 2.5	(12) 33.3 ± 2.3	(58) 24.5 ± 2.6	(12) 22.4 ± 2.4
64	(28) 33.0 ± 2.4	(60) 36.9 ± 3.1	(28) 30.9 ± 4.0	(60) 35.6 ± 3.3
65	(62) 0	(35) 3.4 ± 1.2	(62) 33.3 ± 3.7	(35) 31.8 ± 3.7

by bees and by hand.

It will be seen from the table that on the whole the bumble-bees are efficient pollinators of this species. There was a good correlation between the results of crossing by hand and by the bees. (Cross A, r = + 0.710 * *, Cross B, r = 0.750 * *, see Table IV).

The results of the two modes of crossing cannot be compared on the same basis, because of the different environmental conditions of the glasshouse and the bee-cages. The more equable conditions of the glasshouse were reflected in the general vigour and flowering of the plants growing there. Also in the hand cross-pollination technique unreceptive and unopened florets were often removed from the heads when they were treated, thus allowing more nutrients to be available to the remaining florets. This is known to increase seed-setting per unit number of florets, Atwood (1940). It was not done however, on the heads pollinated by bees.

The bees proved to be the more efficient pollinators in the following cases:- both out-crosses of Clone 19, Clone 31 by 40 and Clone 41 by 60. These exceptions are difficult to explain but in the case of Clone 19, the florets may have been especially attractive to the bees and were consequently well "worked".

Each of the clones in this group of thirty-two has set a definite number of seed per unit number of florets cross-pollinated. In most cases, this ratio of seed set to florets pollinated is similar for both the outcrosses of any clone. The exceptions to this are discussed below.

This inherent ability to set high or low percentages of seed is of fundamental importance in determining total seed yield of a plant.

Ranking the top ten clones for this characteristic with their percentage of seed for the two out-crosses (using the more reliable hand-cross results) the list would be:-

Rank	Clone No.	Cross A	Cross B
1	* 50	80.3 ± 2.0	115.8 ± 2.6
2	1	84,6 ± 2.4	87.5 ± 2.6
3	* 27	82.2 ± 2.7	82.5 ± 2.8
4	* 62	72.2 ± 3.5	97.9 ± 0.9
5	19	75.5 ± 2.7	80.1 ± 2.7
6	12	74.5 ± 3.2	77.3 ± 2.4
7	* 14	74.3 ± 3.2	75.7 ± 3.2
8	* 40	53.2 ± 3.1	71.5 ± 2.3
9	* 31		62.7 ± 2.9
10	38	52.7 ± 3.2	67.0 ± 3.4

* Those marked with an asterick are found in this table and the one below.

If now the clones which produced the most flowerheads are listed on merit, it is possible to decide which are likely to be the best seed yielding clones.

Rank	Clone Number	Total No. of heads	Rank	Clone Number	Total No. of Heads
1	35	382	7	41	214
2	2	370	8	53	205
3	* 40	342	9	61	198
4	* 31	268	11	39	175
5	* 14	267	10	* 50	190
6	* 27	248	12	* 62	160

Those marked with an asterick appear in both lists and are the clones which would probably give the highest total seed yields. The factor of "floret number per head" because of its variability can only be used as a further guide to possible seed-production capacity.

Listed in Table V below are those clones which do show relatively higher seed-setting when crossed with one plant than with another. Most of the differences can be explained in terms of low pollen viability of one of the male parents.

TABLE V.

(Pollen parent number shown in brackets).

Clone No.	Cross 'A' (seed set per 100 florets).		Cross 'B' (seed set per 100 florets).		Difference
17	35.8 ± 3.94	(2)	51.3 ± 3.25	(40)	* *
19	75.5 ± 2.7	(39)	80.1 ± 2.66	(6)	N.S.
22	60.5 ± 2.78	(34)	52.8 ± 2.74	(61)	*
23	45.1 ± 2.84	(3)	52.2 ± 2.82	(59)	N.S.
28	55.0 ± 3.62	(64)	22.8 ± 4.72	(29)	* *
31	45.5 ± 3.0	(40)	62.7 ± 2.9	(27)	* *
34	29.0 ± 2.44	(35)	3.0 ± 0.89	(22)	* *
38	52.7 ± 3.24	(50)	67.0 ± 3.43	(1)	* *
39	38.2 ± 3.28	(53)	26.1 ± 2.78	(19)	* *
40	53.2 ± 3.07	(31)	71.5 ± 2.31	(17)	* *
41	43.3 ± 3.42	(60)	66.0 ± 3.40	(33)	* *
50	80.3 ± 2.02	(38)	115.8 ± 2.59	(14)	* *
53	35.1 ± 2.58	(61)	17.6 ± 2.41	(39)	* *
61	3.03 ± 1.33	(22)	54.0 ± 3.0	(53)	* *
62	72.2 ± 3.52	(2)	97.9 ± 0.93	(65)	* *

The clones showing significant differences in Table V and the probable reason for these differences will be discussed briefly.

Clone 17 by 2. Clone 2 had only approximately ⅓ of its pollen viable.

Clone 22 by 61. Clone 61 had only 55% viable pollen.

Clone 28 by 29. Notes taken at the time of crossing recorded this as a weak plant infected with fungal disease. Where the stem was touched by hand the heads withered and died - thus only two heads were available for counting.

Clone 34 by 22. Clone 22 had sterile anthers.

Clone 39. Both pollen parents had low pollen counts and the plant itself had abnormal florets (twin styles)

Clone 40 This clone had abnormal flowerheads (see photograph 3).

Clone 50 This clone had an exceptionally large number of 2-seeded pods. The high seeding ability of this clone is all the more remarkable when the relative low pollen viability of both the pollen parents is considered.

Clone 53 Both the pollen parents in these crosses had low pollen counts, perhaps the volume of pollen applied to the stigma was important here.

Clone 61 by 22.	Clone 22 had sterile anthers.
Clone 62 by 2	Clone 2 had very low pollen viability, yet in this cross there was still a high seed-set. Clone 62 like Clone 50 appears to be a naturally high seed-setter even when pollinated with pollen of low percentage viability. Again the number of two-seeded pods is remarkable.

The differences between the two crosses of Clones No. 31, 38 and 41 cannot be explained by the writer.

5. Two-seeded Pods

In the following clones a relatively large number of two-seeded pods were recorded:- Clones No. 1, 12, 19 and 22 (self-fertile) and 14, 50 and 62 (self-incompatible). This character was entirely absent from many of the clones. It has been associated with plant vigour by workers in other species (Lucerne, Engelbert 1931).

6. Floral abnormalities Two forms of floral abnormality were found in the clones studied.

Clone 39 had two, apparently normal styles; possibly only one of which, however, was functional. This clone was a relatively poor seed-setter which was probably the result of the abnormal styles.

Clones No. 40, 55, 56 and 60 all had abnormal flowerheads, in that the raceme continued to extend and produced many more florets than was normal (see photograph 3 page 23). If the lower florets (about 45) had set seed this did not happen and the upper undeveloped floret buds died off. If, however, the bottom florets were not fertilized, the raceme produced florets until perhaps 100 or more were formed.

Clones, 39, 40, 55, and 56 came from the same region near Hastings.

These abnormalities are probably the result of recessive mutants which have come to expression after inbreeding.

7. Abnormal Seed Small and wizened seeds were included in the results with the normal seed. No attempt was made to determine the reason for their abnormality. Williams believed that they were the result of apomictic development and excluded them from normal seeds in his results. Engelbert, however, considered that they were normal ~~embryos~~ zygotes that had aborted through lack of adequate

nutrient supply. Cooper and Brink showed that there was a strong tendency for inbred embryos and endosperms to die at an early stage of seed development resulting in the formation of these small seeds.

In the plants studied here, the formation of these seeds appeared to be at random, with the exception of Clone No. 1, which was self-fertile and produced many of these aborted seeds both after selfing and cross-pollination. In the reciprocal crosses of this clone these aborted seeds did not occur, so that the phenomenon was a characteristic of the clone itself and not the result of chromosomal abnormality.

8. Pollen Viability The percentages of normal pollen for each clone are given in ~~the~~ appendix, XVIII. Omitting Clones No. 3, 7, 22 and 42, there is a good correlation between any two clonal propagules ~~plants~~ within a clone for percentage pollen viability (r = + 0.908 * *).

