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Concomitant Determination of Folar Nitrogen Loss, Net Carbon Dioxide Uptake, and Transpiration[1]

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ABSTRACT

A closed system was designed for concomitant determination of net CO_2 uptake, transpiration, and foliar nitrogen (N) loss in soybeans (*Glycine max* [L.] Merr.). The CO_2 uptake was monitored by infrared analysis of system air removed periodically in aliquots. Leaf vapors were trapped in calibrated tubes at Dry Ice temperature, and transpiration rate was determined from the quantity of condensate. Subsequent pyrochemiluminescent analysis of this condensate revealed reduced N forms, although a s⁻'all percentage (4 to 15%) of oxidized forms was found.

Maximum CO_2 uptake (22.3 milligrams per square decimeter per hour), transpiration (2.5 grams H_2O per square decimeter per hour), and the total (4.9 micrograms per square decimeter per hour), reduced (4.1 micrograms per square decimeter per hour), and oxidized (0.7 microgram per square decimeter per hour) N loss rates were measured between 6.5 and 10.5 hours (30 C) of a simulated 13-hour photoperiod in leaves of V4 to V5 (three to four trifoliolate stage) soybeans. During a temperature study (20, 25, 30, and 35 C) with these plants and several leaf positions of older vegetative soybeans (V9 to V11), total and reduced N loss were maximum at 30 C (V4 to V5: 9.8 and 9.3 micrograms per square decimeter per hour; V9 to V11: 3.3 and 2.9 micrograms per square decimeter per hour); transpiration was maximal at 30 C in V4 to V5 plants (2.6 grams H_2O per square decimeter per hour) and at 35 C in V9 to V11 plants (2.4 grams H_2O per square decimeter per hour); and CO_2 uptake was maximal at 25 and 30 C during both sampling dates (V4 to V5: 22.7 to 23.5 milligrams CO_2 per square decimeter per hour: V9 to V11: 14.1 to 14.5 milligrams CO_2 per square decimeter per hour). At 30 and 35 C these parameters were highest in the youngest tissue of V9 to V11 plants.

In V9 to V11 plants at all four temperatures, reduced N loss was correlated with total N loss ($r = 0.99$ at 20, 25, and 30 C and 0.97 at 35 C), and CO_2 uptake with transpiration ($r = 0.47$ at 20 C, 0.75 at 25 C, 0.85 at 30 C, and 0.81 at 35 C). Transpiration was correlated with both total and reduced N loss at 30 C (total N: $r = 0.69$; reduced N: $r = 0.70$) and 35 C (total N: $r = 0.58$; reduced N: $r = 0.54$). In addition, CO_2 uptake was directly related to total ($r = 0.69$ at 30 C and 0.56 at 35 C) and reduced ($r = 0.67$ at 30 C and 0.56 at 35 C) N loss at these two temperatures.

Plant foliage has been found to be a site for significant losses of N (6, 8, 21, 22, 24). This loss was greater in senescing or stressed tissue (8, 24) and in younger vegetative tissue than in the older vegetative tissue or the foliage of plants in reproductive stages (22). On a unit area basis, weed species generally evolved more N than crop species (21). Nitrogen dissipation also seemed to be correlated directly with temperature and transpiration (21, 22), and most of the N evolved as a reduced form (8, 24), although oxidized forms could be detected (24).

Data on the relationship of this N loss and CO_2 utilization are limited. Farquhar *et al.* (8) have found significant ammonia losses (about 3 $\mu g/dm^2 \cdot h$) from senescing, yet photosynthetically active, leaves of maize (*Zea mays* L.) cultured in low N (3.4 meq/liter). Younger leaves, as well as older leaves of plants cultured in high N (35.0 meq/liter), had smaller foliar N losses but greater photosynthetic assimilation of CO_2. No loss could be detected with their technique from the younger leaves of maize grown with high N.

