Interaction of Plant Hormones

Abstract. *The usage of the word* interaction *by physiologists has led to a consensus that gibberellin and abscisin interact in affecting plant responses. However, no such consensus is admissible if one uses the statisticians' definition which is more physiologically sound than that used by physiologists.*

Statisticians define interaction as the failure of a response to one agent to be the same at different amounts of a second agent (*1*). Graphically this means that a plot of response against agent A at two amounts of agent B will yield two curves that are not equidistant from each other in the direction of response at every value of A. For no interaction the curves would be equidistant and, when response is a single-valued function of A, parallel.

If one were to impart an upward vertical velocity to a ball and to measure the peak height attained, the peak would describe a parabola when plotted against various initial velocities. If the ball were propelled from a platform, the peak height would depend on the platform height as well as the initial velocity. However, the two agents, platform height and initial velocity, would be acting independently and would produce independent effects. The parabolas obtained at two platform heights would be parallel to one another. According to the definition of interaction above, it would be incorrect to say that there was an interaction between platform height and initial velocity, or that lowering the platform height reversed the effect of initial velocity. Since the case of no interaction is one of rigid uniformity, accidental anomalies in an experiment will tend to mask this uniformity and appear as evidence of interaction.

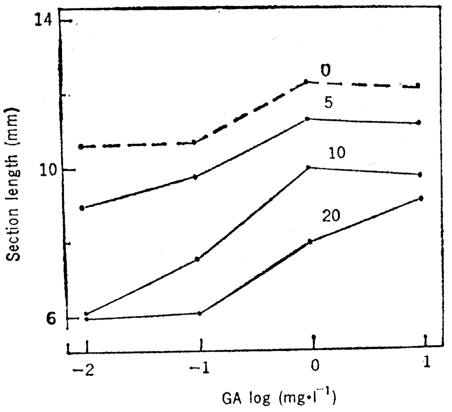

Fig. 1. "Interaction between the inhibitor from nonviable rice seeds and GA_3 in wheat coleoptile section test. All sections received 10.0 mg/l of IAA. Abscissa: log concentration of GA. Curves: 0, control; the others relative concentration of inhibitor." [Reproduced with permission of the editors of *Physiologia Plantarum* (*3*, figure 4)].

Physiologists are inclined to define interaction as the relationship between two agents which affect the same response in algebraically opposite ways. Consequently each agent is said to reverse the effect of the other. The outcome of applying this definition to the example above depends on the algebraic sign one applies to platform height. Lowering the platform results in interaction with initial velocity and the reversal of the effect of initial velocity. Raising the platform results in no interaction, but it would involve a promotion of the effect of initial velocity, if consistency in the use of terms were maintained. However, two agents affecting the same response in algebraically the same way are said to act independently if their effects are additive, while each is said to promote the effect of the other only if their effects are synergistic.

It should be noted that the statisticians' definition of interaction is not statistical, while the physiologists' definition is not physiological. Rather, both definitions are in terms of analytical geometry.

The application of the physiologists' definitions to the action of gibberellin and abscisin on various plant responses has led to a consensus that gibberellin and abscisin interact and reverse the effect of one another (*2–5*). This consensus is fully in accord with these definitions. However, no such consensus is warranted by the data presented when these are evaluated with respect to the statisticians' definition.

Thomas *et al.* (*5*) reported that *Avena* coleoptile section length was affected by gibberellin concentration in the presence of but not in the absence of abscisin. Their data when charted thus consisted of two nonparallel curves. The synthesis of α-amylase by barley endosperm measured in terms of sugar concentration or logarithm of sugar concentration, when plotted against gibberellin concentration also failed to yield parallel curves with different abscisin concentrations (*5*). These results thus indicate interaction by the statisticians' definition.

In contrast, section length of wheat coleoptiles plotted against the log of gibberellic acid (GA) concentration yielded a set of essentially parallel curves at different levels of an abscisin-like factor from rice seeds (Fig. 1) (*3*). The length obtained at the values, inhibitor = 10, log GA = −2, would probably best be ascribed to an accidental experimental anomaly. Also, the response of α-amylase synthesis by aleurone layers of barley to gibberellin and abscisin yielded a set of approximately parallel curves in a study of hormonal control of enzyme synthesis (Fig. 2) (*2*). Using the statisticians' definition, inspection of these two sets of data as presented by the authors admits of only two conclusions: (i) insufficient information was obtained, or (ii) gibberellin and abscisin do not interact in these two phenomena.

