LANDTECHNIK 70(4), 2015, 139–148
DOI:10.15150/lt.2015.2669

LANDTECHNIK
AGRICULTURAL ENGINEERING

Studies on mould prevention in viticulture by means of UV-C application of vines (*Vitis vinifera* L.)

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From an economic perspective, the regulation of grey mould (*Botrytis cinerea*) in viticulture is of great importance, since bunch rot leads to a decrease in terms of must quality and harvest quantity. On two sites, the application of UV-C light, a new approach to control *B. cinerea*, was performed. With a UV-C dose of 160 mWs/cm² canopies and cluster zones were treated on both sides several times. The results have shown that UV-C applications used as a complementary treatment to fungicide applications could significantly reduce the disease severity of *B. cinerea* (up to 82 %). Accordingly, this method is suitable to improve the phytosanitary status in the cluster zone, whereby the ripening of the berries can be extended and consequently the grape quality can be optimised.

Keywords
UV-C treatment, crop protection, *Botrytis cinerea*, viticulture

Controlling fungal pathogens in viticulture practice is an important element for the quality oriented grape production. The use of pesticides in viticulture ranks among the highest along with hops and apple. Indeed, fungicides in viticulture have the highest treatment index (Rossberg 2013). Most of the fungicides applied are used to control *Plasmopara viticola* (downy mildew), *Erysiphe necator* (powdery mildew) and *Botrytis cinerea*, the causal agent of grey mould, since they may lead to considerable yield losses (Mohr 2012).

Although in viticulture intensive control measures are practised, it emerged a deterioration in the grape quality in recent years resulting often in an early harvest, which in turn prevents complete maturation of berries and hence affects storage of key ingredients in the berries. In case of advanced infection the postponement of harvest is difficult. Therefore, the harvest is no longer determined by the ripeness of the berries, but by their health status. The main problems regarding must quality and harvest quantity are caused by *Botrytis cinerea* and acetic acid bacteria. In many places, it is therefore spoken of a race between bunch rot and maturity (Lawnik et al. 2014). The factors responsible for this are the altered temperature and precipitation as a result of climate change (Potsdam Institute for Climate Impact Research 2014).

Based on the aforementioned conditions and challenges, chemical treatments with excellent application quality are important. Beside treatments with botryticides, indirect measures play an important role for maintaining the grape health status. These include specific pruning methods for canopy design, defoliation, achieving a loose cluster structure and a moderate fertilization (Molitor et al. 2011, Baus and Berkelmann-Löhnertz 2014). These interventions are to impair the development conditions for micro-organisms and thus allow a longer ripening time for grapes. Nevertheless, the

received 7 January 2015| accepted 18 May 2015| published 14 August 2015

past years have shown that the combination of scheduled plant protection measures, well aerated canopy, moderate soil management and intervention in the grape architecture were not always suitable to maintain a longer maturity period. In particular, in the years 2006, 2010, 2011 and 2013 a strong bunch rot epidemic of more than 60 % disease severity was observed (Molitor et al. 2014). Moreover, the extreme increase in population density of the spotted-wing drosophila *Drosophila suzukii* (Cini et al. 2014) and the mycotoxin problem due to additional occurrence of secondary fungi (*Penicillium* sp. and *Aspergillus* spp.) (Walter 2012) urges the development of new methods to improve the health status of grapes.

In this project, it was investigated whether a repeated application of UV-C light can complement the currently used measures to control *Botrytis*. Unlike chemical pesticides, irradiation with UV-C leaves no residues so that UV-C treatments can be carried out in the critical time period between the last chemical treatment and harvest.