In order to characterize the relationships of these separate processes, an apparatus for concomitant measurement of net CO_2 uptake, transpiration, and foliar N loss from field, growth chamber, or greenhouse plants has been prepared. With this apparatus, correlated N loss, CO_2 uptake, and transpiration in soybean (*Glycine max* [L.] Merr.) leaves at several vegetative maturities, over a simulated solar day, and at several temperatures have been investigated. By analysis of chemical and physical properties of several N compounds, we can deduce possible forms in which this N evolves.

MATERIALS AND METHODS

Soybean (*Glycine max* [L.] Merr. cv. Davis) seed from a single plant (20) were germinated in horticultural Vermiculite moistened daily with tap water in a controlled environment. Fluorescent and incandescent lighting gave an intensity of 3.0 × 10^4 lux that included 22 w/m^2 of blue, 12 w/m^2 of red, and 6 w/m^2 of far red light at the plant apex as measured with an International Light, Inc.[2] plant growth photometer. To simulate a solar day, a noninductive 13-h photoperiod started at 0630 with 1.5 h of one-third maximum irradiance, progressed through 1.5 h of two-thirds maximum to maximum, and then ended with 1.25 h of two-thirds and 1.0 h of one-third maximum irradiance. Day/night RH was 50/70%, and maximum/minimum air temperatures were 30 C during the day and 18 C during the night.

At the V1 (unifoliate) stage of development (9) plants of similar appearance were transferred to a nitrate hydroponic culture of mineral nutrient solution as described by Stutte *et al.* (20). Plants were grown in separate containers with aeration (20–30 ml/min·liter) in 3.5 liters of the solution. This volume was maintained throughout the experiment by daily additions of one-eighth- to one-fourth-strength nutrient solution.

Leaf vapors were collected in a calibrated tube at Dry Ice temperatures (about −50 C) in a closed system impermeable to CO_2 (Fig. 1). A leaflet was encased in a Saranex bag (Automated Packaging Systems, Inc., Twinsburg, Ohio) secured with a twist-tie. Directed holes through the inlet valve into the bag mixed the

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[2] Trade names are included for the benefit of the reader and do not imply endorsement by the Arkansas Agric. Exp. Stn.

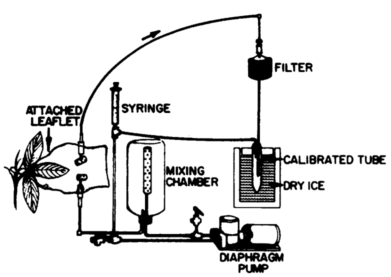

FIG. 1. Diagram of closed system used to determine net CO_2 uptake, transpiration, and foliar N loss.

incoming air throughout this flexible leaf chamber. The system, with an internal volume of 2.80 liters, also included a diaphragm pump (5.0 liters/min), an additional 1.8-liter mixing chamber, Millipore filter (3-μm pores), 0.48-cm (i.d.) Teflon tubing, and 0.64-cm (i.d.) aluminum pipe. Five such units were operated simultaneously.

Two experiments consisted of sampling the center leaflet of the fourth node leaf (numbered acropetally) from five plants (V4–V5) over 1 simulated solar day at 75-min intervals and at four temperatures (20, 25, 30, and 35 C). In addition, the center leaflets of the five uppermost expanded leaves were sampled from five plants (V9–V11) at the same four temperatures. For plant temperature acclimation, 2 days were allowed from the 30, 25, and 20 C runs and 3 days between the 20 and 35 C run. Except for the simulated solar day study, sampling was conducted between 1300 and 1530 h. The upper three leaves sampled during the leaf position study received direct chamber light. The lower two leaves were shaded (3–6 w/m^2 of blue, 3–6 w/m^2 of red, and 2–3 w/m^2 of far red light). A wire support to each sampling bag was used to maintain leaf position in the plant canopy. Temperature of the nutrient culture was approximately that of the chamber air during each sampling period. The internal air temperature of any of the closed sampling systems did not fluctuate from the chamber temperature during the course of an experiment.

