AN ABSTRACT OF THE THESIS OF

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Title: THE PHYSIOLOGY OF SEASONAL GROWTH IN TALL FESCUE VARIETIES (FESTUCA ARUNDINACEA SCHREB.)

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The winter and summer growth of varieties of tall fescue (Festuca arundinacea Schreb.) that had originated from the Mediterranean region (Oregon 1000 and Tunisia) and northern Europe (Alta) were studied outdoors in the Mediterranean climate of western Oregon, and in greenhouses and controlled environment chambers. Treatments of temperature, photoperiod and gibberellic acid (GA) were imposed in order to establish the limitations to winter and summer forage production. Measurements of photosynthesis and the distribution of assimilates were made by growth analysis and the level and nature of reserve carbohydrates by the copper-iodometric method.

In western Oregon, the varieties that had originated from the Mediterranean region made more foliar growth during the winter but less during the summer than Alta. These two different patterns of seasonal growth were interpreted as ecotypic variation that had enabled the species to survive seasons of stress in the regions of

origin--freezing winters of northern Europe and summer droughts of the Mediterranean.

Cubes of sod taken during the winter months from swards of Alta and Tunisia showed that Alta had a greater density of tillers and buds, and a higher level of reserve carbohydrates than Tunisia. This result suggested that Tunisia utilized photosynthate during the winter for the production of leaves rather than storing it as carbohydrates.

During the winter of 1967 the foliar growth of both Alta and Oregon 1000 was increased by a temperature of 15 to 20C, a foliar spray of 0.1% GA, and possibly by a long photoperiod. The suggestion was that winter growth was limited more by the internal growth regulator balance than the external weather, and that relative rather than true dormancy was involved. Although the chlorophyll concentration in the foliage was reduced by GA the total chlorophyll was actually increased by 10 to 30% and, consequently, the chlorosis may not be deleterious to pasture production.

The rate of regrowth of Tunisia was greater at 7/3C than Alta but this difference was eliminated by GA. Rate of regrowth may be an important determinant of winter growth of ecotypes of forages grasses and GA may promote the mobilization, or utilization, or both, of reserve carbohydrate--reserves that were found to be higher in Alta than Tunisia.

At the end of a prolonged exposure of 18 weeks in a simulated winter environment of 7/3C and 19 ly/day (400 to 750 nm) the total

weight of Tunisia was greater than Alta and proportionately more dry weight was present in the leaves than roots. The total available carbohydrates (TAC) extracted with 0.2N H_2SO_4 was similar for Alta and Tunisia; it was 20% of the dry weight of stems. Gibberellic acid increased the concentration of TAC 6% in Alta and 4% in Tunisia. The total water soluble carbohydrates (WSC) were 14 and 9% of the stems of Tunisia and Alta respectively, and 6% of the leaves and 5% of the roots of both varieties. Fructosans were the main component of WSC, and sucrose and reducing sugars were of lesser concentrations. The WSC were increased by GA in all parts of the plants and the increase was greatest in Alta; sucrose showed the greatest increase. Subtraction of WSC from TAC gave an estimation of starch; it was unexpectedly high with concentrations as high as fructosans and indicated that 0.2N H_2SO_4 may have extracted structural carbohydrates.

The superior growth rate of Alta in the summer was established in June. At the end of June 1968 the relative growth rate of both Alta and Tunisia was increased by GA due to a stimulation of net assimilation rates. The WSC were highest in the stems and were 14 and 9% of the stems of Alta and Tunisia respectively. Fructosans were the main WSC and the higher level of WSC in Alta was due to a higher level of fructosans. Gibberellic acid increased the WSC by 1% of the dry weight during the treatment time of 8 days.

The measurement of photosynthesis of leaf segments of tall fescue by manometry was unreliable. The activity of isolated

chloroplasts was also unreliable although some improvement was obtained by isolating in the presence of polyvinylpyrrolidone.

The concept developed in this thesis was that when an ecotype is introduced to a new environment, or used as a basis for a new variety, the production and utilization of photosynthate may be controlled by an internal growth regulator balance which is not well coordinated with the weather. In such a circumstance, the possibility exists that the growth regulator balance may be adjusted by a timely application of the appropriate growth regulators in order that the variety may grow to the limit of its genetic capacity during periods of favorable weather.

The Physiology of Seasonal Growth in Tall Fescue Varieties (Festuca arundinacea Schreb.)

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Despite the relatively mild winters of western Oregon the growth of forage grasses declines during the winter months. However, introductions of tall fescue (Festuca arundinacea Schreb.) from the Mediterranean region have been observed to make more foliar growth during the winter than varieties developed from north European introductions. In contrast, however, the summer growth of the Mediterranean varieties is less than the north European varieties.

The seasonal growth patterns observed in varieties of tall fescue in Oregon indicated differences brought about by genetic changes to adapt the species to the environment of its geographic origin. Turesson (112) coined the word ecotype to describe the product arising from the genotypic response of a species to a particular habitat. And Clausen, Keck, and Hiesey (22) concluded that the important characteristic of an ecotype was the ability to synchronize growth with the diurnal and seasonal changes of the environment. Clausen (21) considered ecotypes to be a stage in the evolution of a species; they may be physiologically or morphologically distinct, or distinct in both attributes, but gene exchange could still occur. These aspects of an ecotype became a central theme to the study of seasonal forage production--a theme that received support

as the study progressed.

The studies reported in this thesis were an attempt to understand the physiological basis for the differences in seasonal growth of varieties of tall fescue that had different geographic origins. It was considered that such an understanding would assist in designing a program aimed at breeding a variety of tall fescue with the ability to produce forage of a less seasonal nature, as well as suggest management practices to obtain maximum production from existing varieties.

II. THE PHYSIOLOGY OF SEASONAL GROWTH--A SELECTIVE REVIEW OF THE LITERATURE

The success of a species depends on its ability to grow and reproduce in the presence of inter- and intra-specific competition. Broadly, there are three methods by which a plant species may achieve success. Firstly, a plant species may achieve success by possessing a greater rate of growth than its competitors. Secondly, a successful species may escape competition by growing during a period of the year when other inhabitants of the environment are not active. A third requirement of a successful species is the ability to survive extremes of the physical environment. In this review only the first and third attributes of a successful species will be considered, and they will be considered only as they relate to the study of seasonal growth in tall fescue varieties.

1. Dormancy

It is well to begin this review with a discussion of dormancy because it not only is the most dramatic example of changes in seasonal growth but a discussion of seasonal growth can be confusing if a definition of dormancy is not established at the outset.

Vegis (119) pointed out that the onset, duration, and termination of dormancy was once believed to be a genetic characteristic of the

plant. However, since the turn of the century evidence has accumulated to show that dormancy is a state of inactivity of the plant induced and terminated by environmental factors. It is now generally believed that plants evolved a phase of dormancy in their life cycle in order to withstand seasonal stresses of temperature or drought in the area of geographic origin. However, because all plant species do not have a dormant stage in their life cycle while others continue to exhibit dormancy even when removed from their area of geographic origin it is apparent that the specific response to the environment is genetically controlled.

1.1 Stages and Types of Dormancy

Neither the onset nor the termination of dormancy is sudden. Rather, there is a gradual narrowing and widening of the range of the restricting climatic factor over which growth can occur. In the case of temperature, the onset of dormancy occurs over a period of time called early rest (119). During early rest, species adapted to a climate which experiences seasonal periods of hot, dry weather may exhibit a gradual loss of ability to grow at high temperatures. On the other hand, species adapted to a climate of freezing winters may lose the ability to grow at low temperatures during early rest. The third type of early rest shows a gradual loss of ability to grow at both high and low temperatures and is an advantage to species in a climate

where both summers and winters are unfavorable for growth.

In the middle rest phase of dormancy a state of true dormancy may be entered during which "normal growth cannot be resumed whatever the external conditions" (119). However, the middle rest phase may involve a state of relative dormancy during which growth is only limited by the external conditions. In order to distinguish between true and relative dormancy Vegis (118) stressed the need to test for growth at several different temperatures.

Following middle rest there is a period, known as after-rest, during which the ability to grow over an extended range of temperature is recovered. After-rest is the familiar period during which dormancy is broken and new growth appears. This phase of dormancy is referred to as after-ripening when applied to seeds.

1.2 Induction and Termination of Dormancy

The environmental conditions which induce or break dormancy include temperature, photoperiod, moisture, and a combination of these factors (119). Furthermore, in order for a plant to be in an insensitive state by the time the seasonal stress occurs it may be necessary for the plant to be induced by the environmental conditions preceding the stress period (118). Thus winter dormancy may be induced by the preceding autumn - early winter in order to survive winter temperatures, and summer dormancy induced by the preceding

late winter - early summer conditions.

1.21 Summer Dormancy

The inter-play of preceding and prevailing conditions in establishing summer dormancy is illustrated by the findings of Laude (62) who determined conditions sufficient to induce summer dormancy in the perennial grass Poa scabrella. Plants in the field continued to grow during the summer when the temperature did not exceed 25C. However, if plants grown under long days were exposed to a temperature of 54C for 4 hr on 3 alternate days dormancy was established. Dormancy was not established by the high temperature treatment unless the plants had been grown under long days. Plants also had a juvenile period of 15 weeks during which dormancy could not be induced. In order to break dormancy in the middle of summer it was necessary to apply water to the soil and to lower the air temperature to 24C; plants in the field remained dormant when the average daily temperature was 18C for 3 weeks but initiated new growth when the first autumn rains occurred.

The results of Laude (62) showed that during the middle rest period of dormancy P. scabrella had lost the ability to grow at high temperatures. Because growth resumed within 4 days after the temperature was lowered and water was applied it is probable that P. scabrella entered a state of relative dormancy rather than true

dormancy. However, relative dormancy would have been as effective as true dormancy in protecting the species from summer drought as low temperature and rainfall rarely occurred during the summer.

The ability to survive summer drought may be associated with the loss of ability of the seed to germinate at high temperatures. This type of relative dormancy was found by Cooper (27) in ecotypes of Phalaris tuberosa. Embibed seeds of ecotypes from Morocco were able to survive 38C whereas seeds of ecotypes from Israel germinated and were killed by high temperature. Morley (75) found a similar genetical variation in seeds of subterranean clover (Trifolium subterranean). It was concluded by Cooper, and by Morley, that the loss of ability to germinate at high temperatures was associated with adaption of the species to an environment which received an erratic summer rainfall.

Soil moisture alone may control summer dormancy (62). However, the mere lack of growth when moisture is limiting can not be referred to as dormancy. It is implied in discussions of dormancy that the middle rest period enables the species to survive stresses of the environment in which it evolved. Stresses of moisture and temperature may be seasonal or of a short term. Hence, dormancy may have evolved to infer long-term or short-term survival of stress. Therefore, the survival of a stress by a plant needs to be interpreted with considerations of the ecology of the species before dormancy

is invoked.

1.22 Winter Dormancy

The most obvious example of winter dormancy is that of deciduous trees. Garner and Allard (40) proposed that photoperiod controlled the onset of dormancy in certain perennials and this has been frequently verified (79). Buds of Fagus silvatica and Betula spp. are maintained in a state of dormancy by low temperatures and a short photoperiod and bud-break occurs only if high temperatures are associated with a long photoperiod (118).

Studies of winter growth of forage grasses have revealed ecotypic differences between populations from Mediterranean and North European regions (25). The slow growth of the north European populations, with the concommitant biochemical changes (32), is believed to infer tolerance to the freezing temperatures of the winter. This loss of ability to grow at low temperatures is genetical and it may be considered a condition of relative dormancy. It has been found that north European ecotypes of orchardgrass (Dactylis glomerata) and perennial ryegrass (Lolium perenne) quickly respond to elevated temperatures in the winter even in the presence of a short photoperiod, and Robson et al. (90) also found this to be the case with ecotypes of tall fescue.

In order to determine whether the retarded growth of the forage grasses during the winter can be considered relative dormancy, as

defined by Vegis (118), it would be necessary to show that exposure to low temperatures resulted in a gradual loss of ability to grow at low temperatures. Such experiments have not been reported. However, there are two observations which suggest that the retarded winter growth is due to relative dormancy. Firstly, ecotypic variation indicates that retarded growth is under internal genetical control and the winter growth rate is not determined by the external environment alone. Secondly, results reported in a later section of this thesis show that winter growth was increased by application of the plant hormone gibberellic acid (GA)[1]. This latter observation clearly indicated that winter growth rate was controlled more by the internal growth regulator balance of the plant than by the external weather. Both these observations indicate relative dormancy because winter growth rates were under internal control and not merely determined by the weather.

1.3 Perception of Changes in Climate

In order for dormancy to be established it is necessary for seasonal changes to be perceived and translated into biochemical terms. The perception of daylength is believed to be due to the

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[1] The abbreviation GA will be used for GA_3 which is commonly referred to as gibberellic acid.

phytochrome system (48). Phytochrome is a biloprotein capable of existing in two forms--a form capable of absorbing radiation in the wave lengths of red light (Pr), and another form capable of absorbing radiation in the far-red wave lengths (Pfr). The two forms are interconvertible depending on the wave length of radiation absorbed. During the day Pr is converted to Pfr and at night Pfr slowly decays back to Pr. It is envisaged that length of day is perceived by the length of night which determines how much of the inactive Pr is formed. The above description of the perception of daylength is no doubt an oversimplification (51). Furthermore, there is evidence that plants possess an endogenous circadian rhythmn which may also be involved in photoperiodism (46).

No specific mechanism for perceiving seasonal changes in temperature has been invoked. Vegis (118) reviewed some evidence which suggested that the synthesis of DNA and RNA may be more sensitive to temperature than other organic compounds which make up the bulk of plant tissue. Furthermore, it is known that enzymes have more-or-less well defined optimum temperatures. Emphasis or blockage of metabolic pathways and the accumulation of metabolites may arise from changes in temperatures. Vegis (118) suggested that the reduced oxygen supply to seeds and buds brought about by the development of impermeable seed coats and bud scales formed during periods of slow growth may be involved in establishing dormancy.

Lipids are frequently observed in dormant seeds and may accumulate because their metabolism via acetyl-coenzyme A and the Kreb's cycle may be limited due to the slow re-oxidation of reduced pyridine nucleotides under limited oxygen supply. It would appear, however, that it is necessary to establish whether these observed biochemical changes are a cause or an effect of dormancy.

1.4 Growth Regulator Balance

The perception of the photoperiodic stimulus elicits biochemical changes in the plant (79). Under short days, trees which are dormant in the winter show a rise in the level of extractable inhibitors and a low level of auxin and gibberellins. Nitsch (78) was able to prevent the onset of winter dormancy of sumac (Rhus typhina) under a short photoperiod by the application of GA. In the same study, Nitsch was able to detect an increase in extractable inhibitors when sumac was placed under a short photoperiod. Nitsch (79) concluded that the onset and termination of dormancy, and other seasonal activity such as rooting, bulbing, tuberization and flowering, involved the dual mechanism of changes in the level of chemical growth promotors and the level of inhibitors.

2. Growth Analysis

Some of the earliest attempts to describe growth quantitatively were successful (12) and have been used extensively in the ensuing 50 years. By the relatively simple process of measuring plant weight and leaf areas at the beginning and end of a short interval, usually

7 to 10 days, the following components of growth can be computed:

$$\overline{RGR} = \frac{\ln W_2 - \ln W_1}{t_2 - t_1} \qquad [1]$$

$\overline{RGR}$ is the mean relative growth rate and is defined as the mean increase of plant material per unit of material present per unit of time. The initial and final weights of plant material over the time interval $t_2 - t_1$ are represented by W_1 and W_2 respectively. The initial and final weights for a whole plant study are whole plant weights but, in the study of a sward, W may be total dry weight per unit area of ground.

$$\overline{NAR} = \frac{W_2 - W_1}{A_2 - A_1} \frac{\ln A_2 - \ln A_1}{t_2 - t_1} \qquad [2]$$

$\overline{NAR}$ is the mean net assimilation rate and is defined as the mean increase of plant material per unit of assimilatory material per unit of time. The initial and final assimilatory material, A_1 and A_2 respectively, is the total leaf area, in the case of whole plant studies, or the total leaf area per unit area of ground, in the study of a stand of plants. Expression [2] is true for the simplest relationship between A and W, i.e. linearity, and Radford (85) developed appropriate expressions should the relationship be exponential; if the interval $t_2 - t_1$ is sufficiently short, such as 1 to 2 weeks, then [2] would be suitable although the long-term relationship between

A and W may be exponential (124).

$$\overline{LAR} = \frac{1}{2}\left[\frac{A_1}{W_1} + \frac{A_2}{W_2}\right] \qquad [3]$$

$\overline{LAR}$ is the mean leaf area ratio and is defined as the mean ratio of assimilatory material per unit of plant material. Watson (124) proposed that the appropriate ratio when yield per unit area of land was being analysed was the area of leaf surface per unit area of land surface; this ratio he called the leaf area index (LAI). Expression [3] is only correct if LAR is linearly related with time (85). It is apparent that LAR consists of two components:

$$LAR = \frac{LW}{W} \times \frac{A}{LW} \qquad [4]$$

i.e. the ratio of leaf weight, LW, to total plant weight and called the leaf weight ratio, and the specific leaf area, $\frac{A}{LW}$. It can be shown that the following relationship can hold between [1], [2], and [3]:

$$\overline{RGR} = \overline{NAR} \times \overline{LAR} \qquad [5]$$

Radford (85) pointed out that [5] is correct only if the assumptions underlying the derivations of [1], [2] and [3] are met and that if A and W vary exponentially they have the same exponent. This difficulty only arises if mean values are computed, equality [6] is correct for

any instant in time:

$$RGR = NAR \times LAR \qquad [6]$$

It can be seen that growth rates of individual plants or swards varying in initial weights can be compared on an initial unit weight basis by computing RGR's. Furthermore, by "Growth analysis," as this method has become known (125), differences in RGR can be ascribed to differences in NAR or LAR, or both. Furthermore, differences in LAR can be ascribed to differences in leaf weight ratios or differences in specific leaf areas. The physiological interpretations of these components of relative growth rate can be succinctly stated:

> NAR is the photosynthetic efficiency per unit of leaf area (125). Expressed as $mg/cm^2/week$, for example, it avoids the complications of photorespiration (8) inherent in gasometric methods of measuring net CO_2 exchange. However, it does require destructive harvesting over a period of time and is not adaptable to instrumentation. The conclusion of Etherington (34) that infra-red gas analysis makes other methods of measuring photosynthesis obsolete is debatable.
>
> Leaf weight ratios indicate the distribution of assimilates between leaf tissue and the remainder of the plant. This component is comparable to the ratio of shoot weight to

root weight which is also useful in the diagnosis of plant growth (17).

Specific leaf area indicates the leaf area per unit weight of leaf tissue and has been shown to be directly related to the light intensity required to saturate the photosynthetic capacity of a leaf, and may be inversely related to the mesophyll resistance to CO_2 diffusion (52).

It can be seen from the above discussion that growth analysis can be a useful method for determining the physiological basis for the differences in growth between plants or swards. Some results of growth analysis pertinant to the thesis problem will now be considered.

2.1 Growth Analysis of Seasonal Growth

The earlier studies correlating seasonal changes in temperature and light with the changes in RGR, NAR and LAR have been reviewed by Blackman (12). A recent paper by Warren Wilson (123) illustrated the variation among species in their reaction to changes in climate. He grew spaced plants of rape (Brassica napa), sunflower (Helianthus annus) and corn (Zea mays) in solution culture at Deniliquin, Australia. Plants were harvested throughout the year at the same stage of development in order to eliminate changes due to age (12, 56, 76). The experiment was thus designed so that growth was limited by climate alone. The relationship between radiation,

measured by a Kipp solarimeter, and mean daily temperature with the components of relative growth rates was established by curvilinear regression. The quadratic equation used with the two climatic variables accounted for 87 to 95% of the total variation observed in RGR and NAR and 52 to 85% of that observed in LAR. The results were checked with the growth made in controlled environments.

Warren Wilson (123) found that NAR for rape was independent of temperature. Consequently, the seasonal change in NAR coincided with the seasonal change in radiation and was maximal in mid-summer and minimal in mid-winter. In contrast, the NAR of sunflower and corn was increased with both seasonal increases in temperature and radiation. Because temperature reached a maximum slightly after mid-summer the maximum NAR for sunflower and corn was also slightly after mid-summer.

Relative growth rate was less dependent on radiation and more dependent on temperature than NAR and, consequently, the maxima and minima for all 3 species were about 1 month after those of NAR.

For all species, LAR was minimal in spring when there was relatively high radiation and low temperature, and maximal in the autumn when the reverse was true.

Blackman (12) reviewed evidence which showed that light intensity had a decisive effect on LAR. In experiments outdoors during the summer where light intensity was altered by shading, the

RGR and NAR of sunflower increased with increasing light intensity while LAR decreased. The increase in NAR was sufficient to offset the decrease in LAR and hence their product, RGR, showed an increase with increasing light intensity. Robson and Jewiss (90) found that tall fescue also showed a decrease in RGR due to shading. In contrast, cocoa (Theobroma cacao) is an obligate shade species and NAR did not show an increase when light intensity was increased; RGR declined above 0.5 daylight because the decline in LAR was not offset by an increase in NAR (12).

Leaf area ratio is composed of two components--the leaf weight ratio and the specific leaf area [4]. It has been shown that the increase in LAR at low light intensities is usually associated with an increase in the specific leaf area (12, 52) and this may be associated with anatomical changes including the number of pallisade and mesophyll cells as well as cell volume (24). Björkman and Holmgren (10) and Holmgren (52) showed that shade ecotypes of Solidago virgaurea are more efficient photosynthetically at low light intensities and photosynthesis by the leaves was saturated at lower light intensities than those of ecotypes from exposed habitats. Thus, an increase in LAR at low light intensities, brought about by an increase in specific leaf area, enabled the species to adapt to reduced light intensities. Blackman (12) pointed out that a plant may show a decrease in specific leaf area as rapidly as 4 days after a change in

light intensity. The second component of LAR, leaf weight ratio, may also be important in explaining differences in the seasonal growth among species and ecotypes and will be considered in the next section.

2.2 Ecotypic Variation in Forage Grasses

Species of forage grasses which are distributed over a wide range of latitude and elevation have been shown to possess ecotypic variation in seasonal growth (25). Thus, ecotypes from the Mediterranean region have become adapted to grow during the relatively mild winters of that region while active growth during the winters of continental or northern Europe would predispose the plant to injury by freezing temperatures. However, the summers of the Mediterranean region are characterized by the lack of rainfall whereas the summers of northern Europe can receive favorable rainfall together with the warm temperatures. Consequently, ecotypes of northern Europe are adapted to grow during the summer months whereas those of the Mediterranean region survive the dry summers in a vegetatively inactive state.

