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RESEARCH PAPER

Germicidal effect of UV light on epiphytic fungi isolated from blueberry

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Abstract

B.A. Latorre, S. Rojas, G.A. Díaz, and H. Chuaqui. 2012. Germicidal effect of UV light on epiphytic fungi isolated from blueberry. Cien. Inv. Agr. 39(3): 473-480. The present study examined the inactivation effect of ultraviolet (UV) light on the conidia of *Botrytis cinerea Cladosporium cladosporioides, C. herbarum* and *Pestalotiopsis clavispora*, common pathogens of blueberry (*Vaccinium corymbosum*), often found as epiphytes. The fungi were grown at 20ºC in acidified potato dextrose agar (APDA) in the dark; conidial suspensions (10^6 conidia mL^{-1}) were placed the bottom of 5 cm glass Petri plates with a maximum height of 0.5 mm. Uncovered plates were immediately exposed to either UVA ($\lambda = 361$ nm), UVB ($\lambda = 302$ nm) or UVC ($\lambda = 254$ nm) at doses between 40 and 110 $mJ \cdot cm^2$. The results were expressed as survival ratios Nt/N0 (the number of colonies obtained after conidia were exposed to UV irradiance/ the total number of fungal colonies in the non-irradiated controls). The fungal-dependent rate constants (k), a measure of the mortality rate, were estimated from an exponential model. The species in order of greatest to least resistance to UV light were *Cladosporium cladosporioides*, *C. herbarum, P. clavispora* and *B. cinerea*. The type of fungal species and the dose of UV irradiance had a significant ($P \leq 0.001$) influence on Nt/N0. The interaction between the fungal species and the UV irradiance dose was significant ($P \leq 0.004$) only when the conidia were exposed to UVB or UVC. The resistance of *Cladosporium* spp. to UV radiation may explain the ubiquity of *Cladosporium* spp. in nature and could allow for the abundant populations of *Cladosporium* spp. often found on the foliage, flowers and fruits of blueberries.

Key words*: Botrytis cinerea, Cladosporium,* blueberry, fungi, *Pestalotiopsis*, UV radiation, *Vaccinium corymbosum.*

Introduction

Several fungal diseases of blueberry (*Vaccinium corymbosum* L.), such as gray mold (*Botrytis cinerea*) and Cladosporium rot (C*ladosporium cladosporioides* and *C. herbarum*), can result in serious economic losses in production. The damage caused by these pathogens is associated with the inoculum potential in the field. These fungal species exist on apparently healthy aerial organs of blueberry plants (Latorre *et al*., 2011a) as well as grapes and other hosts (Briceño and Latorre, 2008; Duncan *et al*., 1995; Guimaraes *et al*., 2011; Latorre *et al*., 2011b; Montealegre *et al*., 2003).

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The detrimental effects of ultraviolet (UV) irradiance on living organisms have been studied for many years and it has been associated to UVB irradiance in previous studies. UV radiation has received particular attention due to the ongoing depletion of the stratospheric protective ozone layer and the possibility that highly energetic UV (UVC, λ = 100-280 nm) irradiance can reach the Earth's surface. UV light directly affects DNA and inhibits translation and transcription; therefore, it has the potential to be highly mutagenic and detrimental to living organisms (Gao and García-Pichel, 2011; Klein, 1978).

The germicidal activity of UV irradiance is well known (Menetrez *et al*., 2010). The effect of solar radiation, primarily UVB, on airborne fungi has been studied previously (Ulevicius *et al*., 2004). A few hours of direct exposure to UV irradiance at an intensity frequently encountered in the environment can be sufficient to completely inactivate the conidia of several fungal species (Braga *et al*., 2001, 2002; Caldwell *et al*., 1998, 2007; Rotem and Aust, 1991). The effect of UV irradiance on the epiphytic fungi commonly found on the surface of the aerial plant organs of blueberries is unknown. This study aims to determine the effects of UV irradiance on the conidia of three fungal genera that are commonly associated with blueberry foliage and fruits.

Materials and methods

Fungi

The conidia of *B. cinerea, C. cladosporioides* and *C. herbarum* isolated from blueberry were used in this study. The partially melanized conidia of *Pestalotiopsis clavispora*, a common pathogen of blueberry (Espinoza *et al*., 2008), were also included as a reference isolate. Stock cultures were maintained at 20 ºC in potato dextrose agar acidified with 0.5 mL per liter of 92% lactic acid (APDA).

