Effect of Temperature Regimes on Photosynthesis, Respiration, and Growth in Alfalfa

Safaa Al-Hamdani and Glenn W. Todd
Department of Botany and Microbiology, Oklahoma State University, Stillwater, OK 74078

The influence of three temperature regimes (34/25, 21/8, and 12/2 °C with 14 hr day/10 hr night) on alfalfa (*Medicago sativa* L.) growth, photosynthesis, respiration, and total nonstructural carbohydrate levels in roots was determined. The plants were grown in a greenhouse for 3 months, and then the tops were removed to the crown. When growth from the crown reached 5 to 8 cm in height, the plants were placed in growth chambers. Photosynthetic photon flux density was 520 μmol m^{-2} s^{-1} during the 14 hr day-period. Plant height, shoot dry weight, and leaf area were greatest from plants at 21/8 °C regime, followed in descending order by those of plants at 34/25 and 12/2 °C. Root dry weight was highest for the plants at 21/8 °C followed by that of plants at 12/2 °C. The concentration of chlorophyll a and b in leaves increased as the temperature increased. Net photosynthesis of the alfalfa canopy increased from 12.9 μmol CO_2 m^{-2} s^{-1} at 12 °C to 18.9 μmol CO_2 m^{-2} s^{-1} at 21 °C, but did not significantly increase from 21 to 35 °C. Shoot and root respiration was increased in response to an increase in temperature. Total nonstructural carbohydrate in roots was the highest for the plants grown at 21/8 °C followed in descending order by that of plants at 12/2 and 34/25 °C.

INTRODUCTION

Experimental evidence is lacking, but Sholar *et al.* (1), Reynolds (2), and Mays and Evans (3) suggest that the mild daily maximum temperature and green leaves on alfalfa plants during winter months in the south-central U.S. might allow some photosynthetic activity, thereby increasing carbohydrates in the roots. The low growth during summer, the "summer slump", has been attributed to high temperatures.

When alfalfa is grown at high (e.g. >30 °C) rather than lower temperatures (4-9), flowering occurs earlier, and the herbage yield is lower at specified growth stages. There is still some controversy, however, about the response of photosynthesis to temperature. Murata *et al.* (10*)* observed a wide range of optimum temperatures from 5 to 30 °C for photosynthesis in whole alfalfa seedlings. Brown and Radcliffe (11) found that the optimum temperature for net photosynthesis in stem tips was between 25 and 30 °C. Pearson and Hunt (12) observed a continuous decline in net carbon dioxide intake with short-term exposure of shoots to increasing temperature from 10 °C to 40 °C. These divergent observations require clarification.

The present study was conducted to determine the influence of temperature on growth, photosynthesis, and respiration of alfalfa shoots and on respiration and total nonstructural carbohydrate (TNC) in roots. The temperatures were selected on the basis of average daily maximum and minimum temperatures during winter, spring, and summer months in central Oklahoma.

MATERIALS AND METHODS

Alfalfa cultivar Cody, which is adapted to the southern plains, was used for this study. Scarified seeds were treated with the fungicide Captan [*cis*-*N*-((trichloromethyl)-thio)-4-cyclohexene-1,2-dicarboximide] and planted in 11-cm diameter, 14.5-cm deep plastic pots. The pots were filled with a 2:1:1 mixture of sand:vermiculite:perlite. Nutrients were added to the soil mixture so that each pot contained 64 mg of available N, 126 mg of available P, and 309 mg of exchangeable K, and had a pH of 7.2. Seedlings were thinned to one plant per pot two weeks after germination. The plants were grown in a greenhouse for three months with a day length of 12 hr and an average photosynthetic photon flux density of 850 μmol m^{-2} s^{-1}. Temperature was between 20 and 30 °C. During the last month of establishment, when plants were at the 50% bloom stage, the shoots were clipped at the crowns and roots of 12 randomly selected plants were sampled for later determination of total nonstructural carbohydrates (TNC). Six days after the shoots were excised, when growth of the crown reached 5 to 8 cm in height, thirty randomly selected plants were placed in each of three controlled environment chambers. Day/night temperatures during a 14-hr

photoperiod in the growth chambers were 12/2, 21/8, and 34/25 °C. Photosynthetic photon flux density was 520 μmol m^{-2} s^{-1}.

The plants were harvested after 30 days. Total leaf areas of seven plants were measured with a LI-COR 3000 leaf area meter (Lincoln, NE). The heights of ten random plants were recorded, and the shoots were severed from the roots at the crown. Roots were washed in tap water, and each of the shoot and root samples was dried at 70 °C and weighed. For measurement of TNC, root samples were ground in a Wiley mill to pass through a 2-mm screen. A representative portion of 0.2 g from each sample was placed in a 250-mL beaker with 50 mL of 0.2 N HCl and the mixture boiled for one hour. The solution was filtered, and deionized water was added to bring the sample to a 100-mL volume. A 0.1-mL aliquot was combined with 0.9 mL of deionized water and 5.0 mL of anthrone reagent (13). The samples were placed in a water bath at 100 °C for 15 min, and then in a water bath at 0 °C for 20 min. Absorbance of the samples was determined with a spectrophotometer (B and L Spectronic 20) at 620 nm. Chlorophyll concentration was determined for seven plants from each treatment. For each determination, 0.3 g sliced leaves were homogenized in 25 mL of 800 mL/L acetone in water. The mixture was filtered through Whatman #1 filter paper and the total volume was brought to 50 mL with the same aqueous acetone. Chlorophyll a and b were measured spectrophotometrically (14).

