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Results of two consecutive years on mould prevention in viticulture by means of UV C application of vines (*Vitis vinifera* L.)

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Abstract. In the last decade, impacts of climate change on cool climate viticulture were most obvious concerning cluster infections caused by *Botrytis cinerea*, the causal agent of grey mould. The control of primary and secondary fungal pathogens is economically most important since bunch rot may severely affect the annual harvest with regard to quality and quantity. Trials with UV C applications, a new approach to control bunch rot, were conducted in 2013 and 2014, representing years with intensive bunch rot epidemics. UV C irradiation was applied by a prototype at an experimental site of Hochschule Geisenheim University. At both canopy sides, leaves and cluster zones were irradiated repeatedly at a dose of ≤160 mWs/cm^2 alone or in combination with chemical control. The results of our experiments indicated that irradiation with UV C, in addition to fungicide treatments at reduced number of applications, was able to significantly decrease infection with grey mould to a degree of efficiency of 82% compared to standard chemical treatment that achieved 51% efficiency in 2014. Accordingly, the new technology is suitable for reducing cluster infection with mould fungi allowing a prolonged grape ripening time, which leads to a better harvest quality and a decreased yield loss.

1. Introduction

At present, the three economically important pathogens *Plasmopara viticola* (causal agent of downy mildew), *Erysiphe necator* (causal agent of powdery mildew) and *Botrytis cinerea* (causal agent of grey mould) require high fungicide application frequencies [1]. In spite of intensive crop protection measures, significant deterioration in terms of grape quality became apparent in cool climate viticulture regions. The biggest problems regarding must quality as well as grape yield are caused by *B. cinerea* and acetic acid bacteria. Due to severe bunch rot, early harvest was necessary in most of the years in the last decade. The race "decay vs. maturity" particularly in the years 2000, 2006, 2010, 2011, 2013 and 2014 showed significantly increased disease severity (more than 60%) in favor of the decay [2]. One major disadvantage of early harvest is the prevention of full ripening of the berries and, therefore, the potential lack of key components responsible for high quality wines regarding flavor and sensory. Owing to this situation, harvest dates are no longer determined by the ripeness of berries, but by the grape health status.

Based on the background of the described conditions and challenges, direct chemical treatments with optimal application dates are very important. In addition to the application of anti-*Botrytis* sprays, indirect measures play an important role in the maintenance of healthy grapes. These include special pruning for canopy design, defoliation methods, achieving a loose cluster architecture and moderate fertilization [3,4].

Nevertheless, the past years have shown that such measures were not always suited for maintaining the health status of grapes for a longer period. Additionally, population densities of the spotted-wing drosophila *Drosophila suzukii* were increased extremely in the last years [5] and the potential hazard of mycotoxins of secondary fungi (*Penicillium* and *Aspergillus* species) still represents a problem [6]. Therefore, it seems essential to use new approaches to improve grape berry health.

In this project it was investigated whether repeated applications of UV C light can complement the existing methods for controlling *Botrytis*. Unlike chemical agents (pesticides), there are no residues after irradiation with UV C. This offers to use UV C treatments also during the critical time period between the last fungicide treatment and harvest. The technology is based on irradiating (micro-) organisms with UV C light. For many years, different UV C approaches are applied mainly in the food industry. In this sector, UV C is used for surface and air disinfection and drinking water treatment [7, 8]. Generally, the UV C treatment of plant surfaces for reduction of phyllosphere and carposphere pathogens is difficult due to the risk of phytotoxic reactions [9]. In the greenhouse UV C irradiation was used successfully in

Figure 1. UV C prototype. Foto: W. Schönbach.

some crops to reduce the population density of various pathogens. The UV C treatment of scion and rootstock during propagation and treatment of harvested grapes as a post-harvest treatment – both with the objective of reducing infestation with *Botrytis* – belong to other applications of this technology in the crop production sector [10, 11]. For this reason, it was encouraging to use irradiation with UV C to reduce fungal infection on grapevine in the field.

The optimal (most effective) wavelength to be used against microorganisms lies between 240–270 nm [12], where the main spectrum of the amalgam lamp used in this experiments is 254 nm. The efficiency of UV C is dose dependent. That means 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) [9, 12, 13].

