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Tipburn in head lettuce – the role of calcium and strategies for preventing the disorder.

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Tipburn in head lettuce – the role of calcium and strategies for preventing the disorder

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In the present study the efficiency of preventive methods for the reduction of tipburn incidence was examined in head lettuce. Foliar applications of calcium chloride or short-term irrigation treatments were carried out in order to decrease tipburn incidence in the field. On the basis of root pressure measurements under controlled environment approaches should be made to explain the tipburn reducing effect of a reduction in VPD or the use of unsusceptible genotypes.

1. The efficiency of Ca-sprays depending on plant development and frequency of the application were examined in iceberg lettuce. Plants that were treated to the time of head formation showed a reduction in tipburn severity. An elevated foliar uptake due to the Ca-sprays could not be manifested by leaf mineral analysis.
2. Tipburn symptoms can be induced under controlled environmental conditions by a constant high VPD. Tipburn incidence could be reduced by a diurnal reduction of VPD. Contrary to the expectation this could not be explained by an encouraged root pressure flow during night time. Rather the concentration of mineral constituents in the root pressure exudate (K/Ca) seemed to be related to reduced tipburn incidence.
3. Shortly after the onset of short-term irrigation VPD within the crop stand was decreased. Tipburn incidence was influenced by the irrigation treatment depending on temperature and daily intervals. This diurnal reduction of VPD lead to a recurrent tendency of the efficiency of the short-term irrigation treatments throughout the planting sets. Although tipburn incidence could be reduced about 20 % by the irrigation treatments, however, the differences could not be calculated by statistical analysis.
4. Head lettuce genotypes expressed symptoms under controlled environmental conditions according to their variation in tipburn susceptibility. Higher growth rates during during early stages of plant development, lower specific leaf weight and also smaller cells per area could be observed in tipburn sensitive cultivars. Differences between the genotypes regarding tipburn incidence could not be explained by variations in the amount of root pressure flow. However, there was an effect on the qualitative compounds in the root pressure exudate, in particular during night time. This was most probably revealed by an increase of the K/Ca ratio during night period for the sensitive cv. ‘Herman’.

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Abbreviations

%	percent
°C	degree centigrade
µmol	micromole
^{45}Ca	radiolabelled calcium isotope
Ca	calcium
$CaCl_2$	calcium chloride
cm	centimetre
cv.	cultivar
d	day
DM	dry matter
$d_{T>24}$	days with temperature >24°C
g	gram
h	hour
H_2O_2	hydrogen peroxide
HNO_3	nitric acid
I_{SUM}	sum of global irradiation
K	potassium
km	kilometre
kPa	kilo pascal
LA	Leaf area
LSD	least significant difference
Mg	magnesium
mg	milligram
mM	millimolar
mm	millimetre
mmol	millimole
n	number
n.s.	not significant
r^2	coefficient of determination
RZ	Rijk Zwaan
S&G	Syngenta Seeds®
SE	standard error
T_{SUM}	sum of temperature
VPD	vapour pressure deficit

A. INTRODUCTION

1. Ca-deficiency symptoms in horticultural crops

Symptoms of calcium deficiency disorders were first described by Thomson in 1926. Only forty years later, these symptoms were attributed to calcium deficiency disorders for the first time by Kruger (1966) and THIBODEAU and MINOTTI (1969). At present, calcium deficiency is regarded as an important disorder in various horticultural crops, such as bitter pit in apple, blossom end rot in tomato or pepper and tipburn in cabbage and lettuce, respectively (EVERAARTS and BLOM-ZANDSTRA 2001; COLLIER and TIBBITTS 1982; SAURE 2001). Calcium related disorders are known for further horticultural crops such as cauliflower (ROSEN 1990), cabbage (WIEBE 1975), collard (JOHNSON 1991), poinsettia (MATTHÄUS and FREUND 2001) and chervil (KLEEMANN 1999; KLEEMANN 1999).

Calcium deficiency in lettuce plants can be categorized into absolute, induced or physiological Ca-deficiency. Absolute Ca-deficiency is due to a poor calcium supply to the substrate, which does practically not exist in soils used for horticultural production in Germany [Middle Europe]. Ca-deficiency can be induced under circumstances where calcium concentration in the substrate is sufficient, but concentrations of antagonistic cations are overcompensating. Also, physiological calcium deficiency can occur while calcium supply in the substrate is sufficient. This disorder is often locally restricted and is assumed to be a result of an uneven distribution of calcium in the sensitive organs (COLLIER and TIBBITTS 1982; MATTHÄUS and FREUND 2001). A symptom of calcium deficiency is caused by the disintegration of the cell walls and the collapse of the surrounding tissue. Cell components can leak out and the tissue of the affected leaf parts will turn brown and necrotic.

2. Function of calcium, calcium concentrations in the leaves

A high proportion of the total calcium is located in the apoplasm due to the large number of binding sites. About 90 % of the calcium can be derived from cell-wall bound calcium where it is fixed to the carboxylic groups of the pectins. Its main function is the stabilization of the cell walls. High concentrations were found further in the exterior surface of the plasma membrane, the endoplasmatic reticulum (ER) and in the vacuole. The water soluble fraction is mainly located in the vacuole accompanied by organic (malate) or inorganic anions (nitrate, chloride). The calcium concentration of the cytosol is very low. Variations of the free calcium concentration in the cytosol are important for the transmission of information signals (MARSCHNER 1995; WISSEMEIER 1996; MENGEL 1991). Calcium functions as a second messenger by the activation of calcium channels in the membranes which lead to an increase of calcium influx and cytosolic free calcium. Thereby certain enzymes such as calmodulin were being activated (MARSCHNER 1995).

The uptake of calcium by the plant mainly takes place via the root tips. Calcium penetrates the cytoplasm by the plasmalemma passively. Due to a low concentration in the cytosol an active transport into the apoplasm or the vacuole occurs. In the phloem calcium concentration is extremely low because of low permeability for calcium ions through the membranes of the sieve cells. The transport of calcium into the growth sinks takes place almost exclusively via the xylem along the transpiration flow (MARSCHNER 1995; MENGEL 1991). Under conditions of high transpiration rates for the plants, the xylem volume flow is almost completely directed towards the higher transpiring organs. Therefore under these circumstances, calcium supply via the xylem flux can be insufficient for younger leaves, enclosed leaves or fruits having only low transpiration rates. This can lead to a localized calcium deficiency of the affected area. Generally, high calcium concentrations were found in older leaves, whereas calcium concentration in the younger leaves was low. Regarding calcium distribution within the leaf blade, lowest calcium concentrations were found in the leaf margins. Leaf calcium concentrations increase from the leaf margins towards the midrib (BARTA and TIBBITTS 1991; BARTA and TIBBITTS 2000).

In contrast to calcium, potassium is very mobile in the phloem. Certain plant parts can be supplied for 100 % with potassium by the phloem. For magnesium, supply to the plant organs via the phloem varies between 25 and 40 % (MARSCHNER 1995).

3. Factors promoting tipburn symptoms

Tipburn frequently occurs during periods of high temperatures. MISAGHI and GROGAN 1978 reported that temperatures above 24°C were relevant for the induction of tipburn. In greenhouse experiments various temperature treatments were given in order to enhance the onset of tipburn symptoms successfully (MISAGHI and GROGAN 1978; WISSEMEIER 1996; KLEEMANN 2000; NAGATA and STRATTON 1994). In certain regions lettuce production is discontinued during the warm summer months in order to avoided the risk of tipburn symptoms. Tipburn incidence in the field is enhanced particularly after a series of days and nights with high temperatures in combination with low relative humidity (COLLIER and TIBBITTS 1982; SAURE 1998). This coincidence indicates that apart from temperature and relative humidity special attention has to be directed to vapour pressure deficit (VPD). The VPD can be related directly to the transpiration rates of the plants (BAAS et al. 2003).

Light is another important factor for the development of tipburn in greenhouse grown lettuce. Whereas light is usually not a limiting growth factor in the field, it is rather decisive under conditions of controlled environment regarding length of light period, intensity and quality (TIBBITTS and RAO 1968). Prolonged light periods were associated with high growth rates and an increased demand for calcium (KLEEMANN 2000).

Examinations of BRUMM and SCHENK (1994) showed that an increase of the nitrogen supply of lettuce plants leads to a higher rate of tipburned plants. The enhancement of tipburn was explained by a higher growth activity of the plants and therefore excessive demand for calcium.

The factors described above, e.g. temperature or light intensity, were generally related to increased growth rates of the plants. Several authors agree that tipburn incidence increases with increased growth rate. In field grown plants tipburn mainly occurs at maturity of the heads, immediately before harvest time (SAURE 1998; COLLIER and HUNTINGTON 1983; BRUMM 1992). In greenhouse or growth chamber plots tipburn symptoms can be induced early after emergence of the plants (SAURE 1998; COX et al. 1976; PALZKILL and TIBBITTS 1977). TIBBITTS and RAO 1968 found that the higher the growth rates the earlier tipburn symptoms appeared. However, experiments in which growth rates were inhibited by one of the factors described above are discussed controversially in literature (SAURE 1998).

A genotypic variation in susceptibility to calcium deficiency disorders has been described by numerous authors (COX et al. 1976; HAGEL et al. 2002; MAROTO et al. 1985; MISAGHI et al. 1981; OHLS 1989; THEILER et al. 2003; WISSEMEIER 1996). The susceptibility to tipburn of a cultivar is genetically determined but influenced by environment (SAURE 1998). Despite improvements in cultural practices, the use of a resistant or tolerant cultivar is often considered as the most effective preventative measure against tipburn (COLLIER and TIBBITTS 1982; JOHNSON 1991; ROSEN 1990; SAURE 1998; WISSEMEIER 1996). It has been suggested that faster-maturing cultivars were susceptible to tipburn. But several cultivars of iceberg lettuce with a short period of development were rather insensitive to tipburn (COLLIER and TIBBITTS 1982). In addition, susceptible cultivars may not be identified by lower internal calcium concentrations (HAGEL et al. 2002). For fruit vegetables, such as tomato, Ca-use efficient genotypes were known. It could be observed the genetic control of Ca-use efficiency is modified to a large extent by physiological factors (CAINS and SHENNAN 1999). Interpretation of the genetic factor still remains unclear. In order to improve investigations in this complex field it would be helpful if a suitable screening method could be developed (COLLIER and TIBBITTS 1982).

4. Occurrence of tipburn, preventive methods

Preventive methods to reduce tipburn occurrence in lettuce can be derived from the factors stated above. Up to now the most frequently recommended and safe method to reduce the risk of tipburn in lettuce, especially during the warm summer months, is the usage of tolerant cultivars (COLLIER and TIBBITTS 1982; JOHNSON 1991; ROSEN 1990; SAURE 1998; WISSEMEIER 1996). There are reports that tipburn in lettuce could be prevented by foliar application of calcium salts (ABD EL-FATTAH and AGWAH 1987; SONNEVELD and VAN DEN ENDE 1975; THIBODEAU and MINOTTI 1969). On the other hand this method is discussed controversially regarding butterhead lettuce. Various studies showed that calcium sprays had no reducing effect on tipburn (CORGAN and COTTER 1971; MISAGHI et al. 1981;). While using calcium sprays in order to reduce tipburn symptoms in lettuce it has to be taken into account that only a small proportion of the calcium applied will be taken up by the leaf tissue (SCHÖNHERR 2000). It has also to be considered that the sensitive organs may not be affected by spray applications in head lettuce due to its fast-growing tissue and the incapability of re-translocating calcium to the sensitive regions. At present any precise information on an optimized procedure for the application of Ca-salts as prophylactic measure against lettuce tipburn is lacking.

5. Aim of the present investigations

It was the aim of the investigations presented here to develop a procedure for an efficient preventive treatment against tipburn injury in butterhead lettuce in the field. The efficiency of $CaCl_2$-applications as influenced by plant development and frequency of the application was studied in field grown iceberg lettuce. Further, butterhead lettuce plants were submitted to short-term irrigation treatments in order to influence the VPD within the crop stand. By reducing VPD during night time calcium supply to the sensitive leaves should be maintained via enhancement of root pressure flow. This hypothesis will also be tested by studies on greenhouse grown lettuce. Quantity and quality of the root pressure exudate were examined by decapitating plants according to an elaborated time pattern. This should help to understand the effects of the treatments observed o the plants.

The genotypic variation in tipburn susceptibility is supposed to have effects on calcium distribution in lettuce. In addition, plant characteristics that could help to identify a tipburn susceptible cultivar of lettuce should be determined. This aims at the development of a method to characterise tipburn susceptible cultivars in a short period of time under controlled environmental conditions without the expression of symptoms. The studies conducted so far should help to understand the causes for tipburn development in lettuce.

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B. EFFECT OF EXOGENOUSLY APPLIED CALCIUM CHLORIDE ON CALCIUM DISTRIBUTION IN HEAD LETTUCE (*LACTUCA SATIVA* VAR. *CAPITATA* L.)

1. Introduction

Calcium related disorders, such as tipburn of lettuce, are a serious problem in commercially grown lettuce. A localized calcium deficiency leads to the collapse of leaf tissue and consequently to necrotic lesions, veinal necroses or necrotic margins, which can result in total loss of the crop. Various investigations were conducted in order to prevent lettuce tipburn by foliar applications of Ca-salts (ABD EL-FATTAH and AGWAH 1987; ASHKAR and RIES 1971; MISAGHI et al. 1981), Ca-salts combined with adjuvants (HOLTSCHULZE et al. 2001) or other chemicals (CORGAN and COTTER 1971). However, the results obtained in head lettuce are contradictory. There are several reports that a reduction in tipburn incidence due to the application of Ca-salts could not be accomplished (CORGAN and COTTER 1971; MISAGHI et al. 1981). On the other hand it was reported that tipburn injury could be reduced by Ca-application (ABD EL-FATTAH and AGWAH 1987; SONNEVELD and VAN DEN ENDE 1975; THIBODEAU and MINOTTI 1969). It should be mentioned that in the successful approaches Ca-treatments were applied in a high frequency. For other vegetable crops like Chinese cabbage (IMAI et al. 1987; MAROTO et al. 1985; VAN BERKEL 1988), chervil (KLEEMANN 1999), collard (JOHNSON 1991), cauliflower (ROSEN 1990) similar observations were made. In tomato fruits weekly sprays of calcium chloride reduced the occurrence of blossom end rot in tomato fruits (SCHMITZ-EIBERGER et al. 2002).

At present numerous commercial growers are using applications of Ca-salts as prophylactic measures against lettuce tipburn while lacking any precise information of an optimized procedure. Therefore, in this study we focused on the effectiveness of exogenously applied $CaCl_2$ depending on plant developmental stage and frequency of the application. Leaf samples were sectioned into margins and remainder in order to reveal the influence of $CaCl_2$ sprays on calcium distribution and relative cation concentrations (K/Ca, Ca/Mg) within the leaves.

2. Material and Methods

2.1. Site of experimentation

Experiments were conducted at the Experimental Station Marhof associated with the Institute of Horticulture Research, of the University of Bonn. The experimental fields were located 15 km north of Bonn between 'Vorgebirge' and 'Kölner Bucht' at 50° 49" northern parallel (6° 59" eastern longitude). Soil was a base-rich brown earth with silt loam to sandy-silt loam. With an average temperature of >10°C and 700 mm of annual rainfall this region represents a typical location for horticultural farming in the Rhineland / Germany.

2.2. Plant Material

Iceberg lettuce 'Chianti' was seeded in compressed blocks of Stender A280 (Stender, Schermbeck, Germany) propagation medium, consisting of 20% white peat and 80 % mixed peat. It contained 14 % nitrogen, 16 % phosphorus, 18 % potassium and microelements at a pH of 5.5 – 6.2. The seedlings were planted on 06.06.2001. The plots consisted of 5 rows per beet (10-12 plants m^{-2}), while the center three rows were determined for examination in order to reduce side effects. 120 kg N ha^{-1} were applied as CAN (calcium ammonium nitrate) additional to N_{min} contents of the soil before planting.

2.3. Treatments

Plants were sprayed twice a week with $CaCl_2$ $2H_2O$ (Merck, reagent grade) at a concentration of 0.04 M with Tween 20 (Merck) added at a concentration of 0.1 % until run-off. Ca-treatments were varied according to plant development. Frequency of application was adjusted (Tab. 1). Applications were carried out with a knapsack sprayer in the early evening in order to ensure leaf moisture for an enhancement of cuticular cation uptake. Previous studies have shown that the application of the adjuvant alone (here: Tween 20) did not have any effect on tipburn or plant characteristics (HOLTSCHULZE et al. 2001).

Tab. 1: Periods and frequency of calcium chloride applications for preventing tipburn incidence in iceberg lettuce. $CaCl_2$applications were carried out twice a week.

Treatment	Period of application	*Frequency*
Control	untreated	*[0]*
Ca1	$CaCl_2$ (0.04 M) from one week after planting until harvest	*[12]*
Ca2	$CaCl_2$ (0.04 M) after head formation until harvest	*[4]*
Ca3	$CaCl_2$ (0.04 M) during head formation	*[4]*

2.4. Tipburn assessment and preparation of samples for analysis of Ca^{2+}, Mg^{2+} and K^+ in the leaf tissue

When iceberg lettuce was harvested, wrapper leaves were cut off prior to the determination of fresh weight. Ten plants per plot and 10 leaves per plant, from outer to inner leaves, were examined for tipburn incidence (%) and severity (number of tipburn spots). Plants having one or more leaves with tipburn symptoms were scored as tipburn plants.

Leaves were washed with distilled water to eliminate adherent calcium, cut into 0,5 cm wide stripes and divided into three samples: leaf margins with and without tipburn symptoms and leaf blade. Tissue samples were dried at 60-80°C for 24 h and at 105°C for 2 h before grinding. An aliquot of 0.3 g was digested with HNO_3 and H_2O_2. Concentrations of total calcium, magnesium and potassium were estimated by atomic absorption spectrometry (AAS).

2.5. Statistical analysis

The experiments were established in a randomized block design with each treatment replicated six times. For statistical analysis of the data the statistic program 'SAS 8.0' (SAS Institute Inc., Cary, USA) was used. Data were subjected to analysis of variance. Comparisons of mean values were performed by Tukey's HSD. Statistical differences were accepted at 5 % level of significance. Graphs were prepared with SigmaPlot 8.0 (SPSS Science Inc.)

3. Results

3.1. Effects of foliar application of calcium chloride on

3.1.1. head weight of iceberg lettuce

Plants were harvested after 55 days of vegetation (on 06/30/2001) with an average head weight of 672 g as related to the marketable plant product. Compared to the untreated control, there was no effect of the calcium chloride treatments on head weight (Tab. 2).

Tab. 2: Effect of foliar application of calcium chloride on weight (g) of marketable heads. Mean values (±) standard error; different letters indicate statistical differences at $p<0.05$ ($n=10$). Treatments: Ca1=1 week after planting until harvest; Ca2=following head formation until harvest; Ca3=during head formation.

Treatment	head weight (g)		
Control	671.4	± 18.8	*a*
Ca1	708.9	± 13.7	*a*
Ca2	648.8	± 17.9	*a*
Ca3	685.6	± 13.8	*a*

3.1.2. tipburn incidence in iceberg lettuce

Tipburn symptoms were characterized by necrosis of leaf margins and veinal necrosis. At harvest, severe tipburn incidence was found in the control plants with almost 93 % of the plants injured (Fig. 1). Foliar applications of calcium chloride from stage of head formation until harvest (Ca2) resulted in a slight reduction of tipburn incidence. These differences were not statistically significant due to a high standard deviation caused by an irregular occurrence of tipburn symptoms within the field plots.

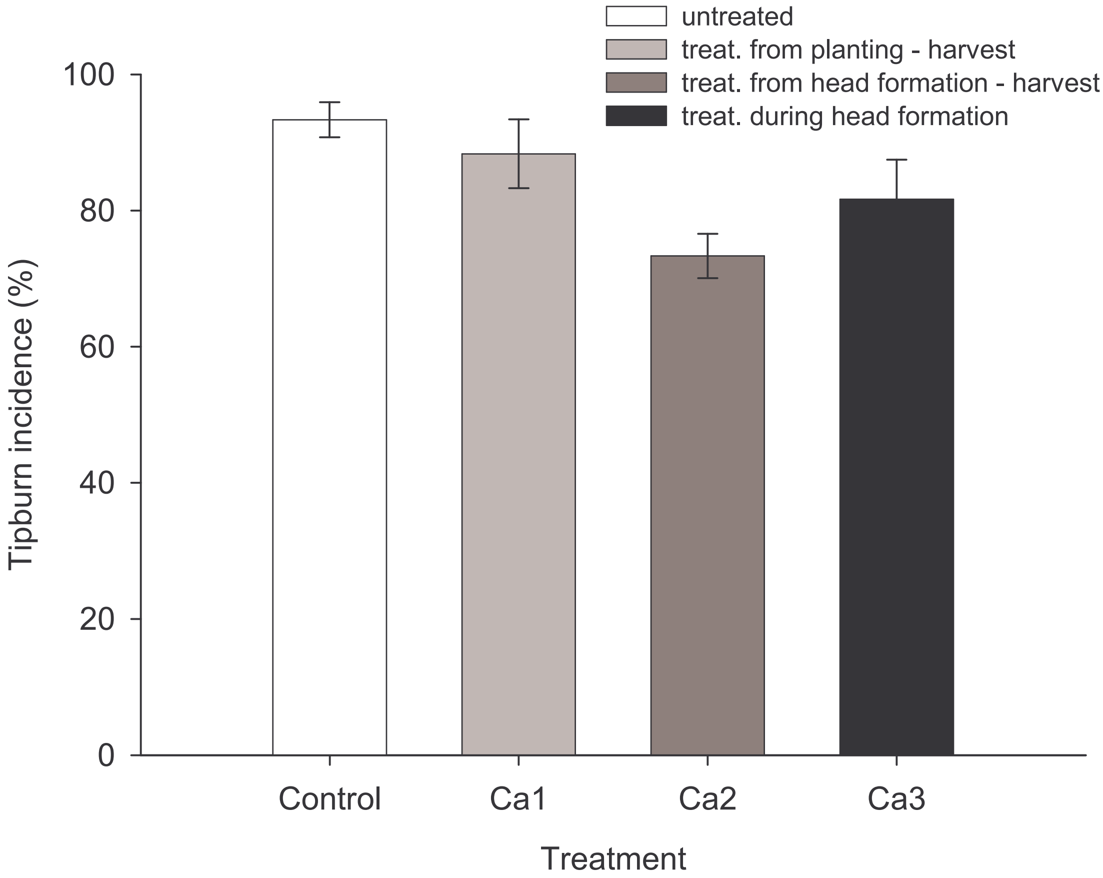

Fig. 1: Tipburn incidence (%) in iceberg lettuce ‘Chianti’ at harvest time as influenced by foliar applications of calcium chloride. Error bars are indicating standard error, differences between the treatments were not significant.

After cutting-off wrapper leaves the ten following inner leaves were examined for severity of tipburn symptoms, which depended on leaf position within the lettuce head (Fig. 2). Outer and therefore bigger leaves were affected more severely than inner leaves. This relationship is negatively correlated (r^2=0.88).Tipburn symptoms were mainly induced directly underneath the wrapper leaves, whereas the younger leaves (No. 8-10) developed the lowest number of symptoms per leaf. Differences between the mean values were expected to be found within leaf position 1-3 (counted from the wrapper leaves) due to greater variances.

