Research Article

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Deficit irrigation reduces postharvest rib pinking in wholehead Iceberg lettuce, but at the expense of head fresh weight

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Abstract

BACKGROUND: Postharvest pinking is a serious issue affecting lettuce quality. Previous studies suggested the possibility of using deficit irrigation to control discolouration; however, this approach may also affect yield. This study investigated the effect of varying irrigation deficits on iceberg lettuce (*Lactuca sativa* L.) to determine the relationship between irrigation deficit, pinking and fresh weight.

RESULTS: The deficit imposed and head fresh weight obtained depended on both the duration and timing of withholding irrigation. Withholding irrigation for a period of 2 or 3 weeks in the middle or end of the growth period significantly reduced rib pinking compared to well-watered controls. Withholding irrigation for 2 weeks at the start of the growth period or 1 week at the end did not significantly reduce pinking. Withholding irrigation also reduced head fresh weight such that minimising pinking would be predicted to incur a loss of 40% relative to well-watered controls. However, smaller benefits to pinking reduction were achieved with less effect on head fresh weight.

CONCLUSION: Deficit irrigation could be used to provide smaller but higher quality heads which are less likely to be rejected. The balance of these factors will determine the degree of adoption of this approach to growers.

Keywords: lettuce; *Lactuca sativa*; deficit; irrigation; pinking; discolouration; postharvest

INTRODUCTION

Product appearance is a key performance trait in fresh produce, with both pre- and postharvest quality important for shelf life and consumer consideration. In wholehead lettuce (*Lactuca sativa* L.), discolouration of the ribs, a problem known as rib pinking, is a major concern in maintaining product quality.

Pinking is thought to represent a physiological response of the plant and often occurs after wounding.[1–3] Upon wounding, the production of ethylene and phenolics by the plant is increased, together with an increase in the activity of the enzymes phenylalanine ammonia-lyase (PAL) and polyphenol oxidase (PPO).[2,4] Levels of phenolics, PPO and PAL activity have been found to associate with the amount of rib discolouration in some studies though not in all.[1,5] However, rib pinking can often occur without prior tissue damage and this type of discolouration has received relatively little attention to date.

A number of factors have been found to affect the incidence of pinking in lettuce, such as time of transplanting, head maturity[6] and lettuce type or genotype.[1,7,8] Heat stress can also induce lettuce discolouration, the incidence and severity of which is affected by the timing of the stress but is not affected by stress duration or night time temperature.[9]

Climate uncertainty, reduced water supplies and increasing drought frequency mean that raising the water use efficiency of crops is becoming of increasing importance.[10] As well as increasing water use efficiency, precision irrigation may also act to improve product quality. Indeed, high levels of irrigation have been found to increase PAL and PPO activity and microbial load at harvest and to reduce the visual quality and fresh weight of lettuce.[5,11] Reduced irrigation (30–50 mm water deficit) has been shown to increase shelf life[7] and reduce postharvest browning;[11] however, it can also lead to reduced fresh weight, leaf number, leaf area index and dry matter accumulation.[11–13] The high water content of lettuce (around 95%), means that a loss of around 5% fresh weight due to reduced water content can affect lettuce appearance and saleability.[14]

Previous studies have investigated the effect of deficit irrigation on yield and browning.[5,11] To our knowledge, this is the first study that considers both duration and timing of deficit irrigation and the effects on postharvest pinking. Using a number of irrigation deficit schemes imposed on polytunnel-grown lettuce in three experiments, the feasibility of this approach was investigated to minimise postharvest rib pinking in wholehead lettuce whilst also determining the potential effects on yield. It was hypothesised that: (1) withholding irrigation for periods of

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Table 1. Summary data for the three irrigation deficit experiments

Experiment	Irrigation withheld	Accumulated relative deficit (mm)	Relative head fresh weight (%)	Head moisture content (%)	Pinking score*
1	Wk 4–6	151.06	44.98	90.83	1.08
	Wk 5–6	95.45	57.29	93.09	1.58
	Wk 6	21.63	95.29	94.48	2.17
	WW	0.00	100.00	94.94	2.00
2	Wk 1–2	16.21	89.86	95.28	1.55
	Wk 3–4	21.44	88.55	95.37	1.25
	Wk 5–6	50.79	49.33	90.81	1.17
	WW	0.00	100.00	95.30	1.67
3	Wk 3–4	32.07	78.38	93.15	1.27
	Wk 4–6	105.87	49.93	87.71	1.00
	Wk 6	37.60	98.66	93.48	1.80
	WW	0.00	100.00	93.56	2.27

