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# Ultralow oxygen treatment for postharvest control of western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae), on iceberg lettuce I. Effects of temperature, time, and oxygen level on insect mortality and lettuce quality

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**Abstract**

Ultralow oxygen (ULO) treatments with different oxygen levels, treatment times, and temperatures were studied to determine effects on western flower thrips mortality and postharvest quality of iceberg lettuce. Thrips mortality increased with reduced oxygen level and increased treatment time and temperature. At 0.003% oxygen, over 99.6% mortality rates of thrips were achieved in three ULO treatments of 2, 3, and 4 d at 10, 5, and 1 °C, respectively. No treatments caused injury to lettuce surface leaves and there was no reduction in visual quality for treated lettuce. However, about 9–33% of lettuce heads sustained injury to heartleaves. The 2 d ULO treatment with 0.003% oxygen had the lowest injury rate to heartleaves and the injury increased with increased treatment duration. The amount of injured leaves was small (<2 g per head). There were also some variations among the lettuce cultivars in susceptibility to heartleaf injury by ULO treatments. Four out of eight cultivars tested tolerated the 2 d ULO treatment at 10 °C without any injury. Therefore, ULO treatment has potential to be developed as an alternative postharvest treatment for western flower thrips on iceberg lettuce.
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*Keywords:* Controlled atmosphere; Ultralow oxygen; Phytosanitary treatment; Lettuce; Western flower thrips; Postharvest quality

## 1. Introduction

Western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is a common pest of lettuce but is quarantined in Taiwan and therefore affect the export of U.S. lettuce. The current method of methyl bromide fumigation for postharvest pest control on lettuce not only causes injury to lettuce but is also unsustainable as global production of methyl bromide is being phased out in accordance to the 2001 Montreal Protocol. Therefore, developing alternative postharvest treatments for pest control is critical for the export of U.S. lettuce and other fresh commodities to overseas markets.

Controlled atmosphere (CA) has been studied for many years as a potential alternative treatment for postharvest insect control on fresh fruits and vegetables (Whiting et al., 1992; Carpenter and Potter, 1994; Mitcham et al., 2001; Liu, 2003, 2005, 2007, 2008). A typical CA treatment is to store a commodity under an atmosphere with reduced oxygen and/or increased carbon dioxide for a certain length of time at a certain temperature to disinfest the commodity of a target pest. Iceberg lettuce, however, is very susceptible to injury by elevated $CO_2$ (Lipton et al., 1972; Brecht et al., 1973a,b,c; Stewart and Uota, 1976). $CO_2$ causes brown stains on or near the midribs of lettuce leaves and as low as 1% $CO_2$ can cause injury to susceptible iceberg lettuce (Lipton et al., 1972). The length of CA treatment required to kill insects often exceeds the limits of lettuce tolerance (Mitcham et al., 2001).

Oxygen level ≤1% is referred as ultralow oxygen (ULO). ULO has been studied for postharvest storage and pest control of fresh commodities (Zheng et al., 1993; Mitcham et al., 2001; Shellie, 2002; Legnani et al., 2004). However, low $O_2$ levels can also result in anaerobic metabolism and damage to fresh commodities (Beaudry, 2000). Low oxygen injury to lettuce can occur in the form of gray dead patches on wrapper leaves or brownish discoloration of heartleaves (Lipton et al., 1972). Storage under 0.25% $O_2$ at 2 °C for 7 d causes injury

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to iceberg lettuce (Lipton, 1967, 1971; Lipton et al., 1972). CA with 0.02% $O_2$ was found to kill aphids in 7–21 d at 0 °C (Zheng et al., 1993; Mitcham et al., 2001). Lettuce, however, can tolerate 0.02% $O_2$ CA for up to 13 d at 0 °C (Cantwell et al., 1995, 1996; Mitcham et al., 2001).

