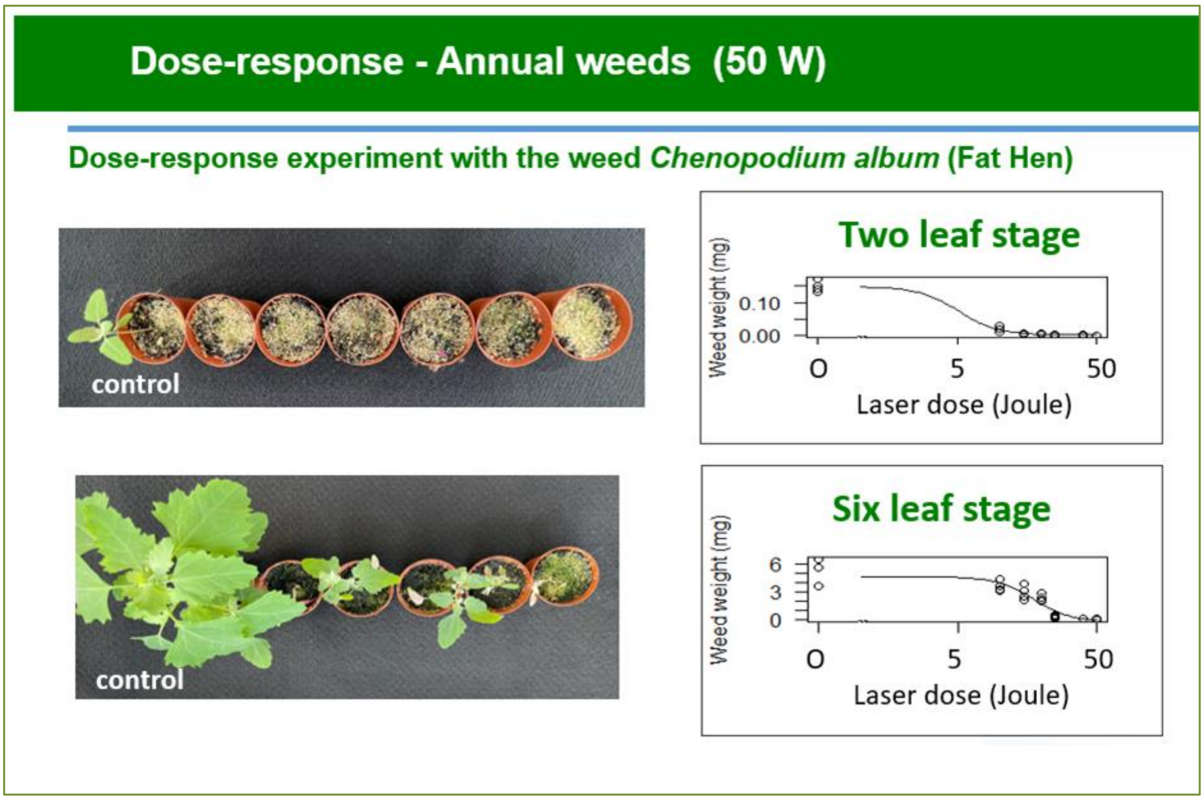
WeLASER

Eco-innovative weeding with laser

Sustainable Weed Management in Agriculture with Laser-Based Autonomous Tools

D2.3 - Impact of laser doses on living organisms and the environment



CSIC, FUTONICS, LZH Laser Zentrum Hannover e.V., University of Copenhagen, Agreenculture, COAG, Alma Mater Studiorum Università di Bologna, IETU Instytut Ekologii Terenów Uprzemysłowionych, Ghent University, van den borne aardappelen

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EXECUTIVE SUMMARY

This deliverable is devoted to experimental studies of the effect of different laser doses on target and non-target living organisms. The activity, carried out in Task 2.5, is split into four subtasks. Subtask 2.5.1 – Dose-response experiments with weeds and crops: We studied the effect of different laser energy dosages on weed and crop plants. The aim was to optimize energy use. Plants were grown in a greenhouse until they obtained a particular growth stage. Then, they were moved to a laser box and exposed to different doses of laser energy. Three weeks after the treatment, the biomass of the plants was measured. The larger the growth stage, the more power was necessary to harm the plants. Large plants with more than four permanent leaves were difficult to control with the laser. Different plant species reacted differently to the laser treatment. The laser generally controlled small plants well (cotyledon stages, two-permanent-leaf stage). Also, seeds were exposed to laser treatments, and afterwards, their germination ability was investigated. Seeds reacted very differently to the treatment, depending on their size. In subtask 2.5.2, dose-response experiments with non-target small organisms (insects, earthworms) were conducted. Different soil types containing earthworms were exposed for varying doses of laser irradiation, and afterwards, the mortality of the worms was recorded. In all experiments, the earthworms were unaffected by the laser exposure of the soil in which they lived. Different life stages of insects were also exposed to varying laser dosages, and afterwards, the effect and behavior of the organisms were recorded. All life stages were significant harmed even when exposed for very small energy doses from the laser. Subtask 2.5.3 concerned risk assessment of large organisms. The risk assessment of larger organisms like rodents and humans was based on a literature review. Subtask 2.5.4 concerned field tests. The intention was to use a prototype of the integrated equipment and make field tests in a cereal field and a row crop (e.g., wheat and maize). However, the WeLASER prototype robot was not fully functioning in the first 36 months, and field tests involving UCPH were therefore postponed.

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Table of Contents

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1. EXPERIMENTAL SET-UP

We used a thulium-doped 50 W fiber laser with a wavelength of 2 µm with a collimated beam (Ø: 2 mm) manufactured by Futonics Laser GmbH, Katlenburg-Lindau, Germany. We constructed a steel box for the laser experiment (Figure 1). The steel box has a door with a metal interlock. On laser activation, the door locks automatically to avoid risk of laser exposure.

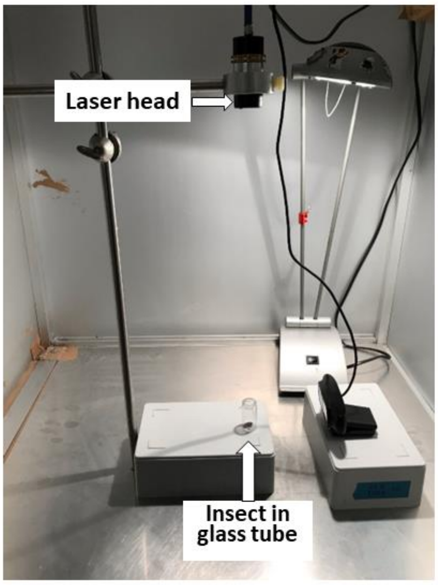

Figure 1: The laser was place inside a steel box, and the targets were placed below the laser head.

The target organisms were placed below the laser head and exposed to increasing dosages of laser energy (from 0 to 235.71 J mm^{-2}).

2. DOSE-RESPONSE EXPERIMENTS WITH WEEDS AND CROPS

We focused on plants with different characteristics for example annual and perennials weeds, weeds with different growth types, and seeds sizes and weight. The reasons choosing the species and some of the dose-response experiments have already been published (Andreasen et al., 2022**;** Andreasen, 2021 a, b; Andreasen 2023). During the last three months of the project, we will focus on publishing the results in peer-reviewed journals.

2.1. Monocotyledonous plants

Two annual weed species (*Alopecurus myosuroides and Lolium multiflorum*), two crop species (*Zea mays* and *Lolium multiflorum*) and a perennial weed species (*Elymus repens*) were exposed to different laser doses at different growth stages (Table 1). Plants were established from seeds except for *E. repens* plants which were established from rhizomes.

Table 1. Experiments with monocotyledonous species exposed to different laser doses

Plant species	Dosages (ms)	Number of experiments	Developmental stage	Number of nodes and shoots
Alopecurus myosuroides	0, 25, 50, 100, 200, 400, 800	2 per developmental stage	1st, 2nd, 3rd leaf	1 node 1 shoot
Elymus repens	0, 50,100, 300, 500, 800,1000	2 per developmental stage and nodes (Total 18 experiments)	1st, 2nd, 3rd leaf	1 node 1 shoot, 2 nodes 1 shoot, 2 nodes 2 shoots
Lolium multiflorum	0, 25, 50, 100, 200, 400, 800	1 per developmental stage	1st, 2nd, 3rd, and 4th leaf	
Zea mays	0, 25, 50, 100, 200, 400, 800, 900,1000	1 per developmental stage	1st, 2nd, 3rd, and 4th leaf	

2.2. Dicotyledonous plants

Fourteen weed species established from seeds and one perennial weed species (*Cirsium arvense*) established from root fragments were exposed to laser treatments (Table 2).

