0038-075X/85/1405-0333$02.00/0
SOIL SCIENCE

November 1985
Vol. 140, No. 5
Printed in U.S.A.

SOIL OXYGEN EFFECTS ON TWO DETERMINATE SOYBEAN ISOLINES[1]

R. E. SOJKA[2]

Because a large portion of southern determinate soybean is grown on land frequently subject to poor soil aeration, an understanding of soybean responses to this condition is needed. It was hypothesized that soybean nutrient status, growth, and stomata would respond in a manner similar to that observed in many other species and, to test this hypothesis, grew soybean [*Glycine max* (L.) Merr., cv. Lee] in the greenhouse in sealed root chambers in equal volumes of soil and perlite. The soil was a Norfolk loamy sand (fine-loamy, siliceous, thermic, Typic Paleudult). Soil water was kept near field capacity; N, P, and K were added at equivalent field recommendation rates. Humidified gas containing 21, 4, 2, or 0% oxygen was passed over the soil at 500 ml/min beginning 46 d after planting. When soil-O_2 diffusion rate (ODR) fell below 40×10^{-8} g cm^{-2} min^{-1}, leaf diffusive resistance (R_s) increased sharply. Plants were harvested 14, 22, and 29 d after treatment initiation. Adaxial stomatal density (SD) of 10/mm^2 was unaffected by soil O_2. Abaxial SD was inversely related to soil O_2, rising from 55/mm^2 at 21% O_2 to 90/mm^2 at 0%. All growth parameters were negatively affected by reduction in soil O_2. The increase in SD was approximately proportional to the loss in leaf area in the absence of soil O_2, indicating that changes in aperture rather than SD account for the rise in R_s. In general, concentrations of K and Ca declined in all tissue with declining soil O_2. Magnesium concentrations were not consistently affected by O_2 treatment.

Soybean and other crops grown in the southeastern Coastal Plain are frequently subjected to low soil O_2 availability in the root zone resulting from poor surface drainage, high rainfall, poor internal drainage, fluctuating shallow water tables, unpredictable showers after irrigation, or some combination of these (Campbell and Seaborn 1972; Campbell and Phene 1977; Reicosky et al. 1976; Hunt et al. 1981). On Coastal Plains soils (Paleudults, Hapludults, and Paleaquults) both drainage and irrigation systems are usually required for optimum crop production (Doty and Parsons 1979). The negative impact of soil aeration on crop growth and yield, although long recognized, has only recently been linked to causative physiological and biochemical mechanisms.

Sojka et al. (1975) and Sojka and Stolzy (1980) determined that the leaf diffusive resistance (R_s) of several diverse crop species rose rapidly when the soil-O_2 diffusion rate (ODR) fell below threshold values for each species, even when soil matric potential was maintained at optimal levels. Smucker (1975) and Meek et al. (1980) reported similar results for navy bean (*Phaeseolus vulgaris* L.) and cotton (*Gossypium hirsutum* L.). Karlen et al. (1983) reported wilting and high R_s in tomato leaves and accelerated maturation and softening of flood-treated tomato fruit. Bradford and Yang (1981) reviewed the plant responses to flooding, citing studies showing that waterlogging induces stomatal closure in many species.

Mechanisms directly responsible for stomatal closure with poor root aeration are not fully understood (Bradford and Yang 1981; Karlen et al. 1983). Mechanisms suggested have included: plant hormonal responses, changes in K, changes in the plant ontogenetic development resulting in fewer or smaller stomata per unit leaf area of new and expanding leaves, and changes in root membrane permeability to water or nutrients. Particular attention has been paid to K concentrations, since Fischer (1968), Humble and Hsiao (1969), Humble and Raschke (1971), and others demonstrated that stomatal regulation is more sensitive to K concentration than to any other cation. Observations of rhizosphere O_2-stress effects on stomatal distribution, pore aperture, or size have not been previously reported for soybean, which is extensively grown under physiographic conditions conducive to this syndrome.

A greenhouse study with determinate soybean

[1] Contribution of the USDA-ARS, Coastal Plains Soil and Water Conservation Research Center, Florence, S.C. 29502, in cooperation with the S.C. Agric. Exp. Stn., Clemson, S.C. 29631. Received for publication 20 September 1984; revised 22 January 1985.

[2] Coastal Plains Soil and Water Conservation Research Center, USDA-ARS, Florence, S.C. 29502.

was conducted in an attempt to determine: (1) the critical ODR and duration of low ODR required for stomatal closure, (2) changes in the concentrations of K, Ca, and Mg as low ODRs persisted, and (3) the effect of low ODRs on leaf-surface distribution of stomata and on leaf diffusive resistance.

MATERIALS AND METHODS

Trichomatous and nontrichomatous isolines of the determinate soybean cultivar Lee (designated D49-2491 and D62-7812, respectively) were provided by Dr. E. E. Hartwig of Stoneville, Mississippi. For convenience, the isolines will be referred to hereafter as Lee (+) and Lee (−) to indicate the presence (+) or absence (−) of leaf hairs (trichomes).

Norfolk loamy sand (fine-loamy, siliceous, thermic, Typic Plaeudult) was fumigated with methyl bromide and mixed with an equal volume of coarse perlite. Perlite was mixed with the soil to prevent the soil from settling in the cylinders upon watering, thereby maintaining porosity and making imposition of O_2 treatments easier. Nontrafficked, stable bulk densities above 1.6 Mg m^{-3} are not uncommon in these soils. At these bulk densities, soil strength can also severely limit root growth.

Nutrients were added at the field equivalent rates (assuming 2.47×10^6 kg of soil per hectare per 15 cm depth) of 47 kg/ha N, 157 kg/ha P_2O_5, 157 kg/ha K_2O, and 448 kg/ha lime. Cylinders were filled with 3300 g of this mix and packed to a uniform bulk density of 0.88 Mg m^{-3}. A tensiometer was placed in each cylinder at the 40-cm depth to monitor soil water status, prevent waterlogging, and eliminate the need to leave the cylinder bottoms open for drainage. Cylinder dimensions were 46 cm high and 10 cm in diameter. Each cylinder was uniformaly watered and allowed to equilibrate overnight.

