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Responses of *Phlox subulata* aboveground to root-feeding Scarabaeidae larvae and drought stress

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Abstract

The ornamental perennial *Phlox subulata* and its varieties are often affected by root-feeding Scarabaeidae larvae, leading to dieback. These larvae are harmful insects that are difficult to detect. The relationship between the horizontal distribution of root-feeding Scarabaeidae larvae and the aboveground condition of *P. subulata* was examined in the field. Laboratory experiments were conducted to examine the effects of drought stress, root-cutting, and larvae introduction on the leaf chlorophyll index (LCI) in *P. subulata*. A field survey was also conducted, and the numbers of larvae were found to be higher in plots where *P. subulata* experienced dieback than in plots where the plants did not die, and in plots adjacent to dieback plots. This result indicates that the number of larvae corresponds to the aboveground condition of the plant. Both root-cutting and drought stress treatments significantly decreased the LCI and killed some of the plants, implying that drought stress and physical root injury changed the plants' aboveground condition. The introduction of larvae also significantly decreased the LCI, in a manner similar to the drought stress treatment. The decreases in the LCI due to the drought stress and larvae introduction treatments were detected before the dieback of *P. subulata* in the experiment, which implies that constant monitoring of the LCI is an effective method for early detection of root-feeding larvae.

Key words: Drought, leaf chlorophyll index, leaf weight, *Phlox subulata*, root cutting, SPAD

Introduction

Phlox is a commercially important ornamental plant that is often used in greening and horticulture as cover vegetation (Zale and Jourdan, 2015). The maintenance of ornamental plants usually requires pest control, often using pesticides. However, pest control methods that minimize pesticide use and have a low environmental impact are preferred, as pesticides can have unexpected negative consequences (Md-Meftaul *et al.*, 2020).

Phlox subulata L. and its horticultural varieties, commonly known as *shiba-zakura* (turf cherry) in Japanese, are among the most important ornamental plants in Japan (Ikuyo *et al.*, 2018). *P. subulata* has been planted extensively in parks (*e.g.*, Shibazakura Takinoue Park and Higashimokoto Shibazakura Park in Hokkaido). However, these plantings often show patchy dieback and there have been reports of root-feeding Scarabaeidae larvae (*e.g.*, *Heptophylla picea* or *Anomala* sp.) in the area.

Root-feeders (*e.g.*, scrab beetle or nematode larvae) are considered pests by farmers and gardeners because they are difficult to detect and control (Blackshaw and Kerry, 2008; Johnson *et al.*, 2016). Pesticide spraying after outbreaks has been a common way to manage these pests, but it is not effective in controlling the pathogen and is harmful to the environment. Understanding the ecology of the target insect and developing methods for early symptom detection would help to reduce costs and pesticide use.

Several studies have used the leaf chlorophyll index (LCI) to detect plant stress non-destructively (Yuan *et al.*, 2013; Martinez-Ferri *et al.*, 2016; Liu *et al.*, 2024) based on the relationship between the LCI and leaf chlorophyll contents (Xiong *et al.*, 2015). This index is influenced by stresses such as drought and root disease (Yuan *et al.*, 2013; Martinez-Ferri *et al.*, 2016; Liu *et al.*, 2024). However, the relationship between the LCI and the actual levels of chlorophyll in leaves differs among species (Sanchez *et al.*, 1983). Additionally, the tiny leaves of *P. subulata* shed doubt on whether the LCI is useful for detecting changes in the aboveground condition of the plant.

Phlox subulata (Polemoniaceae) is a perennial herb that is native to North America (Igarashi, 2016). It shows strong resistance to salt, drought, heat, and cold stresses (Qu *et al.*, 2015) and is therefore often planted in gardens and parks. It has been planted extensively throughout Japan. In Hokkaido, it flowers from May to June (Igarashi, 2016).

In the planted area of this species in Shibazakura Takinoue Park, Hokkaido, Scarabaeidae larvae have often been observed by gardeners. Scarabaeidae such as *Heptophylla picea* and *Anomala* sp., which develop underground and feed on plant parts, are agricultural and horticultural pests, impacting a wide range of crops, including potatoes, turfgrass, tea, and other plants (*e.g.*, Teranishi and Imaoka, 1992; Bhuiyan and Nishigaki, 1995; Kakizaki, 1997).