The aim of the present study was to increase grape health by using the UV C technology that allows the extension of the maturity period of the berries, even under unfavorable weather conditions. In two consecutive years (2013, 2014) irradiation trials were conducted in field experiments using a UV C prototype.

2. Material and methods

For this experiment, a UV C prototype was used, which was 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 institutes of Hochschule Geisenheim University worked in collaboration with the company uv-technik meyer gmbh, a market leader for UV irradiation systems in the food industry, to develop a device suitable for practical vineyard conditions. Regarding these requisites, a prototype based on existing elements has been developed.

2.1. UV C prototype

This prototype was built on the basic framework of a three-point equipment with radial fan, normally used for crop protection (Fig. 1). 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 0–1000 mm and was set at about 10 cm from the canopy surface during application. The air generated by the air blower causes turbulence in the foliage 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 have controlled air pressure movable slats with which the UV C radiation units can be shielded as required. The closed slats prevent irradiation of the canopy areas which are not supposed to be treated.

2.2. Experimental site

In the modules multiple high power amalgam lamps are installed with a length of 0.95 m. 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 $\leq$ 160 mWs/cm^2 per canopy side. This dose has been achieved through a defined speed. The continuous compliance of the driving speed was performed using cruise control on the tractor Fendt® Vario.

The field trials in the vineyard "Kellersgrube" were performed according to GEP standard [14]. This vineyard belongs to the experimental site of 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.

2.3. Trellis system and viticultural measures

The trellis system is 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, each containing twelve grapevines. In order to better reach the grapes by UV C irradiation, the cluster zone was defoliated at developmental stage 75 (= BBCH 75). For this purpose, all lateral shoots were removed by hand within the cluster zone and if necessary additional leaves nearby. This measure was performed in all treatments. Table 1 presents the details of the different variants in 2013, while Table 2 provides the data of 2014.

2.4. Disease assessment and statistical analysis

In each replicate, 100 grapes were used to assess disease severity caused by *B. cinerea* (%) so that each treatment included a total of 400 grapes. The results presented here are from the last disease severity assessment date on 15 October 2013 and on 01 October 2014, respectively. Grapes were harvested on 23 October 2013 and on 07 October 2014, respectively.

Statistical analysis of the results obtained was done using STATISTICA software. Duncan's test, with $\alpha = 0.05$, was applied, where means and standard deviations of replicates were used as input. The degree of efficiency in relation to the disease severity of the control plot was calculated according to Abbott [15].

Table 1. Treatments of the UV C experiment in the vineyard "Kellersgrube" on cv. Riesling in 2013 (BBCH = developmental stage).

treatment number	Treatment	description
1	control	Undisturbed development of grey mould without any phytosanitary measure against *B. cinerea*. Treatment of all replications of this plot against other fungal pathogens with fungicides that has no effect on *B. cinerea*.
2	integrated pest management	Standard treatment according to integrated grapevine protection. Six chemical crop protection applications against all pathogenic fungi in the growing season, two of them inclusive botryticides (BBCH 77, BBCH 81).
3	reduced integrated pest management	Reduced number of chemical treatments (50% of "integrated standard"). Three chemical applications against all pathogenic fungi at critical stages of development (late flowering, bunch closure, final injection), two of them inclusive botryticides (BBCH 77, BBCH 81).
4	reduced integrated pest management + UV C (alternating series) + 2 botryticides	Three chemical treatments with fungicides (cf. treatment 3; inclusive botryticides in BBCH 77 and BBCH 81); in alternating series three applications with UV C against all pathogenic fungi.
5	UV C solo <u>until</u> final (chemical) treatment	Three treatments with UV C irradiation at the developmental stages: post-blossom, bunch closure and last (chemical) control measure. Other pathogens were treated by fungicides without side effects on *B. cinerea*.
6	UV C solo <u>after</u> final (chemical) treatment	Treatment as in treatment 5 but instead of UV C post-blossom treatment two additional UV C treatments in the period between the last chemical application and grape harvest

Table 2. Treatments of the UV C experiment in the vineyard "Kellersgrube" on cv. Riesling in 2014 (BBCH = developmental stage).

