

# GENETIC, ENVIRONMENTAL, AND PHYSIOLOGICAL CONTROL OF LEAF ORIENTATION IN *PLANTAGO LANCEOLATA*

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[Manuscript received November 2, 1967]

## Summary

Considerable diversity in genes controlling leaf orientation in *P. lanceolata* has been demonstrated in a population growing in the Sydney area. The expression of these genes is greatly influenced by both light intensity and temperature; low light intensity and high temperature induces erect leaf orientation, while conversely high light intensity and low temperature favours the formation of prostrate leaves. Gibberellin apparently blocks the expression of the genes for prostrate leaf orientation. It is suggested that the seasonal variation in air temperature may be the climatic factor chiefly responsible for the disappearance of prostrate leaf orientation in midsummer and for the reappearance of this character in autumn and winter.

## I. INTRODUCTION

The genetic control of the physiological mechanism determining shoot and leaf orientation in herbaceous plants has been studied in a number of cases by investigating mutants which result in the abnormal orientation of part of the plant. For example, Bendixen, Stanford, and Peterson (1960) reported the action of a single recessive gene mutant of strawberry clover (*Trifolium fragiferum*) which results in the development of erect shoots in place of the normally prostrate ones, characteristic of this clover. They also found that the horizontal orientation of the stoloniferous shoots of strawberry clover depends upon the shoots being exposed to normal daylight; in prolonged darkness these shoots become erect. This is in agreement with conclusions of Palmer (1956, 1958) that bright illumination of the aerial shoot is a common requirement for plagiotropic and stoloniferous growth in herbaceous plants. Van der Veen and Meijer (1959) have shown in *Plantago media* that the leaves become erect at low light intensities while intensities above about 1 mW/cm<sup>2</sup> result in prostrate leaf orientation. Bendixen and Peterson (1962) have shown that treating prostrate shoots of strawberry clover with gibberellin resulted in the development of erect shoots resembling those found in the mutant. Gibberellin has long been known to induce a more erect orientation in dicotyledonous leaves. Photoperiod appears to control shoot orientation in *Proserpinaca palustris* where long days induce an erect habit in contrast to short days where a prostrate form develops (Wallenstein and Albert 1963). Here also prostrate shoots can be converted to erect ones by treatment with gibberellin. In *Helianthus annuus* the epinastic curvature of the petiole, inducing downward orientation of the leaf lamina with increasing age, is

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under correlative control, probably through auxin synthesized in the young parts of the shoot (Palmer and Phillips 1963).

So called "lazy" mutants which result in the shoot and leaves becoming insensitive to gravitational influence have been reported and studied in rice (Jones and Adair 1938), maize (Jenkins and Gerhardt 1961), and teosinthe (Langham 1941).

Barber (1965) has briefly reported that the juvenile leaves of *Eucalyptus obliqua* show a different orientation in the northern and southern parts of its geographic range. In the south, only the first few leaves produced by the seedling are horizontally orientated while the later-formed ones come to hang vertically, whereas in the north, leaves with an approximately horizontal orientation continue to be produced up to the sapling stage. Subsequent progeny testing has shown that the difference is genetic.