Seeds were soaked for 3 h in aerated water and germinated on moist paper towels until root radicals 0.5 to 1.0 cm long appeared. One germinated seed of each isoline was placed in each cylinder (two plants per cylinder), covered with an additional 1 cm of soil, and irrigated immediately with a few additional milliliters of water on 27 December 1981. Plants were then grown for 47 d before treatment initiation. Each cylinder was maintained between 15 and 25 kPa matric potential from planting to harvest by frequent watering (sometimes two or three times daily). Natural lighting was supplemented with incandescent and fluorescent lighting, and photoperiods were held at 12.5 h daily. Mean maximal and minimal temperatures over the treatment period were 35 and 19.5°C, respectively. Relative humidity was not controlled, and means were 43.5 and 35%, respectively.

On the 47th d after planting, the plants (third trifoliate stage of development) had their roots sealed from atmospheric O_2 with a mixture of wax, paraffin, and cotton fiber similar to that used in Sojka et al. (1975). A small (approximately 2.5-cm) air space was left between the soil surface and the cylinder tops to allow humidified gas mixtures to be flushed over the soil surface. Cylinder bottoms were also sealed from the atmosphere with plastic bottoms cemented to the cylinders.

Treatments were in a randomized complete block design with four replications and three sampling dates. Four soil-O_2 treatments (0, 2, 4 or 21% O_2) were created by mixing air and nitrogen gas, using flow meters to regulate the composition of the gas mixtures. Gas mixtures were passed over the soil surface at a rate of 500 ml/min via manifolds servicing each treatment, replicate, and harvest date. Gas mixtures were humidified before flowing through the cylinders to prevent soil water evaporation. Prior to initiation of gas treatments on the 47th d after planting, four untreated, representative (extra) cylinders were harvested. Treated plants were subsequently harvested on days 61, 69, and 76 after planting to characterize time course effects of the treatments. The effectiveness of treatments in altering soil O_2 was evaluated at irregular intervals by measuring soil-O_2 diffusion rate (ODR) using the method of Letey and Stolzy (1964), with a Jensen Instrument's model C ODR meter.[3] Values of ODR used in correlations and analysis of variance were the mean readings from electrodes at the 15-cm and 19-cm depths in each cylinder.

Leaf diffusive resistance of the most recently matured, fully expanded trifoliate on each plant was determined for the abaxial (R_{ab}) and adaxial (R_{ad}) surfaces using a Licor meter and porometer

[3] Mention of trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the U.S. Dept. of Agric. or the S.C. Agric. Exp. Stn. and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

similar to the one described by Kanemasu et al. (1969). Soil ODR was determined in conjunction with measurement of leaf diffusive resistance 47, 48, 49, 52, 53, 54, 55, 59, 65, 69, and 74 d after planting. Parallel leaf diffusive resistance (R_s) was calculated by the formula

$$R_s^{-1} = R_{ab}^{-1} + R_{ad}^{-1} \quad (1)$$

The three harvest dates did not coincide with the 11 ODR and r_s monitoring dates.

Stomatal impressions were made prior to harvest at 61, 69, and 76 d after planting on the most recently matured, fully expanded trifoliate leaf on each harvest date, using the method of Rice et al. (1979). It was initially thought that trichomes on soybean leaf surfaces of the (+) isoline might cause the vinyl cast to adhere to leaves, preventing collection of successful leaf impressions, but no such problems were encountered. Stomatal density (SD) was estimated by randomly positioning each of the four quadrants of the impression under the microscope field and counting the number of stomata in a 0.25-mm^2 area in each quadrant and then summing the four counts for a mean estimate of stomatal population per square millimeter.

Dry weights of leaf blades, stems, petioles, pods, and roots and leaf areas were determined on the four representative cylinders on the day of treatment initiation and again on each of the three harvest dates. The partitioned dry matter components were subsequently ground and digested with a 1:1 nitric:percholoric acid mix. The K concentration was determined by flame emission spectrophotometry, and Ca and Mg concentrations were determined by atomic absorption spectrophotometry. A solution of 1% La was added to prevent anion interference in Ca analysis (Isaac and Kerber 1971).

RESULTS AND DISCUSSION

Effects on growth

Pretreatment plant characteristics (day 47) appear in Table 1. These data give the condition of the plants at treatment initiation. Nutrient concentrations in all plant parts were somewhat higher in the pretreatment sampling than at later dates, due to the immaturity of much of the tissue analyzed. This trend continued, but to a lesser extent into the first complete sampling date in the control (21% O_2) plants. Due to the small size of the pretreatment samples, all four petiole samples of each isoline were combined, and samples were paired for stem analysis. Because both isolines were grown together in a single cylinder, root systems were intimately associated with one another and could not be separated. Root weights are the mean of the two isolines. Only sparse nodulation was observed during root washing (due probably to soil fumigation and N fertilization). Concentrations of K and Ca correspond closely with values reported for field-grown Lee soybean of the same age (Henderson and Kamprath 1970; Bhangoo and Albritton 1972; Terman 1977; Batchelor and Scott 1979), whereas observed concentrations of Mg were somewhat elevated.

The effects of the four soil-O_2 regimes on plant growth are summarized in Tables 2 and 3 for the three harvest dates. On the first harvest date, mean isoline responses to O_2 differed significantly for root and pod weights, and numerical trends for negative response existed in the other parameters, at the lowest O_2 level. Isolines (means of all O_2 treatments) differed significantly in all shoot components on this date at the $P = 0.1$ level or better, and for all components except pod weight, the Lee (−) isoline was

TABLE 1

Pretreatment plant characteristics

	Weight, g plant^{-1}			Leaf area, cm^2 plant^{-1}			K, g kg^{-1}			Ca, g kg^{-1}			Mg, g kg^{-1}		
Isoline	(+)	(−)	Mean	(+)	(−)	Mean	(+)	(−)	Mean	(+)	(−)	Mean	(+)	(−)	Mean
Stem	0.16	0.14	0.15	–	–	–	20.6	19.4	20.0	4.2	3.4	3.8	6.8	7.0	6.9
sd	0.05[a]	0.10		–	–		0.4	0.8		1.2	0.1		0.1	0.0	
Petiole	0.03	0.06	0.05	–	–	–	39.5	39.0	39.3	10.2	10.7	10.5	10.0	9.3	9.7
sd	0.01	0.01		–	–		–	–		–	–		–	–	
Leaf	0.39	0.51	0.45	127	135	131	26.7	26.6	26.7	7.9	7.8	7.9	6.8	6.8	6.8
sd	0.16	0.17		24	31		0.2	0.2		0.8	0.7		0.6	0.2	

[a] Sample standard deviation (sd).