The rate of net CO_2 uptake was determined by monitoring at intervals the disappearance of CO_2 in each system during an experiment. Chamber CO_2 concentration levels ranged between 450 and 650 μl/liter during this time. After each leaflet was sealed in the bag, the air was circulated for 2 min to purge the system of any atmospheric vapors and of the initial leaf vapors. Then all pumps were switched off (time 0), and clean, calibrated gas trap tubes were exchanged for those removing initial vapors. Circulation was then resumed and supplemental CO_2 (about 3.5 ml) added immediately via a syringe through a septum. Five-ml gaseous aliquots were removed with syringes for CO_2 analysis from each system at 3, 5, 7, 9, and 11 min. The CO_2 in each aliquot was determined with a Beckman model 215A IR analyzer. Concentrations of CO_2 at 3 min were usually 700 to 900 μl/l and decreased to 400 to 600 μl/l at 11 min. This sample removal from each system reduced the internal volume 1.0–1.5% which was considered negligible. The decrease of CO_2 over the 8-min sampling period was used for net CO_2 uptake determinations; the CO_2 readings at 5, 7, and 9 min were used to ensure linear removal of this gas. After 12 min, the gas trap tubes were removed and stoppered.

When the tubes had equilibrated to air temperature, the transpiration rate was determined from the volume of condensate collected during the 12-min period. The condensate was then analyzed for soluble N with a pyrochemiluminescent technique which was sensitive to 10 μg N/liter. The technique allowed determination of total N (21, 22) and oxidized N (24) concentrations, and reduced N was estimated by difference.

Nitrogen loss, CO_2 uptake, and transpiration rates were based on leaf area measured at the initiation of each experiment with a LI-COR model LI 3000 area meter.

RESULTS AND DISCUSSION

Rates of CO_2 uptake and transpiration, similar to those derived by other methods, were determined using this technique (Tables I and II). Net CO_2 assimilation of 12–24 (4), 17–19 (23), and 20–30 mg/$dm^2\cdot h$ (15) has been measured for soybeans with closed systems. Similar soybean transpiration rates of 1.2–2.3 (3), 1.4–1.8 (14), and 1.5–2.0 g/$dm^2\cdot h$ (16) employing gravimetric methods and 2.7–3.8 g/$dm^2\cdot h$ (7) using psychrometers have also been reported.

Net CO_2 uptake, transpiration, and N loss were all maximal from 1315 to 1700 of the simulated solar day (Fig. 2). Although CO_2 uptake and transpiration were also high at 1200, when the chamber air temperature had just increased to 30 C, total N loss was near minimum during this time; a similar trend had been noted during an earlier field study with soybeans (22). This decrease in N loss with corresponding increases in CO_2 uptake and transpiration indicates that N loss is a separate process. Regression analysis of all data present in Figure 2 ($r = 0.38$, $0.01 > P > 0.001$, for total N loss and net CO_2 uptake; $r = 0.37$, $0.01 > P > 0.001$, for total N loss and transpiration) suggests that the processes are statistically related. Both reduced and oxidized N losses were correlated to total N loss ($r = 0.99$ and 0.76, respectively) as was reduced N loss to oxidized loss ($r = 0.65$, $P < 0.001$). Additionally, net CO_2 uptake and transpiration were highly correlated over the solar day's samplings ($r = 0.77$, $P < 0.001$). The diurnal changes in CO_2 uptake and transpiration corresponded to those found by Pallas (16) with soybeans.

Highest rates of CO_2 uptake and H_2O loss by V4–V5 plants occurred at 25 and 30 C (Table I). Total, reduced, and oxidized N losses were maximal at 30 C. In the older plants (V9–V11) the greatest rates (although not as great as found in V4–V5 plants) of CO_2 uptake, H_2O transpired, and total and reduced N loss occurred in the younger leaves (Table II) at the higher temperatures (30 and 35 C). Farquhar *et al.* (8) found the highest N losses in the oldest leaves of N-stressed maize grown hydroponically. However, Weiland and Stutte (24) reported the least amount of N loss in the middle foliage of flowering field-grown soybeans (R2). Oxidized N loss appeared minimal in the middle leaves sampled at 30 C; it was maximal in these leaves at 35 C (Table II). Lowest CO_2 assimilation rates were determined from shaded leaves in the plant canopy at all four temperatures.