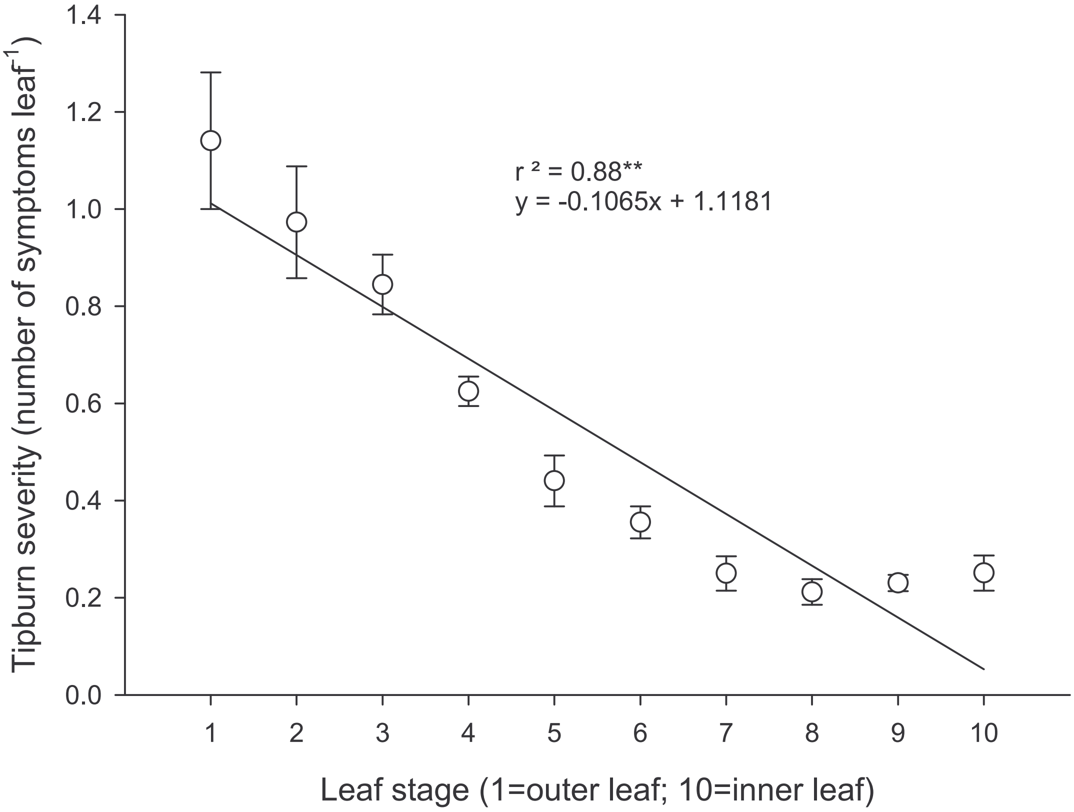

Fig. 2: Severity of tipburn symptoms depending on leaf position in iceberg lettuce 'Chianti' at harvest time. 1=outer leaf, 10=inner leaf, wrapper leaves were not included. Each value represents a mean of 60 plants. Error bars indicate standard error. Correlation is significant to the level $p<0.01$ (**).

As a consequence of the results shown above, number of tipburn symptoms from the first to third leaf (counted from wrapper leaves) were examined in the following section. Spraying calcium chloride following head formation until harvest (Ca2) and during head formation (Ca3) reduced tipburn severity by about 50 % compared to untreated plants (Fig. 3). Calcium spraying at its highest frequency, starting one week after planting until harvest (Ca1), had no effect on number of tipburn symptoms per leaf. Efficiency of Ca-sprayings in reducing tipburn severity therefore was dependant on plant development. Ca sprays in an advanced stage of plant development were more effective in reducing severity of lettuce tipburn.

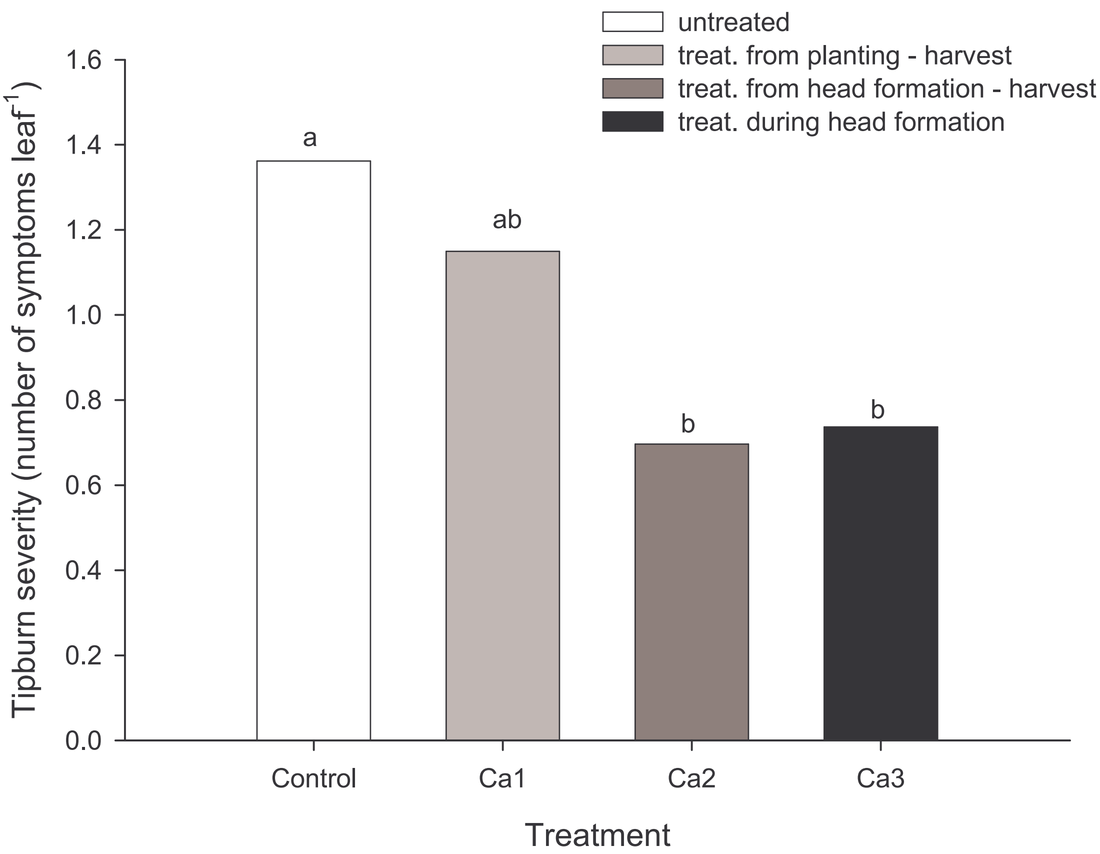

Fig. 3: Effect of foliar applications of calcium chloride on tipburn severity in iceberg lettuce 'Chianti' at harvest. Severity is indicated by the mean number of tipburn symptoms of leaves 1-3, counted from the wrapper leaves. Different letters indicate statistical differences at $p<0.05$.

3.1.3. distribution of Ca^{2+}, Mg^{2+} and K^{+} in iceberg lettuce leaves

Tipburn is caused by local calcium deficiency in the leaf margins. Therefore the influence of spraying $CaCl_2$ to lettuce plants on calcium distribution within single leaves was in the focus of the study. Distribution of minerals within the leaves was examined by separating leaves into blade, margin of unaffected leaves and margin of tipburned leaves. Concentrations of the analysed cations within the sampled leaves are presented in Tab. 3.

Calcium concentrations were highest in the leaf lamina and averaged 0.48 % of dry matter (DM). Towards the margins, a reduction in Ca content was observed. Within the leaf margins Ca content was approximately 0.24 % of DM. A tendency to higher Ca concentrations in tipburned leaves, as documented by our results, is in contrast to the values expected (BARTA and TIBBITTS 2000). Calcium content in the different leaf regions was not affected by applications of $CaCl_2$. The relative concentrations of potassium and calcium (K/Ca) in a total mean were lowest in the leaf blade (9:1), increased significantly in the margins up to 15:1 in unaffected and 14:1 in injured leaves, respectively. This may indicate a larger sensitivity for Ca-deficiencies in the margins due to a competition between Ca^{2+}- and K^{+}-ions. Spraying calcium chloride had no effect on K/Ca ratio within the sampled leaf parts. Regarding over-all values, discrepancies between the results of the present study and the work of BARTA and TIBBITTS (2000) could be explained by examining leaves of different stages of plant development.

Magnesium concentrations were lowest in the leaf lamina and increased towards leaf margins, indicated by a decrease in Ca/Mg ratio. Ca/Mg ratio <1 in tipburned leaves revealed that Mg-concentration was higher than Ca-concentration, whereas an inverse relationship was observed in healthy plant parts. Ca/Mg ratio ≥ 1 corresponds to lower tipburn severity regarding unaffected leaves (Tab. 3).

Tab. 3: Influence of foliar applications of $CaCl_2$ on calcium content as well as K/Ca and Ca/Mg ratio, respectively of selected (% DM) parts of iceberg lettuce leaves at harvest time. Numbers indicate standard error (±), differences between the treatments were not significant. Legend: Ca1=1 week after planting until harvest; Ca2=following head formation until harvest; Ca3=during head formation.

Ca content (% DM)

	blade	unaffected leaf margin	tipburned leaf margin
Control	0.48 ±0.09	0.18 ±0.02	0.28 ±0.02
Ca1	0.49 ±0.09	0.21 ±0.02	0.28 ±0.05
Ca2	0.48 ±0.05	0.19 ±0.04	0.21 ±0.04
Ca3	0.43 ±0.04	0.21 ±0.02	0.27 ±0.04

K/Ca

	blade	unaffected leaf margin	tipburned leaf margin
Control	10.1 ±1.26	16.9 ±2.04	12.9 ±1.42
Ca1	8.9 ±0.59	15.4 ±0.80	13.3 ±2.30
Ca2	8.6 ±0.43	16.3 ±2.03	16.1 ±3.89
Ca3	8.6 ±0.48	14.5 ±1.49	14.0 ±1.48

Ca/Mg

	blade	unaffected leaf margin	tipburned leaf margin
Control	3.02 ±0.54	0.82 ±0.09	0.74 ±0.07
Ca1	2.72 ±0.64	0.88 ±0.17	0.90 ±0.15
Ca2	2.88 ±0.48	1.04 ±0.25	0.79 ±0.17
Ca3	2.49 ±0.46	1.00 ±0.06	0.79 ±0.05

4. Discussion

As demonstrated above (Fig. 1), a reduction in percentage of tipburn affected plants could not be accomplished by calcium treatments in the field trials described, which corresponds to previous results (CORGAN and COTTER 1971; MISAGHI et al. 1981). The most effective treatment, spraying $CaCl_2$ following head formation until harvest, was also applied in field experiments with butterhead lettuce in 2002 (data not presented). However, a decrease in tipburn injury could not be observed either. In some cases, the treatment even partially aggravated the injury (MAROTO et al. 1985). Nevertheless, it could be shown in our study that there were effects on tipburn severity. However, the efficiency of $CaCl_2$ sprays to reduce severity of tipburn symptoms in iceberg lettuce was dependant on plant development. When $CaCl_2$ was applied in the advanced stage of plant development, when heads were almost closed, tipburn severity could be reduced significantly. Higher frequencies of application, starting at an earlier plant developmental stage, were not able to reduce the injury (Fig. 3). These results are contradictory to results obtained in previous investigations, where a tipburn-reducing effect could be achieved by a high frequency of calcium sprayings in lettuce (ABD EL-FATTAH and AGWAH 1987; SONNEVELD and VAN DEN ENDE 1975; THIBODEAU and MINOTTI 1969).

The effectiveness of foliar applied calcium chloride should be enlightened by its ability of influencing the calcium distribution in lettuce leaves. The separation of leaves into tipburn affected and unaffected margins and leaf blade, respectively, was done to elucidate the distribution of mineral nutrients across a lettuce leaf. The concentration gradient of mineral elements from leaf lamina to the margins corresponded to what is documented in the literature (BARTA and TIBBITTS 1991b; BARTA and TIBBITTS 2000; SONNEVELD and MOOK 1983). It could be shown that Ca-sprays had no effect on total calcium content in leaf blade and margins of iceberg lettuce (Tab. 3). THIBODEAU and MINOTTI (1969) obtained similar results in bibb lettuce. Regarding head lettuce, no influence of foliar applications of several Ca-salts could be found, either (ABD EL-FATTAH and AGWAH 1987).

The analysis of total magnesium content revealed that higher concentrations were located in tipburned leaves as compared to healthy margins (BARTA and TIBBITTS 1991b; BARTA and TIBBITTS 2000). In combination with lower Ca concentrations in the margins, magnesium and potassium, as competitive elements to calcium, may have forced tipburn symptom development (ASHKAR and RIES 1971; SONNEVELD and MOOK 1983). In our study, the relative cation concentrations (Ca/Mg, K/Ca) were lower than described in other examinations (BARTA and TIBBITTS 1991a; BARTA and TIBBITTS 1991b; BARTA and TIBBITTS 2000). The K/Ca ratio, which also plays an important role in the development of bitter pit in apple (HAEFS 2001), could not be influenced by calcium chloride application in lettuce leaves, either.

BARTA and TIBBITTS (1991b) pointed out that Ca-content in tipburned leaf margins is lower than in healthy parts. They came to the conclusion that Ca-concentrations below 0.4 mg g^{-1} dry weight led to the collapse of the surrounding tissue. Mineral concentrations were estimated in the youngest leaves by electron microprobe x-ray analysis, which allowed to analyze small discrete areas of the leaf. In the present study Ca^{2+} content was estimated in field grown lettuce by sampling 0.5 cm wide stripes out of older leaf margins. The sample also included veinal tissue and therefor may have resulted in higher Ca-concentrations. Besides, this method of analyzing mineral contents in smaller samples of lettuce leaves proved not to be sensitive enough to detect smallest changes in tissue mineral content compared to the x-ray microprobe analysis mentioned above. Thus, it is critical to give a general threshold value for a tipburn inducing calcium concentration (in DM), when measurements are made in youngest and smallest leaves (30 mm). A collapse of cell walls due to a lack of calcium tissue occurs in further developed leaves as well. These leaves were generally containing higher mineral concentrations. The over-all concentrations in the plant tissue also strongly depend on the nutritional status of the plants. That means, that over-all concentrations are hardly comparable. Therefore, data should preferably be expressed as relative cation content, e.g. K/Ca ratio. The concentration gradient across the leaves was independent from leaf age (BARTA and TIBBITTS 2000). Such data therefore are more comparable to mineral content of leaves of varying developmental stages.

In general, these analytical results correspond to what is documented in literature (ASHKAR and RIES 1971; COLLIER and HUNTINGTON 1983; CRESSWELL 1991; SONNEVELD and MOOK 1983; THIBODEAU and MINOTTI 1969). Regarding magnesium, the concentrations found in the present study were slightly lower. In the case of calcium, higher concentrations were determined as a result of analyzing composite samples, whereas other investigations focused on individual leaves at earlier stages of development (BARTA and TIBBITTS 1991b; BARTA and TIBBITTS 2000).

Due to the inability of calcium sprays to increase Ca-content in lettuce, it has to be discussed if sufficient foliar uptake of calcium occurred due to application.
Leaf cuticles are the main barrier for exogenously applied substances prior to tissue uptake. $CaCl_2$ penetrates the leaf cuticle through aqueous pores. The penetration rate is improved by high relative humidity and higher Ca-concentrations (SCHÖNHERR 2000). These prerequisites were assured by spraying $CaCl_2$ in the evening hours and using a Ca-concentration of 5 g l^{-1}. The uptake rate may have been too small to be detected by this method of leaf sampling and analysis per AAS. However, it was guaranteed by the time of application, that leaves were moistened as long as possible to enhance foliar penetration of Ca^{2+}. At least smallest amounts of $CaCl_2$ should have penetrated into the leaf tissue, as tipburn severity could be reduced in two of the $CaCl_2$ treatment groups. Concerning other horticultural crops, such as chervil (KLEEMANN 1999) or tomato (SONNEVELD and VAN DEN ENDE 1975) a significant effect of Ca-sprays increasing Ca-content in leaves is well documented. By adding an adjuvant, the effect could even be enhanced in apple fruits (HAEFS 2001). Analysis of leaf samples of greenhouse grown lettuce could not confirm this positive effect of the adjuvant (HOLTSCHULZE et al. 2001).
Exogenous calcium chloride applications seemed to be more effective in slow growing organs, such as apple or tomato fruits, than in fast growing tissue, like lettuce leaves. This may lead to the assumption that lettuce growth rate, and thus the requirement for Ca^{2+}, under tipburn promoting conditions is higher than the

velocity of uptake exogenous applied calcium for cell wall stabilization. Besides, apple fruits have the ability to use lenticels as special pathway for the uptake of exogenously applied calcium chloride (SCHLEGEL and SCHÖNHERR 2004). Experimental models describing foliar uptake or cuticular penetration are well documented by the use of isolated cuticles for various horticultural crops (BAUR and SCHÖNHERR 1994; HAEFS 2001; KNOCHE and BUKOVAC 1999; MARZOUK et al. 1998; SCHÖNHERR 2000). The availability of a model similarly suited for the penetration of minerals through lettuce leaves would provide a fundamental prerequisite for advanced application experiments. Concentration studies and the evaluation of penetration promoting substances should be performed. However, it is technically not possible to prepare isolated cuticles from lettuce leaves. In order to examine the tipburn preventive potential of suitable substances, a testing system has to be established in which the performance of experiments under standardized environmental conditions is permitted. In experiments currently planned, lettuce plants are cultivated in a growth chamber under tipburn inducing climatic conditions (Chapter C), with standardized nutrition, temperature, relative humidity and light intensity. Untreated plants will definitely develop tipburn symptoms under such conditions and can be compared to selected treatment groups of calcium application. Due to the constant growing conditions, experiments can be repeated independent from seasonal effects, and a suitable treatment for tipburn prevention can be identified by screening various alternatives.

Tipburn symptoms in iceberg lettuce were found most frequently in the outer leaves, directly underneath the wrapper leaves (Fig. 2). Examinations of tipburn development in butterhead lettuce revealed that symptoms mainly occurred in inner leaves (COLLIER and HUNTINGTON 1983, HOLTSCHULZE unpublished data). The head of iceberg lettuce plants is completely enclosed by the wrapper leaves and transpiration of the inner leaves is therefore hindered. Outer leaves of butterhead lettuce were free of tipburn because transpiration is still possible due to the differing growth habit.

Tipburn assessment in iceberg lettuce can be accomplished by examining the outer 3 leaves with wrapper leaves excluded. Due to the greater variances, differences in tipburn severity between different treatments were to be expected in these leaf positions (Fig. 2). The data of the first three leaves were in agreement with the data of all the leaves examined (data not presented). The standard deviation of symptom severity, which often causes great problems in interpreting the results of investigations on tipburn, could even be reduced. This way of estimating tipburn severity seemed to be more precise than cutting whole heads into half, since smaller necrosis may not be detected then (HAGEL et al. 2002; WISSEMEIER and ZÜHLKE 2002).

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6. Summary

The effectiveness of calcium chloride applications to prevent tipburn incidence was evaluated in field grown iceberg lettuce cv. ‘Chianti’. Calcium chloride was applied at a concentration of 0.04 mM and varied according to the developmental stage of the plants which resulted in differing frequencies of application. Data indicate that severity of tipburn symptoms can be reduced when plants are treated in an advanced stage of plant development, at the time of head formation. Analysis of calcium distribution between differing sections of lettuce leaves gave no evidence of a sufficient foliar uptake of calcium chloride. Due to the lack of a model appropriate for foliar uptake of nutrients in lettuce, an alternative approach of a standardized testing procedure is discussed.

C. EFFECT OF REDUCED VAPOUR PRESSURE DEFICIT DURING NIGHT TIME ON ROOT PRESSURE AND CALCIUM DISTRIBUTION IN LETTUCE PLANTS

1. Introduction

Calcium deficiency in heading vegetables frequently occurs during periods of high transpiration, particularly, when plants are subjected to a high VPD at the time of head formation (PALZKILL and TIBBITTS 1977). Conditions that were promoting calcium deficiency disorders could be diminished by regulation of climatic conditions in greenhouse grown tomato or pepper plants (VOGEL 1996). Trials to reduce tipburn injury in vegetable plants by control of climate factors proved to be successful for Chinese cabbage (VAN BERKEL 1988; KUO et al. 1981). Tomato plants that were subjected to diurnal fluctuations in VPD responded with less severe symptoms of Ca deficiency. Transpiration rates of the plants are supposed to be restricted and root pressure enhanced by a decrease of VPD during dark period in the greenhouse (ADAMS 1991). The enforcement of root pressure during the dark period therefore was regarded as a promising method to prevent tipburn in greenhouse grown lettuce.

In previous studies root pressure enhancement was indicated by the onset of guttation of leaves (PALZKILL and TIBBITTS 1977). However, this method does not allow quantification of root pressure. In order to force exudation by root pressure, the xylem sap pressure has to be increased above the atmospheric pressure by technical means. This can either be achieved by generating pressure at the root system or by decapitation of the shoot system (MARSCHNER 1995; SCHURR and SCHULZE 1995). The utilization of a pressure bomb was not supposed to be practicable in lettuce plants due to their soft hypocotyls (SCHOPFER and BRENNICKE 1999). Regarding the latter, i.e. decapitating plants and collecting xylem sap for a short period of time, the results obtained were comparable to those of intact and transpiring plants (SIEBRECHT 2000). Data on xylem composition in a daily period do not exist for lettuce (WISSEMEIER 1996).

The aim of this study was to examine the effect of a diurnal reduction in VPD on root pressure of lettuce plants. Furthermore, the diurnal course of quantity and quality of root exudates as well as their constituents was recorded and correlated with the occurrence of tipburn symptoms.

2. Material and Methods

2.1. Site of Experiments

The experiments were carried out in a greenhouse at the Experimental Station Marhof associated with the Institute of Horticulture, Science University of Bonn. The station is located 15 km to the north of Bonn between ‘Vorgebirge’ and ‘Kölner Bucht’ at 50° 49” northern parallel (6° 59” eastern longitude) in the Rhineland area, an area of intensive fruit and vegetable production.

The greenhouse climate was controlled and recorded by a RAM (Hersching, Germany) climate control computer. Additionally, air temperature and relative humidity were recorded within the planting by mini data logger (Fa. Meilhaus Electronic, Puchheim, Germany), installed in weather-instrument shelters. Vapour pressure deficit (VPD) was calculated according to FAO Penman-Monteith formula (ALLEN et al. 1998).

2.2. Plant Material

Experimental plots were planted in different sets starting in 02/2002 until 06/2002 and from 10/2002 to 07/2003 respectively. Tipburn susceptible cultivar ‘Herman’ (Fa. S&G, Kleve) was set up in rockwool cubes or in pots containing perlite (granulation 0-3 mm) employing one plant per cube and pot, respectively. Cubes or pots were placed in saucers to which nutrient solution was applied. Each experimental plot consisted of 64 plants per treatment, random samples were taken for each measurement.

Lettuce plants grown in rockwool were determined for root pressure measurements. A 10 mm piece of a PE-tube (18 mm inner diameter) was fixed on top of the cube to ensure homogenous sampling spots due to an upright development of the hypocotyls.

Plants were fertilized with a nutrient solution containing: [mM/L] 13.7 N; 1.65 P; 1.95 K; [µM/L] 650 MgO; 53 Fe; 1200 Ca; 9 Mn; 9 B; 1.5 Cu; 1.5 Zn; 0.25 Mo at a pH of 6.0-6.5. Nutrient solution was supplied according to plant development.

2.3. Treatments

After development of the 3rd-4th leaf, the plants were transferred into separate greenhouse chamber with different climate settings. Corresponding to natural day length a 16-h photoperiod was maintained by additionally applied assimilation light (Philips SON-T agro) at a photosynthetic photon flux of 350±50 µmol^{-2} s^{-1}. Tipburn-inducing climate was established according to BARTA and TIBBITTS (2000). Air temperature and relative humidity averaged 20°C±2 and 65%±10, respectively, throughout light and dark period, and assured a vapour pressure deficit of 0.82 kPa (VPD-1). Alternatively, a diurnal change of VPD was realized by an increase of relative humidity, while air temperature was kept constant in order to avoid temperature effects. VPD was reduced to 0.0 kPa during the dark period (VPD-2) by a high pressure fogging system. As a consequence of the dry season in 2003 rain-water feeding the fogging system was a limiting factor temporarily. During those periods reduction of VPD was achieved by covering the plants with layers of poly-ethylene (KUO et al. 1981; VAN BERKEL 1988). By either way VPD was decreased by increasing relative humidity up to 100%, while keeping temperature constant at 20°C.