TABLE 2

Component weights for Lee soybean isolines with (+) or without (−) trichomes as affected by four root zone oxygen treatments harvested 61, 69, and 76 d after planting

Harvest	% O_2	Total wt[a]	Root wt	Top wt (+)	Top wt (−)	Mean
		g plant^{-1}				
1	21	3.26f	1.09f	2.24fgh	2.09ghi	2.16f
	4	3.04f	0.99fg	1.55hi	2.54fg	2.04f
	2	3.17f	1.00fg	1.22i	3.12f	2.17f
	0	2.72f	0.80g	1.60hi	2.24fgh	1.92f
2	21	4.68f	1.06f	2.21i	5.00f	3.60f
	4	4.66f	0.90g	3.10ghi	4.42fg	3.76f
	2	4.31f	0.86g	3.87fgh	3.02ghi	3.45f
	0	2.74g	0.52h	1.88i	2.57hi	2.23g
3	21	5.55f	0.97f	4.54f	4.64f	4.59f
	4	4.98g	0.84g	4.05f	4.22f	4.14f
	2	4.90g	0.77g	3.66f	4.59f	4.12f
	0	2.29h	0.44h	1.93g	1.75g	1.84g
Mean of 1–3	21	4.50f	1.04f	2.99gh	3.91f	3.45f
	4	4.23f	0.91g	2.90gh	3.73fg	3.25f
	2	4.12f	0.88g	2.92gh	3.58fg	3.25f
	0	2.58g	0.59h	1.81i	2.19hi	2.00g

[a] Means within each column followed by the same letter are not significantly different by P (0.10) by Duncan's multiple range test. Means from both (+) and (−) columns are used to derive significant differences.

TABLE 3

Component weights and leaf areas for Lee soybean isolines with (+) or without (−) trichomes as affected by four root zone oxygen treatments harvested 61, 69, and 76 d after planting

Harvest	Treatment	Stem wt[a]	Pet. wt	Leaf wt	Live L. Area[b]	Dead L. Area	Pod wt
1	21	0.42f[c]	0.27f	1.21f	348f	–	0.26f
	4	0.43f	0.28f	1.19f	354f	–	0.15g
	2	0.44f	0.31f	1.27f	401f	–	0.16g
	0	0.40f	0.25f	1.06f	292f	–	0.22fg
Mean (+)		0.35y	0.19y	0.87y	279y	–	0.24x
(−)		0.50x	0.37x	1.49x	418x	–	0.15y
2	21	0.56f	0.42fg	1.46f	343fg	–	1.17f
	4	0.63f	0.52f	1.65f	390f	–	0.96fg
	2	0.57f	0.45fg	1.32f	326fg	–	1.17f
	0	0.53f	0.25g	0.84g	232g	–	0.61g
Mean (+)		0.45y	0.28y	1.00y	274y	–	1.05x
(−)		0.70x	0.51x	1.64x	371x	–	0.91x
3	21	0.66f	0.47f	1.37f	384f	15g	2.09f
	4	0.66f	0.45f	1.23f	331f	31g	1.79f
	2	0.63f	0.47f	1.18f	326f	41g	1.85f
	0	0.49g	0.24g	0.68g	212g	107f	0.43g
Mean (+)		0.55y	0.35x	0.98x	302x	62x	1.67x
(−)		0.67x	0.47x	1.25x	325x	35y	1.41x

[a] Weights are in g plant^{-1}.

[b] Areas are in cm^2 plant^{-1}.

[c] Means within each column followed by the same letter are not significantly different by P (0.05) by Duncan's multiple range test. Overall (+) and (−) means are treated as separate columns.

superior to the Lee (+) isoline. This is in part related to pretreatment performance (Table 1) and may indicate superior seedling vigor for the Lee (−) isoline seedstock.

In spite of the generally superior vegetative growth of the Lee (−) isoline, pod set and development were significantly less, even at the first harvest, and remained numerically so through the third harvest, although no longer significant at the 5% level for that date. Combined top weight (Table 2) did not differ significantly between isolines on the third harvest, primarily because greater leaf loss of the Lee (−) isoline was further offset by greater pod production of the Lee (+) isoline. Although plant vegetative growth-response patterns were more statistically significant after prolonged exposure to the O_2 treatments (later harvests), numerical trends in growth components were apparent, even in the two earliest harvests, between the three aerated treatments and the 0% O_2 treatment.

The time course effect of gas treatment on ODR is presented in Fig. 1 for the control (21% O_2) and the 0% O_2 (N_2) treatments. The ODR dropped sharply in the 0% O_2 treatment in the first 24 h and more gradually thereafter, indicating a lag in root zone O_2 depletion. Time course observations of ODR in the 4 and 2% treatments indicated that ODR declined only in the final few observations. As predicted by

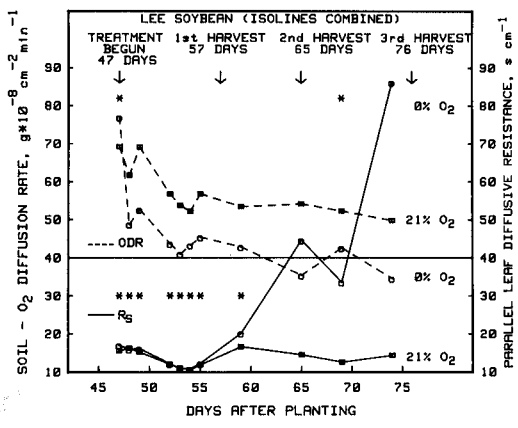

FIG. 1. Time course of parallel leaf diffusive resistance (R_s) and soil O_2 diffusion rate (ODR) for 0 and 21% O_2 treatments. Paired values of ODR or R_s not under asterisks (*) differ significantly at $P \leq 0.05$. Each point is the mean of between 3 and 12 observations.

