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Laser Weed Management: Development of an experimental protocol to ensure reliable dose-response analysis

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Abstract

Effective weed management is crucial for optimizing horticultural and agricultural plant production. This study aims to establish a reproducible and comparable experimental protocol to determine the damage thresholds of weeds subjected to high-energy, coherent laser radiation. The lethal dose is influenced by process parameters, including plant species, growth stage, irradiation point, process precision, and laser wavelength. Recognizing the importance of adapting process parameters to specific use cases in laser weed management (LWM), this research contributes to enhancing the reproducibility and comparability of dose-response analysis for LWM applications.

The study focuses on two plant species: Alopecurus myosuroides (ALOMY) and Amaranthus retroflexus (AMARE). The resulting protocol is a three-stage protocol including classification, parameter study, and detailed measurements. A key methodological insight is the necessity of a two-step parameter study: the first step involves capturing a full range of the sigmoid response curve, while the second focuses on dense sampling around the inflection point to ensure robust data fitting.

1. Introduction

Weeds compete with crop plants in various ways. On the one hand, they occupy the same space, whereby factors such as the availability of root space and light play a vital role. Secondly, they compete for water and nutrients in the soil. This competition can lead to reduced development of the crop plants and thus to considerable yield losses (Bertram 1996).

Weed control is therefore one of the most important measures for ensuring the yield of horticultural and agricultural plant cultivation. Laser weed management can be regarded as an additional non-chemical and soil-conserving method (Bauer et al. 2020). This method essentially involves the rapid heating of critical plant tissue (apical meristem) by high-energy radiation, which leads to temperatures in the target cell tissue reaching lethal levels (Marx 2014). This process can thus be classified as a thermal control method. The merits of thermal methods in weed management are also applicable to the laser method (Coleman et al. 2019). Thermal weeding preserves the soil, is beneficial to phytosanitary conditions due to the non-contact treatment and the tool is not subject to significant wear and tear (Bauer et al. 2020). The laser process is highly selective, given its ability to focus on a single irradiation point of a few millimetres in diameter. This feature enables the precise targeting of weeds in close proximity to crop plants.

Over the last decades, a variety of research projects have been conducted on weed management using lasers, often employing different systems (e.g. laser sources, beam shaping) and process parameters (Matthiassen et al. 2006; Marx 2014; Coleman et al. 2021). The parameter studies conducted on various plants included a range of evaluation criteria for estimating the destruction threshold. In addition to visual assessments, the fresh weight of treated plants is a common criterion used to determine dose-response curves. Given the energy demands of laser processes and the limited energy capacities of mobile field applications, it is a fundamental objective to increase system efficiency. One aspect of achieving this is the utilisation of the minimum necessary dose for the desired effect.

The objective of this study is to develop an experimental protocol that defines a reliable procedure and a uniform evaluation criterion in order to create a standardised framework for further damage threshold/lethal dose experiments.

2. Data, Methods and Procedure

Two plant species were selected for analysis: *Alopecurus myosuroides* (ALOMY), a monocot, and *Amaranthus retroflexus* (AMARE), a dicot. Indicative plant samples are shown in Figure 1.

The plants were grown from seeds using ProLine Potground (Klasmann-Deilmann, 49744 Geeste, Germany) and 30 x 40 cm boxes (AUER GmbH, 83123 Amerang, Germany) at the Laser Zentrum Hannover. Randomised treatments were applied to each box containing 12 plants. The plants were cultivated until growth stage BBCH 12 and then treated.

Figure 1: left: *Alopecurus myosuroides* (ALOMY); right: *Amaranthus retroflexus* (AMARE).

The plants were treated with a thulium fibre laser (model TLR-100-AC) from IPG Photonics (57299 Burbach, Germany). The laser has a wavelength of 1940 nm and an optical power of 100 W, with a beam diameter of 5.1 mm ($1/e^2$ definition). A coaxial pilot laser was used to accurately aim the laser at the plant meristem. The desired dose was set by varying the irradiation duration of the laser at 100% power.