Table 2. Experiments with dicotyledonous species exposed to different laser doses

Plant species	Dosages (ms)	Number of experiments	Developmental stage
Beta vulgaris	0, 25, 50, 100, 200, 400, 800	2 per stage	Cotyledon stage, 2 and 4 leaves
Cirsium arvense	0, 25, 50, 100, 200, 400, 800	2 per stage	1, 2 and 3 leaves
Centaurea cyanus	0, 25, 50, 100, 200, 400, 800	1 per stage	Cotyledon stage, 2, 4 and 6 leaves
Erodium cicutarium	0, 25, 50, 100, 200, 400, 800	2 per stage	Cotyledon stage, 2 and 4 leaves
Geranium molle	0, 25, 50, 100, 200, 400, 800	2 per stage	Cotyledon stage, 2 and 4 leaves
Lamium purpureum	0, 25, 50, 100, 200, 400, 800	2 per stage	Cotyledon stage, 2 and 4 leaves
Myosotis arvensis	0, 25, 50, 100, 200, 400, 800	2 per stage	Cotyledon stage, 2 and 4 leaves
Plantago major	0, 25, 50, 100, 200, 400, 800	2 per stage	Cotyledon stage, 2 and 4 leaves
Rumex crispus	0, 25, 50, 100, 200, 400, 800	2 per stage	Cotyledon stage, 2 and 4 leaves
Silene noctiflora	0, 25, 50, 100, 200, 400, 800	1 per stage	Cotyledon stage, 2 ,4 and 6 leaves
Sonchus oleraceus	0, 25, 50, 100, 200, 400, 800	2 per stage	Cotyledon stage, 2 and 4 leaves
Stellaria media	0, 25, 50, 100, 200, 400, 800	2 per stage	Cotyledon stage, 2 and 4 leaves
Tripleurospermum inodorum	0, 25, 50, 100, 200, 400, 800	1 per stage	Cotyledon stage, 2 and 4 leaves
Veronica persica	0, 25, 50, 100, 200, 400, 800	2 per stage	Cotyledon stage, 2 and 4 leaves
Viola arvensis	0, 25, 50, 100, 200, 400, 800	2 per stage	Cotyledon stage, 2 and 4 leaves

2.3. Seeds

Seeds of five weed species and two crop species (maize and wheat) were exposed to laser irradiation (Table 3). The seeds were irradiated with different doses directly on the surface, but also covered with 2.5 - or 5-mm soil before irradiated.

Table 3. Experiments with seeds. Seeds were exposed directly to different laser doses or covered with soil before they irradiated

Plant species	Dosages (ms)	Number of experiments	Soil depth (mm)
Alopecurus myosuroides	0, 25, 50, 100, 200, 400, 800	2 per depth	0, 2.5, 5
Anisantha sterilis	0, 25, 50, 100, 200, 400, 800	2 per depth	0, 2.5, 5
Avena fatua	0, 25, 50, 100, 200, 400, 800	2 per depth	0, 2.5, 5
Centaurea cyanus	0, 25, 50, 100, 200, 400, 800	2 per depth	0, 2.5, 5
Silene noctiflora	0, 25, 50, 100, 200, 400, 800	2 per depths	0, 2.5, 5
Triticum aestivum (Wheat)	0, 25, 50, 100, 200, 400, 800	2 per depth	0, 2.5, 5
Zea mays (Maize)	0, 25, 50, 100, 200, 400, 800	2 per depth	0, 2.5, 5

The effect of the laser dosages on the germination percentage of the plant species deviated significantly between the species when the seeds were exposed directly to the laser beam (See an example in Figure 2).

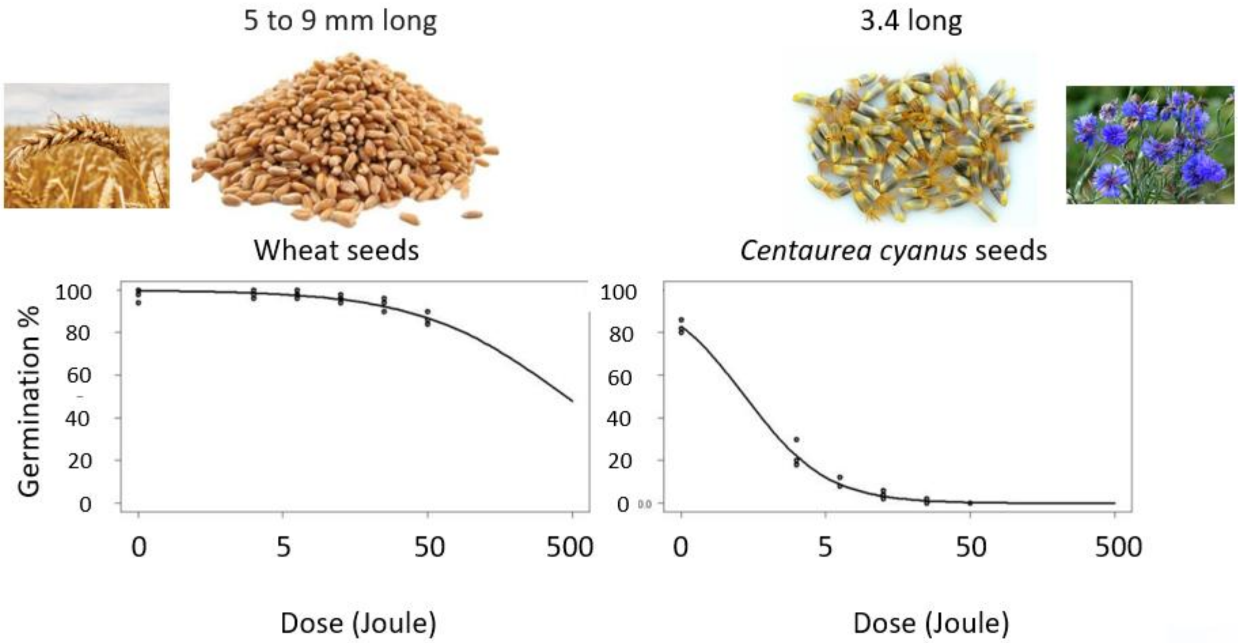

Figure 2. Dose-response experiment with wheat and cornflower.

All species were affected by the highest dosages. *Silene noctiflora,* having the smallest seeds, was completely burned at the highest dose. The larger the seed biomass was, the less affected became the germination percentage of the treatment.

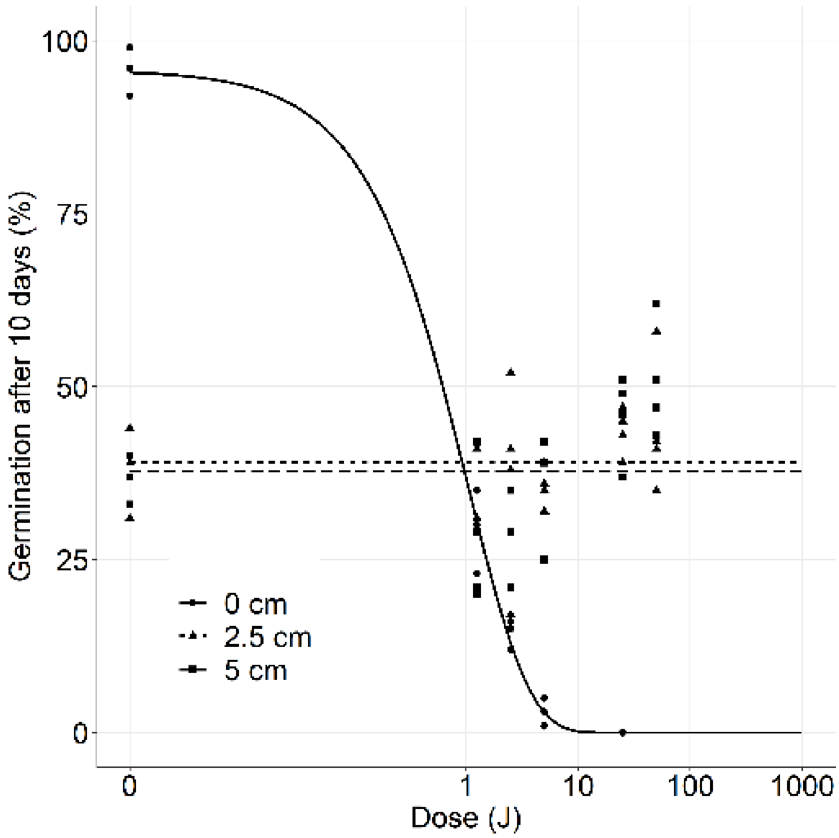

Figure 3. Example of dose-response experiment with *Silene noctiflora* seeds placed in different soil depth.

The laser did not affect the germination percentage of seeds covered with 2.5 mm or 5 mm so il (Se example in Figure 3). The soil was only exposed for one second for the laser beam at the highest dose, which was not enough to transfer the heat 2.5 mm down in the moist soil but had probably coursed soil water evaporation. Generally, seeds placed in 2.5−5 mm depth seemed well-protected from these laser dosages. The heat spread in the soil from the laser beam depends on the water content, soil structure and composition. We cannot exclude that other soil conditions, like a lower or higher water content or other soil types, would result in other germination responses. The 2 mm circular laser beam hit approximately in the middle of the seeds in all experiments. Hence, the laser beam energy only covered a part of the surface of large seeds of *Avena fatua,* wheat and corn, while the whole seed of *Silene noctiflora* was exposed to the irradiation. Therefore, the tiny seeds were more exposed and significantly more affected than the large seeds, even at small dosages. Many common weed species have significantly smaller seeds than *S. noctiflora* (e.g., *Arenaria serpyllifolia* L., *Papaver rhoeas* L, *Stellaria media* (L.) Vill.) (Holm-Nielsen, 1998).