Luxmoore and Stolzy (1972), until root systems were relatively large, their consumption of soil O_2 in all but the 0% O_2 treatment was insufficient to rapidly deplete O_2 supplied at the soil surface by the aerating stream, cylinder leakage, or internal plant diffusion. In addition, fumigating the soil prior to planting probably limited the initial contribution of soil-microbial respiration in this study. Nonetheless, Fig. 1 provides evidence that the ODR of the 0% O_2 treatment was consistently 20×10^{-8} g cm^{-2} min^{-1} below the ODR of the 21% O_2 treatment, and that as respiring root systems grew, ODR gradually declined in all treatments. Observations of growth parameters and plant nutrient concentrations and uptake (Tables 5 and 6) suggest that the 2 and 4% O_2 treatments were effective even before ODR further decreased in these treatments.

Effects on stomata

The existence of an ODR response threshold for stomatal closure is known (Sojka and Stolzy 1980), but the time dependency of exposure to the threshold ODR has not been well defined. Stomata closed in this study upon attainment of an ODR of approximately 40×10^{-8} g cm^{-2} min^{-1} (Fig. 1). These data suggest that closure occurs when exposure to the threshold ODR lasts for some period in excess of 48 h. Although the threshold was nearly encountered briefly on day 53 (6 d after treatment initiation), diffusive resistance (R_s) remained low. When ODR dropped below 40×10^{-8} g cm^{-2} min^{-1} between days 59 and 65, R_s rose sharply. On day 69, R_s fell again when ODR briefly exceeded 40×10^{-8} g cm^{-2} min^{-1} and was high again on day 74 when ODR fell once more below 40×10^{-8} g cm^{-2} min^{-1}. In addition to the apparent need for a 48-h exposure to 40×10^{-8} g cm^{-2} min^{-1} ODR, it should be noted that the response of R_s to ODR in this experiment and in Sojka and Stolzy's (1980) earlier work was related by a power function of the form

$$Y = ax^b \qquad (2)$$

For these reasons, R_s did not rise on day 53 even though ODR came near, but did not cross, the response threshold. In this respect, stomatal response to soil ODR is similar to Raschke's (1975) observation of the nature of stomatal closure caused by soil-water stress. He observed

neglible stomatal closure until a threshold plant water potential (ψ_x) was achieved, after which complete closure occurred rapidly. No measurements of ψ_x were made in this study, although it has been shown earlier that stomatal closure due to poor root aeration is not consistently associated with corresponding changes in ψ_x (Sojka et al. 1975; Sojka and Stolzy 1980; Bradford and Yang 1981).

The distribution of R_s versus ODR for all treatments on all observation dates is shown in Fig. 2. The existence of the 40×10^{-8} g cm^{-2} min^{-1} threshold is readily apparent. Because proper regression analysis requires a normal distribution of data about the mean, a meaningful regression fit of the R_s response to ODR is possible only by limiting the regression of data to the last three observation dates (days 65, 69, and 74). On these days, ODR hovered at or below the threshold response value (using all observations biases the regression to the nonnormal distribution of data in the narrow R_s range prior to attainment of the threshhold ODR across treatments). The relationship of R_s to ODR is presented in Fig. 3 for all treatments on the final three R_s observation dates. Stomatal closure in this study occurred at approximately 40×10^{-8} g cm^{-2} min^{-1} for both isolines. In both Figs. 2 and 3, the threshold response (or "on-off" response) mode is similar to data presented earlier by Sojka and Stolzy (1980). The threshold ODR reported for stomatal closure in this study, however, is approximately double their observed stomatal response threshold and double the critical ODR reported earlier for general plant responses

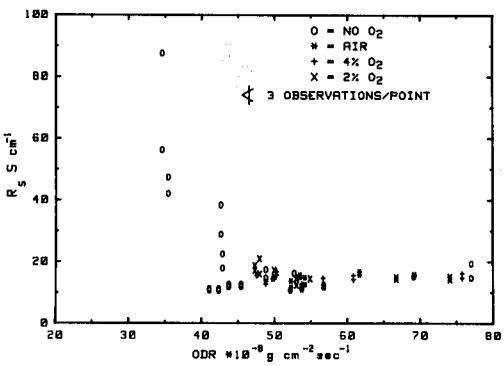

FIG. 2. Parallel leaf diffusive resistance (R_s) as a function of soil O_2 diffusion rate (ODR) for all O_2 treatments and both isolines on all observation dates. Each point is the mean of between 3 and 12 observations.

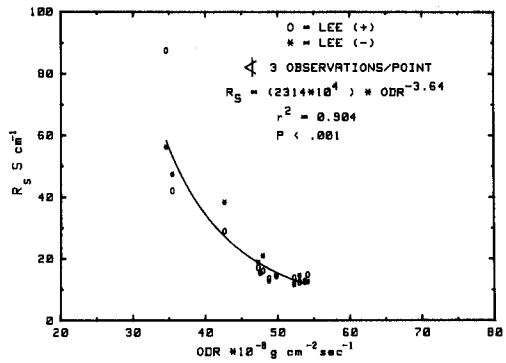

FIG. 3. Parallel leaf diffusive resistance (R_s) as a function of soil O_2 diffusion rate (ODR) for all O_2 treatments and each isoline on the final three observation dates. Each point is the mean of between 3 and 12 observations.

other than stomatal closure (Stolzy and Letey 1962). It would seem that Stolzy and Letey were correct in cautioning against too broad an application of the 20×10^{-8} g cm^{-2} min^{-1} rule of thumb, and that in fact each plant species and, perhaps individual physiological processes within species begin to react at their own response thresholds.