The UV-C irradiation is based on treating (micro-) organisms with UV-C light. Various UV-C applications have mainly been used in the food industry for many years, where it has been applied for surface disinfection, treatment of drinking water and air disinfection (Koutchma 2014, Kirschbaum 2014). The treatment of plant surfaces for reduction of pathogens is much more difficult due to the potential risk of phytotoxic reactions (Klärner et al. 2013). In greenhouse cultivation, UV-C radiation has been used successfully in some crops in order to reduce the population density of various pathogens. The treatment of scion and rootstock with UV-C during propagation and the irradiation of harvested grapes as a post-harvest approach – both with the objective of reducing infestation levels of *Botrytis* – represent other applications of this technology in the crop production sector (Farkas and Kocsis 2013, Romanazzi et al. 2012). For this reason, it was encouraging to use UV-C irradiation to reduce fungal infection on grapevine in the field.

The most effective wavelength to be used against micro-organisms lies between 240 and 270 nm (Renzel 2013). The main spectrum of the amalgam lamp used in this experiment is 254 nm. The efficiency of UV-C is dose-dependent, e.g. a longer duration of low irradiation, for example 10 s at 8 mW/cm (= 80 mWs/cm^2) has the same effect as a short but strong irradiation of 2 s at 40 mW/cm (= 80 mWs/cm^2) (Renzel 2013, Gayán et al. 2013).

The aim of the present study was to maintain the grape health status by using UV-C technology that allows an extension of the maturity period of the berries, even under unfavourable weather conditions. The field trial was carried out under standardised test conditions in the vineyards of Hochschule Geisenheim University (Germany). In addition, it was examined whether these strategies are also suitable under practical conditions (in a pilot estate) to produce healthy and mature grapes.

Material and methods

Two UV-C prototypes were used for this experiment, which were developed as part of a project funded by Hessen ModellProjekte. Since there are no data or previous experiences with the UV-C technology in viticulture, several departments of Hochschule Geisenheim University worked in collaboration with the company uv-technik meyer gmbh (Germany), a market leader for UV irradiation systems in the food industry, to develop a device suitable for practical vineyard conditions.

Figure 1: Prototype UV-C 3 (photo: W. Schönbach)

UV-C prototype 3

To gain experience with the UV-C methods in the field, a prototype based on existing elements was developed (Figure 1). This prototype UV-C 3 was assembled on the basic framework of a mounted air blast sprayer. It was built on the framework of a three-point plant protection equipment with radial fan, normally used for pesticide application. A hydraulic framework that is vertically and horizontally adjustable has been set up. The two UV-C modules are opposing each other, where both can irradiate both sides of the canopy simultaneously. The distance between the UV-C modules can be varied between 0 and 1000 mm and was set at about 100 mm from the canopy surface during application. The air generated by the air blower causes turbulences in the canopy that increases the percentage of surfaces treated with UV-C irradiation and thus reduces shading. To meet the needs of a UV-C application in a randomized trial system, the modules are equipped with air pressure controlled movable slats enabling the UV-C radiation units to be shielded as required. The closed slats prevent irradiation of those canopy areas that should not be treated.

Inside the modules multiple high power amalgam lamps with a length of 0.95 m are installed. The total electrical power consumption of a module is about 1.3 kW. The energy is provided by an engine-driven power generator. In the experiments shown here, the UV-C radiation doses were at 160 mWs/cm^2 per canopy side. This dose can be adjusted through a defined driving speed by cruise control tractor Fendt Vario 211 V where the speed is constant ± 0.1 km/h. In order to achieve the desired dose of UV-C, the driving speed operating this prototype was less than 1 km/h.

Prototype UV-C 4

In order to increase the UV-C performance and to improve the work efficiency, a new prototype was designed (Figure 2). This prototype UV-C 4 includes in addition to the UV-C modules a narrow trailed air blast sprayer with mounted radial fan, which can be towed by a common vineyard tractor. For this, only the base frame and the fan are required, while container, pump, etc. can be dismantled. The prototype UV-C 4 is equipped with two UV-C modules per side. This design allows the simultaneous treatment of two rows of vines. To adapt to the particular row width and height, the modules are hydraulically adjustable laterally and vertically. The power supply, an engine-driven generator, and the electronics for controlling the modules are housed in four rack systems on the trailer. Each of the modules is equipped with mercury-vapour lamps with a radiation length of 1.5 m. The total electrical power per module is 3.8 kW.

