Determining Nutrient Stress in Lettuce Plants with Machine Vision Technology

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SUMMARY. The rate of change of top projected leaf area (TPLA) of lettuce (*Lactuca sativa* L.) seedlings was determined with machine vision technology. Differences of TPLA between control and treatment plants were detectable with this technique within 48 hours from the onset of an imposed nutrient stress. The nutrient stress treatments were 0%, 50%, 150% of the control (100%). There were no differences for the 50% and 150% treatments compared to the control plants, even after a 6-day observation period. However, the 0% treatment caused different TPLA expansion within 48 hours and required a recovery period of 3 or 4 days after being returned to normal EC levels before again attaining prestressed growth rates.

Producing high-quality horticultural crops and timing their maturity to meet the consumer standards requires careful decisions of the grower. Correct decisions can only be made with proper information about the state of the crop and its environment. There are various sensors to measure the environmental condi-

tions that influence the development of the plants. However, measuring the status of the crop, especially in a noninvasive manner, is much more difficult and currently unavailable.

Major environmental factors are relatively easy to measure with readily available commercial sensors and systems. The temperature of the air and root zone can be measured with thermocouples, thermistors, or resistance temperature devices. The relative humidity may be measured with a variety of sensor types such as dry and wet bulb temperature sensors, dewpoint detectors, and electronic devices. Other parameters such as the CO₂ concentration of the air, electrical conductivity (EC) and some of the individual ions of the nutrient water, and the amount of photosynthetically active radiation the plant receives can also be measured by commercially available sensors.

The internal status of the plants, on the other hand, is much more difficult to determine. There are sensors to monitor some processes in the plants such as CO₂ assimilation, sap flow, leaf temperature, and the reactions of photosynthesis (Gensler, 1986; Omasa, 1991). These sensors, however, monitor only one point or a small area of one plant that may not provide representative information of the entire crop and, thus, are not useful for commercial-scale production. A preferred plant monitoring system should be able to determine the status of a large number of plants quickly, noninvasively, and nondestructively. Machine vision technology has the potential of becoming such a sensing system for monitoring the development, appearance, morphology, and health status of plants.

A machine vision system may consist of a camera and a frame grabber board within a properly programmed computer. Other equipment that may be necessary are optical filters and monochrometers to illuminate the object at specific wavebands. Collectively, these components can be a powerful instrument to monitor the morphological and spectral characteristics of plants, which can be correlated to the condition of the plants.

This study concentrated on detecting plant response to nutrient stress, defined primarily as an off-nominal condition (i.e., a deficiency or an excess of nutrients) in lettuce plants. The morphological changes and growth rate changes due to nutrient stress were

regularly measured and examined during the growth of the plant. Previous studies have demonstrated that top projected leaf area (TPLA) is an indicator of the physiological development of lettuce plants. TPLA is the horizontal projection (equivalent to the shadow) when viewed from a position directly overhead. A strong correlation was determined between TPLA and the fresh and dry weight of lettuce seedlings (Ling and Ruzhitsky, 1992). The dynamic change of TPLA is therefore a logical feature to consider for monitoring plant development.

The Controlled Environment Plant Production Systems greenhouse program at Rutgers University, Cook College, has as one of its goals to improve plant quality and maintain predictable productivity in greenhouse crop production by using real-time plant development information and applying it within the greenhouse climate-control strategy. The objective of this study was to determine lettuce plant response to nutrient stress with a machine vision system. The considerations were first to determine whether nutrient stress in lettuce plants would be reflected in the rate of change of TPLA and then second to determine how quickly a measurable difference can be detected after the onset of stress.

Subsequently, the practical concerns of whether various levels of nutrient stress could be recognized and whether recovery from nutrient stress could be measured were to be considered. Should the above objectives be proven attainable, then these subsequent questions would become a critical concern in the development of future control systems.

Materials and methods

'Ostinata' lettuce (*Lactuca sativa*) was chosen for this study because it exhibits nutrient deficiencies relatively quickly (Hetzroni et al., 1994). The lettuce plants were grown within a specially designed, lighted growing area in a controlled-climate chamber where the air temperature and CO₂ concentration, humidity, photoperiod, and intensity of photosynthetically active radiation (PAR) could be controlled and monitored. A 16-h photoperiod (0800 to 0000 HR) with an average spatial intensity of 430 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ photosynthetic photon flux (PPF) with high-pressure sodium lamps was used. The dark period was 8 h. The plant microcli-

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Fig. 1. A schematic diagram of the machine vision image acquisition system.

Table 1. Number of plants, seeding dates, starting date for treatment application, and age of plants in each experiment.