The laser beam covered an area of 3.14 mm^2. Using a larger beam diameter (e.g., 4 mm) would cover a larger area of the seeds (12.57 mm^2) and increase the probability of hitting a seed on the ground, but distributing the energy on a larger area would be less effective or require more energy to obtain the same effect per area. The 2 mm beam diameter was chosen because it has been suggested for weed control (see https://welaser-project.eu/).

Some seeds of corn and wheat got a laser hole in the endosperm region of the seed, but the seeds germinated anyway. The effect on large seeds would probably have been more significant if the laser had targeted the embryo of the seed. Using artificial intelligence to recognize the embryo region on larger seeds may be possible (e.g., cereals and weed seeds like *Avena fatua* seeds).

Although we made a great effort to place the seeds in the middle of the holes in the metal frames to ensure targeting the seeds after they were covered with soil, we cannot be sure that all seeds were just below the laser-exposed spot. Some of the smallest seeds may have been placed on the border or beside the exposed soil area, which could have affected the results.

In general, tiny weed seeds can easily be destroyed with relatively low laser energy, but they are challenging to recognize. They easily roll down between soil particles and disappear. They are difficult to target with the laser under field conditions when an autonomous vehicle with laser equipment moves at a certain speed (e.g., 2−6 km $hour^{-1}$). Large seeds like *Avena fatua* and volunteers (e.g., wheat and barley) are easier to identify and target but require a larger dose to harm or kill.

We do not consider using autonomous vehicles only to control weed seeds on the ground economically feasible. However, targeting larger seeds with a laser while weed seedlings are controlled with lasers may be an option in the future, but it requires developing seed recognition tools based on artificial intelligence. Our result shows, that it may be necessary to use higher laser energy dosages to harm large seeds than to control small weed seedlings.

3. DOSE-RESPONSE EXPERIMENTS WITH NON-TARGET SMALL ORGANISMS (INSECTS, EARTHWORMS)

Two earthworm species (*Enchytraeus albidus* and *E. crypticus),* a pest *(Tenebrio molitor)* and a beneficial insect (*Adalia bipunctata*) were exposed for seven doses of laser (Table 4.)

Some of the experiments have been published in (Andreasen 2023a, b, c, d, e). A peer-reviewed publication is under review (Andreasen et al., 2023a) and is attached as **a supplementary file** with all details of the study (page 18).

The earthworms were mostly unharmed when the soil surface was exposed to laser dosages up to 236 J mm^{-2} as the soil protected them. Laser doses sufficient to kill plants were lethal to the insects, and lower doses that did not kill plants killed or harmed the insects across all life stages tested. The larger *T. monitor* beetle survived higher doses than the smaller *A. bipunctata* beetle. The results indicate that pest control might be a possibility using laser dosages which do not harm plants. In general, the probability of harming insects with dosages used for laser-weeding is small, as only a tiny proportion of the area will be exposed for the treatment, even with a high weed density. Using another type of laser may give other results. Developing a recognition tool using artificial intelligence to differentiate between pests and beneficial arthropods would make pest control possible without harming beneficial organisms at the same time as the laser weeding takes place.

Table 4. Experiments with insects and earthworms. The animals were exposed to different laser dosages.

Species	Dosages (ms)	Number of experiments
Tenebrio molitor	0, 1, 10, 20, 50, 100, and 500	2 for each life stage (larvae, pupae, adult stage)
Adalia bipunctata	0, 1, 10, 20, 50, 100, and 500	2 per adult stage
Enchytraeus albidus	0, 500, 1000, and 1500	2 for each of 3 soil types
Enchytraeus crypticus	0, 500, 1000, and 1500	1 for each of 3 soil types

4. RISK ASSESSMENT OF LARGE ORGANISMS (RODENTS, HUMANS)

The risk assessment of larger organisms like rodents and humans is based on a literature review so far published in the WeLASER paper **(**Andreasen et al., 2022; Andreasen 2022 a, b, c,).

However, the laser equipment is going to be constructed in such a way that neither large animals nor humans have a risk of being exposed to the laser radiation above the exposure limit values according to directive 2006/25/EU. Below is a summary of the risk assessment:

With respect to the autonomous movement and navigation of the robotic platform through a field, laser weeders have the same safety issues as other autonomous vehicles. Although such vehicles are only approved driving on the roads in a few experimental places until now, some robots (e.g., lawnmowers) are widely accepted and used on private and public properties. Also, small autonomous agricultural robots are approved to drive on private and public properties (e.g., in EU and UK) as long as all safety guidelines are followed (Basu et al., 2020).

A collimated laser beam executes high levels of energy in the form of a narrow and non-spreading beam, transmitting into heat energy when it hits a surface. The heat can potentially ignite dry material in the field (e.g., straw, leaves, other organic matter, lost paper) and start a fire. While driving, various sensors (e.g., smoke sensors, cameras) can be mounted to register any sign of uncontrolled heating or fire. However, when the laser weeder has passed, a spark could be hidden and expose a danger. Many fires have been started by leaving a spark after flame weeding (Rask & Kristoffersen, 2007), and this is also a risk with laser weeding. Therefore, surveillance of the laser weeder and the treated area must be considered. Leaving the laser weeder driving without surveillance, for example, during the night, could be too risky. Dry organic matter should be avoided on the soil surface, and therefore ploughing, which may not be sustainable in the long term, might be necessary to reduce fire risks in some fields.

Laser beams can be harmful to humans and animals. Infrared cameras and sensors must be mounted on the vehicle to warn and stop it when it approaches humans, animals or any other obstacles ensuring an appropriate safety distance. Depending on the laser wavelength, the laser can be more or less harmful to the eyes and skin and cause irreversible damage (e.g., blindness). Visible and near-infrared (400–1400 nm) laser light poses a critical hazard on the retina. Since the tissue structures of the retina are unable to undergo any repair, lesions caused by the focusing of visible or near-infrared light on the retina may be permanent. The most critical area of the retina is the central portion, the macula, and the fovea.

Laser light in the ultraviolet or far-infrared spectrum can cause damage to the cornea or the lens. Far infrared (1400 nm − 1 mm; CO_2 lasers, 10,600 nm) can cause thermal damage by the heating of the tears and tissue water of the cornea. Excessive exposure to infrared radiation results in a loss of transparency of the cornea or surface irregularities (OSHA, 2022).

Skin damage is most commonly caused by thermal injury. Thermal damage is associated with lasers operating at exposure times greater than 10 microseconds and in the wavelength region from the near-ultraviolet to the far-infrared. The thermal effects of laser exposure depend on 1) The absorption and scattering coefficients of the tissues at the laser wavelength, 2) Irradiance or radiant exposure of the laser beam, 3) Duration of the exposure and pulse repetition characteristics, where applicable, 4) Extent of the local vascular flow, 5) Size of the area irradiated (OSHA, 2022).

Laser-protecting glasses for the specific laser wavelength can protect the operator of the laser weeder when it is necessary to adjust, clean or work close to the laser weeder. Clothing and gloves can help protect the skin. Screens and curtains can be mounted, avoiding laser beams escaping from the target area caused by reflection from stones, sand, and other reflecting items, which may spread the beam. It is important to stress that the laser beams can be invisible, and only the result of heating can be seen, for example, when it hits a target.

5. FIELD TEST

The development of the WeLASER autonomous vehicle has been significantly delayed due to the corona pandemic and technological challenges. The WeLASER robot was demonstrated in Denmark for one day (see Andreasen, 2023d) and then transported to the Netherlands for another demonstration. Due to the short time the robot was in in Denmark, it has not been possible to conduct fields experiments in the first 36 months of the project, and therefore we have focused on experiments we could do with the laser box in the laboratory.

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Quantifying off-target risks to earthworms (Enchytraeids) and insects (*Tenebrio molitor* and *Adalia bipunctata*). *Frontiers in Agronomy (*resubmitted after revision).

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7. Suplementary file

Side-effects of laser weeding: Quantifying off-target risks to earthworms (Enchytraeids) and insects (*Tenebrio molitor* and *Adalia bipunctata*)

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Adalia bipunctata LSID urn:lsid:zoobank.org:pub:6245285F-7A74-4277-AD4F-E81B501CDE03

Enchytraeus albidus LSID urn:lsid:zoobank.org:pub:508560B7-BD89-472A-B3FD-F48851B024C3

Enchytraeus crypticus LSID urn:lsid:zoobank.org:act:C2B913ED-4259-4191-995B-4A6A1D095F23

Tenebrio molitor LSID urn:lsid:zoobank.org:act:19EE3BC1-AD92-4A15-82F1-EB3DBE81DFE6

Keywords: integrated weed management, laser non-target, non-chemical weed control, site-specific weed management, thermal weed control.