In this study, we examined the distribution of root-feeding larvae in a planted area of *P. subulata* and conducted cultivation experiments to examine the effects of physical damage to roots, the introduction of root-feeding larvae to pots, and drought stress on the LCI of *P. subulata*. In the field, the relationship between the aboveground condition of *P. subulata* and the number of Scarabaeidae larvae in soil was examined. In the cultivation experiments, we analyzed whether the index is effective for the early detection of root damage caused by these larvae. Although diebacks of *P. subulata* have been linked to nematodes (Ikuyo *et al.*, 2018), we focused on root-feeding Scarabaeidae larvae (Redmond and Potter, 2010) because they have often been reported at the study site.

Materials and methods

Study species: **Site descriptions**: A field survey was conducted in Shibazakura Takinoue Park (N44°11'51", E143°4'31") in Takinoue, Hokkaido, and the experimental plants were prepared at the park. The park is located in a valley with a cool temperate climate. Secondary forests in and around the area are composed of deciduous trees such as *Quercus crispula*, *Tilia japonica*, *Acer pictum*, *Ulmus davidiana* var. *japonica*, *Fraxinus mandshurica*, *Sorbus commixta*, *Betula platyphylla*, and *Salix hultenii* var. *angustifolia*. According to data from a nearby meteorological station, the mean annual temperature from January 2010 to December 2022 was 5.7°C and the annual precipitation was 980.2 mm (JMA, 2023).

Potted plants for cultivation experiments were grown in a greenhouse at the park starting 3 months before the experiments began. Cuttings were planted in soil and encouraged to develop roots in a seedbed for 1 month with regular watering and fertilizing. After this period, each cutting was transplanted into a pot and regularly supplied with water and fertilizer.

Horizontal distribution of Scarabaeidae larvae and aboveground condition of *P. subulata*: The horizontal distribution of Scarabaeidae larvae and the aboveground condition of *P. subulata* were examined in the field. The field survey was conducted in the park in early summer (late May and early June) and autumn (October) in 2022. Four 12-m lines were set in the planted areas and 13 quadrats (50 cm × 50 cm) were placed at equal intervals starting at each line. The lines were established in areas in which the plants had partially turned yellow or gray. The aboveground condition of plants was coded for each quadrat as follows: G, no discoloration; Y, plants with yellow or gray leaves; and N, neighboring quadrat was Y. To evaluate the status of Scarabaeidae larvae in the soil below the plants, an area (20 cm × 20 cm) in each quadrat was dug up (to 10 cm depth), and the larvae were counted.

Aboveground response of *P. subulata* to root damage and drought stress: To examine the effects of root damage and drought stress on the aboveground condition of *P. subulata*, we conducted an experiment to evaluate leaf weight and the LCI after root-cutting and drought stress treatments in June 2023. In total, 48 potted plants (16 plants per block) were prepared and grown in an air-conditioned room under high-luminance light-emitting diodes (LEDs) within open chambers for 1–2 weeks before starting the experiments. Each block was set up by allocating four plants to each treatment (WC: irrigation and no root-cutting; DC: no irrigation and no root-cutting; WT: irrigation and root-cutting; DT: no irrigation and root-cutting). The experiments were conducted for each block in succession. For the root-cutting treatment, the rootball of 8 of 16 plants per block was cut longitudinally and latitudinally. Half of the root-cut and uncut plants were cultivated without irrigation, while the others were irrigated every two days. Leaf weight and LCI were measured on days 0, 3, and 6 after starting the experiments. Eight leaves per plant were randomly collected, and the LCI, as determined according to Soil Plant Analysis Development (SPAD) values, was recorded using a SPAD-502 chlorophyll meter (Konica Minolta Japan, Inc., Tokyo, Japan). After the SPAD values were measured, the leaves were dried at 80°C for 72 h and weighed.