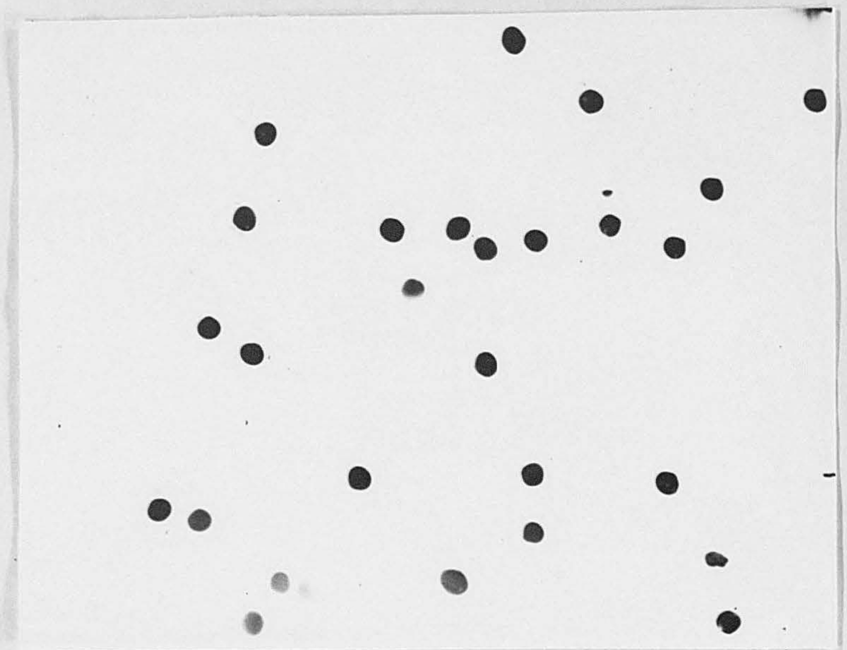
There is however, a wide variation between the clones in the proportion of viable pollen. In selecting plants for future breeding work, therefore, these pollen counts must be taken into consideration, especially where both the total volume and proportion of viable pollen are low.

No estimate of the relative volumes of pollen produced by the clones, was made, but variation in this respect was observed among the clones at the time the pollen viability estimates were made.

The photographs below (7 and 8) illustrate the contrast in numbers of viable pollen in the microscope field, of a clone producing a large proportion of viable pollen (Clone 50, 97% viable) and a clone with poor pollen viability Clone 2 (32% viable).

Photograph 7. Pollen grains of Clone No. 50.

(Note non-viable grain, bottom right)



Photograph 8. Pollen grains of Clone No. 2.

(Only one grain is viable - centre)

TRIALS TO INVESTIGATE POSSIBLE INSECT POLLINATORS IN THE FIELD.

A plant of clone No. 50 which was self-incompatible, had attractive, scented flowerheads and a high pollen count was kept in the glasshouse until about 8 heads were fully open. This plant was then taken to a field at Palmerston North where strawberry clover was in flower. The pot containing the clone was concealed so that the plant and its flowers resembled those surrounding it. The heads were then watched for insect visitations. The heads were differentiated by inconspicuous pieces of coloured wool tied around the stalks. Two heads acted as controls, these were isolated from insects by pieces of cheese cloth, approx. 2" x 2", carefully placed over the heads and tied around the stem. One of the heads was later artificially tripped by rolling, the other left untreated.

This was done on 27th February 1958, and the plant was watched for 6 hours, 9 a.m. to 12 a.m. and 1 p.m. to 4 p.m.. During the period between 12 a.m. and 1 p.m. the plant was isolated from insects. Insects visiting the strawberry clover heads in the stand, were caught, taken to the laboratory, killed, and parts of their body washed with dilute alcohol, the wash being made into a slide stained with basic fuschin as described under "Pollen Counts".

Labels for identification were tied to the heads which were later threshed and the seeds counted.

The weather at the time of this trial was fine, warm but windy and cloudy. The plants in the association were docks, dandelion, giant buttercup, ~~and~~ floating sweet grass (*Glyceria fluitans*) and strawberry clover.

Observations: Honeybees (*Apis*) were observed working the surrounding strawberry clover plants. The first bees to arrive at the flowers on Clone 50 stayed a relatively long period working many florets on each head. The duration of stay became less, the later the bees arrived, as they seemed to sense the florets had already been "worked". Eventually late in the morning bees approached the flowers but did not settle. In the afternoon a few honeybees visited the flowers but stayed only a matter of seconds.

During the afternoon there was a large number of "Drone" or "Drain" fly visits (Eristalis tenax). These insects stayed up to 7 minutes on each head, and as shown later were eating the pollen (See photograph 9).

Between 3 p.m. and 4 p.m. when the weather became dull there were no insect visitations.

Results

Results of seed numbers set are shown below, (Table VI). Apparently either the honey bees or the drone flies or both had been instrumental in cross-pollinating this clone.

Table VI.

Flower Numbers.	No. 1 (Red)	No. 2 (Blue)	No. 3 (Yellow)	No. 4 (Black)	No. 5 (White)	No. 6 (Grey)	Controls No. 7 (Rolled)	No. 8 Isolated and untripped
9 a.m.-12 a.m. No. of honeybee visits	5	7	7	6	4	4	-	-
No. of dronefly visits	1	0	2	1	1	1	-	-
1 p.m.- 4 p.m. No. of honeybee visits.	2	-	1	-	-	3	-	-
Total duration	9 secs	-	53 secs	-	-	42 secs	-	-
No. of dronefly visits	6	1	5	3	4	5	-	-
Total Duration	17 min 33 sec	3 min	3 min 2 sec	3 min 4 sec	2 min 58 sec	1 min 17 sec	-	-
No. of bumble bee visits	-	-	-	-	-	1		
(Duration)	-	-	-	-	-	2 sec		
No. of florets exposed	94	91	81	89	92	74	100	75
No. of seed set	38	9	12	27	19	20	0	0
Seed set per 100 florets exposed	40.4	9.9	14.8	30.3	26.6	27.0	0	0

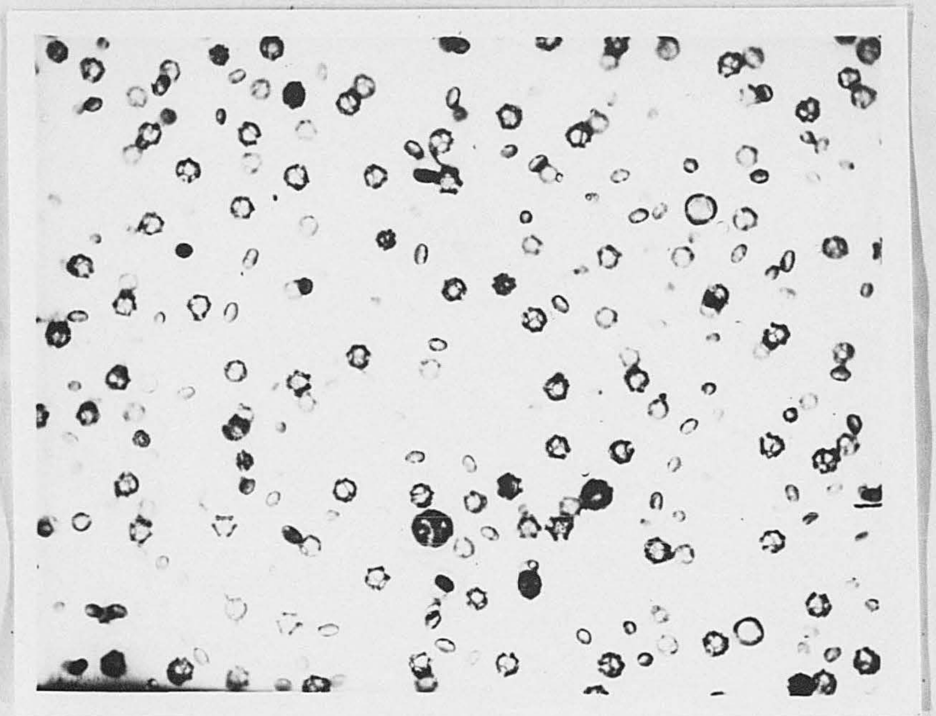
Analysis of the florets on the heads, revealed that most of the seed was set in groups of florets about 2/3 of the way up the raceme.

Slides were made of washes of the heads and probosces of "drone" flies and honey bees and also the anterior part of the gut of a "drone" fly. The pollen on the slides was identified by L.H. McDowell, Biologist, Department of Agriculture. The results were as follows:

"Pollen of T. fragiferum was found to be present on all slides. However, those from the 'fly body' and 'fly tongue' showed very small quantities. That from the body was almost entirely Umbelliferae, with some dandelion, grasses and thistle, and about ten clover grains; the slide from "fly tongue" had very little pollen on it, about four clover grains. The gut preparation was very interesting. Umbellifer and dandelion pollen were present in approximately equal quantities and appeared unchanged by any digestive process. The relatively small number of clover grains were distorted and swollen and in most cases the exine ruptured.

The slides from bees showed larger numbers of T. fragiferum grains. It would seem that the bee is a more efficient pollinator than the fly even though the latter may remain on the flowers longer. From the gut content the fly obviously collected more pollen from flowers in which the anthers were exposed."

Photograph 9. Showing the pollen grains taken from the foregut of Eristalis tenax. The small oval grains are those of Trifolium spp. which have been ruptured by digestive processes.



Cage trials. Two specimens of each of the following species were confined in cages with two unrelated self-incompatible plants of strawberry clover, (a) Eristalis tenax, (b) Apis mellifera workers, (c) Bombus terrestris workers, (as controls).

The clones used were Nos. 50 and 34 and the trial period was 6 days (17.1.59 - 23.1.59).

As the insects died they were replaced by others, five flies, six bumble bees and 12 honey bees were used.

The weather was fine during the trial. The flower heads exposed to the insects were labelled and later the seeds harvested and counted.

