THE EFFECT OF ENVIRONMENTAL CONDITIONS ON THE DEVELOPMENT OF PEA SEEDS

By R. N. Robertson,* H. R. Highkin,† J. Smydzuk,* and F. W. Went‡

[*Manuscript received July 28, 1961*]

Summary

The effects of environmental conditions on pea seeds developing in pods were investigated by growing the plants under controlled conditions in the Earhart Plant Research Laboratory. In one experiment plants were kept under a variety of conditions throughout their period of growth and of seed-pod development. In two experiments plants were grown under standard conditions until they flowered and were then transferred to a series of different environments. The environmental conditions investigated included photoperiod, light intensity, and day and night temperatures. Samples of seeds were taken at different times after flowering and seed growth was recorded by fresh weight and dry weight measurements. The samples were analysed for total sugar, reducing sugar, starch, total nitrogen, and protein nitrogen. The most striking results were the effects of night and day temperatures on the growth and composition of the seeds, particularly on the sugar content. At low temperatures, the conversion of sugar to starch was much delayed and sugar continued to increase in concentration during growth; at higher temperatures the sugar entering the seeds was rapidly converted to starch; thus the carbohydrate composition of seeds grown at different temperatures was markedly different. Protein synthesis was also delayed at lower temperatures. Water uptake and rate of drying out of the seeds were also affected. The possible implications and interrelations of these observations are discussed.

I. Introduction

It has been shown that the very rapid changes which occur during the development of pea seeds in the pod are associated with an increase in protein nitrogen and with an increase in a particular enzyme, starch phosphorylase, during the period when the sugar being translocated to the seed is converted rapidly into starch (McKee, Robertson, and Lee 1955; McKee, Nestel, and Robertson 1955; and Turner and Turner 1957).

This paper sets out the principal results of experiments designed to show the effects of environmental conditions of the plant on the development of seeds in the pod. In two of the three experiments carried out, the plants were grown under a standard condition until they flowered, and were then transferred to a series of different environments. In one of the experiments the plants were kept under a variety of different conditions throughout the period of their growth and seed-pod development. As will be shown, marked differences in composition of the seeds during development occur with as little as 3°C change in the day temperature.

* Division of Food Preservation, C.S.I.R.O., Homebush, N.S.W.

† California Institute of Technology, Pasadena, California.

‡ Missouri Botanic Gardens, St. Louis, Missouri.

II. Materials and Methods

In all experiments the plants were grown in the Earhart Plant Research Laboratory at the California Institute of Technology. A description of this Laboratory and its experimental facilities was published by Went (1957). The analyses were carried out in the Plant Physiology Unit, University of Sydney, by the methods described by McKee, Robertson, and Lee (1955).

The plants used in all these experiments were two varieties of peas, namely "Vinco" and "Unica". Both varieties have been inbred since the early 1930's and are very uniform. Because of the structure of its flower and because pollination takes place even before the flower bud opens, the pea is completely self-pollinated. It is therefore most likely that these two varieties, which have gone through an inbreeding and selection programme extending for some 20–25 years, are as homozygous as plant seed material can be.

(*a*) *Experiment* 1

A set of peas, cv. Vinco, was grown in an air-conditioned greenhouse at 20°C during the day and 14°C during the night. A total of 1080 individual plants were germinated in cups and kept in these conditions until the first flowers opened. After the first flowers opened, the plants were placed in 10 different conditions of temperature and illumination. Most of the treatments were in 16-hr photoperiods of 1000 f.c. intensity, at temperatures of 10, 14, 17, 20, and 23°C. The effect of day length was examined at 17°C under 1000 f.c. intensity, with photoperiods of 8, 12, and 16 hr and continuous light. The effect of light intensity (measured at the top of the plants) was examined at 17°C in 16-hr photoperiods of 1000 and 1500 f.c. The same temperatures were maintained during the night and day.

(*b*) *Experiment* 2

In the second experiment, plants of the variety Unica were germinated and grown under different conditions of temperature throughout their growth cycle; the photoperiod was constant at 16 hr, and the light intensity was 1000 f.c. The temperatures (°C) were: day 10, night 14; day 14, night 14; day 14, night 17; day 17, night 10; day 17, night 17.

(*c*) *Experiment* 3

In this experiment it was decided that more valuable information would be obtained about the development of the seed if the plants, again cv. Unica, were grown under uniform conditions until the time of flowering and then transferred to the different conditions for the development of the pods. Since earlier results had shown that temperature had the greatest influence, it was decided to concentrate on the effect of temperature, particularly day temperature, and to obtain enough samples from any one treatment to establish the curves with some accuracy. Hence a limited range of conditions was chosen. The temperatures (°C) were: day 10, night 10; day 14, night 10; day 17, night 10; day 23, night 10; day 23, night 17.

The light intensity was 1000 f.c. and the photoperiod was 16 hr. A total number of 1000 plants was used and the plants were distributed on the trucks so as to produce minimum shading during the growing period.

III. Results

(a) *Experiment* 1

The results of experiment 1 with cv. Vinco showed that the most interesting effects of the growing conditions on the pea seeds were those of temperature.