Ecotypic variation in the seasonal growth of forage grasses has been observed in perennial ryegrass, orchard grass, Phalaris tuberosa, and tall fescue (25). The physiological basis for the differences in seasonal growth rates has been shown to involve

differences in NAR and LAR. The papers by Robson and Jewiss (88, 89, 90) on tall fescue were published towards the end of the conduct of the research reported in this thesis. However, the results reported in this thesis agree with and add to the findings of Robson et al.

The role of NAR in determining differences among ecotypes in seasonal growth is conflicting. Thus, Chatterjee (20) found that Mediterranean ecotypes of tall fescue had a higher NAR during the winter at Hurley, England, than the Aberystwyth variety S170. Furthermore, the Mediterranean ecotypes responded by increased growth to either increased temperature or increased light whereas S170 only made more growth during the winter when both light and temperature were increased. On the other hand, Robson et al. (90) found that the NAR of two synthetic varieties of tall fescue of North African origin, Syn I and Syn II, only exceeded that of S170 when grown at 5/-3C; when the temperature was 10/0C the NAR of S170 was equal to that of Syn I and Syn II.

Robson et al. (90) studied the response of varieties of tall fescue to decreased light intensity under the simulated winter environment of 6/0C. The light intensity varied from 27 ly/day, the average value for winter radiation at Hurley, to 36, 12 and 4% of this value. It was found that growth continued at all light intensities except the lowest and consequently RGR and NAR were positive. The LAR increased when light intensity was decreased. There were no

differences between varieties in the response to decreased light intensities indicating that, at the simulated winter temperature of 6/0C, the varieties did not differ in their ability to grow at low light intensities. Robson et al. (90) concluded, therefore, that differences in winter growth between the varieties was not determined by different responses to low light intensity. However, it should be noted that the RGR of the 3 varieties did not differ under a regime of 6/0C and the response to changes in light intensity may have differed at lower temperatures where differences in winter growth were observed.

Finally, Eagles (31) found that at 5 and 10C a Norwegian ecotype of orchardgrass had a higher RGR than a Portuguese ecotype, and this difference was attributable to differences in NAR rather than LAR. This result is inconsistant with other studies reviewed which have shown that Mediterranean ecotypes have a higher RGR at low temperatures than north European ecotypes. In fact, MacColl and Cooper (67) found that a Portuguese ecotype of orchardgrass did have a higher RGR during the winter than a Danish ecotype and the higher RGR was due to a higher LAR rather than a higher NAR. Eagles (31) suggested that the higher light intensities he used in the controlled environment chambers may have caused the conflicting result. This seems quite feasible considering the pronounced effect light intensity has on LAR (12); the high light intensity used by MacColl may have prevented an increase in LAR sufficient to offset the decrease in

NAR at low temperatures in the Portuguese ecotype.

It has become increasingly apparent that it is misleading to discuss photosynthetic efficiency of leaves, eg. NAR, as an isolated process. Rather, photosynthetic efficiency may be controlled by a "negative feed-back" mechanism operating between photosynthesis and growth. Thus, photosynthesis may be limited by the rate of removal of photosynthate from the leaf (33, 77, 122, 129, 130). Furthermore, photosynthesis may be influenced by substances translocated to the leaf from other regions of the plant such as growth regulators (9, 63, 113) and many other substances (59). Robson et al. (90) suggested that the failure to consistently measure a difference in NAR's among ecotypes of tall fescue at low temperatures may be due to differences in the amount of photosynthate which had accumulated in the leaf at the time the measurements of NAR were initiated. Thus, the level of photosynthate accumulated may be influenced not only by differences in growth rate at that time, but also by preceding cultural conditions, temperature changes, and how long the plants had been under a low temperature regime.

Growth is generally more sensitive to limitations in the environment than physiological functions of the plant (17). Thus, when growth is limited by low temperatures carbohydrates may continue to accumulate because photosynthesis is not limited to the same extent as growth (6). Eagles (32) found that a Norwegian ecotype of

orchardgrass accumulated higher concentrations of simple sugars and fructosans at 5C than did a Portuguese ecotype, and at 30C the relative concentrations were reversed. These differences were correlated with the differences in NAR. And Robson et al. (89) found that the total water soluble carbohydrate of S170 tall fescue grown in an unheated greenhouse during the winter was higher than that of Syn I and Syn II. Cooper (26) measured the rate of leaf growth of ecotypes of perennial ryegrass and orchardgrass and found that the north European ecotypes had a slower growth rate than Mediterranean ecotypes.

It is apparent from the above discussion that growth of the north European ecotypes is more limited by low temperatures than Mediterranean ecotypes. Furthermore, the retarded growth is initially not accompanied by an equivalent reduction in photosynthesis and carbohydrate concentrations in the plant tissue increases. The higher concentration of carbohydrate may infer cold tolerance to the plant (25, 64). Ultimately, the high concentrations in the leaf tissue may inhibit photosynthesis (33, 130). On the other hand, the Mediterranean ecotypes at low temperatures continue to utilize carbohydrate in the growth of leaves and this results not only in the development of a greater total leaf surface but also a greater photosynthetic efficiency per unit area of leaf. In consequence, analysis

of winter growth may show a greater NAR and LAR, and lower reserve carbohydrate levels for Mediterranean ecotypes than for north European or continental ecotypes.

III. STUDIES ON THE PHYSIOLOGY OF SEASONAL GROWTH IN TALL FESCUE VARIETIES AT CORVALLIS, OREGON

1. Climate of Corvallis, Oregon

Corvallis is 69 m above sea level and is situated in the Willamette valley of western Oregon at a latitude of 44° 38' north. Corvallis, and much of Oregon west of the Cascade mountain range, has a typical Mediterranean climate. The winter temperatures are mild with an average January minimum of -0.6C and a maximum of 5.6C. Summer temperatures are also moderate with an average July maximum of 26.7C and a minimum of 10.0C. Rainfall is sharply winter incident with an average of 7 inches in December and only 0.25 inches in July and August. The photoperiod of Corvallis changes from 8 hr in December to 16 hr in June. The climate of Corvallis is shown in the Appendices.

It is the relatively mild winters of western Oregon that enable livestock to graze outdoors throughout the year. Although low temperatures and radiation limit winter forage production, management and suitable forage varieties could increase production. Furthermore, irrigation of suitable forage varieties during the dry summer would enable utilization of the high light intensities and favorable temperatures for growth of forage.

2. Tall Fescue Varieties Studied

The variety Alta was grown from Foundation Seed harvested in 1966 from block F5 - R12 at Hyslop Experiment Farm, Corvallis.

The original collection of the source material for Oregon 1000 was made by Dr. O. S. Aamodt near Constantine, Algeria, in 1952.[2] The introduction was received by Dr. J. R. Cowan and about 130 plants were established from seed at Hyslop Farm, Corvallis, Oregon. The regrowth of these plants during the first winter was notably superior to Alta and Kentucky 31 and, consequently, an attempt was made to increase the seed supply. However, during at least one winter plants had been killed by freezing temperatures. The seed I used in my study was harvested from the surviving plants and it is likely that the genotypes for more vigorous winter growth had been eliminated by the natural selection of the freezing winters at Corvallis. However, it will be shown in section 3 that Oregon 1000 retained a superior rate of foliar growth during the winter. Therefore, the Mediterranean origin of Oregon 1000 was still apparent in its pattern of seasonal growth.

The variety Tunisia was grown from seed that was received from the International Cooperation Administration at Tunis by the

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[2] Information supplied by Dr. O. S. Aamodt, now retired from the USDA, and Dr. J. R. Cowan, Head, Farm Crops Department, Oregon State University.

New Crops Research Branch of USDA in 1961. The germination of this seed was very low (18%) but the variety had the advantage that no selection subsequent to introduction had occurred because it had not been grown in environments removed from its area of geographic origin. The introduction had the accession number PI 277076 but unfortunately no information is available on the history of the variety at its place of origin.[3] However, this variety did exhibit the typical seasonal growth of a Mediterranean variety and it can be safely assumed that it originated in a Mediterranean or maritime environment.

3. Comparisons of Seasonal Growth in the Field

3.1 Procedure

The three varieties of tall fescue Alta, Tunisia, and Oregon 1000 were included in a field experiment at Hyslop Farm aimed at comparing the seasonal production of forage by a number of species and varieties. The experiment was a joint project of Dr. W. S. McGuire and Dr. R. V. Frakes of the Farm Crops Department, Oregon State University, Corvallis. The experiment was established in June, 1963 when the varieties were sown in 0.01 ha plots on Woodburn soil.

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[3] Letter from Dr. A. Hovin, USDA, ARS, Beltsville, Maryland, November 14, 1967.

The plots were adequately fertilized and irrigated during the summer. Forage yields were determined when the forage reached 30 to 40 cm by harvesting with a mower, weighing the green material, and correcting the fresh weight for oven dry weights.

3.2 Results and Discussion

During 1966 and 1967 the plants were harvested and the forage growth rates of the tall fescue varieties were determined. These results are plotted in Figure 1 and show the typical differences expected of north European and Mediterranean varieties. Thus, the growth rate of Alta was greater than that of the Mediterranean varieties during the summer but during the winter the Mediterranean varieties made harvestable growth whereas Alta did not.

It was apparent that true dormancy was not involved in the summer growth of the two Mediterranean varieties since both made vegetative growth during the summer when irrigated. However, it was not apparent from this experiment whether true dormancy was involved in the lack of winter growth of Alta. It was observed, however, that Alta made more foliar growth during the late winter-early autumn than did the Mediterranean varieties. This response in winter growth of Alta when the photoperiod changed very little (Appendix I) suggested that the winter growth of Alta may respond to short-term changes of light or temperature, or both. Furthermore, it suggested

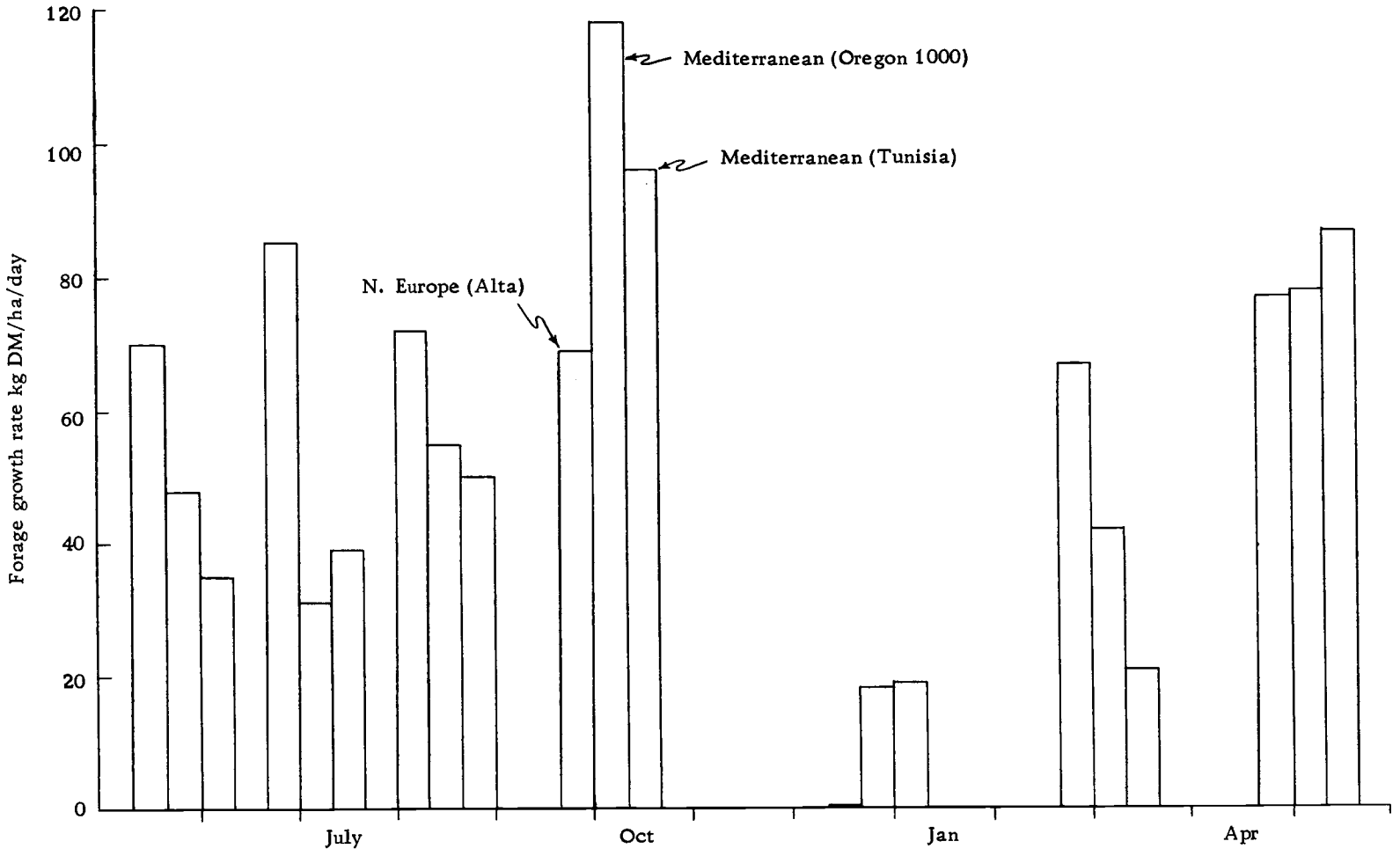

Figure 1. Seasonal growth rates of forage from swards of tall fescue varieties grown at Corvallis, Oregon, September 1966 through August 1967.

that during the winter Alta may be in a state of relative dormancy rather than true dormancy. To further investigate these aspects of winter growth additional experiments were initiated.

4. Changes During the Winter in Tillers, Buds, and Reserve Carbohydrates

4.1 Procedure

At the beginning of the months of November, December and January of the winter of 1967/1968 the plots described in 3.1 of the Alta and Tunisian varieties were sampled for tillers and buds, and also for an estimation of carbohydrate reserves. The plots were sampled with a 15 cm square cutting tool made from 1/2 × 15 cm steel. The bottom edge of the tool was sharpened, and the top edge reinforced so that the tool could be driven into the sward with a heavy hammer.

The cutting tool enabled 15 cm cubes of the sward to be taken from uniform sites in the plots. The cubes for bud and tiller counts were freed from soil and the tillers stripped of dead leaves. After washing, the tillers and macroscopic buds were easily counted.

Reserve carbohydrates present in the roots and tiller bases of the sample cubes were estimated by the dry weight of etiolated regrowth produced in a greenhouse maintained at 20C. Wolf (133) had shown a good correlation between etiolated regrowth and carbohydrates

estimated by chemical methods. The cubes were wrapped in sheets of black plastic film and kept well watered. Each 2 weeks the etiolated regrowth was harvested, dried, and weighed; no further regrowth occurred after the third harvest.

4.2 Results and Discussion

The changes during the winter of the density of tillers and buds is shown in Figures 2 and 3 respectively. It is obvious that a lack of tillers, or macroscopic buds available for tiller development, was not a cause for the lesser winter growth of Alta; the density of tillers and buds of Alta was either greater or equal to that of Tunisia. Consequently, the differences in winter growth between the north European and Mediterranean varieties must be due to differences in the rates of growth of individual tillers. Furthermore, as Mitchell (70) pointed out, once full light interception is achieved pasture growth from a sward is likely to be independent of tiller density.

The amount of etiolated regrowth from the cubes collected during the winter is shown in Figure 4. This result provided an important clue to the physiological basis for the differences in winter growth. The significant varieties × time of sampling interaction indicated that the level of carbohydrates for Alta continued to rise during November-December whereas the level for Tunisia remained rather constant. Some recent work by Robson et al. (89) also found

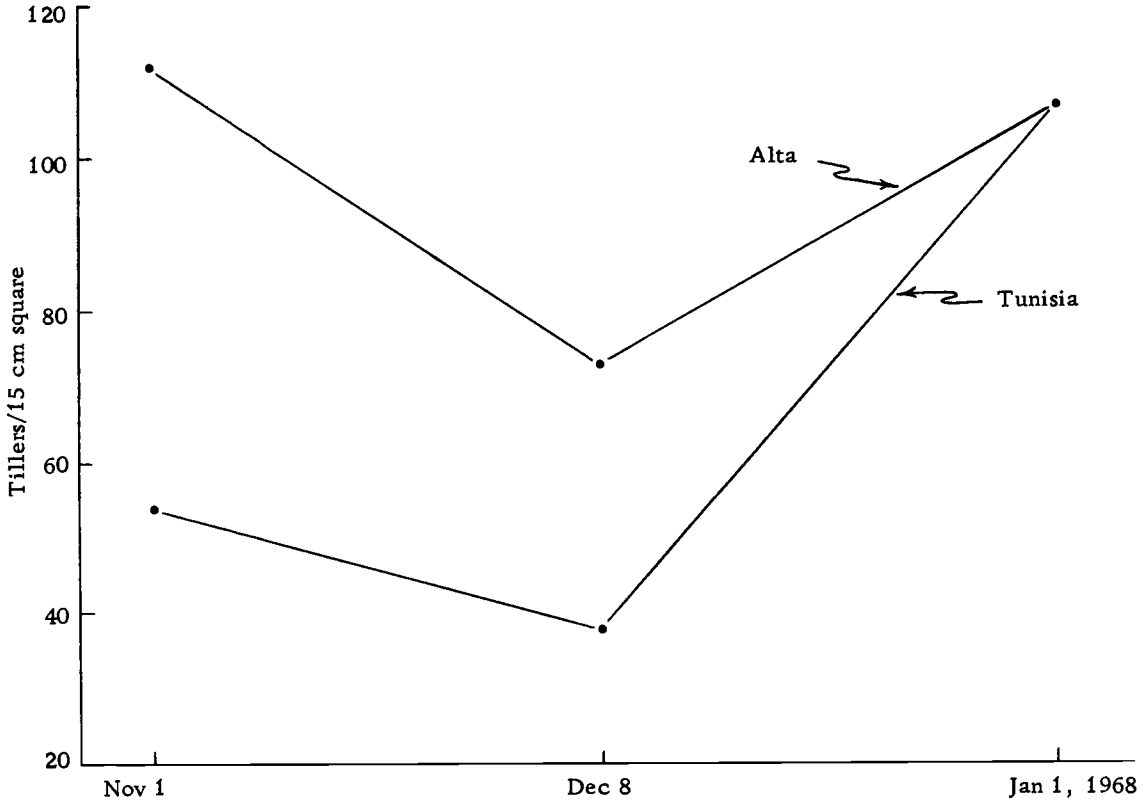

Figure 2. Changes during winter of tiller density of tall fescue varieties grown in a sward at Corvallis, Oregon. Statistical significance: varieties***, harvests***, varieties × harvests**.

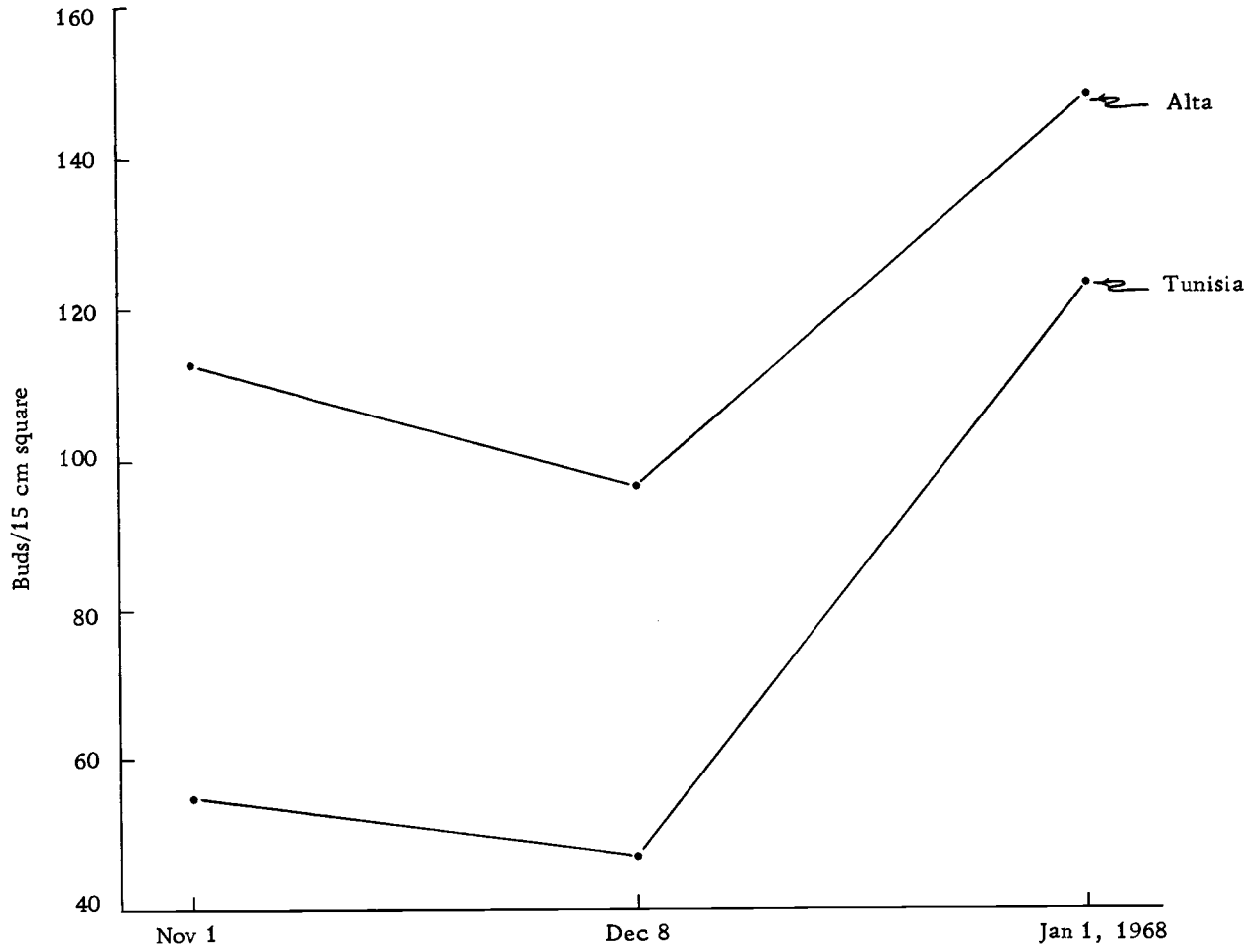

Figure 3. Changes during winter of bud density of tall fescue varieties grown in a sward at Corvallis, Oregon. Statistical significance: varieties ***, harvests ***, varieties × harvests NS.