Linear regression analysis (Table III) of the data in Table II suggested a direct relationship between total and reduced N loss, as well as between CO_2 uptake and transpiration, at all four temperatures. These relationships were also found in the solar day study (Table I). At 30 and 35 C, both CO_2 uptake and transpiration

Table I. *Net CO_2 Uptake, Transpiration, and N Loss Rates Determined in V4–V5 Soybean Foliage at Four Temperatures*

Values are means of five samples.

Temp	CO_2 Uptake Rate	Transpiration Rate	Nitrogen Loss Rate		
			Total N	Reduced N	Oxidized N
C	*mg CO_2/$dm^2\cdot h$*	*g H_2O/$dm^2\cdot h$*		*$\mu g/dm^2\cdot h$*	
20	16.8	1.8	3.2	2.8	0.36
25	23.5	2.4	5.1	4.8	0.31
30	22.7	2.6	9.8	9.3	0.54
35	19.4	2.2	7.7	7.2	0.45
$LSD_{0.05}$	2.5	0.5	1.9	1.8	0.18

Table II. *Net CO_2 Uptake, Transpiration, and N Loss Rates Determined from Five Soybean Leaf Positions of V9–V11 Plants at Four Temperatures*

Values are means of five samples.

Trifoliolate Leaf[a]	Temperature (C)			
	20	25	30	35
	CO_2 uptake in mg CO_2/ $dm^2 \cdot h$			
1	11.0	14.5	14.1	11.0
2	9.4	12.5	12.1	11.3
3	7.6	10.5	8.5	8.4
4	5.2	5.8	6.6	7.6
5	4.2	5.8	9.7	7.7
$LSD_{0.05}$ within any temperature = 2.0; $LSD_{0.05}$ over temperatures = 1.0				
	Transpiration in g $H_2O/dm^2 \cdot h$			
1	0.7	1.0	2.0	2.4
2	0.6	0.9	1.9	1.9
3	0.6	0.8	1.2	1.4
4	0.5	0.7	0.9	0.9
5	0.6	0.7	0.8	0.8
$LSD_{0.05}$ within any temperature = 0.2; $LSD_{0.05}$ over temperatures = 0.1				
	Total N loss in μg N/$dm^2 \cdot h$			
1	0.8	1.6	3.3	2.7
2	0.8	1.4	2.8	2.5
3	0.8	1.5	2.6	2.1
4	0.9	1.6	2.4	2.3
5	0.9	2.0	1.9	2.1
$LSD_{0.05}$ within any temperature = 0.6; $LSD_{0.05}$ over temperatures = 0.3				
	Reduced N loss in μg N/dm^2/h			
1	0.7	1.5	2.9	2.4
2	0.7	1.3	2.5	2.4
3	0.7	1.4	2.4	1.8
4	0.8	1.5	2.1	2.1
5	0.9	1.9	1.6	1.9
$LSD_{0.05}$ within any temperature = 0.5; $LSD_{0.05}$ over temperatures = 0.3				
	Oxidized N loss μg N/$dm^2 \cdot h$			
1	0.07	0.09	0.34	0.16
2	0.06	0.10	0.20	0.14
3	0.06	0.11	0.19	0.31
4	0.06	0.11	0.27	0.24
5	0.04	0.11	0.26	0.19
$LSD_{0.05}$ within any temperature = 0.08; $LSD_{0.05}$ over temperatures = 0.04				

[a] Expanded trifoliolate soybean leaves numbered basipetally from the plant apex.

were correlated to total and reduced N losses. Field studies by Stutte *et al.* (22) have also shown a direct correlation between transpiration and N loss as well as between temperature and N loss, and temperature and transpiration. The similarities in rates of total N loss throughout the plant canopy at 20 and 25 C contribute to a lack of correlation of N loss and temperature with CO_2 uptake and transpiration rates. The reduced CO_2 uptake and N loss rates observed at 35 C compared to 30 C could be the result of high root temperature and/or partial closing of stomates. Preliminary measurements with an adapted sphygmomanometer using clamping scissors (2) indicated that stomatal opening was less at 35 than at 30 C.

