Diurnal Rhythm of Sensitivity of Cotton Seedlings to Herbicides

Abstract. *The inhibition of growth of cotton seedlings* (Gossypium hirsutum, *var. Stardel) varied diurnally to applications of three herbicides [1,1-dimethyl-3-(α,α,α-trifluoro-*m*-tolyl) urea, 3′,4′-dichloro-2-methacrylamide, and ethyl-*N,N*-dipropylthiocarbamate], but not to a fourth [3-(3,4-dichlorophenyl)-1, 1-dimethylurea]. Inhibition was strongest when the plants were treated at about daybreak. The rhythmic response was apparently not endogenously controlled, since most of the diurnal effect was lost under conditions of constant light and temperature.*

Numerous reports of diurnal rhythms of various physiological functions of plants and animals have been summarized by Sollberger (*1*). Among recent reports are rhythms in susceptibility of animals to administered drugs and pesticides, including the response of mammals to several pharmacologic agents (*2*), a rhythm of susceptibility of cotton boll weevils to methyl parathion (*3*), and the diurnal rhythm of sensitivity of two spotted spider mites to dimethyl 2,2-dichlorovinyl phosphate (*4*). Our work extends the range of these observations to include a diurnal sensitivity in the growth response of cotton seedlings to several herbicides.

Greenhouse and field tests clearly show the existence of a rhythm of sensitivity of cotton seedlings grown under natural lighting to applications of several herbicides after emergence. Figure 1 shows the results of a typical greenhouse test. Cotton seedlings (variety Stardel), germinated in sterile soil in flats, were treated at the age of 12 days on a moving spray table with an overhead spray of dicryl (3′, 4′-dichloro-2-methacrylamide) at a rate equivalent to 3 lb of active ingredient per acre (3.4 kg/hectare) [15 ml of emulsifiable concentrate containing 2 lb of active ingredient per gallon (240 g/liter), at a pressure of 30 lb/in.^{2} (211 g/cm^2) and a table speed of 2½ miles per hour (4 km/hr)]. Different groups were sprayed every 6 hours beginning at 6 a.m. Figure 1 shows the percentage of inhibition of elongation of the first internode in the 24-hour period after treatment and the area of the first leaf 4 days after treatment.

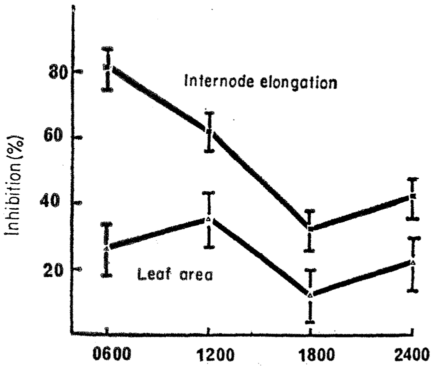

Fig. 1. The relation of time of treatment to inhibition of growth of 12-day-old cotton seedlings in the 24-hour period following application of dicryl. The vertical lines show the 95-percent confidence limits for each point on the graph.

Maximum sensitivity to the herbicide occurs during the early daylight hours, with a marked decrease at night. In four field tests, in which age of the treated plants and environmental conditions varied widely, diurnal differences occurred in sensitivity of cotton seedlings to dicryl, C-2059 [1,1-dimethyl-3-[α,α,α-trifluoro-*m*-tolyl) · urea], and EPTC (ethyl-*N,N*-dipropylthiocarbamate), but not to DCMU [3-(3,4-dichlorophenyl)-1,1-dimethylurea] (Table 1). The seedlings were treated with an overhead spray, with the conventional commercial equipment for application, at 6 a.m. or 6 p.m. Differences were measured as fresh weights of samples (20 plants each) harvested 7 to 12 days after treatment. Again, maximum sensitivity, when it occurred, was in the morning.