Conidia production and survival

The isolates used in this study were grown on APDA in polystyrene Petri dishes under continuous light from a fluorescent lamp (model F36T8, Westinghouse, Philadelphia, PA) that was placed approximately 60 cm above the plates. Plates were kept at 20 ºC for 14 days. The conidia were harvested into aqueous 0.05% (v/v) Tween 80. Conidia concentrations were adjusted to 10^6 conidia per mL with a hemocytometer. An aliquot (1 mL) of the conidial suspension was spread onto the bottom of 5 mm glass Petri plates, with a maximum height of 0.5 mm. The uncovered plates were immediately exposed to a particular UV wavelength. Conidia survival was estimated by plating (in triplicate) 0.1 mL aliquots of the UV exposed conidial suspensions on APDA containing 0.1% (v/v) of Igepal CO- (Sigma630-Aldrich, Atlanta, GA, USA), which was added as a colony restrictor to facilitate colony counting. The plates were incubated at 20 ºC for 7 days. The total number of colonies was counted.

Irradiance chamber

The irradiance experiments were conducted in a room chamber with the temperature set between 20 and 22 ºC. The UVA irradiance, λ = 361 nm, was provided by nine LED lamps (model L5-O-U5TH15-1, Sensor Electronic Technology Inc., [SETI] Columbia, SC) at 25 mA. The UVB irradiance, λ = 302 nm, was provided by nine LED lamps (model UVTOP300 TO39 FW, SETI) at 20 mA. The UVC irradiance, λ = 254 nm, was provided by one fluorescent linear lamp (model F15T 8BLB of 20 W, Philips, Eindhoven, The Netherlands). The wavelength emission of the LEDs was tested by the manufacturer. To confirm the UVC wavelength emission of the fluorescent lamp, the maximum optical power emitted was measured with an optical power meter (model PM100 A, THORLAB, Munich, Germany).

The distances between the samples and the source of irradiance were 80±1 mm, 50±1 mm and 79±1 mm for UVA, UVB and UVC, respectively. These working distances were adjusted based on the optical power of the lamps to minimize variation. An optical power meter with a 3% error was used for this measurement. The samples were located in the middle of the irradiance cone where the optical power ranged from 91.0 to 171.1 µW, 56.3 to 60.9 µW and 450 to 488 µW for UVA, UVB and UVC, respectively. Independent of UV light, the irradiance doses (ID) were 0.0, 40, 80 and 110 mJ·cm^{-2} for UVA, UVB and UVC, respectively. The ID was determined as follows: $ID = (P \cdot T_e) \cdot A^{-1}$, where P = optical power, T_e = exposition time and A = sensor area of the optical power meter. The T_e times were 3.95, 7.90 and 11.85 min for UVA, 14.32, 28.64 and 42.95 min for UVB and 1.15, 2.30 and 3.46 min for UVC. These experiments were repeated twice.

Data analysis

The results were expressed as Nt/N0 ratios, where Nt equals the number of fungal colonies obtained after the exposure of the conidia to UV irradiance and N0 is the total number of fungal colonies obtained in the non-irradiated controls. The data were subjected to a two-way analysis of variance (fungal species x UV irradiance dose) with three replicates; the means were separated according to Tukey's tests, and the standard errors of the differences (SED) were estimated.

The relationship between the UV irradiance dose and the proportion of organisms surviving the UV exposure was adjusted to Nt/N0 = exp (-k x Dose) by regression analysis. In this equation, the dose was equal to the effective irradiance (constant) received by the fungal conidia (W·cm^{-2}), in a period, t (s), and where *k* (cm^2·(mWs)$^{-1}$) was the fungal-dependent rate constant (Menetrez *et al*., 2010). Data analyses were performed using SigmaStat 3.1 (Systat Software Inc., San José, CA, USA).

Results

The fungal species tested and the UV irradiance dose applied had significant (P≤0.001) effects on conidia survival. The interaction between fungal species and UV irradiance dose was also significant (P≤0.004) when the conidia were exposed to UVB or UVC; however, no significant interaction (P=0.08) was seen between fungal species and UVA (Table 1). The conidia of *C. cladosporioides* and *C. herbarum* were the most resistant to irradiance with UV light at doses between 40 and 110 mJ·cm^{-2} (Figure 1). Mean survivals of *C. cladosporioides* conidia were 87, 84 and 78% following exposure to UVA, UVB and UVC, respectively. Mean survivals of *C. herbarum* conidia were 89, 83 and 80% following exposure to UVA, UVB and UVC, respectively. Significant differences (P=0.05) in the survival rates of *C. cladosporioides* and *C. herbarum* conidia were only obtained when exposed to UVB.