Experiment	Plants (no.)	Seeding date	Treatment start date	Plant age (d)
1	48	4 Nov. 1994	5 Dec. 1994	31
2	18	11 Nov. 1994	13 Dec. 1994	32
3	36	9 Dec. 1994	18 Jan. 1995	33
4	36	18 Dec. 1994	30 Jan. 1995	40

mate air temperature was held constant at $23 \pm 1^\circ\text{C}$, and the CO_2 concentration was maintained at 1000 ± 50 ppm only during the photoperiod. The relative humidity of the air ranged from 50% to 65% during the light period and 90% to 95% in the dark period.

The plants were grown in an ebb-and-flood hydroponic system, which consisted of two 1×1 -m, 3-cm-deep trays. The flooding frequency was 2 h throughout the day, with flooding depth at 2 cm and a flooding duration of ≈ 3 min. Nutrient solution was pumped from storage below the trays and returned by gravity flow for reuse. Solution temperature was maintained at approximately 18°C . Seeds were placed in 25-mm rockwool cubes, covered from the light, and watered with tap water. Upon germination (3 to 4 d after seeding), the cover was removed, and the seedlings were provided nutrient water with an EC of $2.0 \text{ mS}\cdot\text{cm}^{-1}$, which was created by adding $1.1 \text{ g}\cdot\text{L}^{-1}$ of Peter's Hydrosol and $0.7 \text{ g}\cdot\text{L}^{-1}$ of calcium nitrate. The seedlings were grown in this manner until testing began for each experiment, ≈ 31 d from seeding.

Lighting conditions were carefully controlled to achieve uniform PPF for all plants within the growing area.

Ninety-eight locations (14-cm grid spacing) were measured with a calibrated LI-COR quantum sensor. To improve the light distribution, a white cloth (55% transmissivity) was attached to the aluminized walls surrounding the growing area. New 400-W bulbs were added to the four high-pressure sodium fixtures, which were positioned 1.2 m above the tray surface and allowed an initial period of burn-in. PAR irradiance ranged from 400 to $460 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, which provided a coefficient of variation of a PPF of 4.3% over the 2-m^2 growing area.

A schematic diagram of the machine vision image acquisition system used in this study is shown in Fig. 1. The system consists of an incandescent light source, a monochrome camera (TM-745; PULNiX America Inc., Sunnyvale, Calif.), a frame grabber (PCVisionplus; Imaging Technology Inc., Woburn, Mass.), and an IBM PC compatible 386 computer. Front lighting was used to illuminate the top view of the plant canopy during image acquisition. The machine vision system was installed adjacent to the plants within the growth chamber. Each plant was moved from the tray to the image acquisition system where the top view images were acquired. To reduce the time nec-

essary to acquire images of all plants, each image was stored on computer hard disk for later image processing. At the beginning of each test, the image acquisition software program was calibrated and the camera was adjusted to obtain focused, high-contrast images. Each lettuce plant image was quantified using image processing techniques, including thresholding (to distinguish the seedling from the background) and blob analysis (algorithms to calculate physical parameters). Threshold values were determined interactively by the operator, and blob analysis automatically determined the area and perimeter of the thresholded object within the image.

Four experiments are reported here. Each experiment was designed as a comparative treatment test with multiple samples. Table 1 contains the information about the pertinent dates and age of the plants for each of the experiments. Experiment 1 was a demonstration that morphological changes of the plants could be measured reliably on a daily basis. Experiment 2 increased the time domain resolution over Expt. 1, and Expts. 3 and 4 examined plant response to different stress levels.

For Expt. 1, 48 plants of visually uniform size and mass were selected and divided into two groups of 24 plants to create a control group and a nutrient stressed group for testing. The control group received nutrient solution with an EC of $2.0 \text{ mS}\cdot\text{cm}^{-1}$. The stressed group had its root zone flushed with fresh tap water and placed in a separate area on the tray, where thereafter it received only fresh tap water. At the same time, every 24 h for 5 d, images of the plants were acquired with the machine vision system and the TPLA was measured. For Expt. 2, 18 plants were used, 9 in the control group and 9 in the stressed group. Similar procedures were followed as described above, except images were recorded and TPLA was measured just before the beginning of

Table 2. Average top projected leaf area (TPLA) of control and stressed groups for 5 d of Expt. 1.

Day	TPLA (cm^2)	
	Control	Stressed
1	149.7	148.9
2	160.8	152.5
3	168.2	157.0
4	180.7	160.2
5	187.3	162.1

Table 3. Average increase and cumulative increase of top projected leaf area (TPLA) for control and stressed groups in Expt. 1.

