Effect of Water Supply and Defoliation on Photosynthesis, Transpiration and Yield of Soybean*

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Received June 24, 1991

Abstract : While increasing leaf area increases CO₂-fixing ability of plants, it also increases transpiration and may cause water deficit within a plant. This study was conducted in order to analyze the combined effects of leaf area and water-supply treatments on photosynthesis, water status, and yield of pot-grown soybeans (*Glycine max* [L.] Merr. cv. Tachinagaha).

When plants reached the R4-R5 stage, the leaf area of a whole plant was altered by defoliating one, both, or none of the lateral leaflets of each trifoliate, and three levels of water-supply treatments (2100, 1000, 500 ml/day) were given until maturity.

Transpiration per plant (Tr/P) was approximately equal to water supply regardless of leaf area under water-restricted conditions (1000, 500 ml/day). With sufficient water supply (2100 ml/day), Tr/P varied with leaf area. Across all the treatments, there was a clear linear relationship ($r=0.994^{**}$) between apparent photosynthetic rate (AP) and stomatal conductance (g_s) at one week after the initiation of water-supply treatment. Increasing water supply increased g_s and AP. In water-restricted plants, decreasing leaf area by defoliation raised leaf water potential and caused higher g_s and AP of the remaining leaves. Successive water stress of water-restricted plants decreased leaf chlorophyll content and AP at the late ripening stage. Grain yield was reduced by defoliation only under well-watered conditions but was not affected by defoliation in water-restricted plants.

The data suggest that water supply proportionate to leaf area is essential to maximizing soybean yield.

Key words : Defoliation, *Glycine max*, Leaf water potential, Photosynthesis, Stomatal conductance, Transpiration, Water stress.

灌水と摘葉がダイズの光合成・蒸散および収量に及ぼす影響：島田信二・国分牧衛・芝田英明**・松井重雄***
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要 旨：葉面積の拡大は作物の CO₂ 固定能力を増大させる一方、蒸散の増大を招き、水ストレスを引き起こしやすくと考えられる。そこで、葉面積と水分供給量の組合せ効果がダイズの葉のみかけの光合成速度 (AP) および収量に及ぼす影響を明らかにするために、ポット栽培したダイズに灌水処理と摘葉処理をそれぞれ 3 水準ずつ組み合わせて、開花後約 2 週間目から成熟期まで処理を行い、作物体の光合成、蒸散および収量に及ぼす影響を検討した。

灌水量が少ない場合、個体当りの蒸散量 (Tr/P) は葉面積の大小にかかわらず灌水量とほぼ同等になり、それは気孔コンダクタンス (g_s) によって調節されていた。灌水量が十分にある場合、Tr/P は葉面積の増大に伴い増大した。灌水量が多いほど g_s 、AP が高かった。一方、灌水量が少ない場合は摘葉程度が大きく葉面積が小さいほど葉身の水ポテンシャル (ψ_l) が高く維持され、 g_s が増し、AP も高かった。継続的な水ストレスは登熟後期の葉緑素含量、光合成能力を低下させた。灌水量が多い場合、子実重は葉面積と相関が高く、葉面積が大きいほど多収となったが、灌水量が少ない場合は葉面積が収量へ及ぼす影響は認められず、灌水量が収量規制要因となっているように思われた。

以上からダイズで多収を得るためには、葉面積の確保だけではなく、葉面積に見合った十分な水分供給を行い、 ψ_l を高く維持し、光合成能力を高く維持することが必要であると考えられる。

キーワード：気孔コンダクタンス、光合成速度、蒸散、ダイズ、摘葉処理、水ストレス、水ポテンシャル。

Farmers, especially of warm regions, tended to grow soybeans at a sparse planting rate or

under less fertilization to avoid lodging or spindly growth. There is evidence, however, that yield increased with increasing LAI up to about 9, when a lodging tolerant cultivar was

* A part of this study was presented at the 191st (April, 1991) meeting of the Crop Science Society of Japan.

used at a drained paddy field¹⁷⁾. Soybean requires more water compared to other cereal crops^{16,20)}, and the water status of soybean plants strongly affects its photosynthesis^{1,7)} and dry matter production^{8,18)}. Although increasing LAI increases carbon dioxide fixing ability, it also increases transpiration^{5,9)} and may cause water stress in a plant. This experiment was conducted to analyze the combined effects of leaf area and water supply on photosynthesis, transpiration and yield of pot grown soybeans.