The damage threshold was determined in the following manner: First, the two plant species were classified. This was followed by the first coarse parameter study. The second detailed parameter study was then carried out. The classification was necessary to identify relevant differences in growth that could affect the damage threshold within a growth stage. For this purpose, the plants were visually classified at growth stage BBCH 12, and the fresh weight of the green parts of the plant was determined using a gravimetric scale (VWR ECN 611-2258, Avantor Radnor PA 19087).

For the parameter studies, the treated plants were assessed after four weeks of cultivation in comparison with the control group on the basis of the fresh weight and the survival rate. In addition to the fresh weight, a weekly assessment was conducted in which the plants were classified into four levels (1. no visible damage; 2. slight damage; 3. severe damage; 4. lethal damage).

First parameter study**:** The objective of a first parameter study is to ensure that the upper and lower plateau of a dose-response curve is adequately represented by data points. The dose values for the second parameter study are then chosen around the estimated turning point off the sigmoidal curve. The upper plateau of a dose-response curve represents 100% survival rate, while the lower plateau represents 0% survival rate. In order to ensure coverage of the upper and lower plateaus, a logarithmic increase in dose was applied to obtain a full sweep of the sigmoid curve. The dose parameter range started at 0.01 J/mm² with logarithmic increments up to 10 J/mm² and was extended with a 0 J/mm² control. Approximately 20 plants were treated per dose level. The number of samples and dose levels were chosen to be the same for both plant species.

Second parameter study: The objective of the second parameter study was to ensure the presence of datapoints in the previously identified transition area of a dose-response curve. The experimental procedure was identical to the first parametric study. Four weeks after treatment, the fresh weight, dry weight and survival rate were determined.

The data was analysed using the statistical software R (version: i386 4.1.1) with the integrated RStudio environment. The fresh weights were analysed using a t-test. The survival rates were analysed using a chi-squared test and a dose-response curve was modelled. This was accomplished through the utilization of the drc function package (https://bioassay.dk/) in R for logistic regression, in accordance with the model proposed by Ritz and Streibig (2005). The model is based on the LD value and is described by the following formula:

$$f(x) = \frac{d}{1 + e^{b(\log(x) - \log(LD50))}}$$

Where d is the upper limit (100%), b is the slope of the curve, x is the dose and LD50 is the dose that leads to 50% lethal damage.

3. Results and Discussion

First parameter study**:** The assessment after four weeks revealed that the dose levels had completely covered both the upper and lower plateaus of the Dose-response curve. For both plant species, the survival rate was 100% at dose levels ranging from 0 to 0.1 J/mm². However, one AMARE plant exhibited sublethal damage after four weeks. The survival rate of plants irradiated with 1.0 J/mm² decreased to 14% for AMARE and 37% for ALOMY. The surviving plants exhibited a significant reduction in growth compared to the control. At the 10 J/mm² dose level, all plants of both plant species were lethally damaged immediately after the treatment. The results of the first parameter study indicate that the upper plateau is covered with data points at dose levels 0 to 0.1 J/mm² and the lower plateau at 10 J/mm². A preliminary dose-response fit was performed to estimate the turning point of the curve. This estimation was used to determine the dose levels for the second parameter study. The dose levels 0.4 - 1.2 J/mm² are selected for AMARE, while 0.6 - 1.4 J/mm² are selected for ALOMY.

Second parameter study: Figure 2 shows the fresh weight of the living plants as a function of the dose four weeks after treatment as a box plot. The number of plants treated and survived per dose are shown in the table in the top right corner. Despite the large deviation within dose levels, it can be seen that the control plants have significantly higher fresh weight than all treated plants. This was also confirmed with a t-test comparison ($p < 0.05$) of the fresh weight of the control and all treated plants. The mean fresh weight of the control is approx. 25 g, which is about three times higher than the dose levels 0.4 and 0.6 J/mm^2. A comparison with the dose levels 0.8 J/mm^2 to 1.2 J/mm^2 is not possible as the number of plants analysed (survived) is too small to make a valid statement. An analysis of significant differences between the doses was carried out, but due to the high deviations and in some cases the small number of plants analysed, this was not considered meaningful. The dry weight (data not shown) shows no systematic difference to the fresh weight, which is why the determination of the dry weight is not recommended for further investigations.

