RESEARCH ARTICLE

Flashes of UV-C light: An innovative method for stimulating plant defences

Jawad Aarrouf[☯], **Laurent Urban**[iD][☯]*

UMR Qualisud, Avignon Université, Avignon, France

☯ These authors contributed equally to this work.
* laurent.urban@univ-avignon.fr

Abstract

Leaves of lettuce, pepper, tomato and grapevine plants grown in greenhouse conditions were exposed to UV-C light for either 60 s or 1 s, using a specific LEDs-based device, and wavelengths and energy were the same among different light treatments. Doses of UV-C light that both effectively stimulated plant defences and were innocuous were determined beforehand. Tomato plants and lettuce plants were inoculated with *Botrytis cinerea*, pepper plants with *Phytophthora capsici*, and grapevine with *Plasmopara viticola*. In some experiments we investigated the effect of a repetition of treatments over periods of several days. All plants were inoculated 48 h after exposure to the last UV-C treatment. Lesions on surfaces were measured up to 12 days after inoculation, depending on the experiment and the pathogen. The results confirmed that UV-C light stimulates plant resistance; they show that irradiation for one second is more effective than irradiation for 60 s, and that repetition of treatments is more effective than single light treatments. Moreover a systemic effect was observed in unexposed leaves that were close to exposed leaves. The mechanisms of perception and of the signalling and metabolic pathways triggered by flashes of UV-C light vs. 60 s irradiation exposures are briefly discussed, as well as the prospects for field use of UV-C flashes in viticulture and horticulture.

Introduction

There is the need to develop alternative or complementary solutions to pesticides (to decrease their use) that are effective, safe and economically viable. Chemical elicitors of plant defences are on the rise but their development is limited by inconsistent efficacy as a consequence of problems of formulation and stability in field conditions [1].

Physical elicitors do not present these drawbacks and have the additional advantage that they can be easily combined with other existing methods of treatment, either chemical or biological [2]. UV-B radiation has been observed to increase plant resistance to pathogens [3, 4]. UV-B light is known to act through signalling pathways, involving notably mitogen-activated protein kinases, which closely resemble those for pathogens [5]. It has also been observed that UV-C light can stimulate plant and crop defences against *Botrytis cinerea* and *Sclerotinia minor* [6–11]. UV-B light requires extensive periods of irradiation (several hours or days) to be

effective. This limits its use in greenhouse conditions, where lamps are in stationary positions, whereas effective doses of UV-C light can be supplied to plants and crops typically in 60 s [2, 12]. However actual use of UV-C light in field conditions will require the capacity to deliver effective doses in much less time than that. Therefore there is a need to design lamps that are able to deliver effective doses that are ideally one second or less. It also needs to be determined whether doses delivered in such a short time still are able to stimulate plant defences.

We tested in this trial the hypothesis that UV-C light in flashes of 1 s are capable of stimulating plant defences against several fungal and oomycete diseases. We moreover tested the idea that flashes of 1 s are more efficient than irradiations for 60 s by using an original device specifically designed for this purpose, i.e. maintaining equal wavelengths and energy among light treatments in a range of exposures from 1 to 60 s. Eventually we evaluated the effects of repetition of light treatments and systemic effects.

Materials and methods

LEDs-based lamp

The lamp used for the trials was made of 15 light emitting diodes (LEDs) on a printed circuit board fitted inside an integrating sphere (Labsphere Inc., North Sutton, NH, USA) (Fig 1). The LEDs consisted of SMD LEDs (Crystal IS Inc., Green Island, NY, USA). These LEDs are made of an alloy between gallium nitride (3.6 eV) and aluminium nitride (6.2 eV) and generate photons at 265 nm (Fig 1). They are capable of delivering more than 20 mW each in the pulse mode, depending on the temperature. A specific power supply and Peltier cooling systems were designed to maximize the light output of the LEDs. Calculation and direct measurement (Hera spectrophotometer, Pro-Lite, Marcillac, France) showed that it was possible to reach 100 mW cm^{-2} (1 kW m^{-2}, corresponding to 2214 μmol photons m^{-2} s^{-1}) at the level of the 5 cm^2 window at the bottom of the integrating sphere [13].

Plant culture

Experiments were conducted in the Avignon University greenhouses, between October 2016 and May 2017 (Table 1). Daily mean maximum and minimum temperatures and relative humidities, and daily cumulated global solar radiation were recorded (Fig 2). Lettuce, tomato and pepper seeds were sown in 1 cm^3 rockwool cubes in a glasshouse at 25 ± 2˚C. One week after sowing, the cubes, each containing one plantlet, were transferred into plastic pots (5 L), filled with a commercial growing medium (Klasmann Deilmann Gmbh, Bremen, Germany) containing 80% organic matter (pH = 6). The plants were then grown for 4 weeks at 24/16 ˚C (day/night temperatures) at ambient CO_2. For grapevines, rooted cuttings, cv. Cabernet Sauvignon, were cultivated in plastic pots (10 L), filled with a commercial growing medium (Platinium, Avignon, France) containing 85% organic matter (pH = 6.5). Plants were grown for 10 weeks under controlled conditions at 25/20 ˚C (day/night temperatures) at ambient CO_2. We used fertilizers with the following compositions: 5% N, 5% P_2O_5, 7% K_2O, 2.5% MgO, 12% SO_3 and 13% CaO (lettuce, 30 g per plant); 5% N, 5% P_2O_5, 8% K_2O, 3% MgO, 11% SO_3 and 14% CaO (tomatoes, 50 g per plant); 6% N, 3% P_2O_5, 3% K_2O, 6% MgO, 2% SO_3 and 14.6% CaO (peppers, 30 g per plant); 7% N, 4% P_2O_5, 7% K_2O, and 6% MgO (grapevines, 60 g per plant). A regular water regime was applied for all of the plants every two days. No pesticides were applied during the whole period of the trials. Control and treated plants were randomly distributed on part of a bench that was selected for being homogeneous in terms of light and temperature.
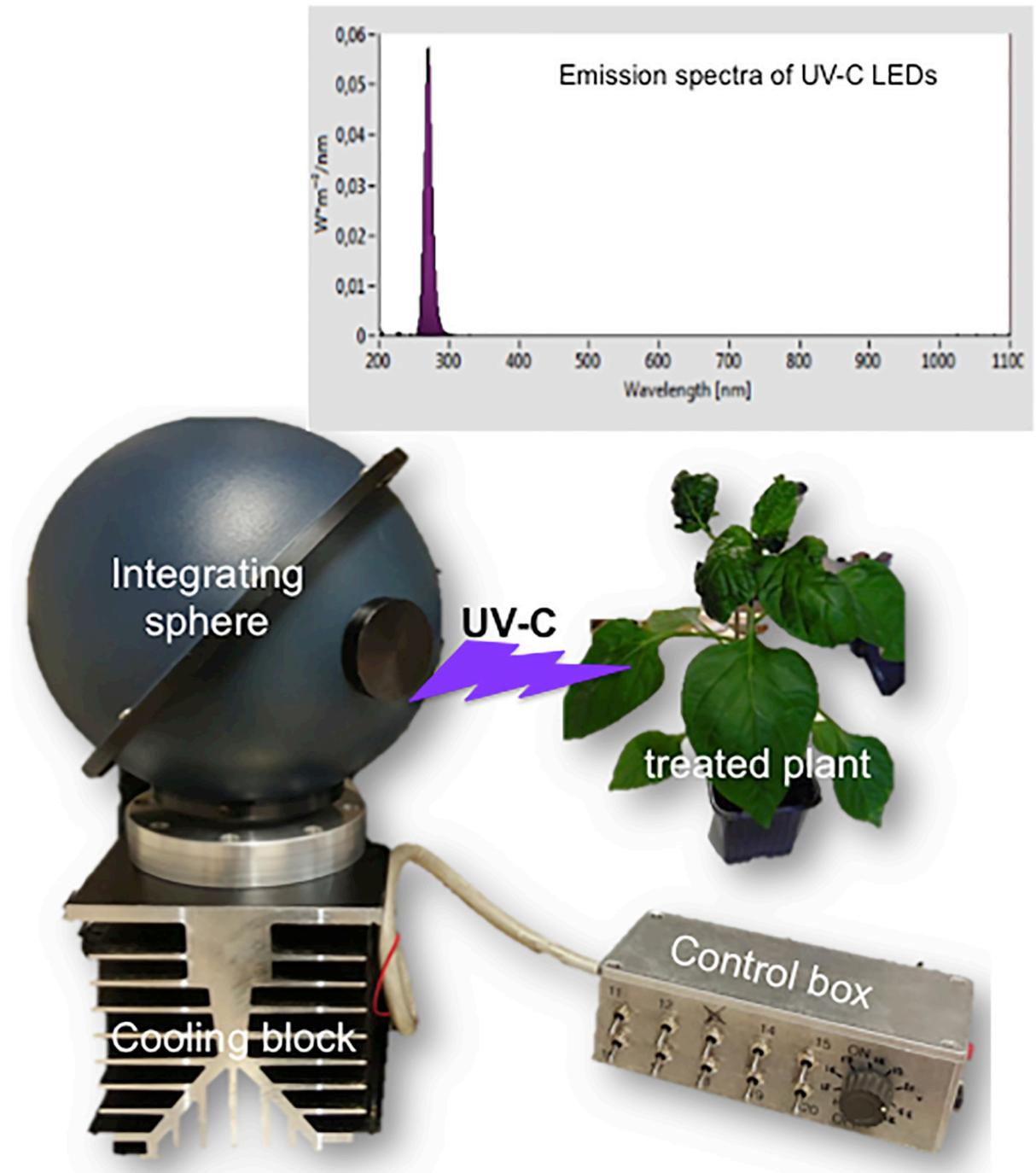
Fig 1. Photo of the device used for supplying UV-C light to plants. The inside of the integrating sphere accommodates 15 UV-C LEDs on a printed circuit board. The cap of the 5 cm^2 window is removed and the window placed directly on the leaves for treatments. A typical light spectrum is shown.