The honeybees quickly died and were not observed to work the heads during the trial. In their efforts to escape from the cage, they ignored the flowerheads and soon became exhausted. Consequently no seed was set on any of the heads exposed to these bees. However, they have been observed many times working this clover in the field and under natural conditions they may be efficient pollinators of the species.

The table below gives the results of seed-setting with the drone flies and bumble bees. This shows beyond doubt that the pollen-eating E. tenax is an efficient cross-pollinator of strawberry clover. This insect lays its eggs in wet situations, which are frequently the natural habitats of T. fragiferum. The rat-tailed maggot of E. tenax may often be seen in cow-shed drains in this country.

Table VII.

Drone Flies (Eristalis tenax).

Clone 50.			Clone 34.		
Head No.	Florets	Seeds	Head No.	Florets	Seeds
1	69	48	1	70	36
2	88	42	2	77	39
3	96	37			
4	70	43			
	323	170		147	75
Seed per 100 florets 52.6			Seed per 100 florets 51.0		

B. terrestris (Workers).

Clone 50.			Clone 34.		
Head No.	Florets	Seeds	Head No.	Florets	Seeds
1	84	57	1	55	20
2	96	65	2	84	48
3	78	71	3	73	46
	258	193		270	148
Seed per 100 florets 74.8			Seed per 100 florets 54.8		

DISCUSSION AND CONCLUSIONS.

The evidence obtained in this work strongly supports the hypothesis that strawberry clover is a cross-pollinating species. Forty-four clones out of fifty-nine were found to be self-incompatible, the remaining fifteen being self-fertile. Furthermore a higher percentage of seed was usually set after cross-pollination of the self-fertile plants than after self-pollination.

Apparently this clover follows a pattern similar to that described for other widely distributed perennial pasture legumes, in that local populations may contain varying proportions of autogamous individuals. For this reason a representative sample of the species would be difficult to obtain.

The spontaneously self-fertile plants almost invariably were non-vigorous and had low fertility. These had, it seems suffered from inbreeding depression for a number of generations. Therefore, further inbreeding as a method of improvement would not be expected to be of any advantage, except where it was desired to make the plants homozygous for certain simply inherited characters. Kirk (1933) with lucerne and Williams (1931) with red clover both used the selfed-line method and discarded it as unsatisfactory.

Probably the best approach to improving this species agronomically, is to combine the best available plants into a synthetic strain, after progeny testing for general combining ability and heterosis.

The variability found in the material used here for factors associated with seed production, indicates that improvement of seed-yielding ability could readily be made by selection.

Before a breeding programme is commenced, however, it is felt that much more material should be obtained from as many overseas sources as possible, especially from the Mediterranean centre of gene diversity. This was the origin of the highly successful, winter-growing, Palestine strain (Tiver 1954).

Some of the plants used here showed some promise and should be studied further, but many could possibly be discarded without further consideration.

The source nursery and the areas where progeny testing is to be carried out should be in localities typical of the country in which the improved strain would be used.

There is the possibility that the difficult areas where the species is normally used in pasture may not be suitable for high seed production and/or harvest. The waterlogged and sandy soils where the species is grown are usually of poor fertility and may not be expected to give maximum seed yield. Selection for agronomic type may have to be made in one locality and seed increase in another.

It has been shown conclusively that bumble bees and other insects do cross-pollinate this species. As the majority of the plants were found to be self-incompatible, the presence of adequate insect numbers at peak flowering period becomes important. This may have been one of the main factors determining low seed yields of this species in the past. Lack of sufficient numbers of pollinating insects in some of the regions where the clover has been grown, may have lead to poor seed-setting and over a long period increased self-fertilization and inbreeding.

Large increases in seed yield have been obtained overseas, from placing honey bee hives in the field where legume seed crops are flowering (F.A.O. Report 1953). This technique might also prove to be efficient in increasing seed-setting in strawberry clover.

For future breeding work, there is the possibility of forming an artificial tetraploid strain. The diploid chromosome number is 16, whereas the more vigorous natural tetraploid, white clover has 32. There is also the possibility with improved techniques of making wide outcrosses with related species.

There is little doubt that this species can be improved by selection and breeding and will play an important role in increasing production in coastal areas, swamp-land and irrigated pastures in New Zealand.

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APPENDICES.

APPENDIX I.

CLONE NO. 1 (Total flowerheads from 5 clonal propagules = 98).

TREATMENTS.

	Left Alone	SELFED Rolled	Hand Tripped	With bees	CROSSED BY BEES WITH- Clone No.38	(Reciprocal of cross)	Clone No.4	(Reciprocal of cross)	CROSSED BY HAND WITH - Clone No.38	(Reciprocal of cross)	Clone No.4	(Reciprocal of cross)
No. of heads examined.	5	5	1	7	5	5	5	7	7	5	4	6
No. of florets " .	305	258	32	308	231	274	225	380	227	188	161	175
Total seed set (normal & abnormal).	4	130	20	111	121	157	122	75	192	126	141	21
No. of normal seed.	2	125	18	67	96	157	72	75	172	126	129	21
No. of abnormal seed.	2	5	2	44	25	-	50	-	20	-	12	-
No. of 2 seeded pods.	0	2	0	5	1	2	0	1	20	16	14	0
No. of all seed set per 100 florets.	1.3	50.4	62.5	36.0	52.4	57.3	54.2	19.7	84.6	67.0	87.5	12.0
Standard error.	±0.7	±3.0	±8.6	±2.7	±3.3	±3.0	±3.3	±2.0	±2.4	±3.4	±2.6	±2.3

CLONE NO. 2 (Total flowerheads from 5 clonal propagules = 370).

TREATMENTS.

	Left Alone	SELFED Rolled	Hand Tripped	With bees	CROSSED BY BEES WITH- Clone No.17	(Reciprocal of cross)	Clone No.62	(Reciprocal of cross)	CROSSED BY HAND WITH - Clone No.17	(Reciprocal of cross)	Clone No.62	(Reciprocal of cross)
No. of heads examined.	5	5	2	5	5	6	5	5	5	5	8	6
No. of florets " .	278	272	118	289	242	194	272	230	191	148	352	162
Total seed set (normal & abnormal).	0	0	3	0	128	32	130	36	102	53	178	117
No. of normal seed.	-	-	3	-	128	32	129	36	101	53	178	117
No. of abnormal seed.	-	-	-	-	-	-	1	-	1	-	-	-
No. of 2 seeded pods.	-	-	0	-	0	0	1	2	1	0	4	3
No. of all seed set per 100 florets.	-	-	2.5	-	52.9	16.5	47.8	15.7	52.8	35.8	50.6	72.2
Standard error.	-	-	±1.4	-	±3.2	±2.7	±3.0	±2.4	±3.6	±3.9	±2.7	±3.5

APPENDIX II

CLONE NO.3 (Total flowerheads from 5 clonal propagules = 120)

TREATMENTS

	Left Alone	SELFED			CROSSED BY BEES WITH				CROSSED BY HAND WITH			
		Rolled	Hand tripped	With bees	Clone No.12	(Reciprocal of cross)	Clone No.23	(Reciprocal of cross)	Clone No.12	(Reciprocal of cross)	Clone No.23	(Reciprocal of cross)
No. of heads examined	5	5	2	5	5	5	5	5	4	4	6	6
No. of florets "	243	234	81	224	245	358	250	353	129	181	230	308
Total seed set (Normal & abnormal)	0	0	0	0	49	189	94	194	47	135	79	139
No. of normal seed	-	-	-	-	49	183	94	193	47	135	79	139
No. of abnormal seed	-	-	-	-	-	6	-	1	-	-	-	-
No. of 2 seeded pods	-	-	-	-	0	3	0	6	1	15	1	1
No. of all seed per 100 florets	-	-	-	-	20.0	52.8	37.6	55.0	36.4	74.5	34.3	45.1
Standard error	-	-	-	-	±2.6	±2.6	+-3.1	±2.6	±4.2	±3.2	±3.1	±2.8

CLONE NO.4 (Total flowerheads from 5 clonal propagules = 65)

TREATMENTS

	Left Alone	SELFED			CROSSED BY BEES WITH				CROSSED BY HAND WITH			
		Rolled	Hand tripped	With bees	Clone No.1	(Reciprocal of cross)	Clone No.58	(Reciprocal of cross)	Clone No.1	(Reciprocal of cross)	Clone No.58	(Reciprocal of cross)
No. of heads examined	4	4	2	4	7	5	5	5	6	4	8	4
No. of florets examined	273	272	130	269	380	225	280	299	175	161	297	188
Total seed set (Abnormal & Normal)	0	0	0	0	75	122	39	28	21	141	35	22
No. of normal seed	-	-	-	-	75	72	37	28	21	129	33	22
No. of abnormal seed	-	-	-	-	-	50	2	-	-	12	2	-
No. of 2 seeded pods	-	-	-	-	1	0	1	1	0	14	0	3
No. of all seed set per 100 florets	-	-	-	-	19.7	54.2	13.9	9.4	12.0	87.5	11.8	11.7
Standard error	-	-	-	-	±2.0	±3.3	±2.1	±1.7	±2.3	±2.6	±1.0	±2.3

APPENDIX III.