Abstract

With challenges posed by chemical and mechanical weed control, there are now several research and commercial projects underway to develop autonomous vehicles equipped with lasers to control weeds in field crops. Recognition systems based on artificial intelligence have been developed to locate and identify small weed seedlings, and mirrors can direct a laser beam towards the target to kill the weed with heat. Unlike chemical and mechanical weed control, laser weeding only exposes a small area of the field for the treatment. Laser weeding leaves no chemicals in the field after the treatment or does not move the soil which may harm crop roots and non-target organisms. Yet, it is well-known that laser beams can harm living organisms; the effect on the environment and fauna should be studied before laser weeding becomes a common practice. This project aimed to study the effect of laser on some living non-target organisms. We investigated the effect of laser treatment on the mortality of two species of earthworms (*Enchytraeus albidus* and *Enchytraeus crypticus*), larvae, pupas, and beetles of yellow mealworm beetles (*Tenebrio molitor*) and the two-spotted lady beetle (*Adalia bipunctata*) for increasing dosages of laser energy.

In all earthworms experiments except one, the mortality rates of the worms living in the uppermost soil layer of clay, sandy, and organic soil exposed to laser heating were not significantly different from the controls even with laser dosages up to 236 J mm^{-2}. Laser doses sufficient to kill plants were lethal to the insects, and lower doses that did not kill plants killed or harmed the insects across all life stages tested. The larger beetles survived higher doses than smaller. Laser weeding is a relatively new technology and not yet widely practiced or commercialized. Therefore, we do not discuss and compare the costs of the different weeding methods at this early stage of the development of the technology.

1. Introduction

Weed control with laser beams has achieved increasing attention as the fast development in artificial intelligence has enabled recognition of the location and identification of plant species precisely and rapidly (Rakhmatulin et al., 2021). Furthermore, laser can be guided by mirrors to target weeds (Rakhmatulin and Andreasen, 2020). When small weeds are hit correctly in the meristem, the heat from the laser can kill the plants (Coleman et al. 2021; Heisel at al., 2001). Lasers are powered by electricity, which can be supplied by batteries, charged from renewable (non-fossil) energy sources reducing CO_2 emission compared to commonly used weed control methods. Suppose a laser beam has a diameter of 2 mm and there are 150 weeds m^{-2}, then only 0.5% of the total area will be exposed to the treatment. A common practice of herbicide application and mechanical weed control exposes most of the field or the whole area to the treatments, and chemicals often stay on the soil surface or in the soil matrix for a while with the risk of affecting non-target organisms and the environment negatively (Mehdizadeh et al., 2021; Thiour-Mauprivez et al., 2019). Consequently, replacing herbicide application and mechanical weed control with laser weeding seems to be a method to reducing some of the negative environmental impact of weed control.

If the laser beam hits non-target organisms, they are likely harmed or killed. That may happen, for example, if insects or other organisms (1) are on the target plant at time of exposure, (2) move into the laser beam, or (3) if unintended platform movement results in the inaccurate position of the laser energy.

The effect of the laser beam on the weed plants depends on physical parameters (*e.g.*, laser wavelength, beam diameter, and dose (J)) (Rakhmatulin and Andreasen, 2020) and biological factors (*e.g.*, plant species, plant size, and developmental stage) (Heisel et al., 2001; Andreasen et al., 2022). These factors may also be crucial for the effect of laser beams on non-target organisms and should be investigated.

It is not economically feasible to study the effect of laser on all potentially exposed organisms, and therefore model organisms are often used. We examined how laser treatment affected the survival rate of two earthworms and two insects.

Enchytraeids (class Oligochaeta, family Enchytraeidae) are ecologically important soil worms due to their activity in bioturbation and decomposition of organic matter in many soil types (Castro-Ferreira et al., 2012; Didden, 1993). Enchytraeids are widespread on moist soil types from the arctic to the tropics, occurring in quantities from 100s to more than 100,000 individuals m^{-2}. Generally, they thrive within a temperature range of 8 °C to 25 °C. Enchytraeids are often used as model organisms in toxicological laboratory tests (*e.g*., Cedergreen et al., 2013; He and van Gestel, 2013; Gomes et al., 2013). *Enchytraeus albidus* and *E. crypticus* are white worms with a long thin body with a soft skin and a fast reproduction rate. They can grow up to 3 cm long. They have been considered suitable to assess ecotoxicity in many different soil types due to their larger tolerance range to pH (4.4–8.2), clay (1–29%) and content of organic matter in the soil (1.2–42%) (Kuperman et al., 2006; Castro-Ferreira et al., 2012).

Tenebrio molitor (Coleoptera: Tenebrionidae) is a holometabolous insect (complete life cycle with egg, larva, pupa, and adult stages) and is considered a pest due to its ability to consume stored flour, grains, or animal feeds. The larvae are called mealworms. The larva is white and reaches 2−2.5 cm in length. They gradually become yellow and then darker brown. They have three pairs of legs and are active crawlers. The *T. molitor* beetle reach 25 mm in length and are the largest insects infesting stored products (Davidson and Lyon, 1979). *Tenebrio molitor* has often been used as a test insect in ecotoxicological studies as it is easy to propagate, feed and keep indoors (*e.g*., Fei et al., 2022; Bednarska and Świątek, 2016; McCallum et al., 2013).

Adalia bipunctata (Coleoptera: Coccinellidae) is an aphidophagous beetle also called two-spotted labybug/two-spotted labybird/two-spotted laby beetle. It is native to North America and Western and Central Europe (Hodek et al., 2012). The larvae and adults feed on aphids and other small insects, so *A. bipunctata* is therefore considered a beneficial insect. It has been commercialized for aphid pest control in protected environments (Khan et al., 2016; Wyss et al., 1999). Adults reach a length of 3.5−5.2 mm (Gordon, 1985).

The aim of this study was to investigate how a fiber laser with a wavelength of 2 µm and a 2 mm diameter affected the mortality of two species of soil worms (*E. albidus* and *E. crypticus*) when the soil surface was exposed to increasing doses of laser energy. The 2 µm wavelength from the fiber laser is mainly absorbed by the water inside the target and is more beneficial for weed control than a CO_2 laser, which energy is primarily absorbed on the surface of the plant (Wieliczka et al., 1989). Therefore, a thulium-doped 2 µm fiber laser has been installed in the autonomous vehicle for laser weeding developed in the EU project WeLASER (https://welaser-project.eu/). A laser energy dose of 236 J mm^{-2} may be used to control seedlings of weeds in agricultural and horticultural fields. Weed plants on the cotyledons and two permanent leaf stages are usually killed when they are exposed to 157 J mm^{-2} (Andreasen et al., 2022; Heisel et al., 2002). We also exposed larvae, pupae, and beetles of *T. molitor* and the *A. bipunctata* beetle to increasing doses of laser energy. We hypothesized that all organisms would be negatively affected if they were exposed to an energy level of 79−236 J mm^{-2}) which may be used to control dicotyledon and monocotyledon weeds at the early stages of development (Andreasen et al., 2022; Coleman et al., 2021).

2. Materials and Methods

2.1 Laser equipment

We used a thulium-doped 50 W fiber laser with a wavelength of 2 µm with a collimated beam (Ø: 2 mm) manufactured by Futonics Laser GmbH, Katlenburg-Lindau, Germany. The laser was placed within a steel box (68 cm × 68 cm × 68 cm) with a door with a metal interlock (Figure 1). On laser activation, the door locks automatically to avoid risk of laser exposure.

(Please insert Fig. 1 here)

The target organisms were placed approximately 20 cm below the laser head and exposed to increasing dosages of laser energy (from 0 to 235.71 J mm^{-2})

2.2 Laser experiments with Enchytraeus spp.

2.2.1 Culturing conditions of Enchytraeus spp.

Enchytraeus albidus and *E. crypticus* were cultured in soil in plastic buckets with lids (length: 13.5 cm; Ø: 13 cm). Three soil types were used: a) sandy soil containing about 9% clay, 10% silt, 32% fine sand, 47% coarse sand, and 2% organic matter, b) clay soil containing about 28% clay, 23% silt, 10% fine sand, 36% coarse sand, and 3% organic matter, and c) an organic soil based on sphagnum (Pindstrup mixture 2 (https://www.pindstrup.dk/professionel/product-details/pindstrup-f%C3%A6rdigblanding-2), Pindstrup mosebrug a/s, Ryomgaard, Denmark). There was one bucket for each soil type and worm species (n=6). The buckets were weighed after adding soil and water, and afterward once a week to check the soil moisture. Water loss was replenished by adding an appropriate amount of deionized water. The buckets were placed in the dark in a climate cabinet at 20 °C ± 2 °C. The soil was kept moist but not wet corresponding to 40−60% of the water-holding capacity. Four holes (2 mm) were made in the lids of the buckets to allow adequate gaseous exchange with the atmosphere. The soil was cautiously broken up with a spatula each week to facilitate aeration. The worms were fed with rolled oats weekly. The rolled oats were ground and autoclaved (121 °C, 105 min, 1200 mbar) before use to avoid infestation with flour mites (*e.g.*, *Glyzyphagus* sp. and *Astigmata, Acarina*) or predacious mites (*e.g.*, *Hypoaspis scimitus* (Cosmolaelaps) and *Gamasida* (Acarina)). If the oat became contaminated with fungi, it was removed and replaced.

