Transpiration and Stomatal Opening with Changes in Carbon Dioxide Content of the Air

Abstract. *Increasing the carbon dioxide content of air reduces the transpiration rate of corn and sorghum plants and, to a lesser extent, of cotton, soybean, and tomato plants by causing the stomata to close. Closure of corn and sorghum stomata occurred when the concentrations of carbon dioxide were 2000 and 3000 parts per million, respectively. Cotton, soybean, and tomato stomata did not close completely at concentrations of carbon dioxide up to 4000 parts per million.*

The movement of stomata is affected by the concentration of CO_2 in the air (*1*); they generally open when subjected to CO_2 concentrations below normal [300 parts per million (ppm)] and close at concentrations above normal (*2*). There is no completely satisfactory explanation of the effect of CO_2, although I incline to agree with Kuiper (*3*) that the answer must be sought in the metabolism of guard cells.

Most experimenters have measured with a porometer the resistance to passage through the leaf of air containing various amounts of CO_2. Porometers do not measure transpiration directly and entail other difficulties (*4*). However, it can be assumed from data obtained with porometers that changes in CO_2 concentration, coupled with the effect of this concentration on stomata, have measurable effects on the transpiration of at least some species. Gaastras (*4*) went one step further and demonstrated a relation between the CO_2 content of the air, transpiration, and the temperature of leaves of sugar beets and turnips at several intensities of light. As the concentration of CO_2 increased from 0 to 1500 ppm, the transpiration rate decreased. No direct observations of stomata were made.

My original purpose was to measure reduction in transpiration when stomata were closed by a change in CO_2 concentration of the air and, thus, to evaluate the potential for reducing transpiration of several field crops by control of guard cell activity (*5*). Individual hybrids, varieties, or lines used in the experiments are either grown extensively in the southeastern United States, or promise to become important crops in that region.

All plants were grown and tested in controlled environmental chambers. Growth and test conditions were: 14 hours of light (Sylvania F96T/CW/VHO cool white fluorescent lamps and supplemental incandescent lamps—approximately 0.5 to 0.6 cal cm^{-2} min^{-1}), temperature 25°C, and 50 to 70 percent relative humidity; and 10 hours of dark, temperature 20°C, and 85 to 95 percent relative humidity. Average wind speed near the plants was 25 m/min. Single plants were grown in asphalted cans containing 3600 g of fertile Cecil clay loam; a plastic cover over each can, snug with the stem, minimized evaporation from the soil surface. Soil moisture was kept high (less than 0.3 bar) to minimize its effect on stomatal operation or transpiration (*6*). Five replicates of each variety of plant were used in the first series of experiments; two were used in the second series.

In the first series of experiments the concentration of CO_2 was raised to 400 ppm or slightly higher to close the stomata; to open them, the concentration was lowered to less than 250 ppm, the exact concentration used depending on the species. The stomata were monitored by microscopic observation of upper and lower leaf surfaces (*7*). This method has disadvantages; it is tedious, and at × 200 magnification one cannot be certain that closure is complete.

Measurements of transpiration by each species were made on several consecutive days, the sequences of high to low or low to high concentrations of CO_2 being alternated. For example, on alternate days at 6:00 a.m., the CO_2 concentration of the air was gradually reduced by chemical washing and maintained below 250 ppm from 8:00 a.m. to 12:00 noon. Water loss was measured by weighing each can with plant before and after the 4-hour period and recording the individual weight difference as attributable to transpiration by that plant. Beginning at noon, enough CO_2 was added to the chamber to maintain the CO_2 concentration between 400 and 500 ppm. Transpiration was again measured by weight difference over a 4-hour period; the period began when stomata closed or when steady-state condition of the stomata was observed. In several species not all the stomata of a particular plant would open (or close) at any one time; stomata of corn and sorghum were the most responsive.