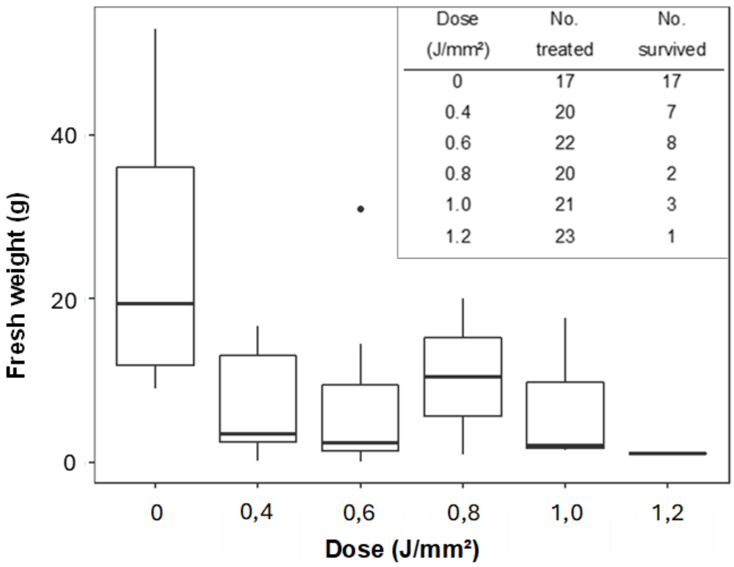

Figure 2: Freshweight of living AMARE plants as a function of the dose four weeks after treatment. The number of treated and survived plants per dose are listed in the tabel inside the figure 2.

The investigated objective of laser weed management (LWM) is not to reduce plant growth but to cause lethal damage to plants. Therefore, the survival rate is a suitable criterion for evaluating the effectiveness of the treatment. In this experiment, the survival rate dropped to 35% at dose levels of 0.4 and 0.6 J/mm² and to below 15% at dose levels of 0.8 and 1.0 J/mm² (Figure 3). There was a significant difference ($p < 0.05$) between the control and doses 0.8, 1.0 and 1.2 J/mm². No significance was found between the individual dose levels.

The survival rates determined in this way from the two parametric studies can be combined in a dose-response curve using a logistic regression model. Figure 3 on the left shows these dose-response curves for AMARE and ALOMY. The curve has a sigmoidal shape with three main areas: 1. upper plateau, 2. lower plateau and 3. transition area between the plateaus. The distribution of measurement points for both plants across the three areas of the curve is provided. It is evident that the dose-response curve of AMARE is shifted to the left on the abscissa in comparison to ALOMY. This indicates that AMARE plants require a lower dose to exhibit the same level of damage as ALOMY plants. Both plant species are at the same growth stage (BBCH 12). This difference is primarily attributable to the damage effect, in conjunction with the anatomical dissimilarities between the two plant species.