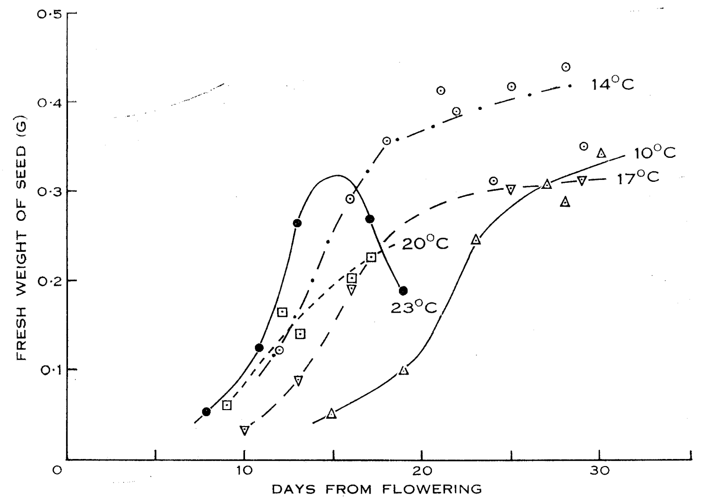

Fig. 1.—Growth of pea seeds on plants (cv. Vinco) held at different temperatures after flowering. Experiment 1. Conditions: 16-hr photoperiod, 1000 f.c. light intensity, night temperatures the same as day temperatures.

Unfortunately, the number of samples obtained from the different light intensities and the different photoperiods was inadequate to follow the effects of these conditions through the whole life cycle. The following general conclusions are indicated:

(1) Increase in the photoperiod caused an increase in the rate of growth of the seed over the early period. The average fresh weights of seeds after 18 days in the photoperiods 8, 12, and 16 hr and in continuous light were respectively 0·17, 0·25, 0·25, and 0·33 g.

(2) Increase in light intensity increased the rate of growth over the early stages. The average fresh weights of seeds after 15 days at 17°C with 16-hr photoperiod at 1000 and 1500 f.c. were respectively 0·16 and 0·28 g.

Since the main interest in the experiment was the effect of temperature and since light intensities of 1000 f.c. gave satisfactory growth, the effects of temperature, with a 16-hr photoperiod and 1000 f.c. light intensity, were compared. The results are shown in Figure 1. The principal effect of low temperature was to delay the period of more rapid growth. This can be seen particularly by comparing the curve at 23°C with the curve at 10°C. The former had an average seed weight of 0·06 g at 8 days, while at the lower temperature the same seed weight was not reached until 15 days. Thereafter the seed weight in both increased rapidly so that the maximum at the high temperatures was reached at about 15 days, whereas the peas grown at the lower temperature were still increasing in weight after 30 days from blossom. The effect of temperature on dry weight was very similar to that on wet weight. Some difference in both fresh weight and dry weight is seen between 14 and 17°C suggesting that the higher temperature here was not quite as efficient in permitting growth as the lower temperature, though whether this is significant is not known.

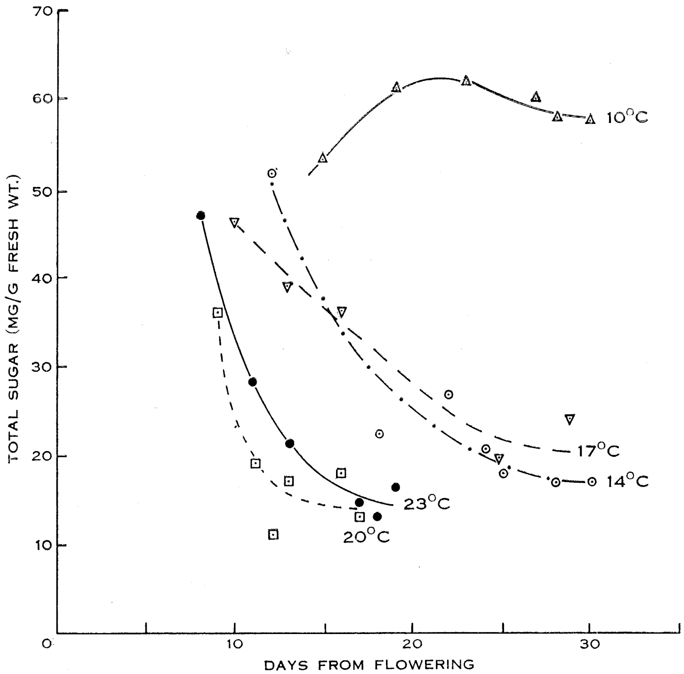

Fig. 2.—Total sugar content (on a fresh weight basis) during seed development. Experiment 1. Conditions as for Figure 1.

The analysis of the seeds for total sugars showed that the sugar concentration tended to build up rapidly at all temperatures and to fall subsequently, the initiation of the fall being more rapid the higher the temperature. This increase in total sugars during the early growth of the seed has been demonstrated in previous work (Bisson and Jones 1932; McKee, Robertson, and Lee 1955). At the lowest temperature of growth, the sugar concentration remained high throughout the period (Fig. 2). Most of the sugar was sucrose, with lower concentrations of reducing sugar. This point is illustrated by the figures in Table 1 for the plants grown at 10°C compared with the plants grown at 23°C.