https://doi.org/10.1371/journal.pone.0235918.g001

UV-C treatments

Three experiments were performed (Table 1). There were five plants per UV-C light treatment and five plants served as untreated controls. Three tagged leaves per plant, of similar age and

Table 1. Cultivation periods of the different species used in the experiments.

	Experiment 1	Experiment 2	Experiment 3
Tomato	03 Oct—07 Nov	19 Dec—23 Jan	6 Mar—10 Apr
Lettuce	17 Oct—21 Nov	09 Jan—13 Feb	20 Mar—24 Apr
Pepper	31 Oct—05 Dec	23 Jan—27 Feb	28 Mar—2 May
Grapevine	-	26 Dec—06 Mar	20 Mar—8 May

exposure to light, were submitted to UV-C treatments or used as controls (n = 15). UV-C treatments consisted in exposing one single 5 cm^2 spot per leaf for either 1 or 60 s. Beforehand we determined the doses of UV-C light that are effective for stimulating plant defences without any negative effects on plants. The dose of UV-C light used in this study is the highest dose that can be delivered in 1 s by the LEDs system described above, i.e.1 kJ m^{-2}. The absence of negative effects on plants was assessed visually and by measurements of chlorophyll fluorescence, ChlF.

Chlorophyll fluorescence measurements

ChlF measurements were performed on treated and control leaves, ca. 48 hours after UV-C treatments, around 2 pm, with a Pocket PEA chlorophyll fluorimeter (Hansatech Instruments, King's Lynn, Norfolk, United Kingdom). Leaves were dark-adapted for 1200 s with a lightweight plastic leaf clip prior to measurements. The transients were induced by 1 s illumination with a single light-emitting diode providing a fully saturating photon flux density of 3500 μmol photons m^{-2} s^{-1} with a peak wavelength of 627 nm at the sample surface, and homogeneous irradiation. The ChlF intensity at 50 μs was considered as F_0 [14]. Several parameters derived from measurements of induction curves of maximal ChlF were calculated, the ratio of variable ChlF (F_v) to maximum ChlF (F_m), F_v/F_m, and the Performance Index (PI_{abs}), a plant vitality indicator [15], which is generally believed to be a more sensitive parameter than F_v/F_m even though there are contradictory observations [16]. We also calculated V_k/V_j, which represents the ratio of variable ChlF at 300 μs (K-step) to variable ChlF at 2 ms (J-step), and S_m, the normalized area above the ChlF induction curve.

Experiment 1: The effect of single UV-C flashes.   15 lettuce plants, 15 tomato plants and 15 pepper plants were used in this experiment between October and December 2016 (Table 1). After four weeks of cultivation, leaf spots of 5 cm^2 were treated by single exposures to UV-C light at 1 kJ m^{-2} supplied over 1 s (1 kW m^{-2}) or at the same cumulative dose supplied over 60 s. Two days later, leaves were detached and placed separately in plastic Petri dishes on moistened filter paper for inoculation.

Experiment 2: The effect of repeated UV-C flashes.   15 lettuce plants, 15 tomato plants, 15 pepper plants and 15 grapevine plants were used between December 2016 and March 2017 (Table 1). After four weeks of culture, leaf spots of 5 cm^2 were exposed to UV-C light at 1 kJ m^{-2} for 1 s (1 kW m^{-2}) in one treatment. UV-C flashes were then repeated three times on different spots of the same leaves, at 48 h intervals. In another treatment, lettuce and tomato leaves were exposed only to a single flash of UV-C light, supplied the same day as the day of the last exposure to UV-C light in the previous treatment. Two days later, leaves were detached and placed separately in plastic Petri dishes on moistened filter paper for inoculation.