Materials and Methods

1. Plant material

Soybean plants (*Glycine max* (L.) Merr. cv. Tachinagaha) were grown in a greenhouse in 1/2000 a Wagner pots containing 10 kg of volcanic ash soil. Combined chemical fertilizer supplying 1.8 g N, 6 g P₂O₅ and 6 g K₂O per pot, 20 g per pot of calcium carbonate and 45 g per pot of fused phosphate were applied, respectively.

Four seeds per pot were sown on 30 June 1990. Following emergence, plants were thinned to one plant per pot. The plants were periodically sprayed with insecticide.

2. Treatments

Three levels of defoliation were made on Aug. 15, followed by the initiation of three levels of water supply treatment on Aug. 16 (at the stages of R4 to R5, according to Fehr et al.²⁾), when water stress reduces yield more severely than other stages^{4,19)}. These treatments were continued until maturity (Oct. 25). For the defoliation treatment, One (1/3 def.) or both (2/3 def.) lateral leaflets, or none (cont.) were removed. For controlling water supply, the amount of water supplied daily and the interval of water application were varied: high water supply (HW, 2100 ml daily by supplying 300 ml every 2h from 6:00 to 18:00); medium water supply (MW, 1000 ml daily by supplying 250 ml every 4h from 6:00 to 18:00); and low water supply (LW, 500 ml daily, 250 ml each at 6:00 and 12:00). Before the initiation of the two treatments, all plants received equally sufficient water supply. The bottom holes of the pots were kept open for drainage of excess water. Since soil surface of HW treated pots was wet during the treatment, water supply always appeared to exceed the amount of water transpired at HW plants.

We did not measure soil moisture contents, but differences of soil moisture contents among the treatments appeared to be less when rainy and cloudy days continued or maturity was approaching.

Eight plots were used for each treatment.

3. Measurements

Apparent photosynthetic rates (AP) and stomatal conductance (g_s) of an intact leaf were measured in the field using a portable photosynthesis measuring apparatus (The Analytical Development Co. Ltd.), consisting of a CO₂ analyzer (LCA2), an air supply unit (ASUM2), a leaf chamber (PLC-B, enclosed area: 6.25 cm²), and a data logger (DL-2). It is capable of detecting CO₂ concentration, temperature and humidity in the chamber, photosynthetic photon flux density incident on the leaf chamber. The data logger provides AP, g_s , transpiration rate, intercellular CO₂ concentration and leaf temperature by calculation. Mesophyll conductance (g_m) was calculated by the function according to Lamoreaux and Chaney¹¹⁾. For measurements of AP and g_s , the terminal leaflet of the second trifoliate leaf from the terminal node was inserted into the leaf chamber and kept vertical to sunlight to obtain light saturation. Air for the chamber sampled through a mast that drew air from 3 m above the plant canopy was supplied at a flow rate of 500 ml min⁻¹. The same leaflets were used for the morning (8:00) and afternoon (14:00) measurements. The upper part of a pot was covered by a vinyl sheet to prevent evaporation from the soil surface, and transpiration per plant was measured by the weighing method. Leaf area was calculated using the function given by the quadratic relationship ($r=0.976^{**}$) between the length of central leaflet and the area of the trifoliate leaf. The relationship was obtained from prior measurement. Chlorophyll contents of terminal leaflets of the second trifoliate from the terminal node were measured by a Minolta SPAD-502 with six replications. AP, g_s and transpiration rate were measured with three replications on two clear days (Aug. 23 and Sep. 21) when the plants reached R5 and R6 stages.

The leaf water potential (ψ_l) of the terminal leaflet at the 3rd node below the terminal node was measured on Aug. 30. Leaf discs, 6 mm in diameter, were sampled and immedi-

Fig. 1. Effects of water supply and defoliation on transpiration per plant between 7 : 00 to 17 : 00 on Aug. 23. The vertical bars represent $\pm$ SE of three replicates.

Fig. 2. Effects of water supply and defoliation on transpiration rate per leaf area. Values indicate transpiration rate per leaf area between 7 : 00 to 17 : 00 on Aug. 23. The vertical bars represent $\pm$ SE of three replicates.

ately placed in a Wescor model C-52 sample chambers, followed by equilibration for two hours at 25°C, then ψ_t was measured with a Wescor model HR-33T dew point microvoltmeter.

At maturity, five plants from each treatment were harvested for grain yields measurement.

Results

1. Transpiration, apparent photosynthetic rate and stomatal conductance at the R5 stage

Transpiration per plant (Tr/P) between

Fig. 3. Effects of water supply and defoliation on apparent photosynthetic rate at 14 : 00 on Aug. 23. The vertical bars represent $\pm$ SE of three replicates.

