

EFFECTS OF THE ENVIRONMENT OF THE MOTHER PLANT ON
GERMINATION BY CONTROL OF SEED-COAT PERMEABILITY
TO WATER IN *ONONIS SICULA* GUSS.*

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Summary

The time course of imbibition in seeds of *Ononis sicula* Guss. was more rapid when the mother plants were grown in 8-hr days (short-day seeds) than in 20-hr days (long-day seeds), outdoors as well as in a greenhouse. Once imbibed, seeds invariably germinated within 48 hr. Desiccated seeds from all these treatments entirely failed to imbibe, but when scarified they all imbibed and germinated fully within 48 hr. The level of seed-coat impermeability to water increased somewhat after storage for 2 years without changing the qualitative effects of day length. Differences in kinetics of imbibition due to outdoors *v.* greenhouse conditions were observed in long-day seeds only when freshly harvested, and in short-day seeds after storage for 2 years.

The results are interpreted in terms of differential environmental control over various partial processes of seed and pod maturation. The ecological implications of the results are discussed.

I. INTRODUCTION

Water-impermeable seed coats are the responsible factor in the delayed germination of many plants and are most prevalent in the Leguminosae.

This characteristic, termed "hard-seededness", is an inherited one, yet large-scale variations in its degree (i.e. percentage of hard seed) occur in seeds of the same species harvested in different localities or in different years (Loftus Hills 1944; Dexter 1955; Grant Lipp and Ballard 1964). Our own observations on seed germination and seedling establishment in the field under desert conditions in the Negev have shown that vast year-to-year fluctuations occur in the floristic composition of the seedling population. This phenomenon is at least partly due to the existence of specific environmental requirements for germination of certain species, or ecotypes. In some cases, these specific requirements may become modified by action of the environment which impinges upon the seed on or inside the soil long before germination, as in certain "hard-seeded" species, where weathering changes the water permeability of the seed coats (Koller and Negbi 1962, 1963, 1965). In other cases the specific requirements may become modified even earlier, i.e. by the environment of the mother plant (Loftus Hills 1944; Lona 1947; Harrington and Thompson 1952; Von Abrams and

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Hand 1956; Koller 1956; Jacques 1957; Delouche 1958; Heslop-Harrison 1959; Enileev and Solovev 1960; Pollock 1961; Evenari 1965a, 1965b). In one instance (*Chenopodium amaranticolor*) this effect was attributable to "characters acquired by the seed coat" (Lona 1947).

The aim of the present study was to find out if the environment to which the mother plant had been exposed might affect hard-seededness. *Ononis sicula*, a desert annual, was selected as the experimental plant. Large year-to-year differences were observed in the occurrence of this species in the Negev highlands (Evenari, unpublished data). The species may apparently be classified as having facultative (quantitative) long-day responses with respect to flowering (Evenari and Gutterman 1966).

II. MATERIALS AND METHODS

Mature seed pods of *Ononis sicula* were collected in Wadi Migra, in the foothill region of the Negev highlands, during April–May 1961. The experiments were carried out at the Hebrew University in Jerusalem. Climatological data are available in meteorological tables.*

Plants were grown in No. 10 flower pots containing terra-rossa soil, Negev loess soil, sand, and humus in the proportions (by volume) 5 : 1 : 2 : 2. Scarified seeds were sown on top of moist soil and covered until germinated by a disk of thin polythene, 65 mm in diameter. The plants were watered to maintain soil moisture as close as possible to field capacity. The plants were gradually thinned out until only 1–3 remained in each pot at time of flowering. The plants grown in short days, indoors and outdoors, are referred to as "S.I." and "S.O.", respectively. Similarly, plants grown in long days, indoors and outdoors, are referred to as "L.I." and "L.O.", respectively. Short-day conditions were obtained by exposing the plants daily to 8 hr natural light (8 a.m.–4 p.m.). In the greenhouse, this light was filtered through the glass and the 16-hr dark period was spent in a temperature-insulated dark chamber. Outdoors, the natural light was unfiltered and the 16-hr dark period was spent under a light-tight tent, made of black plastic fabric. The dimensions of this tent were greater than those of the chamber, to minimize possible effects of heating by the uninsulated walls. Long-day conditions were obtained by extending the natural day length to 20 hr with artificial light in such a way that the plants were kept in darkness from 11.30 p.m. to 3.30 a.m. In the greenhouse, the light source was mixed (incandescent and fluorescent lamps, totalling 600 and 240 watt, respectively), providing 80–90 f.c. light intensity at plant level, from a height of 100 cm. Outdoors, only incandescent flood lamps with internal reflectors were used and provided 80–100 f.c. light intensity from a height of 200 cm. Intensity of natural light in the greenhouse was 50–60% of that outdoors, which reaches maximal values higher than 12,000 f.c. To compensate for positional effects, the positions of the plants were randomly reshuffled at intervals. Records of maximal and minimal temperatures in each of the growing conditions were kept (Evenari and Gutterman 1966). In the greenhouse, differences in maxima and minima between short-day and long-day conditions

