Leaf growth and development are dynamic processes which vary in response to the external environment and to internal factors such as plant size and activity (20, 9). In the sugarbeet these processes are unconstrained by reproductive activities during the first year of growth and leaves are produced throughout ontogeny against a background of increasing plant size. A progressively more massive root sink is an important aspect affecting internal status of the plant.

Integration of leaf growth processes within whole-plant activities was reviewed in detail by Wetmore and Wardlaw (31). Although some of the events are controlled in part by plant hormones, it will be useful here to emphasize nutritional aspects of the problem.

Hussey (10, 11) showed with tomato that leaf initiation rate was relatively constant during ontogeny, but if old leaves were removed, the initiation rate increased and the apex enlarged. He suggested that subapical nutrition was normally limiting to leaf initiation and early growth. Wilson (32) came to a similar conclusion regarding leaf growth after removing the apical meristem of cucumber. With sugarbeet, Das Gupta (6) found that the growth of young leaves was greatly enhanced by removing the apex and the more juvenile unfolding leaves, an observation also made by Loomis and Brookings (unpublished).
Of particular interest is the progression of increasing and then decreasing mature sizes shown by the first 20 to 25 leaves of the sugarbeet. We (13, 14) were able to duplicate this progression with a simulation model based on nutritional control of leaf cell division and expansion. Allsop (1) concludes in his review that such sequences are dependent upon carbohydrate nutritional status and associated changes in the apical meristem; he found no need to postulate that specific juvenile hormones were involved. Ashby (2) and Ashby and Wangerman (3, 4) came to a somewhat similar view after establishing correlations between cell size, mature leaf size, and the number of less mature, competing leaves.

Nutritional status also is strongly affected by the general environment. Conditions favoring photosynthesis relative to growth sink activity will enhance carbohydrate supply and thus alter growth balances (15). Such hypotheses can be used in the interpretation of leaf growth responses to temperature and radiation (16, 18, 21, 26, 29). Leaf growth responses to moisture (24, 25) and nutrient stresses (12, 17, 27) can be analyzed in a similar approach — using nitrogen deficiency, for example — by considering separate limiting nutritional pools of nitrogen and carbon assimilates.

The simulations of juvenile leaf sequences in sugarbeet with a dynamic model based on assimilate limits to growth illustrated importance of variations in leaf initiation rate and leaf morphology to the integration of shoot and storage root activities. But an effort by Loomis, Ng and Hung (13) to simulate responses to variations in plant density failed, in part because that "computer genotype" failed to duplicate the developmental and environmental plasticity of real plants. A better understanding of the physiological controls is needed. The experiment reported here involves a field study of this issue. Emphasis was given to characterization of the responses to light-limited status imposed by variations in plant density.
MATERIALS AND METHODS

The growth and development of sugarbeet leaves as influenced by planting density were investigated in a field experiment conducted at Davis, California, during the summer months of 1974 with a high-performing commercial variety, US H10B. Four density treatments with equidistant (hexagonal) spacing patterns were established. This was accomplished by varying between-row and within-row spacing (Table 1). An excess of seeds, pretreated with PCNB, Dexon, and Lindane, was planted in rows in dry soil with the effective date of planting taken from the first irrigation on 20 May. When the plants had reached the 8- to 12-leaf stage (14-21 June), the rows were thinned carefully to single plants to establish the hexagonal pattern. The treatments were arranged replicated four times in a Latin square design with appropriate borders. Plots were 7.6 cm wide and contained 240 spaced plants. Plot length varied accordingly.

Table 1. -- Space relations used to achieve four plant densities after thinning. Alternate rows were offset to achieve a triangular relationship among adjacent plants.

<table>
<thead>
<tr>
<th>Plant density</th>
<th>Spacing between plants</th>
<th>Within rows</th>
<th>Between rows</th>
</tr>
</thead>
<tbody>
<tr>
<td>pL m⁻²</td>
<td>pL ha⁻¹</td>
<td>cm</td>
<td>cm</td>
</tr>
<tr>
<td>24</td>
<td>240,000</td>
<td>22</td>
<td>18</td>
</tr>
<tr>
<td>12</td>
<td>120,000</td>
<td>30</td>
<td>26</td>
</tr>
<tr>
<td>6</td>
<td>60,000</td>
<td>42</td>
<td>37</td>
</tr>
<tr>
<td>3</td>
<td>30,000</td>
<td>61</td>
<td>53</td>
</tr>
</tbody>
</table>

Prior to planting, 153 kg N ha⁻¹ and 90 kg P ha⁻¹ from a mixture of NH₄NO₃ and ammonium phosphate fertilizer were incorporated uniformly with the soil. Additional N, totalling 52.5 kg ha⁻¹, was applied by hand during the season as needed to maintain soil resources in a nonlimiting condition. During the first 4 weeks water was applied frequently from sprinklers mounted on risers to facilitate emergence and early growth. Later irrigations were applied as needed to prevent water supply from becom-
ing limiting to growth. Weeds were a problem early in the season and were not fully controlled until the end of June. An incidence of powdery mildew was noted in August and controlled with wettable sulphur (9.4 kg ha$^{-1}$ in an 0.5% aqueous suspension). The disease attacked only the older leaves so that possible influences on leaf growth were presumably indirect. The results presented here are from selected situations judged free from confounding influences of the disease.