Aboveground condition of *P. subulata* in response to root feeders and drought stress: To examine the effects of stresses due to root feeders and drought on the aboveground condition of *P. subulata*, we conducted another experiment and monitored leaf weight and the LCI in October 2023. The preparation and experimental setup were the same as for the previous experiment, combining treatments of root-feeder introduction and drought stress (3 blocks, 16 plants per block, 4 plants per treatment per block). The four treatments were WC (irrigation and no root-feeder introduction), DC (no irrigation and no root-feeder introduction), WT (irrigation and root-feeder introduction), and DT (no irrigation and root-feeder introduction). The experiments were conducted for each block in succession. For the root-feeder introduction treatment, five Scarabaeidae larvae were placed in each pot. The larvae were collected from Shibazakura Takinoue Park a week before starting the experiments, reared in large polypropylene boxes, and fed with potatoes. Measurements of 8 leaves weight and the LCI were taken five times on days 0, 3, 6, 9, and 12 after starting the treatment. The weights of the larvae at the beginning and end of the experiment were recorded for each pot to confirm that the larvae were alive and increasing in weight until the end of the experiment. Unfortunately, data were not recorded from the first block.

Statistical analysis: The relationship between the aboveground condition of *P. subulata* and the distribution of Scarabaeidae larvae was examined using a generalized linear mixed model. This model used aboveground condition (G, N, B) as the explanatory variable, larvae population as the dependent variable, and the difference between lines as a random variable, assuming a Poisson distribution for the dependent variable. Likelihood ratio tests compared the model with the explanatory variable to the model without it. To examine differences in the larval population between spring and autumn, a generalized linear mixed model was again used, taking the difference between lines as a random variable. Models with and without the explanatory variable were compared using the likelihood ratio test.

Model selection using a linear mixed model was conducted to examine the effects of root-cutting, root-feeding larvae, drought stress, and their interactions on aboveground growth (LCI, dry weight of 8 leaves). The full model included the effects of root-cutting, irrigation, days, and their interactions as explanatory variables, aboveground growth variables as dependent variables, and blocks as random variables. Models in which each explanatory variable was reduced one by one were created, and differences were evaluated using likelihood ratio tests to detect significant variables. Differences between treatment groups (WC, DC, WT, DT) for each observation day were evaluated using the Tukey method. Significance levels were corrected using Bonferroni's method to manage multiple comparisons for each observation day.

R v 4.2.2 was used for analysis (R Core Team, 2024). The R packages *lme4* (Bates *et al.*, 2015) and *Matrix* (Bates *et al.*, 2022) were used to construct linear mixed and linear models. The R packages *emmeans* (Searle *et al.*, 1980), *pbkrtest* (Halekoh and Højsgaard, 2014), and *lmerTest* (Kuznetsova *et al.*, 2017) were used for multiple comparison tests.

Results

Horizontal distribution of Scarabaeidae larvae and aboveground condition of *P. subulata*: Scarabaeidae populations were larger in Y plots than in G plots in both spring and autumn, and the differences among plot types were significant (Fig. 1, spring, $X^2 = 7.224$, $P = 0.001$; autumn, $X^2 = 60.003$, $P < 0.001$). In May and June, no larvae were found in the G plots. In autumn, many were found in theY plots, significantly more than in the N and G plots. Significantly more larvae were found in autumn (2.6 ± 0.4 N/plot) than in spring (0.3 ± 0.2 N/plot) ($X^2 = 60.832$, $P < 0.001$).

Aboveground response of *P. subulata* to root damage and drought stress: The effects of the root-cutting and no irrigation treatments on the LCI differed significantly between days (Table 1). The LCI did not differ between treatments on day 0; however, on days 3 and 6, those of the DC, WT, and DT treatments were lower than that of the WC plot, significantly so for DT and WT (Fig. 2). The index was lower for both no-irrigation plots than for those that were irrigated (WC,WT) but without significance. There were no significant differences in the dry weights of 8 leaves within or between treatments (Tables 1 and 2).

Aboveground response of *P. subulata* to root feeders and drought stress: The effects of each treatment differed significantly between days (Table 3). LCI values did not differ significantly between treatments on days 0 and 3 but were significantly lower in the larvae-introduction treatments (WT, DT) after day 6 (Fig. 3). The dry weights of 8 leaves differed significantly between days (Table 3), although differences among the days were rather small (Table 4). There were no significant differences between treatments in the dry weights of 8 leaves (Table 3).

Table 1. Statistics of model selection for the leaf weight and leaf chlorophyll index in the root-cutting experiment

Variables	Weight of leaves		Leaf chlorophyll index	
	X^2	P	X^2	P
No irrigation×root-cutting×days (interaction)	0.003	0.955	3.715	0.156
No irrigation×days (interaction)	0.155	0.694	2.877	0.237
Root-cutting×days (interaction)	0.316	0.574	15.163	<0.001
No irrigation×root-cutting (interaction)	2.630	0.105	4.693	0.030
No irrigation	0.005	0.946	3.089	0.079
Root-cutting	2.168	0.141	34.635	<0.001
Days	0.000	1.000	95.420	<0.001

Table 2. Dry weight of 8 leaves (means and standard errors) from day 0 to 6 of *P. subulata* in the root-cutting experiment.