2.2.2 Experiments with *Enchytraeus albidus* and *E. crypticus*

To study the effect of the laser on the earthworms, 10 g of dry soil was moistened with demineralized water to achieve approximately 50% of the water-holding capacity. The moist soil was placed in a 50 ml plastic tube (Length: 11.2 cm; Ø: 30 mm), with 10 holes ((Ø: 1 mm) in the lid to ensure gas exchange.

After the soil was placed in the tubes three worms were transfer to each tube and kept for one day in a climate cabinet in darkness at 20 °C ± 2 °C before laser treatment. For each soil type and laser doses (0 (control), 0.5, 1, and 1.5 seconds corresponding to 0, 78.6, 157,1, and 235,7 J m^{-2}), 10 tubes with three *E. albidus* worms and 10 tubes with *E. crypticus* were used. Three soil types were chosen because the heat transfer from the laser depends on the soil textures. The lid of the tubes was move before the soil surface in the tubes was exposed to the laser beam from above (Figure 1). After the treatment, the tubes were placed with the lids in a climate cabinet in darkness at 20 °C ± 2 °C. The number of immobile, live, dead, or missing enchytraeids in all treatments were recorded by empty the tubes and searching through the soil with a spatula 7 days after the treatment. Worms could be missing because they were dead, decomposed and dissolved during the period. The experiment was repeated four times with *E. albidus*, using the same worms, as they were not affected by the treatment, but only done once with with *E. cryptibus* due the lack of worms.

2.3 Experiments with *Tenebrio molitor*

Tenebrio molitor were bought from the company InsektOrama A/S, Herning, Denmark. Adults and half of the delivered larvae were used for the first experiment and lasered two days after the delivery. The rest of the larvae were reared to produce an adequate number of new adults, larvae (to repeat the experiment) and pupae (for the first experiment and its repetition). When larvae were developed, they were moved to a round open plastic tray (10 cm high; Ø: 29 cm) and placed in a climate cabinet (at 20 °C ± 2 °C in darkness). The bottom of the tray was covered with rolled oats (3 cm high) necessary for the larvae’s rearing. Additional oats were added when necessary to ensure a continuous supply of feed. Four to five slices of fresh potato (Ø: 3−4 cm) were added to the tray every second day to ensure water supply. The tray was checked daily, and as soon as a pupa was observed, it was immediately separated from the larvae and placed in a new, similar tray. Adults were isolated from pupae in the same way to avoid cannibalism. Adults were producing eggs, creating a new generation of larvae required to repeat the experiment. All insect stages were kept and reared under the same conditions until they were lasered.

Ten individuals each of larvae, pupae, and adult *T. molitor* were exposed to the laser beam for 0, 1, 10, 20, 50, 131 100, and 500 ms corresponding 0, 0.16, 1.57, 3.14, 7.86, 15.71, and 78.57 J mm^{-2}. . Ten individuals of each developmental stage of *Tenebrio molitor* were treated with each dose with 4 replicates (10 × 4 individuals). The larvae were retained in a groove in a small piece of wood during the treatment. The pupae were placed on a piece of paper, and the adults were placed in a glass tube (length: 40 mm; Ø: 18 mm) to prevent them escaping the treatment (Figure 1). The laser was aimed at the dorsal surface, approximately halfway along the length of the body, but because they were alive and able to move some were hit in other locations. Afterwards, the insects were carefully moved to four plastic transparent boxes with lids (12 cm × 12 cm × 4 cm) (10 individuals per box) immediately after lasering with lids partially (1/3 of the lid) covered by a net (holes: 1 mm × 1 mm) ensuring gas exchanges.

The bottom of the boxes was covered with approximately 1 cm of rolled oats and slices of fresh potato (Ø: 3−4 cm) was added as feed and water supply. Additional rolled oats and fresh potato was added when necessary to ensure a continuous supply of feed and water. The number of live and dead individuals were counted 8 and 15 days after the laser treatment. Damages and deformities were noted.

2.4 Experiments with *Adalia bipunctata*

Adalia bipunctata, produced by EWH BioProduction ApS, Tappernøje, Denmark was bought via Horticoop Scandinavia A/S, Hinnerup, 143 Denmark. The beetles were kept in transparent plastic containers (28 cm × 20 cm × 22 cm) with lids, partially (2/3) covered by a net (holes: 1 mm × 1mm) allowing gas exchange, and kept in a climate cabinet at 21°C with 12 hours light from 8 a.m. to 8 p.m. The beetles were fed with honey diluted with water (ratio 1:10) during the experimental periods. The diluted honey was supplied via pieces of filter papers (3 cm × 3 cm). Four to five new papers were placed in the box with the beetles every day. The beetles were kept and reared in the same conditions until being lasered.

For each laser treatment, ten beetles were exposed to a laser dose. Each beetle was placed in a plastic tube (length: 50 mm; Ø: 5 mm) during the irradiation preventing the beetle from escaping. We aspired to hit the beetle in the center of their dorsal surface, but they were able to move a little and therefore some were hit in other places. The laser dosages were 0, 1, 10, 20, 50, 100, and 500 ms corresponding to 0, 0.16, 1.57, 3.14, 7.86, 15.71, and 78,57 J mm^{-2}. After exposure, each beetle was moved to a plastic container together with the nine other beetles receiving the same dose and kept exactly the same way as the pretreated adults. The mortality rate was recorded over 15 days. There were four replicates for each dose (total 4 ×10 beetles). Two independent experiments was done twice.

2.5 Statistical analyses

Statistical analyses were done using the statistical program R´ (R Core Team,2021). All data sets were initially analyzed using a dose-response model. If no dose-response trend was present in the data, the lowest observed adverse effect level (LOAEL) and the no observed adverse effect level (NOAEL) were found as the lowest dose showing a significant effect compared to the control group and the highest dose with a non-significant effect compared to the control group, respectively.

2.5.1 *Enchytraeus albidus* and *E. crypticus*

Since no clear dose-response trend were observed for mortality of *Enchytraeus albidus* and *E. crypticus*, data were analysed with a logistic regression model with laser dose as factorial explanatory variable. For *E. albidus* exposed in clay and sandy soil with four repetitions, a logistic mixed model was used instead. For these models, laser dose was included as a factorial fixed effect and repetition was included as random effect. Pairwise comparisons to the control group to find NOAEL and LOAEL were based on the fitted models (Hothorn et al., 2008).

2.5.2 *Tenebrio molitor*

A non-linear dose-response model for binary data was used to describe the association between laser dose and mortality of the individual life stages. A three-parameter log-logistic model assuming an upper limit of 100% mortality was fitted to the data from each repetition and for 8 and 15 days after treatment, individually. The effective dose (ED_x) killing a percentage, x, of the individuals remaining after adjustment for background mortality was estimated from each individual model and combined in a meta-analytic linear mixed model for each ED_{20} and ED_{80}, separately. Each model included the day of observation as fixed effect and repetition as random effect. For plotting, parameters from each model fit were combined in a second step using a meta-analytic linear mixed model (Jensen et al., 2020). The model included the two-way interaction of day of observation and model parameters as fixed effect, repetition as random effect with corresponding standard deviations that were assumed different for each of the three model parameters, and an unstructured variance-covariance matrix. Pairwise comparisons of parameters and ED-values between days of observation were based on the estimated meta-analytic models as post hoc pairwise comparisons (Hothorn et al., 2008).

2.5.3 *Adalia bipunctata*

Data for *Adalia bipunctata* were analyzed in a similar way as data for *Tenebrio molitor* but for five different time points. For day 1 and day 4, there were no background mortality and accordingly a two-parameter log-logistic model was used. For day 8, 11, and 15, a three-parameter log-logistic model was used.

3 Results

3.1 *Enchytraeus albidus* and *E. crypticus*

In all earthworms experiments except one, the mortality rates of the worms living in clay, sandy, and organic soil exposed to laser heating were not significantly different from the controls (Table S1). Consequently, the NOAEL was the highest dose of 235.71 J mm^{-2} and the LOAEL could not be estimated. For *E. crypticus*, the highest laser dose of 235.71 J mm^{-2} was the only dose significantly higher than the control (p=0.0185), and accordingly the LOAEL, making 157.14 J mm^{-2} the NOEAL (Table S1).

3.2 *Tenebrio molitor*

3.2.1 Larvae

In both experiments, the dose influenced the mortality of the larvae. A non-linear function fitted the data well (Fig. 2). Model parameters and ED_{20}, ED_{50}, and ED_{80} values are shown in Table S2.

(Please insert Fig 2 here)

The mortality after 8 days did not change significantly. The larvae from the control group (0 J mm^{-2}) developed into normal pupae and beetles. At the smallest dose (0.16 J mm^{-2}) some larvae and pupae developed normally, but some developed into beetles with wing deformities. When the dose was increased to 3.14 J mm^{-2}, most of the insects (all stages) were living, but the living larvae received a spot burn from the laser treatment while living adults had deformed wings. The dead insects became brown, dark or with a big dark spot from the laser. The living larvae developed into deformed beetles that almost immediately died. At a dose of 7.86 J mm^{-2}, more than half of the larvae died. Most of the dead insects were at the larva stage and very few could complete metamorphosis resulting in severely deformed insects, and in some cases, they became half pupa and half beetle. Most of the dead larvae were dark and brown, and the few living ones received a dark spot burn from the laser (Fig. 2).