* Monthly Weather Report, Meteorological Service, Israel Ministry of Transport and Communications, 1963.

did not exceed 1 degC, while outdoors they did not exceed 2 degC. Differences in maxima and minima between indoor and outdoor conditions did not exceed 3 degC. For physiological uniformity, seed were harvested at the identical phenological stage, just prior to the dehiscence of the fruit, which takes place explosively within 3-4 days of yellowing of the pods.

TABLE 1
ENVIRONMENTAL EFFECTS ON ONSET OF FLOWERING AND ON TIMING OF SEED HARVEST
Plants sown January 29, 1963

Environment	Photoperiod (hr)	First Flowering Node*	Harvest (days from planting)		
			First	Main	Last
Greenhouse	8	19.0 ± 0.0†	126	146	150
Greenhouse	20	7.7 ± 0.5	78	86	131
Outdoors	8	7.0 ± 0.7	115	149	149
Outdoors	20	4.8 ± 0.3	89	103	115

* Means ± S.E. of seven replicates.

† Harvests were made at frequent intervals, just prior to pod dehiscence. Main harvest was the one which contained the largest number of seeds.

‡ Only three replicates.

III. RESULTS

The experiment was started on January 29, 1963. The plants reached full maturity and formed ripe fruit and seeds under long- and short-day conditions, in the greenhouse as well as outdoors, but plant development and the topographical position and time of appearance of the first flower buds differed very much in the different treatments.

All plants produced between four and eight juvenile leaves on the main shoot before branching. In L.I., S.O., and L.O. plants the first flower buds appeared on the main shoot, starting generally with the axils of the first or second trifoliate leaf. Accessory axillary buds on the main shoot then developed into first-order branches carrying axillary flowers. Second-order branching was rare. S.I. plants produced only vegetative first-order laterals in leaf axils of juvenile leaves and of adult trifoliate leaves up to the 19th node. These bore numerous second-order branches. Flower buds appeared simultaneously on the 19th node of the main shoot and generally on the 7th node of the first-order branches.

Both onset and completion of fruit ripening were markedly hastened in long days, as compared with short days, but differences between plants grown outdoors and in the greenhouse were small. These data are summarized in Table 1. Additional information is given by Evenari and Guterman (1966).

The seed populations from the various treatments did not vary in seed dimensions by more than ±5%, but while those obtained from L.I. and L.O. plants were

nearly exclusively composed of yellow seeds, the S.I. population contained more greenish seeds than yellow ones and a small percentage of brown seeds. S.O. plants, however, produced many more brown than yellow and greenish seeds. Yellow seeds weighed slightly but consistently more than greenish ones, while brown seeds weighed much less than either (Table 2). The percentage of the differently coloured seeds per seed population was not only a function of the treatment but also of the time of harvest. In all treatments (in which this property was followed) the fraction of yellow seeds decreased and the fraction of brown and greenish ones increased, the older the plant and the later the harvest (Table 3).

TABLE 2

EFFECTS OF THE ENVIRONMENT OF THE MOTHER PLANT ON COLOUR, WEIGHT, AND SIZE OF THE SEEDS
AT TIME OF THE MAIN HARVEST

Environment	Photo-period (hr)	Approx. No. of Seeds Harvested	Seed Colour	Seed Weight (mg)	Seed Length (mm)	Seed Width (mm)
Greenhouse	8	1250	Yellow	0.57 ± 0.03	1.07	0.90
			Greenish	0.51 ± 0.04	1.04	0.89
			Brown	0.35 ± 0.02	1.07	0.94
Greenhouse	20	2500	Yellow	0.61 ± 0.03	1.11	0.92
			Greenish	*	*	*
			Brown	*	*	*
Outdoors	8	300	Yellow	†	1.02	0.86
			Greenish	†	1.03	0.89
			Brown	0.23 ± 0.04	1.01	0.86
Outdoors	20	1650	Yellow	0.62 ± 0.03	1.11	0.91
			Greenish	0.53 ± 0.04	†	†
			Brown	0.23 ± 0.05	†	†

* Not present in main harvest.