Figure 2: Prototype UV-C 4 (photo: W. Schönbach)

The rows of grapevines are treated at a defined speed, the same as Fendt Vario in UV-C 3, so that the desired UV-C dose (160 mWs/cm² per canopy side) can be exactly obtained. The driving speed to operate this prototype is five times higher than its predecessor but is still below the desired target velocity that is at least 5 km/h. To protect the UV-C lamps of the radiator from the "rough" use in the field, the modules are secured by double bars against mechanical damage.

UV-C applications at the experimental vineyard (location "Kellersgrube ") with UV-C 3 prototype

The field trial in the vineyard "Kellersgrube" was performed according to GEP standard (GEP guidelines 2013). The vineyard belongs to Hochschule Geisenheim University (49°58'59.4''N 7°56'51.1''E) and is planted with cv. Riesling. The distance between plant rows is 2 m, while the distance between plants within a row is 1.30 m. The employed trellis system is VSP (vertical shoot position). Every second lane was covered with green lawn. Ten rows were available for this experiment. Each variant consisted of four blocks, which were placed randomly within the field. Twelve vines added up to one replication. In order to better reach the grapes during the UV-C irradiation, the grapes zone was defoliated at the time point BBCH 75 (Lorenz et al. 1994). For this purpose, all lateral shoots were removed within the grape zone by hand and if necessary additional leaf per grape-bearing twig is left. For better comparison, this measure was performed in all variants. Table 1 shows the trial variants in the vineyard “Kellersgrube”.

Table 1: Treatments (short title und details) of UV-C experiment in the vineyard „Kellersgrube“ in cv. Riesling in 2014; V = variant; BBCH = phenological growth stage (according to Lorenz et al. 1994)

Variant No	Variant	Description
V-1	control (= untreated disease control)	Unhindered development of grey mould (*B. cinerea*) without any phytosanitary measure. Cover of the plot against other fungal pathogens using pesticides without side effect on *B. cinerea*.
V-2	integrated standard	Comparison with conventional treatment according to integrated grapevine protection. Seven chemical crop protection applications against all pathogenic fungi in the growing season, two of them were botryticides (BBCH 77, BBCH 81).
V-3	integrated reduced	Comparison with a reduced treatment strategy. Three chemical applications against all pathogenic fungi at critical stages of development (end of flowering, bunch closure, final treatment), two of them were botryticides (BBCH 77, BBCH 81).
V-4	integrated reduced + UV-C (alternate) + 2 botryticides	Three applications involved pesticides as in V-3 and in between four applications with UV-C against all pathogenic fungi.
V-5	integrated reduced + UV-C (alternate) + 1 botryticide	Three applications involve pesticides as in V-3 but with only one botryticide at bunch closure (BBCH 77) and in between four applications with UV-C against all pathogenic fungi.
V-6	UV-C solo until final treatment	Treatment of grey mould (*B. cinerea*) with UV-C irradiation at the developmental time points: at the end of flowering, bunch closure and final treatment. Other pathogens were treated with pesticides without side effects on B. cinerea.
V-7	UV-C solo after final treatment	Treatments as in V-6 but instead of UV-C post-bloom treatment two additional UV-C treatments in the period between final treatment and grape harvest

For each replicate 100 grapes were used to assess disease severity by *B. cinerea* (%) so that each treatment had a total of 400 grapes. The results presented here are from the last disease assessment on 1 Oct 2014. On 7 Oct 2014 the grapes were harvested.

