PHYSIOLOGICAL GENETICS OF *PISUM*

I. GRAFTING EXPERIMENTS BETWEEN EARLY AND LATE VARIETIES

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Summary

The grafting of genetically early scions of *Pisum sativum* var. Massey on genetically late stocks (var. Telephone) leads to flowering at a higher node. In reciprocal grafts, the scions of the late variety flower at an earlier (lower) node.

Control grafting, i.e. scions to stocks of the same variety, has no effect on Massey scions but leads to flowering at a lower node in the Telephone scions.

Removal of cotyledons as soon as possible after germination (3-7 days) has no effect on Massey but leads to earlier flowering in Telephone.

These results are best explained on the assumption that the genetically later variety produces a flower inhibitor (or delaying) substance which can pass a graft union and alter the flowering behaviour of genetically early scions.

I. INTRODUCTION

The garden pea, *Pisum sativum,* is a classical subject for work in both genetics and physiology. In general, the work in any one of these disciplines has been carried out with little reference to the other. The exceptions to this general statement are few and concern studies on the physiological activity of genes controlling growth in length of the internodes. De Haan and Gorter (1936) attempted to show that the "slender" pea, which is the double recessive with respect to the two multiple loci *La* and *LB* (or, to use Lamm's (1947) symbolism, Cy_1 and Cy_2) differ from the normals in having a lower capacity for the enzymic destruction of auxin.

By means of reciprocal grafting of etiolated plants, Went (1938, 1943) has obtained some evidence that "slender" peas also differ from their normal tall (*Le*) or short (*le*) sisters in the production of, and response to, the caline group of hormones. He has shown that similar differences may exist between different commercial varieties. These last differences have not been investigated genetically. Went (1943) was unsuccessful in obtaining transmission of "acacia-leaf" (*tl*), "stipuleless" (*st*), and "rogue" (plasmagene) characteristics across graft unions between normal and mutant stocks.

Von Abrams (1953) who worked with two commercial varieties, Tall Telephone and Dwarf Telephone, which possibly differ at the *Le* locus, was unable to demonstrate any differences in the enzymic mechanisms controlling auxin production and destruction, although the etiolated dwarf responded more to auxin sprays than the etiolated tall.

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Commercial varieties of peas differ greatly in flowering behaviour. The genetics of these differences is complex but in view of our increasing knowledge of the physiological mechanisms controlling flowering (Lang 1952) a joint physiological and genetical study seems worth attempting. This paper gives an account of the results of grafting experiments between genetically early and late varieties. A preliminary account of the experiments has been published (Barber and Paton 1952). The results are most easily interpreted on the basis of a flower inhibitor (or delaying) substance which is produced by the late varieties and can pass a graft union and delay the flowering of a genetically early scion. The analysis of the genetical differences between the two varieties will be published separately.

II. Material and Methods

(*a*) *Survey of the Flowering Behaviour of Some Commercial Varieties*

Table 1 gives a survey of certain characteristics of the commercial varieties available in Hobart. The data refer to spring sowings in the experimental garden of the University. The characteristics that have been measured are (i) node number (N), exclusive of the cotyledonary node, at which the first flower forms, (ii) number of days (T) from soaking of seed to opening of first flower, and (iii) height of plant (H) to first flower.

It will be seen that the node number for these varieties varies from 9 for Massey to 18 for Telephone. This difference in node number corresponds to a difference of about 22 days in flowering time. While there is a significant linear regression of days to flower on node number ($T = 58{\cdot}7 + 2{\cdot}0\ (N - 14{\cdot}5)$, $P < 0{\cdot}01$) this general comparison shows that some varieties, e.g. Alaska, Canner's Perfection (early), and Latefeast are well off the regression line, Alaska and Latefeast being later than expected on the basis of node number and Canner's Perfection being earlier. This means presumably that time to flower is not determined absolutely by node number. Earlier genetical work which has been reviewed by Wellensiek (1925) and Rasmusson (1935) has given rise to the same general conclusion. Wellensiek assumed at least two types of genetic control, one pair of genes altering the node number and another pair at a different locus controlling flowering time or the rate of development of these nodes. Rasmusson, who unfortunately did not count node number in his progenies, has suggested that half the genic variation of flowering time in his crosses can be explained by two major genes, one linked to the *A* locus (flower colour) and the other, either identical with the *Le* gene (dominant for greater internode length) or very tightly linked to it, the *le* allele being dominant for later flowering. The other half of the genic variation is probably due to modifiers. On the other hand, Pellew (1940) has suggested that a series of three multiple alleles, L, l_1, and l_2 determine the three main classes of flowering behaviour, the dominant L gene determining later flowering (18th node).