Very few larvae survived a dose of 15.71 J mm^{-2}. Most larvae died having a dark dehydrated and burned appearance. At a dose of 78.57 J mm^{-2}, almost all the larvae died the first week after application at larva stage. Only one transformed into a pupa. The dead larvae became completely black or brown with the haemolymph running out from the burned hole in their body just after treatment. (Fig. 3).

(Please, insert Fig. 3 here)

3.2.2 Pupae

In contrast to larvae and beetles, the mortality of the pupae increased between 8 and 15 days (Fig 4, Table S3). Fifteen days after the laser application, all pupae in the control groups developed into beetles with normal appearance and high survival rate (90%). When a dose of 0.16 J mm^{-2} was applied, all pupae developed into beetles with the same survival rate as the control group. However, one third developed deformed wings and/or body (Fig. 5b). When the dose was increased to 1.57 J mm^{-2}, the survival rate declined approximately 68%. The living pupae all developed into adults, which, however, had some wing and body deformities. Most of the non-living pupae and beetles were discoloured and broke into small pieces (Fig. 5c). When the dose was doubled (3.14 J mm^{-2}), the survival rate decreased even more, and the mortality rate rose to 62%. The appearance of non-living adults varied from injured (Fig. 5d), cut into pieces or dead bodies without any indication of abnormal appearance. A dose of 7.85 J mm^{-2} increased the mortality to approximately 85%. Many pupae did not develop into adults as a high number of dead pupae had a brown dark color or completely dark and dehydrated body. A dose of 15.71 J mm^{-2} almost killed all insects after 15 days (mortality ~ 97.5%). Many pupae did not develop into adults due to high mortality. Dead pupae had a brown dark color or a completely dark and dehydrated body. At the highest dose (78.57 J mm^{-2}), all pupae died within the first week after the irradiation with a burned-like appearance. None of them managed to develop further to the adult stage indicating that the death happened a few days after the laser treatment.

(Please insert Fig. 4 & 5 here)

3.2.3 Adults

At the lowest dose (0.16 J mm^{-2}), the mortality did not differentiate from the controls (Fig 6, Table S4). Treatment with 1.57 J mm^{-2} increased mortality. After applying a dose of 3.14 J mm^{-2}, the mortality rate increased to *ca* 2.5 %. Most of the living adults had a spot derived from the laser beam while most of the non-living adults had a small hole from the laser. More than half of the adults died at a dose of 7.86 J mm^{-2} with a hole from the laser. At the highest dose, the mortality was about 92.5 % and most beetles were killed immediately with hole in the body. (Fig. 7).

(Please insert Fig. 6 and 7 here)

3.3 *Adalia bipunctata*

In general, the non-linear model fitted the data well (Fig. 8). Model parameters are shown in Table S5. During the 15 days, some of the non-exposed beetles died. The mortality increased over time and with increasing dosages. Even 0.16 J mm^{-2} affected the shape of the dose-response curve, and the beetle's elytron became brownish in color (Fig. 8). A dose of 7.86 J mm^{-2} severely harmed the beetles, and almost all beetles died during the 15 days. A dose of 78.57 J mm^{-2} immediately killed all beetles burning significant holes in the beetles (Fig. 9).

(Please insert Fig. 8 and 9 here)

4 Discussion

4.1 Earthworms (*Enchytraeus albidus* and *E. cryptibus*)

There were no differences in mortality between the control group and worms exposed to all laser doses, with a single exception with *E. cryptibus* in sandy soil at a dose of 157 J mm^{-2}**.** The spread of the heat in the soil from the laser beam depends on the water content and soil structure and composition. We cannot exclude that other conditions like a higher water content or other soil types would result in other mortalities. We consider a soil water content of 50% of the water capacity to be realistic in the early spring when weed control usually is conducted, but it depends on many factors in the field (*e.g*., variation in soil composition, precipitation, and evaporation). The earthworms in the tubes were living in a very small soil volume (<15 g) during the experiment mimicking the uppermost part of the soil profile. Although heat corresponding to 235,7 J mm^{-2} was executed on a spot with a 2 mm diameter, which easily kills weed seedling, the exposure did not warm up the soil sufficiently to affect the mortality of the worms living close to the soil surface within the seven days. In general, the worms will be spread over the whole area and even if the laser beam did have some impact on their performance and reproduction, there would probably be other worms in the deeper soil matrix and field area ready to invade the tiny area that is exposed to the laser irradiation as there can be up to 100,000 worms per m^2. Measuring the effect of laser on the reproduction rate was not done but would also be relevant.

4.2 Insects

Tenebrio molitor is a large model insect. Larger insects, such as *T. molitor*, appear to be more resistant to the laser than smaller insects such as *A. bipunctata*. In general, the insects were all killed immediately at a laser dose corresponding to what would be appropriate for killing small weed seedlings (78.57−157.14 J mm^{-2}). We aimed to focus the laser on the middle of the body of the insects, but because the insects were able to move a little, there would be variations in where the laser hit. If we had focused the laser on the head or the rear part, the mortality might have been different.

4.3. Laser safety

We used a collimated beam in the experiments to precisely give the wanted dose independent of the precise distance to the target. However, if stones or other reflecting materials are hit with a collimated laser beam, the reflected beam may escape the target area, and humans or larger animals (dogs, hares, etc.) may be exposed, burned, or blinded. Therefore, the laser beam should not be collimated in laser weeding robots, but only be focused and concentrated on the meristem of the weed seedlings. If the laser then hits a reflecting material, the beam will be spread in a cone and the risk of harming the humans, animals and other plants would be significantly reduced due to the lower dose per area. That means that only insects or other organisms placed exactly in the focus point would receive the dose determined for the target plant. The further away from the focus point the lower the dose an organism would receive and the less harmful the exposure.

Some insects benefit the crop like ladybugs, spiders, and predatory beetles, as they can reduce the number of harmful insects (*e.g.*, aphids (Aphididae) and rape beetles (*Meligethes aeneus*)). Some beneficial insects like ladybugs have characteristic colors and can easily be identified with recognition tools (Rakhmatulin et al., 2021). In principle, a laser-weeding robot could be programmed to recognize the different between beneficial and pest arthropods and kill the latter.

On the other hand, small dosages of laser energy could be considered to control harmful arthropods. Flying insects are common vectors for the transmission of pathogens between crop plants (Heck, 2018). Mullen et al. (2016) presented proof of principle for an optical system capable of highly specific vector control using a combination of optical sources, detectors, and sophisticated software to search, detect, and identify flying insects in real-time, with the capability of eradication using a lethal laser pulse. They focused on two insect species: *Diaphorina citri*, a vector of the causal agent of citrus greening disease, and *Anopheles stephensi*, a malaria vector. There seems to be a great potential to use laser technology to protect people and crops from pestilent flying insects (Keller et al., 2020). Our experiments showed that laser dosages, which will not harm the plants, could significantly damage harmful insects.

4.4 Laser weeding compared to herbicide application and mechanical weed control

Laser weeding seems to be a promising tool to replace or supplement herbicides and mechanical control, with only a small treatment area in contrast to other weed control measures. Unlike herbicide spraying, laser weeding leaves no chemicals in the field that may harm non-target organisms after the treatment. Herbicides may evaporate or leach to surface and groundwater and may expose the environment to short or long-term unwanted side effects. Laser weeding only leaves the ash from the burned weed meristem in the field after the treatment, which may be taken up by the crop plants as fertilizer.

In contrast to laser weeding, mechanical weeding impact shallow living worms negatively (and potentially other soil organisms), as the weeding implements are passing through the top layer of the soil (Doran and Zeiss, 2011). Mechanical weeding also harms beneficial organisms on the soil surface, like spiders and predatory beetles (Michalko et al., 2019; Symondson et al., 2022). Therefore, laser weeding seems to have less negative impact on the environment that other weed control measures.

5 Conclusion

Laser doses sufficient to kill plants were lethal to the insects, and lower doses that did not kill plants killed or harmed the insects across all life stages tested. The larger *T. monitor* beetle survived higher doses than the smaller *A. bipunctata* beetle. The results indicate that pest control might be a possibility using laser dosages which do not harm plants. In general, the probability of harming insects with dosages used for laser-weeding is small, as only a tiny proportion of the area will be exposed for the treatment, even with a high weed density. Using another type of laser may give other results. Developing a recognition tool using artificial intelligence to differentiate between pests and beneficial arthropods would make pest control possible without harming beneficial organisms at the same time as the laser weeding takes place.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

C.A. was responsible for funding acquisition and the design of the experiments. E.V. and K.S.J. conducted the experiment with insects. S.M.J. made the statistical analyses. C.A. wrote the first draft of the article, and all authors added to the manuscript, reviewed, edited, and accepted the final manuscript. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement

The datasets presented in this study can be found in the online repository zenodo after the paper has been accepted.