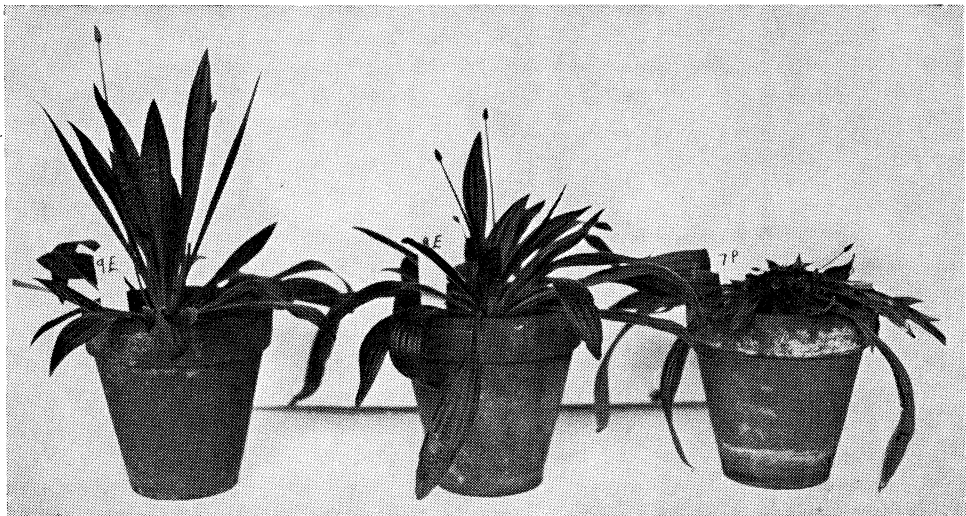


Fig. 1.—Examples of the variation in leaf orientation in *P. lanceolata* between some of the progeny examined. Left to right: 9E, 8E, and 7P plants.

In Australian populations of *Plantago lanceolata*, there appears to be distinct variation in the general orientation of the leaves between individual plants. Plants with markedly prostrate leaves are found near plants with predominantly erect leaves. Plants with most of their leaves orientated between these two extremes are also common (Fig. 1). Observations show that this variation in leaf orientation is not associated with morphological differences, which might indicate the occurrence of distinct varieties of *P. lanceolata*, and also that the prominence with which individual plants show the prostrate leaf condition varies greatly with the seasons, the tendency for prostrate leaves to form being greatest in late autumn and during the winter and least in midsummer. The young leaf of *P. lanceolata* is straight and erect when it emerges from the terminal bud, but as it ages, it moves away from the vertical due to curvature of the short petiole and tends to attain an equilibrium position, then forming a characteristic angle with the vertical in the range 0–90°. It is this

equilibrium position that appears to vary from plant to plant and to change with the seasons.

In this paper a preliminary study is reported on the inheritance of the characters for erect and prostrate leaf orientation in *P. lanceolata*, together with the results of investigating the response of leaf orientation to the application of gibberellin and to variation in two climatic factors, namely light intensity and air temperature.

## II. MATERIALS AND METHODS

Experimental material was collected from an old market-garden site in the Beecroft area of Sydney. Selection was made for plants showing leaves which were either mainly erect (E plants) or mainly prostrate (P plants). After being classified according to leaf orientation the plants were transplanted into pots to form a parental stock. In general, the classification initially arrived at was maintained. However, in early spring, the orientation of the leaves of some plants changed from being predominantly prostrate to mainly erect. Seed for experimental purposes was produced by interpollination in isolation between (1) three parental plants with prostrate leaves (3P, 4P, 7P), (2) three parental plants with erect leaves (1E, 4E, 9E), and (3) two parental plants (8"P"\*, 8E). Baker (1963) states that *P. lanceolata* is self-incompatible. Our limited tests also show that selfing is normally ineffective in Australian plants. Thus, seed from any one mother must have involved pollen from one of two fathers in the first two sets of parents.

The seed from these eight mothers was germinated and the seedlings were transplanted into 4-in. pots in horticultural compost. When the seedlings were 28 days old, the progeny of each mother was divided into two equal halves, one of which was allowed to remain in the glasshouse where the seedlings had been reared and the other was placed on the ground in an area outside the glasshouse. At both test sites the plants were arranged in two 5 × 5 latin squares with 20 plants from each of *four* mothers in each, arranged in plots of four. As a control the fifth set of plots was planted with seedlings derived from open pollinated seed of the same population from which the eight mothers had originally been collected. Control population A was collected in December, whilst control population B was collected late in the season in February. Thus, in two of the squares, progenies 1E, 8E, 3P, 4P, and B were tested in the environment outside and inside the glasshouse, whilst in the other two squares, 4E, 9E, 7P, 8"P", and A were similarly tested. A guard of either A or B was arranged round each latin square. The experiment was commenced in late autumn and allowed to continue into the winter. This time of year was selected in view of earlier observations that the winter climate of Sydney tends to favour the development of the prostrate leaf character.