The varieties we have used also differ in internode length, the extremes again being Massey with a mean internode length of 0·7 in. and Telephone with a mean length of 2·0 in. There is apparently no correlation of internode

length with earliness either measured in terms of node number of first flower or in days to opening of first flower.

The data in Table 1 were obtained by sowing all varieties at the same time. The effect of variations in such environmental factors as photoperiod and temperature, which are known to affect flowering behaviour in many species of plants, have also been investigated in the two extreme varieties, Massey and Telephone. In general, Massey is relatively unaffected in node number by photoperiod and temperature, e.g. vernalization at 4°C, while Telephone behaves as a quantitative long-day plant, the node number of first flower falling from about 20 under short days (*c.* 10 hr) to 15 under long days (*c.* 18 hr). Telephone also shows a small vernalization reaction, germination at 4°C for three weeks lowering the position of the first flower by one or two nodes. These reactions will be described in full in a later paper.

TABLE 1

FLOWERING CHARACTERISTICS OF PEA VARIETIES: SPRING SOWINGS 1951

Variety	No. of Plants *n*	Node No. of First Flower *N*	No. of Days from Sowing to Flowering *T*	Ht. of Plant to 1st Flower (in.) *H*
Massey	45	9·5±0·14	44·8±0·65	6·8±0·31
Alaska	49	10·0±0·08	57·5±1·92	19·4±0·54
Utility	56	11·1±0·08	46·8±0·41	9·2±0·30
King Edward	51	14·6±0·15	62·5±1·93	19·9±0·62
Canner's Perfection (early)	49	14·6±0·12	47·8±1·87	16·3±0·46
Latefeast	33	14·6±0·12	66·9±0·67	19·6±0·53
Stratagem	27	14·7±0·17	62·4±0·90	12·9±0·46
Greenfeast	34	16·1±0·20	62·2±0·65	12·2±0·44
Canner's Perfection (late)	55	16·6±0·14	60·9±0·48	21·4±0·51
Yorkshire Hero	27	16·8±0·25	62·9±0·86	18·2±0·68
Te Oroha	32	17·2±0·16	62·8±0·91	23·3±0·97
Telephone	34	18·2±0·18	66·4±0·91	35·9±1·29

(*b*) *Experimental Techniques*

For the grafting experiments, the two extreme varieties William Massey (early, dwarf) and Telephone (late, tall) were used in most experiments. For some of the first experiments, Richard Seddon, an intermediate variety flowering at the 13th or 14th node, was also used.

The dry seeds were surface sterilized in a weak solution of bleaching powder, washed, and soaked in aerated tap water for 8 hr at 23-25°C. Soaking ensures uniform germination and tests conducted during the course of the present experiments have shown that over these short periods, the leaching action which Bonner, Haagen-Smit, and Went (1939) and Eyster (1940) have studied, does not affect germination, growth, or development of the peas.

Germination conditions were standardized, the turgid disease-free seeds being sown in fresh moistened sphagnum moss in seed boxes kept in a heated glasshouse at a temperature of 15-20°C. At the end of 3-4 days, when the plumules have grown to approximately 1 cm, healthy uniform seedlings were planted in 6 in. pots, six plants being allowed to mature. We have found it advantageous to leave the cotyledons exposed on the surface to facilitate easy access to the cotyledonary buds during post-graft inspections and also to reduce susceptibility to attack by pathological organisms. The plants were grafted 1-2 days later, when the epicotyl was approximately 2 cm and the second internode is just visible.

A cleft graft technique was used, the stock plant being decapitated immediately below the first leaf, care being taken to remove all bud tissue, which, if left on the stock, may proliferate from the cut surface. A median longitudinal cut for approximately half the length of the stock epicotyl allowed the wedge-shaped scion to be easily pushed into position. For strapping the cut surfaces together, thin rubber rings cut from bicycle valve rubber tubing is all that is required, the rubber ring being slipped over the stock just before insertion of the scion. With this method, it has been possible to obtain over 90 per cent. successful grafts and to complete an individual graft in less than a minute.

The grafted plants were protected from excessive transpiration for one to two days, by which time partial tissue union had taken place and water transport to the scions established. Even though tissue union was relatively rapid, there was no marked scion growth for 10-14 days. Indeed, establishment of full physiological union, including the organization and transport of factors responsible for apical dominance over lateral shoot growth, required a period of the order of 3-4 weeks (cf. Went 1943). Until apical dominance had been established by the scion apex, scion growth was slow, and the cotyledonary buds of the stock produced vigorous basal shoots. These shoots were removed daily until the scion had established dominance.