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Captions

FIGURE 1

The laser was placed within a steel box (68 cm × 68 cm × 68 cm) with a door with a metal interlock. The organisms were placed below the laser head.

FIGURE 2

The mortality (%) of *Tenebrio molitor* larvae 8 and 15 days after exposure to increasing dosages of laser energy (J) from a thulium-doped 2 µm 50 W fiber laser with a collimated beam with a diameter of 2 mm. Points show mean values (n=20).

FIGURE 3

Deformities observed during the study of *T. molitor* (larvae experiment): 2a) *T. molitor* control adult and larva 2b) 0.57 J m^{-2}): Larva becomes a deformed alive adult. 2c) 3.14 J m^{-2}: Larva developed into a deformed adult and died. 2d) 7.56 J m^{-2}: Larva did not complete metamorphosis (severely deformed insect: half pupa, half adult). 2e) 15,71 J m^{-2}: Burned (brownish-dark) larva 3f) 78 J m^{-2}:. Larva were rapidly killed after exposure, and there was significant damage caused to the insects, e.g., hemolymph flowed out of the laser hole in the insects' body.

FIGURE 4

The mortality (%) of *Tenebrio molitor* pupae 8 and 15 days after exposure to increasing dosages of laser energy (J m^{-2}) from a thulium-doped 2 µm 50 W fiber laser with a collimated beam (Ø = 2 mm). Points show mean values (n=20).

FIGURE 5

Typical deformities that were observed on *Tenebrio molitor* after laser application (pupae experiment): 4a) *T. molitor* control pupa 4b) 0.57 J m^{-2} (1 ms): Pupa developed into adult with deformed wings 4c) 1.57 J m^{-2}: Non-living adult and adults broken into pieces. 4d) 3.14 J m^{-2}: Pupa transformed to an injured adult. 4e) Pupa died few days after laser application having a brown-burned appearance.

FIGURE 6

The mortality (%) of *Tenebrio molitor* beetles 8 and 15 days after exposure to increasing dosages of laser energy (J) from a thulium-doped 2 µm 50 W fiber laser with a collimated beam (Ø = 2 mm). Points show mean values (n=20).

FIGURE 7

Typical deformities that were observed on *Tenebrio molitor* adults after laser application (adults' experiment): 6a) 0.15 J m^{-2}: *T. molitor* adults were alive with normal appearance 6b) 3.14 J m^{-2}: a living adult with a spot from the laser. 6c) 78,57 J m^{-2}: *T. molitor* adult died immediately with a large hole in its body.

FIGURE 8

The mortality (%) of *Adalia bipunctata* beetles 1, 4, 8, 11 and 15 days after exposure to increasing dosages of laser energy (J) from a thulium-doped 2 µm 50 W fiber laser with a collimated beam (Ø = 2 mm). Points show mean values (n=20).

FIGURE 9

Malformations on the body of the adults *Adalia bipunctata* observed in the study. a) 0.05 J (0.16 J mm^{-2}): Brownish color on the outer shell. b) 0.1 J (78 J mm^{-2}) Small damage on an alive adult. c) 2.5 J (50 ms J mm^{-2}) severe damage, dead adult d) 25 J (500 ms J mm^{-2}) Acute damage: dead adult one day after lasering. e) 78.57 J mm^{-2}), fungal infection.