Leaf orientation in each plant was measured using a protractor. The angle subtended by the leaf with the horizontal was measured for each of the six youngest leaves, excluding those which were less than 5 cm long. The values obtained were used to classify the leaves as (1) prostrate (0–30°), (2) intermediate (31–60°), (3) erect (61–90°). The degree to which the prostrate character was present in the progeny of each of the mothers was made quantitative by scoring the numbers of leaves in each case. A single score for each plant was then calculated by giving 2 for each prostrate leaf, 1 for each intermediate leaf, and 0 for each erect leaf, i.e. a plant with wholly prostrate leaves had a score of 12 and an erect one a score of 0. The mean scores of the four plants in each plot were used in the analysis of variance of the latin squares.

## III. GENETIC CONTROL OF LEAF ORIENTATION

Table 1 gives mean scores of the progenies tested in the four latin squares. It will be seen that the glasshouse environment resulted in a uniform growth of erect leaves in all progenies, suggesting that any genetic differences present were

\* 8"P" was a plant in which the leaves were initially prostrate, but subsequently became erect.

unable to express themselves in this environment. In contrast, the outside test site allowed the expression of differences between progenies, extremes being progeny 8E with a score of only 3 and 7P with a score of 10. These progeny differences are highly significant statistically ( $P < 0.001$ ).

TABLE 1

MEAN LEAF SCORES FOR PROGENY TESTED IN LATIN SQUARES, AND ANALYSIS OF VARIANCE WITHIN SQUARES

Progeny in latin squares 1 and 3 were grown outside, whilst those in latin squares 2 and 4 were grown in the glasshouse until June 28, 1966, when they were transferred outside. Score 0 indicates all leaves are erect (61–90°), whilst score 12 indicates that all leaves were prostrate (0–30°)

Progeny	Date of Scoring				
	23.vi.66	29.vi.66	23.vi.66	4.vii.66	18.vii.66
	Latin Square 1		Latin Square 2		
1E	7.0	7.2	0	8.5	11.7
8E	2.0	3.9	0	5.0	11.5
3P	8.3	7.7	0	9.3	11.0
4P	10.4	8.7	0	8.5	11.4
B*	7.6	7.1	0	7.0	11.3
Analysis of variance: progeny effect					
Error variance	1.75	1.875	—	5.26	0.156
Variance ratio	20.73	8.147	—	2.27	1.43
Probability	<0.001	<0.001	—	n.s.	n.s.
	Latin Square 3		Latin Square 4		
4E	7.5	5.9	0	6.2	10.9
9E	7.5	7.2	0	3.2	10.9
7P	10.4	9.2	0	7.7	11.3
8"P"	5.9	4.5	0	4.7	11.8
A*	9.2	7.0	0	7.0	11.1
Analysis of variance: progeny effect					
Error variance	1.92	1.448	—	1.76	0.179
Variance ratio	7.84	10.48	—	10.29	3.70
Probability	<0.001	<0.001†	—	<0.001	n.s.

\* Control populations.

† Column effects significant at  $P < 0.05$ , i.e.  $F = 3.61$ . In no other cases were column or row effects significant.

The two squares in the glasshouse were then transferred to the outside site on June 28, 1966. There followed a spectacular change in leaf orientation, most leaves becoming prostrate during the following 3 weeks. The rate at which this change occurred appeared to vary among the progenies. All the progenies were scored on July 4, 1966 after the plants had been in the open for 7 days. The means of the scores obtained agreed fairly closely with those recorded for the same progenies which had been continuously outside. In one square, progeny differences did not reach significance ( $F = 2.27$ ;  $F$  for 5% level = 3.26); in the other square, differences were significant at the 0.1% level.