Recently, controlled atmosphere with ultralow oxygen (referred as ULO treatment thereafter) was found to be safe and effective for postharvest control of lettuce aphid, *Nasonovia ribisnigri* (Homoptera: Aphididae), on lettuce and western flower thrips on broccoli (Liu, 2005, 2007). In the present study, ULO treatments with different treatment times, temperatures, and oxygen levels were evaluated to determine their effects on survival of thrips and postharvest quality of different lettuce cultivars in an effort to development a safe and effective ULO treatment for control of western flower thrips on iceberg lettuce.

## 2. Materials and methods

### 2.1. Insects

Western flower thrips were reared on lettuce plants in a greenhouse. Potted lettuce plants were placed in Petri dishes on a layer of sandy soil in trays to allow thrips to pupate in the soil. Plants were watered daily. Thrips (larvae and adults) were collected in plastic vials (2.5 cm diameter × 7 cm high) with lettuce leaf pieces using a vacuum powered aspirator for ULO treatment. Each vial contained 10–20 thrips. The vials were sealed with screened lids lined with Kimwipe tissue.

### 2.2. Effects of temperature, time, and oxygen level of ULO treatments on insect mortality

Ultralow oxygen was first established in a large box chamber with lettuce. The ULO atmosphere was then circulated through a series of plastic jars with western flower thrips in plastic vials for different time periods at different temperatures to accomplish ULO treatments. The procedures for establishing ULO in the treatment chamber was the same as those described by Liu (2007). The box chamber (107 cm × 74 cm × 71 cm) was modified by adding a trough to the outside of the rim. The trough was filled with water. When the box was covered with the lid, the lip of the lid extended down into the water in the trough to achieve airtight seal of the box. Three cartons of commercial head lettuce were used in each box chamber. A dish of about 100 g of sodasorb (Grace & Co., Atlanta, GA) was placed on top of a lettuce carton to absorb $CO_2$. A 12VDC fan was set up in the chamber to circulate air constantly. The chamber was initially flushed with generated nitrogen gas with about 0.2% $O_2$ from a nitrogen generator (Balston 75-7820, Parker Hannifin Co., Tewksbury, MA). Once oxygen level was reduced below 0.5%, pure nitrogen in compressed cylinders was released into the chamber to establish a desired ULO level. After the ULO level was established in the treatment chamber, pure nitrogen was released into the box chamber continuously at 16.7 mL $s^{-1}$. Oxygen level was monitored with an oxygen analyzer (Series 800, Illinois Instruments, Inc., Johnsburg, IL). Generated nitrogen with 0.2% oxygen was released into the nitrogen flow to increase the oxygen level in the chamber once the oxygen level in the chamber fell below a set level. This was controlled by a solenoid valve and an alarm relay of the oxygen analyzer. A carbon dioxide analyzer (model 302M, Nova Analytical Systems, Inc., Niagara Falls, NY) was used to monitor carbon dioxide levels in the treatment chambers periodically and carbon dioxide levels were below 0.1% in all ULO treatments.

Once the desired ULO level was established, a series of jars with thrips in plastic vials and an air pump (SP6000, Smart Products, Inc., Morgan Hill, CA) were flushed with nitrogen and connected to two ports of the box chamber. A vial with moist paper towel was also added in each jar to ensure moist condition in the jars. The ultralow oxygen atmosphere in the box chamber was circulated by the air pump through the treatment jars for certain lengths of time to complete specific ULO treatments. Individual jars in a series were taken out at different times to achieve different treatment durations. This was done by turning off the air pump and disconnecting a jar from a series and quickly reconnecting the tubing to allow air circulating from the box chamber through the jar series to continue the ULO treatments.