A direct correlation between total N and oxidized N loss was found at 25 and 30 C, but not at 20 and 35 C. As in V4–V5 plants highest rates of oxidized N loss were at the two highest temperatures. Weiland and Stutte (24) also observed that under stress conditions (*i.e.* H_2O or temperature) a higher rate of oxidized N loss occurs. Similarly, Klepper (13) has shown herbicide-treated soybeans to evolve significant quantities of oxidized N forms.

Several inorganic N compounds were introduced into vapor entrapment systems to test possible entrapment of forms or chemical products of these compounds. Five-ml aliquots of nitrogen dioxide, nitrous oxide, nitric oxide, and ammonia were injected into separate closed systems containing 0.2 ml of reagent water and ambient air. Only nitrous oxide was not detected in the water after 10 min and thus would not be removed under conditions of the experimental procedures.

Because nitrous oxide (b.p. −88.5 C), as well as nitric oxide (b.p. −151.8 C), has a condensation point lower than the temperature of the Dry Ice, this organic form would not be entrapped. Also, nitrous oxide is stable in air. Nitric oxide is rapidly oxidized

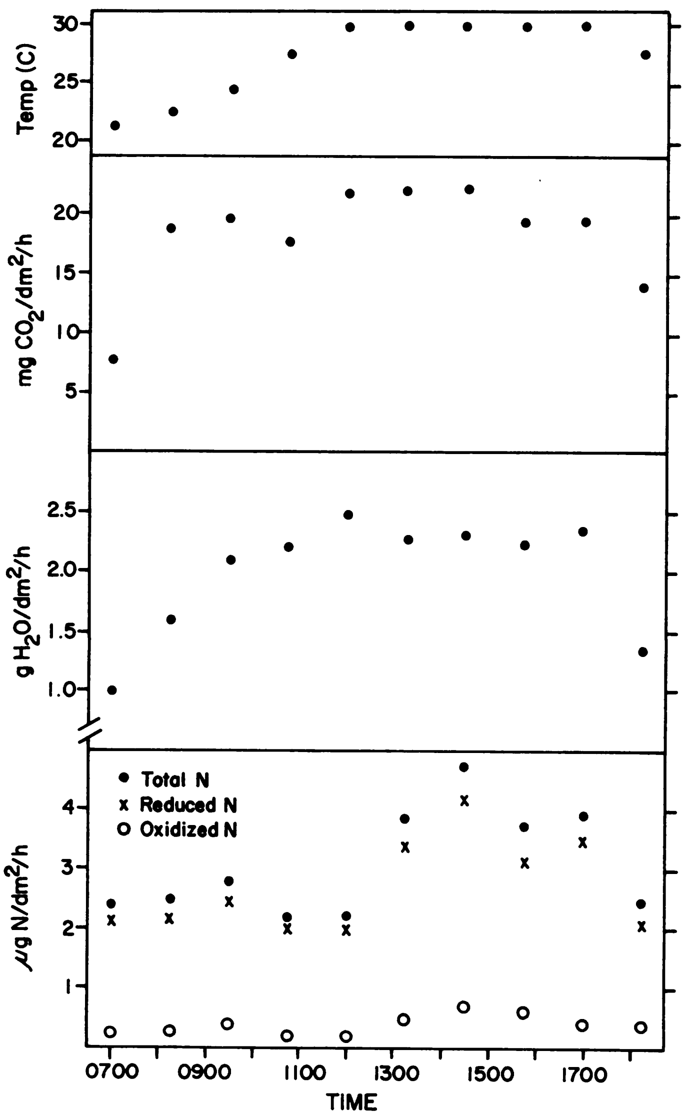

FIG. 2. Net CO_2 uptake, transpiration, and total, reduced, and oxidized N loss rates over 1 simulated solar day. The 13-h photoperiod started at 0630. Values are the mean of five samples. $LSD_{0.05}$ for CO_2 uptake = 2.2 mg/$dm^2 \cdot h$, for transpiration = 0.3 g $H_2O/dm^2 \cdot h$, for total N loss = 0.7 μg/$dm^2 \cdot h$, for reduced N loss = 0.7 μg/$dm^2 \cdot h$, and for oxidized N loss = 0.2 μg/$dm^2 \cdot h$.

Table III. *Linear Regression Coefficients of Parameters Determined from Five Leaf Positions of Five Plants at Four Temperatures*

Regression	Temperature (C)			
	20	25	30	35
PS[a] *vs* TR	0.47*[b]	0.75***	0.85***	0.81***
PS *vs* N	−0.14	−0.30	0.69***	0.56**
PS *vs* RN	−0.17	−0.28	0.67***	0.56**
PS *vs* ON	0.34	−0.24	0.37	−0.11
TR *vs* N	0.18	0.14	0.69***	0.58**
TR *vs* RN	0.15	0.15	0.70***	0.54**
TR *vs* ON	0.24	0.18	0.20	−0.03
N *vs* RN	0.99***	0.99***	0.99***	0.97***
N *vs* ON	0.29	0.56**	0.45*	0.13
RN *vs* ON	0.20	0.51**	0.33	0.22

[a] PS = net CO_2 uptake; TR = transpiration; N = total N loss; RN = reduced N loss; ON = oxidized N loss.