The squares were scored again a fortnight later. The leaves had now almost all become prostrate with no significant differences between progenies. It thus appears that the expression of the genetic differences in leaf orientation depends not only on the environmental conditions prevailing at the time, but also on the previous environmental history of the plants.

Control populations A and B behaved similarly under all conditions in the four latin squares. There is, thus, no evidence that any selection for genes capable of altering leaf orientation had occurred in the 3-month period which elapsed between the collection of these two random samples of seed from the same population.

TABLE 2  
FREQUENCY DISTRIBUTION OF PROSTRATENESS AMONG 20 PLANTS OF EACH PROGENY GROWN CONTINUOUSLY IN THE NATURAL ENVIRONMENT

Progeny	Leaf Score												Variance	Mean Leaf Score	
	0	1	2	3	4	5	6	7	8	9	10	11			12
A							5	1	1	3	1	6	3	5.432	9.2
B	1	—	—	—	1	1	4	2	4	3	1	1	2	7.945	7.55
1E	1	—	—	1	1	2	5	2	2	3	—	1	2	8.526	6.95
4E					1	2	7	2	1	1	4	1	1	5.316	7.5
9E			1	1	1	—	7	1	2	—	3	2	2	8.579	7.5
8E	6	2	1	5	1	—	3	—	—	1	1	—	—	9.263	3.0
3P		1	—	1	—	1	2	—	3	3	5	3	1	8.134	8.35
4P							1	—	—	3	6	6	4	2.029	10.35
7P								1	1	4	3	6	5	2.187	10.35
8“P”				1	4	3	7	—	4	1				2.766	5.85

Table 2 gives progeny means and variances for the 20 plants of the squares grown continuously in the outside environment. It is interesting that interpollination of the three erect mothers, 1E, 4E, and 9E leads to no significant change in progeny variance over the control populations A and B. However, for the three prostrate mothers, 3P, 4P, and 7P, which were interpollinated, the variance is greatly reduced in two of the progenies. The explanation may be in the prostrate habit being largely a recessive character. However, as might be expected, no simple, monofactorial model appears to explain all the results.

#### IV. PHYSIOLOGICAL CONTROL OF LEAF ORIENTATION

##### (a) *Light Intensity*

The effect of light intensity upon leaf orientation in the various progeny was tested in a glasshouse experiment carried out in March 1966, when maximum noon light intensity inside the glasshouse ranged up to 1.0 cal/cm<sup>2</sup>/min, and mean minimum and maximum glasshouse shade temperatures were 18 and 26°C respectively. Progeny from 3P, 4P, and 7P mothers raised in the glasshouse under these conditions showed predominantly prostrate leaf orientation. In the experiment, five plants from each of the progeny groups were shaded by sarlon cloth for 2 weeks, reducing the sunlight intensity by about 25% (measured at noon with a short-wave solarimeter).

At the end of the period of shading, all the plants appeared healthy and it was found that in every plant from "prostrate" groups the majority of the leaves had changed their orientation from prostrate to erect. Only old leaves showed no change. Plants of "erect" progeny did not develop any new leaf orientation during the period of the experiment, and there was also no change of leaf orientation among the unshaded control plants. This experiment shows that the prostrate leaf character disappears in *P. lanceolata*, under summer temperature conditions, when the light intensity is reduced by shading.