CLONE NO. 6 (Total flowerheads on 5 clonal propagules = 89).

TREATMENTS.

	Left Alone	SELFED.			CROSSED BY BEES WITH-				CROSSED BY HAND WITH-			
		Rolled	Hand tripped	With bees	Clone No.19	(Reciprocal of cross)	Clone No.14	(Reciprocal of cross)	Clone No.19	(Reciprocal of cross)	Clone No.14	(Reciprocal of cross)
No. of heads examined.	5	6	5	5	6	5	7	5	5	5	9	6
No. of florets " .	275	294	259	250	320	302	383	221	197	226	389	183
Total seed set (normal & abnormal).	5	1	0	0	95	265	165	150	74	181	170	136
No. of normal seed.	5	0	-	-	89	246	162	143	73	181	160	123
No. of abnormal seed.	-	1	-	-	6	19	3	7	1	-	10	13
No. of 2 seeded pods.	0	-	-	-	0	24	0	9	3	24	0	12
No. of all seed per 100 florets.	1.8	0.34	-	-	29.7	87.7	43.1	67.9	37.6	80.1	43.7	74.3
Standard error.	±0.8	-	-	-	±2.6	±1.9	±2.5	±3.1	±3.4	±2.7	±2.5	±3.2

CLONE NO. 12 (Total flowerheads on 5 clonal propagules = 41).

TREATMENTS.

	Left Alone	SELFED			CROSSED BY BEES WITH-				CROSSED BY HAND WITH-			
		Rolled	Hand tripped	With bees	Clone No.3	(Reciprocal of cross)	Clone No.63	(Reciprocal of cross)	Clone No.3	(Reciprocal of cross)	Clone No.63	(Reciprocal of cross)
No. of heads examined.	5	4	5	-	5	5	5	9	4	4	7	8
No. of florets " .	386	294	228	-	358	245	352	417	181	129	317	290
Total seed set (normal & abnormal).	177	203	163	-	189	49	309	139	135	47	245	65
No. of normal seed .	174	203	163	-	183	49	308	139	135	47	243	65
No. of abnormal seed.	3	-	-	-	6	-	1	-	-	-	2	-
No. of 2-seeded pods.	12	4	0	-	3	0	22	1	15	1	17	0
No. of all seed set per 100 florets.	45.9	69.1	71.5	-	52.8	20.0	87.8	33.3	74.5	36.4	77.3	22.4
Standard error.	±2.5	±2.7	±3.0		±2.6	±2.6	±1.7	±2.3	±3.2	±4.2	±2.4	±2.4

APPENDIX IV.

CLONE NO. 14 (Total flowerheads on 5 clonal propagules = 267).

TREATMENTS.

	Left Alone	SELFED			CROSSED BY BEES WITH -				CROSSED BY HAND WITH -			
		Rolled	Hand Tripped	With bees	Clone No.6	(Reciprocal of cross)	Clone No.50	(Reciprocal of cross)	Clone No.6	(Reciprocal of cross)	Clone No.50	(Reciprocal of cross)
No. of heads examined.	5	5	4	5	5	7	5	5	6	9	5	7
No. of florets examined.	222	222	164	221	221	383	235	393	183	389	177	273
Total seed set (Normal & abnormal).	0	0	0	0	150	165	178	269	136	170	134	316
No. of normal seed.	-	-	-	-	143	162	178	265	123	160	133	316
No. of abnormal seed.	-	-	-	-	7	3	-	4	13	10	1	-
No. of 2 seeded pods.	-	-	-	-	9	0	7	18	12	0	9	112
No. of all seed per 100 florets.	-	-	-	-	67.9	43.1	75.7	68.4	74.3	43.7	75.7	115.8
Standard error.	-	-	-	-	±3.1	±2.5	±2.8	±2.4	±3.2	±2.5	±3.2	±2.6

CLONE NO. 17 (Total flowerheads on 5 clonal propagules = 132).

TREATMENTS.

	Left Alone	SELFED			CROSSED BY BEES WITH -				CROSSED BY HAND WITH -			
		Rolled	Hand Tripped	With bees	Clone No.2	(Reciprocal of cross)	Clone No.40	(Reciprocal of cross)	Clone No.2	(Reciprocal of cross)	Clone No.40	(Reciprocal of cross)
No. of heads examined.	5	4	5	5	6	5	5	6	5	5	8	10
No. of florets " .	192	146	173	210	194	242	184	243	148	191	236	382
Total seed set (normal & abnormal).	4	1	0	3	32	128	63	117	53	102	121	273
No. of normal seeds.	4	1	-	3	32	128	63	117	53	101	121	272
No. of abnormal seeds.	0	-	-	-	-	-	-	-	-	1	-	1
No. of 2 seeded pods.	0	-	-	0	0	0	0	7	0	1	0	25
No. of all seed set per 100 florets.	2.1	0.7	-	1.4	16.5	52.9	34.2	48.2	35.8	52.8	51.3	71.5
Standard error.	±1.0	±0.7	-	±0.8	±2.7	±3.2	±3.5	±3.2	±3.9	±3.6	±3.2	±2.3

APPENDIX V.

CLONE NO. 19 (Total flowerheads on 5 clonal propagules = 78).

TREATMENTS.

	Left Alone	SELFED: Rolled	SELFED: Hand Tripped	SELFED: With bees	CROSSED BY BEES WITH - Clone No.39	(Reciprocal of cross)	Clone No.6	(Reciprocal of cross)	CROSSED BY HAND WITH - Clone No.39	(Reciprocal of cross)	Clone No.6	(Reciprocal of cross)
No. of heads examined.	5	6	-	4	5	5	5	6	5	6	5	5
No. of florets " .	299	366	-	176	299	370	302	320	253	249	226	197
Total seed set (normal & abnormal).	134	220	-	102	263	21	265	95	191	65	181	74
No. of normal seed.	134	220	-	98	263	21	246	89	188	65	181	73
No. of abnormal ".	-	-	-	4	-	-	19	6	3	-	-	1
No. of 2-seeded pods.	21	13	-	18	23	0	24	0	12	1	24	3
No. of all seed per 100 florets.	44.8	60.1	-	58.0	88.0	5.7	87.7	29.7	75.5	26.1	80.1	37.6
Standard error.	±2.9	±2.6	-	±3.7	±1.9	±1.2	±1.9	±2.6	±2.7	±2.8	±2.7	±3.4

CLONE NO. 22 (Total flowerheads on 5 clonal propagules = 80).

TREATMENTS.

	Left Alone	SELFED: Rolled	SELFED: Hand Tripped	SELFED: With bees	CROSSED BY BEES WITH - Clone No.34	(Reciprocal of cross)	Clone No.61	(Reciprocal of cross)	CROSSED BY HAND WITH - Clone No.34	(Reciprocal of cross)	Clone No.61	(Reciprocal of cross)
No. of heads examined.	5	5	4	5	5	5	5	5	8	7	9	4
No. of florets " .	235	243	189	206	248	334	249	281	309	366	331	165
Total seed set (normal & abnormal).	28	19	1	0	146	0	104	1	187	11	175	5
No. of normal seed.	28	19	1	-	146	-	104	1	183	11	173	5
No. of abnormal seed.	-	-	-	-	-	-	-	-	4	-	2	-
No. of 2-seeded pods.	2	0	-	-	9	-	26	-	19	0	13	0
No. of all seed set per 100 florets.	11.9	7.8	0.5	-	58.9	-	41.8	0.4	60.5	3.0	52.8	3.03
Standard error.	±2.1	±1.7	-	-	±3.1	-	±3.1	±0.4	±2.8	±0.9	±2.7	±1.3

APPENDIX VI.

CLONE NO. 23 (Total flowerheads on 5 clonal propagules = 68).

TREATMENTS.

	Left Alone	SELFED			CROSSED BY BEES WITH -				CROSSED BY HAND WITH -			
		Rolled	Hand Tripped	With bees	Clone No.3	(Reciprocal of cross)	Clone No.59	(Reciprocal of cross)	Clone No.3	(Reciprocal of cross)	Clone No.59	(Reciprocal of cross)
No. of heads examined.	5	5	-	5	5	5	7	1	6	6	7	6
No. of florets " .	344	295	-	311	353	250	390	22	308	230	314	330
Total seed set (normal & abnormal).	0	0	-	0	194	94	206	3	139	79	164	118
No. of normal seed.	-	-	-	-	193	94	205	2	139	79	164	115
No. of abnormal seed.	-	-	-	-	1	-	1	1	-	-	-	3
No. of 2-seeded pods.	-	-	-	-	6	0	13	0	1	1	3	0
No. of all seed per 100 florets.	-	-	-	-	55.0	37.6	52.8	13.6	45.1	34.3	52.2	35.8
Standard error.	-	-	-	-	±2.6	±3.1	±2.5	±7.3	±2.8	±3.1	±2.8	±2.6

CLONE NO. 27 (Total flowerheads on 5 clonal propagules = 248).

TREATMENTS.