At the end of each ULO treatment, the jars with thrips were held in an environmental chamber at 24 °C and 14:10 (L:D) photoperiod for 1 d before being scored for mortality. Thrips that failed to walk or move appendages in response to repeated probes with a soft brush were classified as dead. ULO treatments with oxygen levels of 0.0015%, 0.004%, and 0.01% were tested at 4 °C and 6 °C for 2 and 3 d and each test was replicated three times. The 0.004% oxygen level was also tested at 8 °C for 2 and 3 d and at 10 °C for 2 d and each test was replicated three times. For each test, untreated thrips in plastic vials were stored in a cooler as controls. A total of 3085 thrips were used in ULO treatments and a total of 643 thrips were used as controls.

### 2.3. Effects of ULO treatments on insect mortality and lettuce quality

ULO treatments were conducted in the large box chambers to determine effects on survival of western flower thrips and postharvest quality of head lettuce. Oxygen levels of 0.003% and 0.005% were used in different ULO treatments. The ULO levels were established using the same procedures as detailed above. Vacuum-cooled commercial head lettuce was obtained from Tanimura & Antle Co. (Salinas, CA) on the day of harvest with cultivars identified. Three cartons of commercial head lettuce were used in each test in one box chamber. Lettuce cultivars used in each test varied depending on availability. All lettuce heads were wrapped individually in perforated plastic sleeves. Plastic vials with thrips were added in each carton. For each test, a minimum of six heads for each cultivar was stored at 2 °C as controls. At the end of each ULO treatment, thrips mortality was scored using the procedures stated above and lettuce was stored for 2 weeks in a cooler at 2 °C before being evaluated for postharvest quality.

Lettuce visual quality for marketability was evaluated using the scoring system of Kader et al. (1973) after removing the plastic wrapping sleeves. The visual quality scores ranged from 1 (extremely poor) to 9 (perfect) with 3, 5, and 7 representing poor, fair, and good quality respectively. Lettuce heads were weighed individually. They were then cut into halves to inspect for injury to heartleaves. All heartleaves with injury by ULO treatments were removed and weighed.

At the 0.003% oxygen level, ULO treatments tested included: 4 d at 1 °C, 3 d at 5 °C, and 2 d at 10 °C. At the 0.005% oxygen level, 2 d ULO treatments were conducted at 6, 8, and 10 °C. Each treatment was replicated three to five times. For each test, 12–20 treated heads and 6–12 untreated heads from each cultivar were evaluated for visual quality and injury by the ULO treatment. A total of 4061 thrips were used in ULO treatments and 1042 thrips were used in controls. A total of 136 cartons of lettuce from 11 cultivars were tested. The cultivars tested were: “Corona”, “Dallas”, “Durango”, “Green Beauty”, “Hallmark”, “Liberty”, “Oso Flaco”, “Sniper”, “Telluride”, “Titan”, and “Venus”.

### 2.4. Data analysis

All insect mortality data were adjusted for control mortality using Abbott’s formula. For small scale ULO treatments in plastic jars, regression analyses were conducted between oxygen level and insect mortality for 2 and 3 d ULO treatments at 4 and 6 °C to determine effects of oxygen levels. Regression analyses were also conducted between temperature and insect mortality for 2 and 3 d ULO treatments with 0.004% oxygen to determine temperature effects. For ULO treatments in the large box chambers, insect percent mortality was transformed by arcsine$\sqrt{x}$ prior to analysis of variance (ANOVA). Tukey–Kramer multiple range test was used to compare insect mortality rates among different ULO treatments using one-way platform of JMP Statistical Discovery software (SAS Institute, 2002). For selected ULO treatments, visual quality scores, weights of lettuce, and weights of heartleaves with injury were analyzed and compared using ANOVA and Tukey–Kramer multiple range test. Likelihood ratio Chi-square test was used to analyze the percentages of lettuce heads with heartleaf injury from different ULO treatments (SAS Institute, 2002).