† Not measured.

Tests of seed coat permeability to water were made on September 9, 1963 (expt. I), and again two years later, on September 9, 1965 (expt. II), by imbibing at 20°C, at which temperature scarified seed of the parent generation germinated fully within 48 hr. The seeds were stored from harvest in polythene bags at room temperature in darkness. Only seeds of the main (i.e. largest) harvest were used in these tests. In experiment I, the course of attainment of seed-coat permeability and subsequent germination was followed in quadruplicate samples of 25 seeds during 84 days. Despite the small size of each sample, differences between replicates were quite small. In this experiment, seeds invariably germinated within 48 hours of imbibition. The results in Figure 1 therefore show that seeds of plants grown in short days attained permeability at a much more rapid rate than those grown in long days. In seeds produced in short days, no marked differences in the time course of attainment of

permeability were observed, which could be related to greenhouse or outdoor conditions. In seeds produced in long days, permeability characteristics appeared to be affected by their being grown outdoors or in the greenhouse. In the former, a small proportion of the population imbibed soon after sowing and this proportion increased only slightly with time. In the latter, no imbibition occurred for a considerable time, but after this initial lag-phase the percentage of imbibing seeds increased at about the same rate as in seeds produced on plants growing in short days.

TABLE 3

DISTRIBUTION OF YELLOW, GREENISH, AND BROWN SEEDS IN THE SEED POPULATION, AS AFFECTED BY AGE AT HARVEST AND BY ENVIRONMENT OF THE MOTHER PLANT

Mother plants planted January 29, 1963

Environment	Photo-period (hr)	Approx. No. of Seeds Harvested	Age at Time of Harvest* (days)	Distribution of Seed Population*		
				Yellow Seeds	Greenish Seeds	Brown Seeds
Greenhouse	8	900	126	52	46	2
		1250	146	31	59	10
Greenhouse	20	700	78	100	< 1	0
		150	131	86	4	10
Outdoors	8	300	149	17	42	41
Outdoors	20	1650	89	97	2	1
		150	115	64	18	18

* Entire population of indicated harvest counted.

Concurrently with this experiment, seeds harvested from all treatments were desiccated over granulated CaCl_2 for a period of 6 days, after which they were tested for permeability as described. Not a single seed had imbibed within 67 days after sowing. All these seeds were fully viable and germinated promptly and equally (within 48 hr) after scarification.

In experiment II, the coat permeability of seeds of each colour was tested separately. In Figure 2, the kinetics of imbibition of the greenish and yellow fractions are presented by the time course of germination. Those of the brown-seeded fractions are not shown in Figure 2, because though nearly all of them had imbibed, only few germinated and most of them decayed. This fraction must have deteriorated in storage, as the data in Figure 1 show that S.O. seeds reached 90% germination, even though about 40% of them were brown (Table 3). Fungal hyphae were found between the seed coat and embryo of the dry brown seeds.

The results in Figure 2 show that four groups are distinguishable on the basis of the kinetics of imbibition. One group consists of the yellow fraction of the L.O. and L.I. seeds, whose germination did not exceed 5%. Another group consists of both

greenish and yellow fractions of S.O. seeds, where most of the overall germination took place rather early, and then levelled off. The third group consists of both greenish and yellow fractions of S.I. seeds, whose germination increased gradually throughout the experiment. The last group is represented only by the small fraction (about 2%)

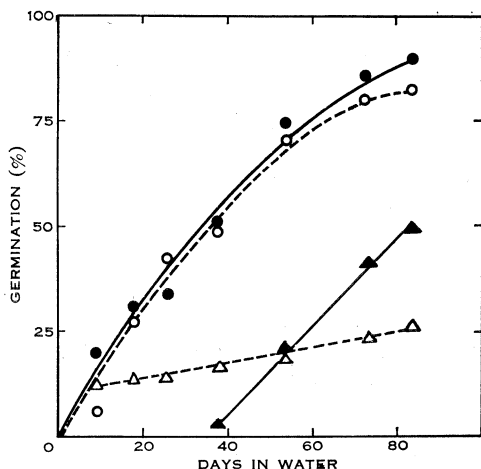


Fig. 1.—Time course of germination of intact seeds of *Ononis sicula* grown in a greenhouse for photoperiods of 20 hr (▲) and 8 hr (●), and also for intact seeds grown outdoors for photoperiods of 20 hr (△) and 8 hr (○).

of greenish L.O. seeds, whose behaviour resembled that of both greenish and yellow fractions of the S.O. seeds, though final percentages of germination were somewhat higher. No greenish L.I. seeds were available for comparison, as the small fraction of green seeds produced indoors in long days were not found in the main harvest (Table 2).

