quina clams bore the same color and pattern, it would be possible for their predators to learn to distinguish them quickly by these signs. However, the virtually endless variation makes it impossible for a sandpiper to learn that all "pebbles" of any particular appearance are probably little clams for the very good reason that no two clams have the same appearance. The situation is somewhat analogous to making a code by randomizing the components.

A similar explanation would hold good for the massive variation in the brittlestars and in such other species showing it as Cerianthus americanus (6) among coelenterates, the Bermuda Sabella and the Woods Hole Hydroides among tubiculous polychaetes, and Acridium arenosum and possibly other grasshoppers (locusts) among insects (7).

The type of selection postulated for massive polymorphism does not easily fit into the familiar categories as stabilizing, directional, or disruptive (8). It is not disruptive selection, at least in any ordinary sense, since it does not break the population into two or more incipient new species. Perhaps it should be regarded as a special form of stabilizing selection which produces not uniformity but continual diversity. This seems a contradiction in terms. At the same time it does not seem to be "balancing selection" (9) where, for example, heterozygotes producing sickle cell anemia, or the MN blood group, are maintained because the heterozygote is superior to the homozygote by conferring resistance to a specific disease or in some other way. The term reflexive selection suggests itself because it is the variation per se which is adaptive, and the frequency of any one type is determined by a feedback relationship with all the other types.

GAIRDNER B. MOMENT Department of Biological Sciences. Goucher College, Baltimore, Maryland

## **References and Notes**

- 1. E. B. Ford, "Genetics of polymorphism in Lepidoptera," in Advances in Genetics 5, 43
- (1953).
   H. J. Turner, Jr., and D. L. Belding, Limnol. and Oceanogr. 1, 120 (1957).
   C. C. Sperry, U.S. Dept. Agr. Wildlife Re-search Bull. No. 1 (1940).

- search Bull. No. 1 (1940).
  U. Dahlgren, personal communication.
  H. B. Cott, Adaptive Coloration in Animals (Oxford Univ. Press, London, 1940).
  G. B. Moment, Bull. Mount Desert Island Biol. Lab. No. 30 (1939).
  C. D. Darlington and K. Mather, The Ele-ments of Genetics (Allen and Unwin, London, 1940)
- 1949). Mather, Evolution 9, 52 (1955).
- K. Mather, Evolution 9, 52 (1955).
   T. Dobzhansky, Am. Scientist 49, 285 (1961).
- 27 November 1961
- 20 APRIL 1962

## **Transpiration Rate Reduction** in Plants with Atrazine

Abstract. When treated with atrazine, a herbicide that acts as an inhibitor of photosynthesis, both tolerant and susceptible plants showed a reduction in transpiration rate. This occurred within 1 to 3 hours of application and usually reduced transpiration by 50 percent or more after about 6 hours.

Atrazine (2-chloro-4-ethylamino-6isopropylamino-s-triazine) has gained widespread use as a herbicide, especially for the control of annual weeds and quackgrass (Agropyron repens) in corn. Recently, we noticed an interesting plant response to atrazine. Young plants showed a dramatic reduction in transpiration rate soon after treatment. In solution culture this took place within 1 to 2 hours. This effect seems to correspond in time to the effect of simazine (2-chloro-4,6-bis-ethylamino-s-triazine) on photosynthesis which was observed by Ashton et al. (1), who found a drastic reduction in C14O2 fixation in red kidney bean leaves within 3 hours after they placed the roots in a 1 ppm solution of simazine. The first visible evidence of damage by the herbicide to susceptible plants did not occur until about 3 days after treatment under the conditions of our experiments. The reduction in transpiration rate was established in plants grown in soil, as well as in solution culture, and occurred in a wide variety of crop plants.

Our experiments were conducted under controlled light (2500 ft-ca) and temperature (light 76°F, dark 65°F) conditions in a controlled environment room with a light period of 15 hours. Measurements of transpiration rate were made with a modification of the potometer described by Grobbelaar (2). In experiments involving soil the method of weighing pots was used.

In most experiments with the potometer, treatment was accomplished by replacing the distilled water with previously prepared solutions of atrazine. Foliar sprays with suspensions ranging from 14 ppm to 12,000 ppm, in which the leaves were thoroughly wetted, all gave significant reductions within 6 hours. In the case of soil experiments atrazine was applied in water solution.

Figure 1 shows the transpiration response of corn, a species known to be very tolerant of atrazine, and of soybeans, a susceptible species. The difference in magnitude of response is not necessarily due to the difference in susceptibility of the two

crops. Maximum reduction of transpiration ranged from 44 percent of the normal rate in corn to 67 percent in soybeans. In canning peas a significant reduction occurred within 1 hour after treatment. Maximum reductions were generally obtained 4 to 6 hours after treatment.

In order to estimate the degree of stomatal closure after treatment with atrazine, the leaves were infiltrated with gentian violet in absolute ethanol after a method described by Williams (3). The leaves of control plants showed much greater injection of the dye than leaves of treated plants. This difference indicated that the reduction of transpiration rate resulted from decreased stomatal aperture. This was further substantiated by determining the water content of leaves 6 hours after the plants had been treated with atrazine. Leaves of treated plants showed no decrease in moisture content. This would indicate that inhibition of water uptake occurred at the stomata. If water movement were blocked further down the plant, loss of water from leaves would have proceeded until wilting occurred.