	Left Alone	SELFED			CROSSED BY BEES WITH -				CROSSED BY HAND WITH -			
		Rolled	Hand Tripped	With bees	Clone No.31	(Reciprocal of cross)	Clone No.33	(Reciprocal of cross)	Clone No.31	(Reciprocal of cross)	Clone No.33	(Reciprocal of cross)
No. of heads examined.	5	5	5	5	5	8	5	6	5	8	5	5
No. of florets " .	270	263	250	258	236	445	230	253	202	348	189	176
Total seed set (normal & abnormal).	65	126	126	64	163	297	110	133	166	218	156	84
No. of normal seed.	65	122	126	49	156	290	102	131	164	218	153	81
No. of abnormal " .	-	4	-	5	7	7	8	2	2	-	3	3
No. of 2-seeded pods.	6	0	0	0	0	0	0	7	14	1	0	0
No. of all seed set per 100 florets.	24.0	47.9	50.4	24.8	69.1	65.2	47.8	51.8	82.2	62.7	82.5	47.7
Standard error.	±2.6	±3.1	±3.2	±2.7	±3.0	± 2.3	±3.3	±3.1	±2.7	±2.9	±2.8	±3.8

APPENDIX VII.

CLONE NO. 28 (Total flowerheads on 5 clonal propagules = 88).

TREATMENTS.

	Left Alone	SELFED Rolled	Hand tripped	With bees	CROSSED BY BEES WITH – Clone No.64	(Reciprocal of cross)	Clone No.29	(Reciprocal of cross)	CROSSED BY HAND WITH – Clone No.64	(Reciprocal of cross)	Clone No.29	(Reciprocal of cross)
No. of heads examined.	5	5	5	5	5	8	5	5	6	4	2	2
No. of florets " .	232	209	203	195	218	369	205	231	189	136	79	77
Total seed set (normal & abnormal).	0	0	1	0	74	122	44	73	104	42	18	31
No. of normal seed.	-	-	-	-	72	122	44	69	103	42	18	28
No. of abnormal seed.	-	-	1	-	2	-	-	4	1	-	-	3
No. of 2-seeded pods.	-	-	-	-	0	0	0	0	1	0	0	0
No. of all seed per 100 florets.	-	-	0.5	-	38.9	33.0	21.2	31.6	55.0	30.9	22.8	40.2
Standard error.	-	-	±0.5	-	±3.3	±2.4	±2.8	±3.1	±3.6	±4.0	±4.7	±5.6

CLONE NO. 29 (Total flowerheads on 5 clonal propagules = 53)

TREATMENTS

	Left Alone	SELFED Rolled	Hand tripped	With bees	CROSSED BY BEES WITH – Clone No.28	(Reciprocal of cross)	Clone No.59	(Reciprocal of cross)	CROSSED BY HAND WITH – Clone No.28	(Reciprocal of cross)	Clone No.59	(Reciprocal of cross)
No. of heads examined.	5	3	5	5	5	5	6	5	2	2	3	3
No. of florets " ,	247	119	215	240	231	205	286	301	77	79	98	145
Total seed set (normal & abnormal).	1	0	0	0	73	44	72	20	31	18	43	55
No. of normal seed.	1	-	-	-	69	44	72	18	28	18	43	54
No. of abnormal " ,	-	-	-	-	4	-	-	2	3	-	-	1
No. of 2-seeded pods.	-	-	-	-	0	0	0	0	0	0	0	4
No. of all seed set per 100 florets.	0.4	-	-	-	31.6	21.2	25.1	6.6	40.25	22.8	43.9	37.9
Standard error.	±0.4	-	-	-	±3.1	±2.8	±3.0	±1.4	±5.6	±4.7	±5.0	±4.0

APPENDIX VIII

CLONE NO.31 (Total flowerheads on 5 clonal propagules = 268)

TREATMENTS

	Left Alone	SELFINGS Rolled	Hand tripped	With bees	CROSSED BY BEES WITH- Clone No.40	(Reciprocal of cross)	Clone No.27	(Reciprocal of cross)	CROSSED BY HAND WITH- Clone No.40	(Reciprocal of cross)	Clone No.27	(Reciprocal of cross)
No. of heads examined	5	4	1	5	8	5	8	5	6	6	8	5
No. of florets "	266	194	46	235	359	245	445	236	312	265	348	202
Total seed set (normal & abnormal)	0	0	0	0	261	133	297	163	142	144	218	166
No. of normal seed	-	-	-	-	261	133	290	156	138	141	218	164
No. of abnormal seed	-	-	-	-	-	-	7	7	4	3	-	2
No. of 2-seeded pods	-	-	-	-	2	3	0	0	0	14	1	14
No. of all seed per 100 florets	-	-	-	-	72.7	54.3	65.2	69.1	45.5	53.2	62.7	82.2
Standard error	-	-	-	-	±2.4	±3.2	±2.3	±3.0	±3.0	±3.1	±2.9	±2.7

CLONE NO.33 (Total flowerheads on 5 clonal propagules = 66)

TREATMENTS

	Left Alone	SELFINGS Rolled	Hand tripped	With bees	CROSSED BY BEES WITH- Clone No.27	(Reciprocal of cross)	Clone No.41	(Reciprocal of cross)	CROSSED BY HAND WITH- Clone No.27	(Reciprocal of cross)	Clone No.41	(Reciprocal of cross)
No. of heads examined	6	5	5	5	6	5	5	5	5	5	7	5
No. of florets "	355	236	208	215	253	230	206	247	176	189	208	194
Total seed set (normal & abnormal)	0	0	0	0	133	110	108	123	84	156	110	128
No. of normal seed	-	-	-	-	131	102	101	123	81	153	109	128
No. of abnormal "	-	-	-	-	2	8	7	-	3	3	1	-
No. of 2-seeded pods	-	-	-	-	7	0	0	0	0	0	3	14
No. of all seed set per 100 florets	-	-	-	-	51.8	47.8	52.4	49.8	47.7	82.5	52.8	66.0
Standard error	-	-	-	-	±3.1	±3.3	±3.5	±3.2	±3.8	±2.8	±3.5	±3.4

APPENDIX IX.

CLONE NO.34 (Total flowerheads on 5 clonal propagules = 112).

TREATMENTS.

	Left Alone	SELFINGS			CROSSED BY BEES WITH-				CROSSED BY HAND WITH -			
		Rolled	Hand tripped	With bees	Clone No.35	(Reciprocal of cross)	Clone No.22	(Reciprocal of cross)	Clone No.35	(Reciprocal of cross)	Clone No.22	(Reciprocal of cross)
No. of heads examined.	5	2	2	5	5	7	5	5	7	8	7	8
No. of florets " .	358	148	147	292	348	314	334	248	348	326	366	309
Total seed set (normal & abnormal).	0	0	0	0	126	181	0	146	101	182	11	187
No. of normal seed.	-	-	-	-	126	159	-	146	99	176	11	183
No. of abnormal seed.	-	-	-	-	-	22	-	-	2	6	-	4
No. of 2-seeded pods.	-	-	-	-	9	0	-	9	5	0	0	19
No. of all seed per 100 florets.	-	-	-	-	36.2	57.6	-	58.9	29.0	55.8	3.0	60.5
Standard error.	-	-	-	-	±2.6	±2.8	-	±3.1	±2.4	±2.8	±0.9	±2.8

CLONE NO.35 (Total flowerheads on 5 clonal propagules = 382).

TREATMENTS.

	Left Alone	SELFINGS			CROSSED BY BEES WITH-				CROSSED BY HAND WITH -			
		Rolled	Hand tripped	With bees	Clone No.34	(Reciprocal of cross)	Clone No.65	(Reciprocal of cross)	Clone No.34	(Reciprocal of cross)	Clone No.65	(Reciprocal of cross)
No. of heads examined.	7	5	5	5	7	5	6	5	8	7	7	5
No. of florets " .	358	235	241	248	314	348	266	243	326	348	223	157
Total seed set (normal & abnormal).	0	14	11	0	181	126	67	8	182	101	120	50
No. of normal seed.	-	14	11	-	159	126	62	8	176	99	120	50
No. of abnormal seed.	-	-	-	-	22	-	5	-	6	2	-	-
No. of 2-seeded pods.	-	0	0	-	0	9	0	0	0	5	1	0
No. of all seed set per 100 florets.	-	6.0	4.6	-	57.6	36.2	25.2	3.4	55.8	29.0	53.8	31.8
Standard error.	-	±1.6	±1.4	-	±2.8	±2.6	±2.7	±1.2	±2.8	±2.4	±3.3	±3.7

APPENDIX X.

CLONE No. 38 (Total flowerheads on 5 clones = 142).

TREATMENTS.

	Left Alone	SELFINGS			CROSSED BY BEES WITH -				CROSSED BY HAND WITH -			
		Rolled	Hand tripped	With bees	Clone No.50	(Reciprocal of cross)	Clone No.1	(Reciprocal of cross)	Clone No.50	(Reciprocal of cross)	Clone No.1	(Reciprocal of cross)
No. of heads examined.	5	5	4	-	5	5	5	5	6	7	5	7
No. of florets " .	275	298	211	-	256	438	274	231	237	386	188	227
Total seed set (normal & abnormal).	0	1	3	-	139	296	157	121	125	310	126	192
No. of normal seed.	-	1	3	-	138	269	157	96	125	303	126	172
No. of abnormal seed.	-	-	-	-	1	27	-	25	-	7	-	20
No. of 2-seeded pods.	-	-	-	-	3	8	2	1	1	53	16	20
No. of all seed per 100 florets.	-	0.3	1.4	-	54.3	67.6	57.3	52.4	52.7	80.3	67.0	84.6
Standard error.	-	±0.3	±1.2	-	±3.1	±2.2	±3.0	±3.3	±3.2	±2.0	±3.4	±2.4

CLONE No. 39 (Total flowerheads on 5 clonal propagules = 175).