## 3. Results

### 3.1. Effects of temperature, time, and oxygen level of ULO treatments on insect mortality

Oxygen level, temperature, and treatment time of ULO treatments had significant effects on mortality of western flower thrips. Mortality of thrips increased with decreasing oxygen level and increasing temperature and time (Figs. 1 and 2). There were significant correlations between oxygen level and thrips mortality for both 2 and 3 d treatments at both 4 and 6 °C (Fig. 1). At 4 °C, thrips mortality increased from 18.5% at 0.01% oxygen to 67.5% at 0.0015% oxygen in the 2 d ULO treatment ($y = -5940.6x + 78.9$, $R^2 = 0.986$, $P < 0.001$) and increased from 72.4% at 0.01% oxygen to 97.6% at 0.0015% oxygen in the 3 d ULO treatment ($y = -3028.5x + 103.0$, $R^2 = 0.993$, $P < 0.001$). At 6 °C, thrips mortality increased from 16.6% at 0.01% oxygen to 76.7% at 0.0015% oxygen in the 2 d ULO treatment

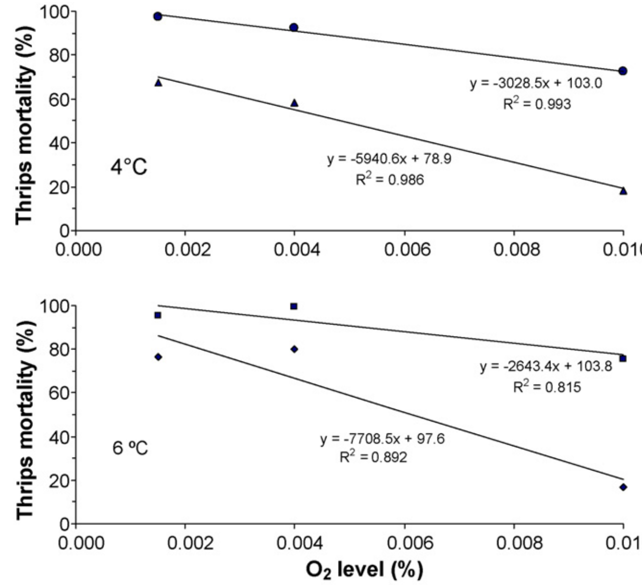


Fig. 1. Relationship between oxygen level and mortality rate of western flower thrips in ultralow oxygen treatments of 2 and 3 d at 4 and 6 °C.

($y = -7708.5x + 97.6$, $R^2 = 0.892$, $P < 0.001$) and increased from 75.6% at 0.01% oxygen to 95.5% at 0.0015% oxygen in the 3 d ULO treatment ($y = -2643.4x + 103.8$, $R^2 = 0.815$, $P < 0.001$) (Fig. 1).

There were also significant correlations between temperature and thrips mortality for 2 and 3 d ULO treatments with 0.004% oxygen (Fig. 2). In the 2 d ULO treatment, thrips mortality increased from 58.5% at 4 °C to 100% at 10 °C ($y = 6.787x + 34.896$, $R^2 = 0.956$, $P < 0.001$). In the 3 d ULO treatment, thrips mortality increased from 92.2% at 4 °C to 100% at 8 °C ($y = 1.958x + 85.452$, $R^2 = 0.805$, $P < 0.001$). Complete control of thrips was achieved in the 2 d treatment at 10 °C and the 3 d treatment at 8 °C (Fig. 2).

### 3.2. Effects of ULO treatments on insect mortality and lettuce quality

There were significant differences among different treatments in thrips mortality (Table 1). For the ULO treatments with 0.003% oxygen in large box chambers, 4 d treatment at 1 °C, 3 d treatment at 5 °C, and 2 d treatment at 10 °C killed > 99.6% of thrips. At 0.005% oxygen, ULO treatment of 3 d at 6 °C killed

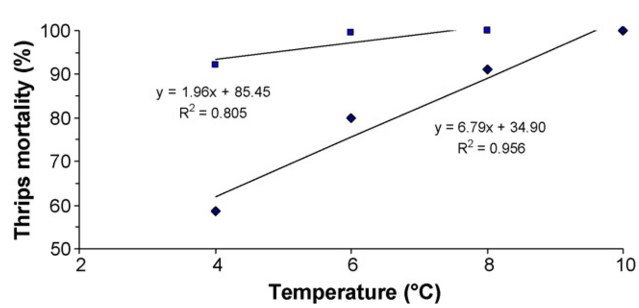


Fig. 2. Relationship between temperature and mortality rate of western flower thrips in 2 and 3 d ultralow oxygen treatments with 0.004% oxygen.