The experiments were established as complete randomized design, with each treatment replicated 4 times.

2.4. Evaluation of Tipburn, Recording root pressure and transpiration rate as well as preparation of sample for analysis of minerals

Tipburn symptoms were evaluated weekly and recorded as percentage of tip-burned plants. Plants with at least one leaf showing tipburn symptoms were scored as "tipburned plants". Since under greenhouse conditions the heads of lettuce plants did not close completely, evaluation of tipburn incidence was possible without destroying the plants.

Measurements of transpiration rates were conducted with a Ciras-I portable porometer (PP-Systems, Hitchin, U.K.). VPD in the sample chamber was analogous to the surrounding atmosphere. Transpiration rate was recorded as mmol $m^{-2} s^{-1}$.

Root pressure was measured from decapitated plants that were grown in rockwool. Released latex was washed off with distilled water and remaining water carefully removed by tissue paper. A silicon pipe was fixed and sealed to the stump as reservoir for root pressure exudate, which was pipetted and weighed after 1 h in order to determine the amount of exudate flow in g h^{-1} (MORAD et al. 2000). Four plants were probed individually every four hours six times a day. These samples were combined for further examination of relevant exudate constituents. They were frozen and stored at -20°C until analyses of calcium, magnesium and potassium content by atomic absorption spectrometry (AAS) were conducted.

2.5. Sample preparation for analysis of minerals

Plants grown in perlite were used for analysis of minerals in various plant parts. Ten plants representing the treatment were taken out of each plot. They were divided into outer leaves, inner leaves and roots. Roots were washed with distilled water in order to remove adherent substrate. Samples were dried at 60-80°C (24 h) and for 2 h at 105°C before grinding. An aliquot of 0.3 g was digested with HNO_3 and H_2O_2. Total calcium, magnesium and potassium content was analyzed by AAS.

2.6. Statistical analysis

Plants were cultivated on different greenhouse benches. Since between the benches homogeneity could be assumed for all the environmental factors relevant to the experiment the test plants were arranged in completely randomized plots. In order to realize treatments with two different humidity regimes the plants had to be assigned to different cabins (or tables, respectively). Samples were randomly chosen out of 65 single plants per treatment. Statistical data were calculated with the statistic program 'SAS 8.0' (SAS Institute Inc., Cary, USA). Data were subjected to analysis of variance. Comparisons of mean values were performed by Tukey's HSD or t-test. Statistical differences were accepted at 5 % level of significance. Graphs were prepared with SigmaPlot 8.0 (SPSS Science Inc.).

3. Results

3.1. Effects of decreasing VPD during night time on

3.1.1. tipburn incidence in lettuce

Tipburn inducing climate was maintained in the greenhouse according to BARTA and TIBBITTS (2000). The setpoint-temperatures were realized in the greenhouse (20°C, 65% relative humidity). Corresponding with global irradiation, air temperature exceeded the setpoint temporarily. Likewise relative humidity declined below 65 % (VPD-2 treatment) in periods of dry conditions (VAN BERKEL 1988). Consequently, VPD was slightly increased. Tipburn injury developed in the first mature leaves which are involved with the beginning of head formation. Exposed and enclosed leaves were equally damaged. Particularly in plots planted from March onwards typical tipburn symptoms could be observed. As shown by the data given in table 1 tipburn incidence could be reduced by lowering VPD during dark period (VPD-2). In the first set tipburn was reduced by about 55% compared to plants at a constant VPD level of 0.82 kPa (day and night). In the second set tipburn was reduced by about 25%. Therefore, it was concluded that for plants susceptible to tipburn the risk of symptom development could be significantly reduced by specific control of climate. Suggestions for the underlying processes will be given in the following.

Tab. 1: Effect of reduced VPD during dark period (VPD-2) on tipburn incidence (%) in lettuce cv. 'Herman'. VPD-1 = 0.82 / 0.82 kPa (day/night); VPD-2 = 0.82 / 0.0 kPa (day/night). Letters indicate statistical differences between the treatments at $p<0.05$.

Planting date -	harvest date	VPD-1	VPD-2
03/27 -	05/13/02	92.9 % *a*	38.5 % *b*
03/24 –	05/14/02	87.5 % *a*	62.5 % *b*

3.1.2. transpiration rate and root pressure of head lettuce

Transpiration rates were measured during periods of assimilation light or when days were cloudless in order to avoid effects of changing light intensities on plant transpiration. Due to the increase of relative humidity during night time in the VPD-2 treatment, leaves were moistened and therefore for technical reasons not suitable for measurements with the Ciras-I. Transpiration rates of butterhead lettuce 'Herman' (Fig. 1) indicated that diurnal changes in VPD (VPD-2) significantly decreased transpiration during daytime compared to plants that were constantly kept at conditions of high transpiration demands (VPD-1).

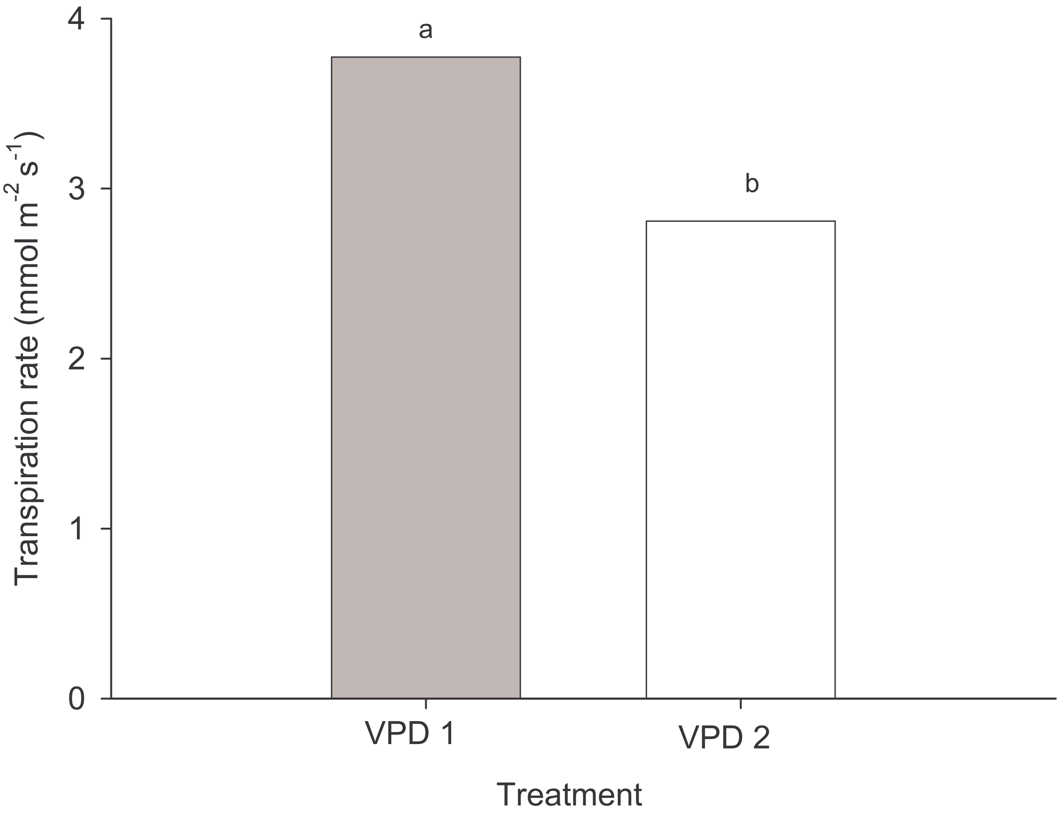

Fig. 1: Transpiration rates of head lettuce cv. 'Herman' as affected by reduced VPD during night time. Measurements on 10 plants (2 leaves plant-1) were conducted at 04:00 p.m. (±0.5 h). VPD-1 = 0.82 / 0.82 kPa (day/night); VPD-2 = 0.82 / 0.0 kPa (day/night). Letters indicate significant differences at p=0.05 (t-test).

There is general agreement that high relative humidity during night time should force root pressure of plants and consequently, calcium transport to inner leaves improved (COLLIER and TIBBITTS 1984; KUO et al. 1981; VAN BERKEL 1988; PALZKILL and TIBBITTS 1977). In the present study root pressure flow was determined in order to measure the impact of a diurnal change in VPD on root pressure in lettuce plants during night time. When plants were subjected to reduced VPD (VPD-2) they did not respond with increased root pressure flow during the dark period (Fig. 2), but elevated amounts of root pressure exudate were found in the light period (18:00 h), prior to the onset of VPD-2 treatment.

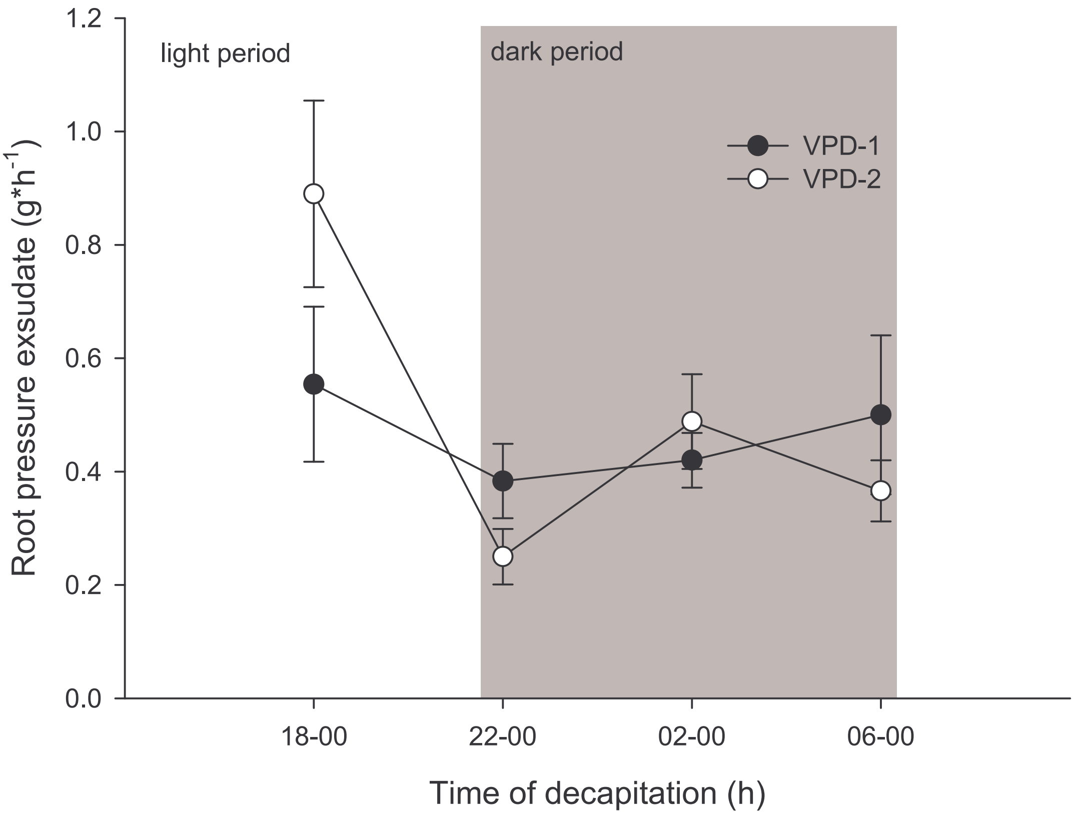

Fig. 2: Effect of reduced VPD during night time on root pressure of head lettuce, indicated as amount of root pressure exudate (g h^{-1}) of decapitated plants. VPD-1 = 0.82 / 0.82 kPa (day/night); VPD-2 = 0.82 / 0.0 kPa (day/night). Onset of experiment (= treatment) on 03/27/02, measurements were taken after 34 days of treatment. Bars indicate standard error.

3.1.3. mineral content of root pressure exudate and distribution of Ca^{2+}, Mg^{2+}, and K^{+} within the plant

Since the amount of root pressure exudates varied most at 06:00 p.m. (Fig. 2), effects of the VPD treatments were to be expected rather during light than during dark period. Therefore, measurements of root pressure exudates followed the same method throughout a 24 hours period. It was known from previous measurements that probing four plants and combining samples gave more reliable material for analysis of cations. Particularly during night time, when root pressure flow declined, the sample size required was critical but guaranteed each time by combining four samples.

The diurnal course of root pressure flow is presented in Fig. 3A. It is obvious that root pressure flow was at its minimum during dark period. Maximum values occurred when the measurements were started in the light period (10:00 a.m.). Afterwards they declined constantly. Plants that were subjected to conditions of continuously high transpiration (VPD-1) developed slightly higher root pressure flow during daytime.

Therefore, the reduction in tipburn incidence parallel to decreasing VPD during night time as mentioned above (Tab. 1), could not be explained by elevated root pressure flow. The lower root pressure of the VPD-2 treatment corresponded well to the lower transpiration rates as presented above (Fig. 1).

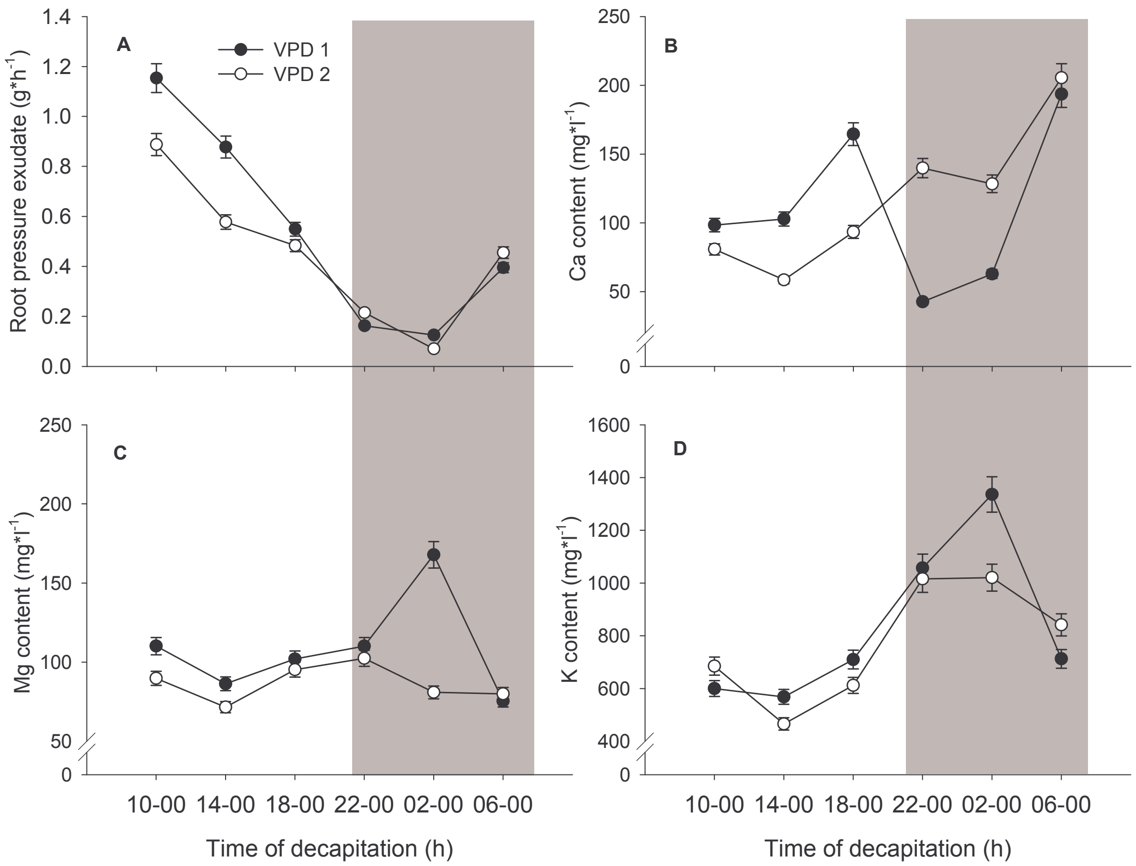

Fig. 3: Effect of reduced VPD during dark period on root pressure (A) and mineral content of root pressure exudate (B-D) in head lettuce. Exudate was collected for 1hour after decapitation every 4 hours and monitored for 24 hours. Each value represents a mean out of 2 planting sets.
VPD-1 = 0.82 / 0.82 kPa (day/night); VPD-2 = 0.82 / 0.0 kPa (day/night). Shaded areas refer to night period. Error bars indicate LSD (5%)

Mineral content of the root pressure exudate is described in figure 3B-D. The concentration in the exudate was highest, when root pressure flow declined. Lowering VPD during night time resulted in lower cation concentrations in the root pressure exudate during daytime. Ca^{2+} and K^{+}-concentrations increased during the night period, whereas a diurnal rhythm could not be obtained for Mg^{2+}. If plants were cultivated at a constant level of VPD, Mg^{2+}- and K^{+}-concentrations were higher during dark and light period. The Ca^{2+} concentrations in the exudate decreased considerably during the night. Plants exposed to a constant level of VPD seemed to be capable of transporting higher amounts of exudate and minerals during daytime.

However, ion competition in the xylem sap during the night seemed to be of greater consequence for potential tipburn induction.

The K/Ca ratio in the xylem sap of the VPD-1 plants increased remarkably at the beginning of the night period and dropped afterwards to a similar level as at daytime (Fig. 4). Plants that were subjected to a diurnal change of VPD had a constantly lower K/Ca ratio for both light cycles. Furthermore, the Ca/Mg ratio was higher during the night, indicating higher concentrations of Ca^{2+} than Mg^{2+}. At daytime K/Ca ratio varied between 4.5 and 6.2 in the VPD-1 treatment as well as between 7 and 10.6 in the VPD-2 treatment, respectively.

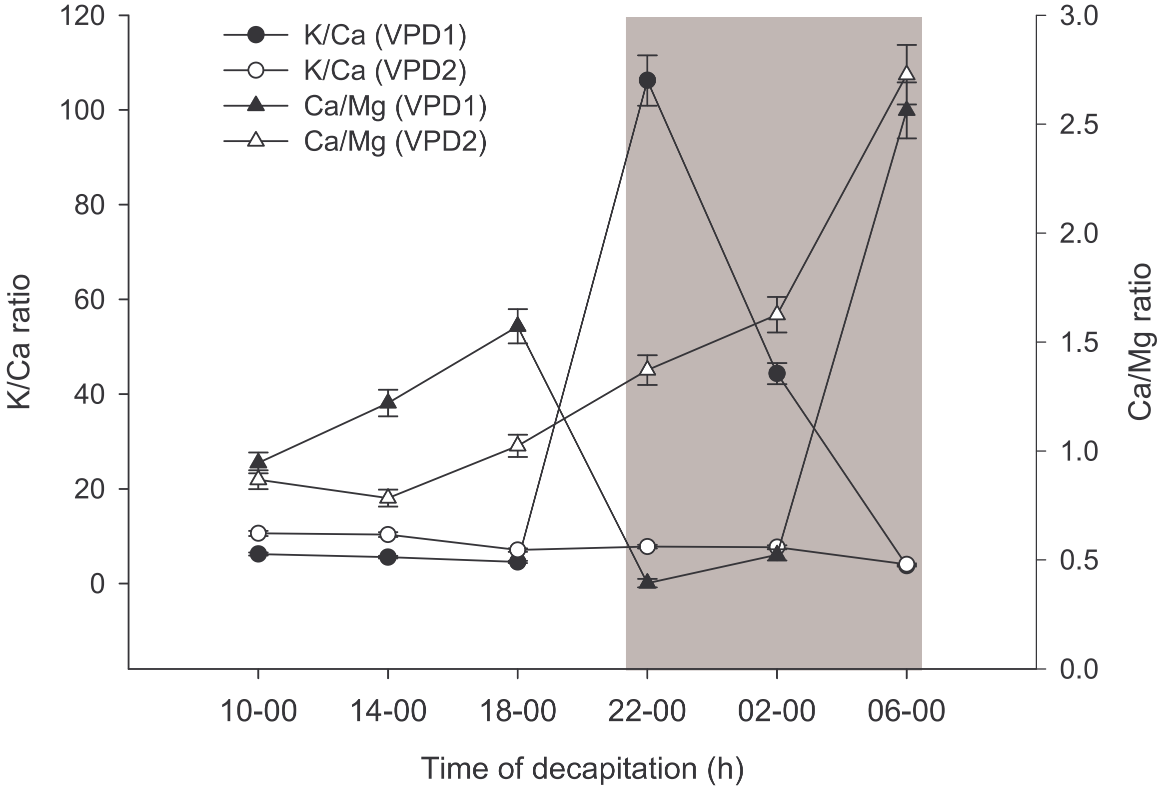

Fig. 4: Effect of reduced VPD during dark period on K/Ca and Ca/Mg ratios in the root pressure exudate of head lettuce. Exudate was collected for 1 hour after decapitation every 4 hours and monitored for 24 hours. Each value represents a mean out of 2 planting sets. VPD-1 = 0.82 / 0.82 kPa (day/night); VPD-2 = 0.82 / 0.0 kPa (day/night). Shaded areas refer to night period. Error bars indicate LSD (5 %).

By examination of nutrient concentrations in diverse parts of the plant tissue, information is to be expected on the significance of cation competition in the xylem exudate for mineral distribution in different plant parts. The plants were sectioned in outer leaves, inner leaves and roots for determination of dry matter and mineral content (Tab. 2). A decrease of VPD during night time (VPD-2) lead to lower DM in the plant parts probed. However, Ca-, Mg- and K-contents were significantly higher in outer and inner leaves, whereas concentrations in the roots were only slightly higher. Lower leaf Ca-concentrations in the VPD-1 treatment group corresponded to a higher incidence of tipburn (Tab. 1). Since the cation concentrations were generally increased in the VPD-2 variant, a significant effect of the VPD on the relative cation concentrations could not be found. The Ca/Mg ratio of the leaves was high (between 4.1 and 5.1) compared to values found in iceberg lettuce leaves (Chapter B). This in fact did not indicate an ion competition with reference to calcium. A lower Ca/Mg ratio in the roots corresponded to the values found in the exudate (Fig. 4). The K/Ca ratios were almost homogenous throughout the leaf sections and were only slightly lower in the roots. Measurements of the xylem sap revealed up to two times higher K/Ca ratios.

Tab. 2: Effect of reduced VPD during night time on dry matter production and distribution of calcium, magnesium and potassium in butterhead lettuce. VPD-1 = 0.82 / 0.82 kPa (day/night); VPD-2 = 0.82 / 0.0 kPa (day/night). Numbers (±) indicate standard error, differences between the VPD treatments were significant at p=0.05 according to t-test.

	VPD level	Dry matter (%)		Ca (% DM)		Mg (% DM)		K (% DM)		K/Ca	Ca/Mg
outer leaves	VPD-1	7.2	±0.22	1.52	±0.05	0.30	±0.01	5.6	±0.18	3.7	5.1
	VPD-2	4.9	±0.18	1.70	±0.09	0.39	±0.01	7.0	±0.25	4.1	4.4
inner leaves	VPD-1	8.9	±0.08	0.87	±0.04	0.21	±0.01	3.5	±0.11	4.0	4.1
	VPD-2	5.2	±0.67	1.09	±0.09	0.26	±0.02	4.8	±0.35	4.4	4.2
roots	VPD-1	11.4	±0.64	0.66	±0.05	0.25	±0.02	1.9	±0.18	2.9	2.6
	VPD-2	7.1	±0.22	0.69	±0.05	0.35	±0.03	2.1	±0.16	3.0	1.9

4. Discussion

Tipburn symptoms can be induced in lettuce plants that were cultivated in the greenhouse under tipburn favoring conditions (BARTA and TIBBITTS 2000). Assimilation light was applied in order to achieve a 16-h light period. While using an outdoor lettuce cultivar, the onset of tipburn symptoms was, besides the VPD, dependant on meteorological day length, too. Therefore symptoms occurred in plots that were planted with the beginning of March. The utilization of this method to induce tipburn in the greenhouse without air-conditioning is restricted by higher day temperatures from the beginning of July. In general, the cultivation of plants in greenhouse cabins for research purposes gives the advantage of larger sample sizes. For the experiments described above this approach proved to be successful for two years.