The decrease in total sugar was shown in earlier work to be related to the period of starch increase. The peas in this experiment showed that the higher the temperature, the earlier the increase in rate of starch formation. These results are illustrated in Figure 3. After 8 days, the starch content at 23°C was 8 mg per gram fresh weight of seed, and by 12 days had risen to 58 mg; by contrast, the starch content at 10°C was only 5 mg after 19 days and only when the experiment ended

Table 1

COMPARISON OF SUCROSE AND REDUCING SUGAR CONTENT OF PEA SEEDS GROWN AT DIFFERENT TEMPERATURES

All values expressed as mg/g fresh weight of seed

Days from Blossom	10°C		23°C	
	Sucrose	Reducing Sugar	Sucrose	Reducing Sugar
8	—	—	42·8	4·2
11	—	—	27·3	1·1
13	—	—	20·4	1·1
15	44·8	8·5	—	—
17	—	—	12·0	2·7
18	—	—	11·0	1·9
19	54·3	7·4	14·8	1·7
23	53·7	8·6	—	—
27	55·8	5·1	—	—
28	54·9	3·6	—	—
30	54·8	3·3	—	—

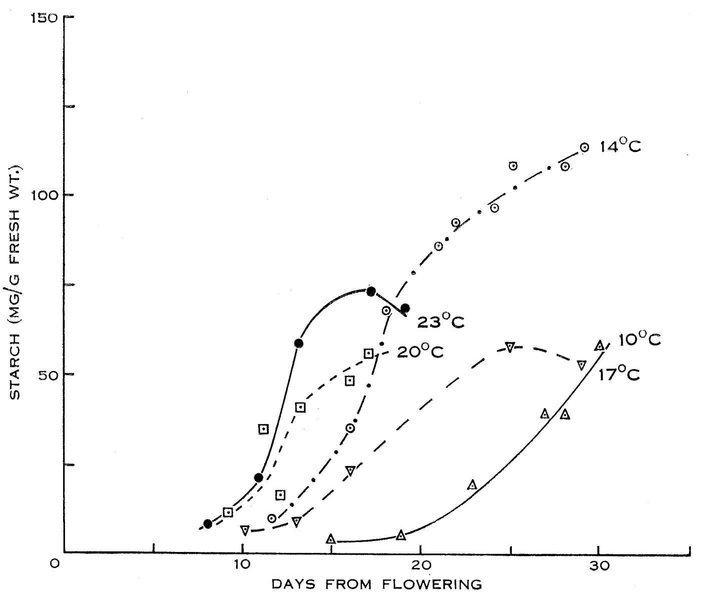

Fig. 3.—Starch content (on a fresh weight basis) during seed development. Experiment 1. Conditions as for Figure 1.

after 30 days had it risen to 58 mg. It appears that the long delay in the increased rate of starch formation is the main factor associated with the build up of total sucrose concentration in the seeds at 10°C. It is particularly noticeable, however, that in the period from 23 to 30 days, when the rate of synthesis of starch was at its maximum in these peas, the reducing sugar concentration fell from 8·6 mg per gram fresh weight to 3·3 mg per gram fresh weight. Thus it appears that while the sucrose continues at low temperature to be imported into the seed, the formation of starch brings about a preferential decrease in the concentration of reducing sugar. Similar effects, but not so striking, occurred in the intermediate temperatures, where the decrease in reducing sugar occurred more rapidly than the corresponding reduction in sucrose.

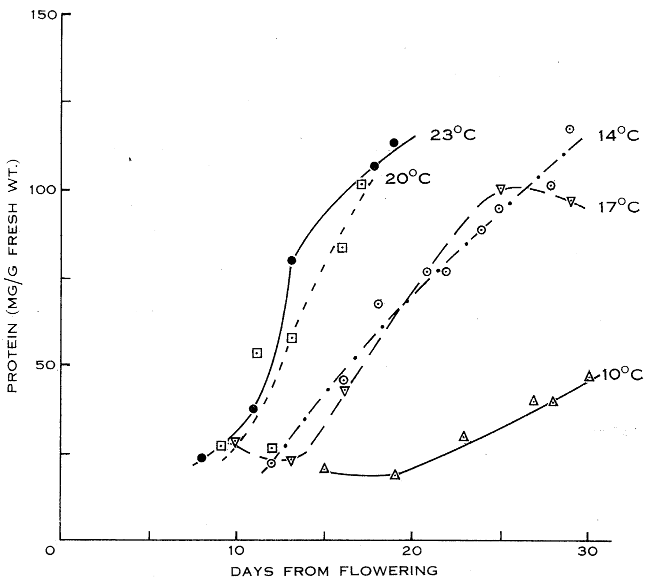

Fig. 4.—Protein content (on a fresh weight basis) during seed development. Experiment 1. Conditions as for Figure 1.

The delayed growth of the pea at low temperatures is reflected in the delay in the rate of protein synthesis. The increase in protein nitrogen is shown in Figure 4. At any time the soluble nitrogen is only a small fraction of the total nitrogen and, though the soluble nitrogen is reduced during the growth of the seeds, the protein nitrogen increases obviously at the expense of the nitrogen being brought into the seed all the time. The long delay in starch synthesis at low temperature is paralleled by the delay in protein synthesis.

One of the most striking features of these results is the marked difference between peas grown at 14 or 17°C and peas grown at 10°C. In the peas grown at the higher temperatures the sugar concentration never reaches the high concentration of the peas at the lower temperature and is certainly reduced markedly late in the life of the pea. The significance of this will be discussed subsequently.