The effect of soil O_2 treatment on stomatal density (SD) is presented in Fig. 4 and quantified in Table 4. There was a numerical trend for the higher Lee (+) SD on the abaxial surface and for higher Lee (−) SD on the adaxial surface, although not significant at the 5% level when comparing response on individual dates separately. The abaxial SD of the 0% O_2 treatment increased 43% over time, whereas the SD of the 21% O_2 treatment remained nearly constant. As soil O_2 levels dropped, the abaxial SD (Table 4) increased, whereas leaf area (Table 3) decreased. It appears that abaxial SD was affected primarily by a reduction in leaf expansion associated with inadequate soil aeration.

Failure to see a corresponding trend in adaxial SD was related to adaxial stomatal distribution. Adaxial stomata were unevenly distributed across the leaf surface, occurring primarily in close association with conductive tissue (so-called leaf veins, Fig. 4C). Therefore, greater sampling error was associated with the random positioning of impressions in the microscope field for stomatal counts. In some instances, interveinal areas were counted, resulting in few or no stomata per mm^2; at other times veinal tissue in the microscope field resulted in SDs similar to the abaxial surface. Precise determi-

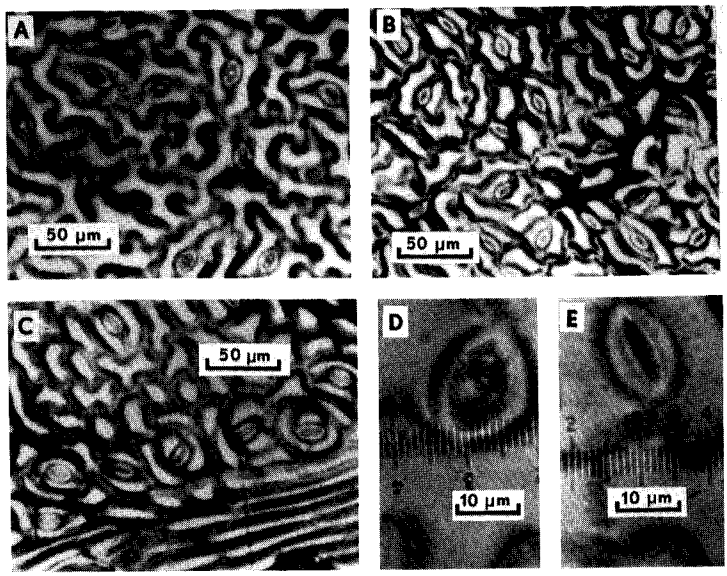

FIG. 4. Photomicrographs of vinyl leaf impressions showing: (A) open, well-aerated (21% O_2) abaxial stomata; (B) closed, more densely distributed abaxial stomata of the poorly aerated (0% O_2) treatment; (C) grouping of adaxial stomata along leaf xylem; (D) enlarged impression of an open (21% O_2) stomate, and (E) enlarged impression of a closed (0% O_2) stomate.

TABLE 4

Stomatal density of abaxial (Ab) and adaxial (Ad) leaf surfaces as affected by soil oxygen treatment of the Lee cultivar as observed on three harvest dates (61, 69 and 76 d after planting)

%O_2	Stomata/mm^2					
	Harvest 1		Harvest 2		Harvest 3	
	Isoline mean		Isoline mean		Isoline mean	
	Ab	Ad	Ab	Ad	Ab	Ad
21	58.6f[a]	12.3f	62.5g	9.8f	54.8h	6.9f
4	64.3f	9.1f	68.0fg	10.4f	65.3g	10.8f
2	62.4f	10.0f	72.6f	11.0f	62.8gh	6.6f
0	63.4f	9.8f	71.8f	14.8f	90.4f	8.4f

[a] Means within each column followed by the same letter are not significantly different by P (0.05) by Duncan's multiple range test. Population per mm^2.

nation of adaxial SDs would require a considerably larger sample population than used in this study. That R_s increases with falling ODR, even though abaxial SD rises, clearly indicates that reduction in individual stomatal aperture was the immediate mechanism responsible for the rise in R_s. Although sizes of individual stomata were not compared quantitatively, there were no striking visual dissimilarities in stomatal dimensions (Fig. 4).

Cation concentrations

Various growth components (Tables 2 and 3) and nutrient concentrations (Table 5) were affected before a detectable change in stomatal performance. This suggests that some physiological processes were affected at their own specific response thresholds of ODR, and that these thresholds are largely above 40×10^{-8} g cm^{-2} min^{-1}. Concentrations of K were numerically less for all plant components (except pods on the first and third harvests). The concentration of K of the 0% O_2 treatment were numerically less than the 21% treatment for all plant components on all harvests and significantly less at $P \leq 0.10$ in 9 of the 12 analyses across the three harvest dates and in all analyses when the three dates were averaged. Significant and consistent changes in Ca concentration were observed in the last two harvests and were more or less parallel to changes in K concentration. Concentrations of Mg were not consistently affected by aeration treatment throughout the study. Total uptake of plant nutrients is presented in Table 6. These data indicate that, although uptake of K, Ca, and Mg progresses on all three harvests for the 21% O_2 treatment, their uptake is supressed in the 4% and 2% O_2 treatments and unchanged between the second and third harvest for the 0% O_2 treatment.