A tipburn inducing climate was maintained by a constant VPD of 0.82 kPa. Plants that were subjected to these conditions developed tipburn symptoms before head formation, as soon as inner leaves started to close. A decrease of VPD during the night reduced tipburn incidence of the lettuce plants (Tab. 1). Similar observations were described previously regarding other vegetable plants (COLLIER and TIBBITTS 1984; PALZKILL and TIBBITTS 1977; VAN BERKEL 1988). A complete prevention of tipburn was strongly dependant on the cultivar (KUO et al. 1981; own observations: data not shown). Young, rapidly expanding tomato leaves may develop Ca-deficiency symptoms in high humidity atmosphere. When plants were cultivated with diurnal fluctuations in VPD, the tomato leaves showed less severe Ca-deficiency symptoms (ADAMS 1991). Tipburn enhancement through a constantly high VPD can partly be explained by higher transpiration rates. Higher incidence of leaf tipburn at strawberry leaves could be associated with higher transpiration rates (MASON and GUTTRIDGE 1975). Higher transpiration rates of the plants can generally be related to a higher VPD (BAAS et al. 2003). Therefore, a reduction in tipburn incidence can be related to lower transpiration rates of the VPD-2 plants (Fig. 1). If mean values of VPD-1 = 0.82 kPa and VPD-2 = 0.55 kPa throughout a 24 hours period were assumed, lower transpiration rates correspond to the lower VPD-2 treatment. In the present study transpiration rates could not be measured during the night due to porometer calibration problems at high relative humidity and leaf wetness. For further investigations it would be recommended to measure the diurnal course of transpiration rates by weight-loss method.

It has further been suggested that tipburn incidence could be reduced by an improved Ca transport to the younger leaves as a result of lower transpiration rates and root pressure enhancement (COLLIER and TIBBITTS 1984). In previous trials, root pressure enhancement was indicated indirectly by an increase of guttation within high humidity atmosphere during night time (VAN BERKEL 1988; KUO et al. 1981; PALZKILL and TIBBITTS 1977). In the present experiment root pressure flow was examined by decapitating plants and determining xylem exudate release rate (SCHURR and SCHULZE 1995; MORAD et al. 2000;). Further effects that may have had an influence on xylem exudate flow, e.g. transpiration, could be excluded with this method (MARSCHNER 1995). By reducing VPD (increase of relative humidity respectively) during dark period no strengthening of root pressure flow could be observed (Fig. 2). Exudate flow was even reduced during the light period compared to the VPD-1 plants (Fig. 3A). The prevention of tipburn injury in lettuce by reducing VPD during night time therefore cannot be explained by an enhancement of root pressure flow only. These results were in contrast to what was suggested by other authors (COLLIER and TIBBITTS 1982; KUO et al. 1981; PALZKILL and TIBBITTS 1977; VAN BERKEL 1988).

Mineral nutrient contents, above all calcium, of the exudates examined can be attributed almost completely to xylem sap. It may contain certain amounts of shoot derived potassium, though (MARSCHNER 1995). The dynamics of mineral nutrient concentrations in the root pressure exudate of lettuce was monitored for 24 hours. Nutrient transport to the xylem is strongly dependant on intact phloem transport to the roots. More than one hour after detopping the plants, nutrient concentrations of the xylem began to decline (SCHURR and SCHULZE 1995; own observations). When collected for a short time after decapitation, exudate concentrations were similar to those of intact transpiring plants (SIEBRECHT 2000). Sampling root pressure exudate of lettuce plants for one hour after decapitation every four hours therefore was found to be an appropriate approach for the examination of the cation concentrations in the root pressure exudate.

A diurnal fluctuation in the xylem sap concentration of the cations was observed (Fig. 3B-D). High mineral concentrations were estimated around the beginning of daytime . During light period concentrations declined and increased again at the beginning of night period. Nutrient concentrations were enhanced when xylem volume flow decreased (MARSCHNER 1995; SCHURR and SCHULZE 1995). Ca^{2+} concentration in the xylem exudate of lettuce was enhanced by a diurnal decrease of VPD compared to plants subjected to a constantly high VPD. Mg^{2+} and K^{+} concentrations were decreased at the same time. The qualitative composition of the xylem exudate therefore seemed to be of greater importance for explaining the reduction of tipburn injury through daily fluctuations of VPD. A cation competition particularly during night period, indicated by the K/Ca ratio of the VPD-1 variant, could be responsible for an insufficient uptake of calcium. In general, the observed mineral composition of the xylem sap did not correspond completely to what has been examined previously in *Populus ssp.* Seedlings (SIEBRECHT 2000). The analyzed nutrients were undergoing similar daily fluctuations, when plants of the VPD-2 treatment group were concerned. However, the K^{+} concentration declined during the night in *Ricinus communis* plants (SCHURR and SCHULZE 1995). These deviations are attributed to the different plant species.

Occasionally lower mineral concentrations in the root pressure exudate of the VPD-2 plants (Fig. 3) could not be confirmed by analysis of leaf tissue (Tab. 2). Dry matter production declined and mineral contents were higher when plants were subjected to low VPD during night time. These observations are confirmed by experiments with Chinese cabbage plants (KUO et al. 1981). Lower mineral concentrations in combination with higher dry matter percentage may be resulting from a dilution process within the leaf tissue. Diurnal fluctuations in relative humidity showed contrary results in tomato: low VPD (high relative humidity) at night initiated lower concentrations of Ca^{2+}, Mg^{2+} and K^{+} in the leaves (ADAMS 1991). Possibly the fruits instead of the leaves may have been benefiting from the decrease in VPD, due to a change in their source-sink relationship. Observations regarding the onset of blossom end rot or the mineral contents of the fruits were not given. TORRE et al. (2001) found lower Ca^{2+} concentrations in leaves and flowers when roses were cultivated at a constantly low VPD. Petal necrosis was initiated as consequence of a competition between flowers and higher transpiring leaves. Chinese cabbage plants accumulated lower amounts of ^{45}Ca when leaves were exposed to a constantly low VPD as well (PALZKILL and TIBBITTS 1977). The absence of a diurnal change in VPD leads to continuously lower transpiration rates, which could affect the amount of calcium transported via the xylem flow.

The ion competition in the exudate (Fig. 4), which is manifested by an increase of the K/Ca ratio at the time of 10:00 p.m., is not reflected in the leaves (Tab. 2). Root pressure samples were taken just after the onset of tipburn. The cation competition in the exudate of the VPD-1 plants may have had an effect on tipburn development, but was not lasting for sufficient time to influence tissue concentrations. On the basis of the data given above conclusions from root pressure exudate content cannot be drawn on the distribution of leaf minerals. A translocation rate from the root to the shoot could only be calculated if leaf analyses were carried out on the basis of fresh plant material (SIEBRECHT 2000). The decrease in leaf calcium concentration which was found in the VPD-1 treatment (high VPD day/night) could be related to a higher susceptibility to lettuce tipburn compared to plants of the VPD-2 treatment.

The K/Ca ratio in the leaves was unaffected due to the increase of both, Ca^{2+} and K^{+}, concentrations. The increase of the K^{+} concentration in the leaves is not understood, since it should have been unaffected by a fluctuation of VPD (MARSCHNER 1995). On the other hand, found an effect of VPD on K^{+} content of tomato leaves as well. The K/Ca ratio of complete tomato leaves, however, remained unchanged when plants were subjected to lower VPD at night. The K/Ca ratio in lettuce leaves at an advanced stage of maturity (Tab. 2) was lower compared to those of young developing leaves (BARTA and TIBBITTS 2000). OHLS (1989) again found relative cation concentrations in cabbage leaves that were comparable to matured lettuce leaves. Plants that were affected by leaf necrosis had a higher K/Ca ratio than unaffected leaves. It has been shown previously that a higher K/Ca ratio in tipburn affected mature lettuce leaves could not be found (Chapter B). It may possible that this gradient is only reflected when leaves were sectioned into smaller discrete areas. In faster growing tissue, such as lettuce leaves, possible changes in the K/Ca ratio (through a decrease of calcium) may be detected by the analysis of whole leaves. With the inclusion of veinal tissue, which

generally contains high amounts of calcium, smallest changes in the cation contents can be overlaid (BARTA and TIBBITTS 1991; JESCHKE and PATE 1991).
Compared to plants cultivated under diurnal changes of VPD, plants that were subjected to a continuously high VPD (day and night) had higher transpiration rates and root pressure volume flow. They transported xylem sap with higher concentrations of cations at certain times of the day, and dry matter production was enhanced. Their leaf calcium concentration (in % of DM) was significantly reduced. This data indicated that the occurrence of tipburn symptoms could possibly be a consequence of a dilution process in the leaf tissue. Generally it is suggested that dilution effects occur during periods of higher growth rates, such as conditions of continuously high transpiration (COLLIER and HUNTINGTON 1983; COX et al. 1976; EVERAARTS and BLOM-ZANDSTRA 2001). The cation competition in the root pressure exudate of the VPD-1 plants may have had additional effects on progress of tipburn.

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6. Summary

The reduction of tipburn incidence in lettuce by a diurnal decrease of vapour pressure deficit was investigated. The diurnal course of quantity and quality of root pressure exudates as well as the leaf mineral content was recorded.

Tipburn symptoms could be induced successfully in greenhouse grown lettuce plants that were cultivated at continuously high VPD (VPD-1=0.82 kPa day/night). When plants were submitted to a low VPD during night time (VPD-2=0.82/0.0 kPa day/night) tipburn incidence could be reduced significantly. Measurements of root pressure revealed that the exudate flow is not enhanced during night periods when VPD was reduced. The reduction of tipburn by decreasing VPD at night therefore could not be explained by root pressure flow only. The concentration of the mineral constituents in the root pressure showed a diurnal fluctuation. When VPD was low, calcium content was increased whereas magnesium and potassium contents were reduced. K/Ca ratio of the VPD-1 plants showed a significant increase at the beginning of night time, while the K/Ca ratio of the VPD-2 plants was at a constantly lower level throughout the daily course. Lettuce plants that were cultivated within the VPD-2 treatment had lower mineral concentrations in outer and inner leaves as well as in the roots. Likewise the content of dry matter was reduced.

Approaches will be made to explain the reduction of tipburn incidence by a reduced VPD during night time.

D. EFFECT OF SHORT-TERM IRRIGATION ON TIPBURN INCIDENCE IN BUTTERHEAD LETTUCE (LACTUCA SATIVA VAR. CAPITATA L.)

1. Introduction

A satisfying control of tipburn has not yet been reported for field grown vegetable plants. Tipburn incidence could often be decreased by using non-susceptible cultivars (HAGEL et al. 2002; JOHNSON 1991; MAROTO et al. 1985; MISAGHI et al. 1981a; SCHLAGHECKEN and STROHMEYER 1997). Furthermore, exogenous application of Ca-salts provides a suitable, but controversially discussed prophylactic measure to control this injury in vegetable plants. It has frequently been applied by several scientists and horticultural growers as well (ABD EL-FATTAH and AGWAH 1987; HOLTSCHULZE et al. 2001, Chapter B, CORGAN and COTTER 1971; KLEEMANN 1999; MISAGHI et al. 1981b; THIBODEAU and MINOTTI 1969).

Alternative preventive measures against tipburn injury in leaf vegetables, such as decreasing vapour pressure deficit (VPD) within the field crop stand, have hardly ever been examined. Under controlled environmental conditions several experiments were carried out before, where a decrease of VPD was mainly achieved by increasing relative humidity. SONNEVELD and MOOK (1983) and SONNEVELD and VAN DEN ENDE (1975) applied overhead irrigation in greenhouse lettuce. However, tipburn reduction was obtained only in combination with $CaCl_2$-sprayings. While comparing different irrigation methods, tipburn could be reduced slightly by overhead irrigation depending on lettuce cultivar (BERT and HONMA 1975). A complete prevention of tipburn could be achieved in Chinese cabbage by decreasing VPD by polyethylene sheeting of the plants, especially during dark period. It could be shown for lettuce as well that prevention of tipburn was strongly dependant on the cultivar (KUO et al. 1981, Chapter C, VAN BERKEL 1988). Misting Chinese cabbage for 15 minutes daily starting at the beginning of head formation, could not reduce tipburn injury significantly (ALONI et al. 1986).

As could be demonstrated above (Chapter C) in previous greenhouse experiments a significant reduction of tipburn incidence was achieved by a diurnal decrease in VPD. The objective of the present study was to investigate if these effects could be transferred to plants grown in the field. It was supposed that by lowering VPD

during night time the transpiration rate of the plants should be reduced and Ca-distribution into low transpiring organs, such as inner leaves, should be enhanced (BAAS et al. 2003; MARSCHNER 1995; PALZKILL and TIBBITTS 1977; VAN BERKEL 1988). A decrease of VPD should be realised by short-term irrigation with time and frequency of application defined prior to the treatment. Under these circumstances it was of special interest to take into account that lettuce rot risk could be elevated by increasing humidity of leaves and soil surface. Therefore, this aspect was also examined.
The efficiency of the treatments was correlated to frequency and intensity (l m^{-2}) of irrigation as well as air temperature. The trials were carried out in field grown plots of tipburn receptive butterhead lettuce cv. ‘Herman’.

2. Material and Methods

2.1. Site of experiments

The experiments were carried out at the Experimental Station Marhof belonging to the Institute of Horticulture, University of Bonn. The station is located 15 km to the north of Bonn between ‘Vorgebirge’ and ‘Kölner Bucht’ at 50° 49” northern parallel (6° 59” eastern longitude). Soil was a base-rich brown earth with silt loam to sandy-silt loam. With an average temperature of >10°C and 700 mm of annual rainfall this region represents a typical location for horticultural farming in the Rhineland.
Air temperature and relative humidity were recorded within the crop stand by mini data logger (Meilhaus Electronic, Puchheim, Germany), installed in weather-instrument shelters. Vapour pressure deficit (VPD) was calculated according to FAO Penman-Monteith formula (ALLEN et al. 1998).

2.2. Plant material and cultivation

Tipburn susceptible butterhead lettuce cv. 'Herman' was seeded in compressed blocks of Stender A280 (Stender, Schermbeck, Germany) propagation medium, consisting of 20 % white peat and 80 % mixed peat. It contained 14 % nitrogen, 16 % phosphorus, 18 % potassium and microelements at a pH of 5.5 – 6.2. The experiments were repeated in four sets according to the following planting dates: 06/05/02, 07/01/02, 08/14/02 and 07/11/03 The successive plots of the first year were planted adjacent to the previous plot within the same field. In 2003 the experiments were carried out with a reduced number of variants. The plots consisted of 5 rows per bed (10-12 plants m^{-2}), considering only the three center rows for examination in order to avoid side effects. In addition to the nitrate contents of the soil determined by analysis of N_{min} 120 kg N ha^{-1} was applied as CAN (calcium ammonium nitrate).

2.3. Short-term irrigation treatments

It has been reported that the incidence of lettuce rot seemed to be increased by irrigation treatments during the period of heading (Schlaghecken, personal communication). Temperatures >24°C were reported to increase tipburn risk in lettuce (MISAGHI and GROGAN 1978). Short-term irrigation was carried out at previously defined threshold values with reference to air temperature and relative humidity. The irrigation treatments were thereby reduced to the critical days for tipburn development. Threshold values were determined at the time of 07.00 p.m. immediately before short-term irrigation was initiated. Two irrigation intensity levels (1l m^{-2} and 3l m^{-2}) were applied depending on temperature (>24°C) in combination with low relative humidity (<65%). Additionally, short-term irrigation was carried out at daily intervals, starting one week after planting each at 1 l m^{-2} and 3 l m^{-2}, respectively. Short-term irrigation was only carried out on days without natural rainfall.

Since a computer controlled self-propelled irrigation system was available, a randomised distribution of the irrigation subplots could be established within the test field in a randomised block design.

Tab. 1: Additional water supply (in liters) and number of applications [in parentheses] of short-term irrigation within the planting sets 1-3. *Daily irrigation treatments were only carried out when natural rainfall did not occur. Due to extraordinary high temperatures during vegetation in 2003, the variants '1l_temp' and '3l_temp' were carried out daily; they will therefore be described as '1l_pday' and '3l_pday' in the following. n.n.: not applied

Planting date	1l_temp	3l_temp	1l_pday*	3l_pday*
06/05/02	3 [3]	9 [3]	7 [7]	21 [7]
07/01/02	6 [6]	18 [6]	17 [17]	51 [17]
08/14/02	4 [4]	12 [4]	22 [22]	66 [22]
07/11/03	n.n.	n.n.	16 [16]	48 [16]

2.4. Assessment of tipburn symptoms

During vegetation period tipburn incidence was recorded weekly. Symptoms were estimated from intact heads in the field. From the time of head formation onwards, symptoms of the inner core as well as enclosed leaves could not be counted. Plants having one or more leaves with tipburn were scored as tipburn plants. At harvest time, 15 plants per treatment were weighed. Tipburn incidence (%) and tipburn severity (number of tipburn symptoms per plant) were determined by defoliating the heads. Senescent leaves were removed and left in the field.

2.5. Statistical analysis

The experiments were established as randomised block design, each treatment was replicated 6 times. Statistical data were calculated with the statistic program 'SAS 8.0' (SAS Institute Inc., Cary, USA). Data were subjected to analysis of variance. Mean comparisons were performed by Tukey's HSD. Statistical differences were accepted at 5 % level of significance. Graphs were constructed with SigmaPlot 8.0 (SPSS Science Inc.)

3. Results

3.1. Effect of short-term irrigation on

3.1.1. the vapour pressure deficit within the crop stand

Previous experiments revealed that tipburn incidence could be reduced in greenhouse grown lettuce by a decrease of VPD during dark period (Chapter C). Short-term irrigation was considered to influence the microclimate within the crop stand in the field. Plants were to be exposed to conditions of low transpiration due to reduced VPD. Following short-term irrigation in the present study temperature was decreased rapidly within the crop stand, while relative humidity was increased. Consequently a rapid decrease of VPD could be observed (0). This decrease of VPD was independent of the irrigation intensity of 1 or 3 l m^{-2}, respectively. In the graph given below data of a summer day with average values of mean temperature (21.2°C) and maximum temperature (28.7°C) are presented. In the control plot VPD was reduced to the same level later in the course of the night period.

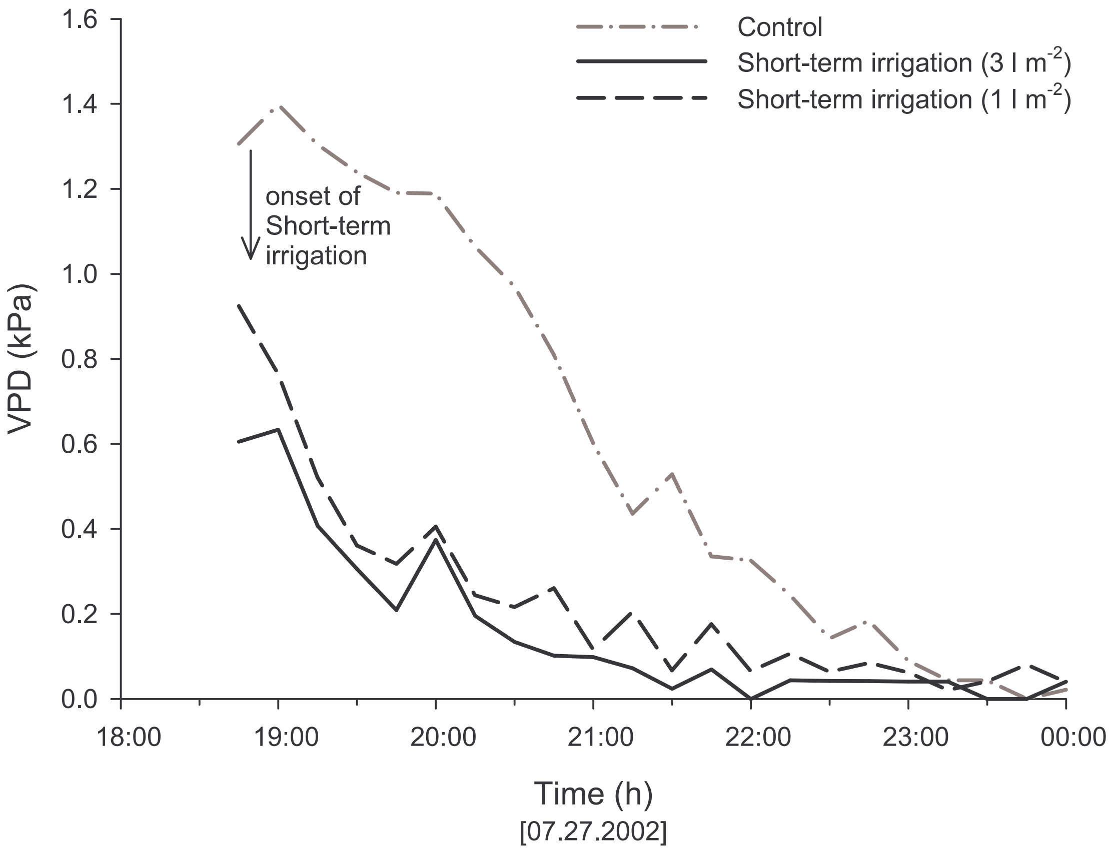

Fig. 1: Effect of short-term irrigation (onset at 07:00 p.m.) on VPD within the crop stand. VPD was calculated from temperature and relative humidity measured within the field. Data given here were

recorded within the second planting set (07/01/02) on an average warm summer day; mean temperature was 21.2°C, maximum temperature was 28.7°C.

Short-term irrigation after a warm day with temperatures exceeding average values, had a longer lasting effect on VPD during night period. The course of VPD following irrigation on a day with a mean temperature of 26.7°C and a maximum temperature of 35.4°C is shown in Fig. 2. A rapid decrease of VPD down to 0.0 kPa could be observed under the short-term irrigation treatment. VPD in the control plot declined only later in the course of the night period. However, a complete saturation of the air (0.0 kPa) was to be observed only for a very short period (04:00-04:45 a.m.). In the control plants compared to the plants under irrigation treatments this possibly may have led to continuously higher translation rates during the night period.