Fig. 4. Effects of water supply and defoliation on stomatal conductance at 14 : 00 on Aug. 23. The vertical bars represent $\pm$ SE of three replicates.

7 : 00 and 17 : 00 at R5 stage (Aug. 23) is illustrated in Fig. 1. With MW and LW treatments, soybean plants transpired almost the same amount with the volume of water supplied, regardless of defoliation treatment. With HW treatment, Tr/P decreased with decreasing leaf area due to defoliation. Transpiration rate per leaf area (Tr/L) increased as water supply increased except for in MW-2/3 def. treatments (Fig. 2). Decreasing leaf area by defoliation generally increased Tr/L within the same water supply treatment.

Trends for AP and g_s across defoliation and water-supply treatments were similar for both

Fig. 5. Effects of water supply and defoliation on afternoon declines of stomatal conductance on Aug. 23.

● : HW-cont., ○ : MW-cont., □ : MW-1/3 def.,
△ : MW-2/3 def., ⊙ : LW-cont.

Fig. 6. Effects of defoliation on the diurnal change in water potential at middle water supply treatment (1000 ml/day) on Aug. 30. The vertical bars represent $\pm$ SE of three replicates.

Table 1. Effect of water supply on apparent photosynthetic rate, stomatal conductance and mesophyll conductance of control plants (no defoliation) at R6 stage (Sep. 21).

Water supply (ml/day)	Apparent photosynthetic rate		Stomatal conductance		Mesophyll conductance	
	($\mu\text{mol m}^{-2} \text{sec}^{-1}$)	%*	(cm sec^{-1})	%*	(mm sec^{-1})	%*
Low (500)	0.92 a	19	0.185 a	61	0.073 a	15
Mid. (1000)	1.87 a	19	0.184 a	29	0.154 a	15
High (2100)	7.94 b	34	0.587 b	42	0.724 b	26

Values within a column followed by the same letter are not significantly different at the 0.05 probability level, according to Duncan's new multiple range test.

* : The percentages of the values (measured at 8:00 AM) of Sep. 21 to those of Aug. 23.

morning and afternoon measurements.

Increasing water supply tended to increase both AP (Fig. 3) and g_s (Fig. 4). With MW and LW plants, decreasing leaf area by defoliation increased AP and g_s , whereas no significant changes were observed in HW plants. Diurnal changes of g_s from 8:00 to 14:00 are shown in Fig. 5. The g_s of HW-cont. and MW-2/3 def. plants were higher at 8:00 and remained at the same level at 14:00, whereas g_s of the other treatments was lower and decreased further at 14:00.

There was a clear correlation between AP and g_s ($r=0.994^{**}$) at both measurement times.

2. Apparent photosynthetic rate, stomatal conductance and mesophyll conductance at R6 stage

At the R6 stage (Sep. 21), low-water supply

(MW and LW) decreased AP, g_s and g_m of control plants (Table 1), whereas plants with sufficient water supply had higher AP, g_s and g_m compared to less watered plants. Reductions of AP and g_m from Aug. 23 to Sep. 21 were smaller with HW treatment than with MW and LW treatments.

3. Leaf water potential

Defoliation effects on ψ_l are shown in Fig. 6. Both cont. and 2/3 def. leaves had around -0.9 MPa at 7:00. ψ_l decreased gradually with time and the difference between control and defoliated plants increased, reaching a maximum level at 13:00 when ψ_l of cont. was below -1.7 MPa, whereas that of 2/3 def. had about -1.4 MPa. After 13:00, ψ_l recovered and the difference between the two treatments became less. Since defoliated plants had consistently higher ψ_l , it appears that

Fig. 7. Effects of water supply and defoliation on reduction of chlorophyll content (SPAD value) at ripening period. Values indicate the percentages of the values of Sep. 19 to those of Aug. 23.

defoliation reduced the degree of the midday water deficit.

4. Chlorophyll content

Fig. 7 shows the ratio of leaflet chlorophyll content measured on Sep. 19 to that of Aug. 23. Chlorophyll content decreased from Aug. 23 to Sep. 19 in all treatments, especially in less water supply. However, the reduction in MW plants appeared to be eased by defoliation to some extent.

5. Grain yield

Grain yield was increased by increasing water supply (Fig. 8), and it was not significantly affected by defoliation in MW and LW plants, even if leaf area was decreased by about two-thirds. On the contrary, grain yield decreased as leaf area decreased in HW plants. The number of ripened pods, seeds per pod and seed size were also affected by both water supply and defoliation, resulting in the difference in grain yield by the treatments.