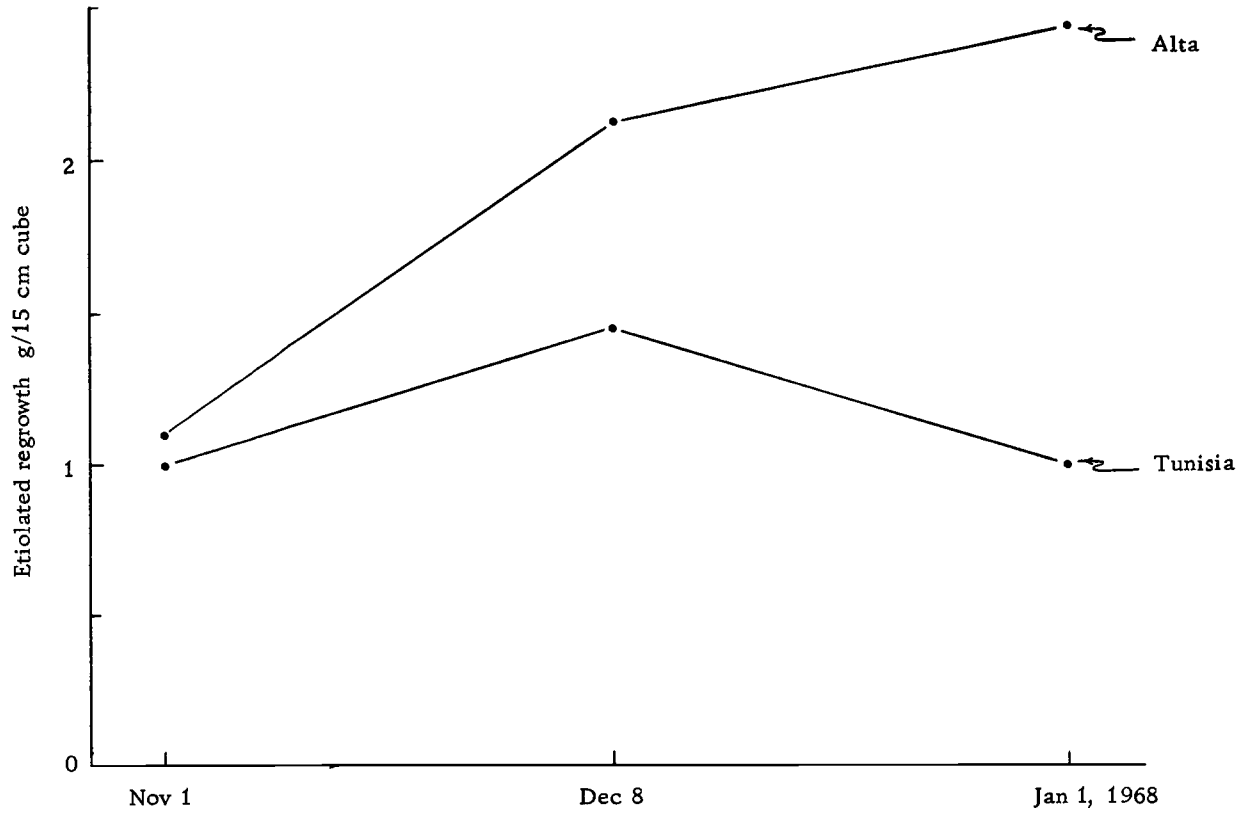

Figure 4. Changes during winter of reserve carbohydrates of tall fescue varieties grown in a sward at Corvallis, Oregon. Statistical significance: varieties**, harvests**, varieties × harvests**.

the level of carbohydrates higher in the winter inactive S170 tall fescue than Syn I and Syn II of Mediterranean origin.

The etiolated regrowth test not only estimated the amounts of carbohydrates present but also the utilization of the reserves for growth at 20C. It was found that Alta was able to utilize the reserve carbohydrates for etiolated growth when the temperature was increased. This indicated that the inability to use reserve carbohydrates when the temperature is low may be an important determinant of the winter growth of the varieties. The alternative, that differences in photosynthesis during the winter determined the differences in winter growth, is unlikely because a product of photosynthesis, carbohydrates, was higher for Alta than for Tunisia. Cooper (26) did find that leaf growth of Mediterranean and maritime varieties of perennial ryegrass and orchard grass was greater at low temperatures than that of north European and Scandinavian varieties.

5. Influence of a Preceding Summer on Winter Growth

Morgan (72) suggested that it was necessary to expose tall fescue plants to long days before differences in the winter growth of S170, and Syn I and Syn II could be observed. Morgan interpreted his results as a need for plants to experience a preceding summer before winter growth rates could be established. Initially, I was not able to

observe differences in growth between Alta and Oregon 1000 at cool temperatures, 16/10C, and an 8 hr photoperiod and decided to investigate the need for an exposure to a preceding summer in order to establish winter growth rates. It should be noted here that the temperatures in the controlled environment chamber of my experiments were not low enough and later experiments produced meaningful differences in growth rates with temperatures of 6/3C.

5.1 Procedure

Seed of Alta and Oregon 1000 was sown in 2.5 liter cans of soil. Several seeds were sown in each pot and the seedlings were later thinned to one average-plant per pot. The soil was a prepared potting soil with finely ground limestone and N, P and K fertilizer mixed with the soil prior to filling the cans. Urea fertilizer was added at infrequent intervals to the pots but particularly after harvests of foliar growth. The pots were punctured around the base to allow free drainage, and were surface irrigated when required. The pots were managed so that water and nutrients were not limiting. The plants were harvested when they had made sufficient foliar growth such that harvesting would have been contemplated under practical conditions.

Seed was sown at intervals throughout the summer beginning with May 6, 1967, and ending with the late sowing on October 6, 1967. There were 12 to 15 plants of each variety for each sowing date and

the pots were left outdoors to respond to the natural environment. The purpose of the sequential sowings was to have plants growing during the winter which had experienced differing proportions of the preceding summer.

5.2 Results and Discussion

The winter growth of plants for May 6, July 7 and October 6, 1967 sowings is shown in Figure 5. The winter growth from all sowing dates was less from Alta than from Oregon 1000. Because plants from the October 6 sowing would have received virtually no exposure to the long photoperiods and high temperatures of summer it seems unlikely that an exposure to a preceding summer was necessary to establish the different winter growth rates observed. Robson et al. (90) also found it unnecessary to expose tall fescue varieties to a preceding summer before differences in winter growth rates were observed. They studied the same 3 varieties as Morgan and found that plants grown from seed in a simulated winter environment exhibited differences in growth rates. The chambers had an 8 hr photoperiod, winter light intensities (25 ly/day) and 10/0C or 5/-3C. At both these temperatures, S170 had a lower RGR than Syn I or Syn II. Thus, neither a preceding long photoperiod exposure nor a high temperature exposure were necessary to establish differences in winter growth rates.

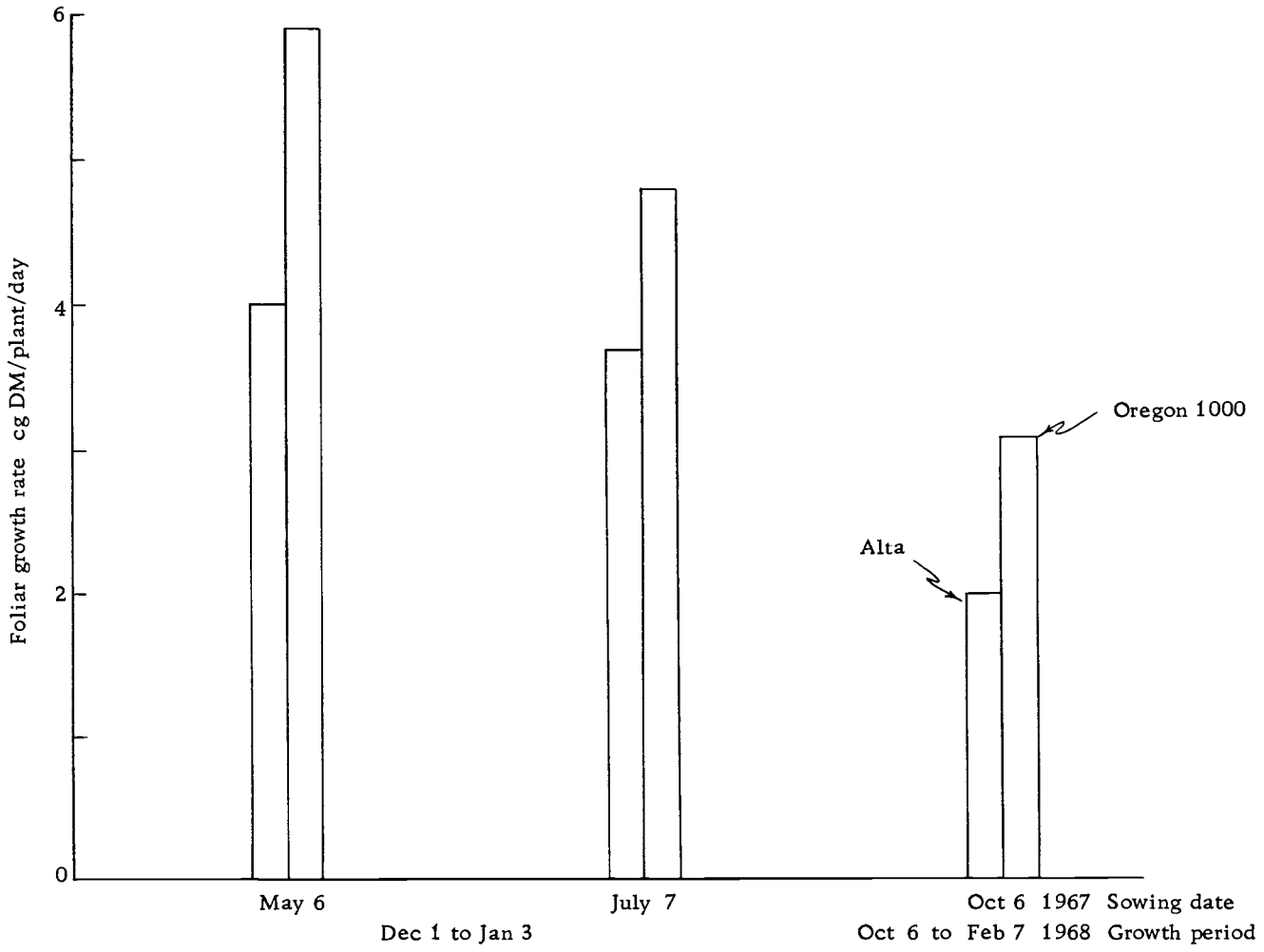

Figure 5. The influence of a preceding summer exposure on the winter growth of tall fescue varieties at Corvallis, Oregon.

A possible explanation of Morgan's results is suggested from his experimental conditions. Under unspecified "low" light and low temperature conditions and an 8 hr photoperiod, the growth of S170 at the end of 11 weeks was greater than that of Syn II. However, if under the low light and low temperature conditions the plants were exposed to a 16 hr photoperiod for the final 6 weeks, then the growth of S170 was less than Syn II at the end of the 11 weeks. Morgan's data suggested, however, that the photoperiod was extended merely by leaving the "low" light intensity lights on for the additional 8 hr because Syn II made more than twice the growth under the 16 hr photoperiod than it did under the 8 hr photoperiod. Morgan's results, therefore, may be a response to the total energy received rather than a photoperiodic response and if he had grown his plants longer than 11 weeks S170 may have eventually fallen behind Syn II. Parenthetically, it should be noted that Robson et al. harvested as early as 5 weeks and as late as 12 weeks but a comparison of the energy regimes of the two studies is not possible. A physiological explanation, which will be developed in this thesis, is that Syn II in Morgan's study was able to utilize the additional carbohydrate from photosynthesis under the greater radiation regime whereas S170 was unable to do so under the low temperatures. Ultimately, a high carbohydrate level could limit photosynthesis.

6. Factors Limiting Winter Growth

It was not established from the results of the field experiment presented in Figure 1 whether Alta made any foliar growth during December-January 1966/1967. Therefore, it seemed desirable to grow plants in pots and more completely harvest the foliar growth during the winter months. In addition, the presence of true dormancy could be established by determining whether Alta would respond to changes in temperature during winter. It will be recalled, that Vegis (118) defined true dormancy as the inability to grow under any external conditions, i.e. an absence of growth due to internal controls, whereas relative dormancy was restricted growth controlled by the external environment.

6.1 Procedure

Plants were grown as described in 5.1. On January 3, 1968 plants of Alta and Oregon 1000 had obviously different growth rates (Figures 6 and 7). At this time the foliage was harvested and three experiments initiated. Firstly, the May sown plants described in section 5 were divided into 2 groups. One group remained outdoors while the other group was moved into a heated greenhouse maintained at 15 to 20C.

Figure 6. Growth during December 1968 of tall fescue varieties at Corvallis, Oregon. Left: Alta, right: Oregon 1000.

The second experiment initiated on January 3, 1968 was to test the response of the plants during winter to an extended photoperiod. Six plants of each variety from the June sowing date of experiment 5 were placed under a 50 watt incandescent light bulb suspended 3 m above the pots. The pots remained outdoors and the light was turned on automatically at 6 a.m. and off at 10 p.m. to give a photoperiod of 16 hr. The other half of the plants of this sowing date were maintained as controls.

The third experiment initiated on January 3 was to test the response of the plants to GA. The July sown plants described in

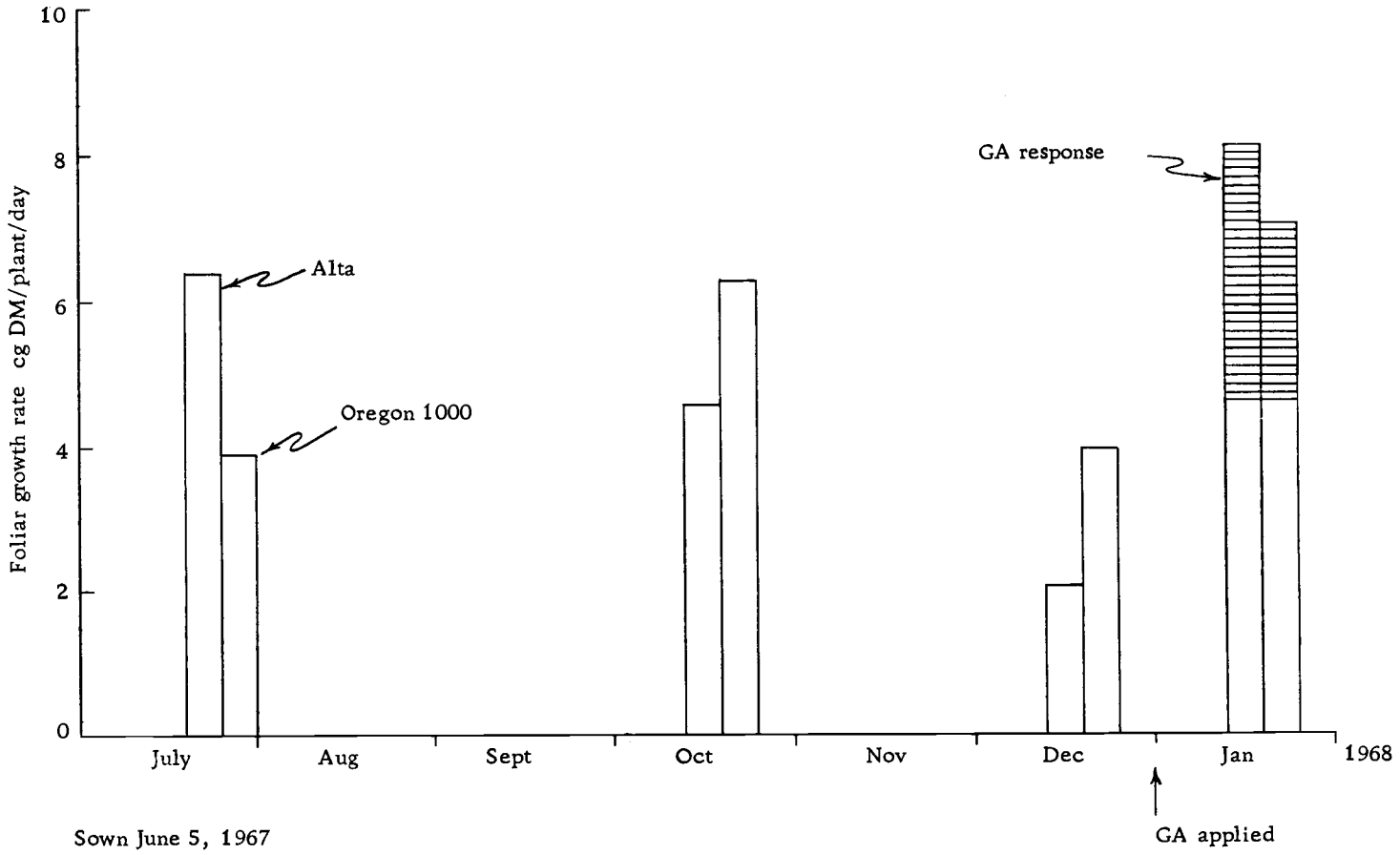

Figure 7. Seasonal growth rates of tall fescue varieties at Corvallis, Oregon, and the response to a winter application of gibberellic acid.

section 5 were divided into two halves. Half were sprayed with 0.1% w/v GA[4] with 0.1% v/v Tween 20[5] as an adjuvant (11) while the other half was used as a control. All plants remained outdoors under the natural conditions of winter.

On February 3, 1968 the 3 experiments were harvested by clipping the foliage about 4 cm above the soil surface. The harvested foliage was oven dried and weighed. A few grams of the fresh foliage from the GA experiment was extracted for chlorophyll determination according to the method of Arnon (4) by grinding in a pestle and mortar with 80% acetone.

6.2 Results and Discussion

The results shown in Figure 8 revealed that the winter growth of both Alta and Oregon 1000 was stimulated by an increase in temperature, an extension of the photoperiod, and also by GA. The suggestion was that Alta responded more to each of the three treatments than Oregon 1000. These experiments established that the limitation of winter growth was chiefly temperature and this limitation was overcome by GA or by extending the photoperiod.

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[4] "Gibrel" supplied by Merck and Company Incorporated, Rahway, New Jersey. Sample number 67 RTS1074 which contained 80.1% potassium gibberellate.

[5] Registered trade name of Atlas Chemical Industry, Wilmington, Delaware. Contains polyoxyethylene (20) sorbitan monolaurate.

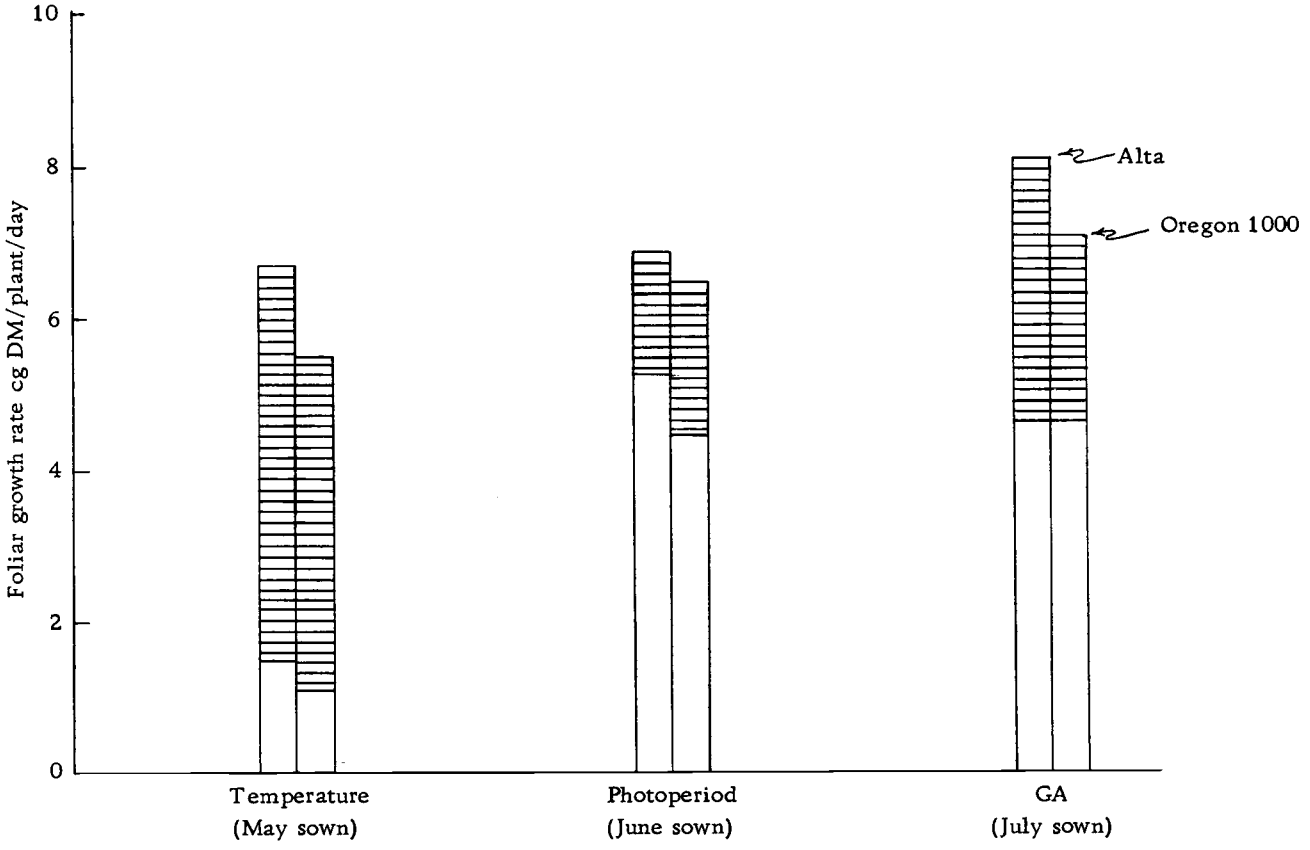

Figure 8. The response of tall fescue varieties to changes in weather, climate, and growth regulator balance during January 1968 at Corvallis, Oregon.

6.21 Relative vs True Dormancy

It can be seen in Figure 7 that Alta did make some growth during the early winter but, as expected, growth rate was less than that of Oregon 1000. Furthermore, the growth rate of Alta declined from its superior summer level to below that of Oregon 1000 sometime during late autumn-early winter. Because Alta made some growth during December and January this experiment showed that Alta did not enter true dormancy during the winter at Corvallis. The lack of winter growth of Alta shown in Figure 1 was, therefore, more likely to have been a function of the harvesting procedure rather than true dormancy.