Period (d)	Control		Stressed	
	Increment ($\text{cm}^2 \cdot \text{d}^{-1}$)	Increment from start (cm^2)	Increment ($\text{cm}^2 \cdot \text{d}^{-1}$)	Increment from start (cm^2)
1-2	11.1	11.1	3.6	3.6
2-3	7.5	18.6	4.5	8.0
3-4	12.5	31.0	3.2	11.3
4-5	6.7	37.7	1.9	13.2

Fig. 2. Average top projected leaf area (in cm^2) of nutrient stressed and control plants in Experiment 1. The control group and stressed groups are represented using symbols ■ and x, respectively. Solid and dotted lines represent the means and the 95% confidence limits of the measurements, respectively.

Fig. 3. Cumulative 24-h relative percent change of top projected leaf area of nutrient stressed and control plants in Expt. 1. The control group and stressed groups are represented using symbols ■ and x, respectively. Solid and dotted lines represent the means and the 95% confidence limits of the measurements, respectively.

the light period (0800 HR), in the middle (1600 HR) and at the end of the light period (0000 HR). The experiment lasted for 3 d.

For Expt. 3, four groups of nine lettuce plants each were selected. Three groups were stressed by constant levels of nutrients of 0%, 50%, 150% of the normal EC. The control group received the normal EC of $2.0 \text{ mS} \cdot \text{cm}^{-1}$. All nutrient elements were proportionally increased to attain the EC 150%. The plants were stressed for 6 d and then returned to normal EC conditions for another 5 d. TPLA images were acquired at the same time every 24 h for the first 8 d, then again at 8.5 and 11 d from the onset of stress conditions.

In Expt. 4, four groups of nine plants each with three nutrient stress levels [EC concentrations of 0%, 50%, and 200% of the normal EC, and one control group ($\text{EC} = 2.0 \text{ mS} \cdot \text{cm}^{-1}$) were measured three times within a 24-h period (0800 HR—just before the light period, 1600 HR, and 0000 HR—just before the dark period) for 5 d. To measure the recovery of the plants after experiencing nutrient stress, the plant group with an EC of 0 was returned to an EC of $2 \text{ mS} \cdot \text{cm}^{-1}$ after a measurable difference was shown from the control group (i.e., after 48 h).

To use the machine-vision-measured TPLA for plant development monitoring, it is important to consider that TPLA is affected by plant movement and plant growth. Meyer and Anderson (1939) reported that many plant species have nastic movements due to disproportionate growth, or increase of turgor in one surface. Alford and Tibbitts (1970, 1971) also reported the natural biorhythm of leaf movement of some plant species. A better understanding of the natural movement of the lettuce would help to improve the accuracy in detecting plant TPLA response to stress by separating actual TPLA expansion from leaf movement. However, by assuming that the plants have a cyclic biorhythm, based on their controlled environment (i.e., light-dark cycle, diurnal temperatures) and acquiring the plant images at the same time each day, the effect of regular plant movements on the measurement accuracy of TPLA could be diminished or eliminated. Therefore, conclusions on growth differences could only be made on time intervals greater than or equal to 24 h for this study. The absolute value of the TPLA measurements may also be

Fig. 4. Cumulative relative change of top projected leaf area of the nutrient stressed and the control plants in Expt. 2. Data were collected on an 8-h interval. The control group and stressed groups are represented using symbols ■ and ×, respectively. Solid and dotted lines represent the means and the 95% confidence limits of the measurements, respectively.

Fig. 5. Cumulative percent change of top projected leaf area of four EC concentrations (EC 100% = 2 mS·cm⁻¹) in Expt. 3. Treatment EC 0% was changed to EC 100% 7 d after the nutrient stress was initiated.

inaccurate; however, the relative values used for comparative evaluation would be only limited by the resolution of the machine vision system.

Results and discussion

The average TPLA (in cm²) for each day of the test for the control and nutrient stressed groups are shown in Table 2. The average incremental daily change and the cumulative daily change from the initiation of stress are presented in Table 3. The average daily

TPLA is depicted in Fig. 2, along with the 95% confidence limits for the control and stressed groups. The cumulative daily percentage increase of TPLA with their 95% confidence limits are shown in Fig. 3. The cumulative daily percentage increases of TPLA for the plants of Expt. 2 are presented in Fig. 4.

NUTRIENT STRESS DETECTION. In Expts. 1 and 2, we found that the rate of increase of TPLA for the stressed plants was consistently less than that of the control plants. Within 48 h, the average TPLA increase of

the control plants had exceeded that of the stressed plants with a confidence of 95%. In Expt. 2, the changes of TPLA within each 8-h segment of the day were not equal, which suggests that the plant canopy expansion rate was variable during the photoperiod, the natural biorhythm of plant movement was occurring, or both. Note that the TPLA measurement is not necessarily determining the absolute growth as indicated only by canopy expansion, but is actually a combination of canopy expansion and leaf movement within the canopy.