Experiment 3: Systemic effects of UV-C flashes.   Ten lettuce plants, ten tomato plants, ten pepper plants and ten grapevine plants were used in this experiment between March and May 2017 (Table 1). After either four weeks of culture (lettuce, tomatoes, peppers) or ten weeks (grapevines), leaf spots of 5 cm^2 were exposed to UV-C light at 1 kJ m^{-2} for 1 s (1 kW
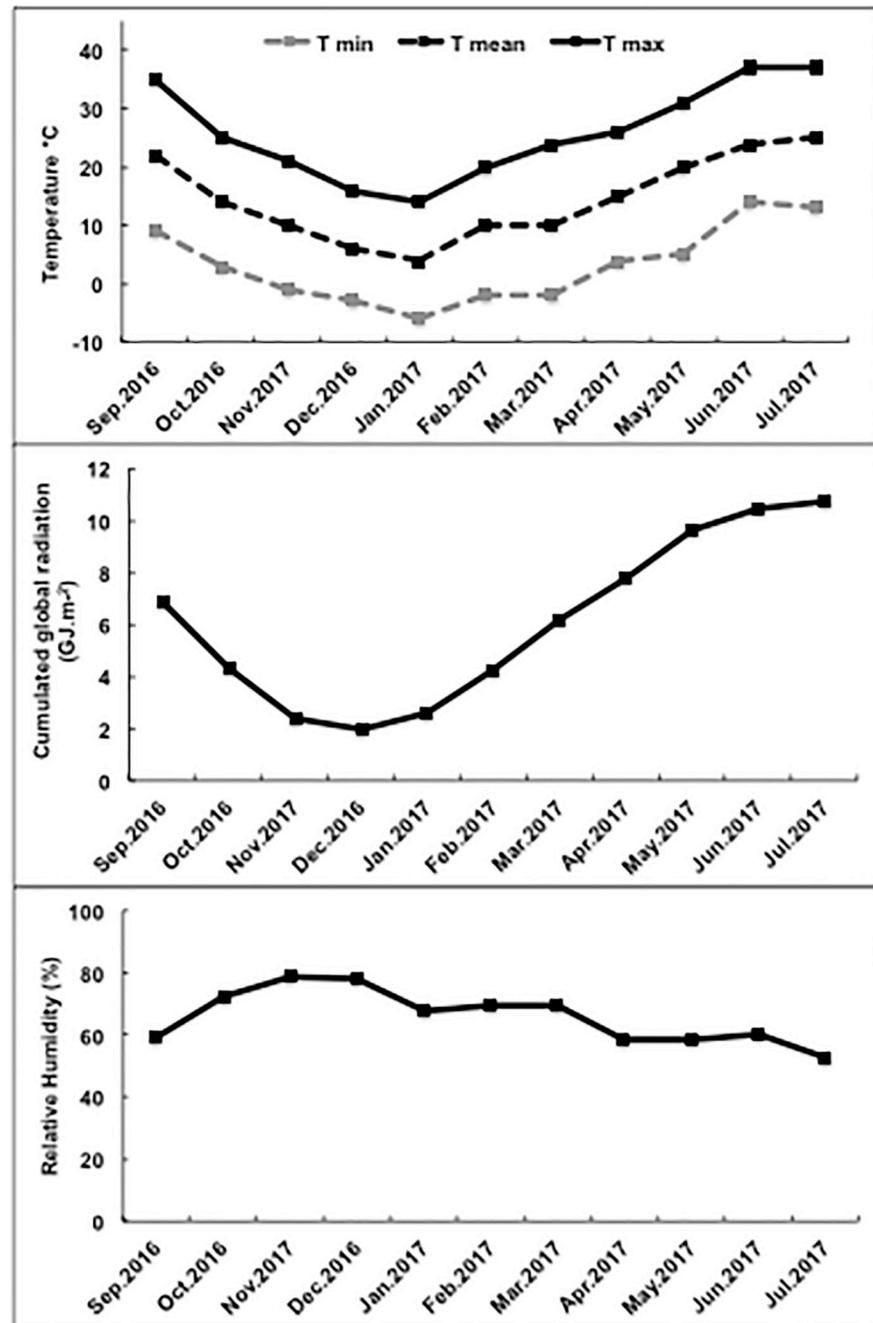
Fig 2. Daily mean, maximum and minimum temperatures and daily cumulated global solar radiation, and daily mean relative humidity from September 2016 to July 2017.

m^{-2}). UV-C flashes were repeated three times on different spots of the same leaves, at 48 h intervals. Two days after the last UV-C treatment, the 15 tagged leaves exposed to flashes of UV-C light were detached. 15 untreated leaves of similar age were also taken randomly among the treated plants, and 15 leaves from the five control plants. Leaves were placed separately in plastic Petri dishes on moistened filter paper for inoculation.

Pathogen culture, inoculation and analysis of necrotic spots

Botrytis cinerea inoculum was produced in three days on a medium made of potato dextrose agar (39 g L^{-1} Difco, Detroit, USA), in a growth chamber at 21 ˚C, with a 14 h/10 h photoperiod. *Phytophthora capsici* mycelium was grown over 8 days on V8 juice agar medium in a growth chamber at 22˚C, with a 12 h/12 h photoperiod. *Plasmopara viticola* inoculum was produced from sporangia derived from a susceptible cultivar of *Vitis vinifera*. The leaves were maintained under moist conditions overnight to induce maximal sporulation. Sporangia were recovered by soaking infected leaves in cold (4 ˚C) distilled water, and the dilution was adjusted to reach a concentration of 5×10^5 sporangia.ml^{-1} using a Malassez haemocytometer.

Leaves were inoculated by depositing either a mycelium plug of *Botrytis cinerea* on the middle of the leaf (lettuce and tomato), or a mycelium plug of *Phytophthora capsici* (pepper) (Fig 3). Grapevine leaves were inoculated by depositing a drop of 20 µl suspension of sporangia of *Plasmopara viticola* on the middle of the leaf (Fig 3).

The leaves were photographed from two to 12 days after inoculation, depending on the species, and the lesion areas were assessed with an image analysis software (ImageJ, US National Institutes of Health, Bethesda, MD, USA), as shown in Fig 3.

Statistical analysis

For each experiment, the Kruskal-Wallis non-parametric statistical test was applied ($n = 15$). The data were expressed as the means ± standard error, and statistical significance was set at $P < 0.05$. All statistical analyses were performed using XLSTAT software (Addinsoft, Deutschland, Andernach, Germany).

Results

Lesions areas increased over time in all control and treated plants, but single 60 s irradiations by UV-C light reduced them by 35% in tomatoes two days after inoculation with *Botrytis cinerea*, by 17% in lettuce three days after inoculation with *Botrytis cinerea*, and by 35% and 21% in peppers, three and four days, respectively, after inoculation with *Phytophthora capsici* (Fig 4). There was no significant or substantial reduction in lesion areas in tomatoes and in lettuce three days after inoculation. Single flashes of UV-C light reduced lesion areas by 41% and 17% in tomato, two and three days, respectively, after inoculation with *Botrytis cinerea*, by 42% and 35% in lettuce, two and three days, respectively, after inoculation by *Botrytis cinerea*, and by 39% and 37% in peppers, three and four days, respectively, after inoculation by *Phytophthora capsici* (Fig 4). For the three pathosystems, single flashes of UV-C light had a more pronounced effect than a single 60 s irradiation (Fig 4).

PI$_{abs}$ did not decrease as a consequence of UV-C light treatments (Table 2). PI$_{abs}$ in the 60 s treatment in tomatoes and in the 1 s treatment in lettuce and grapevines was even higher than in the control. Similarly, F$_v$/F$_m$ did not decrease as a consequence of UV-C light treatments (Table 2). On the contrary, F$_v$/F$_m$ was higher than in the control in the 60 s treatment in peppers, and in the 1 s treatment in lettuce, tomatoes and grapevines. F$_0$ was lower in the 1 s treatment in lettuce and grapevines than in the control, whereas S$_m$ was higher in the 1 s and the 60 s treatments in lettuce and grapevines than in the control. V$_k$/V$_j$ was not affected by any of the treatments.