UV-C applications at the pilot estate vineyard (location “Scharlachwiese”) with UV-C 4 prototype

The pilot estate vineyard “Scharlachwiese” is located in Geisenheim (49°59’49.4’’N 7°59’05.1’’E) and is also planted with cv. Riesling. The distance between plant rows is 1.8 m, while the distance between plants within a row is 1.2 m. The employed trellis system is VSP. Moderate mechanical defoliation of the canopy was done with a leaf remover (Fa. Binger Seilzug, Germany) at BBCH 73. Each second lane was permanently covered with a grass mixture; the intervening rows were planted with a clover mixture during the growing season. For this experiment there were 24 rows available. Since the vines under study could not be randomised within the row due to the use of the prototype UV-C 4, each four adjacent rows comprised a different treatment. To avoid potential pesticide drift from neighbouring treatments, only the two inner rows were used for assessment. These two inner rows were divided into four blocks each with 20 vines. Table 2 shows the different treatments in the vineyard “Scharlachwiese". For economic and epidemiological reasons an untreated control plot was omitted from the pilot operation.

To assess disease severity (%) by *B. cinerea,* 100 grapes from each replicate were used so that each treatment had a total of 400 grapes. The results presented here are from the last disease assessment on 30 Sept 2014. On 1 Oct 2014 the grapes were harvested.

Table 2: Treatments (short title und details) of UV-C experiment in the vineyard „Scharlachwiese" in cv. Riesling in 2014; V = variant; BBCH = phenological growth stage (according to Lorenz et al. 1994)

Variant No	Variant	Description
V-1	integrated standard (2 botryticides)	Control with usual pest control strategy according to integrated grapevine protection protocols. During the growing season, eight chemical treatments against all harmful fungi, two including botryticides were applied.
V-2	UV-C block until final treatment (2 botryticides)	Five chemical applications against all harmful fungi until BBCH 75 and another final treatment, two of them with botryticides. The three UV-C treatments were applied between bunch closure (BBCH 77) and final treatment (BBCH 81).
V-3	UV-C-block before and after final treatment (1 botryticide)	Six chemical applications and three UV-C treatments as in V-2. Here, however, only one botryticide treatment at BBCH 75 and three supplementary UV-C treatments between final treatment and grape harvest.
V-4	UV-C block after final treatment (1 botryticide)	Eight chemical applications as in V-1; however, with only one botryticide treatment during bunch closure (BBCH 77). The three UV-C applications were between the final treatment and grape harvest.
V-5	UV-C block after berries begin to touch (1 botryticide)	Six chemical applications until bunch closure, one of which with botryticide at bunch closure (BBCH 77). Between bunch closure and grape harvest five UV-C treatments were applied.

The weather conditions from the beginning of the growing season until the summer were generally characterised by drought, which kept the disease pressure low. However, in the maturity phase of the grapes a warm and humid weather with partly heavy rain dominated, which led to a rapid and strong spread of *B. cinerea.* Due to these weather conditions and the resulting severe bunch rot epidemic the harvest had to be conducted very quickly and earlier as scheduled.

Results

Biological efficacy against *B. cinerea* in definite field experiment (site "Kellersgrube")

There were significant differences in the efficiency between the different treatments against *B. cinerea* (Figure 3). The control plot V-1 (untreated control) had an infestation rate of 23 %. With the exception of the treatments V-5 and V-6 (integrated reduced + UV-C (alternate) with only one botryticide and UV-C solo until final treatment) all the other treatments showed significantly less infection with *Botrytis.* This effect was particularly pronounced in variant V-4, where the infection was < 5 %.

There were no significant differences between the standard treatments V-2 and V-3. The large discrepancy between the results of the treatments V-4 and V-5 shows the potency of modern botrycides used here. Surprisingly, treatment V-6 (UV-C solo until final treatment; no botryticide) exhibited a significantly worse outcome compared to the untreated control. In this variant, UV-C treatments already started at the end of bloom. In contrast, treatment V-7 – also UV-C solo, but with subsequent irradiation doses until shortly before harvest – showed a slight difference compared to the control plot V-1.