In order to gauge the endogenous component of the rhythmic response to herbicides, further tests were conducted in growth chambers under conditions of controlled temperature and light. In all tests the seedlings were grown in a mixture of sterilized soil and peat at 27°C. When the seedlings were 6 days old, they were treated with dicryl by immersing the plant top in the solution of herbicide. After 2 hours the leaves were thoroughly rinsed in distilled water. When grown in a 12-hour photoperiod, if the treatment was administered in the morning first and followed 12 hours later by the evening treatment, the results were similar to those experienced in the field, that is, the morning treatments were markedly more inhibitory than the evening ones. When treatments were reversed (plants treated first in the evening) much of the difference between treatments was lost. Under continuous light, beginning 12 hours before the earlier treatment, there was no significant diurnal effect, even though the typical "sleep" movements of the leaves showed the persistence of an endogenous rhythm.

Two factors should be considered in interpreting results of tests in the growth chamber. First, the treatments were imposed on a moving base; that is, the plants were actively growing, and treatments 12 hours apart may have been applied to plants with physiologically significant differences in development. When dicryl was applied to plants 6, 8, and 10 days old, they did show a progressive decrease in sensitivity to the morning treatment (Table 2). This may explain why, in studies carried out in the growth chamber, the "diurnal" effect was lost when the evening treatment was administered first. It does not explain the rhythmic response, however, in the field tests, since the plants varied in age when treated, and in two of the tests the evening treatment was administered first without any apparent influence on the results.

A second factor is the nature of the

Table 1. Fresh weight of 30-day-old cotton seedlings 7 days after treatment with herbicides (values are averages of three replications of 20 plants each). This table is representative of four field tests, all with similar results. N.S., not significant.

Herbicide	Weight (in grams) of seedlings treated at 6 a.m.	Weight (in grams) of seedlings treated at 6 p.m.	Analysis of variance (*P*)
Dicryl	53.4	98.4	.01
C-2059	40.6	64.2	.01
EPTC	90.9	199.7	.025
DCMU	46.2	46.9	N.S.

Table 2. Elongation of the first internode and first true leaf of cotton seedlings in 96 hours after treatment with dicryl (27°C; 12-hour photoperiod, 6 a.m. to 6 p.m.). Values are means ± standard error.

Time of treatment	Age of plants (days) at lengths of 6 mm	8 mm	10 mm
	Untreated control		
	27.0 ± 2.2	31.9 ± 2.2	34.3 ± 1.0
	Treated seedlings		
7 a.m.	10.1 ± 1.5	16.4 ± 1.7	21.7 ± 1.0
7 p.m.	21.2 ± 2.0	20.6 ± 1.4	25.1 ± 0.9

herbicides used. Dicryl was used exclusively in the growth chamber studies. Since it is active as an inhibitor of the Hill reaction (*5*) one would expect a greater inhibition of growth during the daylight hours than at night, and this does occur. The occurrence of this phenomenon does not explain, however, why a compound administered at night is less inhibitory the following day than it is when administered during the light period, unless degradation or dilution by translocation occurs during the night. Of the chemicals tested dicryl, C-2059, and DCMU are considered to be inhibitors of the Hill reaction, but, in contrast to the first two, DCMU (the most active of the three) showed no evidence of diurnal activity. The mode of action of the fourth chemical, EPTC, is uncertain, but it is thought to act as a mitotic poison (*6*). The basis of its diurnal selectivity is problematic.

Despite the inconclusive nature of the tests performed in the growth chamber, there is a strong diurnal rhythm of sensitivity to herbicides in the field. The differences are large enough to warrant consideration of the practical importance of the time of day at which chemicals are applied. Preliminary tests fail to show the same diurnal response by soybean seedlings, and this suggests that rhythmic sensitivity might be used as a selective factor in herbicide action. The rhythmic nature of the response to the herbicides tested seems to be caused primarily by one or more rhythmic environmental factors, since under constant temperature and light the rhythm is not clearly defined.