When 1 s UV-C light treatments were repeated four times, with 48 h between exposures, lesion areas were reduced in tomatoes by 40% and 33% two and three days, respectively, after inoculation with *Botrytis cinerea* (Fig 5). There was also an effect of single flashes of UV-C light but a less marked one, since lesion areas were only reduced by 19% and 13%, two and three days, respectively, after inoculation with *Botrytis cinerea* (Fig 5). In lettuce, lesion areas
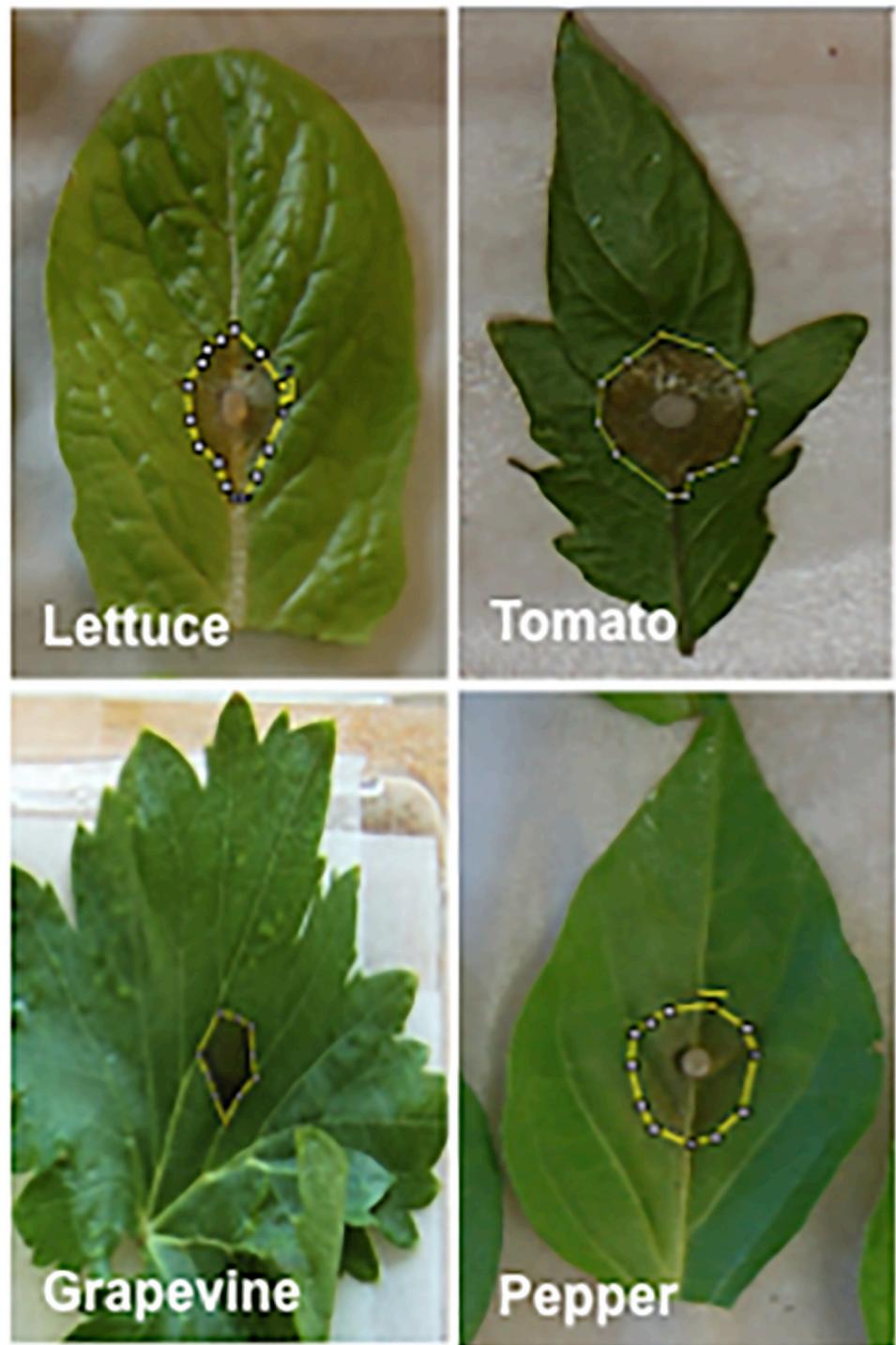
Fig 3. Position of mycelium plugs and typical lesion areas used for image analysis.

https://doi.org/10.1371/journal.pone.0235918.g003

were reduced by single flashes of UV-C light by 24% three days after inoculation with *Botrytis cinerea* (Fig 5). For plants treated four times, the reduction of lesion area was already significant two days after inoculation when compared to either untreated control plants or single

Fig 4. Effect of UV-C light at 1 or 60 s irradiations on plant defences. Leaves were exposed to a single dose of UV-C light of 1 kJ m^{-2}, for either 1 or 60 s. Two days after UV-C treatments, tomato and lettuce leaves were inoculated with *Botrytis cinerea*, and pepper leaves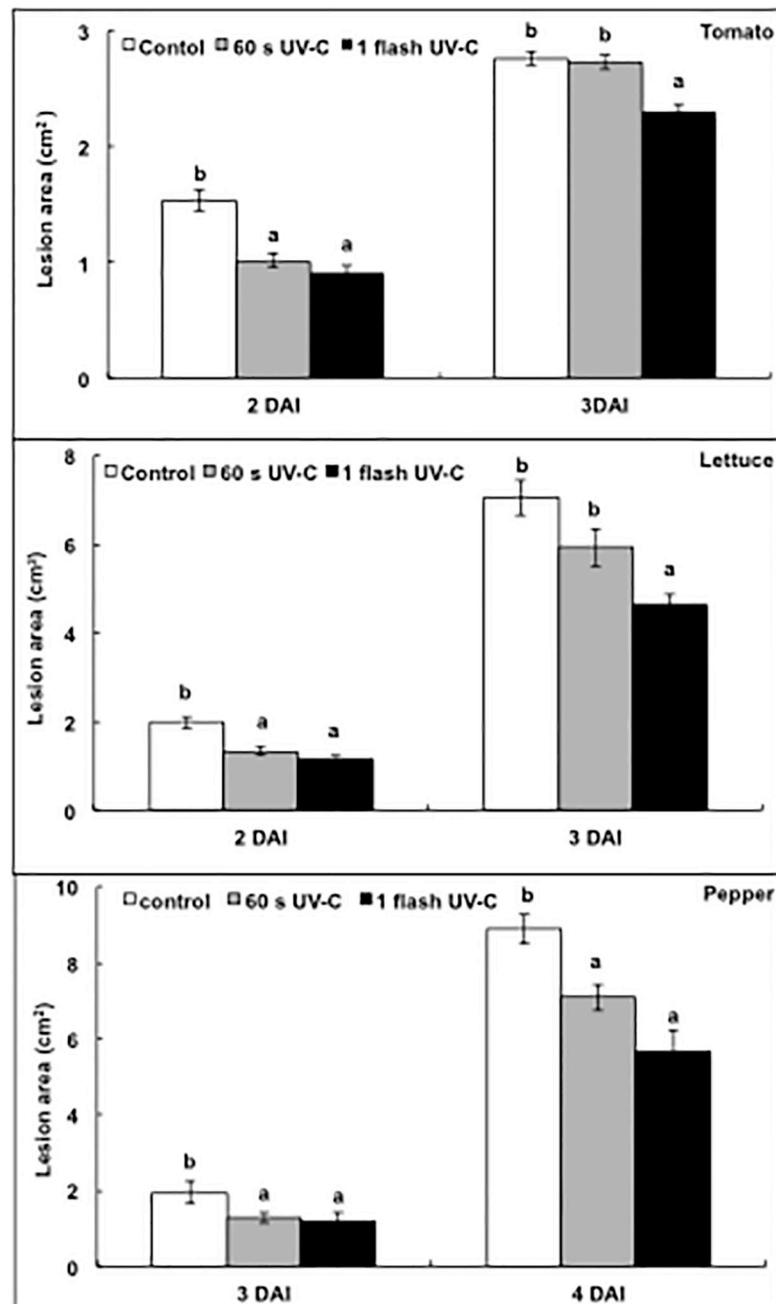 were inoculated with *Phytophthora capsici*. Lesion areas (cm^2) of tomato (A), lettuce (B) and pepper (C) leaves were measured. DAI stands for days after inoculation. The bars represent standard errors ($n = 15$). Different letters indicate significant differences according to the Kruskal-Wallis test (P < 0.05).

https://doi.org/10.1371/journal.pone.0235918.g004

treated plants. It reached 39% when compared to untreated control plants three days after inoculation. Repeated treatments by UV-C light were also tested against *Phytophthora capsici* in peppers and against *Plasmopara viticola* in grapevines. They resulted in a 70% and 48% reduction in lesion areas 7 and 8 days, respectively, after inoculation of peppers with

Table 2. Effect of UV-C irradiation on the Performance Index of Strasser [15] and on the parameters derived from induction curves of maximal ChlF that have been proposed to be damage indicators, including F_v/F_m [16]. Data represent means + SE. n = 18. Different letters for a given species and a given parameter correspond to differences significant at the 5% threshold.