Thus grafting stops growth for a variable period. We have no evidence that the variation in time for a graft to unite directly alters node number. It does, of course, alter flowering time which is more variable in the grafted plants. For this reason, the results of the grafting experiments are analysed solely in terms of node number, exclusive of cotyledonary node, of first flower. Even in ungrafted plants the node number is a less variable quantity than days to flower, the coefficients of variation being 6 per cent. for node number and 11 per cent. for days to flower.

In addition, the effect of removing cotyledons has been investigated. Under the conditions of our experiment, it was found that the removal of cotyledons was fatal if performed earlier than three days after soaking. After this time the embryo can survive and flower in standard potting soil, the survival rate being proportional to the time after germination at which cotyledons were removed.

Spring sowings were used for the experiments. In view of the variation in flowering behaviour caused by environmental factors, a series of controls both grafted and ungrafted was used for each experiment.

III. Results

The results of three grafting experiments are given in Table 2, and graphically in Figure 1. The more extensive data refer to grafting of the varieties Massey and Telephone. In the two experiments with these varieties we can compare the behaviour of ungrafted plants with their behaviour (i) when grafted on stocks of their own variety (control graft), (ii) when grafted on stocks of the other variety (experimental graft), and (iii) when the cotyledons are removed as early as possible and without grafting.

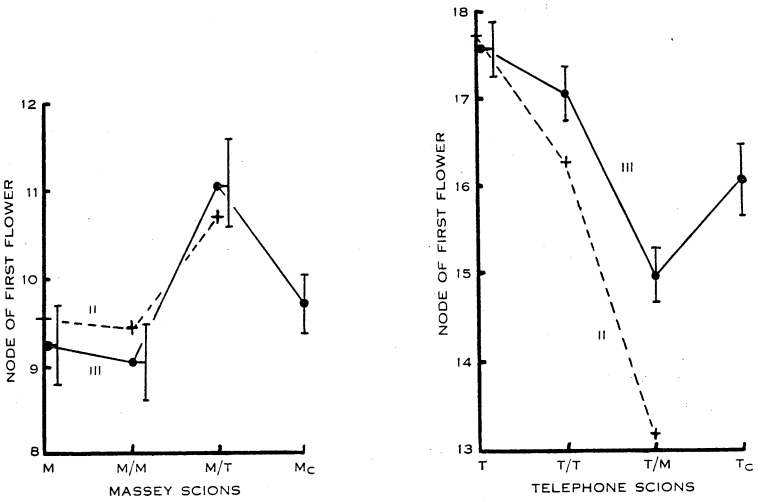

Fig. 1.—Diagrams showing the influence of control grafting (M/M and T/T), experimental grafting (M/T and T/M), and removal of cotyledons (M_c and T_c) on node of first flower of scions of Massey (M) and Telephone (T). The results of experiments II and III are given. The lengths of the vertical lines for experiment III represent twice the standard errors of the mean. For the sake of clarity they are not shown for experiment II but may be seen in Table 2.

In the case of Telephone, all three treatments result in significantly earlier flowering in both experiments. The greatest decrease in flower node is obtained by grafting to Massey stocks, the mean node number being significantly reduced by 4·6 nodes and 2·6 nodes. Control grafting gives the least reduction in node of first flower (1·4 and 0·5 nodes), the smaller of the two differences being statistically significant at the 1 in 50 level of probability.

As regards Massey scions, both experiments show that grafting on Telephone stocks leads to significantly later flowering, whilst control grafting has no significant effect. In the first experiment, removal of cotyledons has apparently caused later flowering, the difference being statistically significant at the 1 in 100 level of probability. However, this result is based on five plants only. In the second experiment, based on 38 plants, there is no significant difference in flowering node between intact plants and plants with cotyledons removed.