(b) *Experiment* 2

The plants (cv. Unica) in experiment 2 were grown under different conditions for the duration of the experiment and samples were taken at intervals after the plants had flowered. The effect of these conditions of growth on the developing pods

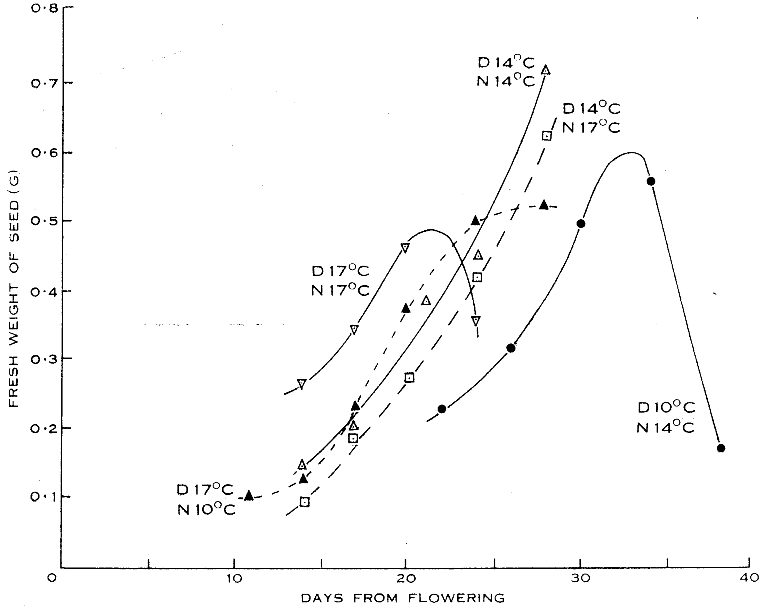

Fig. 5.—Growth of pea seeds on plants (cv. Unica) grown at different temperatures. Experiment 2. Conditions: 16-hr photoperiod, 1000 f.c. light intensity, night and day temperatures as shown on the figure.

was not anticipated and a number of pods dropped off, so the number of samples available from some conditions of growth was insufficient to give reasonable curves. The following are the principal conclusions that can be drawn. With warm days and warm nights the whole growth process of the seeds was accelerated and with cold days and cold nights the whole process was slowed. The seeds of plants grown at 17°C continuous temperature developed more rapidly, but dried out more quickly than those grown at 14°C continuous temperature, or 14°C day and 17°C night. Plants grown at 17°C day with a cold night (10°C) grew for longer and made a greater growth than similar plants with 17°C night and day temperature, and were more rapid in their development than plants with a 10°C day and a 14°C night. The effects of these conditions are compared in Figure 5.

The analyses show results similar to those obtained in the first experiment, though some differences due to the variety are apparent; at comparable temperatures, Unica develops a larger seed than Vinco. The effects of the different treat-

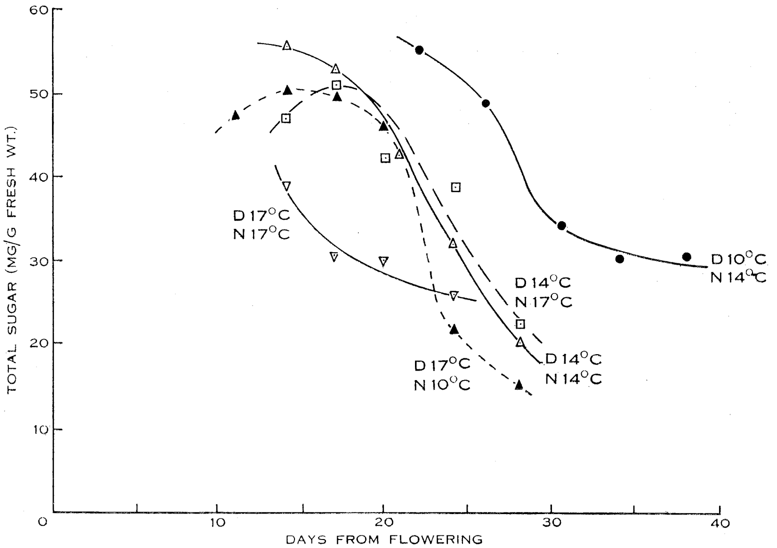

Fig. 6.—Total sugar content (on a fresh weight basis) during seed development. Experiment 2. Conditions as for Figure 5.

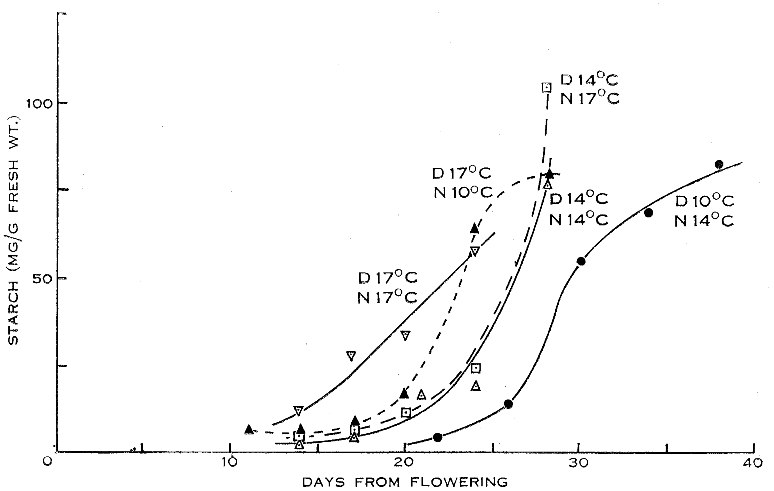

Fig. 7.—Starch content (on a fresh weight basis) during seed development. Experiment 2. Conditions as for Figure 5.

ments on sugar content are shown in Figure 6, on starch content in Figure 7, and on protein content in Figure 8. The slower the growth, the longer delayed was the rise in protein and starch and the reduction in total sugar content.