	PI_{abs}	F_v/F_m	F_0	S_m	V_k/V_j
Lettuce control	2.59 ± 0.18 *a*	0.783 ± 0.004 *a*	5935 ± 158 *b*	23.3 ± 0.4 *a*	1518 ± 52 *a*
Lettuce 1 s	2.91 + 0.17 *b*	0.803 ± 0.003 *b*	5361 ± 71 *a*	24.9 ± 0.5 *b*	1473 ± 34 *a*
Lettuce 60 s	2.70 + 0.13 *ab*	0.793 ± 0.004 *ab*	5696 ± 100 *b*	25.9 ± 0.4 *b*	1470 ± 44 *a*
Tomato control	3.45 ± 0.40 *a*	0.803 ± 0.003 *a*	4798 ± 117 *a*	13.2 ± 0.3 *a*	1658 ± 51 *a*
Tomato 1 s	5.04 ± 0.74 *ab*	0.813 ± 0.004 *b*	5138 ± 161 *a*	13.9 ± 0.5 *a*	1775 ± 85 *a*
Tomato 60 s	5.53 ± 0.73 *b*	0.812 ± 0.003 *ab*	4789 ± 214 *a*	13.9 ± 0.5 *a*	1656 ± 108
Pepper control	3.20 ± 0.33 *a*	0.787 ± 0.004 *a*	6390 ± 144 *a*	16.9 ± 0.6 *a*	1786 ± 51 *a*
Pepper 1 s	2.59 ± 0.23 *a*	0.794 ± 0.004 *ab*	6289 ± 192 *a*	16.6 ± 0.5 *a*	1718 ± 59 *a*
Pepper 60 s	3.00 ± 0.15 *a*	0.799 ± 0.002 *b*	6119 ± 207 *a*	16.7 ± 0.4 *a*	1744 ± 51 *a*
Grapevine control	2.59 ± 0.18 *a*	0.783 ± 0.004 *a*	5935 ± 157 *b*	23.3 ± 0.4 *a*	1518 ± 51 *a*
Grapevine 1 s	2.90 ± 0.17 *b*	0.803 ± 0.003 *b*	5361 ± 70 *a*	24.8 ± 0.5 *b*	1472 + 34 *a*
Grapevine 60 s	2.67 ± 0.15 *ab*	0.793 ± 0.004 *ab*	5691 ± 100 *ab*	25.9 ± 0.4 *b*	1470 + 45 *a*

Phytophthora capsici (Fig 5). They also resulted in a 65% and 41% reduction in lesion areas 10 days and 12 days, respectively, after inoculation of grapevines with *Plasmopara viticola* (Fig 5). This confirmed the potential of repeated flashes of UV-C light to stimulate plant defences against several fungal plant diseases.

Lesion areas were not only reduced in leaves directly exposed to repeated UV-C light treatments, but also in nearby, non-exposed tomato, lettuce, pepper and grapevine leaves taken from the same treated plants (Fig 6). The effect was of similar magnitude in non-exposed tomato, lettuce and grapevine leaves as in the leaves exposed to UV-C treatments, but the effect
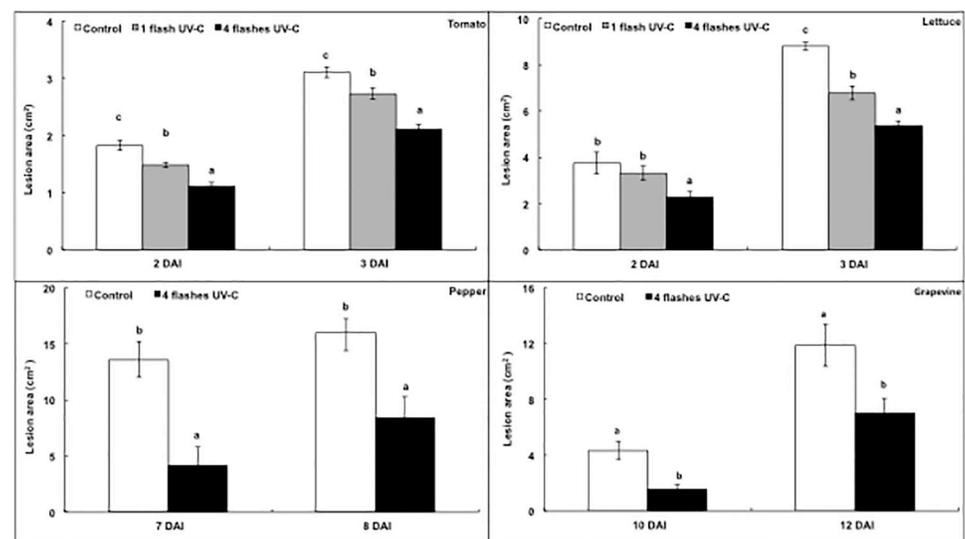

Fig 5. Effect of repeated flashes of UV-C light on plant defences. Tomato and lettuce leaves were exposed to flashes of UV-C light of 1 kW m^{-2} each, either one or four times. Pepper and grapevine leaves were exposed only to a dose of 1 kJ m^{-2}, repeated four times. Two days after UV-C treatments, tomato and lettuce leaves were inoculated with *Botrytis cinerea*, pepper leaves with *Phytophthora capsici* and grapevine leaves with *Plasmopara viticola*. Lesion areas (cm^2) of tomato (A), lettuce (B), pepper (C) and grapevine (D) leaves were measured. DAI stands for days after inoculation. The bars represent standard errors (n = 15). Different letters indicate significant differences according to the Kruskal-Wallis test (P < 0.05).
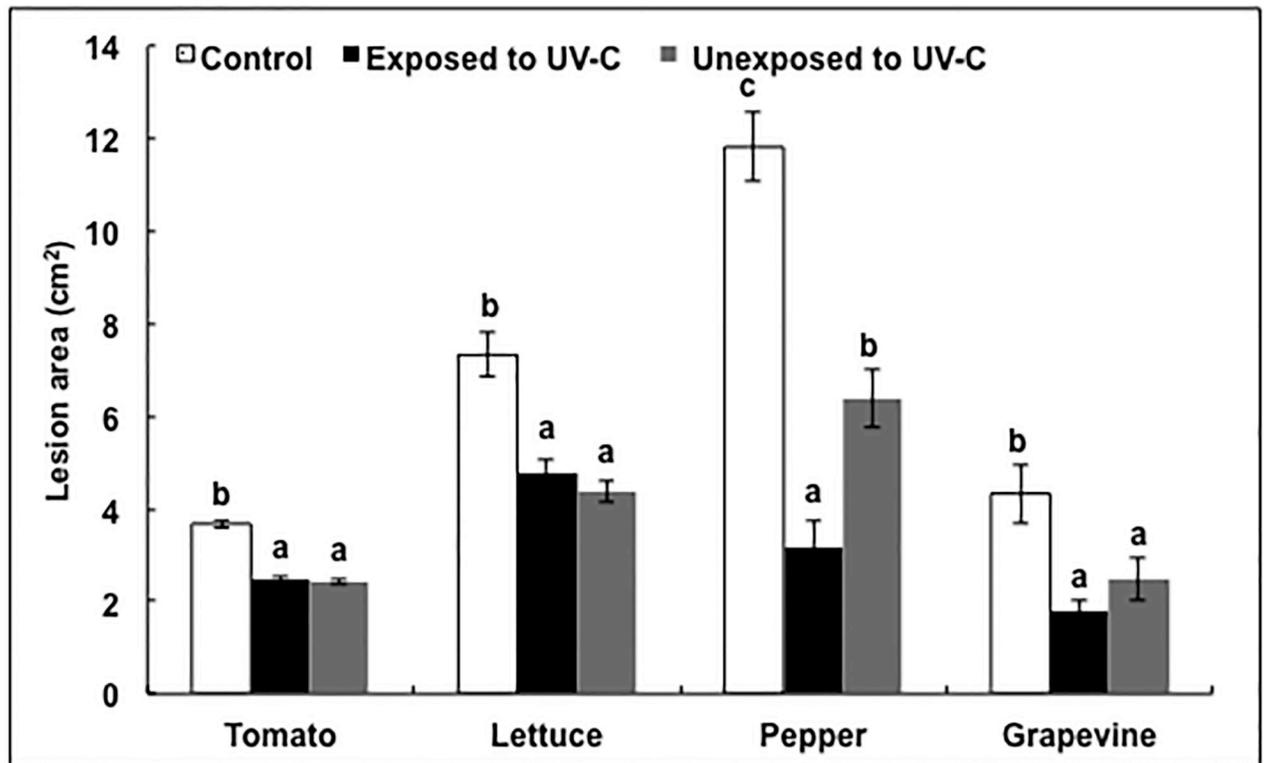
Fig 6. Effect of flashes of UV-C light repeated four times on defences of treated and of nearby untreated leaves. Tomato (A), lettuce (B), pepper (C) and grapevine (D) leaves were exposed to flashes of UV-C light of 1 kW m^{-2}, repeated four times. Two days after UV-C treatments, tomato and lettuce leaves were inoculated with *Botrytis cinerea*, pepper leaves with *Phytophthora capsici* and grapevine leaves with *Plasmopara viticola*. Lesion areas were measured on exposed leaves and on nearby unexposed leaves, after three days for tomatoes, lettuce and peppers, and after 10 days for grapevines. The bars represent standard errors ($n = 15$). Different letters indicate significant differences according to the Kruskal-Wallis test ($P < 0.05$).