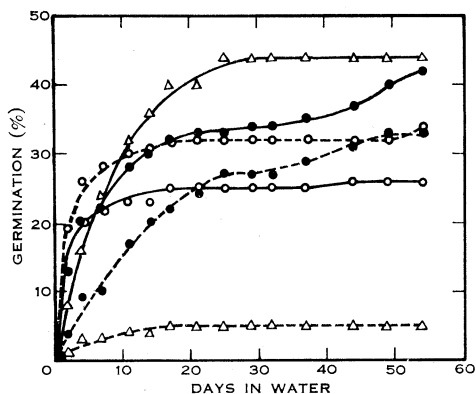


Fig. 2.—Time course of germination of the greenish (—) and yellow (---) fractions of the seed population harvested from mother plants grown in the greenhouse for a photoperiod of 8 hr (●) and for similar fractions of the seed population grown outdoors for photoperiods of 8 hr (○) and 20 hr (△). Seeds grown in the greenhouse for photoperiods of 20 hr did not germinate. Seed tested 2 years after the first experiment.

When differences in colour are disregarded, and the results of the different fractions are pooled, as was done in experiment I, the curves representing the course of germination of each population (Fig. 3) are lower than the corresponding ones in experiment I (Fig. 1), but behaviour of the S.I. seeds is typified by continuous increase in imbibition, while that of S.O. seeds is typified by a rapid increase in imbibition, followed by early cessation.

As in experiment I, seeds were desiccated for 6 days and their germination was tested concurrently with that of non-desiccated ones. None of the desiccated seeds, except the brown fractions, became imbibed. The latter were entirely unaffected by desiccation.

IV. DISCUSSION

In the present experiments, all *O. sicula* seeds germinated within 48 hr of the first visible signs of swelling, with the exception of the brown seeds, which became non-viable after storage for 2 years and decayed after imbibition. Seed coat impermeability to water therefore plays a major role in the control of the germination of this species. There is considerable evidence that in hard-seeded legumes the outermost layers of the seed coat are responsible for impermeability and that water enters the

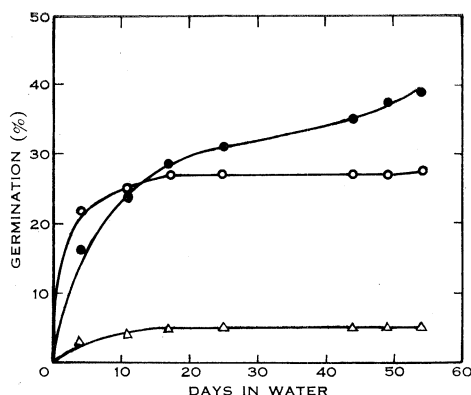


Fig. 3.—As for Figure 1, but for seed tested 2 years later (greenish and yellow seeds only).

seed through distinct pores in these layers. This water is taken up by hydrophilic colloids in the internal tissues, which thereupon imbibe and swell. It is very likely that this swelling in turn causes a progressive decrease in coat resistance to passage of water, by stretching of the impermeable layer. If this analysis is correct, uptake of water into those seeds which eventually imbibe starts immediately upon soaking and the start of visible swelling represents a very late stage. Consequently, the apparent rate of swelling of the population reflects the distribution of initial coat resistances to passage of water in the individual seeds which make up the population.

The degree of seed coat impermeability to water in *O. sicula* seeds was strongly affected by the conditions under which the mother plant had grown. In general, plants grown throughout in short days produced seed populations which, when soaked, took up water more readily than those produced on plants grown throughout in long days (Figs. 1 and 3).

Noteworthy differences between greenhouse and outdoor conditions were observed in seeds from the long-day conditions in experiment I and in seeds from the short-day conditions in experiment II. In both these cases, germination of greenhouse-grown seeds continued to occur at a more rapid rate (L.I. v. L.O. in Fig. 1) or for a longer time (S.I. v. S.O. in Fig. 3).