treatment number	treatment	Description
1	control	Undisturbed development of grey mould without any phytosanitary measure against *B. cinerea*. Treatment of all replications of this plot against other fungal pathogens with fungicides that has no effect on *B. cinerea*.
2	integrated pest management	Standard treatment according to integrated grapevine protection. Seven chemical crop protection applications against all pathogenic fungi in the growing season, two of them inclusive botryticides (BBCH 77, BBCH 81).
3	reduced integrated pest management	Reduced number of chemical treatments (50% of "integrated standard"). Three chemical applications against all pathogenic fungi at critical stages of development (late flowering, bunch closure, final injection), two of them inclusive botryticides (BBCH 77, BBCH 81).
4	reduced integrated pest management + UV C (alternating series) + 2 botryticides	Three chemical treatments with fungicides (cf. treatment 3; inclusive botryticides in BBCH 77 and BBCH 81); in alternating series four applications with UV C against all pathogenic fungi.
5	reduced integrated pest management + UV C (alternating series) + 1 botryticide	Four chemical treatments with fungicides (cf. treatment 3; only one botryticide in BBCH 77); in alternating series four applications with UV C against all pathogenic fungi.
6	UV C solo <u>until</u> final (chemical) treatment	Three treatments with UV C irradiation at the developmental stages: pre-bloom post-bloom, and final (chemical) treatment. Other pathogens were treated with fungicides that have no effect on *B. cinerea*.
7	UV C solo <u>after</u> final (chemical) treatment	Application protocol like treatment 6 but instead of UV C pre-bloom treatment two additional UV C treatments in the period between the final chemical application and grape harvest; a total of four UV C applications.

3. Results

3.1. Results of the experiment in 2013

In 2013, grapevines in the control plot had a disease severity of *B. cinerea* of 31% in average (Fig. 2). The treatment "integrated pest management" reduced disease severity from 31% to 20%. In this experiment, the best phytosanitary results were obtained by treatment 4.

In this treatment, a reduced number of chemical applications including two botryticide treatments and UV C irradiation were combined. The large discrepancy between treatment 4 using a mixed approach and treatments 5 and 6 without any botryticides reflects the potency of modern plant protection agents with anti-*Botrytis* action. To summarize the essential results of 2013: the efficacy of UV C treatments alone without any

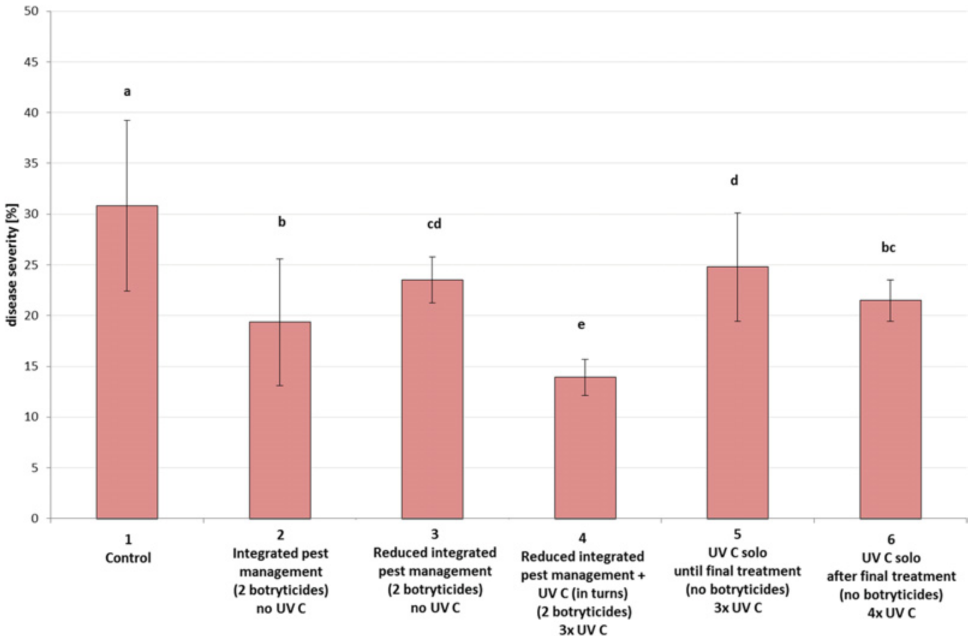

Figure 2. Biological activity against *Botrytis cinerea* on grapes under different fungicide treatments and irradiation with UV C under field conditions on cv. Riesling in 2013. Assessment of disease severity was conducted on 15 October, 2013. Randomization was done in the experimental vineyard "Kellersgrube" of Hochschule Geisenheim University. Statistical analysis: Duncan test, values with the same letter are not significantly different ($p < 0.05$); $n = 400$, mean ± StD.

fungicide application was insufficient to control *B. cinerea* in the vineyard.