	Left Alone	SELFINGS			CROSSED BY BEES WITH -				CROSSED BY HAND WITH -			
		Rolled	Hand tripped	With bees	Clone No.53	(Reciprocal of cross)	Clone No.19	(Reciprocal of cross)	Clone No.53	(Reciprocal of cross)	Clone No.19	(Reciprocal of cross)
No. of heads examined.	5	5	-	5	5	7	5	5	5	5	6	5
No. of florets " .	373	353	-	267	278	323	370	299	220	250	249	253
Total seed set (normal & abnormal).	0	0	-	0	88	73	21	263	84	44	65	191
No. of normal seed.	-	-	-	-	87	71	21	263	84	44	65	188
No. of abnormal seed.	-	-	-	-	1	2	-	-	-	-	-	3
No. of 2-seeded pods.	-	-	-	-	5	0	0	23	8	0	1	12
No. of all seed set per 100 florets.	-	-	-	-	31.6	22.6	5.7	88.0	38.2	17.6	26.1	75.5
Standard error.	-	-	-	-	±2.8	±2.3	±1.2	±1.9	±3.3	±2.4	±2.8	±2.7

APPENDIX XI.

CLONE NO. 40 (Total flowerheads on 5 clonal propagules = 342).

TREATMENTS.

	Not Tripped	SELFINGS: Rolled	SELFINGS: Hand tripped	SELFINGS: With bees	CROSSED BY BEES WITH - Clone No.31	(Reciprocal of cross)	Clone No.17	(Reciprocal of cross)	CROSSED BY HAND WITH - Clone No.31	(Reciprocal of cross)	Clone No.17	(Reciproca of cross)
No. of heads examined.	3	3	1	5	5	8	6	5	6	6	10	8
No. of florets " .	322	218	68	329	245	359	243	184	265	312	382	236
Total seed set (normal & abnormal).	0	0	0	4	133	261	117	63	144	142	273	121
No. of normal seed.	-	-	-	4	133	261	117	63	141	138	272	121
No. of abnormal seed.	-	-	-	-	-	-	-	-	3	4	1	-
No. of 2-seeded pods.	-	-	-	0	3	2	7	0	14	0	25	0
No. of all seed per 100 florets.	-	-	-	1.2	54.3	72.7	48.2	34.2	53.2	45.5	71.5	51.3
Standard error.	-	-	-	±0.6	± 3.2	±2.4	±3.2	±3.5	±3.1	±3.1	±2.3	±3.2

CLONE NO. 41 (Total flowerheads on 5 clonal propagules = 214).

TREATMENTS.

	Not Tripped	SELFINGS: Rolled	SELFINGS: Hand tripped	SELFINGS: With bees	CROSSED BY BEES WITH - Clone No.60	(Reciprocal of cross)	Clone No.33	(Reciprocal of cross)	CROSSED BY HAND WITH - Clone No.60	(Reciprocal of cross)	Clone No.33	(Reciproc of cross)
No. of heads examined.	5	5	-	-	5	5	5	5	5	3	5	7
No. of florets " .	282	212	-	-	272	286	247	206	210	100	194	208
Total seed set (normal & abnormal).	9	1	-	-	157	111	123	108	91	48	128	110
No. of normal seed.	9	1	-	-	157	111	123	101	91	48	128	109
No. of abnormal seed.	-	-	-	-	-	-	-	7	-	-	-	1
No. of 2-seeded pods.	0	-	-	-	6	0	0	0	0	0	14	3
No. of all seed set per 100 florets.	3.2	0.47	-	-	57.7	38.8	49.8	52.4	43.3	48.0	66.0	52.8
Standard error.	±1.0	±0.47	-	-	±3.0	±2.9	±3.2	±3.5	±3.4	±5.0	±3.4	±3.5

APPENDIX XII.

CLONE NO.50 (Total flowerheads on 5 clonal propagules = 190).

TREATMENTS.

	Not Tripped	SELFINGS			CROSSED BY BEES WITH -				CROSSED BY HAND WITH -			
		Rolled	Hand Tripped	With bees	Clone No.38	(Reciprocal of cross)	Clone No.14	(Reciprocal of cross)	Clone No.38	(Reciprocal of cross)	Clone No.14	(Reciprocal of cross)
No. of heads examined.	5	5	2	5	5	5	5	5	7	6	7	5
No. of florets " .	423	432	160	380	438	256	393	235	386	237	273	177
Total seed set (normal & abnormal).	0	1	2	0	296	139	269	178	310	125	316	134
No. of normal seed .	-	1	2	-	269	138	265	178	303	125	316	133
No. of abnormal " .	-	-	-	-	27	1	4	-	7	-	-	1
No. of 2-seeded pods.	-	-	0	-	8	3	18	7	53	1	112	9
No. of all seed per 100 florets .	-	0.2	1.25	-	67.6	54.3	68.4	75.7	80.3	52.7	115.8	75.7
Standard error.	-	±0.1	±0.9	-	±2.2	±3.1	±2.3	±2.8	±2.0	±3.2	±2.6	±3.2

CLONE NO.53 (Total flowerheads on 5 clonal propagules = 205).

TREATMENTS.

	Not Tripped	SELFINGS			CROSSED BY BEES WITH -				CROSSED BY HAND WITH -			
		Rolled	Hand Tripped	With bees	Clone No.61	(Reciprocal of cross)	Clone No.39	(Reciprocal of cross)	Clone No.61	(Reciprocal of cross)	Clone No.39	(Reciprocal of cross)
No. of heads examined.	4	5	1	5	8	5	7	5	7	5	5	5
No. of florets " .	263	247	52	268	380	276	323	278	342	276	250	220
Total seed set (normal & abnormal).	0	0	0	1	116	139	73	88	120	149	44	84
No. of normal seed.	-	-	-	1	114	139	71	87	120	149	44	84
No. of abnormal seed.	-	-	-	-	2	-	2	1	-	-	-	-
No. of 2-seeded pods.	-	-	-	-	0	3	0	5	-	2	-	8
No. of all seed set per 100 florets.	-	-	-	0.4	30.5	50.4	22.6	31.6	35.1	54.0	17.6	38.2
Standard error.	-	-	-	±0.4	±2.4	±3.0	±2.3	±2.8	±2.6	±3.0	±2.4	±3.3

APPENDIX XIII.

CLONE NO. 58 (Total flowerheads on 5 clonal propagules = 34).

TREATMENTS.

	Not Tripped	SELFINGS Rolled	Hand Tripped	With bees	CROSSED BY BEES WITH Clone No.63	(Reciprocal of cross)	Clone No.4	(Reciprocal of cross)	CROSSED BY HAND WITH Clone No.63	(Reciprocal of cross)	Clone No.4	(Reciprocal of cross)
No. of heads examined.	5	4	4	-	4	10	5	5	5	8	4	8
No. of florets " .	314	211	231	-	287	393	299	280	303	273	188	297
Total seed set (normal & abnormal).	10	3	0	-	17	159	28	39	31	67	22	35
No. of normal seed.	10	3	-	-	17	159	28	37	31	67	22	33
No. of abnormal seed.	-	-	-	-	-	-	-	2	-	-	-	2
No. of 2-seeded pods.	1	-	-	-	1	1	1	1	2	0	3	0
No. of all seed per 100 florets.	3.2	1.4	-	-	5.9	39.5	9.4	13.9	10.2	24.5	11.7	11.8
Standard error.	±1.0	±0.8	-	-	±1.4	±2.5	±1.7	±2.1	±1.7	±2.6	±2.3	±1.9

CLONE NO. 59 (Total flowerheads on 5 clonal propagules = 104).

TREATMENTS.

	Not Tripped	SELFINGS Rolled	Hand Tripped	With bees	CROSSED BY BEES WITH Clone No.29	(Reciprocal of cross)	Clone No.23	(Reciprocal of cross)	CROSSED BY HAND WITH Clone No.29	(Reciprocal of cross)	Clone No.23	(Reciprocal of cross)
No. of heads examined.	5	6	2	5	5	6	1	7	3	3	6	7
No. of florets " .	331	323	102	298	301	286	22	390	145	98	330	314
Total seed set (normal & abnormal).	0	0	0	6	20	72	3	206	55	43	118	164
No. of normal seed.	-	-	-	6	18	72	2	205	54	43	115	164
No. of abnormal " .	-	-	-	-	2	-	1	1	1	-	3	-
No. of 2-seeded pods.	-	-	-	0	0	0	0	13	4	0	0	3
No. of all seed set per 100 florets.	-	-	-	2.0	6.6	25.1	13.6	52.8	37.9	43.9	35.8	52.2
Standard error.	-	-	-	±0.8	±1.4	±3.0	±7.3	±2.5	±4.0	±5.0	±2.6	±2.8

APPENDIX XIV.