In Expt. 3, the TPLA values are relative to the TPLA initially measured values at day 0. They are independent of the starting plant sizes; thus, they can be compared with similarly treated groups within other experiments. Plant growth within each of the three treatments (EC = 50%, EC = 100%, and EC = 150%) remained similar throughout the 11-d period (Fig. 5). The treatment with no fertilizer began to deviate from the other three groups with a reduced rate of increase of TPLA after only 2 d. On day 7, these plants (at EC = 0%) were again returned to EC = 100%, and they responded with a dramatic increase in their TPLA during the following 5 d. This suggested that near-normal canopy expansion rates can resume following a period of subnormal nutrient regime. The influence on the plant is very small, as detectable by machine vision, for nutrient concentrations ranging from a 50% reduction to a 100% increase (Expt. 4) compared to the normal EC level (EC = 2 mS·cm⁻¹).

The TPLA development difference between no nutrient (EC = 0%) and the control group of the Expt. 4 are displayed in Fig. 6. The percent increase of TPLA for stressed and unstressed plants were measured at intervals of 8 h. This increased sampling frequency gave an improved time domain resolution for detecting plant response to stress by the machine vision system.

Early determination of a nutrient stressed plant with the machine vision system is important. The average cumulative increase of TPLA for stressed and control groups is found in Table 4. Statistically significant differences (Student *t* test) at the 95% confidence level were measured from Expts. 1, 2, 3, and 4, respectively, within 48 h, between 32 and 48 h, within 24 h, and between 24 and 48 h. These results indicate that the machine vision system could consistently differentiate between treatments within 48 h.

Fig. 6. Cumulative percent change of top projected leaf area of the nutrient stressed (EC 0%) and the control plants (EC 100%) in Expt. 4. Data were collected on an 8-h interval. The control group and stressed groups are represented using symbols ■ and x, respectively. Solid and dotted lines represent the means and the 95% confidence limits of the measurements, respectively.

PLANT RECOVERY AFTER STRESS. In Expt. 3 (Fig. 5) the EC 0% treatment required >5 d (day 6 to day 11) after returning to a normal EC level before the absolute value of its daily TPLA expansion began to approach that of the control. The group at EC 150% level responded within 2 d (day 6 to day 8) of receiving the normal EC level and increased its TPLA expansion equal to the control group. This suggested that the intensity of the nutrient stress applied to the plants would affect the rate of recovery after the plants were returned to nonstressed conditions.

In Expt. 4, the plants were stressed

for only 48 h before returning them to normal EC levels. One might expect that after only a short period of stress the plant would return more quickly to a normal growth that was comparable to the control group. However, a recovery to a normal TPLA expansion required >4 d (Fig. 6). This suggested that the intensity of the stress was more influential than the duration.

Conclusions

These series of experiments demonstrated the current limitations of using machine vision for determining a change in plant growth. The morphologi-

cal change occurred within 48 h from the onset of the stress. The nutrient stress has to be very dramatic before the plant's reaction is detectable with the existing machine vision system. Even after a 6-d period, a doubling or halving of EC was not detected. A recovery period of ≥4 d is necessary for plants having been severely stressed (no nutrients) before returning to prestressed growth rates.

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Table 4. Cumulative increase of top projected leaf area (TPLA) for the stressed group [electrical conductivity (EC) = 0%] and the control group (EC = 100%) for all experiments. Critical *t* values are *t*(0.95; 24) = 1.711 for Expt. 1 and *t*(0.95; 9) = 1.833 for Expts. 2, 3, and 4.

Time (h)	Expt. 1			Expt. 2			Expt. 3			Expt. 4		
	Control	Stressed	<i>t</i>	Control	Stressed	<i>t</i>	Control	Stressed	<i>t</i>	Control	Stressed	<i>t</i>
0	0	0		0	0		0	0		0	0	
8				1.75	2.8	0.57				5.99	4.42	0.68
16				7.59	4.81	1.27				12.4	9.86	1.28
24	11.1	3.58	1.98	7.98	5.11	1.26	12.6	4.17	2.74	14.9	10.6	1.92
32				9.06	6.13	1.52				20.1	9.69	2.92
40				11.2	6	1.71				29.3	16.3	2.94
48	18.6	8.08	2.51	14.5	7.21	2.52	20.1	9.76	2.55	29.8	16.2	2.86
56				15.4	7.11	2.45				34	16.4	3.01
64				19.8	6.36	3.62				43.6	18.9	3.67
72	31	11.3	4.51	23.6	7.31	3.97	28.5	11.1	3.65	41.6	17	3.8
80										44.6	18.3	3.48
88											49.7	19.5
3.8												
96	37.7	13.2	5.49				40.9	11.2	5.13	46	12.3	4.26