Accumulated relative deficit and relative head fresh weight are calculated relative to the well-watered control (WW, irrigation not withheld) in each experiment.
*Wholehead pinking on the 10 cm visible length of ribs from the butt was scored after 10 days of storage using visual assessment criteria, marking from 1 (absence of pinking), through 2 (pink colouration on 1/3 of rib tissue), 3 (pink colouration on 2/3 of rib tissue) to 4 (pink colouration on all of rib tissue).
Wk = week(s).

varying duration and timing within the growth season would produce a range of imposed water deficits upon the plants; (2) that such deficits may also impact upon head fresh weight, and; (3) that water deficits could reduce the extent of postharvest rib pinking. Finally, the relationship between head fresh weight and pinking was investigated to determine whether it is possible to use deficit irrigation to control postharvest pinking whilst minimising yield loss.

EXPERIMENTAL

Plant growth conditions

The experiments were performed in a polytunnel at the Crop and Environment Research Centre at Harper Adams University (Shropshire, UK). Soil taken from the location of the polytunnel was used to fill plastic bins (40.0 × 44.5 × 76.5 cm, total capacity 136 L), which were sunk into the ground so that the top of the bin was approximately 30 cm above ground level. Holes were drilled in the bin bases for drainage and the soil was allowed to settle and the bins topped up. The soil was analysed by standard procedures and was identified as sandy loam with an average pH of 6.4 and average organic matter content of 15%. The top 30 cm of soil was tilled after each crop and the soil below 30 cm was not disturbed. Six blocks of eight bins each were used in a 6 × 8 arrangement with a total of 12 bins per treatment in each experiment. Nitrogen (125 kg ha^{-1}) was applied to the bins as a liquid feed prior to Experiment 1. Fertiliser at a rate of 50 kg P ha^{-1}, 275 kg K ha^{-1}, 160 kg N ha^{-1} was added to the bins prior to the start of Experiment 2. Prior to Experiment 3, the bins received 150 kg ha^{-1} nitrogen. Four commercially propagated transplants of Iceberg lettuce (*Lactuca sativa* L.) cv. Antarctica (obtained from PDM Produce, Shropshire, UK) at 4–5 true leaf stage were planted in each bin at 30 cm spacing. Experiment 1 began on 23 July 2012, Experiment 2 on 7 May 2013 and Experiment 3 on 3 July 2013. Irrigation treatments (beginning of week 1) began after allowing transplant establishment. The average daily temperature in the polytunnel during the experiments ranged from 12.8 °C and 23.4 °C.

Data collection

Each bin had a central access tube for moisture content recording. Soil moisture content of the top 30 cm of the soil profile in each bin was monitored using a Diviner 2000 Soil Moisture Probe (Sentek Technologies, Stepney, Australia) before and 24 h after irrigation, which was applied weekly. After establishment in the polytunnel bins, the plants were grown for 6 weeks then assessed destructively. To provide the different irrigation treatments, watering was withheld during certain weeks within this 6 week period, as indicated in Table 1. This provided periods of varying duration and timing within the 6 weeks where the plants did not receive water. The treatments were repeated twice for all timings except week 1–2. Irrigation volumes were calculated to return the bins not undergoing deficit to approximately 95% field capacity. Accumulated relative deficit was calculated by comparing the moisture content of the bins during the deficit treatment period to that of the well-watered control bin in the same block.

Plants were grown to commercial maturity then harvested. At harvest, plants were cut at the base, the waste was trimmed and the resulting head fresh weight recorded for each of the four plants per bin and then calculated relative to that of the plants from the well-watered control bin in the same block to allow for seasonal variation in well-watered head weight. The head of one plant per bin was dried in an oven at 80 °C for 5 days in order to determine moisture content. Another head from each bin was wrapped in a perforated bag and placed in an unlit cold store (1–5 °C). Wholehead pinking on the 10 cm visible length of ribs from the butt was scored after 10 days of storage using visual assessment criteria, marking from 1 [absence of pinking (white rib)], through 2 (pink colouration on 1/3 of rib tissue), 3 (pink colouration on 2/3 of rib tissue) to 4 (pink colouration on all of rib tissue). Plants were handled carefully to avoid damage to rib tissue.