CLONE NO. 60 (Total flowerheads on 5 clonal propagules = 127).

TREATMENTS.

	Not Tripped	SELFINGS			CROSSED BY BEES WITH-				CROSSED BY HAND WITH-			
		Rolled	Hand Tripped	With bees	Clone No.41	(Reciprocal of cross)	Clone No.64	(Reciprocal of cross)	Clone No.41	(Reciprocal of cross)	Clone No.64	(Reciprocal of cross)
No. of heads examined.	5	5	1	5	5	5	5	5	3	5	2	5
No. of florets " .	252	225	56	251	286	272	235	249	100	210	55	205
Total seed set (normal & abnormal).	0	0	0	0	111	157	79	92	48	91	20	73
No. of normal seed.	-	-	-	-	111	157	79	92	48	91	18	73
No. of abnormal " .	-	-	-	-	-	-	-	-	-	-	2	-
No. of 2-seeded pods.	-	-	-	-	0	6	0	2	0	0	0	0
No. of all seed per 100 florets.	-	-	-	-	38.8	57.7	33.6	36.9	48.0	43.3	36.3	35.6
Standard error.	-	-	-	-	±2.9	±3.0	±3.1	±3.1	±5.0	±3.4	±6.5	±3.3

CLONE NO. 61 (Total flowerheads on 5 clonal propagules = 198)

TREATMENTS.

	Not Tripped	SELFINGS			CROSSED BY BEES WITH-				CROSSED BY HAND WITH-			
		Rolled	Hand Tripped	With bees	Clone No.22	(Reciprocal of cross)	Clone No.53	(Reciprocal of cross)	Clone No.22	(Reciprocal of cross)	Clone No.53	(Reciprocal of cross)
No. of heads examined.	5	5	5	5	5	5	5	8	4	9	5	7
No. of florets " .	304	261	259	231	281	249	276	380	165	331	276	342
Total seed set (normal & abnormal).	0	2	5	6	1	104	139	116	5	175	149	120
No. of normal seed.	-	2	5	6	1	104	139	114	5	173	149	120
No. of abnormal seed.	-	-	-	-	-	-	-	2	-	2	-	-
No. of 2-seeded pods.	-	0	0	0	-	26	3	0	0	13	2	0
No. of all seed set per 100 florets.	-	0.8	1.9	2.6	0.4	41.8	50.4	30.5	3.03	52.8	54.0	35.1
Standard error.	-	±0.7	±0.8	±1.0	±0.4	±3.1	±3.0	±2.4	±1.3	±2.7	±3.0	±2.6

APPENDIX XV.

CLONE NO. 62 (Total flowerheads on 5 clonal propagules = 160).

TREATMENTS.

	Not Tripped	SELFINGS Rolled	Hand Tripped	With bees	CROSSED BY BEES WITH - Clone No.2	(Reciprocal of cross)	Clone No.65	(Reciprocal of cross)	CROSSED BY HAND WITH - Clone No.2	(Reciprocal of cross)	Clone No.65	(Reciprocal of cross)
No. of heads examined.	5	5	5	5	5	5	7	5	6	8	7	5
No. of florets " .	227	238	213	208	230	272	265	224	162	352	236	162
Total seed set (normal & abnormal).	0	0	0	0	36	130	83	0	117	178	231	54
No. of normal seed.	-	-	-	-	36	129	83	-	117	178	231	54
No. of abnormal " .	-	-	-	-	-	1	-	-	-	-	-	-
No. of 2-seeded pods.	-	-	-	-	2	1	3	-	3	4	68	0
No. of all seed per 100 florets.	-	-	-	-	15.7	47.8	31.3	-	72.2	50.6	97.9	33.3
Standard error.	-	-	-	-	±2.4	±3.0	±2.9	-	±3.5	±2.7	±0.9	±3.7

CLONE NO. 63 (Total flowerheads on 5 clonal propagules = 79).

TREATMENTS.

	Not Tripped	SELFINGS Rolled	Hand Tripped	With bees	CROSSED BY BEES WITH - Clone No.58	(Reciprocal of cross)	Clone No.12	(Reciprocal of cross)	CROSSED BY HAND WITH - Clone No.58	(Reciprocal of cross)	Clone No.12	(Reciprocal of cross)
No. of heads examined.	5	5	1	5	10	4	9	5	8	5	8	7
No. of florets " .	249	295	50	218	393	287	417	352	273	303	290	317
Total seed set (normal & abnormal).	0	0	0	0	159	17	139	309	67	31	65	245
No. of normal seed.	-	-	-	-	159	17	139	308	67	31	65	243
No. of abnormal seed.	-	-	-	-	-	-	-	1	-	-	-	2
No. of 2-seeded pods.	-	-	-	-	1	1	1	22	0	2	0	17
No. of all seed set per 100 florets.	-	-	-	-	39.5	5.9	33.3	87.8	24.5	10.2	22.4	77.3
Standard error.	-	-	-	-	±2.5	±1.4	±2.3	±1.7	±2.6	±1.7	±2.4	±2.4

APPENDIX XVI.

CLONE NO. 64 (Total flowerheads on 5 clonal propagules = 114).

TREATMENTS.

	Not Tripped	SELFINGS			CROSSED BY BEES WITH–				CROSSED BY HAND WITH–			
		Rolled	Hand tripped	With bees	Clone No.28	(Reciprocal of cross)	Clone No.60	(Reciprocal of cross)	Clone No.28	(Reciprocal of cross)	Clone No.60	(Reciprocal of cross)
No. of heads examined.	5	5	3	5	8	5	5	5	4	6	5	2
No. of florets " .	236	253	161	253	369	218	249	235	136	189	205	55
Total seed set (normal & abnormal).	1	1	0	9	122	74	92	79	42	104	73	20
No. of normal seed.	1	1	-	9	122	72	92	79	42	103	73	18
No. of abnormal " .	-	-	-	-	-	2	-	-	-	1	-	2
No. of 2-seeded pods.	-	-	-	-	-	0	2	0	0	1	0	0
No. of all seed per 100 florets.	0.4	0.4	-	3.5	33.0	38.9	36.9	33.6	30.9	55.0	35.6	36.3
Standard error.	±0.4	±0.4	-	±1.2	±2.4	±3.3	±3.1	±3.1	±4.0	±3.6	±3.3	±6.5

CLONE NO. 65 (Total flowerheads on 5 clonal propagules = 91)

TREATMENTS

	Not Tripped	SELFINGS			CROSSED BY BEES WITH–				CROSSED BY HAND WITH–			
		Rolled	Hand tripped	With bees	Clone No.62	(Reciprocal of cross)	Clone No.35	(Reciprocal of cross)	Clone No.62	(Reciprocal of cross)	Clone No.35	(Reciprocal of cross)
No. of heads examined.	5	6	3	-	5	7	5	6	5	7	5	7
No. of florets " .	271	326	181	-	224	265	243	266	162	236	157	223
Total seed set (normal & abnormal).	0	7	2	-	0	83	8	67	54	231	50	120
No. of normal seed.	-	7	2	-	-	83	8	62	54	231	50	120
No. of abnormal seed.	-	-	-	-	-	-	-	5	-	-	-	-
No. of 2-seeded pods.	-	1	-	-	-	3	0	0	0	68	0	1
No. of all seed set per 100 florets.	-	2.1	1.1	-	-	31.3	3.4	25.2	33.3	97.9	31.8	53.8
Standard error.	-	±0.8	±0.8	-	-	±2.8	±1.2	±2.7	±3.7	±0.9	±3.7	±3.3

APPENDIX XVII.

SUMMARY OF CLONES NOT USED IN MAIN EXPERIMENT.