Selected parameters of leaf-area expansion and of partitioning plasticity within leaves were measured as follows:

**Leaf Appearance Rate**

Leaf appearance rate (where leaf appearance was arbitrarily defined to be attainment of 5 cm in length) was assessed on five plants in each plot at weekly intervals between 53 and 130 days after planting. The smallest leaf greater than 5 cm in length was tagged for reference and the number of leaves appearing since the previous week's tag was recorded.

**Individual Leaf Expansion**

Leaf area expansion was estimated using the linear regression method of Owen (1957). As indicated in Figure 1, estimated area, A', was taken as the product of the coordinates, X and Y. Owen calculated $A' = kXY$, where k is the coefficient of regression of estimated on actual leaf area. In present study, a sample of 500 leaves yielded a regression coefficient (k) of 1.01 and we took $A' = XY$. The 1% underestimate was well within the error of planimetry. At 1- to 2-week intervals starting on day 53, the youngest leaf over 5 cm long was tagged on each of five plants per plot. These leaves were then measured (X and Y) every 3 to 5 days until they reached maturity.
Figure 1.---Coordinates X and Y used in estimating the area of sugarbeet leaves.

**Mature Leaf Area**

Ten plants in each plot were reserved for measurement of mature leaf area. At 7 to 10 day intervals, every fifth leaf (10, 15, 20, 25, etc.) on these plants was tagged for reference. When these tagged leaves were fully mature, as estimated by their age and appearance (close to senescence), their areas were determined by the linear regression technique.

**Components of Partitioning Plasticity within the Leaf**

At 59, 91 and 129 days after planting, three representative plants were harvested from each plot for partitioning measurements. Plants were harvested prior to 9 a.m. and were maintained within plastic bags in a cold room until separated into components. Individual leaves were removed from the plants in ontogenetic sequence. Blades were separated from petioles at the point where the laminar tissue was sharply reduced. The blade area of each leaf was estimated by regression. Individual blade and petiole parts were then dried at 60°C in a forced draft oven and weighed.

Solar radiation and minimum and maximum daily temperatures as measured at a standard weather station (600 m S.E.) are shown in Figure 2. Incident radiation was relatively constant through 7 August (80 days), at which time it began
a gradual decline to about 650 cal cm\(^{-2}\) da\(^{-1}\) by 27 August (100 da) and 500 cal cm\(^{-2}\) da\(^{-1}\) by mid-September (120 days).

Figure 2.---(Upper) Daily total solar radiation on a horizontal surface (Eppley solarimeter) during the growing season. (Lower) Two-day averages of daily maximum and minimum air temperatures during the growing seasons.

RESULTS

Both of the high density plantings (12 and 24 pl m\(^{-2}\)) had attained full cover by early July (estimated by visual inspection); the next intermediate density (6 pl m\(^{-2}\)) reached full interception in early August (Table 2). With 3pl m\(^{-2}\), interception remained incomplete throughout the season. Lesser levels of light interception at the second sampling date (Table 2) are attributable to a reduction in the canopy following the powdery mildew infection (Table 3).

Leaf Appearance Rate

The rates of leaf appearance (LAR) exhibited by the four densities were highly significantly different during the
first 4 weeks of measurement (50 to 80 days; Fig. 3) with 3 pl m$^{-2}$ having the highest rate, decreasing sequentially to 24 pl m$^{-2}$ having the lowest rate.

Table 2.--Percentage transmission of PAR quanta (Lambda sensor) at solar noon, measured at the ground level at 70 and 108 days from planting.

<table>
<thead>
<tr>
<th>Plant Density</th>
<th>Day 70</th>
<th>Day 108</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Transmission $S_x^-$</td>
<td>Transmission $S_x^-$</td>
</tr>
<tr>
<td>24 pl m$^{-2}$</td>
<td>2.0 % 0.23</td>
<td>8.0 % 1.63</td>
</tr>
<tr>
<td>12 pl m$^{-2}$</td>
<td>2.0 % 0.28</td>
<td>14.0 % 2.20</td>
</tr>
<tr>
<td>6 pl m$^{-2}$</td>
<td>8.0 % 2.05</td>
<td>20.0 % 3.06</td>
</tr>
<tr>
<td>2 pl m$^{-2}$</td>
<td>25.0 % 5.10</td>
<td>37.0 % 3.56</td>
</tr>
</tbody>
</table>

Table 3.--Leaf area per plant and leaf area index (LAI) measured at 59, 91 and 129 days from planting.