(b) *Air Temperature*

The effect of air temperature upon the prostrate leaf character was investigated using a CSIRO artificially lit, controlled-environment cabinet. The light source in this cabinet was primarily fluorescent with supplementary incandescent lighting. Using an EEL selenium barrier cell with a green filter, the light intensity was adjusted in the cabinet to give an illumination intensity of 2000 lumens/sq ft at plant level from the fluorescent lamps with an additional 50 lumens/sq ft from the incandescent source. The photoperiod was 14 hr illumination and 10 hr darkness with a "low"

TABLE 3  
EFFECT OF HIGH AND LOW AIR TEMPERATURE UPON THE DEVELOPMENT  
OF PROSTRATE LEAVES IN 9E AND 7P PROGENY

Air Temperature Regime	Progeny	Mean* Leaf Orientation Angle (deg)		
		Initial	Final	Change
15°C day/10°C night	9E	68	54	— 14***
	7P	63	1	— 62***
30°C day/27·5°C night	9E	74	70	— 4†
	7P	69	31	— 38***

\* Mean of 60 values. \*\*\* Significant at  $P < 0.001$ . † Not significant.

air temperature regime of 15°C (light period)–10°C (dark period) in the first experiment, and a "high" air temperature regime of 30°C (light period)–27·5°C (dark period) in the second experiment. Prior to the commencement of each experiment, 10 plants from each of the 9E and 7P progenies were kept under shade in the glasshouse until their leaves had become erect, and then they were placed in the cabinet for 14 days. At the beginning and end of both experiments, the orientation angle of six young leaves on each plant was measured to the nearest 10° using a protractor with a levelling device, the angle measured being that subtended by the leaf and the horizontal (i.e. a vertical leaf = 90°).

The results are given in Table 3. Under the low temperature regime the leaves of the 7P plants became almost completely prostrate. This occurred within about 4 days from the time they were placed in the cabinet. By contrast the leaves of the 9E plants showed only a small degree of movement towards the horizontal during the 14-day period, so that by the end of the experiment they were still inclined

steeply upwards. When the experiment was repeated using new plants and the high temperature regime, the leaves of the 7P plants did not become prostrate, but showed a slow reorientation towards the horizontal during the first week, and little further change occurred in the second. At the end of the experiment the majority of the leaves of this group still showed a pronounced upwards inclination. The leaves of the 9E plants did not show any appreciable change of orientation during the experiment. Similar results were obtained with smaller trials using E or P plants from the following progeny 1E, 4E, 8E, 3P, 4P, and 8P. These experiments therefore show that the ability of plantain leaves to become prostrate under bright illumination is diminished by high air temperature.

(c) *Gibberellin*

Plants used in this experiment were from the progeny of 7P and 8E, and prior to the start of the experiment they were kept under shady conditions so that horizontal leaves did not form, and hence the maximum initial leaf score of any plant was 6. The leaves of three plants from each group were sprayed with gibberellic acid (potassium salt) at a concentration of 10  $\mu\text{g/ml}$  and the spray was re-applied after 7 days. Three untreated plants served as controls. All the plants were kept in a CSIRO controlled-environment cabinet with a 15-hr photoperiod and a temperature cycle of 5°C (dark) and 15°C (light).

The experiment was terminated after 14 days, when all the plants were scored for leaf orientation. The control plants in the 8E group showed no change in leaf orientation or leaf score, in marked contrast to the control plants in group 7P where the mean leaf score increased to 11, as the majority of the young leaves responded to the favourable temperature and light regime by becoming prostrate. The leaves of the gibberellin-treated plants reacted quite differently. In all the plants of both 8E and 7P groups, the young leaves rapidly became vertically erect, and in each case the mean group leaf score decreased to 1. At the same time the leaves became pale green in colour and developed an increased rate of elongation.

These results show that gibberellin can block the expression of the character for prostrate leaf orientation, even when the temperature and light regime favours the development of prostrate leaves in P plants.