3.2. Results of the experiment in 2014

In 2014, disease severity in the control plot was lower compared to the year before (in 2014: 23%) (Fig. 3). The treatment "integrated pest management" reduced disease severity from 31% to 20%. This standard chemical treatment resulted in a degree of efficiency of 51% in 2014. The same efficacy was obtained in treatment 3, where only less than half of specific mildew fungicides were applied. Both results are unsatisfactory concerning the health status of the grapes and the must quality. Like in 2013, best results in terms of berry health were achieved by treatment 4. Again, the combination of reduced chemical input regarding mildew fungicides including two botryticide treatments and UV C irradiation was most effective against *B. cinerea*. The degree of efficacy was 82% in treatment 4. In 2014, another treatment with reduced integrated pest management was conducted (treatment 5). In this plot, only one botryticide was applied. The differences between results in treatments 4 and 5 indicate that number and time of botryticide applications are essential. Like in 2013, UV C solo treatments achieved unacceptable results regarding *B. cinerea* control. In treatment 6, disease severity was even increased compared to the control plot.

4. Summary and outlook

The results of two consecutive years aimed at mould prevention indicated that the variant "integrated pest management" with seven chemical applications in 2014 and six in 2013 including two treatments with botryticides achieved low degrees of efficiency (2013: 33%; 2014: 51%). These results are in accordance with our experience from other experiments. In spite of intense use of anti-*Botrytis* measures, the phytosanitary status of the grapes was not improved, especially in years with unfavorable weather conditions. In 2014, the comparison of treatment 2 (integrated pest management) and treatment 3 (reduced integrated pest management) with less than half of chemical applications (three instead of seven applications) showed no difference concerning the biological efficacy.

In both years, best phytosanitary results were obtained by treatment 4, although 50% of the chemical treatments regarding mildew fungicides were applied. The combination of chemical and UV C applications including two botryticide treatments in turns achieved a degree of efficiency of 82%.

At the same time, it became evident that the intensity of botryticide treatment – once or twice – largely determines the health of the grapes later, which could be seen by comparing treatment 4 with treatment 5 in 2014.

In case of moderate infection pressure (disease severity in control plot in 2013: 31%; disease severity in control plot in 2014: 23%) complementary UV C treatments achieved a significant biological activity against *B. cinerea*. Under these experimental field conditions, irradiation with UV C contributed significantly in producing healthier grapes.

Based on the results obtained from experiments with UV C prototype, we suggest complementary UV C applications in the future that could maintain healthy grapes for a longer period which in turn allows full ripeness of the berries. In some experimental variants, additional applications were necessary causing more