Treatment combinations	Day 0 (mg)	Day 3 (mg)	Day 6 (mg)
Irrigation, no root-cutting (WC)	21.5±1.6	20.8±1.0	21.3±1.0
No irrigation, no root-cutting (DC)	20.6±0.9	20.0±0.8	19.9±1.0
Irrigation, root-cutting (WT)	18.8±1.0	19.7±1.1	19.5±0.9
No irrigation, root-cutting (DT)	20.3±1.2	20.0±1.1	20.4±1.2

Table 3. Statistics of model selection for the weight of leaves and leaf chlorophyll index in the larvae introduction experiment

Variables	Weight of leaves		Leaf chlorophyll index	
	X^2	P	X^2	P
No irrigation×larvae×days (interaction)	0.256	0.612	0.469	0.493
No irrigation×days (interaction)	1.524	0.217	6.069	0.014
Larvae×days (interaction)	0.289	0.591	37.061	<0.001
No irrigation×larvae (interaction)	0.837	0.360	1.889	0.169
No irrigation	2.355	0.125	19.356	<0.001
Larvae	1.179	0.278	177.269	<0.001
Days	16.000	<0.001	123.607	<0.001

Table 4. Dry weight of 8 leaves (means and standard errors) of *P. subulata* from day 0 to day 12 in the larvae introduction experiment.

Treatment combinations	Day 0 (mg)	Day 3 (mg)	Day 6 (mg)	Day 9 (mg)	Day 12 (mg)
Irrigation, no larvae (WC)	11.8±0.8	11.3±0.8	11.4±0.8	12.3±0.9	13.2±0.8
No irrigation, no larvae (DC)	10.8±1.1	11.0±1.0	9.6±0.5	13.4±1.2	13.8±1.0
Irrigation, larvae (WT)	11.6±0.8	11.8±1.1	10.6±0.8	12.5±0.8	13.1±0.9
No irrigation, larvae (DT)	10.1±0.8	10.5±0.7	10.7±0.7	11.0±0.6	12.7±0.7

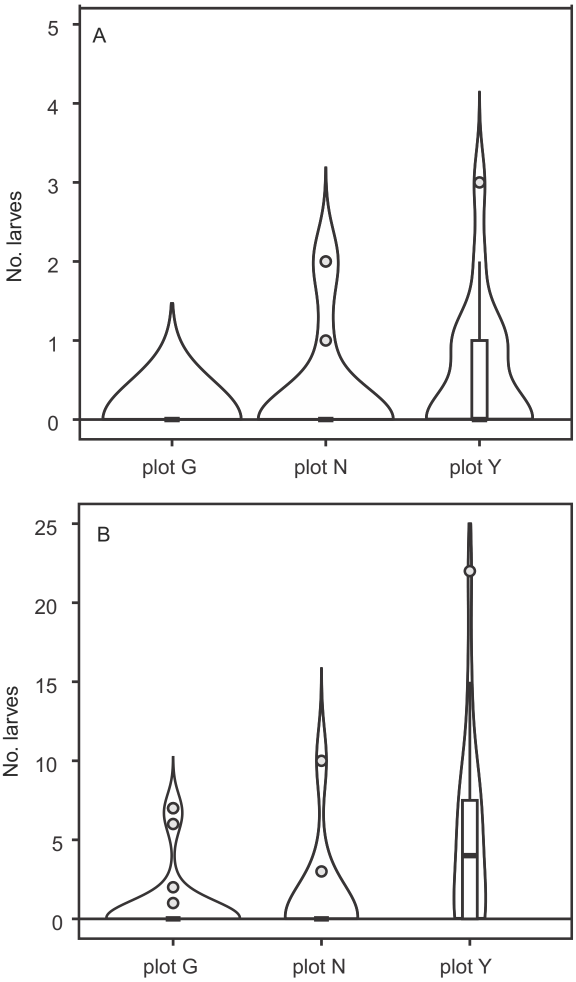

Fig. 1. Numbers and status of leaves in each plot in early summer (A) and autumn (B). G, no discoloration; Y, leaves and stems of most plants yellow or gray; N, neighboring quadrat classified as Y.