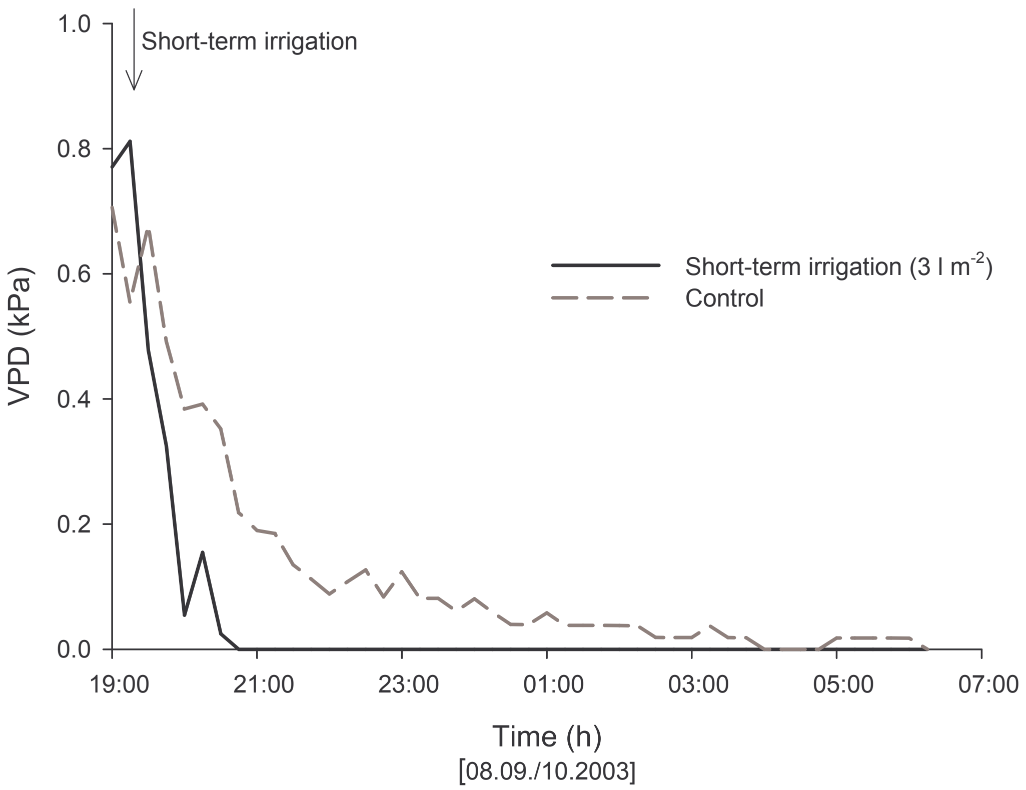

Fig. 2: Effect of short-term irrigation (onset at 07:00 p.m.) on VPD within the crop stand. VPD was calculated from temperature and relative humidity measured within the field. Illustrated data were recorded within the fourth planting set (07/11/03). Mean temperature was 26.7°C, maximum temperature was 35.4°C.

3.1.2. head weight of butterhead lettuce

Plants were harvested when heads were marketable. Dates of harvest as related to the diverse planting dates of the experimental sets are presented in Tab. 2.

Tab. 2: Head weight of marketable plants and period of plant growth depending on different planting dates of butterhead lettuce cv. 'Herman'.

Planting date	Harvest date	Days of vegetation	Head weight (g per head ± SE)
06/05/02	07/18/02	43	542 ± 22.7
07/01/02	08/14/02	44	525 ± 9.6
08/14/02	09/30/02	47	437 ± 8.9
07/11/03	08/14/03	34	420 ± 10.6

Yield was strongly dependant on planting date. Mean head weight of the plots was reduced by about 100 g comparing first (542 g) and third set (437 g). This decline in head weight may be a result of significant changes in growing conditions during later summer, primarily of reduced temperatures and day length. Extraordinarily high temperatures during vegetation in 2003 (meteorological data not presented) resulted in rapid head development, therefore head weight was lowest in the fourth set. Short-term irrigation was only employed as a measure against tipburn incidence. Any impact of the additional water supply on parameters of plant development were to be avoided. Therefore, the additional water supply was related to the head weight of the marketable plants. No side-effect on plant growth parameters was observed (Fig. 3).

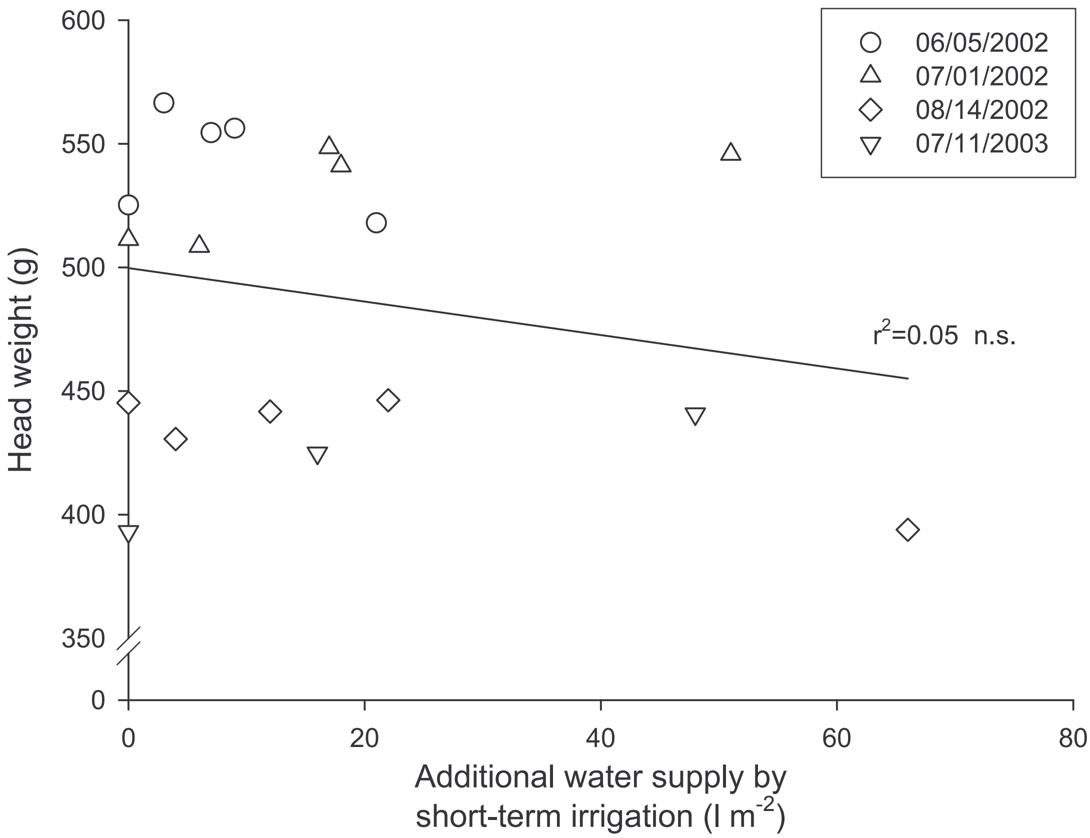

Fig. 3: Effect of additional water supply by short-term irrigation on head weight (stage of marketability) of butterhead lettuce cv. ‘Herman’. Symbols refer to the planting dates, the correlation coefficient (r^2=0.05) was not significant. Differences in amount of water could be related to varying temperature conditions during vegetation (see also Tab. 1).

3.1.3. tipburn incidence of butterhead lettuce

The experimental plots of butterhead lettuce were planted in mid-summer with the intention that tipburn inducing periods, as a consequence of warm temperatures, should be expected during heading of the plants. The tipburn sensitive cultivar ‘Herman’ was chosen in order to increase tipburn risk. Symptoms before harvest were observed within the first (40 days after planting [dap]) and the third set (20 dap). In the first set tipburned leaves first occurred on the exposed leaves just a few days before harvest. The percentage of tipburned plants was not influenced by the short-term irrigation treatments. In the third set tipburn symptoms could be observed at earlier stages of plant development, when leaves were not enclosed and transpiration was not impeded. The percentage of tipburn affected plants was higher than observed in the earliest planted set. In the second and fourth set, however, tipburn symptoms were not to be observed during vegetation by non-destructive rating (Fig. 4).

At harvest time, heads were defoliated, examined for tipburn incidence and tipburn severity, as indicated by number of tipburn symptoms per plant. The occurrence of tipburn symptoms varied between the sets depending on planting date as presented in Fig. 5. Tipburn injury was highest in the earliest planted set, with 43% of the control plants affected. A decrease in tipburn incidence could be observed the later lettuce was planted. In the third set barely 17% of untreated plants developed tipburn symptoms. When plants received short-term irrigation at daily intervals, with 3 l m^{-2} or 1 l m^{-2}, respectively, tipburn injury could have been reduced slightly. The irregular occurrence of tipburn symptoms within the field grown plots resulted in a high standard deviation. Thus, no statistical evidence could be calculated for any of the treatments.

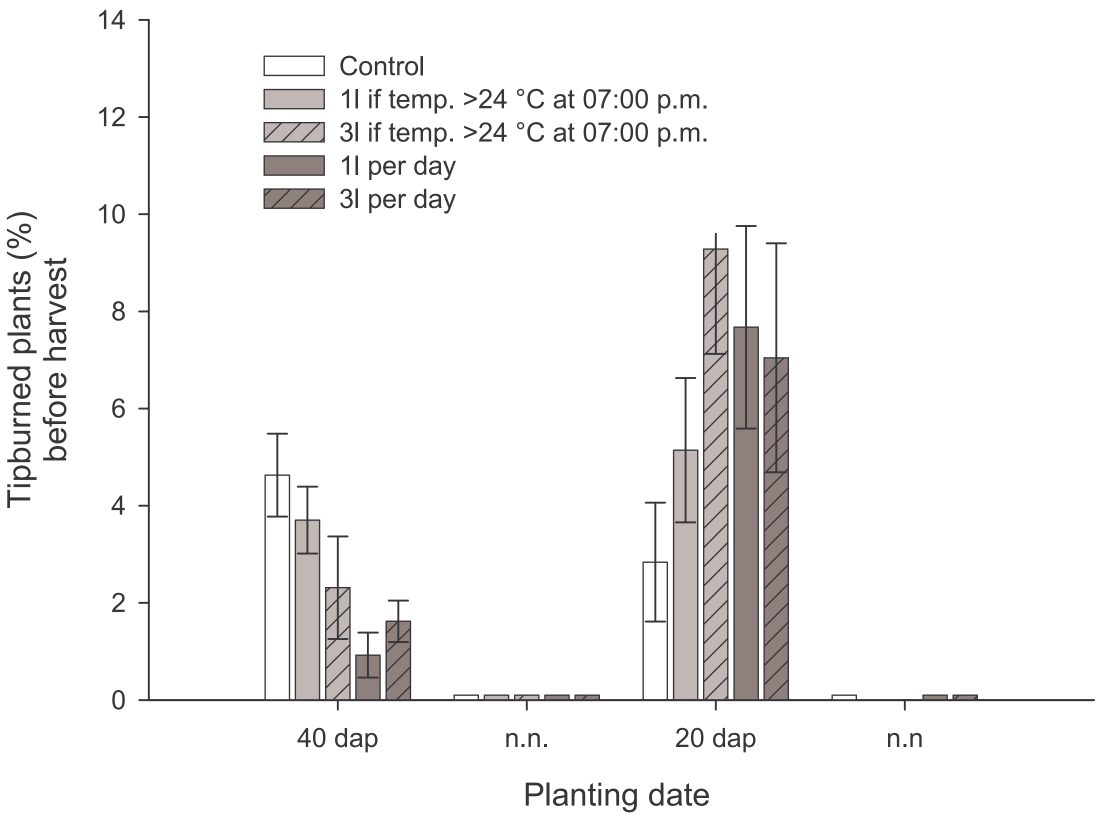

Fig. 4: Effect of short-term irrigation on tipburn incidence in head lettuce ‘Herman’ before harvest depending on planting date. Occurrence of tipburn symptoms before harvest within the sets is

specified in days after planting (dap). Error bars (±) indicate standard error, treatments had no significant effects. Symptoms (n.n.) were not visible in the field by non-destructive grading.

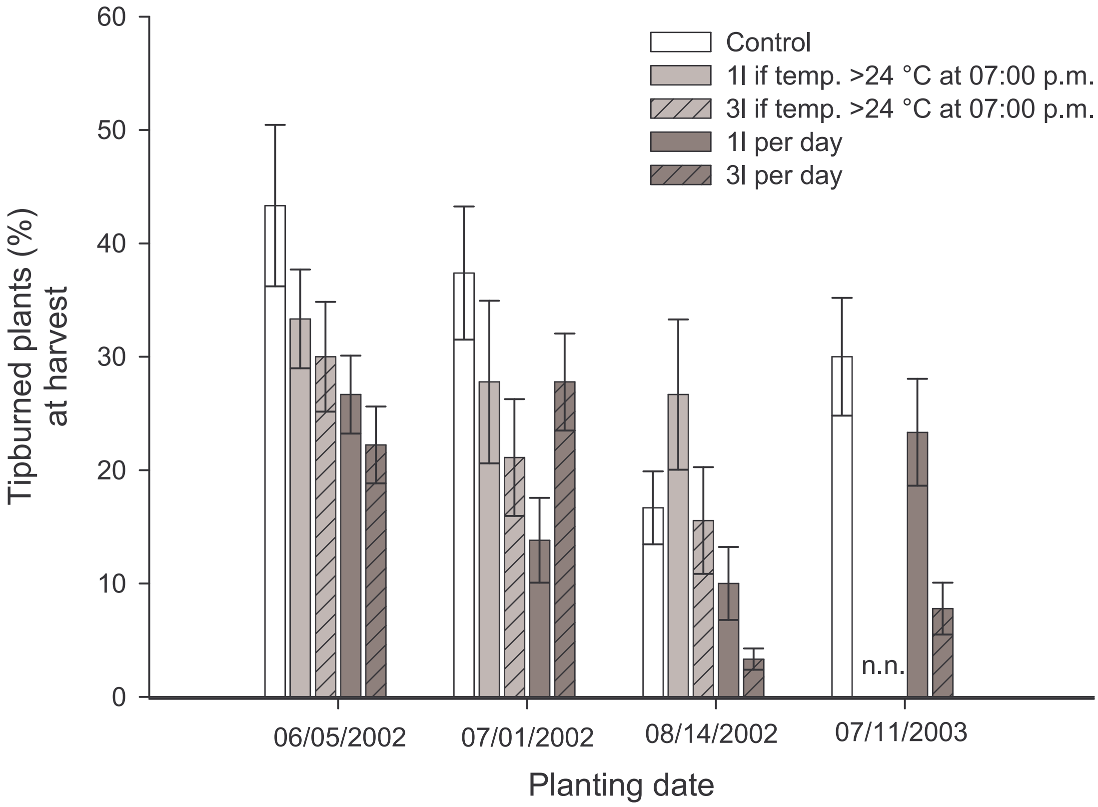

Fig. 5: Effect of short-term irrigation on tipburn incidence in head lettuce ‘Herman’ at harvest time depending on planting date. Error bars (±) indicate standard error, differences between treatments were not statistically significant. The 07/11/03 plot comprises a reduced number of treatment groups (n.n.).

Comparing tipburn incidence at harvest time with the occurrence of symptoms during vegetation, it became obvious that a prognosis of tipburn injury was only valid, when performed in an advanced stage of plant development (Fig. 6). There was a highly significant positive correlation (r^2=0.85) between tipburn symptoms a few days before harvest and tipburn injury at harvest time. The absolute values were remarkably lower. However, the tendency of the treatments applied was analogous to the grading of defoliated plants at harvest.

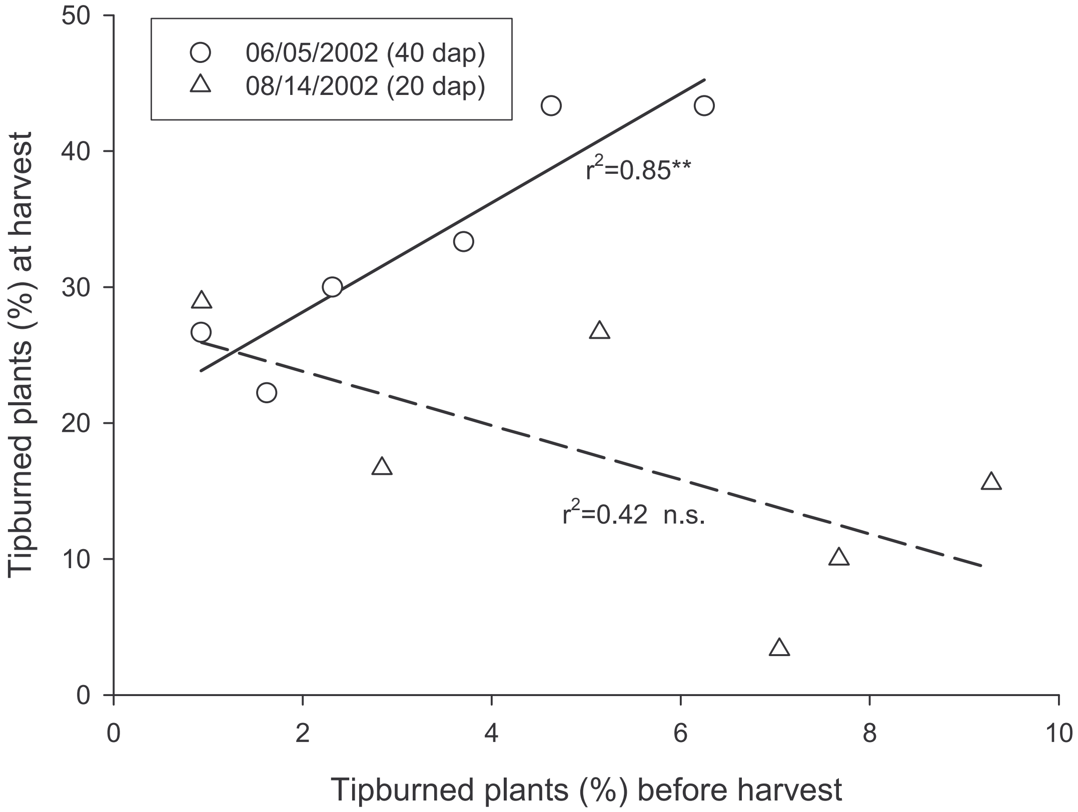

Fig. 6: Correlation between tipburn incidence during vegetation and tipburn incidence at harvest time of head lettuce cv. 'Herman' within to planting sets. Tipburn incidence before harvest was specified in days after planting (dap) from intact plants in the field, tipburn injury at harvest was determined from defoliated plants. The correlation (**) r^2=0.85 was significant at $p \leq 0.001$.

By rating tipburn severity similar trends were revealed as presented before (Tab. 3). In 2002 the number of symptoms per plant was lower in the later established plots. Within the planting sets it could be observed that short-term irrigations slightly reduced tipburn severity of the plants.

Tab. 3: Effect of short-term irrigation on tipburn severity (number of symptoms $plant^{-1}$) in butterhead lettuce cv. 'Herman' at harvest time. Numbers (±) are indicating standard error. Differences between treatments within the same planting set were not significant. The 07/11/03 plot was carried out with a reduced number of treatment groups.

	Treatments				
Planting date	Control	1l if temp. > 24°C at 07:00 p.m.	3l if temp. > 24°C at 07:00 p.m.	1l per day	3l per day
06/05/02	1.3 ± 1.3	1.0 ± 0.9	0.7 ± 0.7	0.5 ± 0.3	0.4 ± 0.3
07/01/02	1.2 ± 0.9	1.3 ± 1.7	0.5 ± 0.4	0.3 ± 0.5	1.1 ± 0.8
08/14/02	0.4 ± 0.3	0.7 ± 0.8	0.4 ± 0.4	0.1 ± 0.1	0.1 ± 0.1
07/11/03	0.5 ± 0.3	n.n.	n.n.	0.5 ± 0.5	0.2 ± 0.3

The impact of the treatments on tipburn incidence in lettuce was compared to untreated plants (Fig. 7). Short-term irrigation treatments revealed a regular pattern concerning effectiveness in preventing the injury. When short-term irrigation was carried out at daily intervals, a reduction of tipburn incidence could be observed in each experimental plot. When tipburn risk was high in 2002, as indicated by a higher tipburn incidence of the control plants (Fig. 5), tipburn injury was reduced by short-term irrigation depending on temperature. Though the treatments were not statistically different, a decline of about 20 % of tipburn affected plants was accomplished. This tendency could be confirmed throughout the experimental trials.

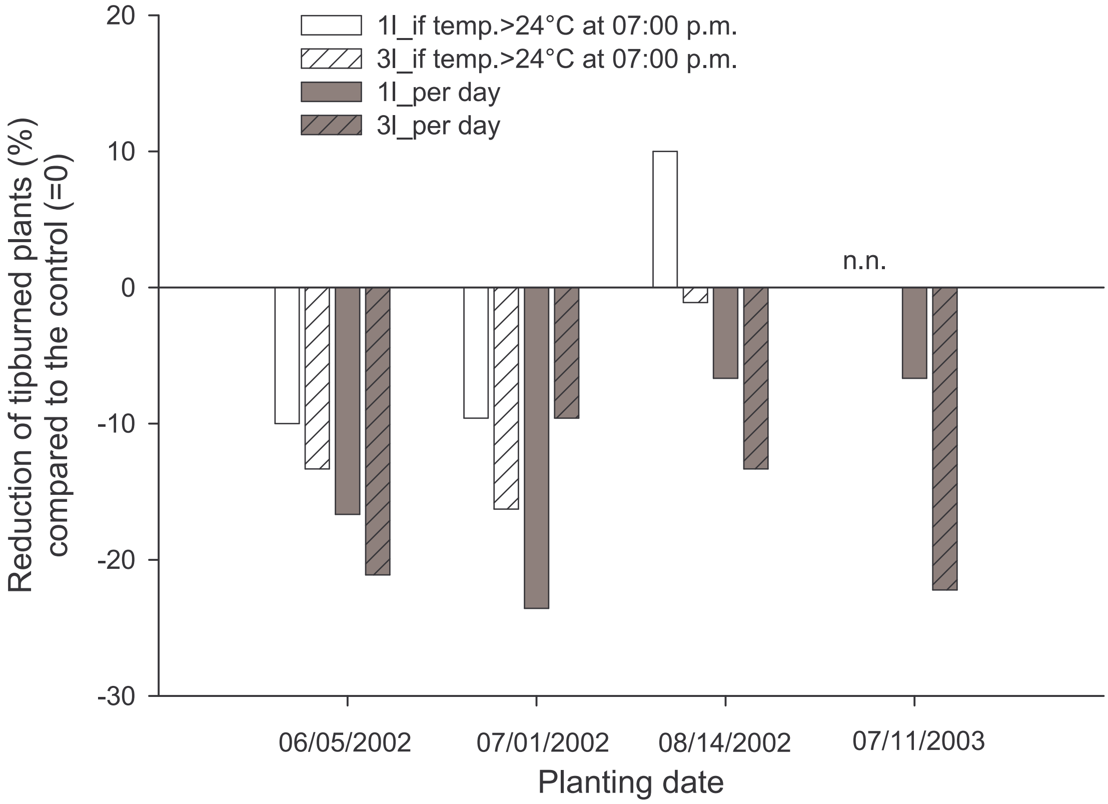

Fig. 7: Effectiveness of short-term irrigation in reducing tipburn incidence in lettuce cv. 'Herman' in comparison to untreated plants (control=0); negative data indicate a reduction in tipburn incidence. Irrigation treatments depending on temperature were not carried out within the plot planted at 07/11/03 (n.n.).

3.1.4. the occurrence of lettuce rot

It has been shown that short-term irrigations had an influence on the microclimate within the crop stand, and it is reported that moistening of leaves and soil surface, particularly during head formation, seemed to enhance the occurrence of lettuce rot (Schlaghecken, personal communication). The occurrence of lettuce rot was strongly dependant on planting date within the present trials. However, a significant increase of lettuce rot due to short-term irrigation compared to untreated plants could not be confirmed (Fig. 8).