(*c*) *Experiment* 3

On the basis of the previous experiments, the number of conditions was reduced. The results are similar to those from earlier experiments. At lower temperatures the growth of the seeds is delayed, while at higher temperatures the growth is rapid after a shorter lag period. A noticeable feature of these results was the effect of a high night temperature, which apparently delayed the growth as compared with a 10°C night temperature but allowed the seeds to reach greater maximum size (cf. 23°C day with 10°C night and 23°C day with 17°C night). The results for fresh weight and dry weight are shown in Figures 9 and 10.

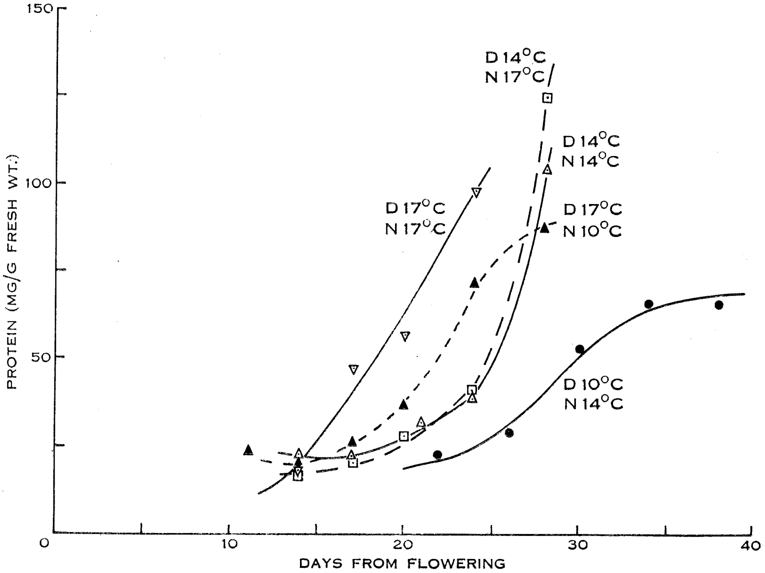

Fig. 8.—Protein content (on a fresh weight basis) during seed development. Experiment 2. Conditions as for Figure 5.

In considering the effect of temperature on the internal composition of the seeds (Figs. 10–14), it may be noted that the final points of several of the curves (where the seeds have already dried out very considerably) are high because the fresh weight has diminished. The total sugars of the seeds grown at lower temperatures were, as in earlier experiments, higher at the peak value and decreased less rapidly than those in plants grown at higher temperatures. In this experiment there is a difference in the sugar content of plants grown at 10°C day and at 14°C day, but this difference is not as striking as the difference between those with the 14°C day and the 17°C day. The difference of 3°C prevented the sugars from rising to anything like the same extent, while at the higher temperatures the sugar content appears to have increased more rapidly initially but also to have fallen rapidly (Fig. 11). In this connection the results for the high night temperature are also interesting, as they suggest that the initial rise in sugar content was greater and the fall more marked (though the evidence for this is based on only one point, at 10 days).

As in the earlier experiments, most of the total sugar was sucrose, and the reducing sugar was a small fraction of the sucrose content. The reducing sugar fell much more rapidly than the sucrose and, at the higher temperatures, the reducing

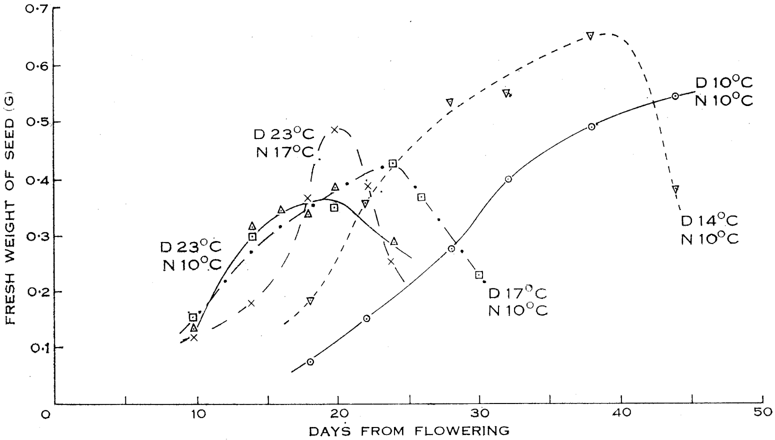

Fig. 9.—Growth of pea seeds on plants (cv. Unica) held at different temperatures after flowering. Experiment 3. Conditions: 16-hr photoperiod, 1000 f.c. light intensity, night and day temperatures as shown on the figure.