For the sake of clarity, statisticians and physiologists should use the same definition of interaction, namely, the statisticians' definition. This would necessitate further examination of effects of gibberellin and abscisin in plant responses already studied, but this is beside the main point. The primary value of the statisticians' definition lies not in its lack of ambiguity but in its physiological insight. If two chemicals do not interact with respect to a given physiological response, one may prop-

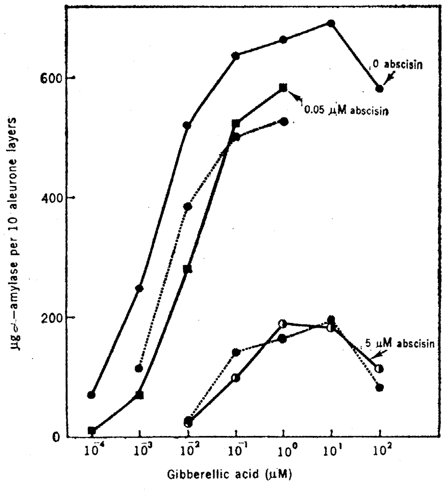

Fig. 2. This figure is adapted from Table 2 of Chrispeels and Varner (*2*). The dashed-line curves, that I have added, are based on the 0-abscisin data reported, and represent curves of no interaction by the statisticians' definition. "Reversal by Gibberellic Acid of the Abscisin Inhibition of α-Amylase Synthesis. Ten half aleurone layers were incubated in buffer with 20 m*M* $CaCl_2$ and the concentrations of GA and abscisin indicated. Activity of α-amylase was measured in the medium and a tissue extract after 24 hours."

erly conclude that their modes of action are physiologically independent within the ranges of concentration of the chemicals used. This information could be available but obscured by the use of the physiologists' definition.

ROBERT E. DRURY
New York State Agricultural
Experimental Station, Geneva 14456

References and Notes

1. R. G. D. Steel and J. H. Torrie, *Principles and Procedures of Statistics* (McGraw-Hill, New York, 1960), p. 198.
2. M. J. Chrispeels and J. E. Varner, *Plant Physiol.* **42**, 1008 (1967).
3. B. Dey and S. M. Sircar, *Physiol. Plant.* **21**, 1054 (1968).
4. B. V. Milborrow, *Planta* **70**, 155 (1966); D. Aspinall, L. G. Paleg, F. T. Addicott, *Aust. J. Biol. Sci.* **20**, 869 (1967); A. A. Khan and R. D. Downing, *Physiol. Plant.* **21**, 1301 (1968).
5. T. H. Thomas, P. F. Wareing, P. M. Robinson, *Nature* **205**, 1270 (1965).

24 January 1969; revised 3 March 1969

Fissioning in Planarians: Control by the Brain

Abstract. *Reduced population densities lead to increased rates of fissioning in planarians whereas higher population densities suppress fissioning. This effect is not primarily due to mucus deposition or substances secreted into the water. Experiments are presented which show a system of population feedback control. In the presence of other planarians, the brain exerts an influence (probably neurohormonal) to suppress fissioning. This influence becomes attenuated with axial distance from the brain.*

Planarians of the species *Dugesia dorotocephala* and *Dugesia tigrina* usually reproduce asexually in laboratory cultures. In such reproduction, the anterior portion of the planarian tears away from the posterior region. Each fission fragment then regenerates the appropriate missing part to yield complete but smaller worms. No morphologically differentiated plane of fracture is evident before the onset of fissioning to account for the fissioning or for the point of rupture. Observation of the fissioning process suggests that it is a behavioral or neuromotor event and thus, most probably, mediated by the central nervous system. Previous results (*1*) indicated that a higher incidence of fissioning occurred with low population densities whereas higher population densities suppressed it. Preconditioning of the water and test container by putting in a number of planarians and removing them did not inhibit the fissioning of planarians subsequently isolated in such test habitats, although it did delay its onset by about a day. Since there is some evidence to suggest that the cephalic ganglion (the brain) of the planarian acts as a morphogenetic organizer (*2*) we conjectured (i) that, in the presence of other planarians, it exerted some influence—probably through the ventral cords which arise from the neuropil (*3*)—to suppress fissioning, and (ii) that this influence becomes attenuated with the distance away from the head. The experiments described here test these conjectures.