was less pronounced in non-exposed pepper leaves than it was in the leaves exposed to UV-C treatments.

Discussion

UV-C light stimulates plant defences

Results obtained as part of this study confirm previous observations showing that UV-C light, either under the form of exposures of 60 s or of 1s (Fig 4), can stimulate plant defences [6–11]. See also Terry and Joyce for older references [17]. Not much is known about the mechanisms of perception and about the signalling and metabolic pathways triggered or stimulated by hormetic doses of UV-C light [2, 12]. It is reasonable to hypothesize that the high energy levels supplied by UV-C light are at the origin of the production of reactive oxygen species (ROS) by either the photosynthetic machinery in the chloroplasts, NADP(H) oxidase activity at the membrane level, xanthine oxidase activity in peroxisomes or NADP-malic enzyme activity in mitochondria [2]. Even though ROS are generally efficiently dealt with by the antioxidants, antioxidant enzymes and antioxidant systems existing in all compartments of the cell, they can be at the origin of oxidative signalling resulting in the triggering or the upregulation of signalling and metabolic pathways associated with the production of secondary defence compounds. In addition, direct lipid damage or peroxidation by ROS can be at the origin of linolenic acid

oxidation products, which serve as precursors in the synthesis of jasmonic acid, an hormone playing important roles in plant responses to biotic stressors [18]. In addition to the ROS hypothesis, photoreceptors could be involved, notably phototropin [19] or UVR8 [20]. The latter protein has a demonstrated role in UV-B perception and an action spectrum overlapping largely over the UV-C domain [21]. In addition, UV-C light was recently found to alleviate transcriptional gene silencing in *Arabidopsis* [22], an indicator that UV-C light has epigenetic effects. We should certainly pay more attention to the latter in the future considering that there is growing evidence for the role played by epigenetic mechanisms in the control of plant immunity [23, 24].

Flashes of UV-C light have a strong potential for stimulating plant defences

One second flashes of UV-C light stimulated plant defences at least as well, and in most cases even better than conventional irradiation exposures of 60 s (Fig 4). There is a strong consensus in the scientific community suggesting that doses (in $J\,m^{-2}$) matter in the observed effects of UV light, not the time of exposure. In other words, it is believed that the response of plants does not change if they are exposed over a short or a lengthy period of time, as long as the dose considered to be effective is delivered. This idea led to the concept of maximal acceptable doses, alias MAD [25]. In plant studies, much like in human health research, authors have also considered that doses matter, not the time of exposure. It has been repeatedly stated that the efficiency of UV-C light is dose-dependent and that a longer duration of low radiance (W m-2) has the same effect as a short but strong irradiation [26–28].

Taking a view opposing that of the scientific community we observed that for a given effective dose of UV-C light, the efficiency is higher when UV-C light is supplied as a flash of one second, rather than under the form of an irradiation of low intensity delivered over an extensive period of time (of 60 s) (Fig 4). The superiority for plant resistance of very short versus long periods of irradiation by UV-C light is intriguing, and suggests that mechanisms of light perception, signalling and metabolic pathways may be different when UV-C light is supplied as flashes instead of prolonged irradiations.

ChlF damage and performance indicators are not affected negatively by treatments

Substantially lower values of F_v/F_m are indicators of photodamage [29]. Similarly, higher values of F_0 suggest damage [30]. We did not observe any decrease in F_v/F_m nor any increase in F_0 as a consequence of UV-C light treatments (Table 2). Limitation/inactivation, possibly through damage to the oxygen-evolving complex (OEC), may be observed and assessed through the increase in V_k/V_j [31, 32]. We did not observe any increase in V_k/V_j either as a consequence of UV-C light treatments (Table 2). By contrast, a slight increase in S_m was observed in lettuce and grapevine as a consequence of the 1s and 60 s UV-C light treatments (Table 2). S_m is assumed to be proportional to the pool size of electron carriers, and decreases in S_m are suspected to be indicators of stress-associated damage [16, 33, 34]. On the other hand, PI_{abs} and F_v/F_m data do not support the view that UV-C light exerts negative effects on lettuce and grapevine plants at the doses considered in this study, notably 1 s treatments, since both parameters were increased compared to that of the control.

Flashes of UV-C light can be at the origin of systemic effects

Clearly, the systemic effect of flashes of UV-C light we observed (Fig 6) and the efficacy against several pathogens are consistent with what we know about systemic acquired resistance (SAR). Systemic immune responses, notably SAR, can be activated in plants in response to pathogen

infection [35–37], and they typically confer broad and long lasting resistance at the whole plant level, even against unrelated pathogens [35, 36, 38].

Several chemical inducers have been identified as being directly or indirectly involved in SAR, including salicylic acid and methyl salicylate [39, 40], jasmonic acid [41], azelaic acid [42], auxin [43], glycerol-3-phosphate [44], pipecolic acid [45], and dehydroabietinal [46]. So far the focus has mainly been on salicylic acid and jasmonic acid, which are still believed to be the key-players in SAR. The importance of their role is regularly confirmed even as our view of plant immunity evolves. For instance, it has been found that salicylic acid and jasmonic acid influence epigenetic responses [47], whereas there is also evidence for epigenetic control of the salicylic acid and the jasmonic acid pathways [48]. There have been many attempts to induce SAR by applications of chemical, biological or, more rarely, physical treatments. SAR can notably be induced by exogenous applications of benzo-(1,2,3)-thiadiazole-7-carbothioic acid (BTH), an analogue of salicylic acid [49, 50]. There are not many studies about the effects of UV-C light on SAR but UV-C light stimulated salicylic acid accumulation in tobacco leaves [51]. Therefore, future studies should test whether the stimulating effect on plant defences of flashes of UV-C light involves the salicylic acid pathway.

From a practical point of view, assessing the systemic effects of UV-C light treatments is needed to define the efficient size and position of lamps for crop treatments. Similarly, there is the need to investigate the role of repetition of UV-C light treatments over time, keeping in mind that the running cost of frequent crop treatments is probably not economically acceptable for farmers and growers. If salicylic acid and SAR are proven to be key players in the immunity conferred by flashes of UV-C light to plants, long-lasting effects are to be expected [38], which could represent an incentive for investigating the possibility of increasing the time between treatments.