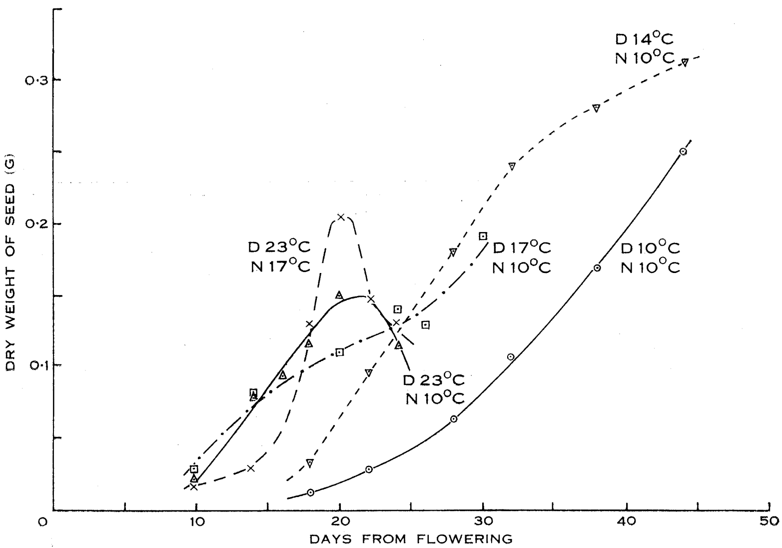

Fig. 10.—Dry weight changes during seed development. Experiment 3. Conditions as for Figure 9.

sugars were low from the beginning of the experiment. The reducing sugar curves are shown in Figure 12.

The sugar content changes in relation to the starch content. Starch at the low temperature remained low and began to rise only slowly after about 22 days from

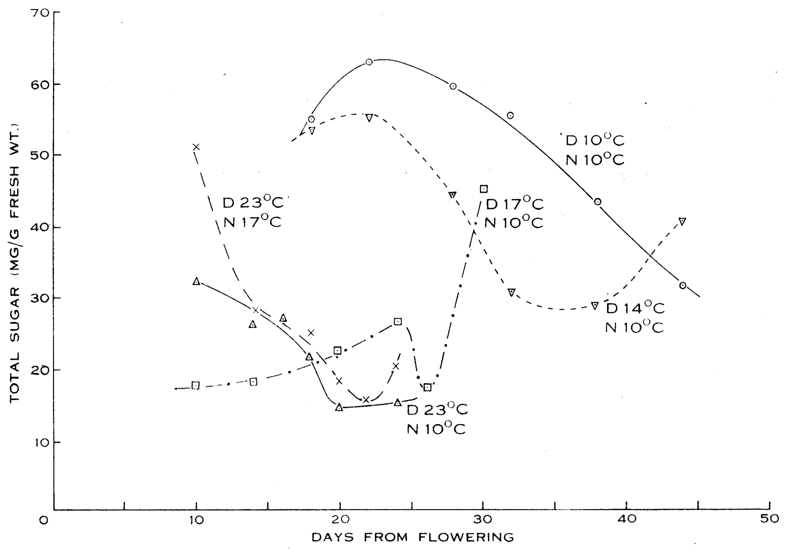

Fig. 11.—Total sugar content (on a fresh weight basis) during seed development; note the effect of drying out towards the end of development, particularly in curves for day 14°C and night 10°C and for day 17°C and night 10°C. Experiment 3. Conditions as for Figure 9.

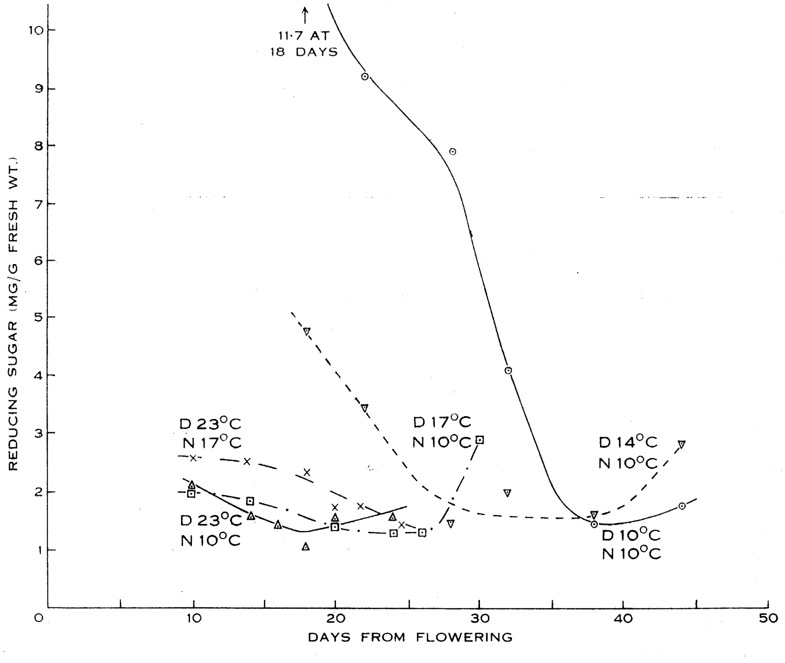

Fig. 12.—Reducing sugar content (on a fresh weight basis) during seed development; note the effects of drying out towards end of development. Experiment 3. Conditions as for Figure 9.

blossom, but with the day temperature of 14°C the starch began its rapid rise 18 days from blossom. Both these results contrasted markedly with those for higher temperatures, where the rapid rise in starch development had already begun at 10 days. The results for the starch analyses are shown in Figure 13.

Protein nitrogen in the seed showed long delay in its formation at low temperatures, as has been observed in the earlier experiments (Fig. 14). The concentration of soluble nitrogen, which at any time represented only a low fraction of that of the protein nitrogen, built up early and later fell, the fall being related to the increase in protein nitrogen, which was continuous and maintained at the expense of new nitrogen compounds imported into the seed.