The values in Table 1 indicate considerable variance among the test species in transpiration at the higher CO_2 concentrations tested. The very decided effect of CO_2 concentration on transpiration is readily apparent from the data. The percentage reduction in transpiration of corn and sorghum plants measured when stomatal closure was

Table 1. Transpiration at CO_2 concentrations of less than 250 ppm, and 400 to 500 ppm (g/dm^2 per 4 hr), and the percentage reduction in transpiration resulting from the higher CO_2 concentration in different plant species and varieties. SD, standard deviation.

Crop	< 250 ppm CO_2		400 to 500 ppm CO_2		Reduction (%)
	Transp.	SD	Transp.	SD	
Corn, 21 days old					
Dixie 82	14.44	.77	4.75	.76	68
MP 399 × MP 311	12.90	.56	4.14	.15	68
MP 305 × T 101	12.57	1.60	4.34	.60	66
MP 305 × MP 307	11.85	.94	3.91	.46	68
Sorghum, 29 days old					
RS–610	11.75	2.2	3.85	1.30	68
NK–210	11.50	1.7	4.92	1.30	58
Amak–R–12	11.00	1.3	4.34	.86	61
Tomatoes, 43 days old					
Marglobe	10.43	1.10	8.24	1.30	21
Rutgers	9.35	0.48	6.32	1.03	33
Marion	9.29	0.51	5.97	1.03	36
Soybeans, 36 days old					
Hampton	10.27	1.7	6.87	2.50	34
Hardee	8.90	1.4	4.27	1.03	53
Beinville	8.83	1.5	5.28	.62	41
Cotton, 56 days old					
Smooth Leaf Empire	7.15	.59	4.75	.62	34
Auburn	7.03	.69	5.05	.73	29
Empire	3.49	.83	2.33	.64	34
Carolina Queen	3.11	.70	2.34	.47	25