The results shown in Figures 1, 7 and 8 all show a recovery of the growth rate of Alta during late winter. This response of Alta may be due to increased radiation and warmer temperatures which, on the average (Appendices), begin to be experienced in late January and February. This response of Alta is a further suggestion that Alta may exist in a state of relative dormancy where winter growth was limited only by the external environment.

6.22 Response to Temperature

Temperature stimulated a five-fold increase in the growth rate during January. Cooper (26) concluded that the difference between

Mediterranean and European varieties of Lolium and Dactylis was due to the inability of the latter varieties to grow leaves at low temperatures. And Robson et al. (88) concluded that the different responses to temperature was also the determinant of growth of tall fescue varieties during the winter.

6.23 Response to Photoperiod

It is tempting to suggest that the extended photoperiod promoted an increase in endogenous GA as postulated by Brian (14). However, the response to photoperiod may rather have been a response to increased light intensity as the incandescent light bulb, albeit of low wattage, was turned on for 16 hr a day. Templeton, Mott, and Bula (110) found an increase in total dry matter of Kentucky 31 tall fescue during the winter when plants were treated in a similar manner to those in my experiments. It would be adviseable, however, to repeat this experiment under controlled environmental conditions where photoperiod could be increased without an increase in total energy received.

Studies in controlled environments have shown that an increased photoperiod can cause a slight increase in the winter growth of ecotypes of forage grasses (26, 90), particularly ecotypes of more northern latitudes (26). And, studies by Ryle (91, 92) on the influence of photoperiods on the growth of leaves at 20/15C showed an increase

in leaf length but a decrease in the rate of leaf production due to long photoperiods; the increase in leaf length was due to increases in cell size and cell number. Ryle concluded that the long photoperiods enhanced NAR only indirectly through promoting more efficient interception of incident radiation. Guttridge and Thompson (43) found that cell length and cell number of strawberry petioles was also increased by long photoperiods and this effect was simulated by GA. The increase in gibberellins under long photoperiods is well recognized (84). Thus, it is possible that the response of tall fescue to the photoperiod-treatment shown in Figure 8 was a real response to an increased daylength and a possible interpretation is that gibberellin biosynthesis was promoted. However, critical studies of this possible interpretation would be necessary before making a definite statement.

6.24 Response to Gibberellic Acid

The evidence for a direct effect of gibberellin on photosynthesis is contradictory. Haber and Tolbert (45) found no influence on CO_2 fixation by leaves of barley, oats, or pea seedlings which had been treated up to 9 days before measurement of photosynthesis with GA. Later, Haber *et al.* (44) presented further evidence, that has been recently quoted (131), which they believed showed that GA had no effect on photosynthesis. In that study, Haber *et al.* germinated

wheat-seeds in the light for 4 days then placed the seedlings in the dark for 4 days with their roots bathed in 5 ppm GA. At the end of the 4 days in the dark the seedlings were detached from their scutella and oven dried. It was found that the seedlings treated with GA had a higher dry weight. Haber et al. concluded that GA, therefore, had no direct or indirect effect on photosynthesis. However, they published shortly before Paleg (83) renewed the interest in the role of GA in promoting de novo synthesis of α-amylase. It is likely that the more rapid growth of the wheat seedlings in the study of Haber et al. was due to GA promoting the synthesis of α-amylase which, in turn, mobilized the starch reserves of the seeds for seedling growth.

Bidwell and Turner (9) measured CO_2 fixation of bean (Phaseolus vulgaris) leaves sprayed with indoleacetic acid (IAA) or GA. Within 30 minutes after spraying the leaf with IAA net photosynthesis increased as much as 100% and then returned to the original level within a further 30 min (113). This rapid response of photosynthesis to IAA was also noted with a wide range of plant species except corn (9). In contrast, bean leaves sprayed with GA showed no increase in net photosynthesis (9). However, earlier Coulomb and Paquin (28) used a very similar experimental procedure to that of Turner et al. (113) to measure the response of tomato (Lycopersicum esculentum) plants to a foliar spray of GA. They found photosynthesis, respiration, and transpiration were increased by GA whether it was

applied to the leaf in the cuvette or to the remainder of the plant excluding the leaf in the cuvette. The response to GA was almost immediate, reached a peak in 3 to 4 hr, and subsequently declined.

The rapid increase in photosynthesis that has been promoted by IAA (9, 113) or GA (28) has been interpreted as an indirect effect. It was suggested that these growth regulators promoted growth and, as a consequence, promoted photosynthesis. (In passing, it should be noted that a suggested action of GA is the promotion of auxin synthesis (84)). Certainly, there is evidence to show that rapid growth promotes more rapid rates of photosynthesis (33). And, Bidwell et al. (9) in their study showed that bud break in beans promoted an increase in photosynthesis of the subtending leaf; an effect which was simulated by removing the bud and applying IAA. If promoted growth was the indirect effect of the growth regulators on photosynthesis it was a remarkably rapid response and apparently required a continued supply of the growth regulator to sustain the promoted growth.

There are two long-term studies involving growth analysis that have shown NAR can be increased by GA. Alvim (2) found that the RGR of bean seedlings was increased over a 10 day interval due to an increased NAR. The increase in plant weight induced by GA was mostly an increase in stem weight and Alvim suggested that the increased demand for photosynthate by the stems enabled photosynthesis to proceed more rapidly in the leaves.

Morgan (73) followed the influence of GA on the summer growth of S170 tall fescue seedlings. Plants were treated at 8 weeks of age and 4 harvests were made at weekly intervals. During the second week after treatment there was a change in the distribution of dry matter; shoot weight increased while root weight decreased. Reduction in root weights has also been recorded in other studies (2, 15, 36, 96). However, the increase in shoot:root ratio was due to a greater increase in shoot weight rather than the growth of the shoot from root reserves; i. e. GA enhanced the competitive ability of the shoot for the common pools of metabolites (17). During the third and fourth week after treatment with GA, Morgan (73) observed an increase in NAR and an increase in leaf weight ratio.

In conclusion, I observed an increase in foliar dry matter in response to GA. Whether this increase in dry matter was derived from root reserves or from enhanced photosynthesis was not investigated in this experiment. There is evidence in the literature which shows that both possibilities exist and it seemed desirable to persue this matter further (see section 7.).

6.241 Chlorophyll Content

The plants treated with GA were obviously chlorotic (Figure 9). Chorosis has been observed in most studies which have recorded an increase in winter production from GA (36, 74, 94, 96, 97, 131, 132).

The chlorophyll content of the foliage of the tall fescue varieties I treated with GA is shown in Table 1. The results showed that the concentration of chlorophyll in the foliage was decreased by GA. However, the total chlorophyll per plant was increased slightly by GA. Therefore, in this experiment, the chlorotic foliage was due to the inability of chlorophyll formation to be promoted by GA to the same extent as foliar growth. Friend (37) found that chlorophyll formation in wheat was temperature dependent with a Q_{10} of 2.0 between 10 and 15C. Apparently, GA had little influence on the temperature dependency of chlorophyll formation in tall fescue.

Figure 9. The response of tall fescue varieties to a winter application of gibberellic acid. Left to right: Alta, Alta + GA, Oregon 1000, Oregon 1000 + GA.

Table 1. The influence of gibberellic acid on the chlorophyll content of tall fescue varieties grown in a natural winter environment.[a]

	GA[b]	Chlorophyll content[c]		
		mg/g fresh wt	mg/g DM	mg/plant
Alta	-	2.8	13.9	23.6
	+	1.8	9.7	31.6
Tunisia	-	2.6	13.0	21.8
	+	1.8	9.6	24.6

[a]Corvallis, Oregon, Jan 3 to Feb 5, 1968.

[b]0.1% foliar spray on Jan 3, 1968.

[c]Error of chlorophyll determination < 5%.

Studies have shown that while production may be related to the total amount of chlorophyll present per unit area of ground surface (13, 16), the concentration of chlorophyll in the foliage may not be a determinant of production (49, 80). However, Gabrielsen (39) showed that chlorophyll concentration can influence the rate of photosynthesis in young leaves or leaves of aurea varieties but only at low light intensities (0.4 cal/dm/hr). No further increase in the rate of photosynthesis was recorded at low light intensities when the chlorophyll concentration was greater than 4 to 5 mg/dm^2. The aurea varieties that Gabrielsen studied were described as greenish yellow and had a chlorophyll concentration of 0.2 mg/dm^2.

Although the concentration of chlorophyll in my experiment was

not measured on a leaf area basis, the concentration of 2.6 and 2.8 mg/g fresh weight is an average value for many different plant species (16). The 30 to 35% reduction in chlorophyll concentration that resulted from the GA treatment may have reduced the rate of photosynthesis per unit area of leaf i.e. reduced NAR. However, leaf area (Figure 9) and total chlorophyll (Table 1) were increased by GA and an increase in total photosynthesis per plant may have actually contributed to the 51 to 72% increase in foliar dry matter recorded (Figure 8). Consequently, the chlorotic foliage of the plants treated with GA may not have been deleterious.

7. Influence of Gibberellic Acid on Shoot Regrowth in a Simulated Winter Environment

It appeared likely that at least part of the energy and structural material for the increased foliar growth in the winter that was promoted by GA was derived from carbohydrate reserves. In addition, the winter growth of pasture that has been stimulated by GA has frequently involved regrowth as well as new growth supported by photosynthesis (3, 74, 94, 97, 131). Consequently, an experiment was set-up in a simulated winter environment to measure the regrowth stimulated by GA when a minimal amount of photosynthetic tissue was present.

7.1 Procedure

Seeds of Alta and Tunisia were sown in "Perlite" November 1, 1967 in a warm greenhouse with supplemental fluorescent light for 16 hr each day. Plants were eventually thinned to 1 plant in each 1 liter can. Plants were watered with a complete nutrient solution and about once a week the pots were leeched of accumulated salts with water. At 18 weeks of age the foliage of some plants was cut to 10 cm and the plants transferred to a chamber set to simulate a winter environment: 8 hr photoperiod, 14 klx light intensity, 19 ly/day in the range of 400 to 750 nm[6] and 6.7/3.3C day/night temperature regime. At the time the plants were transferred to the winter chamber the Tunisian variety had made slightly more growth than Alta but the average number of tillers was 15 for plants of both varieties.

The plants transferred to the winter chamber were acclimatized for 5 weeks before GA was applied. A foliar spray of 100 ppm GA in 0.1% Tween 20 was applied to 4 plants of each variety and 4 plants of each variety were retained as non-treated controls. Eleven days later the foliage was cut back to 1 cm in order to minimize the photosynthetic tissue and to enable regrowth to be measured. Nine days

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[6]Measured with an ISCO Spectroradiometer, Instrumentation Specially Company, Inc., Lincoln, Nebraska, and made available by Dr. W. K. Ferrell, School of Forestry, Oregon State University.

later the first weights of regrowth were recorded and the 1 cm stubble cut back to the crowns of the plants to further reduce photosynthetic tissue. Two days after the first harvest of regrowth the GA treatment was re-applied to the crowns of the plants. Four subsequent harvests of regrowth were taken at intervals of 9 to 21 days. The regrowth was dried at 80C and average rates of regrowth were computed by dividing the weight of regrowth by the elapsed time. The sequence of events in the procedure is shown along the abscissa of Figure 10.

When the experiment was completed, the roots and crowns were extracted with cold water and the reducing power of the extracted carbohydrates after acid hydrolysis was determined by the copper-iodometric method of Somogyi (107). This method of extraction and determination measured the water soluble carbohydrates, including reducing sugars, sucrose, and fructosans, and should have included most of the non-structural carbohydrates found in grasses (30, 81, 105, 127, 128). A more complete description of the carbohydrate analysis procedure is given in section 8.12.

7.2 Results and Discussion

Figure 10 shows that the rate of regrowth of treated and untreated plants of both varieties declined and tended to reach a steady value towards the end of the experiment. This decline was most probably due to the inability of the roots and crowns to maintain the

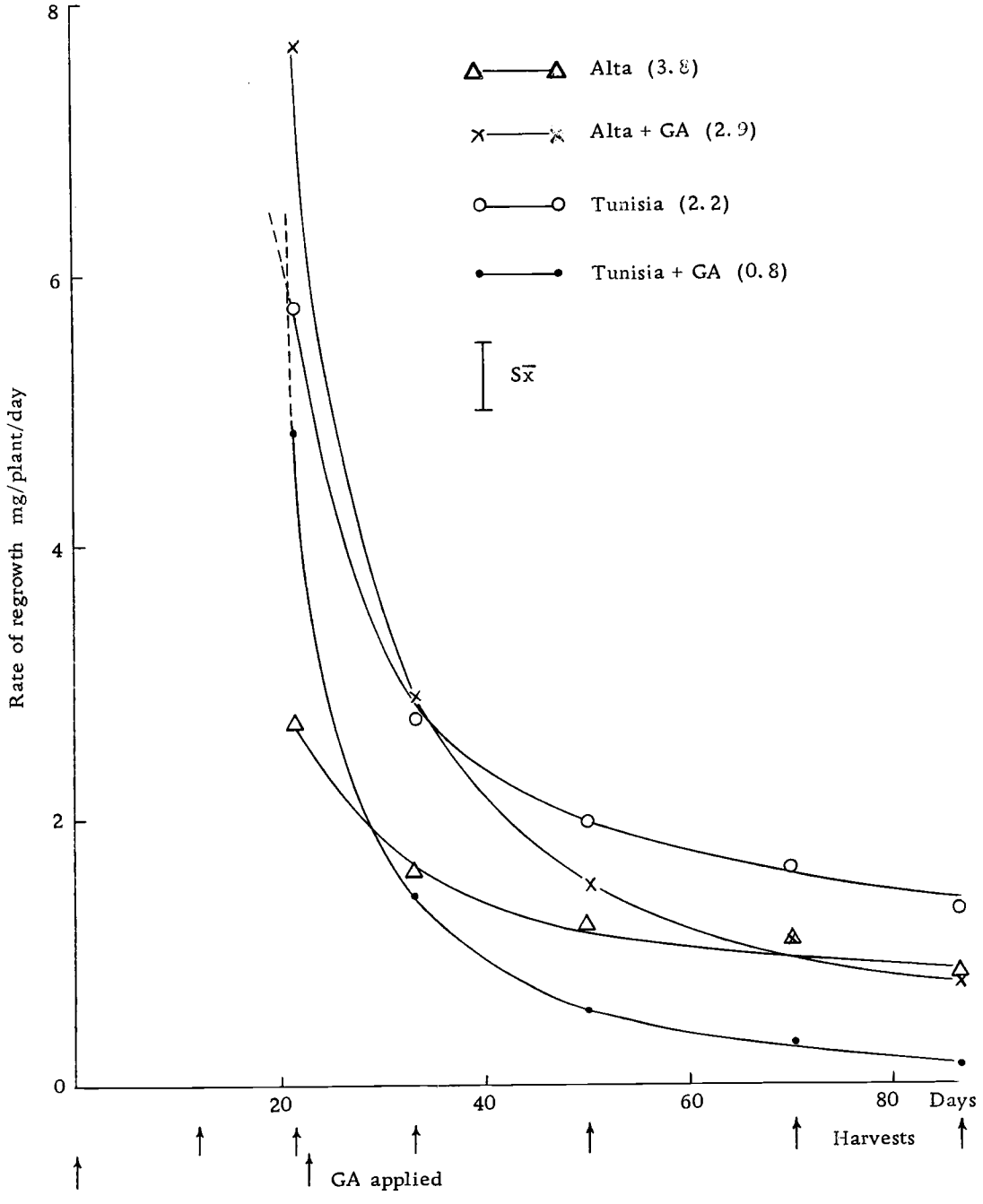

Figure 10. The influence of gibberellic acid on the regrowth of tall fescue varieties grown in a simulated winter environment. Figures in parenthesis are water soluble carbohydrates at final harvest as % of dry weight.

supply of energy and carbon skeletons necessary for regrowth in the near absence of photosynthesis. A decline in the level of carbohydrates of stems and roots following defoliation has frequently been observed and the level was not recovered until sufficient photosynthetic surface was regenerated (68, 127). However, only the regrowth from Tunisia treated with GA approached zero during the 12 weeks the experiment was in progress. The tendency towards a steady state of regrowth that was not zero may have been due to low rates of photosynthesis maintained by the small amounts of green tissue present. It was noted that plants of Tunisia treated with GA had photosynthetic tissue more completely removed at each harvest due to the more upright habit induced by GA.

It can be seen from Figure 10 that GA initially increased the rate of regrowth of Alta. Unfortunately, the initial stimulation of regrowth of Tunisia was apparently removed by the preparative clipping 11 days after the first application of GA. However, extrapolation of the graph in Figure 10 indicated that regrowth of Tunisia was also stimulated initially. The declines in the rate of regrowth, however, differed among varieties. The rate of regrowth of Tunisia treated with GA was less than the untreated plants about 20 days after the first application of GA but this change did not occur with Alta until about 70 days after the initial application.

The total regrowth over the course of the experiment is given

by the area under the rate-curves in Figure 10. It is obvious that GA promoted a greater amount of regrowth of Alta than the non-treated Alta plants. However, it is necessary to include the areas under the extrapolated curves during the first 20 days of the experiment before it could be shown that GA also stimulated a greater amount of regrowth of Tunisia. The lower amount of water soluble carbohydrates in the roots and crowns of Tunisia plants treated with GA supported the suggestion that GA promoted a greater amount of regrowth of Tunisia.

If the total amount of regrowth of both varieties of tall fescue was determined by the amount of reserve carbohydrates present at the beginning of the experiment it would be expected that the non-treated plants would eventually produce as much regrowth as the treated plants. This is suggested by the extrapolated curves for Tunisia shown in Figure 10. Gibberellic acid initially promoted a rapid rate of regrowth but the rate for the treated plants soon declined and approached zero. On the other hand, the non-treated Tunisian plants sustained a higher rate of regrowth--presumably because the reserve carbohydrates were not depleted as rapidly, and ultimately could have produced as much regrowth as the treated plants. The higher level of water-soluble carbohydrates in the non-treated plants supports this interpretation of the regrowth of Tunisia.

The regrowth of Alta did not appear to have depended on the

amount of reserve carbohydrates present when the experiment was initiated as both the treated and non-treated plants approached a common rate of regrowth that was above zero, i. e. 1 mg/plant/day. The final carbohydrate levels of treated and non-treated plants of Alta also tended to a common value which was not zero. It was suggested above that the final rate of regrowth of Alta may have been determined by a slow rate of photosynthesis of the small amount of photosynthetic tissue regenerated between harvests.

In conclusion, it appeared that GA stimulated a rapid utilization or mobilization, or both, of reserve carbohydrates of both varieties. This stimulation appeared to be more immediate with the Tunisian variety than Alta but this was uncertain because regrowth in the first 20 days after GA was applied was not measured. However, it was shown that GA had an effect other than a stimulation of photosynthesis and that promotion of regrowth utilizing reserve carbohydrates may be an important consideration in interpreting the response of pasture growth to GA in the winter. Furthermore, it was shown in Figure 4 and by Robson et al. (89), that during the winter the north European varieties of tall fescue, such as Alta, accumulated carbohydrate to a higher level than Mediterranean varieties, such as the Tunisian variety. Therefore, it may be expected that Alta would show a greater response to GA in the winter than Tunisia, and this is supported by the data shown in Figure 8. A more rapid rate of regrowth

would, in turn, ensure an earlier attainment of a favorable LAI (109).

The mode of action of GA was not established in the studies described to date. However, it was considered that a more detailed determination of the carbohydrates in plants treated with GA may reveal whether GA acts by promoting meristematic activity at low temperatures, or by mobilizing the reserve carbohydrates present in the plant. Consequently a further study in a simulated winter environment was initiated and will now be described.

8. Influence of Gibberellic Acid on Winter Growth and Carbohydrate Composition

8.1 Procedure

8.11 Plant Material

The plants used in this experiment belonged to the same group as those described in 7.1. Alta and Tunisia had been grown for 18 weeks in a warm greenhouse and then transferred to a simulated winter environment. There were 10 plants of each variety and the foliage was cut to 10 cm at the time of transfer. The controlled environment chamber was run at 6.7/3.3C with an 8 hr photoperiod of 14 klx and the radiant energy between 400 and 750 nm was 19 ly/day. After 5 weeks acclimation the foliage was clipped to 8 cm and 5 plants of each variety were sprayed with 100 ppm GA in 0.1%

Tween 20. Three weeks after the first application the plants were resprayed and then a further 3 times at intervals of 7 to 10 days. The plants were transferred to the winter chamber on March 11, 1968 and harvested July 16. At the time of harvest the chlorophyll content of the leaves was determined as described in 6.1, and the carbohydrate content as described in 8.12. Leaf area was calculated by applying the specific leaf area to the leaf weight, and leaf thickness was measured under the low power of a microscope.

8.12 Extraction and Determination of Carbohydrates

The non-structural carbohydrates found in forage plants are frequently referred to as reserve carbohydrates. There is an objection to the word "reserve" in that it signifies teleological thinking. Furthermore, it has not been established that the non-structural carbohydrates have the specific function of reserves for future top and root growth (68). Because of general acceptance, the term "reserve carbohydrates" will be used in this thesis but the above limitations to its use should be born in mind.

The gibberellins are known to influence carbohydrate metabolism. In the last few years, mechanisms of action (84) of GA have been elucidated. The most detailed studies have been concerned with the stimulation of amylase activity in barley seed. It has been shown that in the barley grain GA promoted de novo synthesis of α-amylase

by the aleurone layer (82, 83, 84, 115, 117). The amylase, in turn, diffused to the endosperm to catalyze the hydrolysis of starch to reducing sugars. The aleurone layer of the barley grain was also stimulated to synthesize proteinases which diffused to the endosperm where they activated β-amylase (54, 116). The two amylases together are required for the complete hydrolysis of starch to the reducing sugars glucose and maltose (1). The enzyme maltase is required for the final hydrolysis of the maltose residues from amylolysis and the production of maltase was also stimulated by GA (116). Paleg (84) concluded that there was evidence to show that the system found in cereal endosperm may also exist in developed plants, i. e. that GA promoted enzymatically active protein. It has also been shown that GA promoted an increase in invertase in lentil (Lens culinaris) epicotyl (98) and also in developing Avena internodes. Thus, the hydrolysis of sucrose to glucose and fructose in plant tissue was also promoted by GA. Finally, it will be recalled that the level of gibberellins in the plant are stimulated by long photoperiods (14) and hence enzymatic activity may be under seasonal control.