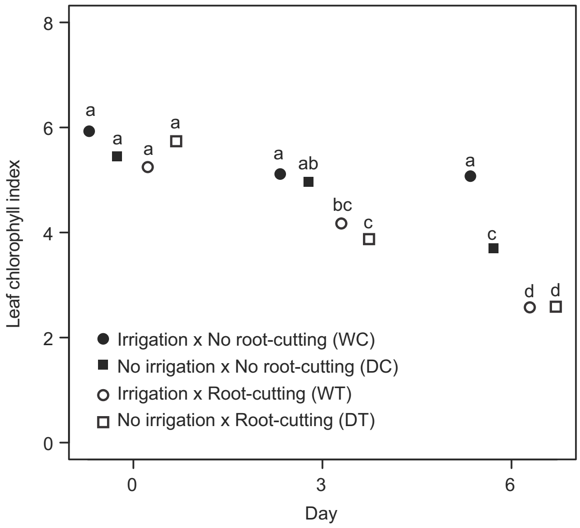

Fig. 2. Changes in the leaf chlorophyll index (based on SPAD values) in a combined experiment featuring no irrigation and root cutting. Symbols indicate means. Different letters indicate that values were significantly different (Tukey's Honestly Significant Difference test, $P < 0.05$).

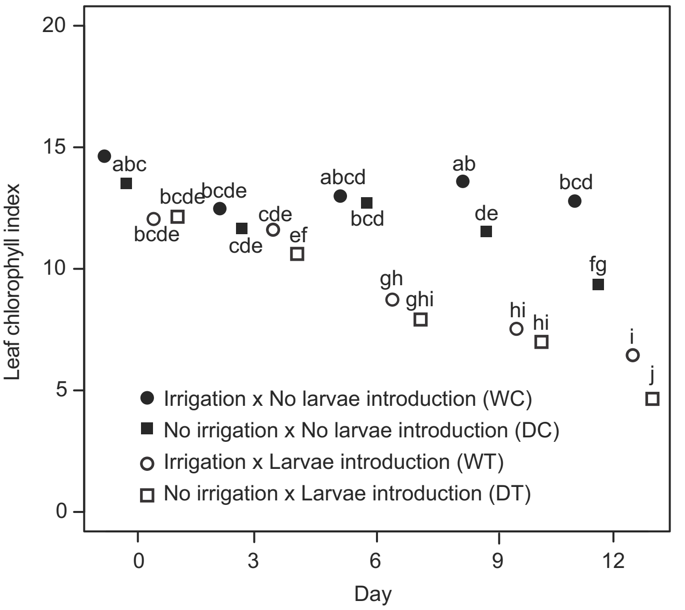

Fig. 3. Changes in leaf chlorophyll index values (based on SPAD values) in a combined experiment featuring no irrigation and the introduction of Scarabaeidae larvae. Symbols indicate means. Different letters indicate that values were significantly different (Tukey's Honestly Significant Difference test, $P < 0.05$).

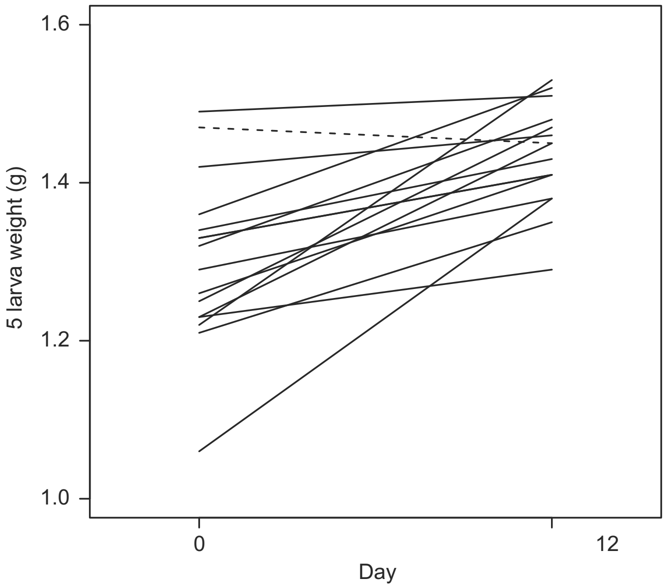

Fig. 4. In the larvae-introduction experiment, five larve in each plot were weighed on day 0 (beginning of monitoring) and 12 (end of monitoring). Soild and broken lines indicate positive and negative slopes, respectively.

