Abscisin II: Inhibitory Effect on Flower Induction in a Long-Day Plant

Abstract. *The flowering response by plants of* Lolium temulentum *after exposure to 1 long day was significantly reduced by single applications of abscisin II to the leaves or near the shoot apex. The time course of this inhibitory effect suggests that abscisin II acts at the shoot apex when the floral stimulus arrives there.*

Abscisin II, a compound of known structure isolated from young cotton bolls (*1*), accelerates petiole abscission in cotton explants and inhibits growth of the oat coleoptile (*2*). It is identical with dormin extracted from leaves of sycamore and birch in increased amount under short-day conditions (*3*), which induces the formation of resting buds when it is applied to birch plants under long-day conditions (*4*).

In the long-day plant *Lolium temulentum* there is evidence of an inhibitor of flower induction, which is exported from leaves in short-day conditions, and acts at the shoot apex in competition with the floral stimulus translocated from leaves exposed to long-day conditions (*5, 6*). The nature of this inhibitor is unknown. In view of the spectrum of biological activity of abscisin II, and the fact that short-day conditions increase the amount of it in leaves, its effect on flower induction in *L. temulentum* was examined.

Plants were grown for 6 weeks in 8-hour photoperiods at 25°C during the day and 20°C at night. All tillers and all leaves except the sixth and seventh on the primary shoot were removed, and the plants were exposed to 1 long day by extending the 8-hour period in daylight with 16 hours of incandescent light (intensity, 660 lu/m^2). The plants were then returned to short days until dissection 3 weeks later. In two experiments random lots of 12 to 14 plants were treated with an aqueous solution of abscisin II (*7*) at various times during photoperiodic induction. Most treatments were made by injection of 0.1 ml of solutions, ranging in concentration from 0.25 to 5 μg/ml, inside the leaf sheaths close to the shoot apex. In two treatments in the second experiment a solution of 1 μg/ml of abscisin II (with 0.05 percent Tween 20) was sprayed over the leaves at the rate of 1 ml per plant. Long-day controls were injected with water or sprayed with the Tween solution. Other groups of plants were transferred to a darkroom at 20°C at various times during the photoperiod extension for determination of the critical photoperiod. Still other plants had their leaves cut off at various times to determine the time of translocation of the floral stimulus out of the leaf blade.

Figure 1 illustrates the results of injections of the abscisin solutions (concentration, 1 μg/ml) for both experiments. The curves for photoperiod response and translocation are from the second experiment; similar curves were obtained in the first experiment. Due to differences in seasonal light conditions, the magnitude of the flowering response differed in the two experiments, but the course of the effect of abscisin was remarkably similar. Applied at or near the beginning of the low-intensity photoperiod extension, abscisin II had no effect on induction, and none when applied 2 days later. On the other hand, all applications between 11 p.m. on the long day and 4 p.m. on the following day were significantly inhibitory, at $P < .001$ to $< .01$. Not only the length of the apex but also the stage of morphological development of the apices at dissection was reduced by applications of abscisin at these times. In the first experiment, flower initiation in some plants was almost prevented by injections of abscisin II at the end of the photoperiod extension, some apices having barely discernible double ridges at dissection, whereas all long-day control plants had differentiated lemma primordia. In both experiments, after injections made at this time, the inhibitory effect increased progressively with an increase in the concentration of abscisin from 0.25 to 5 μg/ml.

Applications to the leaves, which gave each plant 10 times more abscisin than injections inside the leaf sheaths, were only slightly more inhibitory than injections made at the same time. This, together with the fact that applications were highly inhibitory even when made after the critical photoperiod had been passed and some stimulus had been translocated out of the leaves, suggests that abscisin was acting against induction at the shoot apex, rather than against the long-day processes in the leaves. The short-day inhibitor in *L. temulentum* was deduced to act in the same way (*5*). Since applications made at the beginning of the photoperiod extension had no effect, while those made 8 to 12 hours later were highly inhibitory, the applied abscisin II was presumably dissipated in this interval. A similar conclusion was also drawn for the short-day inhibitor in *L. temulentum*.

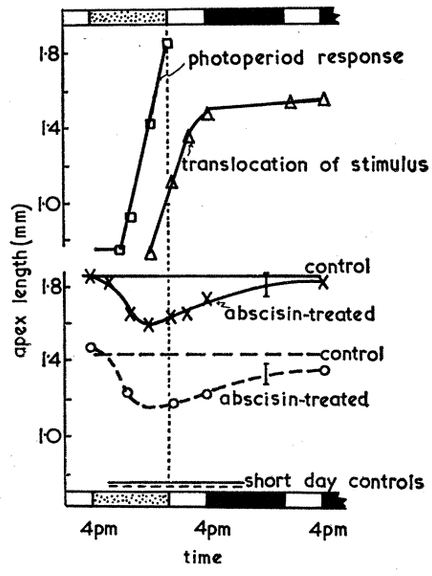

Fig. 1. Effect of the time of injection of 0.1 ml of abscisin II (1 μg/ml) per plant ($\times$ and $\bigcirc$) on the flowering response by plants of *L. temulentum* exposed to 1 long day. Solid lines present data from the second experiment ($\times$, $\square$, and $\triangle$); broken lines, the effect of abscisin treatments ($\bigcirc$) in the first experiment. The upper part of the figure indicates the timing of the photoperiod response ($\square$) and of the translocation of the floral stimulus ($\triangle$) in the second experiment, respectively determined by moving plants to darkness or by cutting off their leaf blades at the times indicated. Mean standard errors for the two experiments are indicated by the vertical lines. Flowering response is given as mean apex length at dissection 3 weeks after the long day, which is indicated by the stippled portion of the horizontal time scale.