Clone number.	Total no. of flower-heads (5 propagules)	Spontaneous self-fertilization.				Artificially tripped.				Cross-pollinated by bees.					
		Heads Examined.	Florets Examined.	Total seed set.	Seed per 100 florets & S.E.	Heads Examined.	Florets Examined.	Total seed set	Seed per 100 florets & S.E.	Heads Examined.	Florets Examined.	Total seed set.	Seed per 100 florets & S.E.	Plants used as pollen parents.	
5	34	4	281	0	-	4	241	1	0.4 ± 0.4	3	109	31	28.4 ± 4.3	47 & 46	I
7	0	N.A. †	-	-	-	-	-	-	-	-	-	-	-	-	-
8	38	5	236	146	61.9 ± 3.2	N.D.*	-	-	-	N.D.	-	-	-	-	SF
9	11	5	243	30	12.3 ± 2.1	N.D.	-	-	-	N.D.	-	-	-	-	SF
10	12	5	318	131	41.2 ± 2.8	N.D.	-	-	-	N.D.	-	-	-	-	SF
11	12	5	278	135	48.6 ± 3.0	N.D.	-	-	-	N.D.	-	-	-	-	SF
13	2	1	77	0	0	1	50	0	-	-	-	-	-	-	I
15	40	3	123	0	0	8	383	0	-	4	258	117	45.3 ± 3.1	66 & 44	I
16	52	5	236	0	0	4	188	1	0.5 ± 0.5	11	289	135	46.7 ± 2.9	57 & 55	I
18	34	5	263	11	4.2 ± 1.2	2	100	28	28.0 ± 4.5	2	87	29	33.3 ± 5.0	52 & 36	SF
20	15	6	273	139	50.9 ± 3.0	2	105	56	53.3 ± 4.9	N.D.	-	-	-	-	SF
21	20	8	400	203	50.8 ± 2.5	N.D.	-	-	-	N.D.	-	-	-	-	SF
24	19	4	158	8	5.1 ± 1.8	N.D.	-	-	-	3	144	65	45.5 ± 4.2	19 & 6	-
25	20	5	239	0	-	1	46	0	-	5	230	120	52.1 ± 3.3	61 & 41	I
26	39	5	234	102	43.6 ± 3.2	4	190	64	33.6 ± 3.4	7	308	167	54.2 ± 2.5	49 & 51	SF
30	13	5	242	0	-	N.D.	-	-	-	N.D.	-	-	-	-	-
32	36	5	278	9	3.2 ± 1.1	2	80	1	1.25 ± 1.2	N.D.	-	-	-	-	I
36	4	2	90	1	1.1 ± 1.1	N.D.	-	-	-	2	85	29	34.1 ± 5.1	18 & 52	-
37	28	5	240	0	-	1	42	0	-	5	171	90	52.6 ± 3.8	46 & 45	I
42	23	5	270	148	54.8 ± 3.0	N.D.	-	-	-	N.D.	-	-	-	-	SF
43	11	5	275	20	7.3 ± 1.6	N.D.	-	-	-	1	53	18	34.0 ± 6.5	Polycross with 8 plants	-
44	36	5	260	104	40.0 ± 3.0	N.D.	-	-	-	4	160	114	71.25 ± 3.6	15 & 51	SF
45	45	5	213	0	-	4	136	0	-	12	404	83	20.5 ± 2.0	37 & 31	I
46	50	4	241	0	-	4	229	0	-	5	215	57	26.5 ± 3.0	37 & 5	I
47	57	4	195	0	-	5	201	0	-	6	244	101	41.4 ± 3.2	55 & 5	I
48	105	2	108	0	-	5	231	0	-	11	510	273	53.5 ± 2.2	54 & 49	I
49	39	4	271	0	-	2	134	0	-	12	798	434	54.4 ± 1.8	26 & 48	I
51	84	4	222	2	0.9 ± 0.6	1	44	0	-	7	258	93	36.0 ± 3.0	44 & 26	I
52	26	5	331	0	-	5	315	1	0.3 ± 0.3	7	331	48	14.5 ± 1.9	18 & 36	I
54	82	5	281	0	-	5	243	4	1.6 ± 0.8	9	295	167	56.6 ± 2.9	48 & 57	I
55	62	4	321	22	6.8 ± 1.4	N.D.	-	-	-	8	289	126	43.6 ± 2.9	16 & 47	-
56	44	5	400	1	0.25 ± 0.2	2	94	0	-	5	380	181	47.6 ± 2.6	34 & 50	I
57	46	3	207	0	-	1	50	0	-	7	285	152	53.3 ± 3.0	34 & 50	I
66	38	4	261	0	-	2	96	0	-	8	321	138	43.0 ± 2.8	45 & 15	I

* N.D. No data available.
† N.A. Not applicable.
I. Self-incompatible.
S.F. Self-fertile.

APPENDIX XVIII

POLLEN COUNTS OF STRAWBERRY CLOVER (FEBRUARY 1958)

At least 5 fields at magnification x 10
Abnormal pollen = small in size, malformed, or collapsed grains.
ND = No pollen counts obtained.

CLONE NO.	Clonal propagule "a". Total No. of grains counted	No. of Normal grains	Percentage Normal of Total & S.E.	Clonal propagule "b". Total No. of grains counted	No. of Normal grains	Percentage Normal of Total & S.E.
1	146	129	88.4 ± 2.6	127	103	81.1 ± 3.5
2	177	58	32.8 ± 3.5	193	61	31.6 ± 3.3
3	229	113	49.3 ± 3.3	N.D.	-	- -
4	185	172	93.0 ± 1.9	191	183	95.8 ± 1.4
5	118	113	95.8 ± 1.9	126	121	96.0 ± 1.7
6	108	100	92.6 ± 2.5	150	138	92.0 ± 2.2
7	N.D.	-	- -	N.D.	-	- -
8	135	127	94.1 ± 2.0	214	160	74.8 ± 3.0
9	134	108	80.6 ± 3.4	164	95	57.9 ± 3.9
10	122	78	63.9 ± 4.3	121	84	69.4 ± 4.2
11	125	55	44.0 ± 4.4	120	43	35.8 ± 4.4
12	125	119	95.2 ± 1.9	225	213	94.7 ± 1.5
13	169	149	88.2 ± 2.5	159	146	91.8 ± 2.2
14	183	68	37.2 ± 3.6	197	87	44.2 ± 3.5
15	137	90	65.7 ± 4.1	217	137	63.1 ± 3.3
16	174	123	70.7 ± 3.5	171	113	66.1 ± 3.6
17	109	100	91.7 ± 2.6	119	112	94.1 ± 2.2
18	161	109	67.7 ± 3.7	180	141	78.3 ± 3.1
19	117	74	63.2 ± 4.5	119	80	67.2 ± 4.3
20	118	112	94.9 ± 2.0	132	120	90.9 ± 2.5
21	105	88	83.8 ± 3.6	124	108	87.1 ± 3.0
22	**On these slides very little pollen was found. A few grains were normal, but most were small and shrunken.**					
23	117	95	81.2 ± 3.6	112	95	84.8 ± 3.4
24	166	156	94.0 ± 1.8	141	117	83.0 ± 3.2
25	132	129	97.7 ± 1.3	152	146	96.1 ± 1.6
26	112	104	92.9 ± 2.4	128	119	93.0 ± 2.3
27	128	126	98.4 ± 1.1	149	128	85.9 ± 2.9
28	111	106	95.5 ± 2.0	153	144	94.1 ± 1.9
29	135	130	96.3 ± 1.6	123	115	93.5 ± 2.4
30	115	112	97.4 ± 1.5	249	244	98.0 ± 0.8
31	134	117	87.3 ± 2.9	142	133	93.7 ± 2.0
32	135	111	82.2 ± 3.3	199	127	63.8 ± 3.4
33	272	143	52.6 ± 3.0	147	74	50.3 ± 4.1
34	210	196	93.3 ± 1.7	134	122	91.0 ± 2.5
35	235	141	60.0 ± 1.0	233	134	57.5 ± 3.2
36	138	43	31.2 ± 3.9	189	51	27.0 ± 3.2
37	347	259	74.6 ± 2.4	357	305	85.4 ± 1.9
38	149	61	40.9 ± 4.0	185	75	40.5 ± 3.6
39	195	138	70.8 ± 3.3	175	104	59.4 ± 3.7
40	130	119	91.5 ± 2.4	122	106	86.9 ± 3.1
41	155	127	81.9 ± 3.1	200	152	76.0 ± 3.0
42	ND	-	- -	ND	-	- -
43	158	100	63.3 ± 3.8	193	106	54.9 ± 3.6
44	184	171	92.9 ± 1.9	123	117	95.1 ± 2.0
45	141	104	73.8 ± 3.7	184	172	93.5 ± 1.8
46	132	117	88.6 ± 2.8	146	101	69.2 ± 3.8
47	141	129	91.5 ± 2.4	124	114	91.9 ± 2.5
48	207	97	46.8 ± 3.5	166	60	36.1 ± 3.7
49	191	100	52.4 ± 3.6	198	102	51.5 ± 3.6
50	139	135	97.1 ± 1.4	194	190	97.9 ± 1.0
51	195	108	55.4 ± 3.6	187	101	54.0 ± 3.6
52	122	100	82.0 ± 3.5	189	178	94.2 ± 1.7
53	142	52	36.6 ± 4.0	155	71	45.8 ± 4.0
54	163	106	65.0 ± 3.7	167	104	62.3 ± 3.8
55	104	69	66.3 ± 4.6	260	130	50.0 ± 3.1
56	116	107	92.2 ± 2.5	144	119	82.6 ± 3.2
57	148	107	72.3 ± 3.7	214	185	86.4 ± 2.3
58	136	96	70.6 ± 3.9	138	110	79.7 ± 3.4
59	128	103	80.5 ± 3.5	111	91	82.0 ± 3.6
60	141	106	75.2 ± 3.6	122	102	83.6 ± 3.4
61	229	128	55.9 ± 3.3	198	108	54.5 ± 3.5
62	125	115	92.0 ± 2.4	102	93	91.2 ± 2.8
63	168	88	52.4 ± 3.8	166	95	57.2 ± 3.8
64	138	131	94.9 ± 1.9	193	186	96.4 ± 1.3
65	113	110	97.3 ± 1.5	140	136	97.1 ± 1.4
66	147	127	86.4 ± 2.8	157	129	82.2 ± 3.0