Table 2 gives the results of grafting the two varieties Richard Seddon and Telephone. Richard Seddon is an intermediate variety flowering at the four-

TABLE 2

RESULTS OF THREE GRAFTING EXPERIMENTS

The table shows the number of plants (n), the node of first flower ($\mathcal{N}$), and the standard error of the estimate of the mean ($S\mathcal{N}$)

GRAFTING EXPERIMENT I. SEPT.-DEC. 1949

Experimental Treatment of Scion	Richard Seddon Grafted on Telephone		Telephone Grafted on Richard Seddon	
	n	$\mathcal{N}\pm S\mathcal{N}$	n	$\mathcal{N}\pm S\mathcal{N}$
Control, ungrafted	40	13·73±0·101	40	16·78±0·141
Control, grafted	19	13·95±0·209	17	15·94±0·218
Experimental graft	13	14·46±0·186***	23	14·83±0·150***

GRAFTING EXPERIMENT II. SEPT.- DEC. 1950

Experimental Treatment of Scion	Massey Grafted on Telephone		Telephone Grafted on Massey	
	n	$\mathcal{N}\pm S\mathcal{N}$	n	$\mathcal{N}\pm S\mathcal{N}$
Control, ungrafted	36	9·53±0·093	23	17·74±0·201
Control, grafted	44	9·45±0·106	34	16·29±0·201***
Experimental graft	18	10·72±0·30***	17	13·18±0·196***
Cotyledons removed, ungrafted	5	10·80±0·583***	2	15·50±0·500**

GRAFTING EXPERIMENT III. NOV.-FEB. 1950-1951

Experimental Treatment of Scion	Massey Grafted on Telephone		Telephone Grafted on Massey	
	n	$\mathcal{N}\pm S\mathcal{N}$	n	$\mathcal{N}\pm S\mathcal{N}$
Control, ungrafted	45	9·27±0·215	63	17·59±0·141
Control, grafted	24	9·04±0·212	36	17·08±0·141*
Experimental graft	31	11·10±0·251***	63	14·98±0·148***
Cotyledons removed, ungrafted	38	9·71±0·164	51	16·06±0·195***

The significance of difference between means of ungrafted controls and treatment means is indicated at the 0·05 level of probability (*), 0·01 level of probability (**), and 0·001 level of probability (***).

teenth node. All treatments lead to significantly earlier flowering in Telephone, but, when Richard Seddon is grafted on Telephone, it flowers significantly

later. The differences in all cases are less than those in the comparable Massey-Telephone experiments.

A few observations have been made on the effect of varying the time interval between germination and grafting. In Experiment II, the time of grafting varied between 7 and 12 days after soaking the seed. There was a gradual loss in the response of Massey scions to grafting on Telephone stocks over this period, 12-day-old scions flowering at the same node as the ungrafted controls or control graft. This loss of flexibility is not shown by Telephone scions, these showing similar decreases in node number whether grafted on Massey at 7 or 12 days after soaking.

Both varieties have 5-7 nodes laid down in the dry seed. Flower primordia are detectable in Massey at the ninth or tenth node 10-15 days after soaking, whereas Telephone takes at least 30 days to form flower primordia. Thus the delaying effect of a Telephone stock on a Massey scion becomes ineffective once the flower primordia are laid down.

IV. Discussion

The results are best interpreted on the assumption that flowering behaviour in these pea varieties is mainly determined by the production of a flowering inhibitor in the cotyledons of late varieties which is then transported to the plumule. There are three lines of evidence in favour of this hypothesis. (i) Grafting of early scions on late stocks delays flowering in the early scion, while in the reciprocal graft, flowering in a late scion is hastened by an early stock. This result could equally well be interpreted in terms of a flowering stimulus produced in Massey. However, the other two lines of evidence show that this action is due largely to a flowering inhibitor in the late varieties. They are: (ii) removal of cotyledons from Massey has no effect on flowering, whereas the removal of cotyledons from Telephone causes flowering at a lower node; and (iii) control-grafting, which must interrupt the supply of inhibitor to some extent, results in earlier flowering of Telephone but has no effect on Massey.

There is some slight evidence that the cotyledons of Massey may also contain a substance promoting flowering. The best evidence is, perhaps, that removal of cotyledons in Telephone is apparently less effective in lowering the node of first flower than is grafting on Massey stocks. However, this evidence is of doubtful significance since in order to obtain sufficient scorable plants after removal of cotyledons, the plumules had to be left longer in contact with their own cotyledons than if the plumules were grafted onto Massey. Haupt (1952) has obtained a slight indication that removal of cotyledons within 8 hr of soaking the seed of the early variety, "Kleine Rheinlanderin" (node number 9-10), may lead to a delay in flowering. His experiments were again based on a small number of plants and were apparently significant only between the 0·05 and 0·01 levels. As indicated above in one of our experiments a similar effect was noted but it has not yet been repeated.