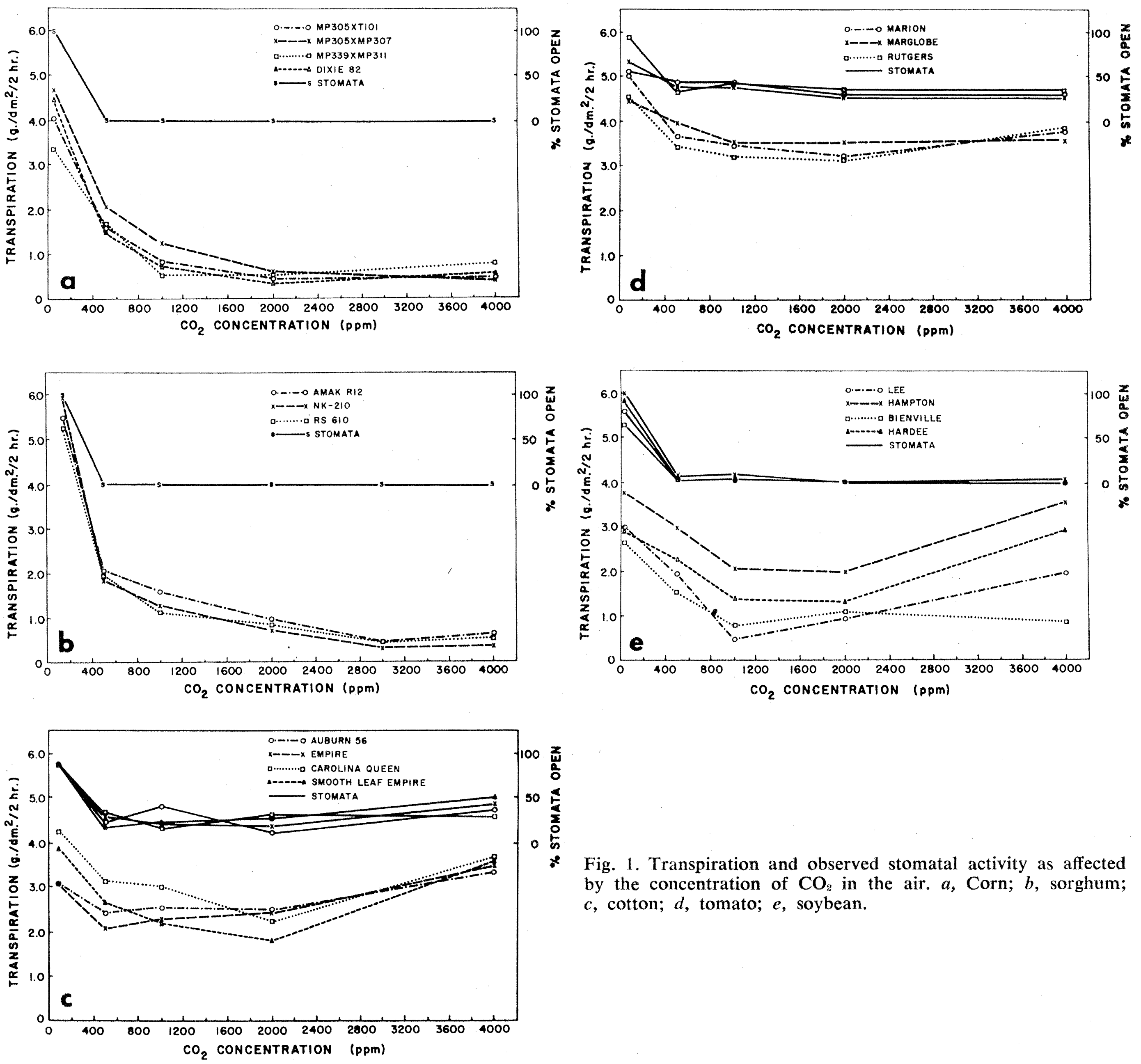

Fig. 1. Transpiration and observed stomatal activity as affected by the concentration of CO_2 in the air. *a*, Corn; *b*, sorghum; *c*, cotton; *d*, tomato; *e*, soybean.

visually apparent is much less than the 90 percent value generally quoted (*8*). From these results it is deduced that a large fraction of the water transpired by such crops may be lost through the cuticle or through stomatal openings too small to be detected with the optical microscope that I used (*9*, *10*).

In a second series of experiments, concentrations of CO_2 up to and including 4000 ppm were used. The plants were grown and tested in essentially the same way as for the first series, with the exception that transpiration at any constant CO_2 concentration was recorded for 2-hour periods so that a more complete sequence of CO_2 changes could be run in a single photoperiod. Because these studies were prolonged and moisture stress might thus influence transpiration (*6*), plants were watered to near 0.05 bar immediately after each final weighing at a constant CO_2 value.

The trends expressed in Fig. 1 indicate that very decisive changes in transpiration were caused by increasing or decreasing CO_2 concentration. With corn and sorghum, transpiration continued to decline after visual stomatal closure was complete. This is considered evidence that the stomata, although visibly closed, were not hermetically sealed, thus seriously limiting any optical assessment of stomatal activity. Methods based on imprints (*11*) have similar limitations. The resolving power of my microscope was considered to be 1 to 2 μ; increased magnification did not help in ascertaining the true stomatal condition.

The steady state of transpiration at several CO_2 concentrations (Fig. 1, *a* and *b*) is considered to be of a cuticular nature, such transpiration amounting to about 10 percent of the total for corn and 18 percent for sorghum. Because of the lack of stomatal closure no values can be determined for the other species. This interpretation that the stomata are closed when transpiration is constant following its exponential decrease resembles the supposition of Hygen and Midgaard (*10*). Their curves for decline of the logarithm of the reduced fresh weight of severed branches run straight and are referred to as the cuticular phase.

Stomata of monocotyledonous and dicotyledenous plants behaved differently at the same CO_2 concentrations. At CO_2 concentrations where corn and sorghum stomata closed completely, stomata of cotton and tomato remained open. Soybean stomata were intermediate in response, showing less tendency than cotton and tomatoes to remain open at high CO_2 values; a small percentage remained visibly open. Diurnal effects on stomatal activity probably mingle with the CO_2 effects; such tendencies were considered to be inherent in these experiments and were not compensated for.