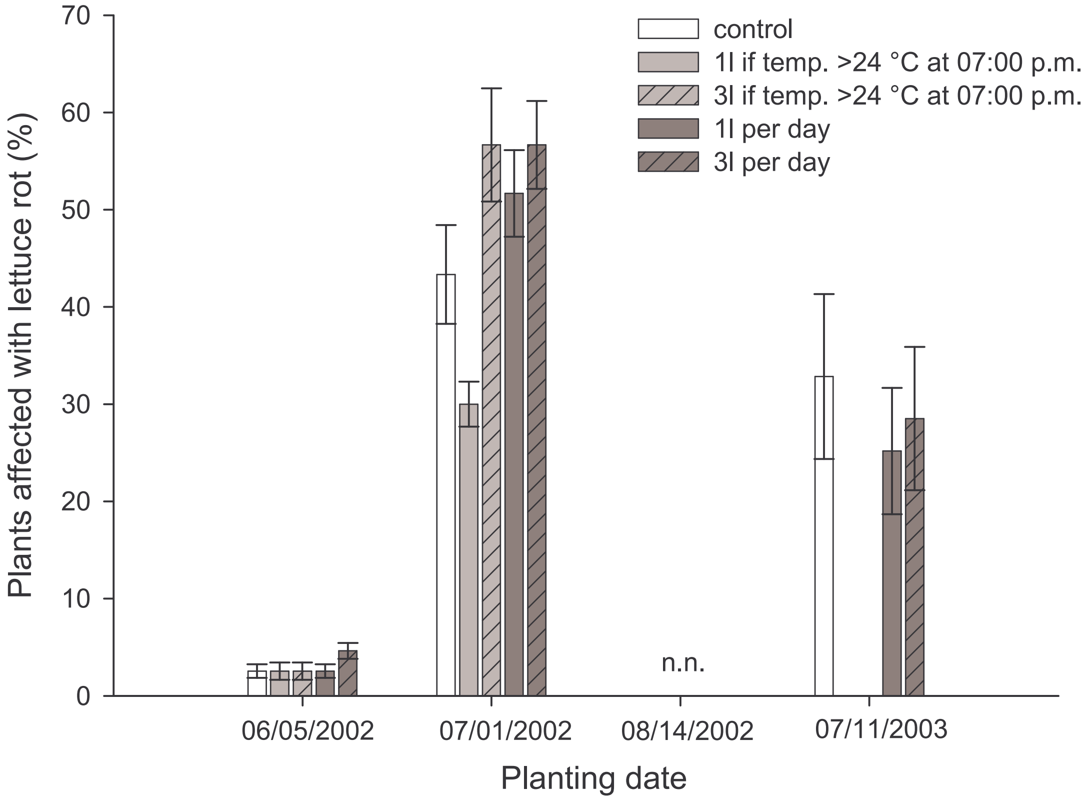

Fig. 8: Influence of short-term irrigation treatments on the occurrence of lettuce rot. Error bars (±) indicate standard error, treatments were not significant. Lettuce rot did not occur within the plot planted on 08/14/02 (n.n.). Irrigation treatments depending on temperature were not carried out within the plot planted on 07/11/03.

3.2. Effect of head weight and growth rate on tipburn incidence

It is generally assumed that accelerated plant growth could be one of the relevant factors causing tipburn injury. Therefore, mean daily plant growth was correlated with tipburn incidence of the respective planting sets (Fig. 9). The correlation indicated that tipburn incidence is dependant on growth rate of the lettuce plants. The coefficient of determination is comparatively low (r^2=0.22) which in fact corresponded to findings of other researchers (WISSEMEIER and ZÜHLKE 2002).

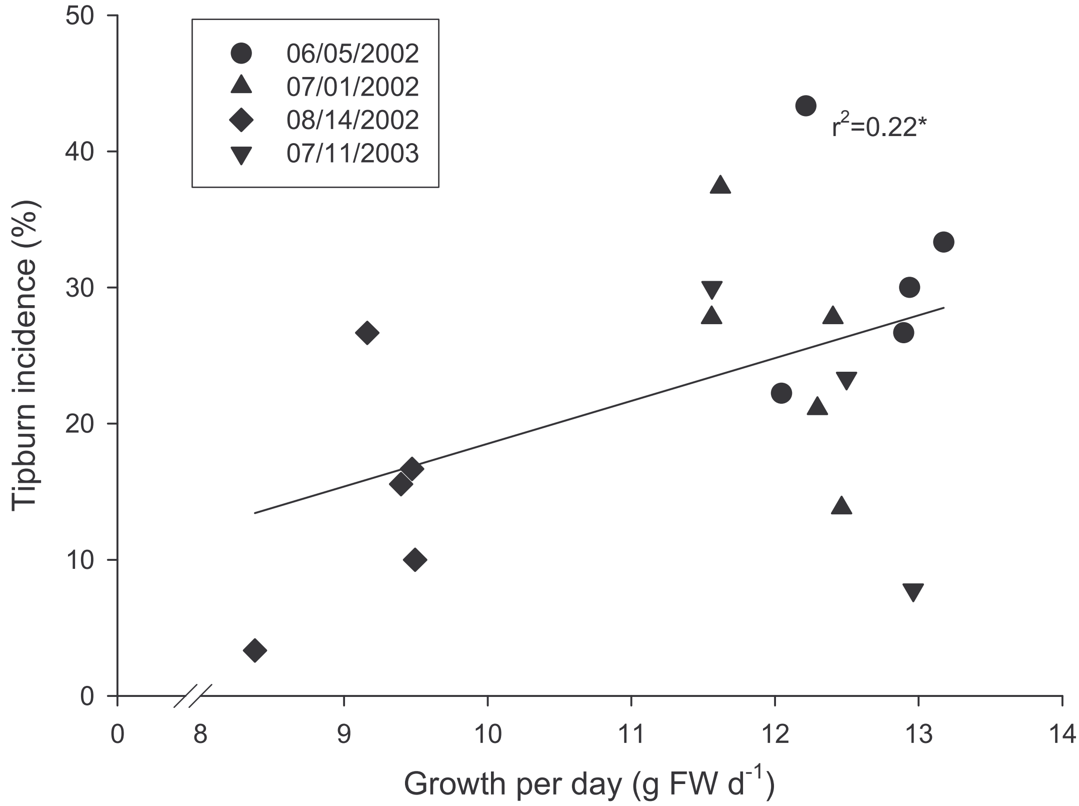

Fig. 9: Effect of daily plant growth (g FW d^{-1}) on tipburn incidence in four different planting sets of butterhead lettuce. Fresh weight was determined from marketable plants at harvest time; (*) correlation is significant at $p \leq 0.05$.

It is suggested further that tipburn incidence might increase when plants are at advanced stages of development. There was a significant correlation between head weight of marketable plants at harvest time and tipburn incidence. However, only about 23 % of the increased tipburn injury could be explained by increment of head weight (Fig. 10).

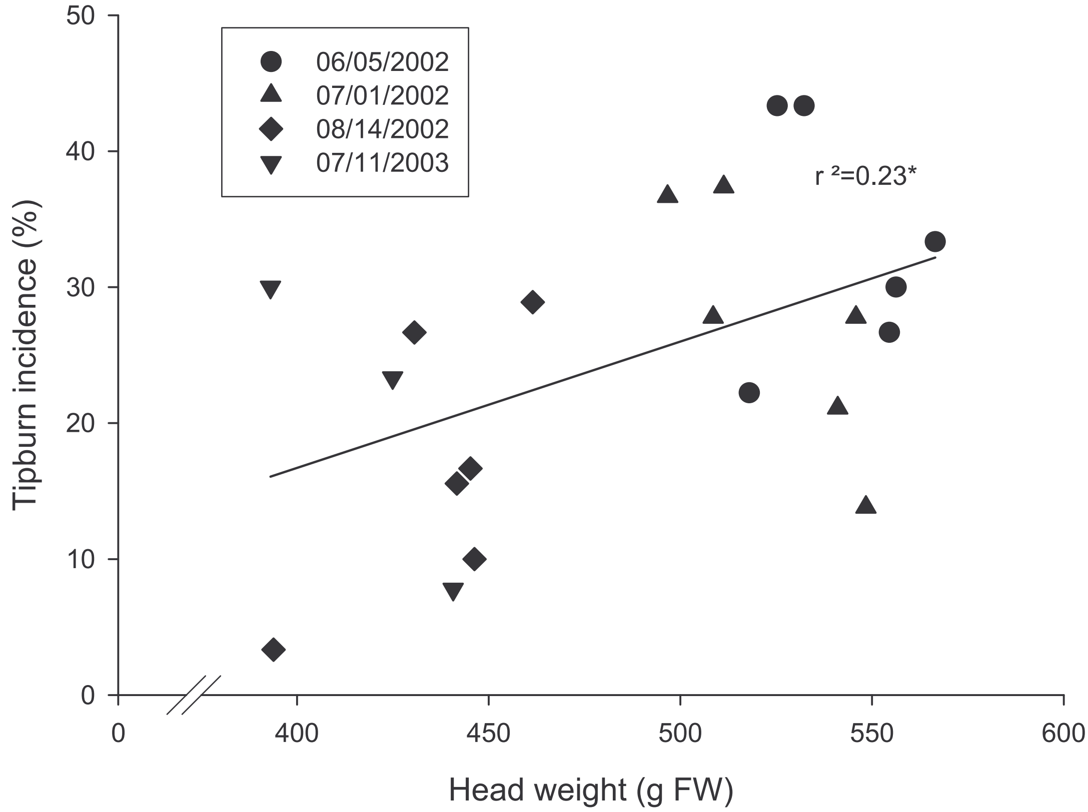

Fig. 10: Effect of head weight (at the stage of marketability) on tipburn occurrence in head lettuce. Symbols refer to the different planting dates of the field trials. The correlation (*) is significant at $p \leq 0.05$.

3.3. The effect of climatic variables and growth parameters on lettuce tipburn

It has often been suggested that tipburn incidence could be related to certain climatic variables, such as sum of global irradiation (I_{SUM}), sum of temperature or else. WISSEMEIER (1996) performed extensive examinations by the means of regression analysis, in which tipburn incidence was correlated to several climatic factors that were assumed to influence the occurrence of tipburn in head lettuce. Regression analysis revealed that the sum of irradiation was the factor with the most favourable correlation coefficient. Therefore, in the present experimental trials the sum of irradiation was correlated to the incidence of tipburn distinguishing three time intervals before harvest (Fig. 11). It became obvious that a homogenous correlation between tipburn incidence and I_{SUM} could not be found. The subdivision of time intervals before harvest (7d, 14d) was not coherent to the incidence of tipburn. However I_{SUM} from planting until harvest at least revealed a positive correlation (r^2=0.52), which in fact was not statistically significant.

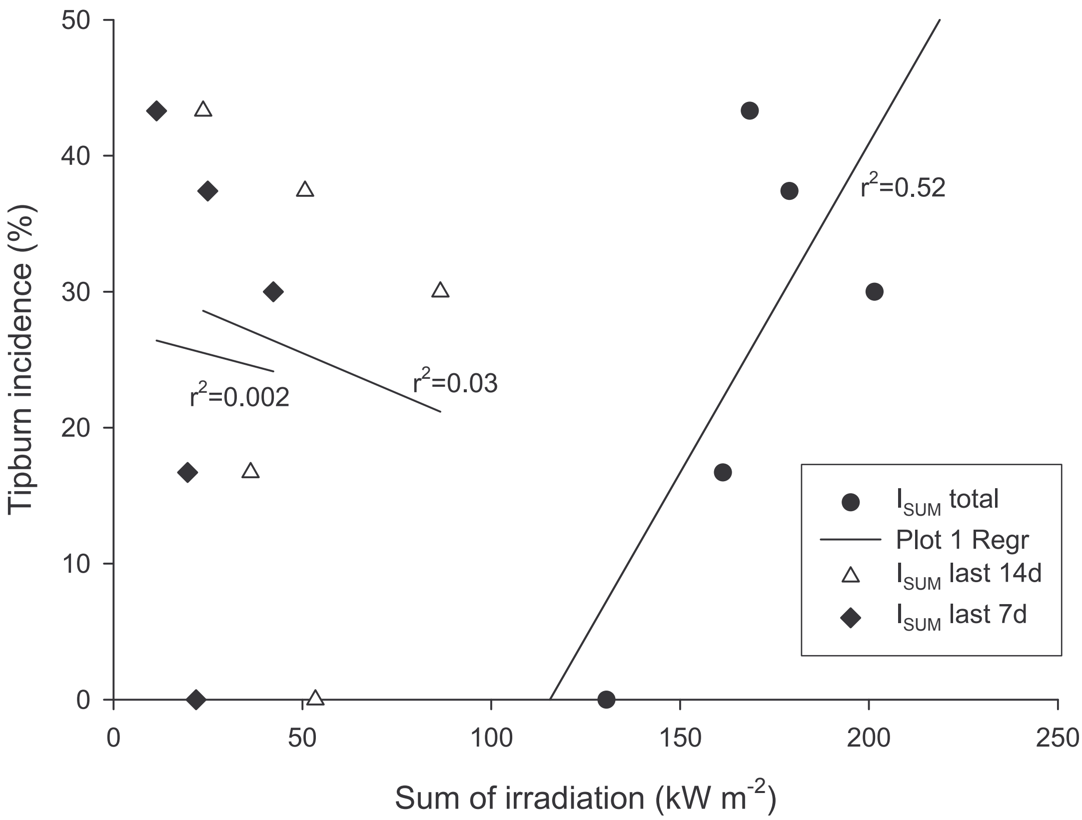

Fig. 11: Influence of Sum of Irradiation (I_{SUM}) on the occurrence of tipburn in field grown butterhead lettuce according to different planting dates. Sum of irradiation was separated into time intervals 'total'=from planting until harvest, 'last 14d'=last 14 days before harvest, 'last 7d'=last 7 days before harvest. Correlations were not significant.

Temperatures above 24°C were reported to enhance tipburn risk in head lettuce, particularly during the time of heading when heads were enclosed (MISAGHI and GROGAN 1978). Consequently tipburn incidence was correlated to the sum of temperature (T_{SUM}), subdivided into three time intervals that were indicating preharvest periods with potential significance for the induction of tipburn symptoms. Data indicated that time intervals chosen did not reveal a reliable correlation to explain the development of lettuce tipburn (Fig. 12).

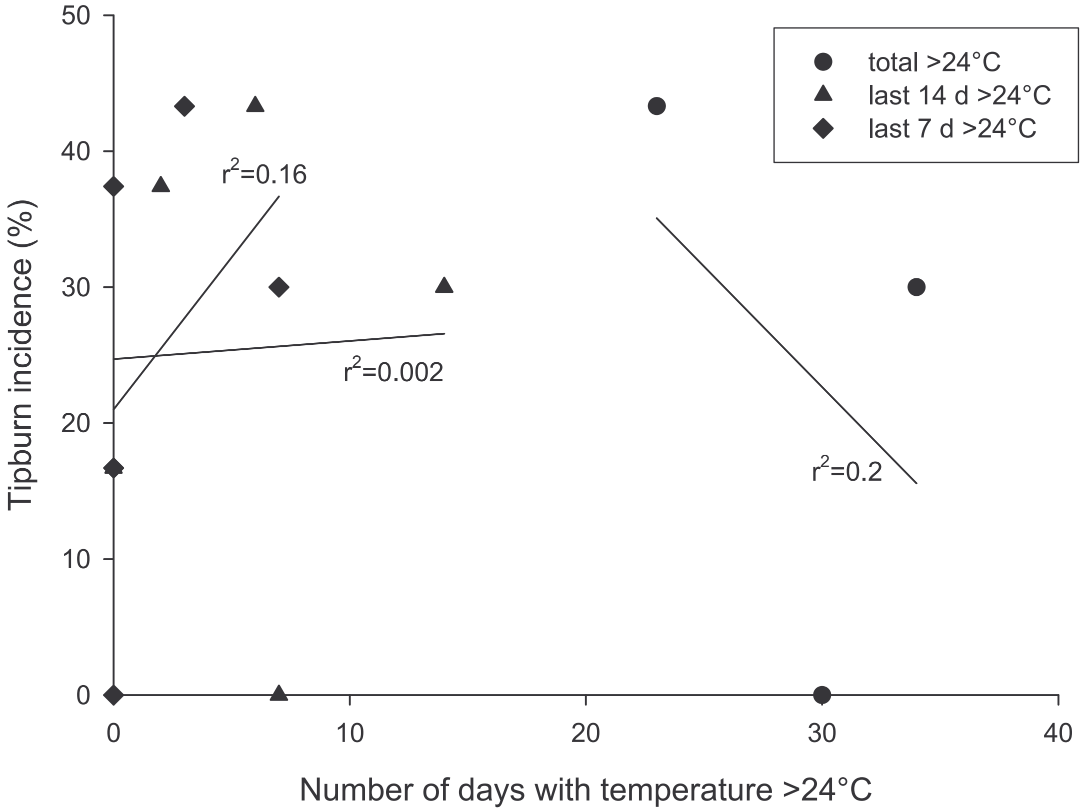

Fig. 12: Effect of number of days with temperatures above 24°C on tipburn incidence in field grown butterhead lettuce. Number of days above 24°C was separated into time intervals: 'total'=from planting until harvest, 'last 14d'=last 14 days before harvest, 'last 7d'=last 7 days before harvest. Correlations were not significant.

4. Discussion

Tipburn incidence in the field is enhanced particularly after a series of days and nights with high temperatures and low relative humidity (COLLIER and TIBBITTS 1982). Under such atmospheric conditions plants are assumed to maintain a continuously high transpiration rate. The xylem flow is encouraged towards the stronger transpiring organs on the disadvantage of lower transpiring organs, which can result in Ca-deficiency disorders (BAAS et al. 2003; BARTA and TIBBITTS 1986). It has been shown that the incidence of Ca-deficiency symptoms could be reduced in greenhouse experiments by a reduced VPD at night (KUO et al. 1981; MASON and GUTTRIDGE 1975; VAN BERKEL 1988, Chapter C). An utilisation of this prophylactic measure for field grown plants seemed to be achievable by short-term irrigation. A randomised distribution of the irrigation subplots within the test field could be accomplished by the use of a computer controlled self-propelled irrigation system. A randomised block design was therefore preferred instead of a split-plot design in order to examine each factor with uniform statistical accuracy (KÖHLER et al. 1994). The length of the short-term intervals of irrigation was chosen just to influence the microclimate within the crop stand. Measurements of relative humidity and temperature within the field crop stand revealed that VPD rapidly decreased following short-term irrigation (0). Even if the soil surface was slightly moistened by short-term irrigation, no influence on water supply of the soil (measurements of soil water tension not presented) could be observed. Short-term irrigations of 1 or 3 l m^{-2}, respectively, were regularly not sufficient to compensate for the evaporative water loss of a summer day, when average values for temperature and relative humidity were implied (ALLEN et al. 1998; PASCHOLD 2001). An additional water supply of the irrigation treatments did not have positive effects on plant growth parameters, since a correlation between additional water supply and yield could not be found within the different planting sets (Fig. 3). This supports the assumption that the main effect of short-term irrigation was the diurnal reduction of VPD within the crop stand of field grown plants (MASON and GUTTRIDGE 1975; VAN BERKEL 1988).

A daily misting of Chinese cabbage plants for 15 minutes at 05:00 p.m. did not lead to a reduction of tipburn injury (ALONI et al. 1986). It has been reported that the plants remained wet for at least 3 h, which implied a decreased VPD. Since no measurements of any relevant factors of the crop microclimate were conducted, the effectiveness of the treatment on VPD could not be judged properly. In general, the results of the present field experiments were corresponding to those of the greenhouse experiments conducted previously (Fig. 4). However, the reduction in tipburn incidence by regular irrigation procedures within field-grown lettuce was not statistically significant (SONNEVELD and MOOK 1983; SONNEVELD and VAN DEN ENDE 1975). It could be shown by a regular tendency throughout the planting sets, that tipburn incidence can somehow be reduced by short-term irrigation (Fig. 7). Particularly when irrigations were carried out at daily intervals, it was the most effective treatment independent from a seasonal tipburn risk. The occurrence of the disorder could be reduced by about 20 % in almost 75 % of the trials. This leads to the conclusion that field-grown plants could be adapted to a diurnal decrease in VPD by the means of short-term irrigation, which resulted in a slight reduction of tipburn injury. However, under greenhouse conditions, a diurnal reduction of VPD had been more effective in preventing tipburn incidence (Chapter C). In the greenhouse experiments VPD could be reduced down to 0.0 kPa more rapidly than in the field plots where it took more time to reach water saturation of the air. Moreover, under greenhouse conditions water saturation of the air (VPD: 0.0 kPa) was lasting for a longer period of time (up to 12 h) compared to the field trials. It is suggested that also the duration of transpiration impediment might have been had a significant influence.

Moistening of the leaves and soil surface did not lead, as it was assumed, to a higher risk of lettuce rot (Fig. 8). For that reason it was not necessary to limit the irrigation treatments to the period critical for tipburn development (COLLIER and TIBBITTS 1982; MISAGHI and GROGAN 1978).

A reliable prognosis of tipburn injury should be accomplished by rating the visible symptoms on intact plants in the field before harvest. Examination of visible symptoms close to harvest time lead to a similar rating of the severity of the necrosis as revealed by the destructive examination at harvest (Fig. 6). This relationship was positively correlated, whereas no correlation between symptom expression earlier in plant development (20 dap, planting set: 08/14/02) with tipburn severity at harvest time could be found. The absolute values of the predicted tipburn injury differed considerably. When rated after early observation of symptoms only about 5 % calcium deficient plants were found, whereas 43 % tipburned plants were noted at harvest time. Nevertheless, the efficiency of the treatments conducted was predicted in an almost similar tendency (Fig. 4).

From the potential of reducing tipburn injury by about 20 % by short-term irrigation it is concluded that the method might be of practical importance. Overhead irrigation could be carried out by using large-scale irrigation systems provided with single nozzles (KUEHLWETTER 2003; ALBRECHT 2003). The frequency of the irrigation treatments is firmly dependant on climatic conditions. In the field trials an application dependant on temperature was carried out 3 to 6 times, whereas the daily variants received irrigations between 7 to 22 times (Tab. 1). Despite their slightly less effectiveness in reducing tipburn, the temperature-dependant variants should be preferred for the sake of economic yield-loss relations.

There is general agreement that growth rate is an important factor in the development of tipburn incidence in lettuce. This also is reflected by the results of the present trials. The occurrence of tipburn was dependant on the daily growth rate of the plants. Besides, head weight of the plants, which is closely correlated to daily growth rate, has also been revealed to influence tipburn incidence (WISSEMEIER 1996). The goodness of the correlations is comparatively low, which is in correspondence to works of other researchers, though (WISSEMEIER and ZÜHLKE 2002). The importance of growth parameters as factors in the development of tipburn has also been revealed in previous trials (Chapter C).

WISSEMEIER (1996) performed extensive examinations by means of regression analysis, correlating tipburn incidence to several climatic factors that were assumed to influence the occurrence of tipburn in head lettuce. He found that the sum of global irradiation during the period of cultivation had high impact on tipburn incidence. Unfortunately, the climatic data had to be monitored in a weather station 8 km away from the field experiments. This may have been a reason for the relatively low correlations (r^2=0.23). Although the correlation was not significant in the present trial the coefficient of determination (r^2=0.52) was higher compared to that of WISSEMEIER (1996). Temperatures above 24°C, that were assumed to increase tipburn risk, could not be confirmed as relevant factor in the present trial (Fig. 12). The short-term irrigation was started as soon as temperatures exceeded 24°C (1I_temp, 3I_temp). The subsequent reduction of temperature below 24°C may therefore not be of any significance, but the reduction of the sum of temperatures. However, regression analysis revealed that T_{SUM} had no effect on tipburn incidence (data not presented).

The data given above lead to the assumption that VPD can be reduced to 0.0 kPa within the crop stand by the application of short-term irrigation. In combination with the natural dew formation the period of time with almost complete water saturation of the air in the crop microclimate can be extended. Transpiration of the plants may be reduced during this period, and the calcium supply to the less transpiring sensitive organs may thereby be enhanced. As a consequence, tipburn severity probably was reduced.

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6. Summary

The present study was aimed at the reduction of tipburn incidence in field grown butterhead lettuce cv. 'Herman' by short-term irrigation. The short-term irrigation treatments were carried out with various irrigation intensity levels (1 or 3 l m^{-2}) either depending on temperature (>24 °C) or at daily intervals, each at 07:00 p.m.. The investigations were conducted in four planting sets.
Following short-term irrigation treatment the VPD within the crop stand could be reduced. On days with temperatures exceeding average values, a reduction of VPD down to 0.0 kPa could be achieved. This was in sharp contrast to the control treatment. The reduction of VPD was independent from the irrigation intensity level. This diurnal decrease of VPD was suggested to prevent tipburn incidence in the plants. Although tipburn incidence could be reduced by about 20 %, no significant differences were established by statistical analysis. However, a recurrent tendency of the efficiency of the short-term irrigation treatments could be observed throughout the planting sets. The moistening of the leaves and soil surface, particularly after the crop stand was closed, did not lead, as assumed beforehand, to a higher incidence of lettuce rot.