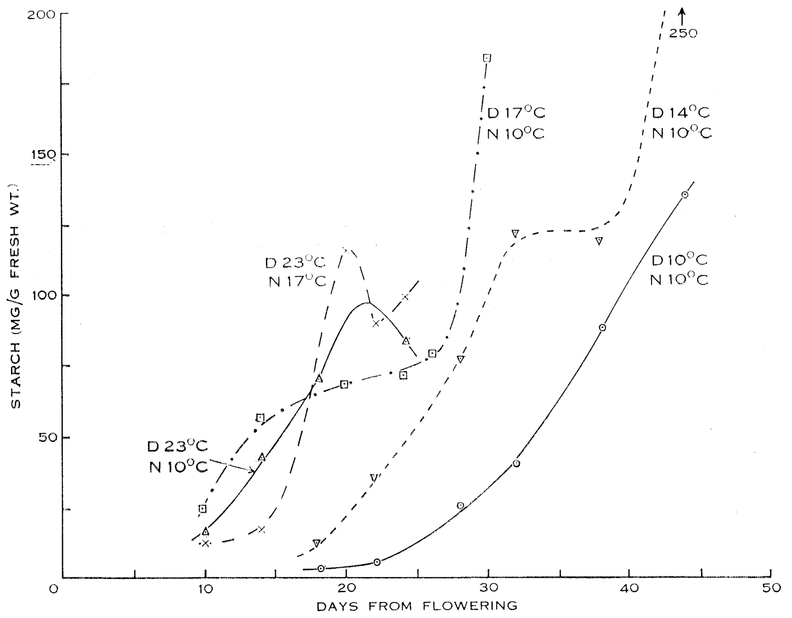

Fig. 13.—Starch content (on a fresh weight basis) during seed development; effects of drying out are seen towards end of development. Experiment 3. Conditions as for Figure 9.

IV. Discussion

The results of these experiments contribute to our knowledge of the processes which bring about synthesis in the seeds and their relation to each other. In earlier work (Bisson and Jones 1932; McKee, Robertson, and Lee 1955) it had been shown that the increase in sugar content of peas grown in the field occurred early in the growth of the seed, but this concentration of sugar was reduced when the starch synthesis occurred. The present work under controlled conditions confirms the results from analyses of peas grown in the field and adds the important information that the sugar content may build up very markedly during the early stages of development at low temperatures—the increase in sugar concentration remaining high in the seeds even though a considerable amount of starch is synthesized. Thus peas of a certain size, grown at low temperature, are completely different in carbohydrate composition from peas of a similar size grown at the higher temperatures. A spectacular difference can be observed in the third experiment between peas which had been grown in day temperature 14°C and night temperature 10°C and peas which had been grown with day temperature of 17°C and night temperature 10°C (Figs. 11–13). This marked difference emphasizes the important effect of microclimate on composition of peas grown under field conditions. From a practical point of view it is apparent that a difference of a few degrees in day temperature during the period when the pods are developing can have a profound effect upon the quality of the pea, since sugar content will be a measure of sweetness.

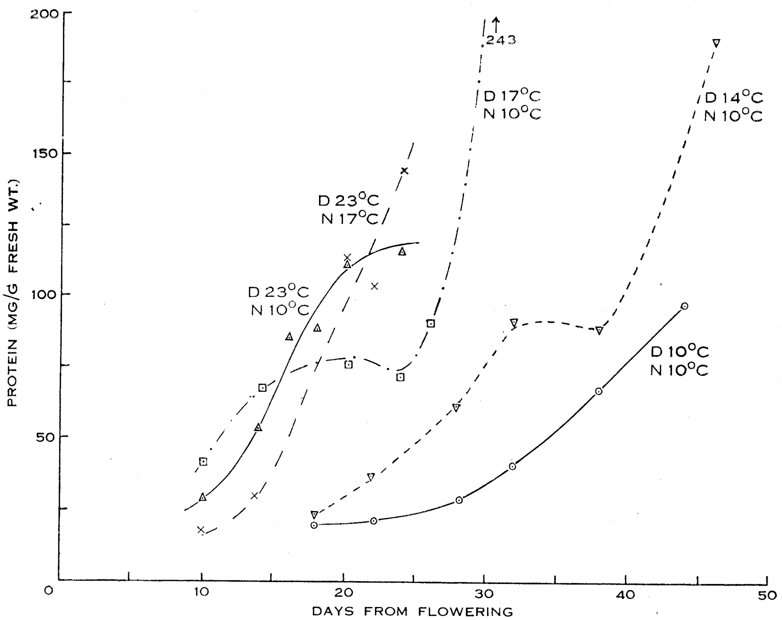

Fig. 14.—Protein content (on a fresh weight basis) during seed development; effects of drying out are seen towards the end of development. Experiment 3. Conditions as for Figure 9.

It is interesting to ask why the sugar content can build up so markedly at low temperature and the conversion to starch can be so long delayed. The amount of sugar synthesized, presumably in the leaves and translocated to the developing seeds, is not decreased in amount by the lower temperatures. On arrival in the seed, the sugar accumulates mainly as sucrose, but with a small amount of reducing sugar. In earlier work it had been shown (Turner and Turner 1957) that the increased rate of starch synthesis was correlated with an increase in the amount of starch phosphorylase. Further, in the period before the rapid synthesis of starch (Rowan and Turner 1957) hexose monophosphate increased but then decreased, along with the reducing sugars. These relationships suggested the possibility that the amount of enzyme governs the rate of starch synthesis and that the starch cannot be formed from its precursor glucose 1-phosphate until the starch phosphorylase has increased sufficiently. A possible interpretation of the results in this paper is that the low temperature delayed the synthesis of starch phosphorylase despite the fact that the sugar content of the seed had increased rapidly. However, we do not know whether the *in vivo* synthesis of starch takes place from glucose 1-phosphate or from uridine diphosphoglucose as suggested by the results of De Fekete, Leloir, and Cardini (1960). The complexities of temperature effects on sugar/starch conversions in plant tissue are illustrated by the work of Arreguin-Lozano and Bonner (1949) on potato tubers.