Besides accelerating abscission, inducing bud dormancy, and inhibiting coleoptile growth, abscisin II also inhibits flower induction in a long-day plant. In this latter case it acts, in several respects, in the manner of the short-day inhibitor. It is not yet known whether plants of *L. temulentum* produce abscisin and whether abscisin is identical with the short-day inhibitor.

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References and Notes

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Food Imprinting in the Snapping Turtle, Chelydra serpentina

Abstract. *Three groups of hatchling snapping turtles, totaling 20, were fed either meat, fish, or worms. When they were tested for preference after 12 daily feedings, each preferred the diet to which it was accustomed. After 12 more days of eating a different food, each still preferred its original diet. A form of imprinting may be operative in the feeding behavior of this species.*

Imprinting, extensively studied in precocial birds, differs significantly from association learning (*1*). This conclusion derives from work with imprinting to a parental object or with social imprinting. Recent work indicates the existence in birds of two other forms of imprinting: environmental or habitat imprinting, and food imprinting (*1, 2*). The latter form was demonstrated in chicks in which the innate pecking preference for a certain color and shape was modified, through reinforcement of pecks, to another color and shape; such modification could occur only at a specific age.

We investigated the existence of food imprinting in turtles, using a different criterion for imprinting: the primacy of early experience. Imprinting has never been reported in turtles or in any other reptile. Turtles are even more precocial than birds; they are completely independent upon hatching, even of parental care or attention, so that social imprinting is unlikely. Food imprinting, however, could be as adaptive in turtles as in birds. Study of imprinting in a species from which the possibility of classical imprinting is absent may help one to understand the basic mechanisms underlying all imprinting.

Twenty snapping turtles (*Chelydra serpentina*) were hatched in captivity in two clutches (*3*). Turtles 1 to 15 and 20 (Table 1) were from a clutch found in Illinois; their mean weight was 10.8 g. The eggs were incubated, with the dirt and sand mixture in which they were found, in a large jar. Turtles 16 to 19 were from eggs removed from a dead female collected in Texas; they were kept first between sheets of moist paper toweling and then in sand; their mean hatched weight was 13.5 g. The incubation media were kept moist, and the air temperature varied from 27° to 30°C by day to somewhat less by night.

Snapping turtles when hatched have an external yolk sac and will not eat for the first few days, being capable of long fasts before the first meal. Animals 1 to 16 and 18 to 20 were approximately 10 days old when the experiment began; No. 17 was 4 days old.

After hatching, most of the turtles were kept in 3 cm of water in several 16-liter aquariums covered with black cloth. After a week they were transferred to individual all-glass tanks measuring 23 by 14 by 17 cm. The exceptions, turtles 17 to 20, were placed in the individual tanks as soon as they were discovered. Each turtle was visually isolated from its neighbors by cardboard partitions. Throughout the experiment each tank contained 2 cm of aged tapwater. The turtles were washed off and the tanks were cleaned and refilled with aged water after the first six feedings, before the first test, after six feedings on a new food, and before the final test.

The turtles were divided into three groups containing 7, 6, and 7, respectively. Turtles in group 1 were fed daily with lean, finely ground horsemeat rolled into balls about 7 mm in diameter; those in group 2 received daily a female guppy (*Lebistes reticulatus*) about 1.7 cm long; and those in group 3 were fed daily a piece of redworm (*Eisenia foetida*) averaging 1.5 cm in length. The weight of each meal was approximately 75 mg. All food was nonmoving; guppies and worms were prekilled by immersion in water at 55°C.

During feeding, a 15- by 20-cm sheet of aluminum was placed between the turtle and the center of the tank where the food was placed with forceps. Because the metal was wider than the tank, which it split diagonally, the turtle was restrained in one corner. With the food in position, the sheet was removed and the animal was allowed to search for and eat it. Once it began to eat, no turtle ever refused its food. After 12 such feedings each turtle was tested for preference by offering it all three foods.

The test procedure was the same as in the regular feedings except that all three types of food were placed in the center of the tank in a straight line parallel with the diagonal metal sheet hiding the turtle; the foods were placed 2 cm apart and the order of placement was systematically varied from turtle to turtle. Almost invariably the turtle was about equidistant from all three foods when it began to head toward one; it was given 10 minutes to make its first choice and then 10 more minutes to make a second choice.

The results (Table 1) show a general first preference for the original diet, which was chosen by 16 ($p < .00003$, the binomial test was used for all statistics; P, .33). After this test, the turtles received 12 feedings on a different food: group 1, originally fed horsemeat, received worms; group 2 (fish) were given worms; and group 3 (worms) were fed meat. Only 11 feedings were given if the first or second choice in the first test was the food to be given during the second series of feedings. After this second series the choice situation was repeated (Table 1), with each order of presentation different from that used in the first test.

Again there was strong preference for the food originally eaten, which was chosen by 16 ($p < .00003$). Even the results for two of the subgroups are significant, which is surprising because of the smallness of the groups (group 1, $p < .001$; group 3, $p < .05$).

The results seem best interpreted in terms of imprinting since the primacy of the early experience has been clearly shown. Although the limited number of turtles available precluded testing for food preference before any prior feeding, the experimental results and informal observations indicate that chopped horsemeat was the most preferred of the three foods. All subjects originally fed horsemeat preferred it on test 1. Turtles that were not fed horsemeat originally invariably took it in test 1 when the originally fed food was not chosen. And no turtle originally fed horsemeat changed its choice. This preference is probably due