The non-structural carbohydrates found in grasses include reducing sugars--mainly glucose and fructose (105)--sucrose, fructosans, and starch. De Cugnac (30) was one of the first to draw attention to the two groups within Gramineae that differed in the types of carbohydrate reserves present. One group, "Graminées

saccharifères," accumulated sucrose with or without starch but no fructosans. The second group, "Graminées lévulifères," accumulated fructosans and usually sucrose but no starch. De Cugnac concluded that the grasses which stored starch were a diverse group but, in general, had their origin in warm climates. On the other hand, the group of grasses which stored fructosans were a large group and of widespread occurrence; in general they originated in cool climates and included Phalarideae, Agrostideae, Aveneae, Festuceae, and Hordeae. Weinmann and Reinhold (128) persued the matter and confirmed the two broad groups in Gramineae proposed by de Cugnac.

A separation of reducing sugars, sucrose, fructosans, and starch in dried plant tissue can be based on the differing solubilities of each in ethanol and water. Reducing sugars and sucrose are soluble in 80% ethanol whereas long-chain fructosans and starch are not. Thus, plant tissue can be extracted with ethanol and only the fructosans and starch remain. A separation of fructosans and starch is based on the solubility of fructosans in water. The remaining starch can be released from the plant tissue by enzymatic hydrolysis with takadiastase (65, 126) or amylase (19, 128), or by acid hydrolysis (19, 87, 102).

The above procedure should be considered only as a general outline since there are a number of modifications that may be necessary. Thus, 80% ethanol may not be the most suitable concentration

for separating reducing sugars and sucrose from fructosans. Smith and Groteluesch en (105) showed that short-chain fructosans are soluble in 80% ethanol and in grasses which contained short-chain fructosans, more concentrated solutions of ethanol were necessary for a satisfactory separation (42). Smith et al. (105) found that 90% ethanol was most suitable for tall fescue, ryegrass, and quackgrass (Agropyron repens) and 92.5% ethanol was required for brome grass (Bromus inermis) which contained a considerable proportion of short-chain fructosans.

The separation of fructosans and starch may also require some modification. Thus, while hot water has been used to extract fructosans (32), Smith et al. (106) showed that hot water may also extract glucosans and, consequently, cold water was preferable. Finally, the extraction of starch may also present some difficulty. For instance, hydrolysis with dilute acid (0.2N H_2SO_4) is the most rapid and simple method and may give good correlation with the more specific enzymatic methods (19, 106). However, Groteluesch en and Smith (41) showed that, whereas 0.2N H_2SO_4 extraction was satisfactory for timothy (Phleum pratense) which was low in starch (6%), there was considerably less starch extracted by 0.2N H_2SO_4 than by takadiastase from alfalfa (Medicago sativa) which stored starch (30%) as the reserve carbohydrate. Groteluesch en et al. (41) concluded, therefore, that dilute acid was a less satisfactory method than

takadiastase for extracting starch from tissue high in starch since it only partially hydrolyzed the starch. Grotelueschen et al. (41) also concluded that direct treatment of tissue with dilute acid can give a high value for non-structural carbohydrates, whether the reserve is fructosans or starch, if structural hemi-celluloses are present since the acid will also hydrolyse hemi-celluloses.

The quantitative determination of the extracted carbohydrates may be based on optical methods (30) colorimetry (99, 135) or the reducing power of the hydrolyzed sugars (47). The method I used was based on the latter using the copper-iodometric method (101) and Somogyi's "new" copper reagent (107). This "new" reagent gave a more rapid reduction of the cupric ion by the reducing sugars than I was able to obtain by Somogyi's older method, described by Heinze and Murneek (47), and used by Smith (104). The standard curve for fructose is given in Figure 11 and shows excellent linearity over the range 0 to 3 mg fructose. A standard curve for glucose was also established and was found to have a slightly larger regression coefficient than fructose--3.25 as against 3.17 for the fructose curve. The curve for glucose was close to that published by Somogyi (107). In applying the regressions to plant extracts which contained an unknown mixture of glucose and fructose a mean regression coefficient of 3.21 was used.

The method finally adopted in determining the non-structural

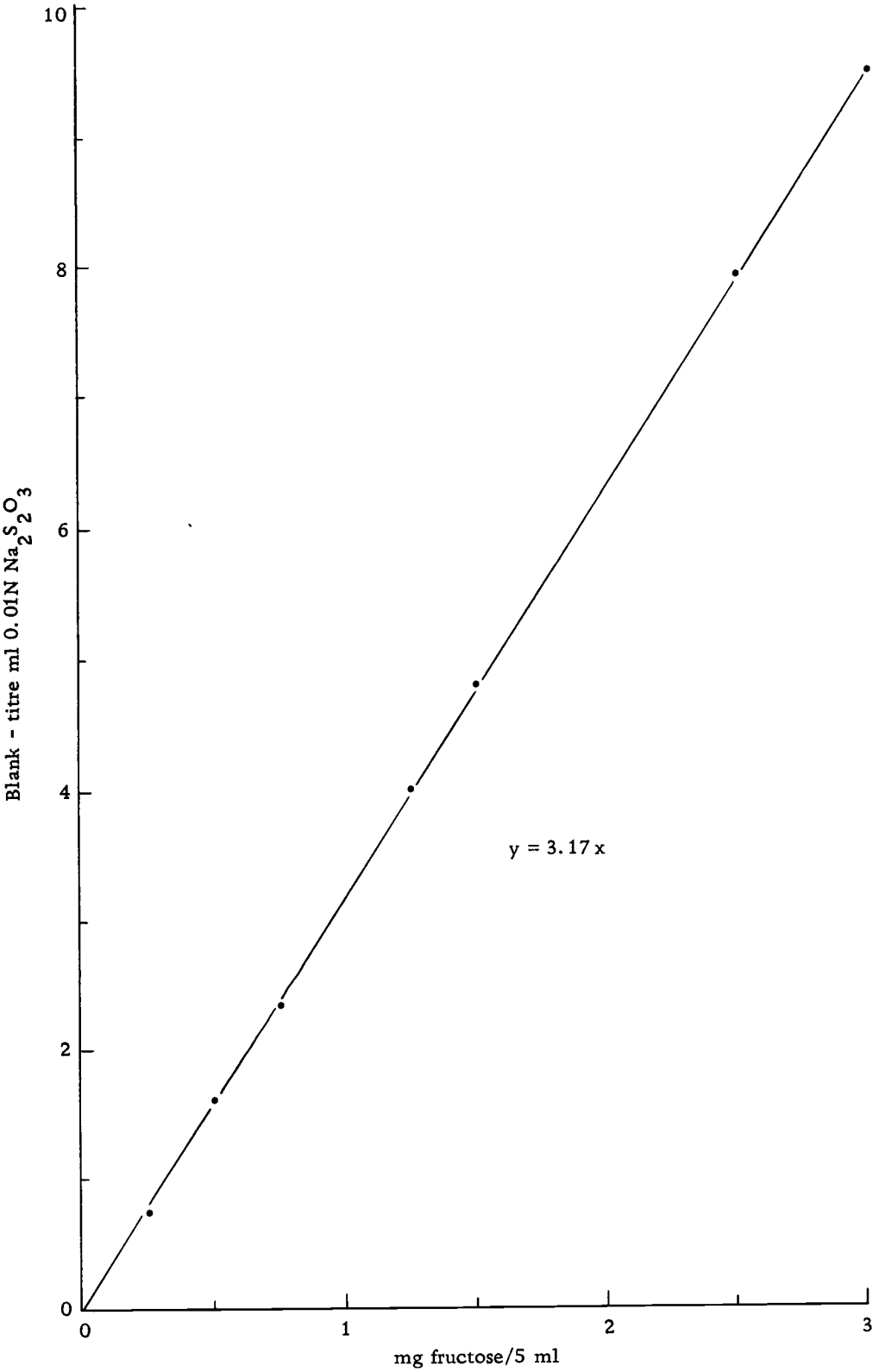

Figure 11. Standard curve for fructose by the copper-iodometric method with Somogyi's new copper reagent.

carbohydrates of the tall fescue plants in my studies was as follows:

(i) Harvested material was separated into leaves, leaf bases, and roots and dried in an oven at 100C for 1 hr, then at 70C for an additional 24 hr (86).

(ii) The dried material for each treatment was bulked together and ground in a Wiley mill to pass through a 40-mesh screen. The ground material was stored in Manila envelopes at room temperature.

(iii) Samples to be extracted were redried at 70C and 300 mg weighed out for ethanol and water extraction or 100 mg for acid digestion.

(iv) Extraction of sugars and fructosans was performed by placing the 300 mg sample together with 50 ml of the extractant in a 125 ml Florence flask, tilting the flask on its side, and shaking with an oscillating lateral motion for 1 hr. Six samples were extracted simultaneously and this enabled the leaves, stems and roots of the two varieties to be extracted simultaneously. This procedure eliminated a replications × treatment interaction, and the variance due to replication was eliminated by an analysis of variance. As a minimum, the extraction and determination of all samples was duplicated.

(v) Ninety percent ethanol was used to extract reducing sugars and fructosans (105). At the end of 1 hr the sample was filtered with a Büchner funnel using Whatman No. 42 filter paper. The alcohol of the filtrate was evaporated over a hot-plate and the volume adjusted with distilled water during evaporation. When the alcohol had been replaced with water the extract was re-filtered and made to 100 ml.

(vi) Fructosans were extracted at room temperature with distilled water (106). At the end of 1 hr shaking time the sample was filtered as described in (v) and the filtrate made to 100 ml.

(vii) The 100 mg samples for the extraction of total non-structural carbohydrates were placed in 2 × 15 cm pyrex tubes with loosely fitting, stainless steel caps. To the sample was added 10 ml 0.2N H_2SO_4 (19, 106) and the tubes were then placed in a boiling water bath for 1 hr. The tubes were shaken occasionally during the hour. At the end of the hour the tubes were cooled and the sample filtered as described in (v). The filtrate was neutralised with 2 ml N NaOH and made to 100 ml.

(viii) Determination of reducing potential.

(a) Reducing sugars: A 5 ml aliquot of the sample with 5 ml of Somogyi's reagent was heated in a 2.5 × 17 cm Pyrex culture tube with a loosely fitting stainless steel cap for 20 min in a boiling water bath. At the end of 20 min the tubes were cooled, acidified with 3 ml 2N H_2SO_4, and titrated with 0.01N $Na_2S_2O_3$. When the iodine color had almost disappeared 1 ml starch indicator was added and the titration continued until the blue color of the starch-iodine complex had disappeared. A blank of 5 ml distilled water was run concurrently and the titre of the unknown subtracted from the titre of the blank was multiplied by the reciprocal of the regression coefficient of 3.21 to obtain the mg reducing sugar present in the 5 ml aliquot. By appropriate calculations the amount of reducing sugar was expressed as a percentage of the dry weight of the plant tissue.

(b) Sucrose: A 5 ml aliquot of the ethanol extract was hydrolyzed with 1 ml 2N H_2SO_4 in a boiling water bath for 15 min. The hydrolysate was neutralised with 2 ml N NaOH and then the reducing power was determined as described in (a). Sucrose as a

percentage of plant dry weight was obtained by subtracting the weight of reducing sugars found in (a) from those found in (b).

(c) Fructosans: A 5 ml aliquot of the water extract of the original plant material was hydrolyzed with 1 ml 2N H_2SO_4 as described in (b) and the reducing power determined as in (a). The water extract contained the soluble sugars of (a) and (b) as well fructosans. Hence, (a) and (b) were subtracted from (c) to enable fructosans to be calculated as a percentage of sample dry weight.

(d) Starch: A 5 ml aliquot of (vii) was treated as in (a). The reducing potential determined was for total non-structural carbohydrate. Hence, the values obtained for (a), (b), and (c) were subtracted from (d) to give a residual value for starch.

8.2 Results and Discussion

8.21 Growth Analysis

The appearance of the plants at final harvest is shown in Figure 12, and the measurements taken are shown in Tables 2 and 3. Considering the non-treated plants first, it can be seen from Table 2

that in the simulated winter environment the Tunisian variety made more growth than Alta. This result agrees with the literature on the comparative growth of north European and Mediterranean varieties of forage grasses (26, 88, 89, 90). This greater growth occurred in the weights of roots, leaf bases, and leaves, and also in leaf area and number of tillers. The 4 to 10 fold increase in tillers of both varieties during the course of the experiment was typical of the response of grasses to short photoperiod and low temperatures. The response of tiller buds was probably due to the increased supply of substrate resulting from the slower growth without a concomittant decrease in photosynthesis (51, 71, 108).

Figure 12. The influence of gibberellic acid on the growth of tall fescue varieties grown in a simulated winter environment.

Table 2. The influence of gibberellic acid on the growth of tall fescue varieties grown in a simulated winter environment. [a, b]

	GA[c]	g DM/plant[d]					S:R	Tillers	Leaf area cm^2	LAR cm^2/g
		Root (R)	Leaf base	Leaf	Shoot (S)	Total				
Alta	-	0.8	0.9	1.0	1.9	2.7	2.3	93	287	106
	+	0.9	1.4	1.2	2.6	3.5	3.1	63	376	107
Tunisia	-	1.4	2.3	2.5	4.8	6.2	3.5	160	599	97
	+	1.7	2.4	2.4	4.8	6.5	3.0	81	554	85
LSD (P = 0.05)		0.5	0.8	0.7	1.4	2.0	0.8	30	168	12

[a] Growth chamber: 8 hr day, 14 klx, 19 ly/day in 400 to 750 nm, 6.7C; 16 hr night, 3.3C. Plants transferred from warm greenhouse at 20 weeks when both varieties had an average of 15 tillers per plant and total plant weights were similar.

[b] For the carbohydrate content of this material see Table 4; chlorophyll content Table 3.

[c] After 5 weeks acclimation sprayed with 100 ppm and 4 subsequent sprays at 1 to 3 week intervals.

[d] Harvested after 18 weeks in the growth chamber.

Table 3. The influence of gibberellic acid on the chlorophyll content and leaf characteristics of tall fescue varieties grown in a simulated winter environment. [a]

	GA[b]	Leaf chlorophyll[c]			Width[d]	Thick[e]	SLA[f]	LWR[g]
		mg/dm^2	mg/gDM	total mg	mm	mm	cm^2/g	g/g
Alta	-	5.4	16.3	16.3	3.7	0.33	302	0.35
	+	3.3	10.0	12.0	2.4	0.35	306	0.35
Tunisia	-	7.5	17.9	44.8	3.2	0.38	238	0.41
	+	3.4	7.7	18.5	2.9	0.35	230	0.37
LSD (P = 0.05)					< 0.1	NS		0.04

[a] Growth chamber: 8 hr day, 14 klx, 19 ly/day in 400 to 750 nm, 6.7C; 16 hr night, 3.3C.

[b] Five spray applications at 1 to 3 week intervals.

[c] Error of determination < 5%.

[d] At mid-section.

[e] At mid-section over the mid-rib.

[f] Specific leaf area means of 30 leaf segments 2 to 3 cm long from mid-section.

[g] Leaf weight ratio.

The LAR for Alta was greater than for Tunisia (Table 2). Robson et al. (90) found the LAR's for S170, a British variety, and the Mediterranean varieties Syn I and Syn II, were about 100 cm^2/g at 5/-3C and 18 ly/day (400 to 700 nm), with that of S170 less than that of the Mediterranean varieties. The LAR's in the experiment of Robson et al. increased to about 200 cm^2/g at 10/0C but S170 was still less than the Mediterranean varieties. Environmental conditions and varieties differed between my experiment and that of Robson et al. and must have contributed to the discrepancy between our results. However, LAR can be a misleading ratio on which to base conclusions since it is a product of SLA (specific leaf area) and LWR (leaf weight ratio). It can be seen from Table 3 that SLA determined the ranking of varieties on the basis of LAR, i.e. leaf weight/unit area of leaf was greater for Alta than Tunisia. The LWR's were also significantly different but the ranking of the varieties was the reverse of that based on LAR and SLA.

The distribution of assimilates in the varieties was measured by S:R (shoot weight to root weight ratio) and LWR. Both these ratios were higher in Tunisia than Alta and indicated that Tunisia utilized proportionately more assimilates in the production of shoot tissue compared to root tissue than did Alta. This was a decided advantage to Tunisia since, in the uncrowded situation, the plant that produced the greater amount of aerial assimilatory tissue made the greatest

growth. Needless to say, the photosynthetic efficiency of the assimilatory tissue, NAR, could not have been appreciably less for Tunisia otherwise, despite its greater leaf area, total growth would not have been greater than Alta as recorded. There is some agreement in the literature that the ability of the Mediterranean varieties to produce more assimilatory tissue than the European varieties during the winter determined the differences in growth rate rather than NAR being the controlling factor (26, 89).

Gibberellic acid failed to significantly increase the total dry weight of either variety. However, it did alter the distribution of assimilates. In Alta, the S:R was increased by GA due to a stimulated increase in shoot growth--mainly leaf bases--while the LWR of Tunisia was decreased. The response of Alta agrees with the results obtained with S170 tall fescue by Morgan (73). The reduction in LWR of Tunisia was due to an increase in root weight rather than a reduction in leaf weight. Thus, the influence of GA on the distribution of assimilates differed with the two varieties; in Alta, it enhanced the competitive ability of the stems for the common pools of metabolites (17) and in Tunisia the competitive ability of the roots was enhanced.

A possible mode of action (84) of GA was that of a true hormone, translocated (136) from the site of application to the root and intercalary meristems where it promoted cell division (43, 134) and,

directly or indirectly via auxin (84), cell elongation (84). The elongated leaf bases and narrower leaves (Table 3) resulting from a treatment of GA have frequently been observed (14, 29, 35, 61, 94, 96). Increased meristematic activity, in turn, may aid the competitive ability of a region to compete for metabolites. The difference in reaction to GA between Alta and Tunisia could then be explained by differences in translocation of GA, or differences in the sensitivity among varieties of the intercalary and root meristems. The increased demand for assimilates by the stimulated meristematic regions may have prevented the development of initiated tillers (69) and caused the reduction in tillers observed in my study (Table 2) and studies of others (35, 57). Ultimately, GA may promote an increase in NAR by promoting sinks for assimilates (77) or better light interception (73) and hence lead to a greater total growth (73).

8.22 Chlorophyll Content

The plants treated with GA were chlorotic (Figure 12). It was found that the concentration of chlorophyll in the leaves was reduced by GA whether expressed on the basis of unit area or unit dry weight (Table 3). The total chlorophyll in the leaves was also decreased. The results are not directly comparable with those in Table 1 because they relate to concentrations and totals on a shoot basis while those in Table 3 refer only to the leaves. Nevertheless, the concentrations

are rather similar and both experiments showed a decrease in chlorophyll concentrations. It is debatable whether the reduced chlorophyll concentrations would have limited photosynthesis. Hesketh (49) found that chlorophyll concentrations from 2.1 to 3.2 mg/dm^2 did not limit photosynthetic efficiency, but Gabrielsen (39) concluded that less than 4 to 5 mg chlorophyll/dm^2 was limiting at low light intensities (0.4 ly/hr). It can be concluded that reduced chlorophyll concentration and total content may not have limited photosynthesis in my experiment. This conclusion is supported by the greater total weights of the plants treated with GA.

8.23 Carbohydrate Content

The influence of GA on the carbohydrate concentrations of the leaves, stems and roots is tabulated in Table 4 and shown graphically in Figure 13. It can be seen that the concentration of total available carbohydrates (TAC) ranged from 11.2 to 27.1% of the dry weight of the plant parts. A scan of the literature indicated these were reasonable values (18, 19, 87, 89, 102, 106, 127, 133). The second order interaction was not significant and indicated that the direction and magnitude of effect of GA on the concentration of TAC in the parts of the varieties was the same for both varieties. Two of the first order interactions were significant, i.e. V × P and V × GA. The significant V × P interaction showed that the stems of Tunisia had a

Table 4. The influence of gibberellic acid on the carbohydrate content of tall fescue varieties grown in a simulated winter environment. [a]

	Plant part	GA[b]	Reducing sugars		Sucrose		Fructosan		Starch		Total	
Alta	Leaf	-	1.7	1.5	1.0	2.2	3.0	4.3	5.5	6.2	11.2	14.1
		+	1.3		3.4		5.5		6.9		17.1	
	Stem	-	1.1	1.1	1.3	3.6	6.5	8.1	8.9	9.3	17.8	21.4
		+	1.0		4.8		9.6		9.6		25.0	
	Root	-	0.9	0.7	1.0	2.9	2.7	4.1	9.6	10.0	14.2	17.6
		+	0.5		4.7		5.4		10.3		20.9	
Tunisia	Leaf	-	1.8	1.5	0.8	1.5	3.2	3.7	6.1	6.2	11.9	13.0
		+	1.2		2.2		4.2		6.6		14.2	
	Stem	-	1.3	1.4	2.3	3.1	10.1	11.4	8.2	8.7	21.9	24.5
		+	1.4		3.9		12.7		9.1		27.1	
	Root	-	1.1	1.1	0.5	1.2	2.8	3.7	10.4	10.8	14.8	16.8
		+	1.1		1.9		4.5		11.3		18.8	
V x P x GA			NS		NS		NS		NS		NS	
V x P				NS		NS		1.1[c]		0.6		1.7
Alta		-	1.2	1.1	1.1	2.7	4.1	5.5	8.0	8.5	14.4	17.7
		+	0.9		4.3		6.8		8.9		21.0	
Tunisia		-	1.4	1.3	1.2	1.9	5.4	6.2	8.2	8.6	16.1	18.1
		+	1.2		2.7		7.1		9.0		20.0	
V x GA			NS		0.7		NS		NS		1.4	
Variety				0.2		(0.2)		(0.7)		NS		NS
	Leaf		1.5		1.8		4.0		6.3		13.6	
	Stem		1.2		3.1		9.7		9.0		23.0	
	Root		0.9		2.0		3.9		10.4		17.2	
Parts			0.2		0.8		0.8		0.4		1.2	
		-	1.3		1.1		4.7		8.1		15.3	
		+	1.1		3.5		7.0		9.0		20.5	
GA			0.2		0.2		0.7		0.3		1.0	

[a] Plants transferred from warm greenhouse at 20 weeks to growth chamber with 8 hr day, 14 klx, 19 ly/day in 400 to 700 nm, 6.7C; 16 hr night at 3.3C. Results expressed as carbohydrate % of dry matter.

[b] After 5 weeks acclimation sprayed with 100 ppm and 4 subsequent sprays at 1 to 3 week intervals. Harvested after 18 weeks in the chamber.