At higher temperatures the increase in sugar content is somewhat offset by the increase in respiration rate of the plant as a whole, and probably of the seeds in particular. This may have contributed to the smaller amounts of sugar which are reached in the early stages of growth, but no detailed information is available.

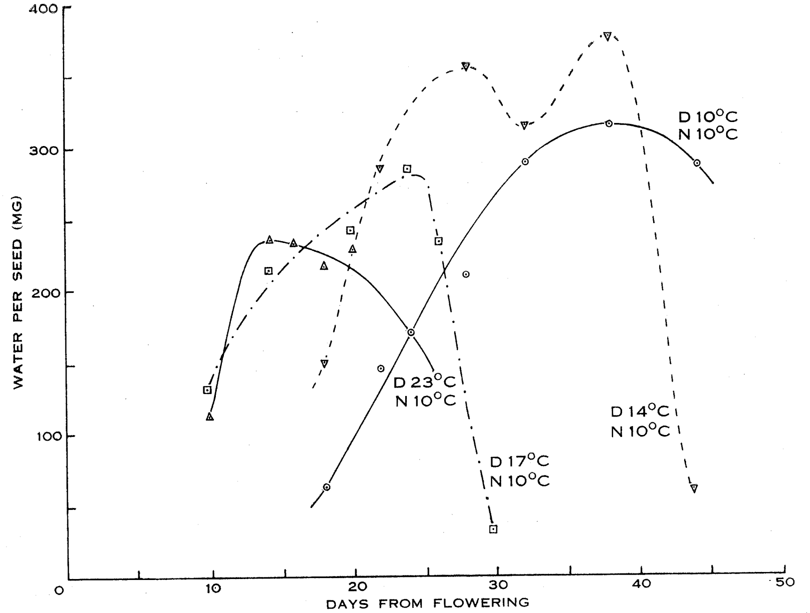

Fig. 15.—Water content per seed shows effects of drying out towards end of development. Experiment 3. Conditions as for Figure 9.

The increase in protein nitrogen is a synthetic process, but differs from the synthesis of starch because the precursors (presumably represented by the soluble nitrogen) are present in small quantity while the protein is being actively synthesized. In contrast to the starch, which increases at the expense of the sugar already present as well as by the conversion of the sugar being imported, the protein increase appears to be mainly due to the conversion of nitrogenous compounds as they enter the seed. This relation had been noted in earlier work. In the current experiments it is seen that, although the soluble nitrogen is a small fraction compared with the protein nitrogen, the delay of protein synthesis at lower temperatures allows more soluble nitrogen to accumulate in the seeds. The relative difference in soluble nitrogen between seeds of low temperature and high temperature is not nearly as great as the difference in total sugars.

Previous experiments (McKee, Robertson, and Lee 1955) had shown that the total water per seed was related to the total sucrose per seed and often ceased to increase at the time that the sucrose content began to fall. The same relation is apparent in these results (the total water content is plotted in Fig. 15). It can therefore be suggested that the interrelated processes of protein synthesis, enzyme synthesis, sucrose decomposition, and starch synthesis, all affect the stage at which the seed ceases to increase in water for osmotic reasons and presumably, since plants and seeds are losing water all the time, influences the rate of drying out of the seed. These observations may have a considerable bearing on the problems both of maturity and of quality in seeds of commercial crops.

V. Acknowledgments

This work arose from a suggestion of Professor Went, during a visit to Australia, for cooperation between the Earhart Laboratory of California Institute of Technology and the Plant Physiology Unit of C.S.I.R.O. Division of Food Preservation and Botany School, University of Sydney. The help of a number of people is acknowledged gratefully. We are specially grateful to Dr. J. F. Turner (Plant Physiology Unit) for his assistance in discussing the work and to Miss Jeanette Huber (Earhart Laboratory) who supervised the growth of the plants and samples for experiments 2 and 3.

VI. References

Arreguin-Lozano, B., and Bonner, J. (1949).—*Plant Physiol.* **24**: 720–38.

Bisson, C. S., and Jones, H. A. (1932).—*Plant Physiol.* **7**: 91–106.

De Fekete, M. A. R., Leloir, L. F., and Cardini, C. E. (1960).—*Nature* **187**: 918–19.

McKee, H. S., Nestel, L., and Robertson, R. N. (1955).—*Aust. J. Biol. Sci.* **8**: 467–75.

McKee, H. S., Robertson, R. N., and Lee, J. B. (1955).—*Aust. J. Biol. Sci.* **8**: 137–63.

Rowan, K. S., and Turner, D. H. (1957).—*Aust. J. Biol. Sci.* **10**: 414–25.

Turner, D. H., and Turner, J. F. (1957).—*Aust. J. Biol. Sci.* **10**: 302–9.

Went, F. W. (1957).—"Experimental Control of Plant Growth." 343 pp. (Vol. 17 of series.) (Chronica Botanica Publ. Co.: Waltham, Mass.)