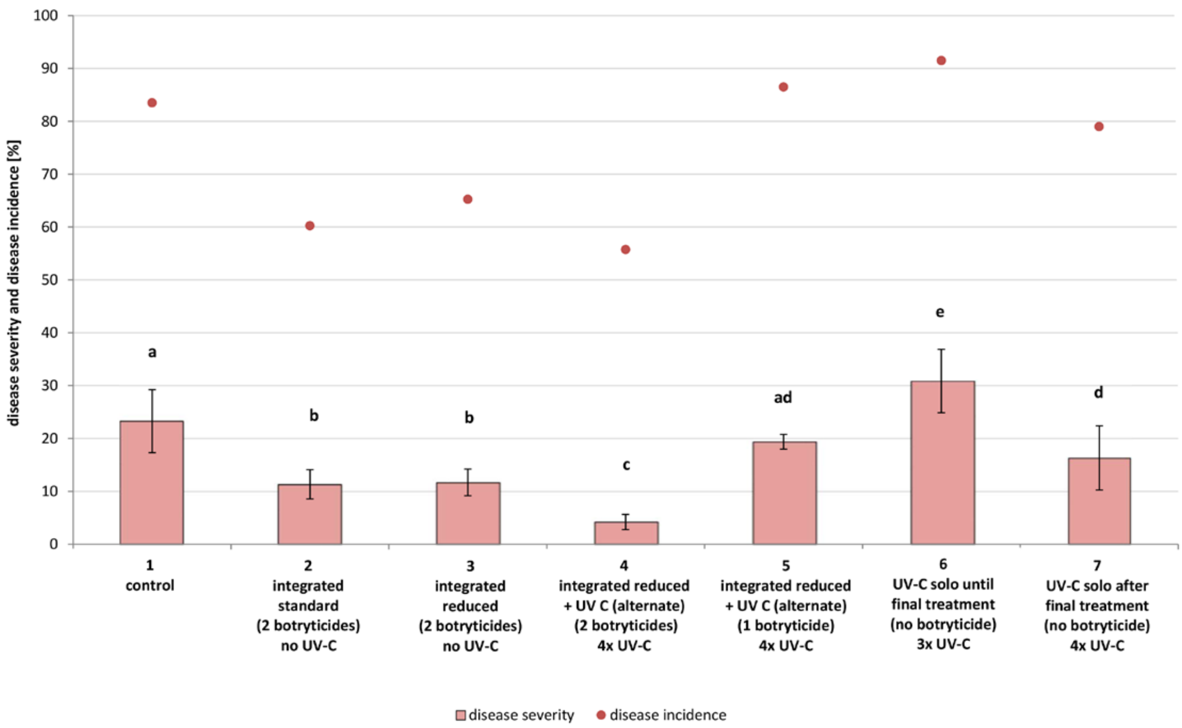

Figure 3: Biological efficacy against *Botrytis cinerea* (causal agent of grey mould) on grapes under different fungicide treatments and irradiation with UV-C under experimental field conditions in cv. Riesling in 2014. Disease assessment on 1 Oct 2014; experimental site: vineyard "Kellersgrube" of Hochschule Geisenheim University. Statistical analysis: Duncan´s test, values with the same letter are not significantly different ($p < 0.05$); n = 400, mean ± SD.

The values of disease incidence of the treatments compared to the control showed a similar pattern as that of disease severity. The relation between the values of disease incidence of treatments was similar to the relations of disease severity to each other.