A contributing factor to stomatal closure may involve a decrease in leaf K concentration. Several recent studies (Drew and Sisworo 1979; Drew et al. 1979; Trought and Drew, 1980*a*,*b*) with barley and wheat have shown the K uptake by roots ceases almost immediately upon loss of adequate root aeration. Their work showed that normal growth and plant function were main-

TABLE 5

Concentration of K, Ca, *and* Mg *in stems, petioles, leaf blades, and pods for Lee soybean isolines with (+) or without (−) trichomes as affected by four root zone* O_2 *treatments harvested 61, 69, and 76 d after planting*

Harvest	Treatment	Cation concentrations, g/kg											
		Stems			Petioles			Leaf blades			Pods		
		K	Ca	Mg	K	Ca	Mg	K	Ca	Mg	K	Ca	Mg
1	21	15.1f[a]	3.4f	5.5f	25.6f	8.3f	6.7g	24.2f	8.3f	6.8f	21.5fg	8.1fg	6.8g
	4	14.2f	3.0f	5.1g	24.1fg	7.5f	6.9f	22.9f	7.3g	6.6fg	22.7f	9.4f	7.8f
	2	14.6f	2.9f	4.9g	23.6g	7.1f	6.8fg	23.4f	7.6fg	6.5fg	22.2f	9.4f	7.7f
	0	12.6g	3.2f	4.8g	18.8h	8.0f	6.5fg	19.5g	7.8fg	6.3g	19.8g	7.8f	7.2fg
Mean (+)		14.2x	3.0x	4.7y	23.7x	7.5x	6.6x	22.0x	7.3y	6.6x	21.4x	7.7y	7.2x
(−)		14.1x	3.3x	5.5x	22.7x	7.9x	6.8x	23.0x	8.1x	6.5x	21.3x	9.7x	7.4x
2	21	12.5f	3.1f	4.2f	17.2f	7.6f	5.0fg	19.7f	8.7f	5.3g	18.5f	6.5f	6.0f
	4	11.7fg	2.1g	3.6fg	16.8fg	6.0g	4.8g	18.4g	7.5fg	5.4g	17.6fg	5.8fg	5.6fg
	2	11.2g	2.1g	3.3g	15.4fg	5.5g	5.3fg	18.6fg	8.0fg	6.1f	17.7fg	5.2g	5.6fg
	0	8.0h	1.8g	3.4g	15.0g	4.8g	5.5f	17.6g	7.3g	6.1f	17.2g	2.9h	5.2g
Mean (+)		11.5x	2.4x	3.1y	17.9x	6.0x	4.8y	18.7x	7.9x	5.9x	18.0x	5.3x	5.7x
(−)		10.3y	2.2x	4.2x	14.8y	6.0x	5.4x	18.4x	7.9x	5.6x	17.5x	4.9x	5.4x
3	21	12.7f	2.8f	3.7g	20.1f	7.1f	5.0fg	22.9f	11.3f	6.0g	20.2f	5.7f	5.7f
	4	11.4g	2.5f	2.5h	17.4g	6.1f	4.8g	20.7f	9.6g	6.2g	20.6f	6.0f	5.6f
	2	11.8fg	2.6f	2.3h	17.5g	6.5f	5.3fg	17.4g	10.0fg	6.1g	20.2f	6.1f	5.6f
	0	9.1h	2.0g	4.2f	18.5fg	4.8g	5.5f	21.8f	8.7g	6.8f	18.2g	2.5g	4.8g
Mean (+)		11.9x	2.6x	2.9y	19.8x	6.1x	4.8y	21.2x	10.0x	6.4x	20.5x	5.1x	5.5x
(−)		10.5y	2.3y	3.5x	16.9y	5.3x	5.4x	10.2x	9.8x	6.1x	19.1y	5.2x	5.4x
Mean of 1–3	21	13.4f	3.1f	4.5f	21.0f	7.7f	5.5f	22.3f	9.4f	6.0g	19.9f	6.6f	6.1f
	4	12.4g	2.5g	3.8h	19.3g	6.4g	5.4f	20.7g	8.1g	6.0g	19.6f	6.5f	6.0f
	2	12.6g	2.5g	3.5h	18.4gh	6.2gh	5.6f	19.8g	8.5g	6.2fg	19.4f	6.1f	5.9fg
	0	9.9h	2.3g	4.1g	17.7h	5.7h	5.6f	19.6g	7.9g	6.4f	18.1g	3.9g	5.5g
Mean (+)		12.5x	2.6x	3.5y	20.1x	6.2x	5.3x	20.6x	8.4x	6.3x	19.8x	5.9x	6.0x
(−)		11.6y	2.6x	4.4x	18.0y	6.8x	5.8x	20.5x	8.6x	6.1x	18.7y	5.6x	5.7x

[a] Means within each column followed by the same letter are not significantly different by P (0.10) by Duncan's multiple range test. Overall (+) and (−) means are treated as separate columns.

tained briefly after this as a result of mobilization of K from older plant tissue to juvenile expanding tissue. In time, this redistribution of nutrients was inadequate to support normal growth and plant functions. Cheeseman and Hanson (1979) analyzed K uptake in corn roots and concluded that anoxia could even reduce passive K influx.

In this study, a general reduction in tissue concentrations of K and Ca was associated with lower O_2 levels. Leaf concentration of K in the 0% O_2 treatment on the last harvest date was still slightly lower than the 21% O_2 treatment on that date, but not statistically so. The lower reduction of leaf K concentration on the last harvest date might be explained in the light of the work discussed above. When lower-leaf abscission is large, as it was in the 0% O_2 treatment on the last harvest (see Table 3), K may have migrated to juvenile tissue prior to abscission. This conclusion is supported by the nutrient uptake data presented in Table 6, for total K values on the second and third harvest dates are essentially the same, meaning that concentration of leaf K in the 0% O_2 treatment could be maintained only through redistribution of K within the plant. With lower leaf abscission, juvenile tissue also becomes a much larger fraction of the remaining total living leaf tissue analyzed. Consequently, mean live-leaf concentrations would be elevated. In this experiment, stomatal observations and R_s determinations were made only on mature leaf blades. From the previously cited work (Drew and Sisworo 1979; Drew et al. 1979; Trought and Drew 1980*a*,*b*), it would seem likely that where leaf drop occurred, K concentrations were probably lower on the physiologically monitored, remaining, mature, expanded leaves than cation analysis of the total leaf mass indicates. Furthermore, it is clear that

where stomatal closure has occurred carbon fixation will decline, reducing the cation dilution effect generally associated with vigorous growth.

Beyond the nutrient analysis alone, the fact that approximately 48 h of exposure to $\leq 40 \times 10^{-8}$ g cm^{-2} min^{-1} ODR seemed necessary to cause stomatal closure indicates that potassium may not act alone in low-O_2-induced closure. The lag time would suggest that another factor, perhaps production of a hormone such as ethylene, may also be involved. Due to the confounding of cation data by abscission in the 0% O_2 leaf sample on the third harvest date, however, conclusive correlation of leaf K concentration to stomatal closure during soil O_2 deprivation is not possible from this experiment. The overall data, nonetheless, generally support the idea that K concentrations may be at least partially involved in the process, and the question still merits further investigation.