Our experiments were independently conceived and executed, although we later discovered that Child (*4*) had inferred a cephalic suppression of fissioning. However, we do not consider that his theoretical or experimental basis for doing so were very compelling. His rationale was based on the concept of an axial metabolic gradient. Child did not recognize the obligatory role of population density in the production of this cephalic suppression, and he made no attempt to control or manipulate this factor. Absence of any surgical controls constituted another serious technical defect in his experimental design.

All of the planaria used in our experiments were *Dugesia dorotocephala* maintained for more than 18 months in the laboratory at 21° ± 1°C in enameled dishpans containing aged tap water. They were fed twice weekly on raw beef liver. After each feeding, the pans were cleaned and the water was changed. Each pan contained 600 to 900 worms kept in the dark except during cleaning time when there was dim illumination. The bowl was processed ("preslimed") in the following way. (i) Nonexperimental planarians were placed in a clean bowl or dish a number of hours before the experiment; (ii) they were allowed to deposit a coat of slime; and (iii) they were then emptied along with the water; (iv) the bowl was gently rinsed without rubbing; and (v) it was then refilled with clean aged tap water.

In the first experiment we used 180 planarians. Sixty were selected in each of three different length classes, (i) 6 to 8 mm, (ii) 10 to 12 mm, and (iii) 15 to 17 mm. Each planarian was isolated into a clean petri dish, 40 mm in diameter, in aged tap water at zero time. Unfissioned planarians were transferred each day to a clean petri dish and water. They were observed at 9:00 a.m. and 3:00 p.m. each day to ascertain whether fissioning had occurred. This experiment was performed in three balanced blocks with 30 subjects in each size class in the first block and 15 in each size class in the second and third blocks. After each fissioning in the second and third blocks the fission fragments were anesthetized with 0.15 percent Chloretone, to relax them in the extended position, and were measured.

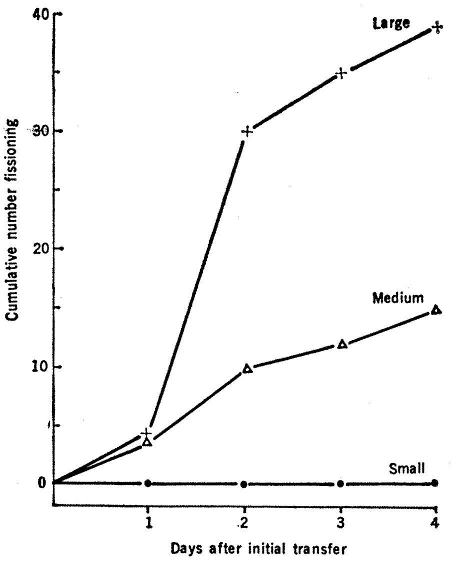

Fig. 1. Cumulative number of fissionings as a function of time after isolation into a new unslimed habitat for three different size classes (15 to 17 mm, 10 to 12 mm, and 6 to 8 mm) of *Dugesia dorotocephala*; 60 planarians were used in each size class.

The cumulative number of fissionings in each size class as a function of the time after the initial transfer is shown in Fig. 1. The lengths of the anterior fragments for the medium and long subjects were 6.1 ± .4 and 7.8 ±

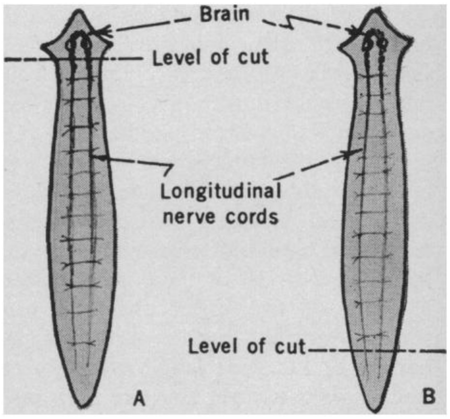

Fig. 2. Schematic diagram of *Dugesia dorotocephala* showing the relation of the nervous system to the levels of surgical transection.