Statistical analyses

Tukey's multiple comparison test was used to determine significant differences between treatment means after analysis of variance (ANOVA, $P \leq 0.05$). All statistical analyses were performed

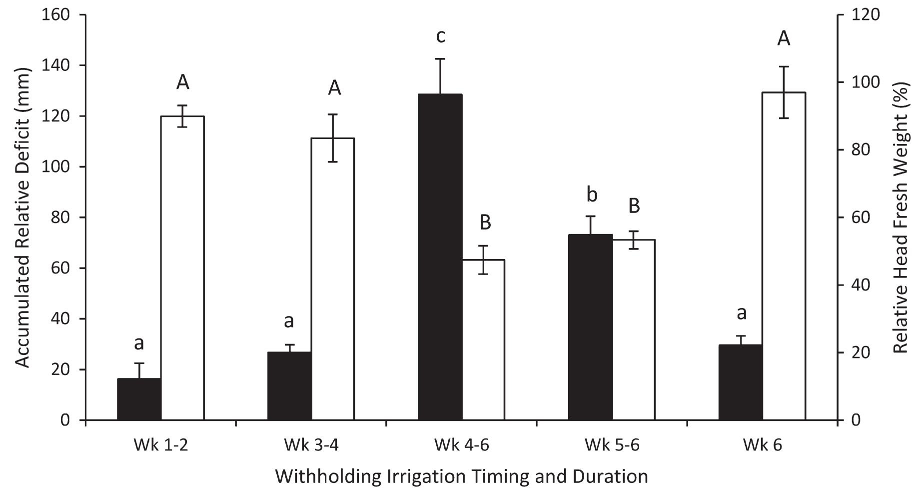

Figure 1. Effect of withholding irrigation on deficit incurred and fresh weight. Effect of timing and duration of withholding irrigation on the accumulated relative deficit and relative head fresh weight (relative to well-watered control) across the three experiments. Black bars = accumulated relative deficit (mm), white bars = relative head fresh weight (%). Bars represent means ± SEM. Within each variable, bars labelled with different letters are significantly different at $P \leq 0.05$ according to Tukey's test (a–c for accumulated relative deficit and A,B for relative head fresh weight). Wk = week. $n = 12$ for Wk 1–2, $n = 24$ for other treatments.

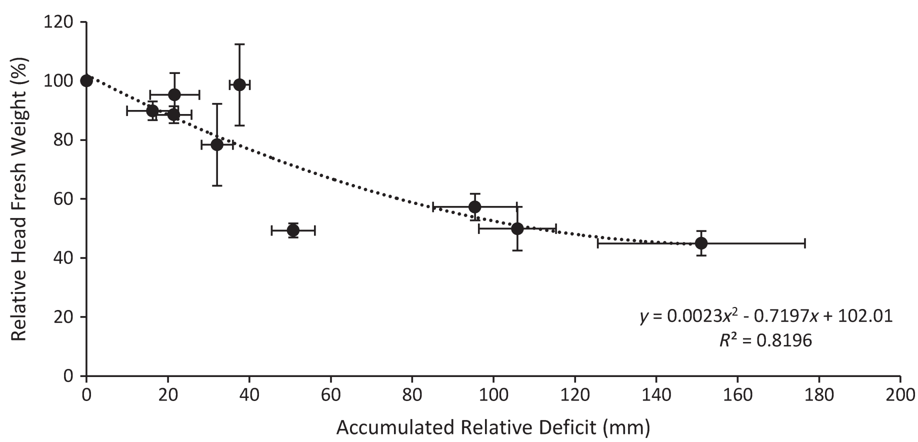

Figure 2. Deficit relationship with head fresh weight. Effect of accumulated relative deficit on relative head fresh weight (relative to well-watered control) across the three experiments. Points represent means ± SEM.

using GenStat 14th Edition software (VSN International Ltd, Hemel Hempstead, UK).

