Influence of Temperature on Growth and Metabolism of Ryegrass Seedlings. II. Variation in Metabolites

Leonard Beevers and J. P. Cooper

IN A previous paper (3), variations in temperature regime were shown to alter the vegetative morphology of ryegrass. Similar observations have been made for these and other species by Mitchell (16), Soper (18), and Friend (10). As plant form is the product of metabolic activity at the cellular level, temperature-induced modifications of morphology should be associated with changes in chemical constituents. The present paper describes a study into the effect of temperature treatment on the level of various carbohydrate and nitrogenous constituents and their relation to the observed differences in growth of ryegrass in the various environments.

MATERIALS AND METHODS

Italian ryegrass (Lolium multiflorum Lam.) and Irish perennial ryegrass (Lolium perenne L.) seedlings were grown in controlled environment chambers as described previously (3). The temperature regimes in respective growth rooms were 12° C. continuously, referred to as (12-12), 25° C. continuously (25-25), and 25° C. day and 12° C. night (25-12).

At 4, 7, and 10 weeks after sowing, leaf material was collected by cutting off material approximately 1/4 inch above the soil surface. After harvesting the samples were stored at —22° C. in sealed polyethylene bags until they were analyzed. At the time of analysis dry matter determinations were made by drying 5 g. of frozen material overnight in an oven at 85° C. The dried material produced during these determinations was subsequently used for total nitrogen estimations.

Alcohol extraction. Five-gram samples of frozen material were cut into 1/8- to 1/4-inch segments and transferred to the beaker of an M.S.E. homogenizer together with 40 ml. of 80% ethyl alcohol and macerated at maximum speed for 5 minutes. The resulting slurry was vacuum filtered in a Buchner funnel and washed twice with 30 ml. of 80% ethyl alcohol. The filter paper and residue were placed in a Soxhlet extraction thimble and extracted for six hours using the filtrate as the extracting medium (Wylam (23)). After this time the alcohol was evaporated off. The remaining solution was filtered and the residue washed with 3 X 20 ml. of hot distilled water. The pale, straw-coloured filtrate was made up to volume; aliquots of this extract were used with appropriate dilutions for the determination of soluble carbohydrate, reducing sugars, nitrate, ammonia, and soluble N-amino nitrogen.

Total alcohol soluble carbohydrates. Total alcohol soluble carbohydrates were determined by the anthrone method (25) using glucose as the standard.

Reducing sugars. Reducing sugars were estimated by measuring the reduction in O.D. at 420 mμ after the diluted extract was heated in a boiling water bath with the alkaline ferricyanide reagent of Burr and Tanimoto (6).

Total nitrogen. Total nitrogen was estimated from the ammonia produced after a Kjeldahl digestion which included the incorporation of reduced iron to convert nitrate to ammonia (Fischer et al., (17)).

Nitrate nitrogen. The nitrate content of the alcohol extract was determined using the phenoldisulphonic acid method described by Bolle-Jones (5).

Ammonia nitrogen. Ammonia N in plant extracts was determined by a direct Nessler's procedure based upon the method of Umbreit et al. (20). Although this method was satisfactory for growth chamber-grown material, ryegrass grown under field conditions contains components which interfere with the colorimetric determination of ammonia.

Soluble N-amino nitrogen. Soluble N-amino N was estimated by the colorimetric method of Yemm and Cocking (24).

RESULTS AND DISCUSSION

Alcohol soluble carbohydrates. As shown by the data (Table 1), the highest carbohydrate contents occurred at 12-12 with lower values at 25-12 and 25-25. Bathurst and Mitchell (2) recorded similar responses in their studies with short rotation ryegrass. In view of this accumulation of carbohydrate in the cool regime it appears unlikely that the slow growth of plants at 12-12 can be totally attributed to a shortage of photosynthate.

The effect of plant age on carbohydrate content varied in the different regimes. Significant decreases in the carbohydrate percentage of the dry matter were observed in the 12-12 regime. No significant changes were observed in the other two regimes although there was a trend for the 25-25 regime plants to increase in carbohydrate content with increasing age.

Chromatographic analyses of the alcohol soluble extracts from 10-week-old plants showed the presence of sucrose,