For 15 out of 16 pots in blocks 2 and 3, the weights of five larvae were greater on day 12 than on day 0 (Fig. 4).

Discussion

Horizontal distribution of Scarabaeidae larvae and aboveground condition of *P. subulata*: The larvae populations were significantly higher in plots with wilted or dead plants (plot Y), implying that dieback was related to the presence of the Scarabaeidae larvae. Dieback is caused not only by pathogens (*e.g.*, Ikuyo *et al.*, 2018) but also by drought stress (Munne-Bosch and Alegre, 2004). This means that, even if the Scarbaeidae larvae do not have an immediate pathogenetic effect on the plants, frequent infestation can cause dieback of *P. subulata* in planted areas. However, as nematodes can also cause dieback in *P. subulata*, the direct cause requires further study.

Some Scarabaeidae larvae were detected in plots that did not experience dieback and in adjacent plots. There may be a delay between when the larvae start feeding and when the aboveground parts show wilting or dieback symptoms.

Response of aboveground *P. subulate* to drought stress: In the two experiments, the LCI values of the DT and DC plots (no-irrigation treatments) were lower than or equal to those of the WT and WC plots, respectively, on day 6 (Figs. 2 and 3). Although it has been reported that the strength of the correlation between LCI values and leaf chlorophyll content differs among species (Sanchez *et al.*, 1983), our results imply that drought stress reduces chlorophyll levels in the leaves of *P. subulate*, in line with data on many other species (Yuan *et al.*, 2013; Xiong *et al.,* 2015).

Response of aboveground *P. subulata* to root damage: In experiments featuring root cutting and drought stress, the treatments led to partial diebacks in the WT and DT plots by day 6, where LCI values were also significantly lower than in the WC and DC plots (Fig. 2). A study that examined the relationship between LCI values and root damage in *Artemisia ordosica* found that LCI values decreased as the degree of root damage increased (Liu *et al.*, 2024). In line with the case in *A. ordosica,* our results indicate that reduced LCI values reflect root damage.

Response of aboveground *P. subulata* to root-feeding larvae: In experiments featuring larval introduction and drought stress, the treatments led to significantly lower LCI values in the DC, DL, and WL plots at day 6, while that of the WC plot remained unchanged (Fig. 3). This result implies that root damage by root-feeding larvae can be detected based on reduced LCI values, in line with studies on an avocado fungus (*Rosellinia necatrex*; Martinez-Ferri *et al.*, 2016) and on Nerica rice impacted by a root-feeding nematode (*Heterodera sacchari*; Akpheokhaia *et al.,* 2014). We also found that the larvae became larger (gained weight) over time in blocks 2 and 3 (Fig. 4), indicating that they were eating the roots of *P. subulata* during the experiments. Therefore, decreased LCI values reflect either the physical root damage caused by feeding or decreased leaf activity due to some secondary effect related to the feeding (*e.g.*, infection by pathogens).

In the root-cutting experiment, partial dieback was observed in the WT, DC, and DT plots on day 6, together with lower LCI values. However, in the larval-introduction experiment, a decrease in LCI values was observed on day 6, but no dieback. This result was partly due to differences in the condition of plants at the beginning of the experiments. In the root-cutting experiment, the LCI values at day 0 were 5.0–5.9, whereas initial values in the larval-introduction experiment were much higher (9.3–13.5). Therefore, it is likely that the plants in the root-cutting experiment were less healthy from the start of the experiment, resulting in the death of some. This result may imply a benefit of using the LCI in the field. Plants may still look healthy even though chlorophyll levels are starting to decrease, meaning that damage cannot be detected visually, while a SPAD meter would provide a direct readout of chlorophyl status. Therefore, regular monitoring of LCI values using a SPAD meter may be an effective method for the early detection of drought stress and root predation by Scarabaeidae larvae.

Root damage is reflected by the LCI. Drought stress also leads to lower LCI values, implying that the lower index values associated with root damage may be due to drought stress. Based on our results, continuous measurement of SPAD values may be useful for early detection of root-feeding damage in *P. subulata* as a means of preventing dieback.

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