Of all the plant nutrient aberrations that have been reported in conjunction with poor root zone aeration, decrease in plant K concentration has been one of the most consistent responses (Sojka and Stolzy 1980; Trought and Drew 1981; Singh and Ghildyal 1980; Thomas and Hipp 1968; Drew et al. 1980; Pessoa da Coasta and Smucker 1981). There are, however, notably few data on the effects of root anoxia on soybean nutrition.

Peaslee and Moss (1968) and Cooper et al. (1967) showed that elevated K levels increased stomatal aperture and affected stomatal population in alfalfa and corn, the former speculating that K migration from guard cells of K-deficient plants resulted in stomatal closure. Peoples and Koch (1979) also found stomatal closure with K deficiency in alfalfa. The work of Wardle and Simpkins (1979), however, showed that elevating plant K concentrations resulted in higher rather than lower R_s in corn.

In the literature, the effect of low soil O_2 on Ca and Mg concentrations is somewhat less consistent than its effect on K. In general, results parallel those from this study; that is, Ca concentrations declined, whereas Mg concentrations remained unaltered.

TABLE 6

Total uptake of potassium, calcium, and magnesium for Lee soybean isolines with (+) or without (−) trichomes as affected by four root zone oxygen treatments harvested 61, 69, and 76 d after planting

Harvest	% O_2	Total cation uptake, g/plant		
		K	Ca	Mg
1	21	0.027f[a]	0.015f	0.013f
	4	0.021f	0.013f	0.012f
	2	0.025f	0.014f	0.013f
	0	0.015f	0.012f	0.011f
Mean (+)		0.020x	0.010y	0.010y
(−)		0.024x	0.017x	0.015x
2	21	0.065f	0.025f	0.019f
	4	0.063f	0.022f	0.019f
	2	0.058f	0.020f	0.018f
	0	0.032g	0.010g	0.011g
Mean (+)		0.047y	0.016y	0.014y
(−)		0.062x	0.023x	0.020x
3	21	0.091f	0.032f	0.025f
	4	0.076f	0.027f	0.021f
	2	0.073f	0.028f	0.021f
	0	0.029g	0.009g	0.009g
Mean (+)		0.069x	0.023x	0.019x
(−)		0.066x	0.025x	0.020x

[a] Means within each column followed by the same letter are not significantly different by P (0.05) by Duncan's multiple range test. Overall (+) and (−) means are treated as separate columns.

CONCLUSIONS

With these soybean isolines, the threshold ODR for stomatal closure was approximately 40×10^{-8} g cm^{-2} min^{-1}. This can be deduced both from simultaneous time-course observations of ODR and R_s and from the regression of R_s on ODR. Exposure to the threshold ODR of approximately 48 h appears necessary to elevate R_s. The increase in R_s was found to be the result of stomatal closure, rather than a gradual change in SD, as SD actually increased substantially over time in the low O_2 treatments.

There are probably several interrelated mechanisms that bring about stomatal closure in response to poor rhizosphere aeration. Some of those implicated by other researchers include hormonal responses and alterations in biochemical activity. Flux of K into and out of guard cells is the cation flux most intimately involved in the regulation of guard cell turgor and stomatal aperture. The data in this paper and the preponderance of substantiating nutrient analyses in the literature show a decline of K concentration and K uptake in the plant with low O_2. The decline of K concentration in leaf blades in this experiment coincides sufficiently with the onset of elevated R_s that one cannot rule out the changes in K concentration as a contributing

factor in this physiological response. The concentration anomaly of the third harvest leaf analysis, however, suggests further work to establish the linkage conclusively.

Finally, soybean was more sensitive to soil O_2 status than a wide range of other plant species previously studied, for which decline in growth and performance has been predicted at 20×10^{-8} g cm^{-2} min^{-1}. If the 40×10^{-8} g cm^{-2} min^{-1} threshold is applicable over a wide range of soybean cultivars and maturity groups, one can infer that, under normal field culture, soybean is less flood-tolerant than heretofore believed. The use of R_s comparisons among cultivars may provide a tool to screen for resistance to soil O_2 stress.

ACKNOWLEDGMENTS

I thank Dr. E. E. Hartwig, for providing the Lee soybean isolines used in this study, and Mr. F. B. Arnold, for his meticulous and energetic technical support.

REFERENCES

Batchelor, J. T., and H. D. Scott. 1979. Effects of irrigation on nitrogen and potassium uptake by soybeans. Arkansas Farm Res. 28:5.

Bhangoo, M. S., and D. J. Albritton. 1972. Effect of fertilizer nitrogen, phosphorus, and potassium on yield and nutrient content of Lee soybeans. Agron. J. 64:743–746.

Bradford, K. J., and S. F. Yang. 1981. Physiological responses of plants to waterlogging. Hortic. Sci. 16:25–30.

Campbell, R. B., and C. J. Phene. 1977. Tillage, matric potential, oxygen and millet yield relations in a layered soil. Trans. ASAE 20:271–275.

Campbell, R. B., and G. T. Seaborn. 1972. Yield of flue-cured tobacco and levels of soil oxygen in lysimeters with different water table depths. Agron. J. 64:730–733.

Cheeseman, J. M., and J. B. Hanson. 1979. Energy-linked potassium influx as related to cell potential in corn roots. Plant Physiol. 64:842–845.

Cooper, R. B., R. E. Blaser, and R. H. Brown. 1967. Potassium nutrition effects on net photosynthesis and morphology of alfalfa. Soil Sci. Soc. Am. Proc. 31:231–235.

Doty, C. W., and J. E. Parsons. 1979. Water requirements and water table variations for a controlled and reversible drainage system. Trans. ASAE 22:532–539.

Drew, M. C., A. Chamel, J. P. Garrec, and A. Fourcy. 1980. Cortical air spaces (aerenchyma) in roots of corn subjected to oxygen stress. Plant Physiol. 65:506–511.