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References and Notes

1. A. Sollberger, *Biological Rhythm Research* (Elsevier, New York, 1965).
2. A. Carlson and F. Serin, *Acta Pharmacol. Toxicol.* **6**, 181, 187 (1950); W. M. Davis, *Experientia* **18**, 235 (1962); S. T. Emlen and W. Kem, *Science* **142**, 1682 (1963); R. J. Ertel, F. Halberg, F. Ungar, *J. Pharmacol. Exp. Therap.* **146**, 395 (1964); E. F. Lutsch and R. W. Morris, *Science* **156**, 100 (1967); P. R. Wiepkema, *Nature* **209**, 937 (1966).
3. C. L. Cole and P. L. Adkisson, *Science* **144**, 1148 (1964).
4. B. Polcik, J. W. Nowosielski, J. A. Naegele, *ibid.* **145**, 405 (1964).
5. N. E. Good, *Plant Physiol.* **36**, 788 (1961).
6. L. J. Audus, *The Physiology and Biochemistry of Herbicides* (Academic Press, New York, 1964).
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Photosynthetic Utilization of Internal Carbon Dioxide by Hollow-Stemmed Plants

Abstract. *The hollow stems of wet-meadow and bog plants often contain relatively large amounts of carbon dioxide. The internal carbon dioxide apparently originates from respiration of root and stem cells. Some of this carbon dioxide is fixed photosynthetically by stem tissues and is thus recycled within the plant. Such internal stem photosynthesis would be advantageous, particularly in the early stages of growth in cold, wet, high-altitude environments.*

Many herbaceous plants characteristic of moist mountain meadows and bogs have hollow stems. Such a hollow stem is part of a system of lacunar air passages which connects the leaves with the roots. Thus, air and individual gases can move internally from one part of the plant to another by diffusion and mass movement through such a porous aerenchyma (*1*). We found that these stems often contain much higher concentrations of carbon dioxide than is present in the external air and that the concentration varies with light and temperature. There is usually a carbon dioxide flux upward inside the stem which originates from respiration in the roots, rhizomes, and in the rapidly growing stems themselves. Our experiments show that much of this carbon dioxide is apparently utilized in photosynthesis by green-stem tissue and is thus recycled within the plant.

We first became aware of the carbon dioxide flux within the stem while measuring photosynthesis and respiration of plants in wet subalpine meadows at 3100-m elevation in the Medicine Bow Mountains, Wyoming. These wet meadows are characterized by many species of grasses, sedges, and dicotyledonous plants. Typical dicots are *Delphinium barbeyi* Huth, *Mertensia ciliata* (James) G. Don., and *Senecio triangularis* Hook. Plants of all of these species grow very rapidly after snowmelt in June, and all have hollow stems with many chloroplasts in the cortical tissue. There are also some chloroplasts in the outer pith cells.

We extracted gas samples, with a hypodermic syringe, from the stems of these plants in the field and injected the samples into a closed, infrared gas-analyzer system. The concentration of carbon dioxide in the stems ranged from two to over fifty times the carbon dioxide content in the ambient air. Shading the plants increased the carbon dioxide concentration in the stem.

Both carbon dioxide and oxygen fluxes and gradients have been previously described in plants growing in wet habitats (*1*); the downward flux of oxygen which is derived both from photosynthesis and from ambient air through the stomates has been emphasized. The oxygen is utilized in root and rhizome respiration; the aerenchyma serves not only as a diffusion pathway during the day, but as a reservior of oxygen at night.

No evidence for the utilization of the internal stem carbon dioxide in photosynthesis has been presented, although such utilization seems likely. For example, Hartman and Brown (*2*) found that the aerenchyma in *Elodea canadensis*, a submersed vascular plant in lakes, acts as a reservoir for carbon dioxide which is utilized in photosynthesis to such an extent that the internal supply is depleted by midafternoon.

To investigate possible relations between internal carbon dioxide and photosynthesis of subalpine meadow plants, we measured carbon dioxide exchange within the stems of plants of *Mertensia ciliata* (grown in the greenhouse) by connecting the internal atmosphere of the plant with an open infrared gas-analysis system (*3*) (Fig. 1). Ambient air, drawn by polyethylene tubing from above our laboratory building, could

Table 1. Photosynthesis within the stem of *Mertensia ciliata*, as indicated by the decrease in detectable carbon dioxide flux with increase in light.

Light intensity on vertical surface (10^3 lu/m^2)	Temperature (°C)			Stem CO_2 flux (μg/min)	Gross photosynthesis (μg cm^{-2} min^{-1})
	Soil	Stem air	Chamber		
Dark	22	18	20	9.7	
33	22	20	20	7.3	0.11
36	22	21	22	6.8	.13
44	22	22	22	6.3	.15