[c] LSD (P = 0.05) Those in parentheses should be interpreted considering significant interactions.

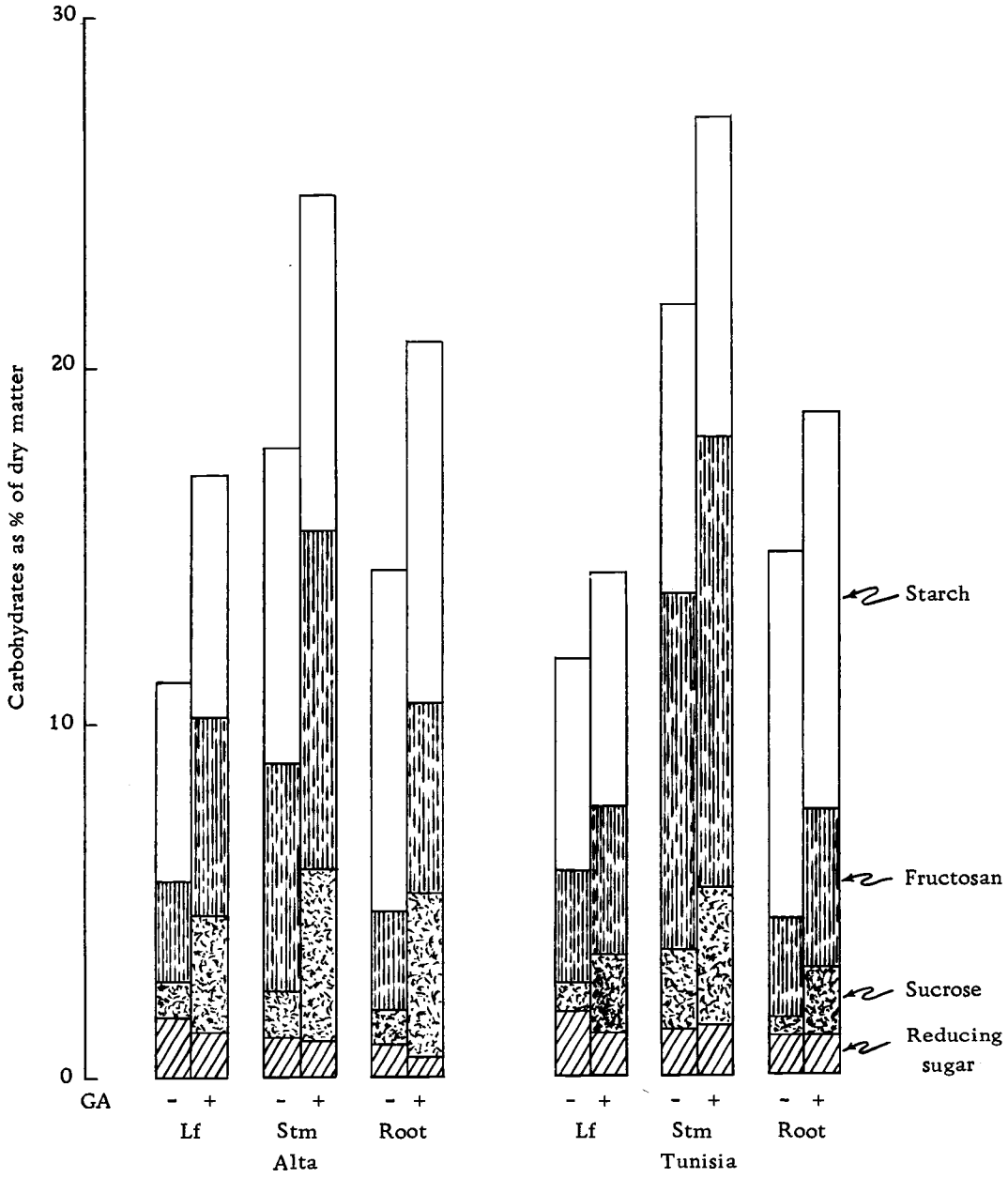

Figure 13. The influence of gibberellic acid on the carbohydrate content of tall fescue varieties grown in a simulated winter environment.

higher concentration of TAC than those of Alta. The significant V × GA interaction showed that the increase in TAC from the GA treatment was greater in Alta than Tunisia. Finally, the main effects of GA and parts were also significant. Although these main effects were involved in significant first order interactions it was considered valid to discuss them as main effects because they were highly significant and the F ratios were at least ten times that of the respective interactions.[7] The significant parts main effect showed that the stems had the highest concentration of TAC followed by roots then leaves. And the GA main effect showed that the concentration of TAC was increased by the GA treatment.

The reducing sugars contributed least to the concentration of TAC. They were always less than 2% of the dry weight and were least in the roots, greatest in the leaves, and intermediate in the stems. Tunisia had a higher concentration than Alta. Finally, the concentration of reducing sugars was decreased by GA.

Sucrose ranged from 0.5 to 4.8% of the dry weight of the parts and was highest in the stems. Gibberellic acid always increased the concentration of sucrose and the increase was greatest in Alta.

The concentration of fructosans ranged from 2.7 to 12.7% and

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[7] Personal communication with Dr. R. G. Petersen, Department of Statistics, Oregon State University.

was greatest in the stems. The significant V × P interaction showed that in relation to other parts of the plants, the concentration in the stems of Tunisia was greater than in those of Alta. Gibberellic acid significantly increased the concentration of fructosans in both varieties.

The carbohydrate fraction called starch in Table 4 ranged from 5.5 to 11.3% of the dry matter of the different parts of the plant. It was greatest in the roots, least in the leaves, and the stems contained an intermediate concentration; the significant V × P interaction indicated that, in comparison to Alta, the Tunisian variety had a lower concentration of starch in the stems but a higher concentration in the roots. Gibberellic acid significantly increased the concentration of starch.

The relatively high concentrations of starch in the vegetative parts of the plants was unexpected. Grasses originating in temperate regions have been shown to store fructosans and sucrose but little starch (30, 128). Frequently, studies of the reserve carbohydrates of temperate grasses have analysed for water soluble carbohydrates (WSC) and ignored the starch content assuming it to be a minor component of TAC (5, 18, 32, 89). Okajima and Smith (81) found only 3.2% starch in the lower 5 cm portion of the stems of tall fescue, and of a number of other temperate grasses the stem bases of timothy contained the highest concentration of starch which was 6.3%. The results shown in Table 4 are higher than the values of Okajima et al.

(81) and furthermore, the concentrations of starch in the stems shown in Table 4 are as high as those of fructosans. Okajima et al. (81) found that starch was 3.2% of the dry weight of the stem bases whereas fructosans were 13.2%. Furthermore, Smith (104) found that the concentration of starch in the roots of timothy was 0.4% and was less than any other part of the plant; the roots of tall fescue in my study contained as much as 11.3% starch and that concentration was higher than any other part of the plant.

It can be concluded, that the high concentrations of starch in the tall fescue plants of my study disagrees with the literature. This may be a valid result or it may be an artifact of the extraction procedure. The TAC values are reasonable. Furthermore, the values for reducing sugars, sucrose, and fructosans are also reasonable. Okajima et al. (81) concluded that tall fescue was an intermediate type of temperate grass in that it stored considerable concentrations of sucrose as well as fructosans--6.9 and 13.2% respectively in the stem bases at anthesis. Higher concentrations of fructosans have been found than those in Table 4 but these have been for mature plants and for stem bases, and both factors contribute to high concentrations of fructosans (81, 103, 104). The completeness of extraction of the fructosans was checked and shaking times of greater than 1 hr failed to yield additional fructosans; Smith (104) routinely uses a shaking time of 1 hr. By a process of elimination, it would appear that the

extraction of starch may be challenged per se. A full discussion of the procedure was given in 8.12. It would be desirable to extract the tissues by the more specific enzymatic methods to verify the results obtained with acid digestion. It should be added, however, that there is considerable justification for choosing the latter method.

I did not expect to find the concentration of TAC as high in Tunisia as in Alta. Results from the field in the winter, depicted in Figure 4, showed that Alta had a higher reserve of carbohydrates than Tunisia. Furthermore Robson et al. (89) have recently published results which showed that S170 tall fescue had a higher concentration of WSC during the winter than Syn I or Syn II tall fescue. A comparison of only the WSC (reducing sugars + sucrose + fructosans) shown in Table 4 and Figure 13, however, also revealed that Alta and Tunisia did not differ appreciably.

The concentration of carbohydrates found in plant tissues is a result of the difference between synthesis and utilization. Usually growth is more sensitive to limitations of the external environment than photosynthesis (17) and carbohydrates accumulate. Since Alta made less growth than Tunisia during the 18 weeks in the simulated winter environment (Table 2) it may have been expected that Alta would have accumulated a higher concentration, and perhaps total amount, of carbohydrates. However, the concentrations of TAC and WSC did not differ between varieties. Furthermore, the total amount

of these two carbohydrate fractions (Figure 14) was greater for Tunisia than Alta. It was apparent, therefore, that photosynthesis tended to maintain a stable concentration of TAC and WSC in the parts of both varieties. These stable concentrations for leaves, stems, and roots were 13.6, 23.0 and 17.2% for TAC and 7.3, 14.0, and 6.8% for WSC. The greater total amounts of TAC and WSC among varieties shown in Figure 14 was explained by the greater growth of Tunisia accompanied by increased photosynthesis that maintained the concentrations of TAC and WSC. The stable concentrations may have been limiting concentrations and prevented higher concentrations from accumulating by some negative feed-back mechanism (77). Perhaps Robson et al. (89) may have found the concentration of WSC in S170 increased to that of the Mediterranean varieties if the exposure to winter conditions had been long enough--my plants were exposed to a simulated winter of 6.7/3.3C for 18 weeks.

Gibberellic acid markedly affected the carbohydrate concentrations, amounts, and relative proportions (Figures 13, 14, 15). It has already been noted that GA decreased the concentrations of reducing sugars but increased the concentration of all other carbohydrate fractions; this effect was consistant for varieties and parts of the varieties. The total amounts of all carbohydrate fractions except reducing sugars was increased; this was mainly due to the changes in concentrations but partly due to the increases in weights

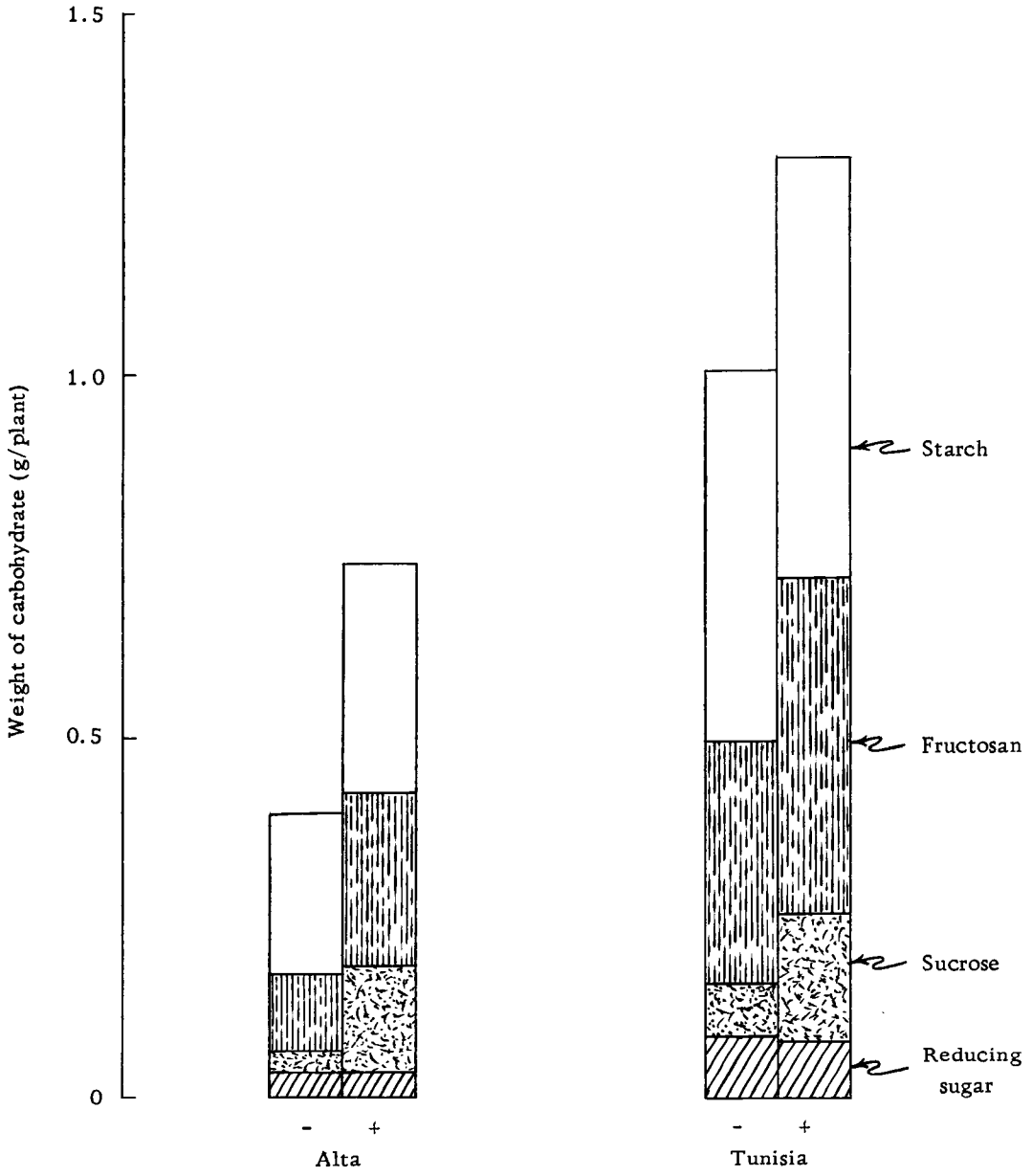

Figure 14. The influence of gibberellic acid on the amounts of carbohydrates produced by tall fescue varieties grown in a simulated winter environment.

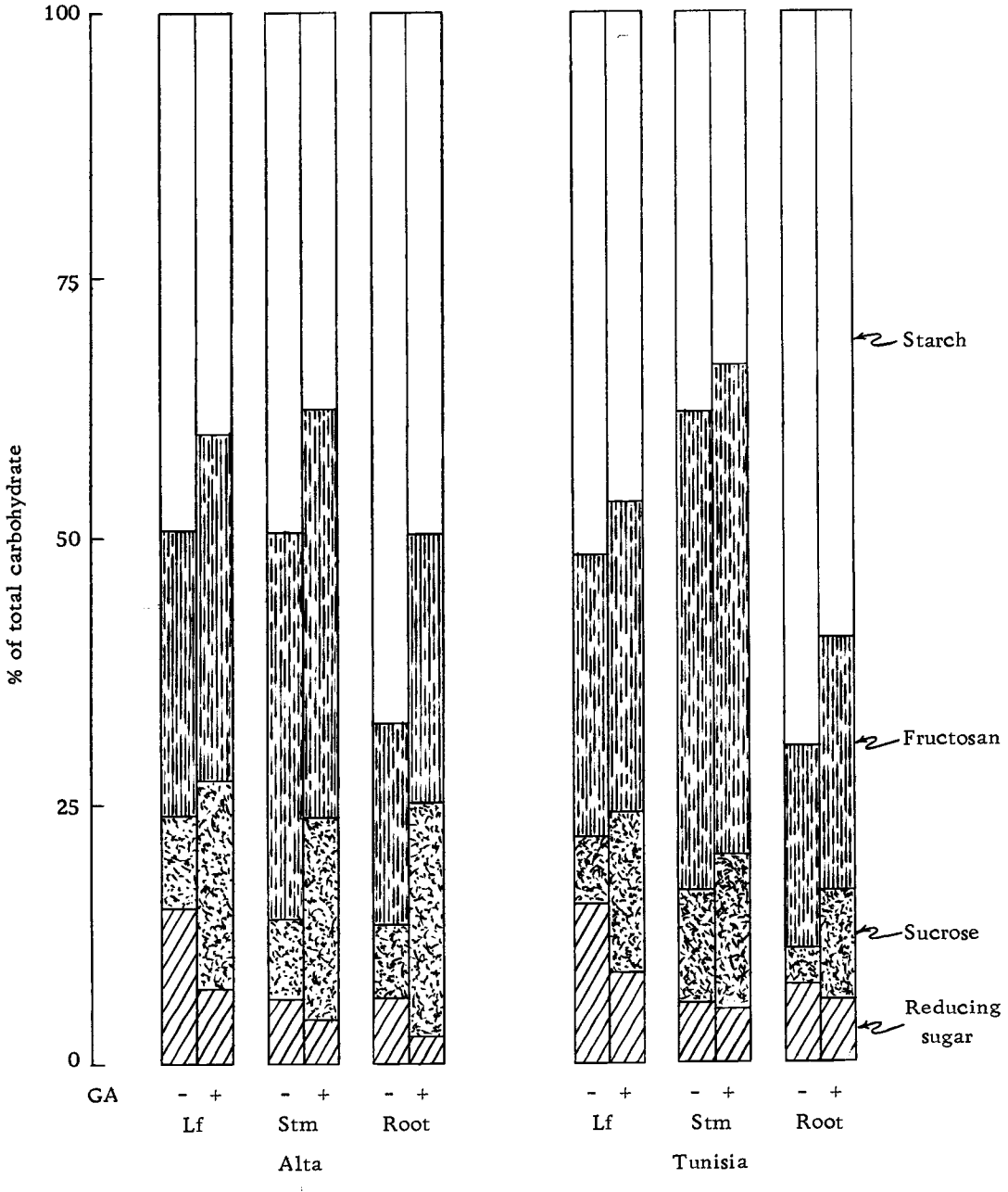

Figure 15. The influence of gibberellic acid on the carbohydrate composition of tall fescue varieties grown in a simulated winter environment.

of the plant-parts stimulated by GA (Table 2).

The relative proportions of the 4 carbohydrate fractions was also altered by GA. It was found, that GA always increased the proportion of sucrose and fructosans but decreased the proportion of starch and reducing sugars. Whether these changes were determined by the effect of GA on photosynthesis (2, 28) or its effect on the synthesis of hydrolytic enzymes (84, 116, 117) could not be established from my experiments. The increase in the less polymerized sugar, sucrose, may indicate mobilization of carbohydrate reserves by GA.

Gibberellic acid promoted growth, increases in carbohydrate concentrations, increases in total weights of the carbohydrate fractions, and changes in the relative proportions of the carbohydrates. Thus, the marked morphological changes induced by GA were accompanied by marked biochemical changes. Using the terminology of Paleg (84), modes of action of GA were the alteration of growth and carbohydrate metabolism; the mechanism, or mechanisms, of action of GA was not established. It should be noted, that Lang and Nitsan (60) showed that a mechanism of action of GA was the promotion of DNA synthesis. If the DNA, in turn, promoted synthesis of enzymatic proteins (84, 117) then the observed modes of action are likely to be varied--not only due to the action of the specific enzymes but also the inter-dependence of metabolites in the same and connected metabolic pathways (95, 114). Consequently, I can but record

the modes of action observed and merely speculate on the mechanisms.

9. Influence of Altitude on Summer Growth

I observed differences in summer growth between Alta and Tunisia (Figure 1). In order to investigate the influence of temperature and photoperiod on summer growth, plants at Corvallis in the Willamette Valley and on the top of a nearby mountain were compared. If changes in the ranking of the two varieties for summer growth at the two locations was observed it could be ascribed to causes other than photoperiod since the location in the valley and the mountain top were at the same latitude.

9.1 Procedure

Plants of Alta and Tunisia were cultured as described in section 5.1. The seed was sown October 6, 1967. On May 13, 1968 a harvest of the foliage was taken and 10 plants of each variety were transferred to the top of Mary's Peak (1249 m ASL) while 10 plants of each variety remained at Corvallis in the Willamette Valley (69 m ASL). Both Mary's Peak and Corvallis are at approximately the same latitude (44°38'N) and during the months of May and June the photoperiod averaged 15 hr. Harvests of foliar dry matter were taken June 4 and July 2, 1968.

9.2 Results and Discussion

The seasonal growth rates during the summer months is shown in Figure 16. The results showed that during May the foliar growth rate of Alta was slightly less than that of Tunisia at both Corvallis and Mary's Peak. However, during June the foliar growth rate of Alta was greater than that of Tunisia and this change in ranking of the two varieties occurred simultaneously at Corvallis and Mary's Peak.

The differences in summer growth of the two varieties was established in June at both locations. During May and June the photoperiod changed relatively little but average temperatures increased (Appendix I). Although the mean temperatures on Mary's Peak were less than Corvallis, indicated by the slower growth rates, the maximum daily temperatures may not have been ameliorated by altitude. Consequently, the plants at both locations may well have been exposed to increasingly high temperatures during May and June when the photoperiod changed relatively little. Laude (62) found that Poa scabrella required an exposure to high temperatures before summer dormancy was established. My experiment indicated that Tunisian tall fescue also required exposure to high temperatures before its growth rate declined relative to Alta. However, growth analysis experiments were established to further investigate the physiology of summer growth.