Relationships between leaf area on Aug. 23 (R5 stage) and grain yield of individual plants are shown in Fig. 9. There was a clear correlation between leaf area and grain yield in HW plants ($r=0.892^{**}$), but not in MW ($r=0.176^{ns}$) and LW ($r=0.538^{ns}$) plants.

Discussion

The results of this experiment have shown that transpiration per plant is affected by leaf area only when water is supplied sufficiently (HW), whereas the amount of transpiration is

Fig. 8. Effects of water supply and defoliation on grain yield. The vertical bars represent $\pm$ SE of five replicates.

Fig. 9. Relationship between leaf area (Aug. 23) and grain yield. Each point indicates one plant.

●: HW ($r=0.892^{**}$), □: MW ($r=0.176^{ns}$),

△: LW ($r=0.538^{ns}$)

** : Significant at 0.01 probability level.

^{ns} : Not significant.

almost equal to the amount of water supplied under water-restricted conditions (MW, LW). Hirasawa et al.⁶⁾ found that the difference between water uptake and transpiration rate, which was controlled by the stomatal aperture, was very small in paddy rice. Soybean plants also appear to regulate transpiration rate by controlling stomatal aperture to equilibrate water uptake and transpiration.

AP and g_s were in many cases increased by increasing water supply, and also by defoliation in water-restricted conditions. Whereas there was little effect of defoliation in AP and g_s with HW treatment. Increased AP might be

due to increased g_s because there was a high correlation between AP and g_s .

AP and g_s decreased in the afternoon except HW-cont. and MW-2/3 def. plants, as shown in Fig. 5. Tr/P of MW-2/3 def. plants was approximately equal to that of HW-2/3 def. plants, suggesting that water supply exceeded transpiration in HW and MW-2/3 def. plants. Well-watered plants of adequate leaf area did not exhibit the afternoon decline in AP and g_s , whereas water-restricted plants had lower AP and g_s in the morning and much lower in the afternoon.

In this experiment, less water supply brought about decreasing g_s , probably because of a decrease in ψ_l , as observed by Boyer¹⁾. Defoliation eased midday water stress in MW plants (Fig. 6), probably by increasing water supply per leaf area. The increased g_s and AP in defoliated plants in MW and LW plots might be explained similarly by the increased ψ_l as a consequence of decreased leaf area.

AP and g_s at late ripening stage (R6) were highest in HW plants and the reduction of AP and g_m from Aug. 23 to Sep. 21 was less in HW plants than MW and LW plants (Table 1). The difference in the degree of decline of chlorophyll content appeared to bring about this different decline of photosynthetic ability. The g_s was also highest in HW plants, but the decline was smallest in LW plants (61%). The lesser reduction in g_s of LW plants with stage reflects the lower value on Aug. 23.

Water stress imposed by the restriction of water supply promoted a decline of leaflet chlorophyll content and photosynthetic ability at a later stage. This result is similar to that reported for maize²¹⁾. Daily occurrence of midday water stress seems to reduce nodule nitrogen fixation¹⁸⁾ and promote protein breakdown and leaf senescence¹²⁾.

Grain yield increased with increasing leaf area only under well-watered conditions. The same tendency was observed in pot-grown rice, where defoliation was shown to be effective in preventing drought damage in some varieties³⁾.

In water restricted-conditions, AP decreased with smaller g_s due to low ψ_l . Defoliation increases water supply to the remaining leaves, thereby improving water status and increasing AP. This adaptation might favor higher grain

yield under water-restricted conditions.

Some reports noted that there was an optimum LAI which maximized yield in field grown soybeans^{10,14,15)}. Irrigation increased the optimum LAI¹⁵⁾, optimum planting density and yield^{13,15)}, and irrigation was more effective in plants of larger growth^{13,15)}.

This evidence along with above-mentioned results seem to indicate that increasing LAI increases transpiration of soybean plants, so that if enough water is not supplied to be proportionate to its LAI, yield may be reduced or limited by water deficit.

Therefore soybeans, when grown in a drained paddy field, seem to be able to produce more yield at higher LAI, compared to non-irrigated upland grown soybeans.

This experiment indicates that sufficient water supply proportionate to leaf area maintains higher ψ_l and AP, and eases reduction of chlorophyll content at ripening stage, resulting in higher yield at larger growth.

From our present and previous results, We conclude that water supply proportionate to leaf area is essential to maximizing soybean yield.

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- * In Japanese with English summary.
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