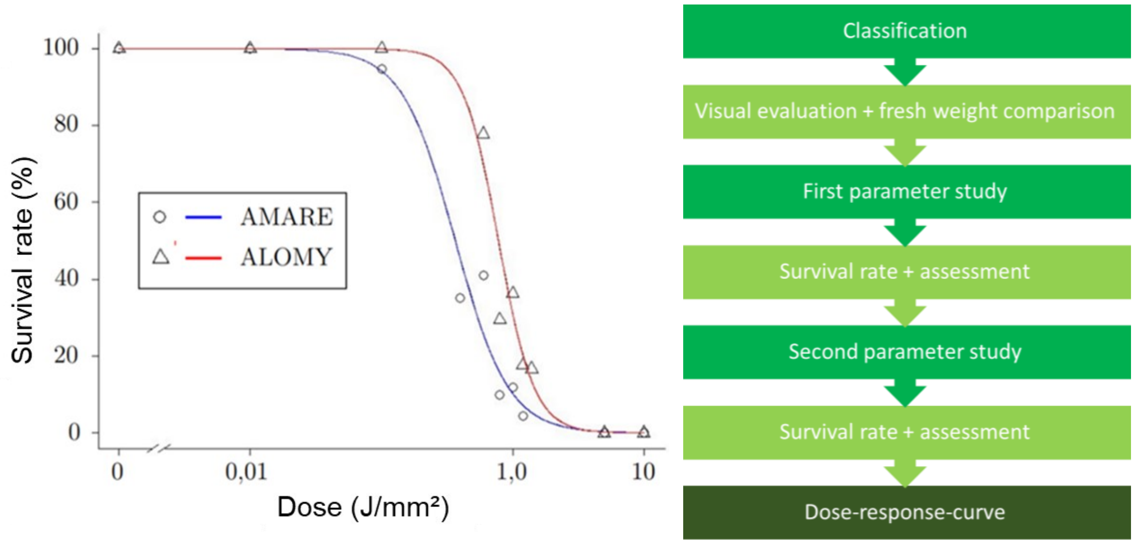

Figure 3: Left: Survival rate as a function of dose of both parameter studies of AMARE (circle) and ALOMY (triangle). Right: Flow chart of the developed protocol.

In AMARE, the meristem is located at the tip of the shoot, exposing it in the irradiation process. In contrast, the meristem of ALOMY is located at the base of the shoot, making thermal energy input into the meristem more difficult due to partial and possibly complete blocking of the radiation by i.e. other plant tissue. A fundamental difference between monocotyledonous and dicotyledonous plants cannot be deduced from the results, as only one plant species was analysed in each case. However, previous studies on thermal weed control have already demonstrated that monocotyledonous plants tend to be more tolerant to thermal damage than dicotyledonous plants (Ascard 1994).

The determination of the lethal threshold, which is referred to as the lethal dose, is dependent upon the defined and targeted growth reduction rate, which can be expressed as either mass reduction or survival rate. The established target values for mass reduction are 90% or 95% (fresh mass or dry mass) in comparison to the control (Matthiassen et al. 2006; Marx 2014; Coleman et al. 2021). In order to determine the destruction threshold and thus to assess the effect of sublethal irradiation, the survival rate is a robust evaluation criterion. With a target survival rate of less than 10% and thus a lethal damage of more than 90%, the lethal dose (LD90) for the plants investigated here is 1.0 J/mm² for AMARE and 1.6 J/mm² for ALOMY for irradiation at 1940 nm. However, the applied dose must be adapted to the experimental design and the targeted crop-weed situation in order to ensure an efficient treatment.

4. Conclusion

A three-stage protocol is a recommended method for creating a valid dose-response curve. The flow chart, shown in Figure 3 on the right, illustrates the three process steps and the respective evaluation steps. The first step, classification, provides a baseline for comparison and ensures comparable results. The second step, the first parameter study, is relevant to identify the upper and lower plateau and to estimate the turning point of the sigmoidal curve. The first parameter study is performed with a logarithmic increase in dose to obtain a full sweep of the sigmoid curve. This enables the third step in the second parameter study with measurements next to the inflexion point. This provides confidence in the fit of the transition region on the sigmoid curve.

The evaluation criteria employed in this experiment are the survival rate and visual assessment, which enable the generation of a complete dose-response curve (see Figure 3, left). This approach can be employed to determine a dose that corresponds to the targeted lethal or sublethal rate. In practice, survival rates of less than 10% are frequently employed, for example, LD90. Given the multitude of factors that influence the survival rate like plant culture, cultivation methods, and environmental conditions, it is likely that a variety of target values will be identified depending on the specific use case.

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