Drew, M. C., and E. J. Sisworo. 1979. The development of waterlogging damage in young barley plants in relation to plant nutrient status and changes in soil properties. New Phytol. 82:301–314.

Drew, M. C., E. J. Sisworo, and L. R. Saker. 1979. Alleviation of waterlogging damage to young barley plants by application of nitrate and a synthetic cytokinin, and comparison between the effects of waterlogging, nitrogen deficiency and root excision. New Phytol. 82:315–329.

Fischer, R. A. 1968. Stomatal opening: Role of potassium uptake by guard cells. Science 160:784–785.

Henderson, J. B., and E. J. Kamprath. 1970. Nutrient and dry matter accumulation in soybeans. N.C. Agric. Exp. Stn. Tech. Bull. 197.

Humble, G. D., and T. C. Hsiao. 1969. Specific requirement of potassium for light activated opening of stomata in epidermal strips. Plant Physiol. 44:230–234.

Humble, G. D., and K. Raschke. 1971. Stomatal opening quantitatively related to potassium transport: Evidence from electron probe analysis. Plant Physiol. 48:447–453.

Hunt, P. G., R. B. Campbell, R. E. Sojka, and J. E. Parsons. 1981. Flooding-induced soil and plant ethylene accumulation and water status response of field-grown tobacco. Plant Soil 59:427–439.

Isaac, R. A., and J. D. Kerber. 1971. Atomic absorption and flame photometry: Techniques and uses in soil, plant and water analysis. *In* Instrumental methods of analysis of soils and plant tissue. L. M. Walsh (ed.). Soil Sci. Soc. Am., Madison, Wis., pp. 17–37.

Kanemasu, E. T., G. W. Thurtell, and C. B. Tanner. 1969. Design calibration and field use of a stomatal diffusion porometer. Plant Physiol. 44:881–885.

Karlen, D. L., R. E. Sojka, and M. L. Robbins. 1983. Leaf diffusive resistance and softness of tomato fruit in response to excess soil-water at three N rates. Commun. Soil Sci. Plant Anal. 14:699–708.

Letey, J., and L. H. Stolzy. 1964. Measurement of oxygen diffusion rates with the platinum microelectrode: 1. Theory and equipment. Hilgardia 35:545–554.

Luxmoore, R. J., and L. H. Stolzy. 1972. Oxygen diffusion in the soil-plant system: 6. A synopsis with commentary. Agron. J. 64:725–729.

Meek, B. D., E. C. Owen-Bartlett, L. H. Stolzy, and C. K. Labanauskas. 1980. Cotton yield and nutrient uptake in relation to water table depth. Soil Sci. Soc. Am. J. 44:301–305.

Peaslee, D. E., and D. N. Moss. 1966. Stomatal conductivities in K-deficient leaves of maize (*Zea mays* L.). Crop Sci. 8:427–430.

Peoples, T. R., and D. W. Koch. 1979. Role of potassium in carbon dioxide assimilation in *Medicago sativa* L. Plant Physiol. 63:878–881.

Pessoa da Coasta, G. T., and A. J. M. Smucker. 1981. Interactions of oxygen-nitrogen-salinity stresses on plant growth and mineral content of sunflower (*Helianthus annuus* L.) in sand culture. J. Plant Nutr. 3:887–903.

Raschke, K. 1975. Stomatal action. Annu. Rev. Plant Physiol. 26:309–340.

Reicosky, D. C., R. B. Campbell, and C. W. Doty. 1976. Corn plant water stress as influenced by chiseling, irrigation, and water table depth. Agron. J. 68:499–503.

Rice, J. S., E. M. Glenn, and V. L. Quisenberry. 1979. A rapid method for obtaining leaf impressions in grasses. Agron. J. 71:894–896.

Singh, R., and B. P. Ghildyal. 1980. Soil submergence effects on nutrient uptake, growth, and yield of five corn cultivars. Agron. J. 72:737–741.

Smucker, A. J. M. 1975. Interactions of soil oxygen and water stresses upon growth, disease, and production of navy beans. Report of the Bean Improvement Cooperative, pp. 72–75.

Sojka, R. E., and L. H. Stolzy. 1980. Soil-oxygen effects on stomatal response. Soil Sci. 130:350–358.

Sojka, R. E., L. H. Stolzy, and M. R. Kaufmann. 1975. Wheat growth related to rhizosphere temperature and oxygen levels. Agron. J. 67:591–596.

Stolzy, L. H., and J. Letey. 1962. Measurement of oxygen diffusion rates with the platinum microelectrode: 3. Correlation of plant response to soil oxygen diffusion rates. Hilgardia 35:567–576.

Terman, G. L. 1977. Yields and nutrient accumulation by determinate soybeans, as affected by applied nutrients. Agron. J. 69:234–238.

Thomas, G. W., and B. W. Hipp. 1968. Soil factors affecting potassium availability. *In* The role of potassium in agriculture. V. J. Kilmer, S. E. Younts, and N. C. Brady (eds.). Am. Soc. Agron., Madison, Wis., pp. 269–291.

Trought, M. C. T., and M. C. Drew. 1980*a*. The development of waterlogging damage in wheat seedlings. (*Triticum aestivum* L.): 1. Shoot and root growth in relation to changes in the concentrations of dissolved gases and solutes in the soil solution. Plant Soil 54:77–94.

Trought, M. C. T., and M. C. Drew. 1980*b*. The development of waterlogging damage in wheat seedlings (*Triticum aestivum* L.): 2. Accumulation and redistribution of nutrients by the shoot. Plant Soil 56:187–199.

Trought, M. C. T., and M. C. Drew. 1982. Alleviation of injury to young wheat plants in anaerobic solution cultures in relation to the supply of nitrate and other inorganic nutrients. J. Exp. Bot. 32:509–522.

Wardle, K., and I. Simpkins. 1979. Stomatal responses of *Phaeseolus vulgaris* L. seedlings to potassium chloride in the nutrient solution. J. Exp. Bot. 30:1195–1200.