Table 1
Mortality of western flower thrips in response to ultralow oxygen (ULO) treatments with different combinations of oxygen level, temperature, and treatment time

| ULO treatment | | | $N$ | Mortality ± S.E. (%) |
|---|---|---|---|---|
| $O_2$ (%) | Temperature (°C) | Time (d) | | |
| 0.003 | 1 | 4 | 711 | 99.86 ± 0.14a |
| | 5 | 3 | 343 | 99.61 ± 0.39ab |
| | 6 | 2 | 755 | 97.08 ± 0.93bc |
| | 10 | 2 | 1064 | 99.74 ± 0.18a |
| 0.005 | 6 | 2 | 283 | 86.71 ± 4.52d |
| | 6 | 3 | 678 | 99.87 ± 0.13a |
| | 8 | 2 | 97 | 93.67 ± 2.64cd |
| | 10 | 2 | 130 | 95.02 ± 3.30abcd |

Mortality rates were adjusted for control mortality using Abbott's formula and transformed by arcsine$\sqrt{x}$ prior to analysis of variance. Mortality rates followed by the same letter were not significantly different (Tukey–Kramer multiple range test; $P > 0.05$, SAS Institute, 2002).

99.97% of thrips. All other treatments killed between 86.71% and 97.08% of thrips (Table 1).

For the three ULO treatments with 0.003% oxygen that killed > 99.6% thrips, postharvest quality of treated lettuce and the control were compared (Table 2). There were no significant differences in visual quality among the 4 d treatment at 1 °C, the 2 d treatment at 10 °C, and the control. The visual quality for the 3 d treatment at 5 °C was significantly lower than for the control. There were no significant differences in head weight among treatments. Heads from controls did not have any injury to heartleaves and therefore were not included in comparison for heartleaf injury. Among the three ULO treatments, there were significant differences in percentage of heads with injured heartleaves based on Chi-square test ($\chi^2 = 79.239$, $P < 0.0001$). The injury to heartleaves declined with reduced treatment time and increased temperature. The 2 d treatment at 10 °C had the lowest percentage of heads with heartleaf injury (8.4%) and the 4 d treatment at 1 °C had the highest percentage of heads with heartleaf injury (33.3%). Amount of heartleaves with injury also varied significantly among the three treatments with the highest for the 3 d treatment at 5 °C. The average weight of heartleaves with injury for each head was 1.1–2.7 g (Table 2).

When lettuce heads with and without injured heartleaves were compared, there were no significant differences in visual quality between heads with injured heartleaves and heads without heartleaf injury, indicating that heartleaf injury had no relationship with visual quality of lettuce (Table 3). However, in general, lettuce heads with heartleaf injury weighed significantly more than heads without heartleaf injury, implying that heavier heads were more susceptible to ULO injury to heartleaves. For individual ULO treatments, significant difference in head weight between heads with heartleaf injury and heads without heartleaf injury was only found in 4 d treatment at 1 °C but not in other treatments due to the small numbers of heads with heartleaf injury and large variation in head weight among the heads with heartleaf injury (Table 3).

Among the nine cultivars subjected to the 4 d ULO treatment at 1 °C, only one cultivar was free of heartleaf injury. However, among the eight cultivars treated for 2 d at 10 °C, four cultivars were free of heartleaf injury (Table 4). The data in Table 4 only included cultivars that were replicated at least two times. Cultivars without replication were included in pooled analysis but not analyzed for cultivar variations. For the 2 d ULO treatment at 10 °C, Corona, Liberty, Telluride, and Venus did not sustain any injury to heartleaves. Other cultivars including Dallas, Durango, and Oso Flaco sustained lower percentage of injury in the 2 d ULO treatment at 10 °C as compared with injury in the 4 d treatment at 1 °C.