Biological efficacy against *B. cinerea* under practical conditions (site "Scharlachwiese")

In the pilot study, in which the chemical treatments were applied according to practical conditions, the results showed that the grapes in the treatment where pesticides were used as recommended for integrated pest control (V-1, integrated standard) had a relatively high *Botrytis* infestation rate (31 %) despite intensive pesticide treatment (Figure 4). By using UV-C irradiation as complementary method in the treatments V-2, V-3 and V-5, a significant reduction of *B. cinerea* compared to the usual method (V-1; integrated standard) could be achieved, regardless of the number of applications with botryticides. Between treatments V-2 and V-3 there was no significant difference. Therefore, under current experimental conditions the timing of the UV-C treatments seemed to play an important role: should botryticides have been used, additional UV-C applications after the final chemical treatment would result in comparable biological efficacy as with two botryticide applications. However, three additional UV-C applications were needed to achieve this result, i.e. 6 x UV-C compared to 3 x UV-C. Furthermore, the results indicate that there is no efficacy of only one botryticide application against *B. cinerea* in combination with three UV-C treatments (V-4).

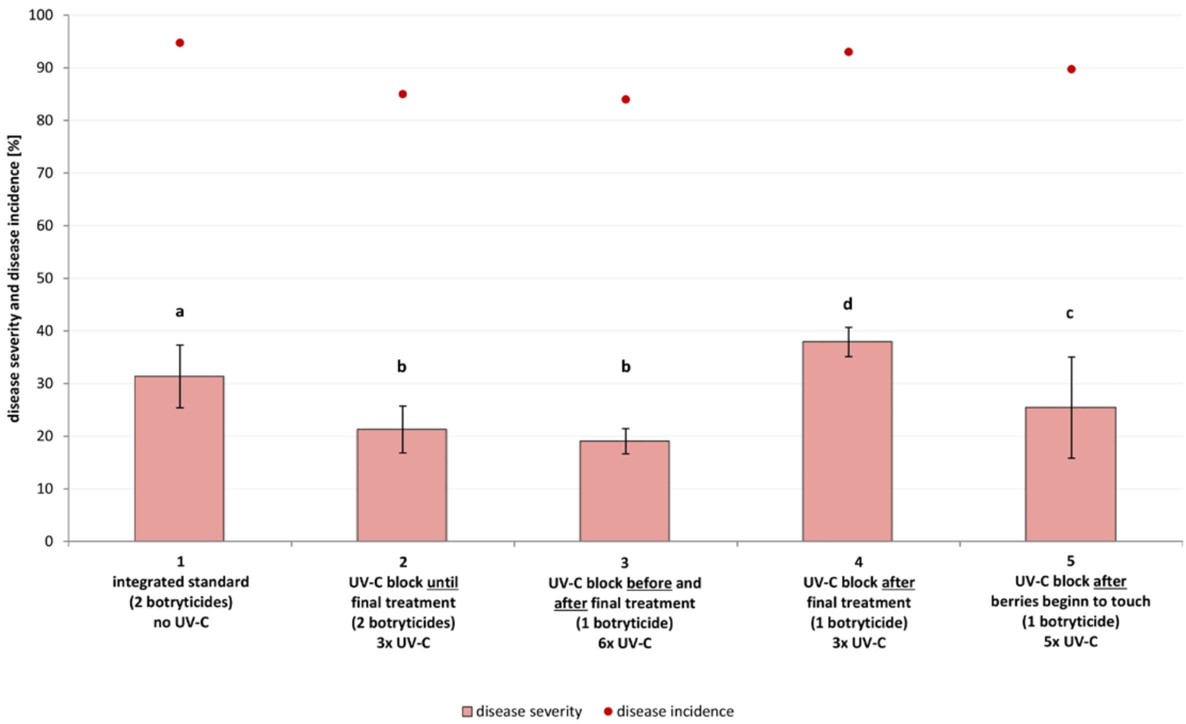

Figure 4: Biological efficacy against *Botrytis cinerea* (causal agent of grey mould) on grapes under different fungicide treatments and irradiation with UV-C under practical field conditions in cv. Riesling in 2014. Disease assessment on 30 Sept 2014; vineyard site: location "Scharlachwiese" of pilot estate. Statistical analysis: Duncan´s test, values with the same letter are not significantly different ($p < 0.05$); n = 400, mean ± SD.