E. EVALUATION OF HEAD LETTUCE GENOTYPES FOR SUSCEPTIBILITY TO TIPBURN UNDER CONTROLLED ENVIRONMENTAL CONDITIONS

1. Introduction

Tipburn injury, i.e. the development of necrosis at the leaf margins due to a localised Ca-deficiency, in head lettuce is a serious problem in commercial vegetable production. It is not tolerated by the consumers. Therefore, it can lead to a reduction in marketable quality up to a total loss of the economic value of the crop.
Despite improvements in cultural practices, breeding and selection for resistant or tolerant cultivars is still considered as the most effective precaution against tipburn (COLLIER and TIBBITTS 1982; JOHNSON 1991; ROSEN 1990; SAURE 1998; WISSEMEIER 1996). The uncertainty of tipburn incidence in field trials makes the process of breeding difficult. Therefore, it is not to be excluded that susceptible lines are overlooked, despite extensive screening of genotypes (NAGATA and STRATTON 1994). An objective and practicable procedure for evaluation of tipburn response has not been established, yet. Previous investigations aimed at the expression of tipburn symptoms by changing the atmosphere conditions for the plants, i.e. elevated temperatures (MISAGHI et al. 1981) or high relative humidity (COLLIER and TIBBITTS 1984). Tipburn injury was encouraged by a reduction of the calcium nutrition as well (ASHKAR and RIES 1971). BARTA and TIBBITTS (2000) successfully induced tipburn in lettuce plants by cultivation under temperatures of continuously 20°C and 65 % relative humidity. In case of greenhouse trials, these atmosphere conditions could be established without difficulty.
Genotypes to be screened will not only vary in severity of tipburn incidence. Variations in other plant characteristics also are to be expected. For different cultivars within the same species, deviations in nutrient uptake and translocation within the plant could be observed (MARSCHNER 1995). Despite the genotypic effect on calcium distribution in lettuce, plant characteristics should be determined additionally that can indicate a tipburn susceptible lettuce cultivar. Those studies should help to understand the causes for tipburn development in lettuce.

2. Material and Methods

2.1. Site of experimentation

The experiments were carried out in a greenhouse at the Experimental Station Marhof belonging to the Institute of Horticulture, University of Bonn. The station is located 15 km to the north of Bonn between ‘Vorgebirge’ and ‘Kölner Bucht’ at 50° 49” northern parallel (6° 59” eastern longitude). It represents a typical location for horticultural farming in the Rhineland area.
Greenhouse climate was controlled and recorded by a RAM (Hersching, Germany) climate control computer. Additionally air temperature and relative humidity were recorded within the crop stand by mini data logger (Meilhaus Electronic, Puchheim, Germany), installed in weather-instrument shelters.

2.2. Plant Material

Experimental plots were planted in different sets starting in 02/2002 until 06/2002 and from 10/2002 to 07/2003. Three cultivars of butterhead lettuce were chosen for variations in susceptibility to tipburn (Tab. 1). Tipburn sensitivity of the genotypes tested was graded according to the results of numerous field trials conducted by the horticultural extension service of the state North Rhine Westphalia.
Plants were set up in rockwool cubes or in pots containing perlite (granulation 0-3 mm) consisting of one plant per cube and pot, respectively. Planting density was 10 plants m^{-2}. Cubes or pots were placed in saucers to which nutrient solution was applied. Each experimental plot consisted of 64 plants per treatment, random samples were taken for each measurement.
Lettuce plants grown in rockwool were determined for root pressure measurements. A 10 mm piece of a PE-tube (18 mm inner diameter) was fixed on top of the cube to ensure homogenous sampling spots due to an upright development of the hypocotyls.
Plants were fertilized by nutrient solution containing: [mM/L] 13.7 N; 1.65 P; 1.95 K; [µM/L] 650 MgO; 53 Fe; 1200 Ca; 9 Mn; 9 B; 1.5 Cu; 1.5 Zn; 0.25 Mo at a pH of 6.0-6.5. Nutrient solution was supplied according to plant development.

Tab. 1: Lettuce cultivars varying in susceptibility to tipburn according to the horticultural extension service and used for the experiments.

Cultivar	Tipburn susceptibility	Breeder
Herman [3]	high	(S&G, Kleve)
Cormoran [2]	moderate	(S&G, Kleve)
Nadine [1]	low	(RZ, Welver)

2.3. Greenhouse climate

Plants were cultivated in tipburn-inducing climate according to BARTA and TIBBITTS (2000). Tipburn-inducing climate was maintained at the following levels for air temperature and relative humidity: 20°C±2 and 65%±10 throughout light and dark period. Corresponding to natural day length a 16-h photoperiod was maintained by additionally applied assimilation light (Philips SON-T agro) at a photosynthetic photon flux of 350±50 µmol $^{-2}$ s^{-1}.

2.4. Evaluation of tipburn and preparation of samples for analysis of minerals

Tipburn symptoms were evaluated weekly and recorded as percentage of tipburned plants. Plants with at least one leaf showing tipburn symptoms were scored as "tipburned plants". Since under greenhouse conditions the heads of lettuce plants did not close completely, evaluation of tipburn incidence was possible without destroying the plants.

Plants grown in perlite were used for analysis of minerals in various plant parts. Ten plants representing the treatment were taken out of each plot. They were divided into outer leaves, inner leaves and roots. Roots were washed with distilled water in order to remove adherent substrate. Samples were dried at 60-80°C (24 h) and 105°C for 2 h before grinding. An aliquot of 0.3 g was digested with HNO_3 and H_2O_2. Total calcium, magnesium and potassium content was analysed by atomic absorption spectrometry (AAS).

2.5. Measurement of root pressure exudate and its mineral constituents

Root pressure was measured from decapitated plants that were grown in rock-wool. Released latex was washed off with distilled water and remaining water was carefully removed by tissue paper. A silicon pipe was fixed and sealed to the stump as reservoir for root pressure exudate, which was pipetted and weighed after 1 hour in order to determine the amount of exudate flow in g h^{-1} (MORAD et al. 2000). Four plants were probed individually every four hours six times a day. These samples were combined for further examination of relevant exudate constituents. They were frozen and stored at -20°C until analysis of calcium, magnesium and potassium content by (AAS).

2.6. Determination of specific leaf weight

To the time of symptom development 6 leaf disks (16 mm diameter) per plant from the tipburn sensitive leaves were taken from each of 10 plants per cultivar. The leaf samples were weighed for fresh weight, dried at 60-80°C (24 h) and weighed for the determination of dry weight. The specific leaf weight was calculated by relating the dry weight of a single leaf disk to the area of the sampled leaf area (LA).

2.7. Sample preparation for determination of cell density

Five plants representing the treatment were chosen randomly for determination of cell density. Leaf samples were embedded in epoxy resin using a complete system for embedding and cutting histological specimen (Technovit 7100/3040 supplied by Hereaus Kulzer, Hanau, Germany). Sample preparation was carried out according to the procedure recommended by the supplier. Histological cuts were accomplished parallel to the leaf surface by a rotary microtome (Microm, Germany). 10-µm slices were stained with 0.1 % Toluidinblue, number of cells per area was determined in the palisade parenchyma using a light microscope at a

100-fold magnification (Olympus, Germany). Three measurements, each within 0.01 mm^{-2}, were taken, out of each specimen and averaged.

2.8. Statistical analysis

Plants were cultivated on different greenhouse benches. Since between the benches homogeneity could be assumed for all the environmental factors relevant to the experiment, the test plants were arranged in completely randomised plots. In order to realize treatments with two different humidity regimes the plants had to be distributed on different cabins (or benches, respectively). Samples were randomly taken out of 65 single plants per treatment. Statistical data was calculated with the statistic program 'SAS 8.0' (SAS Institute Inc., Cary, USA). Data were subjected to analysis of variance. Comparisons of mean values were performed by Tukey's HSD or t-test. Statistical differences were accepted at 5 % level of significance. Graphs were constructed with SigmaPlot 8.0 (SPSS Science Inc.).

3. Results

3.1. Responses of lettuce genotype on

3.1.1. tipburn incidence

Three head lettuce cultivars differing in susceptibility to tipburn were chosen with 'Nadine' described as insusceptible and 'Herman' as sensitive to tipburn injury. Tipburn susceptibility of 'Cormoran' was considered to be moderate. Tipburn incidence depending on genotype and planting date is presented in table 2. The incidence of tipburn corresponded with the previously described tipburn sensitivity of the genotypes. However, under greenhouse conditions with high tipburn risk 'Cormoran' responded similar to 'Nadine'. That means, there was hardly any difference between "low" and "moderate" susceptibility and even the non-susceptible cultivar expressed tipburn symptoms.

Tab. 2: Tipburn incidence in head lettuce cultivars differing in susceptibility to tipburn. Numbers in parentheses [1-3] indicate tipburn sensitivity of the cultivars: 1=low, 2=moderate, 3=high. Plants were submitted to tipburn inducing conditions (VPD 0.82 kPa day/night). Differences between cultivars were significant at $p \leq 0.05$. Cv. 'Cormoran' was not tested within the first planting set *(n.n.)*. Data given here is representative for the experiments repeated within two years.

		Tipburned plants (%)		
Cultivar		(03/27/-05/13/02)	(03/24/-05/14/03)	(04/24/-06/23/03)
Nadine	*[1]*	52.1 *a*	0.0 *a*	16.7 *a*
Cormoran	*[2]*	*n.n.*	18.75 *b*	8.33 *a*
Herman	*[3]*	92.8 *b*	87.5 *c*	75.0 *b*

3.1.2. plant growth during early stages of plant development

It is generally suggested that accelerated plant growth might be one of the relevant factors causing tipburn injury. Therefore, daily plant growth throughout post seedling development was related to tipburn incidence of the respective planting sets. Furthermore, it was the objective of the study to evaluate the possibility for detecting tipburn susceptible genotypes at early stages of plant development.

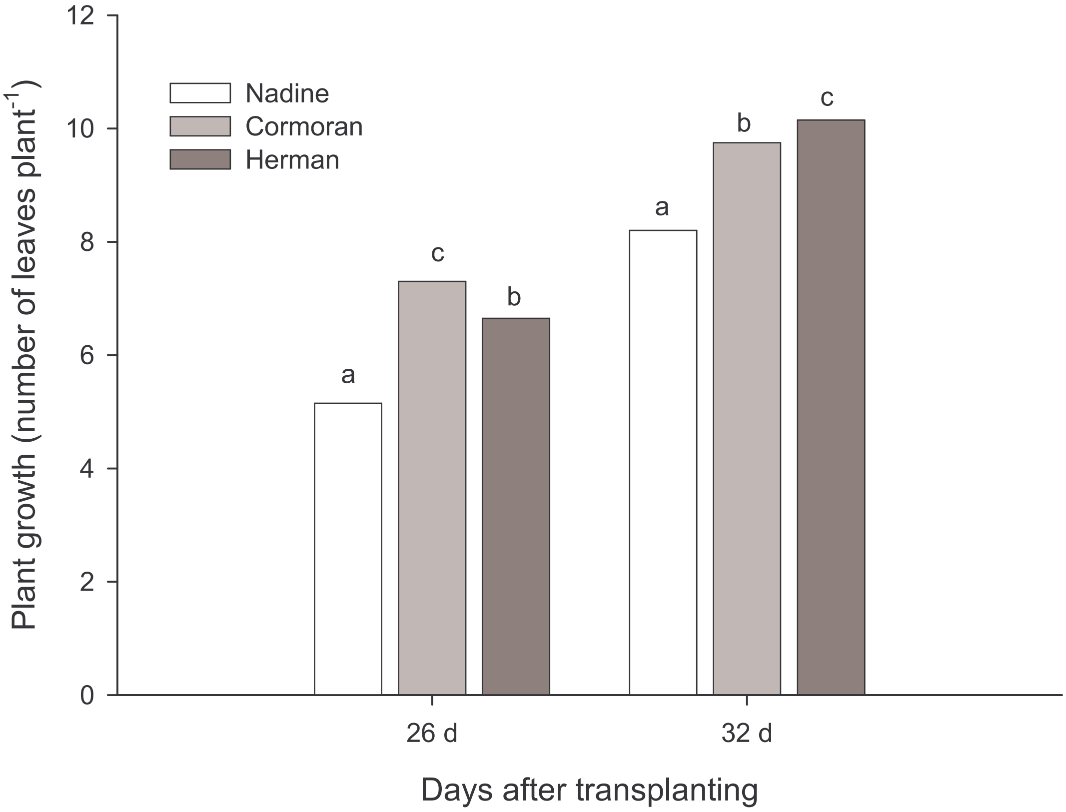

Fig. 1: Effect of head lettuce cultivar on plant growth given as number of leaves per plant counted above the cotyledons. Differences between cultivars within the same sampling date were significant at $p \leq 0.05$. The data is representative for the experimental plots repeated within two years. Tipburn sensitivity of the cv.: Herman=high, Cormoran=moderate, Nadine=low.

Within several planting sets the tipburn insusceptible cultivar (Nadine) developed significantly lower numbers of leaves per plant (Fig. 1). Regression analysis revealed that daily plant growth (during post seedling development) had a significant effect on tipburn incidence of the lettuce cultivars. Higher incidence of tipburn therefore can be attributed by about 55 % to a higher growth rate (Fig. 2). The importance of plant growth as relevant factor for tipburn induction is again highlighted by this result. An option to detect tipburn susceptible plants before head formation is thereby suggested.

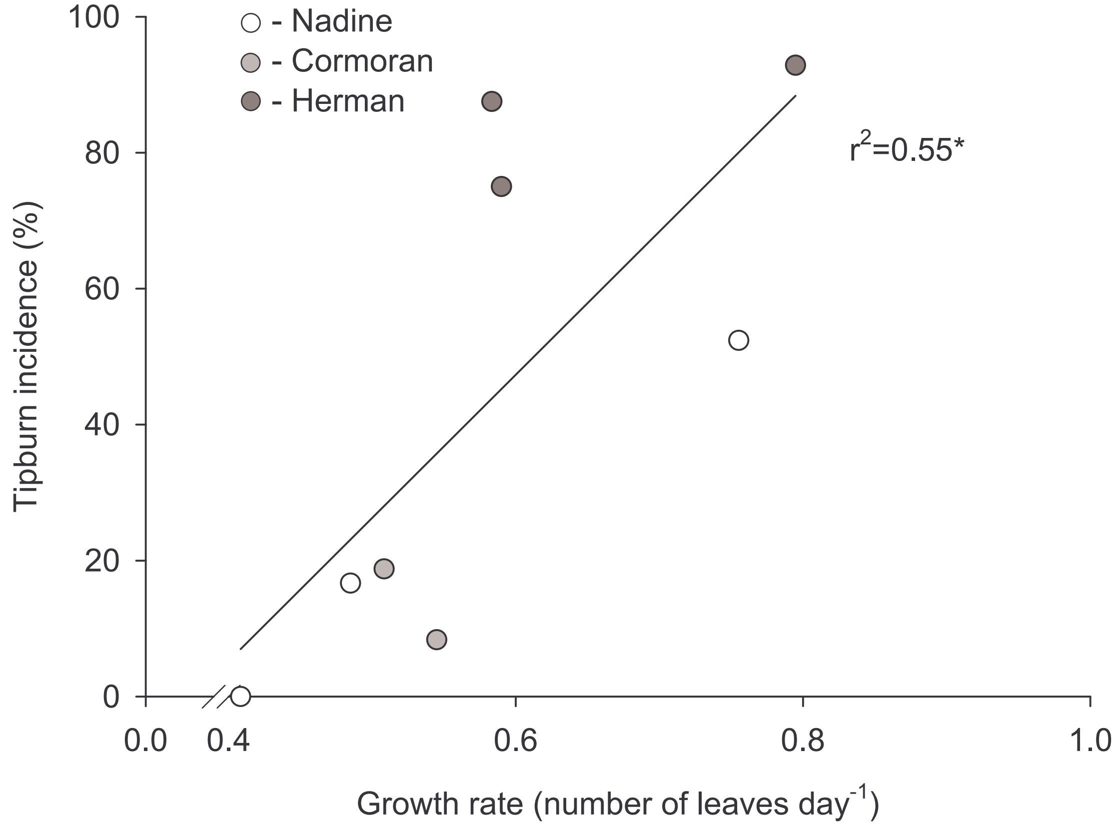

Fig. 2: Effect of growth rate given as number of leaves day^{-1} on tipburn incidence of head lettuce leaf injury was estimated during post seedling development. Correlation (r^2=0.55*) is significant at $p<0.05$. Tipburn sensitivity of the cv.: Herman=high, Cormoran=moderate, Nadine=low.

3.1.3. specific leaf weight and cell density

Specific leaf weight (mg DM cm^{-2} LA) was determined from randomly selected plants at harvest time. The tipburn insusceptible genotype (Nadine) had a higher specific leaf weight than the sensitive cultivar (Fig. 3). Higher specific leaf weight (based on dry matter) can be explained by about 50 % by a higher cell density. This suggestion is supported by regression analysis (r^2=0.53) which is presented in Fig. 4. The tipburn resistant insusceptible head lettuce genotype developed more and consequently smaller cells per leaf area. Generally the demand for calcium is higher during cell enlargement than for cell division. Therefore, this data provides information that plants with smaller cells were assumed to be less sensitive to cell wall destabilisation due to a lack of calcium.

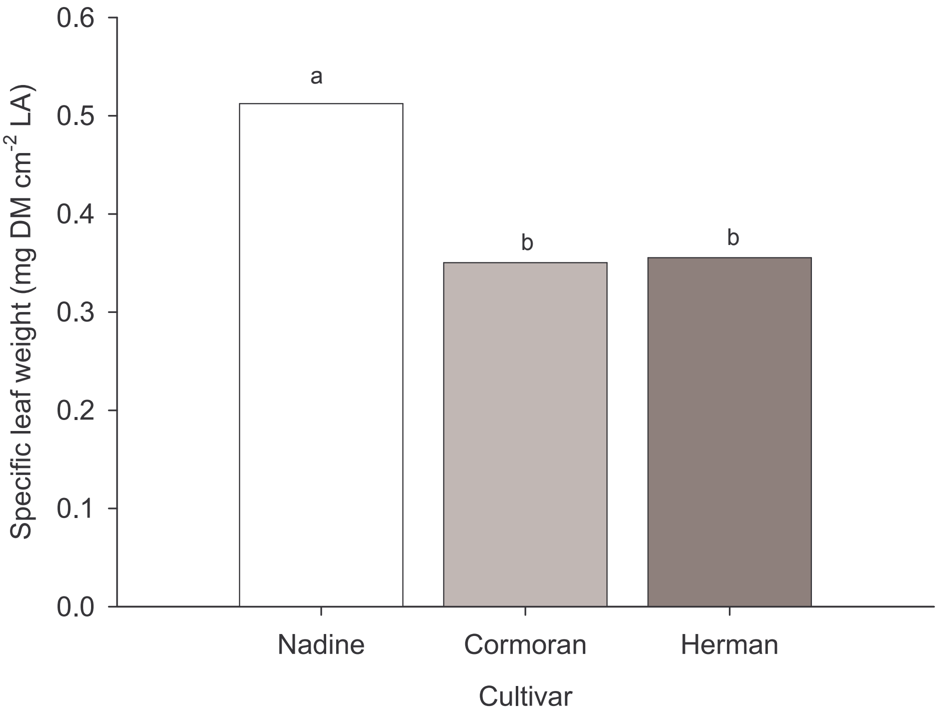

Fig. 3: Specific leaf weight (mg DM cm^{-2} LA) of head lettuce cultivars varying in tipburn susceptibility. Differences between cultivars (n=10) were significant at $p\leq0.05$. The data is representative for the conducted experiments. Tipburn sensitivity of the cv.: Herman=high, Cormoran=moderate, Nadine=low.

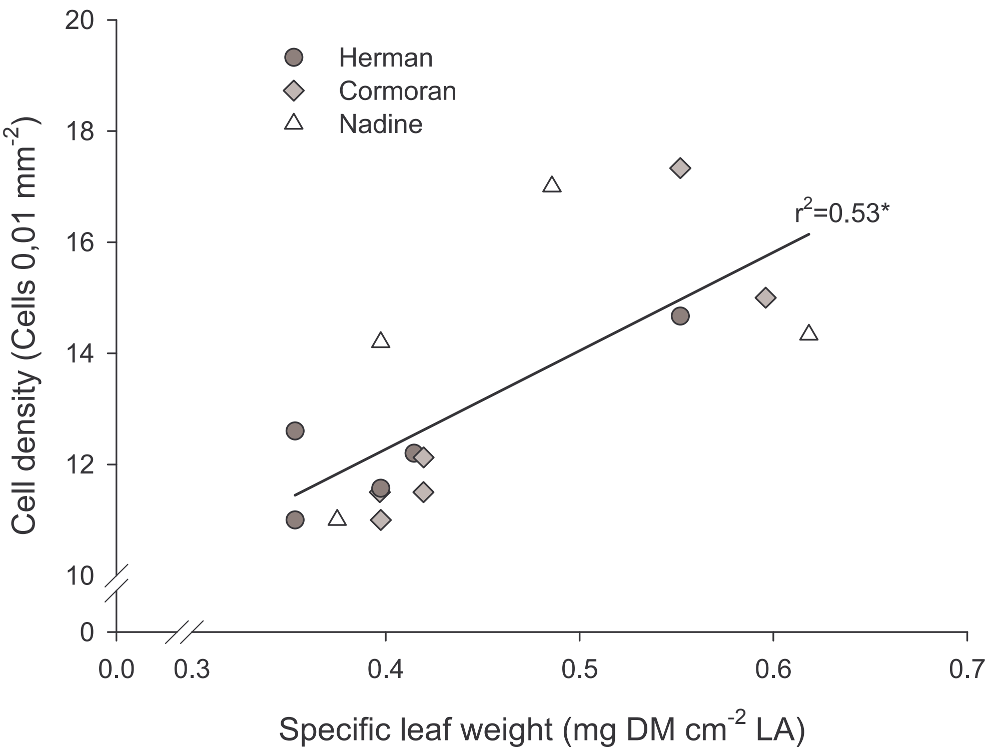

Fig. 4: Specific leaf weight (mg DM cm^{-2} LA) as influenced by cell density (no. of cells 0.01 mm^{-2}) of head lettuce leaves. Cell density was determined in the palisade parenchyma. Correlation (r^2=0.53*) is significant at $p\leq0.05$. Tipburn sensitivity of the cvs.: Herman=high, Cormoran=moderate, Nadine=low.

3.1.4. dry matter and mineral content of the leaves

The results of preceding experiments (Chapter C) revealed that the content of dry matter in the leaves declined as a consequence of reduced VPD at night. The influence of head lettuce genotype on dry matter content and mineral constituents in the leaf tissue is presented in Tab. 3. In respect of mineral constituents and dry matter content of the leaves there was no effect of the tipburn susceptibility of the genotypes. But it is worth to mention that the relative cation ratios of the sensitive cv. ‘Herman’ were lower in the outer leaves and similar in the inner leaves compared to the insusceptible cv. ‘Nadine’. The assumption of a cation competition existing in the leaf tissue, which would be indicated by a wide K/Ca ratio, could not be confirmed by the data of the tipburn susceptible genotype. This data provides information that the lettuce cultivars could not be categorised regarding tipburn sensitivity on the basis of leaf mineral status.

Tab. 3: Dry matter (DM) and leaf mineral composition as influenced by head lettuce genotype (±SE). Letters indicate statistical differences at p<0.05. Each value is a mean out of 10 plants. Tip-burn sensitivity of the cultivars: Nadine=low, Cormoran=moderate, Herman=high.