As regards the properties of the inhibitor, it is clear that a vegatative plumule can remain reactive to it only over a relatively short time, or that the cotyledons soon stop producing inhibitor. The inhibitor cannot alter flowering behaviour in Massey once flower primordia have appeared, i.e. 10-12 days after soaking. Telephone scions apparently remain reactive for more than 12 days. As regards the time during which the cotyledons produce inhibitor, preliminary experiments indicate that production stops after about 14 days, i.e. when most of the food reserves have been removed from the cotyledons.

This short period over which inhibitor production is active provides the explanation for the earlier flowering of control grafts in Telephone. While water transport is established within 3 days of grafting, full growth rate and apical dominance in the scion are not established for a period of 2-3 weeks. Usually apical dominance, as shown by cessation of growth of cotyledonary buds, is established before maximum growth rate is reached. It is unlikely that a 3-day interruption in supply of inhibitor would result in an earlier flowering of 1-2 nodes, so it appears probable that the transport of the inhibitor requires the slower and more intimate physiological union associated with the development of apical dominance. No direct attempts have been made to see whether the inhibitor can pass a water barrier in sufficient quantities to alter the flowering behaviour of early scions.

Comparison of the effect of Richard Seddon and Massey stocks on Telephone scions shows that Richard Seddon is about half as effective in lowering the node of first flower. This presumably means that Richard Seddon stocks produce some inhibitor but only about half as much as Telephone stocks.

The inhibitor is probably of a hormonal nature. Haupt (1952), using "Kleine Rheinlanderin," has shown that extracts of yeast, pea cotyledons, etc., contain substances which can delay the flowering of embryos from which the cotyledons were removed after a few hours of soaking in water. The embryos without cotyledons in these experiments were then grown to the stage of flower initiation on a synthetic medium of agar, sucrose, and mineral salts to which the various extracts were added. Cruickshank (unpublished data) has repeated some of Haupt's experiments using Massey and isolating the embryo aseptically from the cotyledons in the dry seed. He usually finds no delay in flowering if the cotyledons are removed in this way and the embryos grown in artificial light on the synthetic agar medium. He confirms that yeast extracts and extracts from Massey and Telephone seeds contain inhibitors increasing the node number from about 9·5 to 12. However, no differential effect of extracts from the two varieties could be demonstrated and attempts to fractionate the extracts into ether and water soluble fractions have failed. These experiments are continuing with the use of diffusion methods of extraction similar to those used by Bonner *et al.* (1939) in their search for leaf growth factors.

As regards chemical treatments, which might give some indication of the nature of the inhibitor, there appears to be increasing evidence that auxins (indoleacetic acid (IAA), or naphthaleneacetic acid (NAA)), will inhibit flowering in short-day and photoperiodically indeterminate plants (von Denffer 1950; Bonner and Liverman 1953). In long-day plants responses are more

variable, although here an inhibition appears to be usual when treatments with auxins are given under long days. However, under short-day conditions just below the flowering threshold, auxin application may promote flowering (Bonner and Liverman 1953). Earlier work, e.g. Leopold and Thimann (1949), has indicated that an optimum auxin concentration may exist in certain species. Borgstrom's (1939) results with an early variety of pea grown in darkness, appear to indicate that a concentration of IAA of 1 p.p.m. may be the optimum concentration as regards earliness and number of flowers under these growth conditions. Leopold and Guernsey (1953, 1954) using the early variety Alaska have shown that the effect of auxin treatment depends in a complex way on the temperature and light intensity at which the plants are grown after treatment. The auxin (NAA) was applied by soaking the seed in solutions for 4-18 hr. If the seeds are then grown at 20°C, the auxin over a concentration range of 10^{-7}-10^{-3}M, delays flowering by 2-3 nodes. If the auxin treatment at 10^{-5}M is followed by growth for a few days at 10°C, the flowering node is lowered. This effect is evident only at low light intensities (400 f.c. and below). Higher auxin concentrations are inhibitory no matter what the temperature and light regimes are.

Slade and Paton (unpublished data) have obtained no consistent results following auxin (IAA) and anti-auxin (2,3,5-triiodobenzoic acid) applications to seed and growing plants (lanolin paste or spray) of Massey and Telephone. The plants in these experiments were grown under ordinary glasshouse conditions. These observations make it doubtful whether auxin is responsible for the graft transmissible inhibition. Moreover, Haupt (1952) has shown that the floral inhibition in embryos without cotyledons by yeast extracts, is not replaced, nor is the inhibitory action of the extracts altered by addition of IAA at concentrations of 0·25 p.p.m. Thus, although we cannot at present exclude the possibility that an auxin activity is responsible for most of the graft transmissible flower inhibition, it appears more likely that the inhibitor formed by genetically late peas is not the natural auxin, IAA.

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