Rates of net photosynthesis at each day temperature were measured for six plants. A 7-liter air-sealed cylindrical glass chamber was used to enclose the shoots of each intact plant. Air containing 0.34 mL/L CO_2 was passed through the system for 45 min with a flow rate of 2 L/min. The difference in CO_2 concentration between inlet and outlet was measured with a Horib PIR-2000 infrared gas analyzer. The soil surface of each plant sample was covered with a circular glass plate that was divided into halves with a hole in the center. In addition, the base of the shoot and the bottom of the pot were sealed with modeling clay to ensure total enclosure of the root medium. The same system was used in measuring day and night shoot respiration for the same plants. Following the net photosynthetic measurement, lights were turned off and after 45 min the day-respiration measurement was obtained. Night respiration measurements were taken during the dark period. Root respiration was also measured on the same plants. Atmospheric air with a flow rate of 1 L/min was passed through the root medium and the difference in CO_2 concentration between inlet and outlet on opposite sides of the pot was determined by infrared gas analysis.

This experiment was replicated three times and the data were analyzed as a 3 × 3 Latin square. Mean separations were based upon the LSD test.

RESULTS AND DISCUSSION

The largest plant height, shoot dry weight, and leaf area were obtained from the plants grown at the 21/8 °C regime, followed in descending order by those at the 34/25 and 12/2 °C regimes (Table 1). Root dry weight was also greatest in plants growing at 21/8 °C, with less in those plants growing at 12/2 °C and least in those kept at 34/25 °C. Plants at the 34/25 °C regime had the highest shoot/root ratio, followed in order by the 21/8 °C and 12/2 °C regimes.

The temperature optimum for dry weight accumulation in tops was reported to be about 20-25 °C (5) or 15-20 °C (8). Our results are in better agreement with Pearson and Hunt (8), although none of the others include our lowest temperature.

Our highest net photosynthesis was at 34 °C, which is not in agreement with the results of Pearson and Hunt (12), who reported the highest rate at 10 °C with continuously decreasing values to 40 °C. Brown and Radcliffe (11) reported greatest net photosynthesis at about 30 °C with 10 °C yielding 57% of the maximum, which agrees with our observation. Little change in net photosynthesis occurred at 34 °C over that at 21 °C.

There was an increase in respiration in both shoots and roots (Figs. 1 and 2) as the growth temperature increased from 2 to 34 °C. Since dark respiration increases nearly twofold in both tissues between 21 °C and 34 °C, net accumulation of stored carbohydrates in the roots should be less at the higher temperature. In addition, photorespiratio increases with increased temperature and thus further depletes storage carbohydrates (12).

TABLE 1. The effect[a] of temperature regime on several growth components, TNC of roots, and leaf chlorophyll in alfalfa.

	Temperature, °C (day/night)		
	12/2	21/8	34/25
Plant height, cm	32.3	68.8	58.5
Shoot dry weight per plant, g	3.5	6.4	4.8
Root dry weight per plant, g	4.2	5.6	3.1
Shoot/root ratio	0.8	1.1	1.6
Leaf area per plant, cm^2	369	812	584
Root TNC, g/kg dry wt.	280	323	188
Chlorophyll a, mg/g fr. wt.	2.6	3.4	4.0
Chlorophyll b, mg/g fr. wt.	0.6	0.8	1.0
Net photosynthesis, μmol CO_2 m^{-2} s^{-1}	12.9	18.9	19.8

[a]Components for all treatments were found significantly different from each other at the 0.05 level of probability by using the LSD test except for chlorophyll a, where the 12/2 and 34/25 treatments were different from each other but neither was different from the 21/8 treatment, and net photosynthesis, where the 21/8 and 34/25 treatments were different from 12/2 but not each other.

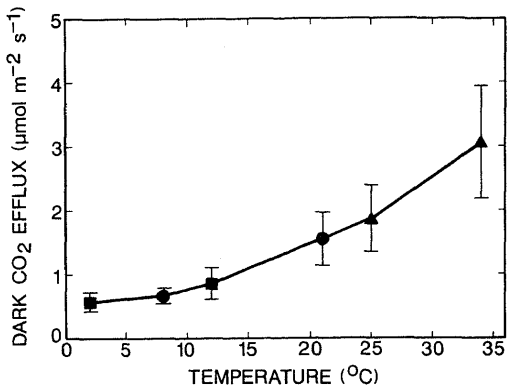

Fig. 1. Respiration rate of alfalfa shoots grown under day/night temperature regime of 12/2 (■), 21/8 (●), or 34/25 (▲) °C. Each value represents the mean of six measurements. Vertical bars represent ±SD.