## 4. Discussion

High mortality rates (>99.6%) of western flower thrips in several ultralow oxygen (ULO) treatments with different treatment durations and temperatures indicate the potential of ULO treatments for postharvest control of the pest on lettuce. However, the shorter treatment of 2 d at 10 °C was safer to lettuce than the longer treatment times at lower temperatures. One half of cultivars (four out of eight) tolerated the 2 d treatment at 10 °C without any injury. Although, thrips mortality did not reach 100% for the 2 d ULO treatment, additional mortality will likely occur during transit as we found high mortality rates of thrips after prolonged cold storage, and total control of thrips was achieved with a combination of cold storage and ULO treatment (Liu, 2008). Therefore, the 2 d ULO treatment at 10 °C has potential to be developed for quarantine control of western flower thrips on iceberg lettuce.

The significant correlations between oxygen level and thrips mortality, and between temperature and thrips mortality, indicated the importance of maintaining selected oxygen levels and temperature to ensure effective ULO treatment for insect con-

Table 2
Effects of time and temperature combinations of ultralow oxygen (ULO) treatments with 0.003% oxygen on postharvest quality of iceberg lettuce of all the cultivars

| ULO treatment | $N$ | Visual quality, mean ± S.E. | Head weight, mean ± S.E. (g) | With injury[a], mean ± S.E. (%) | Weight of injured leaves | |
|---|---|---|---|---|---|---|
| | | | | | $N$ | Mean ± S.E. (g) |
| 4 d at 1 °C | 435 | 7.01 ± 0.02ab | 576.9 ± 6.2a | 33.3 ± 2.3 | 145 | 1.3 ± 0.1b |
| 3 d at 5 °C | 193 | 6.98 ± 0.03b | 559.9 ± 8.1a | 16.1 ± 2.6 | 31 | 2.7 ± 0.6a |
| 2 d at 10 °C | 346 | 7.03 ± 0.02ab | 589.3 ± 8.5a | 8.4 ± 1.5 | 29 | 1.1 ± 0.3b |
| Control | 175 | 7.10 ± 0.03a | 585.7 ± 11.7a | – | – | – |

The values in the columns followed by the same letter were not significantly different (Tukey–Kramer multiple range test, $P > 0.05$, SAS Institute, 2002).
[a] There were significant effects among the three ULO treatments in percentage of lettuce heads with injury to heartleaves ($\chi^2 = 79.239$, $P < 0.0001$, Likelihood ratio $\chi^2$ test, SAS Institute, 2002).

Table 3
Comparisons of visual quality and head weight between lettuce heads with heartleaf injury and lettuce heads without heartleaf injury at 14 d after different ultralow oxygen (ULO) treatments with 0.003% oxygen

| ULO treatment | Heartleaf injury | *N* | Visual quality, mean ± S.E. | Head weight | | | |
|---|---|---|---|---|---|---|---|
| | | | | Mean ± S.E. (g) | d.f. | *F* | *P* |
| All ULO tests | – | 769 | 7.00 ± 0.02a | 572.4 ± 5.0b | | | |
| | + | 205 | 7.03 ± 0.03a | 598.8 ± 9.6a | 1, 972 | 5.944 | 0.0149 |
| 4 d at 1 °C | – | 290 | 7.00 ± 0.03a | 566.3 ± 7.6b | | | |
| | + | 145 | 7.03 ± 0.02a | 598.0 ± 10.7a | 1, 433 | 5.830 | 0.0162 |
| 3 d at 5 °C | – | 162 | 6.97 ± 0.04a | 556.6 ± 8.9a | | | |
| | + | 31 | 7.06 ± 0.04a | 577.2 ± 20.3a | 1, 191 | 0.865 | 0.3535 |
| 2 d at 10 °C | – | 317 | 7.03 ± 0.04a | 586.0 ± 8.9a | | | |
| | + | 29 | 6.97 ± 0.03a | 625.4 ± 29.4a | 1, 344 | 1.648 | 0.2002 |

For each treatment, the values of visual quality score and mean head weight between lettuce heads with injured heartleaves and lettuce heads without injured heartleaves followed by the same letter were not significantly different ($P > 0.05$, ANOVA, SAS Institute, 2002).