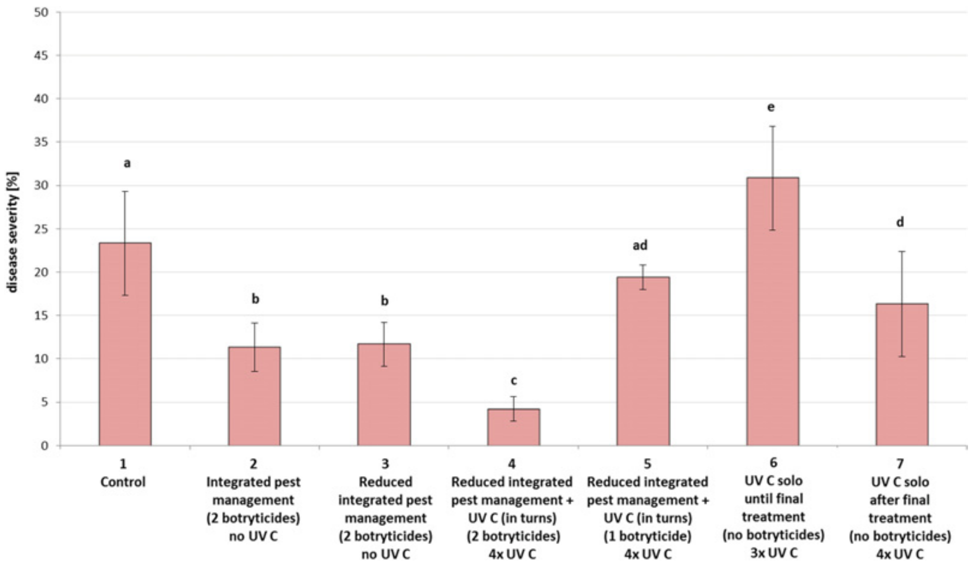

Figure 3. Biological activity against *Botrytis cinerea* on grapes under different fungicide treatments and irradiation with UV C under field conditions on cv. Riesling in 2014. Assessment of infection was conducted on 01 October, 2014. Randomization was done in the experimental vineyard "Kellersgrube" of Hochschule Geisenheim University. Statistical analysis: Duncan test, values with the same letter are not significantly different ($p < 0.05$); $n = 400$, mean ± StD.

tractor passages which leads to soil compaction. However, the conception of the new technology aims at using a complementary approach in addition to chemical treatments so that passages would take place anyway.

Since irradiation with UV C is a non-selective method, it can be assumed that other (micro-) organisms can also be targeted. This applies to both pathogenic [6] and antagonistic microorganisms, larval stages of animal pests [5] as well as beneficial organisms. According to previous laboratory tests on the fecundity and fertility of beneficial organisms in the field of entomology, the exposure to the UV C dose of 160 mWs/cm^2 achieved minor effects, but so far there is no evidence of long term damage. Due to the simultaneous reduction of microbial antagonists in the phyllosphere it makes sense to consider adding plant strengtheners (plant activators) that contain fungal and/or bacterial antagonists to future application protocols. However, it should be investigated if there are such supplementary compounds and whether they may alter wine quality.

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References

[1] H.D. Mohr, *Farbatlas Krankheiten, Schädlinge und Nützlinge an der Weinrebe* (Eugen Ulmer KG, Stuttgart, 2012)
[2] D. Molitor, O. Baus, M. Beyer, Julius-Kühn-Archiv **447**, 430 (2014)
[3] D. Molitor, M. Rothmeier, M. Behr, S. Fischer, L. Hoffmann, D. Evers, Vitis **50** (2), 81–87 (2011)
[4] O. Baus, B. Berkelmann-Löhnertz, Der Deutsche Weinbau **6**, 32–35 (2014)
[5] A. Cini, G. Anfora, L.A. Escudero-Colomar, A. Grassi, U. Santosuosso, U., G. Seljak, G., A. Papini, J Pest Sci **87**, 559–566 (2014)
[6] R. Walter, J für Kulturpflanzen **64** (10), 378–383 (2012)
[7] T. Koutchma, *Food Plant Safety – UV applications for food and nonfood surfaces*. (Elsevier, Amsterdam, 2014)
[8] A. Kirschbaum, HYGIENE Rep **5**, 4–6 (2014)
[9] S. Klärner, B. Berkelmann-Löhnertz, B. Flemming, M. Pfliehinger, O. Löhnertz, R. Keicher, H.-P. Schwarz, SZOW **149** (21), 4–6 (2013)
[10] L. Farkas, L. Kocsis, Int. J Hort Sci **19** (3-4), 53–56 (2013)
[11] G. Romanazzi, A. Lichter, F.M. Gabler, J.L. Smilanick, Postharvest Biol Technol **63** (1), 141–147 (2012)
[12] A. Renzel, Desinfektion mit UV C. Produktinformation uv-technik meyer gmbh, `http://www.uv-technik.de/uvc-anwendung.html` (2013)
[13] E. Gayán, P. Mañas, I. Álvarez, S. Condón, Appl Environ Microbiol, **79** (14), 4465–4473 (2013)
[14] GEP-Leitlinie `http://www.bvl.bund.de/SharedDocs/Downloads/04_Pflanzenschutzmittel/zul_dok_GEP.pdf?__blob=publicationFile&v=2`, (2013)
[15] W.S. Abbott, J. Econ. Ent. **18**, 265–267 (1925)