RESULTS AND DISCUSSION

Across the three experiments, withholding irrigation from growing lettuce plants for a range of durations and at different stages of growth provided a range of imposed water deficits when compared to well-watered controls (Fig. 1, Table 1). Comparing the withholding of irrigation for weeks 1–2, 3–4 or 5–6, indicated that for periods of the same duration (2 weeks), the later in the growth period the irrigation is withheld, the larger the resulting deficit imposed, significantly so by weeks 5–6 (Fig. 1). This is likely due to an increased water demand of larger, more mature plants possessing a greater leaf area for transpiration. In addition, by comparing the accumulated relative deficit imposed by withholding irrigation for a period of 1, 2 or 3 weeks during the second half of the growth period, as the duration of irrigation withholding increased, the deficit imposed upon the plants undergoing treatment increased significantly (Fig. 1).

Deficits imposed later in the growth period resulted in a greater weight loss, with the relative head fresh weight being significantly lower for plants with irrigation withheld in weeks 5–6 than in weeks 1–2 or 3–4 (Fig. 1). In addition, the relative head fresh weight was significantly lower when irrigation was withheld for a period of 2 or 3 weeks compared to 1 week. It appears that withholding irrigation for 1 week late in the growing period (week 6) has a similar effect to the two week treatments imposed earlier in the growth period (weeks 1–2 or 3–4), probably due to the higher rates of evapo-transpiration occurring from plants with greater leaf area later in the growth period (Fig. 1).

The effect of accumulated relative deficit on relative head fresh weight indicated a negative relationship such that, for example, an accumulated relative deficit of 30 mm water was sufficient to lower the relative head fresh weight of lettuce heads by 18%, while a deficit of 100 mm water lowered the head fresh weight by 47% (Fig. 2). This finding agrees with those of other studies where deficit irrigation impacted negatively on yield.[11–13] However, as the accumulated relative deficit increased further, so the effect on relative head fresh weight decreased such that even at the highest deficit imposed in our experiments (151 mm), relative head fresh

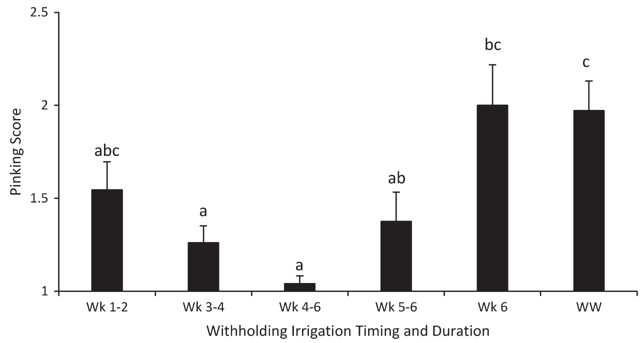

Figure 3. Irrigation scheme effect on wholehead pinking. Effective of timing and duration of withholding irrigation on pinking score across the different treatments in the three experiments. Points represent means ± SEM. Bars labelled with different letters are significantly different at $P \leq 0.05$ according to Tukey's test. Wk = week. WW = well-watered control. $n = 12$ for Wk 1–2, $n = 24$ for other treatments.

weight remained at around 45% of the well-watered plants. It appears that even a modest irrigation deficit can reduce head fresh weight; however, the plant appears to be able to limit this loss to a maximum of around 55%. The plants were grown in uniform soils to a depth of 70 cm and it is likely that lettuce roots accessed water deeper in the profile in response to drying soils in the top of the profile to limit head fresh weight loss at high deficits.[15]

Comparison of the rib pinking scores of lettuce in the different deficit irrigation schemes (Fig. 3) indicated that withholding irrigation for a period of 2 or more weeks in the mid to late growth period (weeks 3–4, 5–6 and 4–6) significantly reduced pinking when compared to well-watered controls. Withholding irrigation either early in the growth period (weeks 1–2) or for 1 week at the end of the growth period (week 6) did not significantly reduce pinking. It appears that deficit irrigation is less effective in reducing pinking when used early in the growth period or when used for a short period of time, such as one week. This may reflect the imposition of an insufficiently large water deficit in these treatments to affect pinking development. This finding is in agreement with an earlier study that found reduced postharvest discolouration in lettuces which had undergone reduced irrigation.[11] However, another report found that the cessation of irrigation for up to 16 days prior to harvest did not significantly affect pinking of lettuce ribs after up to 21 days in cold storage.[16] It is interesting also to note that pinking appears to be unaffected by whether drip or overhead irrigation is used.[6]