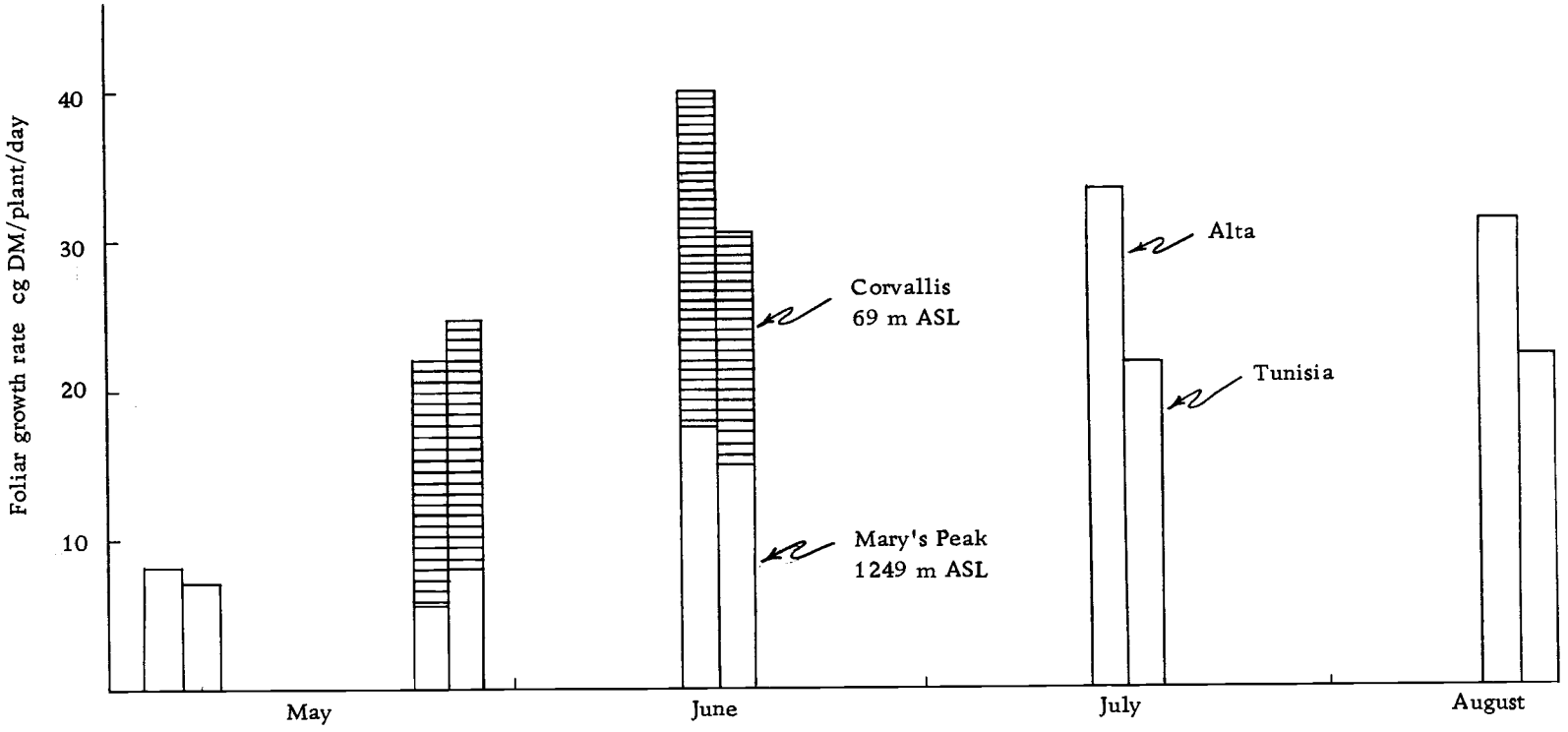

Figure 16. The summer growth rates of tall fescue varieties grown under two weather regimes with the same photoperiod (44°38'N).

10. Growth Analysis of Summer Growth in a Warm Greenhouse

In order to study the physiological basis of differences in summer growth, growth analysis was performed on plants growing in a warm greenhouse. Growth analysis can show whether differences in RGR are due to differences in photosynthetic efficiency, NAR, or to differences in the distribution of assimilates, LAR or S:R.

10.1 Procedure

Seed of Alta and Tunisia was sown on March 8, 1968 in 2.5 liter cans of "Perlite." The seedlings were thinned to 10 seedlings in each pot and nutrient solution was applied as described in section 7.1. The greenhouse was operated at an average day temperature of 32C and an average night temperature of 18C; the photoperiod was maintained for 16 hr with flourescent lights. Harvests were taken at the beginning and end of the eighth week of growth.

At harvest, leaf areas were calculated by measuring the length of the leaves and width at the mid-point of the leaf and then multiplying this width by 0.905 times the length. I checked this method of obtaining leaf areas of tall fescue by making a tracing onto graph paper of an X-ray of the leaves and agreed with Kemp (58) that it was a good approximation. After measuring the leaves, the shoots and roots were oven dried at 80C. Growth analysis was carried out as

described in II, 2 with 10 replications of each variety.

10.2 Results and Discussion

The results obtained from the growth analysis are shown in Table 5. The total weight of Alta was greater throughout the eighth week. However, the RGR was greater for Tunisia than Alta during this period. Therefore, under the high temperature and long photoperiod Tunisia recovered during the eighth week and had a higher RGR than Alta. This was an unexpected result as field studies had shown that the summer growth rates of Tunisia were less than Alta (Figures 1 and 16). In the greenhouse, the higher RGR of Tunisia during the eighth week was due to a higher NAR. The long photoperiod was the most constant aspect of the environment and it was obvious that it was not able to maintain a control over summer growth rates--a conclusion that was also drawn in section 9. A study of the temperature records did not reveal any obvious departure during the eighth week from that maintained during the preceding 7 weeks. Furthermore, the same nutrient solution was used throughout the experiment. Therefore, there was no obvious change in the environment to explain the results.

There is evidence in the literature to show that Mediterranean varieties can have a higher RGR than north European varieties at high temperatures. Eagles (31) found that a Portuguese variety of

orchard grass had a higher RGR at 30C than a Norwegian variety and this was due to a higher NAR. Eagles concluded, therefore, that the Portuguese variety actually had a higher optimum temperature for growth than the Norwegian population. And, Cooper et al. (27) concluded that the reduced rates of growth of Phalaris tuberosa were not established at 31/26C and may have required higher temperatures as shown by Laude (62). Therefore, the Tunisian variety in my study may also be genetically adapted to grow at high temperatures unless some internal control operates.

Table 5. An analysis of the growth of tall fescue varieties grown in a warm greenhouse under a 16 hr photoperiod. [a]

	Growth of 8th week		Leaf area		Shoot	LAR	NAR	RGR
	mg DM/plant		cm^2		root			
	W_1	W_2	A_1	A_2	ratio	cm^2/mg	$mg/cm^2/week$	g/g/week
Alta	258	744	53.5	127.4	3.86	0.19	5.75	1.07
Tunisia	189	717	33.5	98.5	3.45	0.16	8.88	1.32
LSD (P = 0.05)					0.41	0.02	1.55	0.17

[a] Analysis for the week of April 27, to May 4, 1968. Average temperatures of the greenhouse were 32/18C.

11. The Influence of Gibberellic Acid on Summer Growth

In order to further investigate the physiological basis for differences in summer growth, plants growing outdoors were studied by growth analysis. In addition, the influence of GA on the components

of RGR was included.

11.1 Procedure

Seed of Alta and Tunisia was sown in 2.5 liter cans of soil on April 5, 1968; three average seedlings were selected in each pot. On June 24, 1968 there were obvious differences in growth (Figure 17) and a foliar spray of 500 ppm GA in 0.1% Tween 20 was applied to 9 plants of each variety while 18 plants of each variety were left unsprayed. On June 30 a harvest of 9 untreated plants was taken. Leaf areas were computed from a factor for the specific leaf area calculated from 48 segments each about 3 cm long from the mid-section of leaves selected at random. Leaves, stems and roots were dried at 100C for 1 hr then 70C for 24 hr. This same procedure was carried out on 9 treated and 9 untreated plants of each variety on July 8, 1968. Growth analysis was then carried out for the 8 day interval by the methods described in section II, 2. The material harvested on July 8 was also analysed for carbohydrates as described in section 8.12.

11.2 Results and Discussion

11.21 Growth Analysis

The results in Table 6 show that during the week of June 30 to July 8, 1968 the RGR of Alta and Tunisia did not differ significantly.

However, the total weight of Alta was greater at this time (Table 7, Figure 17) indicating that it had made more absolute growth at some stage during the late spring-early summer than Tunisia. There was a suggestion that the RGR of Tunisia may even have been greater than Alta due to a higher NAR during the 8 day interval, however, this was not statistically significant.

Figure 17. Summer growth of tall fescue varieties at Corvallis, Oregon. Left: Alta; right: Tunisia.

Gibberellic acid increased the RGR of both varieties by increasing the NAR. Alvim (2) showed that GA increased the NAR of bean seedlings and suggested this was due to accelerated movement

Table 6. The influence of gibberellic acid on the components of growth of tall fescue varieties grown under a natural summer environment. [a]

	GA[b]	RGR	NAR	LAR		LWR		SLA	
		g/g/week	mg/cm^2/week	cm^2/g		g/g		cm^2/g[c]	
				June	July	June	July	June	July
Alta	-	0.28	2.67	100.0	113.9	0.52	0.47	193.5	241.0
	+	0.39	4.13	100.0	96.2	0.52	0.47	193.5	205.8
Tunisia	-	0.35	3.42	102.1	103.5	0.48	0.49	212.1	211.6
	+	0.56	6.07	102.1	89.3	0.48	0.45	212.1	199.9
LSD (P = 0.05)		0.12	1.46	7.9		NS			

[a]Corvallis, Oregon June 30 to July 8, 1968. Some reproductive tillers of both varieties were elongating.

[b]Foliar spray of 500 ppm on June 24, 1968.

[c]Conversion factors used in determining leaf area.

Table 7. The influence of gibberellic acid on the summer growth of tall fescue varieties. [a]

	GA	Harvest dates	
		July 8, 1968[b] g/plant	August 10, 1968[c] g/shoot
Alta	-	9.9	6.3
	+	11.1	5.9
Tunisia	-	7.0	3.9
	+	8.7	3.5
LSD (P = 0.05)			2.3
Alta		NS	
Tunis		1.7	

[a]Corvallis, Oregon. Seedlings transplanted outdoors April 18, 1968.

[b]June 24, 1968 application of 500 ppm as foliar spray; see Table 6.

[c]Growth from July 23, GA 500 ppm applied July 31.

of photosynthate from the leaves. And Coulomb et al. (28) showed a stimulation of photosynthesis of tomato plants when sprayed onto the leaves and they also suggested this stimulation was due to an indirect effect of stimulating growth and hence utilization of photosynthate. Morgan (73) showed that GA increased the NAR of S170 tall fescue during the summer but attributed this increase to the more upright habit of the treated plants and hence more effective interception of light. Growth of the tall fescue varieties in my experiment was also stimulated by GA during the 8 days from June 30 to July 8 (Table 7) and the increase in NAR may have been due to an increased utilization of photosynthate. However, growth of the same 2 varieties of tall fescue was not stimulated when applied later in the summer on July 23, 1968 (Table 7).

11.22 Carbohydrate Content

The influence of GA on the concentration of carbohydrates in the parts of the two varieties is shown in Table 8, and Figure 18. These data correspond with the growth analysis experiment detailed in Table 6.

The second order interactions of reducing sugars, starch and TAC were statistically significant. Thus, the influence of GA on the concentration of these 3 carbohydrate fractions in the parts of the plants depended on the variety. These interactions are apparent in

Table 8. The influence of gibberellic acid on the carbohydrate content of tall fescue varieties grown in a natural summer environment.

	Plant part	GA[a]	Reducing sugars		Sucrose		Fructosan		Starch		Total	
Alta	Leaf	-	1.1	1.5	2.7	2.5	3.2	3.5	6.7	6.6	13.7	14.1
		+	1.8		2.3		3.9		6.4		14.4	
	Stem	-	1.4	1.6	3.5	3.8	9.1	10.0	10.9	9.7	24.9	25.1
		+	1.7		4.1		11.0		8.5		25.3	
	Root	-	0.3	0.5	1.4	1.8	1.3	1.0	8.8	8.9	11.8	12.1
		+	0.6		2.1		0.7		9.0		12.4	
Tunisia	Leaf	-	1.8	1.7	1.0	1.5	2.6	2.5	6.2	6.0	11.6	11.7
		+	1.5		2.0		2.5		5.8		11.8	
	Stem	-	0.9	1.5	2.9	3.3	5.6	5.2	11.0	10.2	20.4	20.1
		+	2.0		3.7		4.7		9.4		19.8	
	Root	-	0.7	0.7	0.6	0.9	0.6	0.6	9.2	9.5	11.1	11.6
		+	0.6		1.1		0.5		9.8		12.0	
V x P x GA			0.6[b]		NS[c]		NS		0.6		0.5	
V x P				NS		NS		0.9		0.4		0.4
Alta		-	0.9	1.1	2.5	2.7	4.5	4.9	8.8	8.4	16.7	17.0
		+	1.3		2.8		5.2		8.0		17.4	
Tunisia		-	1.1	1.3	1.5	1.9	2.9	2.8	8.8	8.5	14.4	14.4
		+	1.4		2.3		2.6		8.3		14.5	
V x GA			NS		NS		NS		NS			(0.3)
Variety				NS		0.4		0.5		NS		0.2
	Leaf		1.5		2.0		3.0		6.3		12.8	
	Stem		1.5		3.6		7.6		9.9		22.6	
	Root		0.5		1.3		0.8		9.2		11.8	
Parts			0.4		0.5		0.7		0.3		0.3	
		-	1.0		2.0		3.7		8.8		15.6	
		+	1.4		2.6		3.9		8.1		15.9	
GA			(0.3)		0.4		NS		(0.6)		(0.2)	

[a]A foliar spray of 500 ppm was applied on June 24, 1968. Plants were harvested July 8, 1968 when a few reproductive tillers were elongating. Results are expressed as carbohydrate % of dry matter.

[b]LSD (P = 0.05). Those in parentheses should be interpreted considering significant interactions.

[c]Not statistically significant.

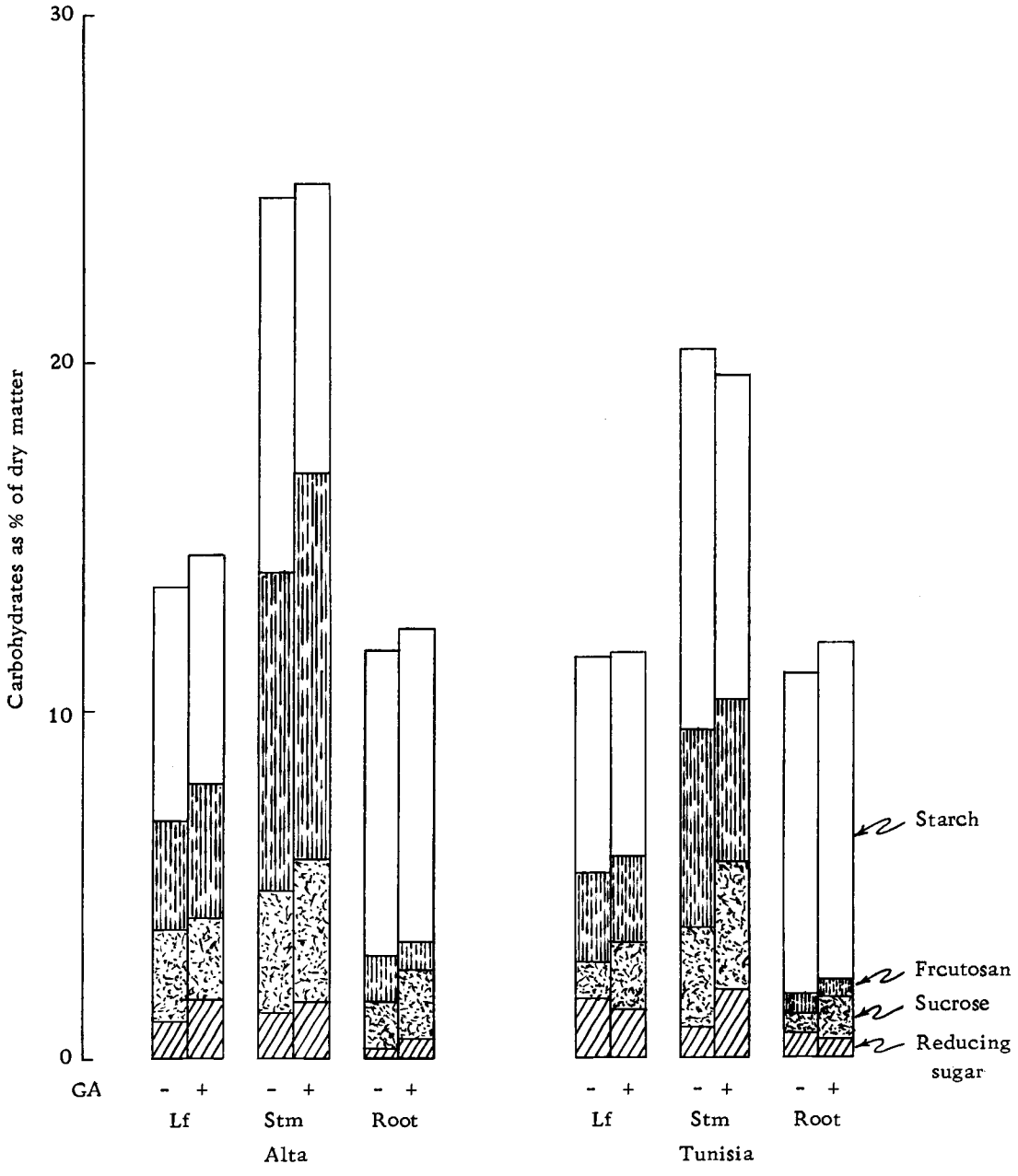

Figure 18. The influence of gibberellic acid on the carbohydrate content of tall fescue varieties grown in a natural summer environment.

Figure 18, they are small and of doubtful importance.

The concentration of reducing sugars was generally the least of all the carbohydrates analysed. The leaves and stems averaged 1.5% and the roots 0.5%. The influence of GA on the reducing sugars was slight and averaged an increase in both varieties.

The concentration of sucrose was greatest in the stems, least in the roots, and leaves contained an intermediate amount. Gibberellic acid stimulated a slight increase in sucrose and the increase did not differ significantly between varieties.

The stems of Alta contained double the concentration of fructosans than those of Tunisia. The concentration of fructosans in the leaves was less than that in the stem, and the roots contained the least concentration; the leaves of Alta had a higher concentration of fructosans than leaves of Tunisia. Gibberellic acid had no significant effect on the concentration of fructosans.

As in the analysis of plants grown in a simulated winter environment (section 8.23), the plants grown under a natural summer environment had a surprisingly high concentration of starch in their tissues. This may have been an artifact of the extraction procedure as discussed in section 8.23. However, it should again be noted that the concentrations of TAC are not unreasonable. The concentrations of starch were generally larger than the total WSC and this does not agree with the literature (30, 81, 128). Until the concentrations of

TAC are checked by enzymatic extraction (65, 126) it may be advisable to place more reliance on the total WSC rather than TAC.

It can be seen from Figure 18 that the concentration of total WSC was increased by GA in all parts of the plants of both varieties; it was higher in all parts of Alta than Tunisia. Table 6 showed that Tunisia had a higher RGR than Alta due to a higher NAR. Furthermore, GA increased the RGR of both varieties by increasing the NAR. It is generally believed that plants with slower growth rates show a higher accumulation of carbohydrates (18, 89). My study of Alta and Tunisia agreed with this. However, studies reported in Figure 1 and Table 6 showed that Alta usually makes more growth during the summer than Tunisia.

It would appear from Table 6 and Figure 18 that, at least for short periods of time, Tunisia can have a higher RGR than Alta during the summer. Eagles (31) and Cooper et al. (27) have shown that, unless dormancy is induced, Mediterranean varieties may have higher growth rates at high temperatures than north European varieties; this conclusion was supported by the experiment reported in Table 5. Therefore, it is possible that during brief periods of cool weather at Corvallis, Tunisia can have a higher RGR than Alta during the summer--this may not be apparent in pasture growth as a variety may have a higher RGR but a lower total production, and the interval between harvests of a pasture may be long enough to enable

a transient superiority in the RGR of Tunisia to be reversed. It can be seen from Table 7 and Figure 19 that although Alta had a lower RGR than Tunisia the total weight of dry matter and WSC or TAC was greater in Alta than Tunisia; the greater weight of WSC and TAC was due to the greater concentration and the greater total weight of Alta.

Obviously, further investigations are necessary before a general statement can be made on the influence of GA on the summer growth of tall fescue varieties. A variety can only be expected to produce dry matter to the limit of its genetic capacity and the limitations of the external physical environment. The growth and development of ecotypes well adapted to a particular environment is likely to be well coordinated with the physical environment. This coordination we believe to be due to the internal growth regulator balance which, in turn, is coupled to changes in the external environment (79). When an ecotype, or the variety derived from it, is transferred to an environment to which it is not genetically adapted the possibility exists that the growth regulator balance may no longer be optimal for growth. Hence, the plant may respond to an application of a growth regulator but it does not necessarily follow that the plant will respond to that growth regulator at any time of the year. Thus, the inconsistent results shown in Table 7 may be an indication that time of application of GA was important in promoting summer growth.

It should be noted in passing, that plants in a greenhouse at

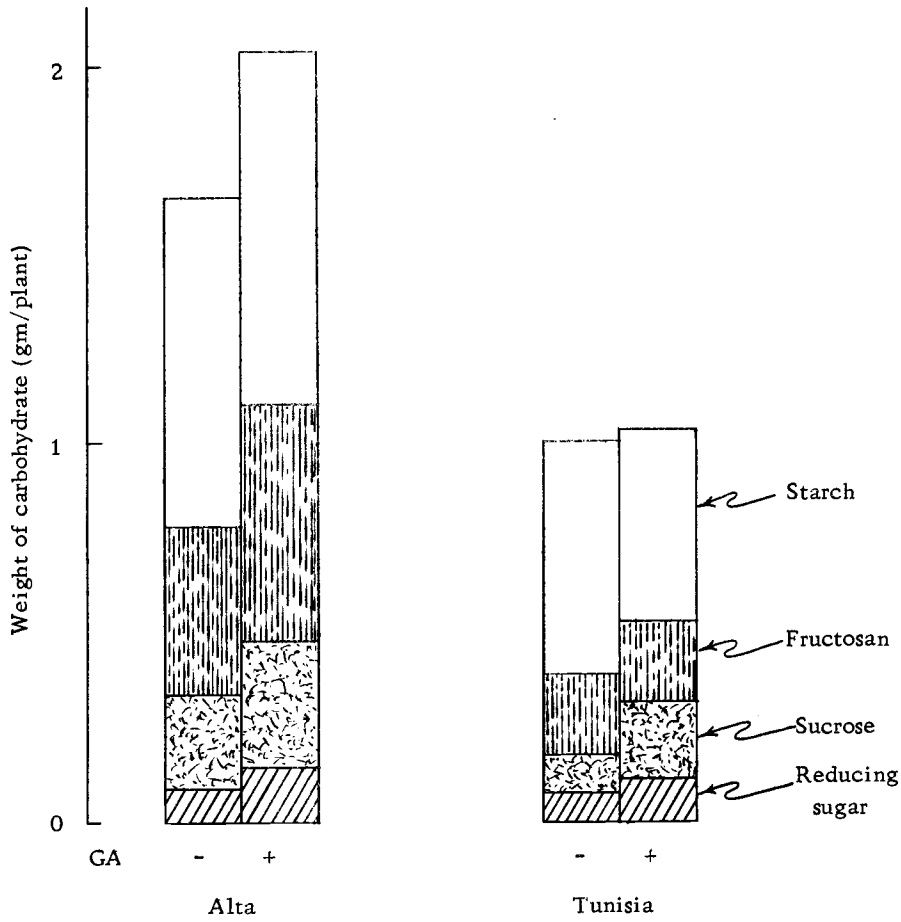

Figure 19. The influence of gibberellic acid on the amounts of carbohydrates produced by tall fescue varieties grown in a natural summer environment.