Conclusions

Our observations clearly show that flashes of UV-C light of 1 s have the potential to drive plant defences, probably over a large range of crops and pathogens, opening the way for field and greenhouse treatments. Moreover, our observations show that flashes have the additional and unexpected effect of being more efficient than prolonged irradiations. We found evidence for systemic effects in the four species studied. At this stage it seems very tempting to treat crops with flashes of UV-C light. To develop flashes of UV-C light as a technique for field treatments, more studies are needed to better characterize systemic effects. The duration of resistance will also need to be assessed since it will determine the frequency of treatments. More fundamental research should be performed to unravel the mechanisms of perception of UV-C flashes and the signalling and metabolic pathways triggered or stimulated by them. It is indeed surprising that UV-C flashes of one second are more effective than 60 s irradiation, hinting at the existence of specific physiological responses.

Supporting information

S1 Data.
(XLSX)

S2 Data.
(XLSX)

S3 Data.
(XLSX)

S4 Data.
(XLSX)

S5 Data.
(XLSX)

S6 Data.
(XLSX)

S7 Data.
(XLSX)

S8 Data.
(XLSX)

Acknowledgments

Author Contributions

Conceptualization: Jawad Aarrouf, Laurent Urban.

Data curation: Jawad Aarrouf, Laurent Urban.

Formal analysis: Jawad Aarrouf, Laurent Urban.

Funding acquisition: Jawad Aarrouf, Laurent Urban.

Investigation: Jawad Aarrouf, Laurent Urban.

Methodology: Jawad Aarrouf, Laurent Urban.

Project administration: Jawad Aarrouf, Laurent Urban.

Resources: Jawad Aarrouf, Laurent Urban.

Supervision: Jawad Aarrouf, Laurent Urban.

Validation: Jawad Aarrouf, Laurent Urban.

Visualization: Jawad Aarrouf, Laurent Urban.

Writing – original draft: Jawad Aarrouf, Laurent Urban.

Writing – review & editing: Jawad Aarrouf, Laurent Urban.

References

1. ACTA. Fiche 24. Stimulateurs de défense des plantes (SDP), technique à l'étude. Guide pour la conception de systèmes de production fruitière économes en produits phytosanitaires. 2014. Pages 104–106.

2. Urban L, Chabane Sari D, Orsal B, Lopes M, Miranda R, Aarrouf J. UV-C light and pulsed light as alternatives to chemical and biological elicitors for stimulating plant natural defenses against fungal diseases. Sci Hort. 2018; 235:452–459.

3. Ballaré CL, Caldwell MM, Flint SD, Robinson SA, Bornman JF. Effects of solar ultraviolet radiation on terrestrial ecosystems Patterns mechanisms and interactions with climate change. Photochem Photobiol Sci. 2011; 10:226–241. https://doi.org/10.1039/c0pp90035d PMID: 21253661

4. Demkura PV, Ballaré CL. UVR8 mediates UV-B-induced Arabidopsis defense responses against Botrytis cinerea by controlling sinapate accumulation Mol Plant 2012; 5:642–652. https://doi.org/10.1093/mp/sss025 PMID: 22447155

5. Holley SR, Yalamanchili RD, Moura DS, Ryan CA, Stratmann JW. Convergence of signaling pathways induced by systemin, oligosaccharide elicitors, and ultraviolet-B radiation at the level of mitogen-activated protein kinases in Lycopersicon peruvianum suspension-cultured cells. Plant Physiol 2003; 132:1728–1738. https://doi.org/10.1104/pp.103.024414 PMID: 12913131

6. Charles MT, Benhamou N, Arul J. Physiological basis of UV-C induced resistance to Botrytis cinerea in tomato fruit: III Ultrastructural modifications and their impact on fungal colonization. Postharvest Biol Technol. 2008; 47:27–40.

7. Forges M, Vàsquez H, Charles F, Chabane Sari D, Urban L, Lizzi Y et al. Impact of UV-C radiation on the sensitivity of three strawberry plant cultivars (Fragaria x ananassa) against Botrytis cinerea. Sci Hort. 2018; 240:603–613.

8. Ouhibi C, Attia H, Nicot P, Urban L, Lachaâl M, Aarrouf J. Effect of UV-C Radiation on Resistance of Romaine Lettuce (Lactuca sativa L) Against Botrytis cinerea and Sclerotinia minor. J Phytopath. 2015a; 163:578–582.

9. Ouhibi C, Attia H, Nicot P, Lecompte F, Vidal V, Lachaâl M et al. Effects of nitrogen supply and of UV-C irradiation on the susceptibility of Lactuca sativa L to Botrytis cinerea and Sclerotinia minor. Plant Soil 2015b; 393:35–46.

10. Vàsquez H, Ouhibi C, Lizzi Y, Azzouz N, Forges M, Bardin M et al. Pre-harvest hormetic doses of UV-C radiation can decrease susceptibility of lettuce leaves (Lactuca sativa L) to Botrytis cinerea L. Sci Hortic. 2017; 222:32–39.

11. Windram O, Madhou P, McHattie S, Hill C, Hickman R, Cooke E, et al. Arabidopsis defense against Botrytis cinerea: chronology and regulation deciphered by high-resolution temporal transcriptomic analysis The Plant Cell 2012; 24:3530–3557. https://doi.org/10.1105/tpc.112.102046 PMID: 23023172

12. Urban L, Charles F, Miranda M, Aarrouf J. Understanding the physiological effects of UV-C light and exploiting its agronomic potential before and after harvest. Plant Physiol Biochem. 2016; 105:1–11. https://doi.org/10.1016/j.plaphy.2016.04.004 PMID: 27064192

13. Chabane Sari D, Urban L, Aarrouf J, Orsal B. Dispositif d'émission lumineuse à LEDs émettant dans le domaine des UVC. Patent claim registered on May 12th 2017 under the number FR1754163

14. Strasser RJ, Srivastava A. Polyphasic chlorophyll a fluorescence transient in plants and cyanobacteria. Photochemistry and Photobiology, 1995; 61, 32–42.

15. Strasser RJ, Tsimilli-Michael M, Srivastava A. Analysis of the Chlorophyll a Fluorescence Transient, in Chlorophyll a Fluorescence, Advances in Photosynthesis and Respiration, vol 19, eds Papageorgiou G. and Govindjee (Dordrecht, Springer Netherlands), 2004; 321–362.

16. Ripoll J, Bertin N, Bidel LPR, Urban L. A User's View of the Parameters Derived from the Induction Curves of Maximal Chlorophyll a Fluorescence: Perspectives for Analyzing Stress. Frontiers in plant science. 2016; 7: 1679. https://doi.org/10.3389/fpls.2016.01679 PMID: 27891137

17. Terry LA, Joyce DC. Elicitors of induced disease resistance in postharvest horticultural crops: a brief review. Posthar Biol Technol. 2004; 32:1–13.

18. Browse J. Jasmonate passes muster: a receptor and targets for the defense hormone. Ann Rev Plant Biol. 2009; 60:183–205.

19. Mageroy MH, Kowalik EH, Folta KM, Shinkle J. Evidence of physiological phototropin1 (phot1) action in response to UV-C illumination. Plant Signal Behav. 2010; 5:1204–1210. https://doi.org/10.4161/psb.5.10.12413 PMID: 20861684

20. Favory JJ, Stec A, Gruber H, Rizzini L, Oravecz A, Funk M et al. Interaction of COP1 and UVR8 regulates UV-B induced photomorphogenesis and stress acclimation in Arabidopsis. EMBO J. 2009; 28:591–601. https://doi.org/10.1038/emboj.2009.4 PMID: 19165148

21. Jiang L, Wang Y, Björn LO He J-X Li S. Sensing of UV-B radiation by plants. Plant Signal Behav. 2012; 7:999–1003. https://doi.org/10.4161/psb.20815 PMID: 22751358

22. Xu W, Wang T, Xu S, Li F, Deng C, Wu L et al. UV-C-Induced alleviation of transcriptional gene silencing through plant–plant communication: Key roles of jasmonic acid and salicylic acid pathways. Mutation Res/Fund Mol Mech Mutagen. 2016; 790:56–67.