These results lead us to distinguish several processes in the maturation (or ripening) of the seeds of *O. sicula*. The first of these is concerned with maturation of the embryo itself, at least with respect to germinability. It appears that these processes are unaffected by either photoperiod, or indoor *v.* outdoor conditions, as in all cases germination of viable seed stocks took place within 48 hr of visible swelling. Treatment-dependent differences which may have been expressed in rates of germination or in seedling vigour were not studied. Immaturity of the embryo is only indicated in brown seeds by their low capacity to maintain viability in storage, and their high susceptibility to fungal attack. Furthermore, where comparative values of seed dry weight were available, the brown seeds were much lighter than all other categories, though their dimensions were comparable (Table 2). The proportion of these seeds did not appear to be affected by treatment, but in all treatments it increased as the plants became older. If this is correct, embryo maturation is independent of environment within the experimental conditions, but is adversely affected by plant age at time of seed set (cf. Grant Lipp and Ballard 1964).

Another set of processes of maturation in these seeds is concerned with the seed coat, and more specifically with the development of the property of impermeability. These processes appear to be most strongly affected by photoperiod, and to a lesser degree by indoor *v.* outdoor conditions. The effects of indoor *v.* outdoor conditions appear to differ from those of photoperiod not merely by the magnitude of the effect, but by the very nature of the processes which are affected. Thus, while photoperiod appears to determine the proportion of the population with fully mature (i.e. impermeable) coats, the complex environmental differences between outdoor and indoor conditions appear to determine the rate of coat maturation of individual seeds. The gradually continuing imbibition which was so typical of seeds grown indoors is interpreted as resulting from coats whose resistance had almost, but not quite, reached its final (infinite?) value.

The third process of maturation also takes place in the seed coat, and is concerned with coloration. Disregarding the brown fraction of the seed population whose coat colour could not be determined, it seems that as the coat matures, its colour changes from greenish to yellow. However, the processes which control the change in coat coloration appear to be independent of those which control the attainment of coat impermeability. This appears from the results in Figure 2, which show that in seeds from both short-day conditions (S.O. and S.I.) nearly the same course of imbibition was followed by green- and yellow-coated seeds, while those from L.O. conditions differed greatly; i.e. the course of imbibition of the green-coated seeds was much higher and the yellow much lower than the corresponding short-day grown seeds.

Changes occurred in the imbibitional pattern of the seeds during the time which passed between experiments I and II. These changes may be explained on the basis of moisture content of the seed colloids. The fact that in both experiments a relatively short but drastic desiccation caused complete impermeability (except in brown seeds), without affecting viability, may have resulted from loss of moisture from the interior of the seed, which in turn increased, to infinity, coat resistance to passage of water. Whether or not this mechanism involves a hydrodynamic valve, as described by Hyde

(1954) for *Trifolium repens*, *T. pratense*, and *Lupinus arboreus*, remains to be seen. On the basis of the results with desiccated seeds, the changes which occurred between the two experiments may have resulted from a more prolonged, but less drastic desiccation during storage. This not only reduced permeability in all treatments, but increased the low coat resistances in seeds from short-day plants so that differences between outdoor and indoor conditions became evident, and analogously eliminated differences in resistance between indoor and outdoor conditions in the long-day plants. The ineffectiveness of desiccation with respect to the brown seeds supports the view of the immaturity of their coats, as well as of their embryos, despite their having reached normal dimensions.

The time interval from harvest till the imbibition tests differed in the various treatments, and this may have contributed to the differences in the kinetics of imbibition by affecting the degree of desiccation. However, as the differences remained qualitatively unchanged during two years of storage, the magnitude of these effects could not have been very large.

The results show that in *O. sicula*, effects of the environment to which the mother plant is exposed, on the germination behaviour of its seed progeny, operate by affecting seed coat permeability to water. Of the various environmental factors, the one which was controlled in the present experiments and the one which also produced the major effects, was day length. As to the way by which day length affects coat permeability, it appears that long-day conditions cause the developmental processes of the seed to overtake those of the fruit, so that pods dehisce and seeds are shed when entirely mature. Short-day conditions, on the other hand, cause developmental processes of the seed to fall behind those of the fruit, so that pods dehisce and seeds are shed before complete maturation of the coat (coloration and permeability) of the entire population.

In conclusion, the vast year-to-year differences in occurrence of *O. sicula* in the Negev highlands are quite probably determined primarily by the environment to which the mother plant is exposed, i.e. the season during which it had grown, secondly by the degree of desiccation after seed dispersal, thirdly by certain as yet undefined field conditions which might increase permeability prior to rainfall (Hyde 1954; Koller and Negbi 1962), and lastly by the conditions impinging on the imbibed seed.

The environmental mechanism which was described above and which may play a part in determining the degree of abundance of *O. sicula* in nature may have its counterpart in other hard-seeded legumes.

A possible practical outcome of this study is that it indicates growing practices by which the level of hard-seededness may be controlled during seed production, e.g. by selection of growing area and season.

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