For deficit irrigation to be considered a useful means to reduce postharvest pinking, negative effects on yield must be minimised. In order to determine the feasibility of this approach, the relative head fresh weight and lettuce pinking scores obtained from the deficit experiments was compared (Fig. 4). This showed that reducing the rib pinking score to a minimal level would be associated with a head fresh weight loss of 40%, a level of yield reduction that would not be commercially viable. However, smaller reductions in pinking could be achieved with a smaller reduction in head fresh weight by using a milder deficit treatment. For example, a reduction in pinking score from 1.86 for the well-watered control to 1.33 would be predicted to be achieved with a head fresh weight loss of 20%, corresponding to an accumulated relative deficit of 35 mm water. The balance of the beneficial effect on pinking and negative effect on head fresh weight when using deficit irrigation remains to be decided by the grower. Whilst deficit irrigation leads to smaller heads, they show reduced pinking, and would be predicted to lead to a lower proportion of rejected heads, meaning that the resulting overall yield and financial cost of using deficit irrigation may not be as large as first appears, particularly for processed lettuce.

Pinking may appear to be related solely to head size and therefore an earlier harvest would be predicted to show the same effect on pinking as deficit irrigation and would be interesting for future investigation. However, the plants grown with deficit irrigation in this study were the same age as the well-watered controls and the number of leaves in the head at harvest did not differ significantly from the well-watered controls (mean of 23.7 leaves for well-watered and 23.6 leaves for deficit irrigation schemes), suggesting that they were at the same developmental stage. The effect of deficit irrigation on pinking therefore does not appear to simply represent slower development of the plants. If all plants were at the same age and developmental stage, then the difference in head fresh weight between well-watered and deficit irrigation plants will mainly reflect a difference in head moisture content, and postharvest discolouration has been associated with high head moisture content at harvest.[11] When pinking severity was compared to head moisture content, it was found that lower head moisture contents were associated with lower pinking scores (Table 1) and that pinking scores were more variable at high moisture contents. As pinking is often associated with wounding of the plant tissue ,[1–3,11] so reducing moisture content may act to reduce pinking by limiting damage as the rib tissue is less turgid. Indeed, lower moisture content has been found to be associated with better maintenance of visual quality during storage

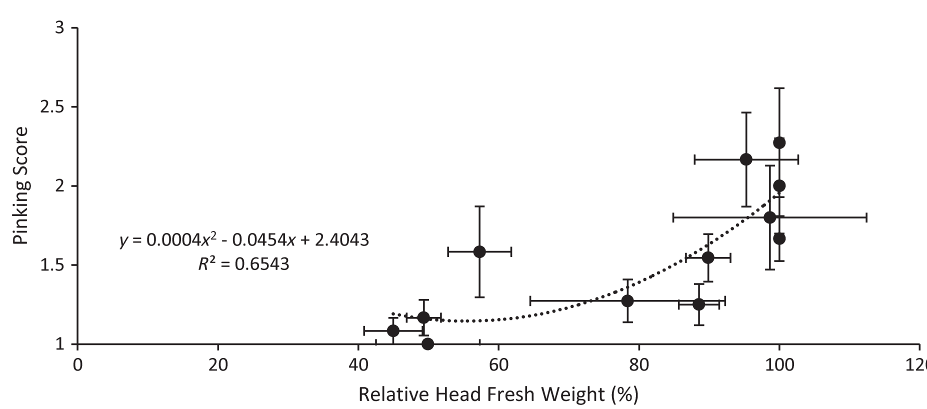

Figure 4. Head fresh weight relationship with wholehead pinking. Relationship between relative head fresh weight (relative to well-watered control) and observed pinking score. Points represent means ± SEM.

and lower percentage of damaged leaves in minimally processed babyleaf spinach (*Spinacia oleracea* L.)[17] and butterhead lettuce.[18] Pinking has also been associated with the presence of the bacterium *Pseudomonas marginalis*.[7] This species was isolated from pink rib legions while injection into the rib led to pinking symptoms. Wounding may therefore aid colonisation by bacteria and subsequently increase pinking in this manner also.

CONCLUSIONS

Whilst deficit irrigation does not appear to be feasible for the complete elimination of rib pinking in whole head lettuce, it is still able to contribute to improving postharvest quality in lettuce when used in moderation. Growers will therefore need to balance the potential beneficial effects of deficit irrigation on rib pinking and the associated risk of rejected lettuce heads on quality parameters, with a commercially acceptable reduction in head fresh weight.

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