## V. DISCUSSION

The physiological experiments show that *P. lanceolata* resembles *P. media* and other plants with prostrate leaves or stems in requiring exposure to relatively high light intensity for this character to develop. The new finding reported here is that this response is apparently enhanced by low temperatures, and reduced by high temperatures. The nature of this interaction between light intensity and temperature remains to be investigated, but this finding will provide an explanation for the common observations in New South Wales that leaves of *Plantago* tend to lose their prostrate orientation and become partially erect in midsummer, although sunlight intensities are generally very high then, while conversely the prostrate leaf character is most commonly seen in winter time when natural illumination levels are lowest. Variation in day length does not seem to be a relevant factor under natural conditions

since long days or continuous illumination has been found to promote the prostrate condition. The way in which leaf orientation can be affected by light intensity and air temperature is unknown, but unpublished findings of one of us (J.H.P.) suggest that the leaf orientation is geotropically controlled and that in some unknown way light and temperature can influence the plagiotropic equilibrium position of the leaf, which in turn is genetically determined.

The adoption of an erect leaf form following the application of gibberellin, which has been found to occur in *P. lanceolata*, appears to be a common response to gibberellin application, but as yet it is not known in what way gibberellin induces this change. Possibly it is a side effect associated with an increase in the rate of cell division or cell size, or enhanced hydrolysis of starch grains—all well known gibberellin responses. There appears to be no information regarding the presence of natural gibberellins in *Plantago* spp. but since these are commonly present in flowering plants, it might be speculated that the genes controlling leaf orientation operate by influencing the gibberellin physiology of the plant, either by modifying the rate of gibberellin synthesis or by varying the sensitivity of leaf cells to gibberellin.

This investigation was initiated by one of us (H.N.B.) with the object of elucidating whether a natural population of *Plantago* subject to a change in selection pressure, in the form of regular mowing of the plants, shows a detectable change in the frequencies of genes controlling leaf orientation. Under the new environment, plants with prostrate leaves should have an advantage. It is hoped to compare leaf orientation in populations which have been regularly mown for 2–3 years with unmown populations.

## VI. REFERENCES

- BAKER, H. G. (1963).—Evolutionary mechanisms in pollination biology. *Science*, N.Y. **139**, 877–83.
- BARBER, H. N. (1965).—Selection in natural populations. *Heredity*, Lond. **20**, 551–72.
- BENDIXEN, L. E., and PETERSON, M. L. (1962).—The physiological nature of gene controlled growth form in *Trifolium fragiferum*. II. Auxin gibberellin relationships to growth form. *Pl. Physiol.*, Lancaster **37**, 245–50.
- BENDIXEN, L. E., STANFORD, E. H., and PETERSON, M. L. (1960).—The physiological nature of gene controlled growth form in *Trifolium fragiferum*. I. Inheritance of growth form. *Agron. J.* **52**, 447–9.
- JENKINS, M. T., and GERHARDT, F. (1931).—A gene influencing the composition of the culm in maize. Res. Bull. Iowa Agric. Exp. Stn No. 138.
- JONES, J. W., and ADAIR, C. R. (1938).—Lazy mutation in rice. *J. Hered.* **29**, 315–18.
- LANGHAM, D. G. (1941).—Effect of light on the growth habits of plants. *Am. J. Bot.* **28**, 951–6.
- PALMER, J. H. (1956).—The nature of the growth response to sunlight shown by certain stoloniferous and prostrate tropical plants. *New Phytol.* **55**, 346–55.
- PALMER, J. H. (1958).—Studies in the behaviour of the rhizome of *Agropyron repens* (L.) Beauv. I. The seasonal development and growth of the parent plant and rhizome. *New Phytol.* **57**, 145–59.
- PALMER, J. H., and PHILLIPS, I. D. J. (1963).—The effect of the terminal bud, indoleacetic acid and nitrogen supply on the growth and orientation of the petiole of *Helianthus annuus*. *Physiologia Pl.* **16**, 572–84.
- VEEN, R. VAN DER, and MEIJER, G. (1959).—“Light and Plant Growth.” (Philips Technical Library: Eindhoven.)
- WALLENSTEIN, A., and ALBERT, L. S. (1963).—Plant morphology: its control in *Proserpinaca* by photoperiod, temperature and gibberellic acid. *Science*, N.Y. **140**, 998–1000.