23. Conrath U, Beckers GJ, Langenbach CJ, Jaskiewicz MR. Priming for enhanced defense Ann Rev Phytopath. 2015; 53.

24. Ramirez-Prado JS, Abulfaraj AA, Rayapuram N, Benhamed M, Hirt H. Plant immunity: from signaling to epigenetic control of defense. Trends Plant Sci. 2018; 23:833–844. https://doi.org/10.1016/j.tplants.2018.06.004 PMID: 29970339

25. Söderberg PG, Michael R, Merriam JC. Maximum acceptable dose of ultraviolet radiation: a safety limit for cataract. Acta Ophthalmol. 2003; 81:165–169.

26. Renzel A. Desinfektion mit UV-C Produktinformation uv-technik meyer gmbh URL: http://wwwuv-technikde/uvc-anwendunghtml%20Zugriff%20am%202422015%202013

27. Gayán E, Mañas P, Álvarez I, Condón S. Mechanism of the Synergistic Inactivation of Escherichia coli by UV-C Light at Mild Temperatures. Applied Environ Microbiol. 2013; 79:4465–4473.

28. Klärner S, Flemming B, Berkelmann-Löhnertz B. Studies on mould prevention in viticulture by means of UV-C application of vines (Vitis vinifera L). Landtechnik—Agric Engin. 2015; 70:139–148.

29. Adams WW, Zarter CR, Mueh KE, Amiard V, Demmig-Adams B. Energy dissipation and photoinhibition: A continuum of photoprotection, in: Photoprotection, Photoinhibition, Gene Regulation, and Environment. Advances in Photosynthesis and Respiration, vol. 22, eds Demmig-Adams B., Adams W.W III. and Mattoo A.K., (Dordrech: Springer). 2006; 49–64.

30. Wingler A, Marès M, Pourtau N. Spatial patterns and metabolic regulation of photosynthetic parameters during leaf senescence. New Phytologist. 2004; 161, 781–789.

31. Srivastava A, Guissé B, Greppin H, Strasser RJ. Regulation of antenna structure and electron transport in Photosystem II of Pisum sativum under elevated temperature probed by the fast polyphasic chlorophyll a fluorescence transient: OKJIP. Biochimica et Biophysica Acta (BBA)-Bioenergetics. 1997; 1320, 95–106. 32.

32. Kalachanis D, Manetas Y. Analysis of fast chlorophyll fluorescence rise (O-K-J-I-P) curves in green fruits indicates electron flow limitations at the donor side of PSII and the acceptor sides of both photosystems. Physiologia plantarum. 2010; 139, 313–323. https://doi.org/10.1111/j.1399-3054.2010.01362.x PMID: 20149129

33. Christen D, Schönmann S, Jermini M, Strasser RJ, Défago G. Characterization and early detection of grapevine (Vitis vinifera) stress responses to esca disease by in situ chlorophyll fluorescence and comparison with drought stress. Environmental and Experimental Botany. 2007; 60, 504–514.

34. Yordanov I, Goltsev V, Stefanov D, Chernev P, Zaharieva I, Kirova M, et al. Preservation of photosynthetic electron transport from senescence-induced inactivation in primary leaves after decapitation and defoliation of bean plants. Journal of Plant Physiology. 2008; 165, 1954–1963. https://doi.org/10.1016/j.jplph.2008.05.003 PMID: 18586352

35. Gao QM, Kachroo A, Kachroo P. Chemical inducers of systemic immunity in plants J Exp Bot. 2014; 65:1849–1855. https://doi.org/10.1093/jxb/eru010 PMID: 24591049

36. Gao QM, Zhu S, Kachroo P, Kachroo A. Signal regulators of systemic acquired resistance. Frontiers Plant Sci. 2015; 6:228.

37. Kachroo A, Robin GP. Systemic signaling during plant defense. Curr Opin Plant Biol. 2013; 16:527–533. https://doi.org/10.1016/j.pbi.2013.06.019 PMID: 23870750

38. Shine MB, Xiao X, Kachroo P, Kachroo A. Signaling mechanisms underlying systemic acquired resistance to microbial pathogens. Plant Sci. 2019; 279:81–86. https://doi.org/10.1016/j.plantsci.2018.01.001 PMID: 30709496

39. Durrant WE, Dong X. Systemic acquired resistance Ann Rev Phytopath. 2004; 42:185–209.

40. Park SW, Kaimoyo E, Kumar D, Mosher S, Klessig DF. Methyl salicylate is a critical mobile signal for plant systemic acquired resistance. Sci. 2007; 318:113–116.

41. Truman W, Bennett MH, Kubigsteltig I, Turnbull C Grant M. Arabidopsis systemic immunity uses conserved defense signaling pathways and is mediated by jasmonates. Proc Nat Acad Sci. 2007; 104:1075–1080. https://doi.org/10.1073/pnas.0605423104 PMID: 17215350

42. Jung HW, Tschaplinski TJ, Wang L, Glazebrook J, Greenberg JT. Priming in systemic plant immunity. Sci. 2009; 324:89–91.

43. Truman WM, Bennett MH, Turnbull CG, Grant MR. Arabidopsis auxin mutants are compromised in systemic acquired resistance and exhibit aberrant accumulation of various indolic compounds Plant Physiol. 2010; 152:1562–1573. https://doi.org/10.1104/pp.109.152173 PMID: 20081042

44. Chanda B, Xia Y, Mandal MK, Yu K, Sekine KT, Gao QM, et al. Glycerol-3-phosphate is a critical mobile inducer of systemic immunity in plants. Nature Gen. 2011; 43:421.

45. Návarová H, Bernsdorff F, Döring AC, Zeier J. Pipecolic acid an endogenous mediator of defense amplification and priming is a critical regulator of inducible plant immunity. Plant Cell 2012; 24:5123–5141. https://doi.org/10.1105/tpc.112.103564 PMID: 23221596

46. Chaturvedi R, Venables B, Petros RA, Nalam V, Li M, Wang X, et al. An abietane diterpenoid is a potent activator of systemic acquired resistance. Plant J. 2012; 71:161–172. https://doi.org/10.1111/j.1365-313X.2012.04981.x PMID: 22385469

47. Latzel V, Zhang Y, Karlsson Moritz K, Fischer M, Bossdorf O. Epigenetic variation in plant responses to defence hormones. Ann Bot. 2012; 110:1423–1428. https://doi.org/10.1093/aob/mcs088 PMID: 22543179

48. Alvarez E, Nota F, Cambiagno DA. Epigenetic control of plant immunity. Mol Plant Pathol. 2010; 11:563–576. https://doi.org/10.1111/j.1364-3703.2010.00621.x PMID: 20618712

49. Friedrich L, Lawton K, Ruess W, Masner P, Specker N, Rella MG, et al. A benzothiadiazole derivative induces systemic acquired resistance in tobacco. Plant J. 1996; 10:61–70.

50. Anand A, Uppalapati SR, Ryu CM, Allen SN, Kang L, Tang Y, Mysore KS. Salicylic acid and systemic acquired resistance play a role in attenuating crown gall disease caused by Agrobacterium tumefaciens. Plant Physiol. 2008; 146:703–715. https://doi.org/10.1104/pp.107.111302 PMID: 18156296

51. Yalpani N, Enyedi AJ, León J, Raskin I. Ultraviolet light and ozone stimulate accumulation of salicylic acid pathogenesis-related proteins and virus resistance in tobacco Planta 1994; 193:372–376.