Table 4
Responses of different lettuce cultivars to three ULO treatments with 0.003% oxygen and different temperatures and durations

| Cultivar | 4 d at 1 °C | | | 3 d at 5 °C | | | 2 d at 10 °C | | |
|---|---|---|---|---|---|---|---|---|---|
| | *N* | Head weight, mean ± S.E. (g) | With injury. mean ± S.E. (%) | *N* | Head weight, mean ± S.E. (g) | With injury, mean ± S.E. (%) | *N* | Head weight, mean ± S.E. (g) | With injury, mean ± S.E. (g) |
| Corona | 70 | 531.9 ± 15.9 | 15.7 ± 4.4 | – | | | 30 | 538.6 ± 19.6 | 0 |
| Dallas | 26 | 499.4 ± 21.2 | 69.2 ± 9.2 | – | | | 30 | 751.7 ± 20.0 | 10.0 ± 5.6 |
| Durango | 97 | 592.1 ± 13.5 | 59.8 ± 5.0 | 75 | 544.3 ± 10.9 | 17.3 ± 4.4 | 112 | 570.5 ± 13.9 | 8.0 ± 2.6 |
| Green Beauty | – | | | – | | | 50 | 475.4 ± 9.8 | 16.0 ± 5.2 |
| Liberty | 22 | 578.8 ± 21.0 | 40.9 ± 10.7 | – | | | 30 | 604.9 ± 28.2 | 0 |
| Oso Flaco | 31 | 663.1 ± 18.4 | 19.4 ± 7.2 | 28 | 563.0 ± 24.9 | 0 | 55 | 563.2 ± 16.5 | 16.4 ± 5.0 |
| Sniper | 24 | 743.1 ± 14.5 | 58.3 ± 10.3 | – | | | – | | |
| Telluride | 26 | 513.8 ± 12.9 | 0 | – | | | 24 | 869.6 ± 21.6 | 0 |
| Titan | 45 | 548.2 ± 18.4 | 40.0 ± 7.4 | – | | | – | | |
| Venus | 94 | 576.2 ± 12.4 | 11.7 ± 3.3 | 45 | 557.2 ± 14.3 | 24.4 ± 6.5 | 30 | 520.3 ± 16.6 | 0 |

trol. The significant difference in head weight between lettuce heads with injury to heartleaves and heads without such injury indicated that smaller and softer heads were more suitable for ULO treatment as compared with larger and compact heads. This is likely related to poorer air exchange in larger and more compact heads that lead to severer deprivation of oxygen and accumulation of carbon dioxide at the center of lettuce heads as compared with smaller and softer heads. There were two types of symptoms of ULO injury to heartleaves. Tissue death along the edge of very small leaves occurred in some lettuce heads indicating low oxygen injury. However, brown spotting resembling carbon dioxide injury or ethylene injury on heartleaves was more common.

For lettuce heads with injured heartleaves, most injured leaves were located at apical points and only about 1 g of injured leaves were found per head for the 2 d treatment. This part of the lettuce head is not consumed. In processed salads, lettuce heads were coned and heartleaves would be removed. Therefore, on a practical level, injury to heartleaves by ULO treatment would not result in any loss of usable lettuce. The results of this study showed promise of the 2 d ULO treatment at 10 °C for postharvest control of western flower thrips on head lettuce. More studies are needed to further reduce susceptibility of lettuce to injury by insecticidal ULO treatment.

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