<table>
<thead>
<tr>
<th>Plant Density</th>
<th>Day 59</th>
<th>Day 91</th>
<th>Day 129</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaf area $dm^2 pl^{-1}$</td>
<td>LAI $b$</td>
<td>Leaf area $dm^2 pl^{-1}$</td>
</tr>
<tr>
<td>24 pl m$^{-2}$</td>
<td>24.5</td>
<td>5.9</td>
<td>32.4</td>
</tr>
<tr>
<td>12 pl m$^{-2}$</td>
<td>39.1</td>
<td>4.7</td>
<td>48.6</td>
</tr>
<tr>
<td>6 pl m$^{-2}$</td>
<td>55.1</td>
<td>(2.8)$^a$</td>
<td>77.7</td>
</tr>
<tr>
<td>3 pl m$^{-2}$</td>
<td>68.7</td>
<td>2.1</td>
<td>120.0</td>
</tr>
</tbody>
</table>

$a$ Standard errors of the treatment means at each harvest are shown in parentheses.

$b$ Calculated from the leaf area ($dm^2 pl^{-1}$) data.

Beyond 80 days, the LAR's converged to a common level and generally were not significantly different from each other for the remainder of the season.

The convergence of LAR among densities to a common level tended to parallel the trend of the various densities towards full cover. The occurrence of the lowest LAR values in each density corresponded with time of infection and buildup of the powdery mildew disease. LAR values increased following an application of sulphur on day 87.
Figure 3.--Leaf appearance rate (LAR) as a function of time for different plant densities.

Leaf Expansion and Mature Area

Leaf expansion measurements were begun on day 53 (17- to 24-leaf stage) with 2 or 3 leaves already senescent. The leaf expansion process was more rapid and the area of mature leaves greater with low densities (Fig. 4). In addition, the rates of expansion in all densities declined as the season progressed.

The differences in final area attained by comparable leaves from densities (Fig. 4) are particularly striking. The areas attained by the leaves with 3 pl m⁻² were the largest, with those of the other densities following in regular order: 6 > 12 > 24 pl m⁻².

The sugarbeet leaves continued to expand, albeit slowly, until just prior to senescence (Fig. 4). The final mature areas (Fig. 5) were determined when the leaves were quite old as judged by their overall appearance and chronological age. Differences among densities in mature leaf areas were highly significant for each measured leaf number. The area reached by successive leaves followed a common pattern in each density -- rising to a maximum, then falling throughout the remainder of the season. The numbered leaf with the maximum area varied with density: leaf 15 with the two higher densities, and leaf 20 at the low densities.
Figure 4.--The course of leaf blade expansion of leaves appearing on various dates for different plant densities.

Figure 5.--Blade area of mature leaves measured just prior to their senescence for various densities. Numbering proceeds from first-appeared to later-appeared leaves.

Partitioning Within the Leaf

Dry matter partitioning within leaves was assessed using plants harvested at 50, 91, and 129 days from planting. The patterns were similar at each harvest; data obtained at 91 days are presented. The chronological leaf distributions of each plant were used to select similarly aged leaves for the construction of Figures 6 and 7, showing
the course of leaf ontogeny between day 53 and day 88. Mean incident radiation declined from 750 to 700 cal cm\(^{-2}\) da\(^{-1}\) during this period.

Specific leaf area (SLA; cm\(^2\) leaf surface g\(^{-1}\) blade dry weight) was smaller with increasing leaf age and with decreasing density (Fig. 6). Young leaves from plants of all densities exhibited high SLA values which were maintained for varying lengths of time and then declined as the leaves increased in weight per unit area during development. After the first week, the SLA values of leaves from each density assumed a consistent order, with density 24 pl m\(^{-2}\) always highest, followed by 12 > 6 > 3 pl m\(^{-2}\).

The allocation of dry matter to blade and to petiole parts with leaf ontogeny is depicted in Figure 7 for the highest and lowest densities (the intermediate densities fell between these two in all parameters measured).

![Figure 6](image)

Figure 6.--Specific leaf (blade) areas (SLA) observed on day 91 as a function of leaf age at various densities.

Resource allocation was markedly influenced by density. Rates of dry matter accumulation were strikingly different after 2 weeks time, when the curves for the lowest density turn sharply upward while those of the highest density remain essentially linear. Blade and petiole tissues differed in this regard. At the lowest density, petiole weight paralleled blade weight for the first 3 weeks, then
leveled off while blade weight continued to increase linearly. At the highest density, however, increases in blade and petiole weight were essentially linear and parallel over the whole period, showing no tendency to level off; petiole weight exceeded blade weight at all leaf ages. At the low density, blade weight exceeded petiole weight with the magnitude of the difference increasing with leaf age.