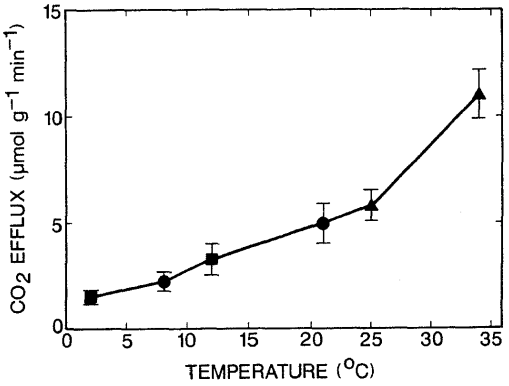

Fig. 2. Respiration rate of alfalfa roots grown under day/night temperature regime of 12/2 (■), 21/8 (●), or 34/25 (▲) °C. Each value represents the mean of six measurements. Vertical bars represent ±SD.

The concentration of total nonstructural carbohydrate (TNC) of the roots was significantly different among the three temperature regimes (Table 1). Roots of plants in the 21/8 °C regime had the highest TNC followed in descending order by those in the 12/2 °C and 34/25 °C regimes. Feltner and Massengale (15) previously reported a depletion in TNC in alfalfa roots grown during the warm summer months compared to low temperature periods. Cooper and Watson (16) also observed a faster depletion in TNC of alfalfa root following cutting during summer than during cooler periods. However, Ueno and Smith (17) found that the percentages of TNC were higher in alfalfa roots grown at 32/27 °C (day/night) than at the 21/15 °C regime.

The TNC of roots of alfalfa plants growing at temperatures higher or lower than 21/8 °C was lower than the 319 g/kg dry weight obtained prior to treatment (Table 1). This indicates that the reduction of root TNC during the hot summer months of the southern plains may result from the increase in both shoot and root respiration (Figs. 1 and 2). As expected, the TNC in roots of plants grown at 34/25 °C was the lowest of the 3 treatments, being nearly one-half that of 21/8 °C. The reduction in TNC in the roots at low winter temperatures (18) may be explained by the low photosynthetic rate (Table 1). However, a decrease in photosynthesis of about 32% in plants at 12 °C versus 21 °C led to a decrease of TNC in the roots of only 13%.

Smith and Young (19) first reported a threshold temperature exists for chlorophyll formation. The concentration of both chlorophyll a and chlorophyll b in alfalfa leaves increased with increasing temperatures (Table 1). No significant difference in the chlorophyll a/chlorophyll b ratio was observed among the treatments. Millerad and McWilliam (20) obtained similar results in corn (*Zea mays* L.) seedlings over the temperature range of 12 to 27 °C.

These data provide a partial explanation for the generally low alfalfa yields when the crop is grown under high temperatures. Plants grown at temperatures of 34/25 °C exhibited higher shoot and root respiration and lower leaf area than plants growing at lower temperatures of 21/8 °C. In addition, plants grown under the coolest temperatures, 12/2 °C, maintained relatively high TNC in roots. These observations are consistent with low respiration in both shoots and roots and the slow growth in the cold.

REFERENCES

1. J.R. Sholar, J.L. Caddel, J.F. Stritzke, and R.C. Berberet, Agron. J. 75:619-622 (1983).
2. J.H. Reynolds, Crop Sci. 11:103-106 (1971).
3. D.A. Mays and E.M. Evans, Agron. J. 65:390-392 (1973).
4. N. Arbi, D. Smith, and E.T. Bingham, Agron. J. 71:573-577 (1979).
5. R.J. Bula, Crop Sci. 12:663-686 (1972).
6. P.L. Greenfield and D. Smith, Agron. J. 65:871-874 (1973).
7. E.H. Jensen, M.A. Massengale, and D.O. Chilcote, Nevada Agric. Exp. Stn. Bull. T9 (1967).
8. C.J. Pearson and L.A. Hunt, Can. J. Plant Sci. 52:1007-1015 (1972).
9. L.R. Vough and G.C. Marten, Agron. J. 63:40-42 (1971).
10. V. Murata, J. Iyama, and T. Honma, Proc. Crop Sci. Soc. Jpn. 34:154-158 (1965).
11. R.H. Brown and D.E. Radcliffe, Crop Sci. 26:1208-1211(1986).
12. C.J. Pearson and L.A. Hunt, Can. J. Bot. 50:1925- 1930 (1972).
13. E.W. Yemm and A.J. Willis, Biochem. J. 57:508-514 (1954).
14. D.I. Arnon, Plant Physiol. 24:1-15 (1949).
15. K.C. Feltner and M.A. Massengale, Crop Sci. 5:585-588 (1965).
16. C.S. Cooper and C.A. Watson, Crop Sci. 8:83-85 (1968).
17. M. Ueno and D. Smith, Agron. J. 62:764-767 (1970).
18. S. Al-Hamdani and G.W. Todd, Proc. Okla. Acad. Sci. 69:11-14 (1989).
19. J.H.C. Smith and V.M.K. Young, in *Radiation Biology*, A. Hollaender (Ed.). McGraw-Hill, New York, 1956, pp. 393-442.
20. A. Millerad and J.R. McWilliam, Plant Physiol. 4:1967-1972 (1968).