31/18C and a 16 hr photoperiod also failed to produce an increase in foliar dry matter although stem elongation was promoted. Furthermore IAA failed to cause any change in summer growth in the warm greenhouse. A cytokinin, N-6-benzyladenine, also failed to consistently increase summer growth in the greenhouse but there was a suggestion that dormant plants responded and further study with a cytokinin may prove informative. Sankhla and Sankhla (93) showed that the inhibition of seed germination and seedling growth of lettuce by abscisic acid was reversed by kinetin. And Arnold, Bennett and Williams (3) showed that kinetin and naphthalene acetic acid (NAA) had a synergistic effect on the promotion of winter growth of pasture by GA. Arnold et al. (3) also found that the time of application and the existing growth rates were important in obtaining a response to GA--an actively growing pasture responded the least to GA. Thus, there is evidence in the literature that the adjustment of the growth regulator balance of a plant may require more than the mere application of one growth regulator ad libitum. Further studies in the field with temperature and radiation adequately monitored, and in controlled environment chambers are needed.

12. Photosynthesis by Chloroplasts and Leaf Segments

These studies were largely unsuccessful due to the obdurate nature of tall fescue. My observations are recorded here for future

reference.

12.1 Chloroplasts

A purpose in studying the isolated chloroplasts was to determine whether the organelles from tall fescue varieties when removed from possible restrictions within the plant (77) and placed in an "optimum" chemical environment differed in their seasonal activity of the Hill reaction and photophosphorylation. A further study envisaged with the chloroplasts was the effect of GA at the organelle level.

A procedure used to obtain active chloroplasts from spinach (Spinacea oleracea) was not successful in isolating active chloroplasts from tall fescue. Ultimately, some encouraging results were obtained using soluble polyvinylpyrrolidone (PVP 40). Loomis and Battaile (66) reviewed the use of PVP in isolating enzymes and organelles. Polyvinylpyrrolidone is capable of forming complexes by hydrogen-bonding with phenolic compounds, and possibly other inhibitors that are released from the vacuole during isolation. No "browning" reactions were obvious during the isolation of tall fescue chloroplasts and it is possible that the beneficial effect of PVP was due to hydrogen-bonding with inhibitors.

12.11 Procedure

A successful isolation is described:

Leaf tissue from tall fescue growing actively in the greenhouse was used. Two lots of leaves each of 2.5 g were ground in a pestle and mortar with 20 ml basal medium. The basal medium consisted of 0.4M sucrose, 0.01M NaCl, 100mM ascorbate, and 50mM Tris buffer pH 8.1 at 25C. One 2.5 g lot was ground with 2.5 g soluble PVP 40 and the other lot was ground in the basal medium alone. Preparative work was carried out in an ice bath in a cold room at 4C. The chloroplast-pellet, obtained by centrifugation between 500 and 1000 × g in a refrigerated centrifuge, was suspended in 10 ml basal medium without ascorbate. Warburg flasks were set-up to contain the following:

Main compartment: 2 ml H_2O, 1 ml chloroplast suspension.

Sidearm: 1 ml 24mM $K_3Fe(CN)_6$

This gave a desirable chlorophyll concentration of 0.025 mg in each flask (23).

12.12 Results and Discussion

The results in Figure 20 show that isolation in the presence of soluble PVP greatly enhanced the activity and longevity of the chloroplasts. The rates of O_2 evolution were 346 and 199 µl/mg chlorophyll/hr for the chloroplasts isolated in the presence and absence of PVP respectively. Furthermore, oxygen evolution

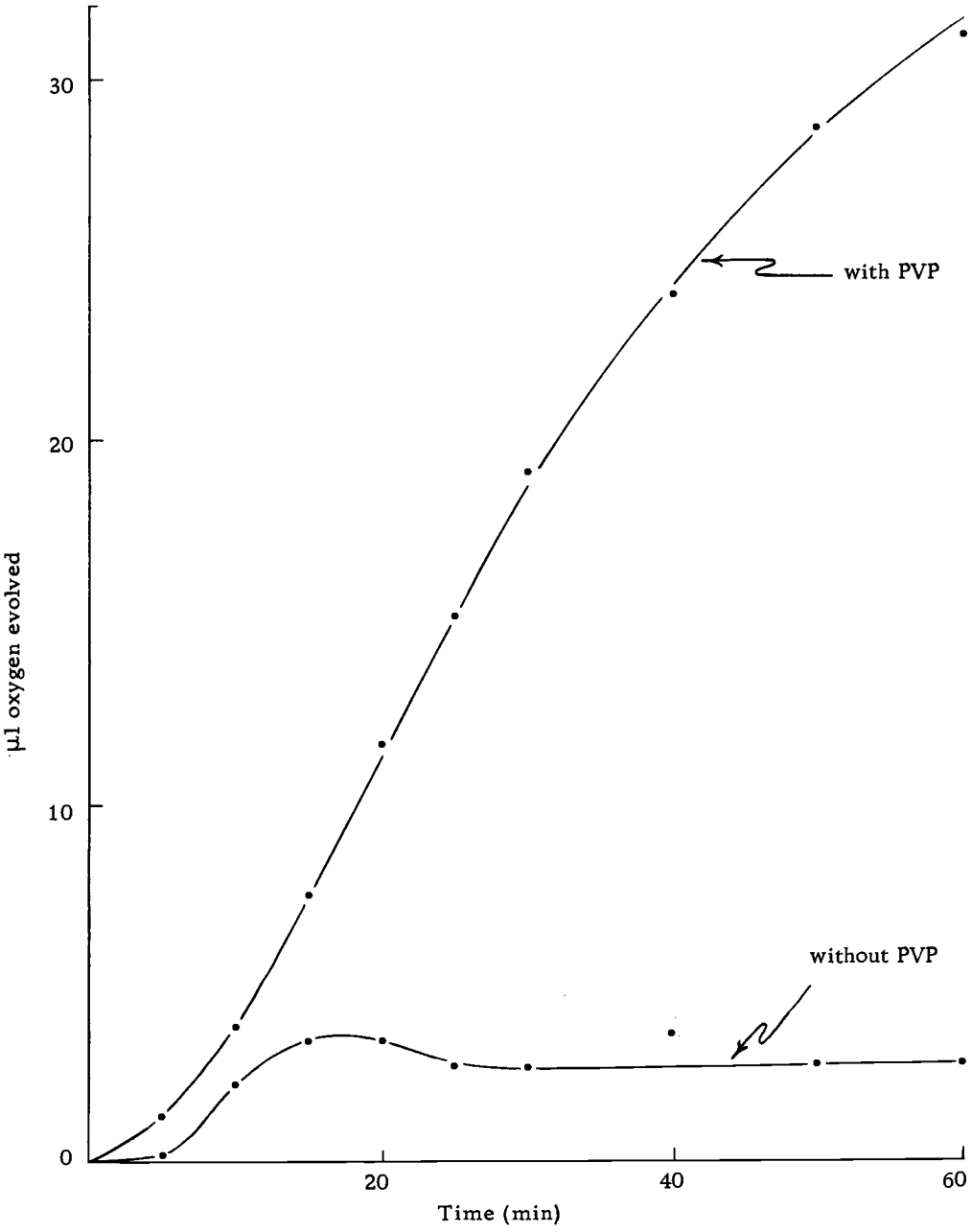

Figure 20. The influence of isolation in the presence of soluble PVP on the performance of the Hill reaction by tall fescue chloroplasts.

occurred for only about 10 min by chloroplasts isolated in the absence of PVP but continued for an hour by the chloroplasts isolated in the presence of PVP with little tendency for the rate to decline over that time. By comparison, I obtained rates of O_2 evolution by spinach chloroplasts of 248 or 417 μl O_2/mg chlorophyll/hr with $K_3Fe(CN)_6$ and benzoquinone as the electron acceptor respectively. And Clendenning et al. (23) obtained a rate of 350 μl O_2/mg chlorophyll/hr for spinach chloroplasts isolated in 0.5M sucrose using benzoquinone as the electron acceptor.

Unfortunately, the respectable activity of the tall fescue chloroplasts was not reproducible. Some isolations yielded inactive chloroplasts and it was considered essential in a study comparing varieties with differing growth rates to be fully confident of the isolation procedure. Consequently, this phase began to develop as a major part of my research, which was not intended, and was, therefore, discontinued.

12.2 Leaf Segments

The purpose of this part of the study was to measure photosynthesis manometrically with a Gilson Differential Respirometer that was adapted to provide a variable source of red light to the flasks. Warburg flasks with a constant CO_2 atmosphere maintained with a carbonate:bicarbonate buffer could be useful in comparative studies of:

(i) Net photosynthesis at different temperatures and light intensities.

(ii) Photosynthetic efficiency by establishing the slopes of the curves of photosynthesis at low light intensity (49).

(iii) The influence of GA on net photosynthesis.

Tall fescue was unsuitable for this type of study. The leaf segments gave erratic rates of photosynthesis which were not always quantitatively related to the area of the particular leaf in the flask. The anatomy of tall fescue leaves was studied and the stomata were found to be sunken and confined to bottom of the trough of the prominent ridges on the adaxial surface. It was concluded, therefore, that the still air boundary above the sunken stomata was a major resistance to gaseous diffusion (121) and probably contributed to the unsuitability of segments of tall fescue leaves to studies of photosynthesis in still air. It should be recorded, that manometric measurement has been used successfully. Homann and Schmid (53) found the method suitable for tobacco (Nicotiana tabaccum), and Fuess and Tesar (38) found it suitable for alfalfa. Treharne et al. (111) found for orchardgrass a correlation coefficient of 0.85 between net photosynthesis determined manometrically and by $C^{14}O_2$ uptake.

It would appear that the remaining method to measure photosynthesis of tall fescue leaves was in a moving stream of air monitoring changes in CO_2 with an infrared gas analyzer. This method was

evaluated[8] and found to be satisfactory but it was difficult to coordinate my studies with the availability of the gas analyzer. It was at this stage that I resorted to conventional growth analysis (sections 8.21, 10 and 11.21).

[8] The unit was made available by Dr. W. K. Ferrell, School of Forestry, Oregon State University.

IV. CONCLUSIONS

This thesis should be concluded by returning to the concepts annunciated in the introduction. The concept of an ecotype provided the rationale for this thesis and the findings can be interpreted on that basis.

The varieties of north European and Mediterranean origin were found to differ in seasonal growth when grown at Corvallis, Oregon. The seasons of retarded growth in Oregon coincided with the seasons of environmental stress in the regions where the varieties originated. Although the ecotypic variation in forage grasses has been studied during the past decade (25), at the time my study was initiated there was little published information on the physiology of seasonal growth of tall fescue ecotypes (20, 72). In addition, investigations of the role of growth regulator balance in determining differences in seasonal growth had not been published.

Recent papers by Robson et al. (88, 89, 90) have added to our understanding of the physiology of growth of tall fescue varieties under winter conditions. And a recent paper by Morgan (73) showed a response of a British variety of tall fescue to GA during the summer. However, the implicit assumption of most studies to date is that growth rates of forage grasses is determined by the prevailing weather. This is particularly so with winter growth where true

dormancy may not be involved. But even in studies of summer growth where true dormancy may be involved (62) dormancy has not been induced (27, 31) and growth has, again, been analysed assuming it was determined by the prevailing weather. On the other hand, my studies showed that winter and summer growth was increased by GA and seasonal growth rates were not, therefore, determined only by the prevailing weather. The role of the growth regulator balance in determining the seasonal growth of woody perennials is well appreciated (79) and it would appear that these concepts should be recognized when studying the seasonal growth of forage varieties.

Differences in the growth of tall fescue varieties were due to differences in photosynthetic efficiency and differences in the utilization of photosynthate for the production of leaves. An ecotype in the environment of its origin may have a growth regulator balance well coordinated with the climate such that its growth rate is determined by the weather. However, when introduced to a new environment, or used as a basis for a new variety, the production and utilization of photosynthate may be controlled by an internal growth regulator balance which is not well coordinated with the weather. In such a circumstance the possibility exists that the growth regulator balance may be adjusted by a timely application of the appropriate growth regulators in order that the variety may grow to the limit of its genetic capacity during periods of favorable weather.

Gibberellic acid promoted the utilization of carbohydrate

reserves and a stimulation of photosynthesis under both winter and summer conditions. It is tempting to suggest that a de-repression of genes by GA promoted de novo synthesis of hydrolytic enzymes (116) and hence mobilization of reserves for growth. In turn, the increased utilization of reserves may have removed an end-product inhibition of photosynthesis (77) and photosynthesis was, consequently, stimulated indirectly by GA.

Studies that have shown a stimulation of the winter production of pastures with GA have generally recorded a decrease in production the second or third cuttings after application (35, 36, 74, 94, 97, 131)--a transfer of foliar production rather than an increase in total foliar production. It is not always appreciated that regrowth may be an essential feature of winter pasture production. My studies showed that the rate of regrowth of the Mediterranean variety in a simulated winter environment was greater than that of the variety of north European origin; this difference was largely overcome by GA. The ultimate decline in growth of plants treated with GA was probably due to the more rapid depletion of carbohydrate reserves. It may be predicted, therefore, that an application of GA to a slowly growing variety that had accumulated high amounts of carbohydrate need not necessarily be followed by a decline in growth. This prediction is supported by a recent study of Arnold et al. (3) who found that the correct timing of an application of GA may avoid a subsequent decline

in production, and that the response to GA was greatest from pastures which had the slowest growth rate.

Reference to the literature suggested that the low chlorophyll concentration of the tall fescue plants treated with GA may not be a serious limitation to NAR. Furthermore, a study outdoors during the winter showed that although the chlorophyll concentration was reduced, the total chlorophyll per plant was not reduced. And, as pasture production has been shown to be a function of the total chlorophyll per unit area of land (14, 16) pasture yield may not be reduced due to the chlorosis. Wittwer et al. (132) found that chlorosis was avoided if the treated plants were heavily fertilized. If chlorosis can not be avoided then the palatability and nutritional value of the chlorotic forage should be considered. To this end, Bidiscombe, Scurfield and Arnold (7) found that animal production was increased during the winter period when the pasture was increased by GA.

The aim of a forage breeder is, to a certain extent, a duplication of what nature has done in the evolution of an ecotype--the development of a high yielding variety well adapted to the local environment. But the plant breeder can not measure time on the same scale as nature. The introduction of genetic variability in the form of ecotypes has been a valuable aid to the plant breeder and the producer of forage. The contribution of the plant physiologist may be to show how the ecotype can be manipulated in a new environment during the

period when the plant breeder is incorporating the genetic material into a new variety well adapted to the local environment. This thesis is, hopefully, a contribution to that end.

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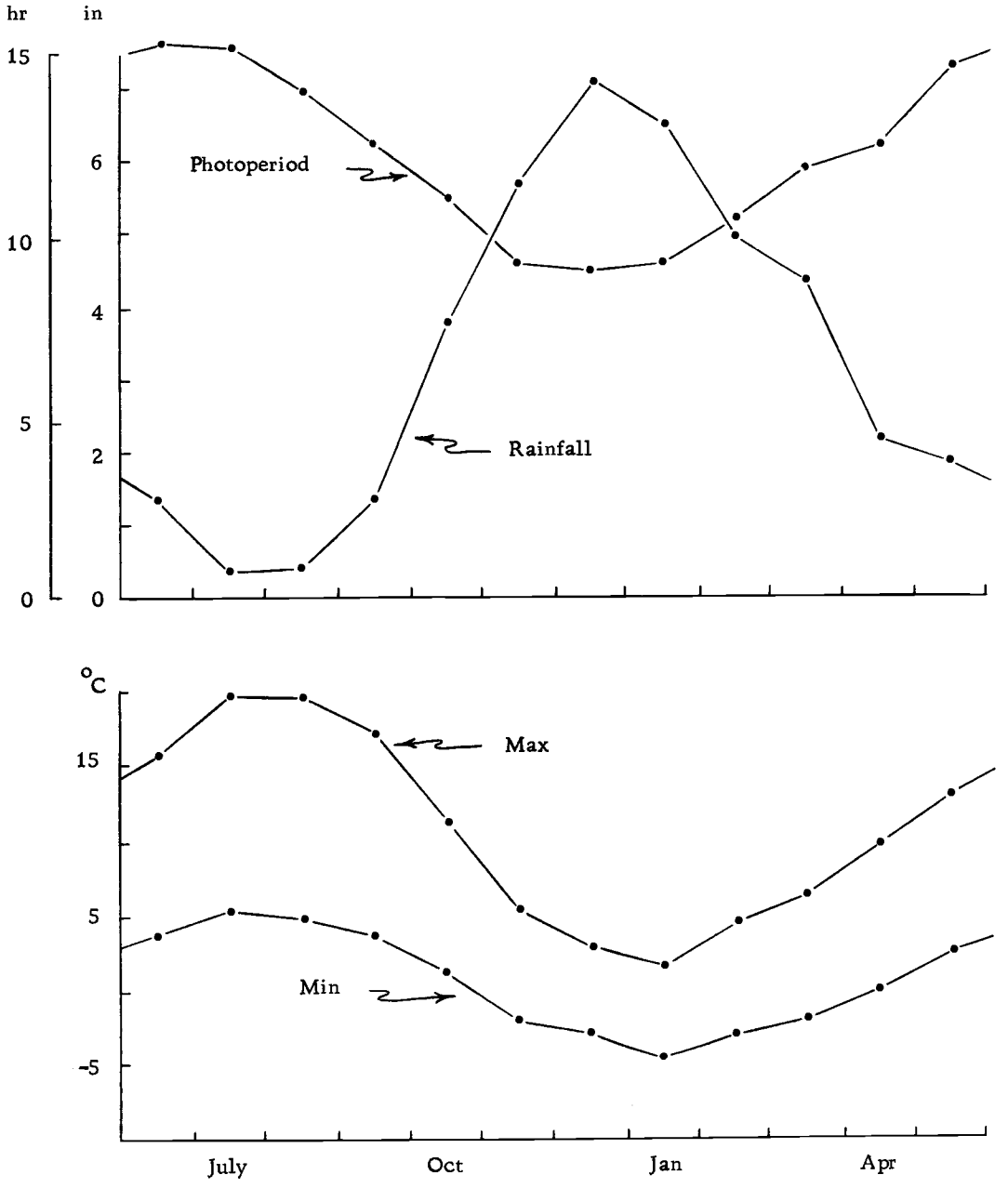
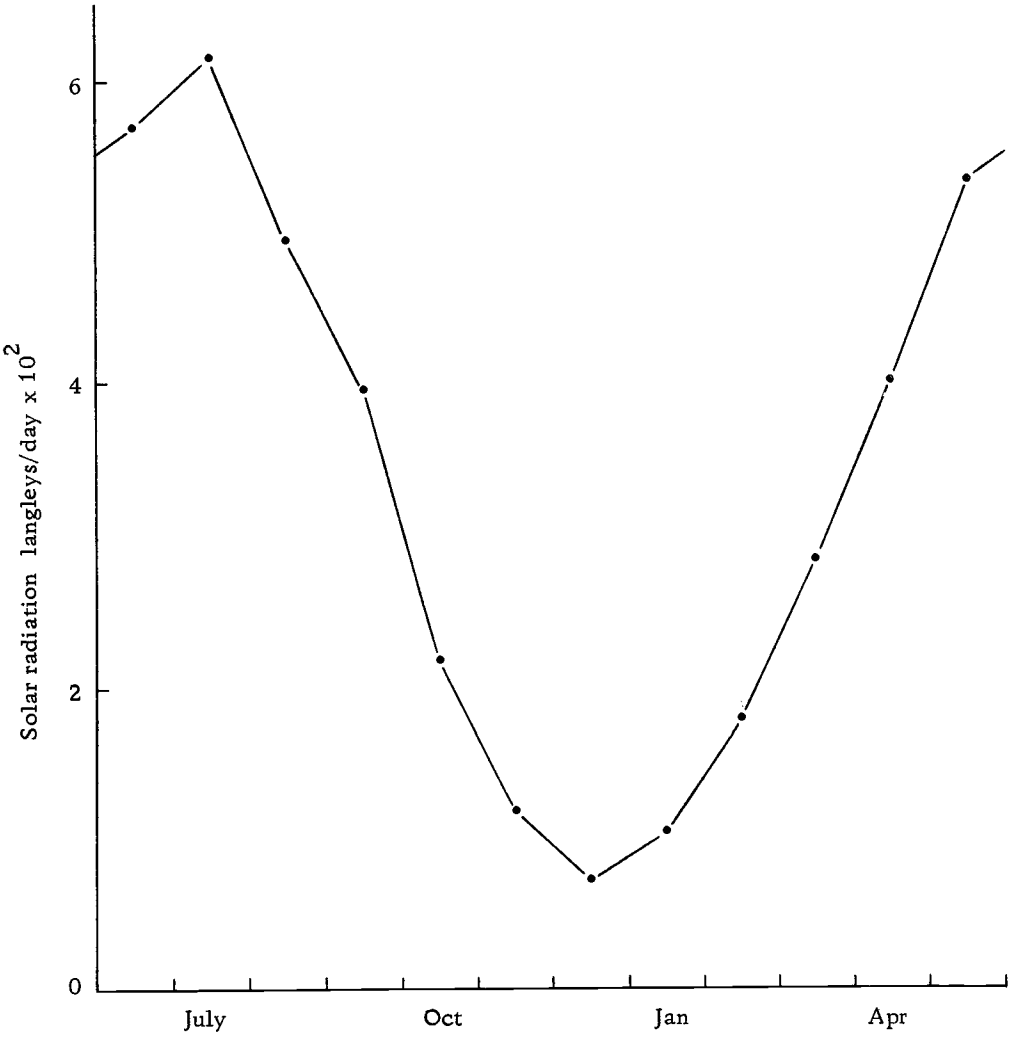
APPENDIX



Appendix Figure 1. The climate of Corvallis, Oregon. Upper: Monthly averages of photoperiod and rainfall. Lower: Monthly averages of maximum and minimum temperatures. (From: Agricultural Experimental Station, Oregon State University. Special Report No. 193, 1965. 30 p.)



Appendix Figure 2. Solar radiation received at Corvallis, Oregon. Measured with an Eppley pyranometer and includes direct and diffuse solar radiation between 350 and 2000 nm. Data is the average for 1961 through 1968 and were supplied by Mr. R. M. Black, Advisory Meteorologist, U. S. Weather Bureau, Corvallis, Oregon.