DISCUSSION

Variations in planting density were used here to elicit growth and developmental plasticity in leaves. The microclimate and the availability of soil resources both vary with population density but with soil resources maintained at nonlimiting levels, we can attribute the observed effects to modifications of the aerial environment. The principal effect of increasing density is a reduction in the area of sunlight per plant and hence a reduction in the potential photosynthate production per plant. The attainment of full cover places a restriction on further increases in photosynthate production per plant and thus in the supply per growing point. Associated with the increasing density of the foliage canopy is increased shading of the apical meristem and the developing leaves,
and, through a greater proportion of the net radiation going to evapotranspiration, lower midday air temperatures within the canopy. Soil temperature fluctuations also are moderated as canopy density increases. These effects of density are to some extent confounded with the normal increase in erectness and tightness of the leaf whorl which occurs during ontogeny.

The principal vegetative response of sugarbeet plants to light and microclimate limitations included variations in storage root growth, leaf surface expansion, and partitioning within leaves. Successively greater growth depression per plant and per organ with increasing density was observed in all measured parameters (Figures 3, 4, 5, 6, and 7).

Assimilate status at the apex has been implicated as a major factor affecting the rate of leaf initiation (30, 18, 5, 11). Leaf appearance rate is determined both by leaf initiation rate (LIR) and by the time interval between initiation and appearance of successive leaves. In sugarbeet, LIR is reduced and the time interval increased in low light (26) as is also the case with cucumber (18), bean (5) and tomato (10). Assimilate status was not measured but we would expect it to vary with density (with light), as well as with the size or age of the plant (increases in root sink activity and mutual shading).

Leaf expansion rate and the final area of mature leaves were affected to a much greater extent by variable plant spacing than was LAR (Figures 3, 4, and 5). This may reflect the relatively small absolute carbohydrate demand, or higher priority, for LIR compared to that for the cell division and expansion activities following initiation. Interpretation is complicated by the fact that the expanding one-third (8) to one-half (23) its mature size. Thrower (28) noted that restricting light incidence on a young, expanding leaf prevented full leaf expansion and resulted in a considerably smaller mature leaf area.
Thus, both LAR and early leaf growth depend on the integrated assimilate status of the plant as a whole and thus density.

The canopy light environment also influenced partitioning in young leaves between blade and petiole tissues. Leaves produced under high density and lower SLA (Fig. 6) and a greater proportion of total leaf weight was used in petiole formation (Fig. 7). In spite of the striking differences between high and low densities in partitioning and in final leaf size, the blade area achieved per unit dry weight allocated to leaf growth was the same at both densities and relatively constant during the later stages of ontogeny (Table 4). The much greater SLA at high density compensated for the smaller fraction of leaf weight going to leaf blades. At the community level, leaf-area index was greater at the high density, despite the smaller leaf size, because of the much greater number and weight of leaves per unit.

Table 4.--Blade area per leaf and per g total leaf weight (blade + petiole) observed on day 91 as a function of leaf age at 3 and 24 pl m⁻². (Blade and petiole weights for these leaves are illustrated in Fig. 7.)

<table>
<thead>
<tr>
<th>Blade area</th>
<th>Plant density</th>
<th>Leaf age (weeks from appearance)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Per leaf (cm²) pl m⁻²</td>
<td>3</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>46</td>
</tr>
<tr>
<td>Per total leaf wt (cm² g⁻¹)</td>
<td>3</td>
<td>133</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>134</td>
</tr>
</tbody>
</table>

As the season progressed, the effects of planting density became less clear, with a tendency for convergence in the individual leaf parameters. Two factors may be involved in controlling this pattern of development: increasing tightness of the whorl which tends to place all developing leaves in a similar environment for light and temperature; and an increasing dominance of the storage
root which competes for a limited supply of assimilates. As the plants approached full cover, competition for light from adjacent plants would limit photosynthetic production per plant to a steady-state level, varying principally with the amount of radiation received to that area each day. As the potential assimilate use capacity of the root sink continued to increase, a generally stable but progressively lower internal photosynthate status would be established. This decline in photosynthate status could serve to restrict vegetative growth of leaves below their genetic potential. The effect would be greater at high densities with a much smaller photosynthate supply per plant.

The depression in vegetative growth during the latter half of the season also involves the increasing requirement for maintenance respiration and the seasonal decline in incident radiation (Fig. 2). Supporting the importance of an active root sink, however, is the fact that leaf growth began to decline well before incident radiation began its seasonal fall (Figures 3, 4, and 6), and the leaves of plants at the lowest density, which never reached full cover, demonstrated the same trends with plant ontogeny as did those of the light-limited plants from the other densities.

LITERATURE CITED