[b] *, **, *** Significant at the 0.05, 0.01, and 0.001 level of probability, respectively.

in air to nitrogen dioxide (b.p. 21.2 C), or its dimer nitrogen tetroxide (N_2O_4), both of which can be condensed at this temperature and are soluble in water (19). Both nitrogen dioxide and tetroxide, as well as nitrogen pentoxide (b.p. 47.0 C), dissolve in water to form nitric acid or nitric oxide. Nitric oxide with some nitrogen dioxide has been reported to evolve from herbicide-treated soybeans (13).

Hydroxylamine is very soluble in water but does undergo slow decomposition in solution to ammonia and even possibly to nitrous oxide or nitrogen; N_2 is not detected by pyrochemiluminescence. More probable reduced N forms which could be volatilizing from the tissue are ammonia (b.p. −33.4 C) and hydrogen cyanide (b.p. 26.0 C). Hydrogen cyanide has been found to be lost from leaves of some cultivars of sorghum (*Sorghum bicolor* [L.] Moench) (10), and ammonia has been detected from plant tissue by Farquhar *et al.* (8) and Hooker *et al.* (11).

Stutte and Weiland (21) and Stutte *et al.* (22) estimated a minimal foliar loss from a soybean field of 45 kg/ha throughout the 1977 growing season. On a per plant basis this loss would amount to at least 5% of the plant's N. A loss of 45 kg of N/ha was also estimated by Daigger *et al.* (5) from the foliage of wheat (*Triticum aestivum* L.) during the growth stages between anthesis and maturity. The significance of this volatilization from plant foliage could explain in part Allison's enigma in his soil N balance studies (1), as well as N imbalance in several other recent experiments (*e.g.* 12, 17, 18).

Although it cannot specifically define N forms, the technique described does allow estimations of total foliar N loss and the amounts in either reduced or oxidized forms. At the same time, it is a quick, repeatable method to determine net CO_2 uptake and transpiration from plants in the field, greenhouse, or environment chamber. The technique is currently being used in a study on nitrate reduction and foliar N loss at several temperatures from plants at different growth stages.

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