The increase in transpiration of the dicotyledonous plants at 4000 ppm CO_2 from that at 2000 ppm is unexplained. It may indicate that CO_2 causes the stomata to begin opening again, or it may indicate an effect only distantly related to stomatal action, such as an increase in cellular permeability to water movement. No deleterious effects were apparent from the CO_2 concentrations used in these studies.

It is easy to see how experiments on plant-water relations may be influenced by the CO_2 content of the plant's environment; little consideration is usually given to CO_2 control in controlled-environment research. On the other hand, the effects of CO_2 on transpiration may be fortunate for those implementing programs of greenhouse fertilization with CO_2 (*12*); they may benefit from increased economy in water used.

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Moth Sounds and the Insect-Catching Behavior of Bats

Abstract. *Captive bats trained to catch mealworms tossed in midair turned away from most of these targets when simultaneously confronted with a recorded train of the ultrasonic pulses generated by an Arctiid moth. When similarly exposed to the recorded echolocation pulses of another bat, presented at the same intensity as the "moth" sounds, they were not so affected.*

Moths of the family Arctiidae have tympanic organs capable of detecting the echolocating cries of bats (*1*). When shaken or otherwise manipulated some members of this family produce trains of clicks with a strong ultrasonic component. These sounds are generated by microtymbal organs on the metathoracic episternites (*2*).

We have confirmed this finding, and in preliminary experiments have found that specimens of *Halysidota tessellaris* and related moths may also be induced to make trains of clicks with a dominant frequency of about 60 kc/sec by being exposed to a series of artificially generated ultrasonic pulses. Freshly captured specimens of *Halysidota tessellaris* and of *Pyrrharctia isabella* were mounted in stationary flight in front of an ultrasonic loudspeaker, and their acoustic and flight behavior was observed. The onset of a train of ultrasonic pulses commonly caused the flying moth to emit a sequence of clicks. At times flight continued with intermittent clicking for many seconds; at others, flying and clicking ceased simultaneously a few seconds after the onset of the stimulus. In one case there was clear evidence of an accelerated frequency of wingbeat accompanied by clicking. In spite of their additional capacity to respond with ultrasonic clicks, Arctiids in fixed flight behave toward ultrasound in a manner similar to Noctuids (*3*).

Because many of these moths are night flyers and are therefore exposed to predation by insectivorous bats, it seems possible that the ultrasonic clicks are normally emitted on the approach of an echolocating bat and somehow protect the moths against attack. The following experiments were designed to determine the reactions of feeding bats to the sounds made by an Arctiid moth.

Five captive *Myotis lucifugus* bats were trained to catch mealworms (*Tenebrio* larvae) tossed into the air from a solenoid-operated "gun" (*4*). A train of ultrasonic pulses recorded on a tape loop running at 1.5 meters per second was turned on upon presentation of some of the mealworms. The loudspeaker from which these sounds came was aimed at the apex of the mealworm trajectory. Tosses with sound were randomly interspersed among those unaccompanied by sounds.

Responses of the bats to the mealworms under these conditions were observed and rated as follows. (i) "Contacts" included those instances in which the bat caught and ate the mealworm, or caught and dropped it, or only hit it, knocking it out of its trajectory. (ii) In a "dodge" maneuver, the bat swerved from its path of flight so as to avoid the

Table 1. The responses of bats to mealworms presented simultaneously with moth sounds. In those instances where the bat's performance was not rated as a contact, dodge, or attempt, the bat continued on its normal path of flight, apparently without paying any attention to the mealworm.

Bat No.	No. of tosses	Number of		
		Contacts (%)	Dodges (%)	Attempts (%)
Targets presented with sound				
1	59	19	73	1
2	49	10	80	4
3	178	5	87	0
4	92	0	100	0
Total	378	8	85	2
Targets presented without sound				
1	71	85	0	8
2	68	80	1	15
3	188	92	0	4
4	94	100	0	0
Total	421	88	1	8