Shaw and Maclachlan showed that C<sup>14</sup>O<sub>2</sub> was fixed by guard cells (4). This gave considerable support to a longheld idea that the guard cells are capable of photosynthesis. Heath proposed that the most important function of photosynthesis in the guard cells is not the production of osmotically active materials, but the removal of carbon dioxide (5). Shaw found that the guard cells in white areas of variegated leaves



Fig. 1. Transpiration rate of soybeans and corn before and after treatment with 1 and 20 ppm of atrazine. The rates are presented as a percentage of the baseline established prior to treatment. The control was defined as 100 percent, and all observations were adjusted according to the absolute transpiration rate of the control plants.

(for example, Coleus) contain relatively small amounts of chlorophyll and are capable of only limited stomatal response (opening) to light (6). Guard cells of albino barley which were completely devoid of chlorophyll did not respond to light. The necessity of chlorophyll for the normal stomatal opening was not conclusively proved, however, because Shaw was unable to observe an opening of the stomata of albino barley leaves at any time. It is also well established that high concentrations of carbon dioxide in the guard cells or substomatal cavities give rise to stomatal closure.

The mode of action of atrazine has been studied by many workers, and Crafts presented a comprehensive review of the literature (7). The action of the chemical apparently is to inhibit CO<sub>2</sub> fixation in photosynthesis and to increase respiration (1). The tolerance of some species, such as corn, appears to be due to the ability of the plant to detoxify the herbicide as rapidly as it is taken up.

There is ample evidence that atrazine is translocated throughout a seedling in a short time (less than 3 hours). The material concentrates first at the leaf margins and accumulates basipetally (8).

Stomatal closure after treatment with atrazine would thus appear to be a result of increased CO<sub>2</sub> in the guard cells and substomatal cavities resulting from the inhibition of photosynthesis and increased respiration rate instituted by the herbicide. This is substantiated by the fact that other rapidly translocated photosynthetic inhibitors such as monuron [3(4-chlorophenyl)-1,1-dimethylurea] and diuron [3(3,4-dichlorophenyl)-1,1-dimethylurea] gave a similar response while herbicides with a mode of action not directly involving photosynthesis, such as dalapon (2,2-dichloropropionic acid) and amitrol (3-amino-1,2,4-triazole) caused no reduction in transpiration rate during the 24-hour test period. The transpiration reduction with the substituted ureas was similar to that observed by Minshall (9;10).

DON SMITH

K. P. BUCHHOLTZ Department of Agronomy University of Wisconsin, Madison 6

## **References and Notes**

1. F. M. Ashton, G. Zweig, G. W. Mason, Weeds 8, 448 (1960).

- N. Grobbelaar, Science 131, 1614 (1960).
   W. T. Williams, Ann. Botany (London) 13, 309 (1949).
   M. Shaw and G. A. Maclachlan, Can. J. Botany 32, 784 (1954).
   O. V. S. Heath, J. Exptl. Botany 1, 29 (1950).
- (1950)
- (1930).
  6. M. Shaw, Can. J. Botany 36, 575 (1958).
  7. A. S. Crafts. The Chemistry and Mode of Action of Herbicides (Interscience, New York, 1961), pp. 115-123.
  8. T. J. Sheets, Weeds 9, 1 (1961).
  9. W. H. Minshall, Can. J. Botany 38, 201 (1960).
- 9. (1960). 10. This report was approved for publication by
- the director of the Wisconsin Agricultural Experiment Station. The project was supported in part by the research committee of the graduate school from funds made available by the Wisconsin Alumni Research Foundation.

27 November 1961

## **Peach Mesocarp Explant Enlargement** and **Callus Production in vitro**

Abstract. Explants from fruits of the peach were stimulated to grow by cell enlargement, cell division, or both when placed on media containing kinetin. Callus cultures have been obtained, even occasionally from ripening fruits, long after cessation of normal cell division.

Tissues from fruits of deciduous orchard trees have not been grown commonly in culture. The cessation of active cell division within 4 weeks after bloom (1) suggests that cells of maturing truit tissue might be incapable of resuming active cell division. Letham, however, reported proliferation of apple fruit tissue from both young fruits and fruits which had been developing for 130 days (2).

Among evergreen fruit species, Schroeder (3) and Schroeder and Spector (4) have reported culturing tissue from the pericarp of the avocado, Persea americana Mill, and the mesocarp of citron, Citrus medica L. Also, small fruits of the cucurbit, Echalium elaterium Rich., have proliferated callus in vitro (5).

The purpose of our investigation (6) was to determine the behavior of explants from the mesocarp of fruits of the peach, Prunus persica (L.) Batsch. Particular attention was given to changes in behavior with advancing fruit age and to the relation of plant growth-regulating substances to length of explant life and the initiation of callus proliferation.

Explants were aseptically cut from peach fruits after the epidermis and a few underlying cell layers had been peeled and discarded. In early experiments, explants were approximately 3 mm<sup>8</sup> in size. In later tests, explants consisted of a cylinder 8 mm in diameter and 8 mm long. Tissue ad-



Fig. 1. Callus production by an explant of Miller's Late peach mesocarp obtained about 4 weeks before the fruit would have been ripe. The photograph was made after 6 weeks in culture. Actual size approximately 5 by 9 mm.