The values of disease incidence of the treatments compared to the control showed a similar pattern as that of disease severity. The relation between the values of disease incidence of treatments was similar to the relations of disease severity to each other.

Conclusions

In the experimental trial site "Kellersgrube", treatment V-2 (integrated standard) that included seven chemical applications and two applications of botryticides achieved a degree of efficiency of 51 %. This result was in accordance with our experience from other experiments. Despite the high use of anti-*Botrytis* measures, the phytosanitary situation was not improved, mostly due to unfavourable weather conditions (warm and rainy). When V-2 (integrated standard) and V-3 (integrated reduced) with less than half of fungicide applications (three instead of seven applications) are compared, no significant difference was observed.

Although in V-4 there were alternating protection (less than 50 % of chemical treatments and UV-C applications) and two botryticide sprayings, a degree of efficiency of 82 % could be achieved with this approach. Regarding this result, V-4 performed significantly better than V-2 (integrated standard; 51 %).

At the same time it became evident that the intensity of botryticide treatment – once or twice – largely determines the health status of the grapes later, which could be demonstrated by comparing V-4 with V-5. For the observed minor effect of V-6 (exclusively UV-C treatment and fungicide application against other pathogenic fungi; no botryticide) on *B. cinerea* there is currently no explanation.

Thus, in the experimental vineyard, it was possible by means of complementary UV-C treatments to achieve a significantly higher biological efficacy against *B. cinerea* compared to the standard application under moderate grey mould disease pressure (untreated control: 23 % disease severity). To summarize these results: under current field conditions, UV-C irradiation contributed significantly to produce healthier grapes. This outcome could be achieved even with less fungicide treatments. The determination of effective application dates – regardless of which manner chemical or physical – is of fundamental concern.

Under practical conditions at the pilot estate, a significant reduction of infection with *Botrytis* could be achieved due to the application of UV-C compared to purely chemical treatment. Indeed, in practical viticulture there is a possibility to reduce *Botrytis* infection by additional UV-C treatments as compared to the usual standard practices based on integrated crop protection protocols.

At both sites, UV-C prototypes were used. A similar process does not yet exist in grape production. In the future, UV-C technology can be used as an important contribution to maintain the health of grapes for a longer period which in turn allows full ripeness. In some experimental treatments additional applications were necessary, which caused burden to soil. However, the conception of the new method aims at using a complementary chemical treatment so that tractor crossings would take place anyway.

Since irradiation with UV-C is a non-selective method of plant protection, it can be assumed that other pathogens that cause rot can be also targeted. This applies on both – pathogenic (Walter 2012) and antagonistic micro-organisms – as well as larval stages of animal pests (Cini et al. 2014) and beneficial organisms. According to previous laboratory tests on fecundity and fertility of beneficial organisms in the field of entomology an exposure to UV-C dose of 160 mWs/cm² achieved minor effects, but so far no evidence of long term damage. Due to the simultaneous reduction of microbial antagonists in the phyllosphere it makes sense to consider adding plant activators that contain fungal and/or bacterial antagonists to future application protocols. However, it should be clarified whether there are such supplementary effects and if those agents alter wine quality.

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Acknowledgements

This project was conducted in the context of Hessen ModellProjekte and funded by the LOEWE (Landes-Offensive zur Entwicklung wissenschaftlich-ökonomischer Exzellenz) conveyor line 3, KMU-Verbundvorhaben. Within this, project responsibility was carried out by HA Hessen Agentur GmbH.

We thank the pilot estate for providing the experimental area with a total of 24 rows of vines. Within Hochschule Geisenheim University the work was done in close collaboration between the Department of Technology as well as the Department of Soil Science and Plant Nutrition.

We are grateful to Mr. Marcel Scherber for his precise implementation of UV-C applications and his professional support for the technical optimisation of prototypes.