Dry matter (%)

	Inner leaves *n.s.*	Outer leaves
Nadine	9.9 ± 0.28	7.4 ± 0.43 *a*
Cormoran	9.3 ± 0.25	8.4 ± 0.29 *b*
Herman	8.9 ± 0.08	7.2 ± 0.22 *a*

Ca (% of DM)

	Inner leaves *n.s.*	Outer leaves
Nadine	0.93 ± 0.04	1.43 ± 0.06 *ab*
Cormoran	0.81 ± 0.04	1.30 ± 0.05 *a*
Herman	0.87 ± 0.04	1.52 ± 0.09 *b*

Mg (% of DM)

	Inner leaves *n.s.*	Outer leaves
Nadine	0.21 ± 0.01	0.31 ± 0.01 *a*
Cormoran	0.21 ± 0.01	0.27 ± 0.01 *b*
Herman	0.21 ± 0.01	0.30 ± 0.01 *ab*

K (% of DM)

	Inner leaves	Outer leaves *n.s.*
Nadine	3.52 ± 0.10 *a*	5.86 ± 0.24
Cormoran	3.87 ± 0.15 *b*	6.13 ± 0.29
Herman	3.46 ± 0.12 *a*	5.64 ± 0.18

Cation ratios

	Inner leaves		Outer leaves	
	K/Ca	Ca/Mg	K/Ca	Ca/Mg
Nadine	3.8	4.4	4.1	4.6
Cormoran	4.8	3.9	4.7	4.8
Herman	4.0	4.1	3.7	5.1

3.2. Effect of the genotype on root pressure flow and mineral content of the root pressure exudate

The test plants were submitted to tipburn inducing climate. Root pressure measurements were carried out when plants of the susceptible cv. developed tipburn symptoms. Root pressure flow of the cultivars revealed a diurnal course within the sampling period. It was highest at the beginning of the light period and declined constantly afterwards. An effect of the genotype on root pressure flow, given as amount of root pressure exudate per hour, could not be found (Fig. 5A). The data presented here correspond to results obtained by earlier trials (Chapter C). Furthermore, the effect of the genotype on the qualitative composition of the exudate was investigated. The tipburn sensitive cultivar ('Herman') showed temporarily higher concentrations of magnesium and potassium throughout light and dark period, whereas the insusceptible cv. 'Nadine' had higher concentrations of calcium during the night (Fig. 5B-D). The course of mineral nutrients concerning calcium and its antagonists, i.e. magnesium and potassium in the exudate particularly during dark period, suggested that nutrient uptake is dependant on lettuce genotype.

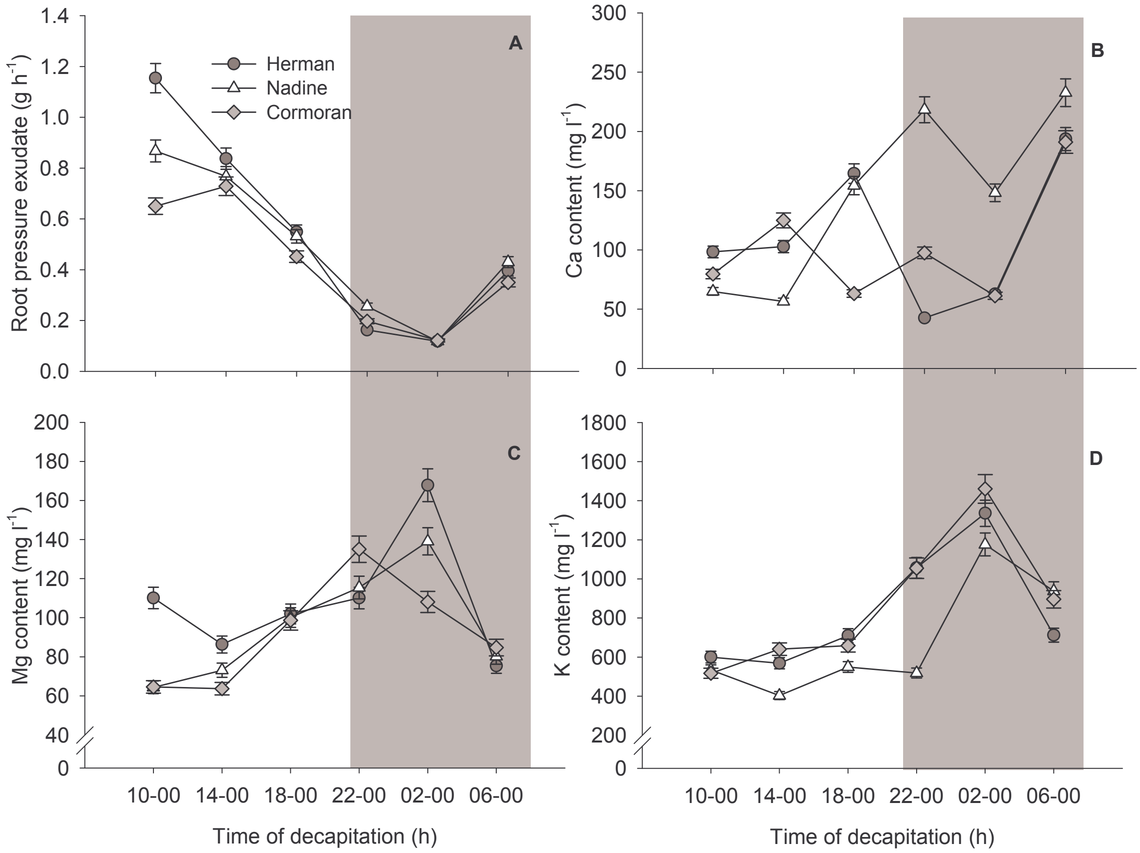

Fig. 5: Root pressure exudate flow (A) and mineral content (B-D) of the exudate in three head lettuce cultivars varying in tipburn sensitivity. Root pressure exudate was collected for 1 hour after decapitation every 4 hours and monitored for 24 hours. Each value represents a mean out of 2 planting sets. Shaded areas indicate night period. Plants were cultivated under constant VPD of 0.82 kPa (day/night). Tipburn sensitivity of the cultivars: Herman=high, Cormoran=moderate, Nadine=low. Error bars indicate LSD (5%).

Higher tipburn incidence of the cv. ‘Herman’ (Tab. 2) could be related to a wider K/Ca ratio (K/Ca>100) during night time (Fig. 6). The lower tipburn sensitivity of the cv. ‘Nadine’ corresponded well to a lower K/Ca ratio in the exudate (K/Ca=8). The K/Ca ratio of ‘Herman’ increased with the beginning of night time and declined afterwards, whereas the ratio of ‘Nadine’ was on a constantly and significantly lower level throughout the time course study. The moderate tipburn sensitivity of cv. ‘Cormoran’ could be confirmed by the K/Ca ratio as well, which varied within the values of the other cultivars. The diurnal course of the Ca/Mg ratio varied indefinitely regarding the cultivars. Ca/Mg ratio of ‘Herman’ was higher at daytime whereas the Ca/Mg ratio of ‘Nadine’ was higher during night time. The tipburn sensitivity of the cultivars therefore could not be classified on the basis of the Ca/Mg ratio.

The date obtained by these experiments provide evidence that calcium uptake of cv. 'Herman' in part of the dark period (10:00 p.m.-02:00 a.m.) seemed to be limited due to an increased concentration of Mg^{2+} and K^{+} in the exudate (Fig. 5). This suggestion is supported by the increased K/Ca ratio of cv. 'Herman' at the beginning of the night time (Fig. 6).

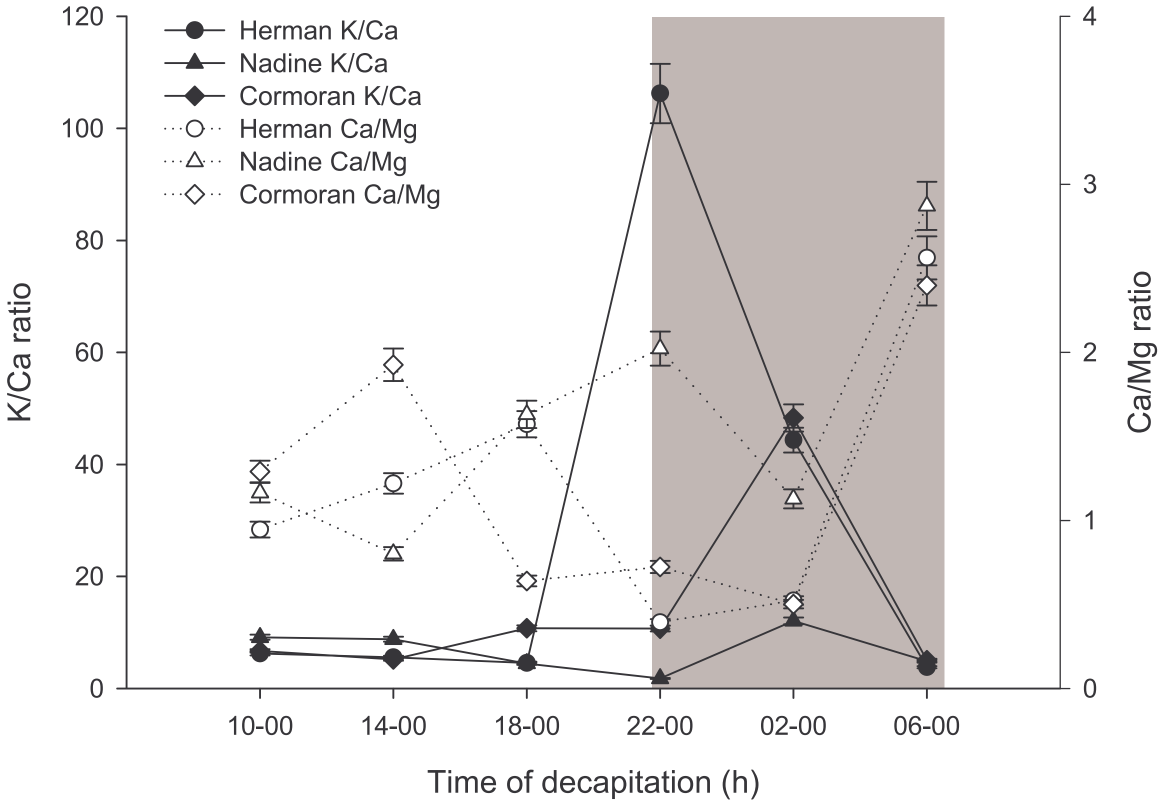

Fig. 6: K/Ca and Ca/Mg ratio in the root pressure exudate of three lettuce genotypes differing in tipburn susceptibility. Each value represents a mean out of 2 planting sets. Root pressure exudate was collected for 1 hour after decapitation every 4 hours and monitored for 24 hours. Shaded areas indicate night time. Plants were cultivated under constant VPD of 0.82 kPa (day/night). Tipburn sensitivity of the cultivars: Herman=high, Cormoran=moderate, Nadine=low. Error bars indicate LSD (5%).

4. Discussion

This investigation mainly aimed at the establishment of a procedure to evaluate head lettuce genotypes under controlled environmental conditions for their susceptibility to tipburn injury. Initially, parameters were to be estimated from several head lettuce cultivars varying in tipburn susceptibility. On the basis of these parameters the tipburn sensitivity of a cultivar should be predictable before head formation. For the trials presented here, plants were cultivated under tipburn inducing climate in order to force the expression of tipburn symptoms (Chapter C; BARTA and TIBBITTS 2000). Tipburn sensitivity of the tested genotypes was graded according to the results of numerous field trials conducted by the horticultural extension service of the state North Rhine Westphalia.

Genotypes

A genotypic variation in susceptibility to calcium deficiency disorders has been described by numerous authors (COX et al. 1976; HAGEL et al. 2002; MAROTO et al. 1985; MISAGHI et al. 1981; OHLS 1989; THEILER et al. 2003; WISSEMEIER 1996). As could be shown by the results described above tipburn incidence also was strongly dependant on head lettuce genotype. The risk for tipburn injury was increased in the greenhouse by conditions of constantly high VPD. The expression of tipburn symptoms within the greenhouse experiments corresponded to the tipburn sensitivity as determined previously on the basis of field trials (Tab. 2). The grading of the tipburn susceptibility of the genotypes remained valid independent of climate or growing substrate. Therefore, an evaluation of open field cultivars is also possible in the greenhouse (WISSEMEIER 1996). However, data of the cv. ‘Herman’ and ‘Nadine’ did not correspond completely to results obtained by a Swiss research group. They carried out field trials for tipburn evaluation in 2002 with several genotypes (THEILER et al. 2003). Tipburn susceptibility of ‘Nadine’ was graded as moderate and ‘Herman’ as low. ‘Cormoran’ was described analogous to the present classification. These trials were carried out within the first year without replication. However, tipburn rating could vary under the influence of varying weather conditions of consecutive years. Deviations in tipburn rating may also derive from differing sampling methods. Since small symptoms can easily be overlooked, the estimation of tipburn severity from longitudinally cut open lettuce heads is generally less precise compared to the examination of defoliated heads (Chapters B and C; THEILER et al. 2003; WISSEMEIER 1996). The elevated tipburn susceptibility of cv. 'Herman' could be verified by various greenhouse and growth chamber experiments (unpublished data), whereas low tipburn susceptibility of 'Nadine' was additionally confirmed by investigations of HAGEL et al. (2002).

In the following section parameters that were suited for a categorisation of the tipburn susceptibility of lettuce plants are considered.

Growth rate

Genotypic differences in tipburn susceptibility can be closely related to the growth rates (BERT and HONMA 1975; WISSEMEIER 1996). The unsusceptible cultivar (Nadine) had lower growth rates compared to the sensitive cv. 'Herman'. A significant correlation between plant growth and tipburn incidence could be established when growth rate was determined at an early stage of plant development (Fig. 2). It is generally agreed that plant growth has a significant impact on the expression of tipburn symptoms (COLLIER 1983). Usually tipburn occurs in leaves that were expanding rapidly (COLLIER and HUNTINGTON 1983). Higher growth rates lead to an earlier initiation of Ca-deficiency symptoms (COX et al. 1976). When leaf initiation exceeded 1.5 leaves day^{-1} tipburn symptoms had been induced (TIBBITTS and RAO 1968). This value is considered to be high, since it could be shown by the results presented above that tipburn symptoms were already expressed when leaf initiation was <0.8 leaves day^{-1}. The sensitive cv. 'Herman' developed 0.7 leaves day^{-1}, whereas leaf development of the insusceptible cv. 'Nadine' was about 0.6 leaves day^{-1}. A threshold value indicating susceptibility could be assumed between 0.7 and 0.6 leaves day^{-1}. WISSEMEIER (1996) suggested that varietal differences could be determined best when growth rates were considered. The determination of growth rate at an early stage of plant development may therefore be a reliable parameter for the differentiation of tipburn susceptible genotypes.

Growth rate has often been considered to influence the calcium content of the leaves (COLLIER 1983). The occurrence of tipburn due to accelerated growth was predominantly associated with an insufficient calcium utilisation (COLLIER and HUNTINGTON 1983). The induction of tipburn symptoms may be depending on calcium supply in relation to the rate of leaf growth (COLLIER and HUNTINGTON 1983). A higher calcium utilisation of Ca-use-efficient lines of tomato genotypes was not contributed to variations in the concentration of different Ca-fractions, such as higher soluble calcium (CAINS and SHENNAN 1999). Therefore, total Ca-content was taken into consideration as a parameter to characterise tipburn susceptible lettuce cultivars. HUETT (1994) found differences in the leaf tissue Ca-concentration which could be related to tipburn. It was suggested that this relation may account for variations in the response of the cultivar. For other vegetable crops, such as cauliflower, a lower Ca-concentration in the leaves could be associated with a higher tipburn incidence in the field. It was concluded that a higher tolerance to Ca-deficiency symptoms is depending on higher Ca-concentrations (MAYNARD et al. 1981). The data presented above gave no evidence for correlations between mineral contents of the leaves and tipburn susceptibility of the genotypes (Tab. 3). These results were corresponding to the findings of other authors (ROSEN 1990; SAURE 1998; WISSEMEIER 1996). They assumed that tipburn susceptibility could not be related to the leaf Ca-content and a prediction of tipburn incidence would not be possible on this basis. A classification of Chinese cabbage cultivars on the basis of leaf mineral content and the relative cation ratios could also not be accomplished. However, K/Ca ratio in the leaves increased with increasing sensitivity of the cultivar (OHLS 1989). This observation could not be confirmed by the investigations presented above (Tab. 3).

<u>Leaf characteristics</u>

The results of earlier experiments showed that in tomato cultivars increased dry weight of the leaves was accompanied by improved Ca-efficiency (CAINS and SHENNAN 1999). Regarding lettuce plants similar observations were made. For the sensitive cv. ‘Herman’ a lower content of dry matter (8.9 %) was found, compared to the insusceptible cv. ‘Nadine’ (9.9 %). However, these differences were not statistically different. Regarding specific leaf weight, differences between the cultivars reflecting their tipburn susceptibility level could be established (Fig. 3). The insensitive cultivar had a significantly higher specific leaf weight. Furthermore, higher cell density and thus, smaller cells per area were determined. The higher specific leaf weight could be explained by about 50 % by the higher cell density. The remaining source of variation may be related to the leaf thickness which for technical reasons had not been determined. Smaller cells of the insensitive genotype may refer to a higher cell stability since the fraction of cell wall is higher in smaller cells.

Mineral content

Leaf mineral content did not prove to be a suitable indicator for a categorisation of the tipburn susceptibility in the tested lettuce cultivars. The variations of the concentrations may have been too small to be detected by AAS. Due to the analysis of undiluted samples from the xylem exudate these differences were expressed noticeably.

A previous study revealed that a decrease of VPD during the night had an effect on the quality of the root pressure exudate (Chapter C). Quantitative and qualitative data of root pressure exudates which would allow to compare lettuce cultivars differing in tipburn susceptibility are not available so far (WISSEMEIER 1996). The present trials revealed that there was no effect of the cultivar on the quantity of root pressure exudate flow (Fig. 5A). These results were in correspondence to suggestions of SAURE (1998), who assumed that root pressure may not be the principle driving force for the import of Ca^{2+} into low-transpiring organs alone. Even though the plants were cultivated under conditions of continuously high transpiration, i.e. VPD 0.82 kPa (day/night), a diurnal course of the root pressure exudation was to be observed. In general, this diurnal course can be related to variations of temperatures (MORAD et al. 2000; SCHURR and SCHULZE 1995). Within the present experiment, a variation between day and night temperature was not intended. Due to the constant temperatures in the greenhouse cabin during day and night, an effect of temperature on the diurnal course of the exudate can be excluded. Unlike quantity the quality of the root pressure exudate within the diurnal course could be related to the tipburn susceptibility of the cultivar (Fig. 5B-D). The lower Ca^{2+} and higher Mg^{2+} and K^{+} concentrations throughout the night as well as the ion transport rate corresponded well with the tipburn sensitivity of the cv. 'Herman'. This indicated an ion competition which in a tipburn sensitive cultivar may impede calcium transport via the xylem. This suggestion is supported by the considerable increase of the K/Ca ratio at the beginning of the dark period. The concentrations of nutrients in the exudate, particularly the relative cation concentrations, are dependant on the nutrient status of the plants and the temperature and humidity conditions during the experimentation (OHLS 1989). For mineral constituents a comparison of total values therefore is not practicable. Since the calcium mobility and content in the phloem is extremely poor, contaminations of the root pressure exudate with calcium of phloem fractions are negligible (JESCHKE and PATE 1991). By sampling xylem exudate from decapitated plants, certain amounts of shoot derived potassium may contaminate the sample (MARSCHNER 1995). If collected for a short time after decapitation, i.e. one hour, exudate concentrations were similar to those of intact transpiring plants (SIEBRECHT 2000). The diurnal course of the analysed nutrients is also reported for other plants, like *Ricinus*, tomato, cucumber and broccoli (HO and ADAMS 1994; SCHURR and SCHULZE 1995; SHELP 1987). High calcium and magnesium concentrations during the night were found in *Ricinus* plants, which at least corresponded to the values of 'Nadine'. HO and ADAMS (1994) calculated for tomato that due to the diurnal course about 80 % of the calcium is absorbed during the day. This leads to the suggestion that an ion competition particularly throughout the period with minor importance for Ca-uptake (night time) would have a significant impact on the calcium distribution within the plant.

<u>Evaluation of tipburn susceptibility</u>

An objective and practicable procedure for the evaluation of tipburn response in lettuce cultivars has not been established, yet. The characterisation of tipburn susceptible genotypes with the help of mineral contents of the leaves was not possible due to insufficient statistical power of prognosis (OHLS 1989). Furthermore, a variation in transpiration rate could also not be related to the tipburn sensitivity of the genotypes (BRUMM 1992). Earlier studies aimed at the expression of tipburn symptoms by varying only one single factor of the atmosphere environment. The variations of a single factor were generally contributed to a change in growth rate (COX et al. 1976). By increasing either temperature (MISAGHI et al. 1981) or relative humidity (COLLIER and TIBBITTS 1984) tipburn symptoms could be induced. Lowering the calcium nutrition has been carried out as well, which is more effective if climatic conditions were suitable for occurrence of tipburn (ASHKAR and RIES 1971). NAGATA and STRATTON (1994) combined these three factors for their experiments. However, they omitted calcium fertilisation totally, which does not allow to discriminate between an over-all Ca-deficiency and a localised Ca-deficiency caused by physiological processes, like tipburn of lettuce. It is not adequate to draw any conclusions from seedlings developing absolute Ca-deficiency symptoms with tipburn symptoms was not valid since a correlation between these symptoms could not be found (WISSEMEIER 1996). The climatic conditions utilised by BARTA and TIBBITTS (2000) during day and night period seemed to be most promising for an utilisation of the testing procedure under controlled environment. The set point values for temperature (20°C) and relative humidity (65 %) could be realised under greenhouse conditions without difficulties.

The cultivation of lettuce plants submitted to tipburn inducing atmosphere conditions in the greenhouse is the basis for a method to evaluate tipburn susceptibility of head lettuce genotypes. In the present study several parameters were determined that were supposed to be suitable to discriminate between tipburn susceptible and unsusceptible lettuce cultivars. Depending on tipburn sensitivity of the genotype the following characteristics became apparent: a tipburn insensitive cultivar had a lower growth rate during early stages of plant development, a higher specific leaf weight and a higher cell density, i.e. smaller cells per area. The mineral constituents were influenced particularly during night period. The calcium contents in the root pressure exudate were higher whereas magnesium and potassium were slightly lower. The K/Ca ratio was on a constantly lower level throughout the diurnal course, whereas the sensitive cultivar showed a noticeable increase of the K/Ca ratio during night period.

This procedure was established to detect susceptible plants before head formation. Under the presumption that correlations between the parameters described above and the respective tipburn susceptibility will be verified by large scale trials with numerous cultivars, plants can be screened before head formation and without the expression of symptoms. In order to standardise the testing conditions, this method is currently being adapted and optimised in a walk-in growth chamber. This will allow the experiments to be independent of seasonal influences, such as global irradiation, and to be carried out throughout the year.

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6. Summary

An objective and practicable procedure for early evaluation of tipburn response of lettuce cultivars has not been established, yet. In the present study head lettuce cultivars varying in tipburn susceptibility were cultivated under tipburn inducing conditions. In order to evaluate and to explain reasons for tipburn susceptibility diverse parameters were investigated and related to tipburn sensitivity of the cultivar.

Under conditions of a constantly low VPD (0.82 kPa) tipburn symptoms were expressed in relation to susceptibility of plants as graded according to the results of field trials. The tipburn sensitivity of the cultivars could be related to a higher growth rate during early stages of plant development, lower specific leaf weight and also smaller cells per area. Differences between the genotypes in tipburn incidence could not be explained by variations in the amount of root pressure flow. However, there was an effect on the qualitative compounds in the exudate, in particular during nigh time. This was revealed by an increase of the K/Ca ratio during night period for the sensitive cv. ‘Herman’. These differences were not found in the leaf mineral concentrations, though.

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