Fig 1

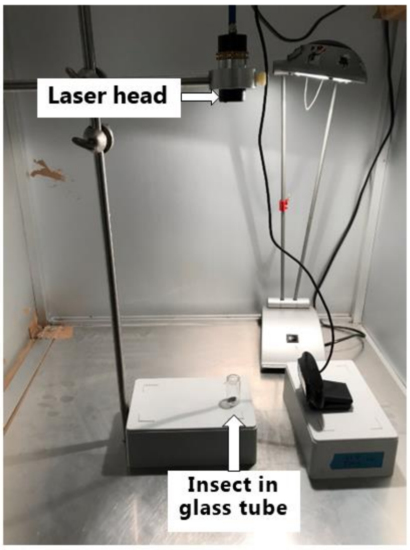

Fig 2 Larvae

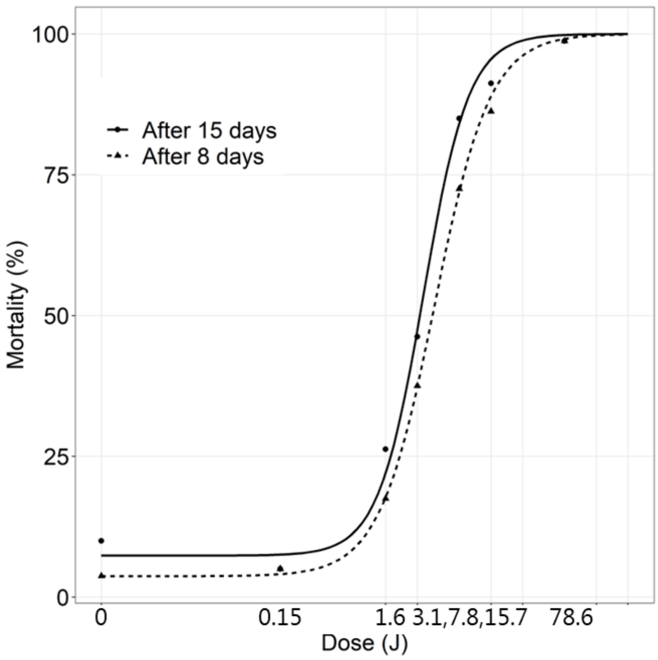

Fig 3

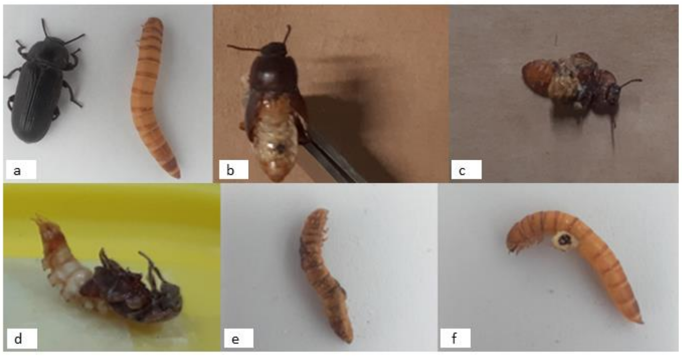

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Fig 4 Pupae

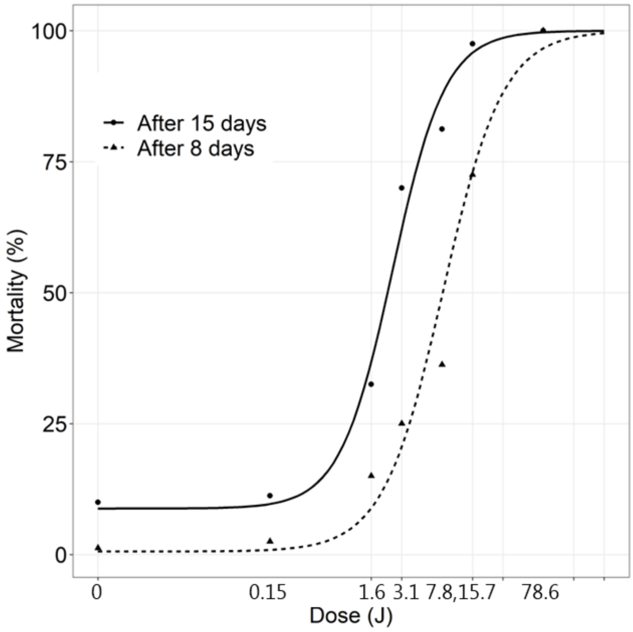

Fig 6 Adult Beetle

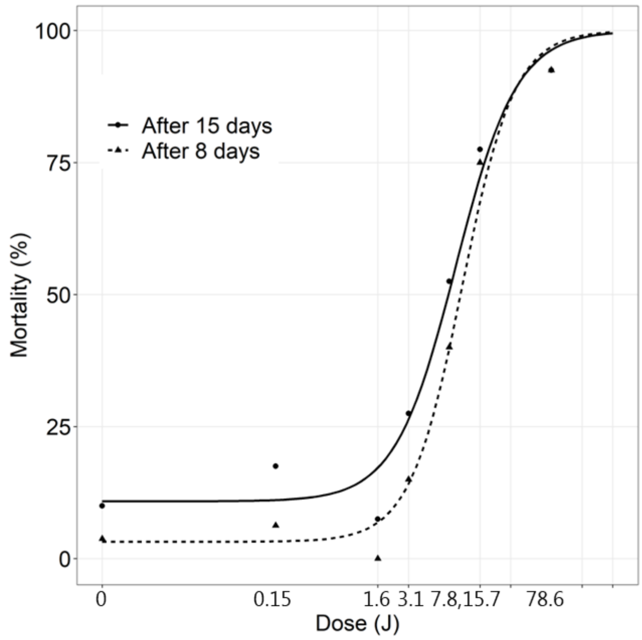

Fig 7

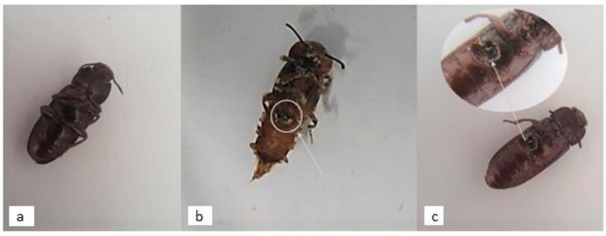

Fig 8 Labybug

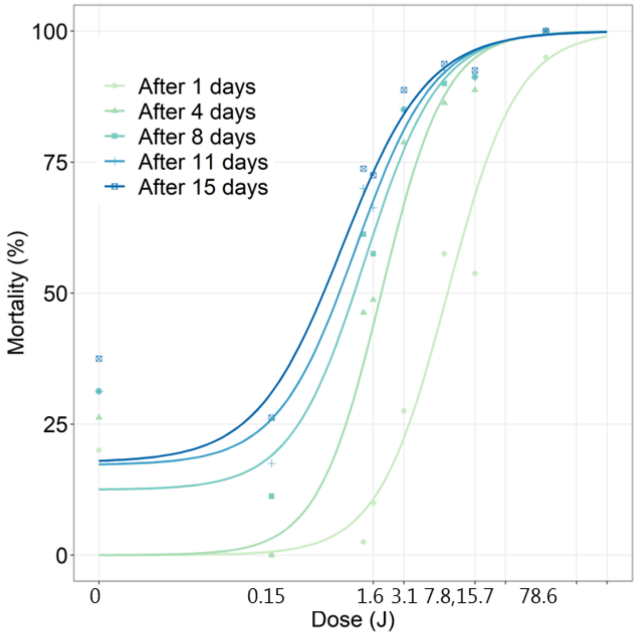

Fig 8

Supplementary Material

Side-effects of laser weeding:

The effect on earthworms (Enchytraeids) and insects

(*Tenebrio molitor* and *Adalia bipunctata*)

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TABLE S1. Estimated mortality (proportion dead) with 95% confidence intervals (95% CI) of *Enchytraeus albidus* and *E. cryptibus* for each dose, species, and soil type. Within species and soil type, dose group marked with * have a significantly different mortality compared to the control group.

Species	Soil type	Dose (J)	Estimate (95% CI)
Enchytraeus albidus	Organic	0	0.25 (0.12 – 0.46)
		25	0.13 (0.04 – 0.32)
		50	0.05 (0.01 – 0.26)
		75	0.10 (0.02 – 0.31)
	Sandy	0	0.07 (0.03 – 0.14)
		25	0.04 (0.02 – 0.10)
		50	0.01 (0.00 – 0.06)*
		75	0.02 (0.01 – 0.08)
	Clay	0	0.04 (0.01 – 0.20)
		25	0.00 (0.00 – 1.00)
		50	0.00 (0.00 – 1.00)
		75	0.01 (0.00 – 0.08)
Enchytraeus crypticus	Organic	0	0.00 (0.00 – 1.00)
		25	0.00 (0.00 – 1.00)
		50	0.00 (0.00 – 1.00)
		75	0.04 (0.01 – 0.23)
	Sandy	0	0.00 (0.00 – 1.00)
		25	0.04 (0.01 – 0.22)
		50	0.04 (0.01 – 0.23)
		75	0.00 (0.00 – 1.00)
	Clay	0	0.05 (0.01 – 0.26)
		25	0.11 (0.04 – 0.29)
		50	0.13 (0.04 – 0.34)
		75	0.40 (0.21 – 0.62)*

TABEL S2. Estimates of parameters of the model describing the relationship between laser dose and mortality of *Tenebrio molitor* larvae. The *b* parameter is a relative slope of the curve at the dose where 50% of the larvae died (ED_{50}). Parameter *c* corresponds to the lower limit of the curve. The effective doses ED_{20} and ED_{80} correspond to doses resulting in a mortality of 20 and 80% (relative to the lower and upper limit), respectively. Test for difference shows differences between observations 8 and 15 days after laser application. Standard errors in parentheses.

Parameter	Day	Estimates	Test for difference (*p*-value)
b	Day 8	-1.65 (0.72)	
b	Day 15	-2.02 (0.76)	0.73
c	Day 8	0.04 (0.02)	
c	Day 15	0.07 (0.03)	0.28
ED_{20}	Day 8	0.62 (0.16)	
ED_{20}	Day 15	0.53 (0.16)	0.51
ED_{50}	Day 8	1.46 (0.14)	
ED_{50}	Day 15	1.14 (0.10)	0.07
ED_{80}	Day 8	3.70 (1.11)	
ED_{80}	Day 15	2.71 (1.08)	0.01

TABEL S3. Estimates of parameters of the model (1) describing the relationship between laser dose and mortality of *Tenebrio molitor* pupae. The *b* parameter is a relative slope of the curve at the dose where 50% of the larvae died (ED_{50}). Parameter *c* corresponds to the lower limit of the curve. The effective doses ED_{20} and ED_{80} correspond to doses resulting in a mortality of 20 and 80% (relative to the lower and upper limit), respectively. Test of difference shows differences between observations 8 and 15 days after laser application. Standard errors in parentheses.

Parameter	Day	Estimates	Test for difference (*p*-value)
b	Day 8	-1.47 (0.36)	
b	Day 15	-1.67 (0.39)	0.71
c	Day 8	0.01 (0.04)	
c	Day 15	0.09 (0.04)	0.16
ED_{20}	Day 8	0.74 (0.18)	
ED_{20}	Day 15	0.36 (0.15)	<0.0001
ED_{50}	Day 8	2.56 (0.28)	
ED_{50}	Day 15	0.81 (0.18)	<0.0001
ED_{80}	Day 8	6.97 (0.97)	
ED_{80}	Day 15	1.90 (0.22)	<0.0001

TABEL S4. Estimates of parameters of the model describing the relationship between laser dose and mortality of *Tenebrio molitor* beetles. The *b* parameter is a relative slope of the curve at the dose where 50% of the larvae died (ED_{50}). Parameter *c* corresponds to the lower limit of the curve. The effective doses ED_{20} and ED_{80} correspond to doses resulting in a mortality of 20 and 80% (relative to the lower and upper limit), respectively. Test for difference shows differences between observations 8 and 15 days after laser application. Standard errors in parentheses.

Parameter	Day	Estimates	Test for difference (*p*-values)
b	Day 8	-1.70 (0.18)	
b	Day 15	-1,46 (0.17)	0.34
c	Day 8	0.03 (0.01)	
c	Day 15	0.11 (0.02)	<0.0001
ED_{20}	Day 8	1.50 (0.17)	
ED_{20}	Day 15	1.13 (0.18)	0.14
ED_{50}	Day 8	3.35 (0.30)	
ED_{50}	Day 15	2.90 (0.33)	0.31
ED_{80}	Day 8	7.53 (0.99)	
ED_{80}	Day 15	7.48 (1.17)	0.98

TABLE S5. Estimates of parameters of the model describing the relationship between laser dose and mortality of *Adalia bipunctata* beetles. The *b* parameter is a relative slope of the curve at the dose where 50% of the larvae died (ED_{50}). Parameter *c* correspond to the lower limit of the curve and *e* corresponds to ED_{50}. The effective dose ED_{20} and ED_{80} corresponding to doses resulting in a mortality of 20 and 80% (relative to the upper and the lower limit), respectively. Statistical differences between the parameters estimated 1, 4, 8, 11, and 15 days after treatment are shown with letters. Within each parameter, Days not sharing a letter are statistically different.

Parameter	Day	Estimate	Sig. letters
b	Day 1	-1.26 (0.13)	a
b	Day 4	-1.38 (0.15)	a
b	Day 8	-1.19 (0.16)	a
b	Day 11	-1.12 (0.16)	a
b	Day 15	-1.03 (0.13)	a
c	Day 8	0.12 (0.17)	a
c	Day 11	0.17 (0.18)	a
c	Day 15	0.18 (0.17)	a
e	Day 1	2.68 (0.26)	a

e	Day 4	0.52 (0.07)	b
e	Day 8	0.41 (0.08)	bc
e	Day 11	0.34 (0.07)	c
e	Day 15	0.25 (0.05)	c
ED_{20}	Day 1	0.97 (0.11)	a
ED_{20}	Day 4	0.17 (0.03)	b
ED_{20}	Day 8	0.12 (0.03)	bc
ED_{20}	Day 11	0.09 (0.03)	bc
ED_{20}	Day 15	0.06 (0.02)	c
ED_{50}	Day 1	2.68 (0.26)	a
ED_{50}	Day 4	0.52 (0.05)	b
ED_{50}	Day 8	0.41 (0.07)	bc
ED_{50}	Day 11	0.34 (0.07)	bc
ED_{50}	Day 15	0.25 (0.04)	c
ED_{80}	Day 1	6.45 (0.99)	a
ED_{80}	Day 4	1.47 (0.19)	b
ED_{80}	Day 8	1.22 (0.19)	bc
ED_{80}	Day 11	1.08 (0.20)	bc
ED_{80}	Day 15	0.82 (0.15)	c