A strong negative effect of UV irradiance on the conidial survival of *B. cinerea* was observed. The mean conidia viability were 38, 31 and 19% when the conidia were exposed to UVA, UVB or UVC, respectively (Figure 1). Complete growth inhibition was obtained with both 80 and 110 mJ·cm^2 of UVC irradiance. The mean survival of *P. clavispora* conidia was 49, 45 and 34% when the conidia were exposed to UVA, UVB or UVC, respectively (Figure 1).

The data collected for each fungal species were adjusted to an exponential model, generating equations in which R^2>0.93 and P<0.038 for *C. cladosporioides*, R^2>0.93 and P<0.025 for *C. herbarum*, R^2>0.91 and P<0.020 for *B. cinerea* and R^2>0.95 and P<0.011 for *P. clavispora* (Figure 1). The *k* (cm^2·(mWs)$^{-1}$) values obtained were in the following ranges: 3.16 x 10^{-3} to 16.83 x 10^{-3} for UVA, 4.18 x 10^{-3} to 21.13 x 10^{-3} for UVB and 6.25 x 10^{-3} to 57.28 x 10^{-3} for UVC (Table 2).

Table 1. Effect of ultraviolet irradiance on the survival of conidia of *Botrytis cinerea, Cladosporium cladosporioides, C. herbarum* and *Pestalotiopsis clavispora* isolated from blueberry.

	Survival proportion, Nt/N0[1]		
Treatments	UVA (λ = 361 nm)	UVB (λ = 302 nm)	UVC (λ = 254 nm)
Fungi (F)			
B. cinerea	0.38c[2]	0.31	0.19
C. cladosporioides	0.87a	0.84	0.78
C. herbarum	0.89a	0.83	0.80
P. clavispora	0.49b	0.45	0.34
d.f.	3	3	3
F	92.99	141.19	180.73
P	<0.001	<0.001	<0.001
SED[2]	0.038	0.032	0.032
UV radiation dose (UV), mJ·cm^2			
0	0.93a	0.93	0.93
40	0.73b	0.66	0.53
80	0.56c	0.49	0.39
110	0.42d	0.34	0.27
d.f.	3	3	3
F	66.20	122.66	155.54
P	<0.001	<0.001	<0.001
SED[2]	0.038	0.032	0.032
F x UV interaction			
d.f.	3	3	3
F	1.95	3.47	6.82
P	0.08	0.004	<0.001
SED[2]	0.076	0.064	0.065

[1]Survival proportion, where Nt=the colonies obtained after the conidia were exposed to UV irradiance and N0=the colonies obtained before the conidia were exposed to UV irradiance.
[2]Means followed by the same letter in each column did not differ significantly according to Tukey's test (P=0.05). SED=the standard error of the difference.

Discussion

The conidia of *C. cladosporioides*, *C. herbarum*, *P. clavispora* and *B. cinerea,* which are pathogens frequently found on blueberries (Caruso and Ramsdell, 1995; Espinoza *et al*., 2008; Latorre *et al*., 2011b), were sensitive to UV irradiance. Considerable differences in the sensitivity of conidia to UV light were observed among these fungal species, with *C. cladosporioides* and *C. herbarum* being the most resistant species.

As seen in previous studies, *C. cladosporioides* and *C. herbarum* were able to survive the strong germicidal effects of UVB and UVC irradiance (Moody *et al*., 1999; Menetrez *et al*., 2010; Valero *et al*., 2007). This relative resistance to UV irradiance may explain, at least in part, the high ubiquity and relatively high populations of *Cladosporium* spp. often found on the foliage and fruits of blueberries (Latorre *et al*., 2011a; Valero *et al*., 2007) and other hosts. A previous study (Latorre *et al*., 2011b) on grapevines demonstrated that *Cladosporium* spp. are abundant on grape clusters and that these *Cladosporium* populations increased considerably on heavily defoliated vines where their clusters were exposed to direct sunlight. *C. cladosporioides* has been identified as a predominant fungal species that persists in the air after exposure to UVB solar irradiance (Ulevicius *et al*., 2004).

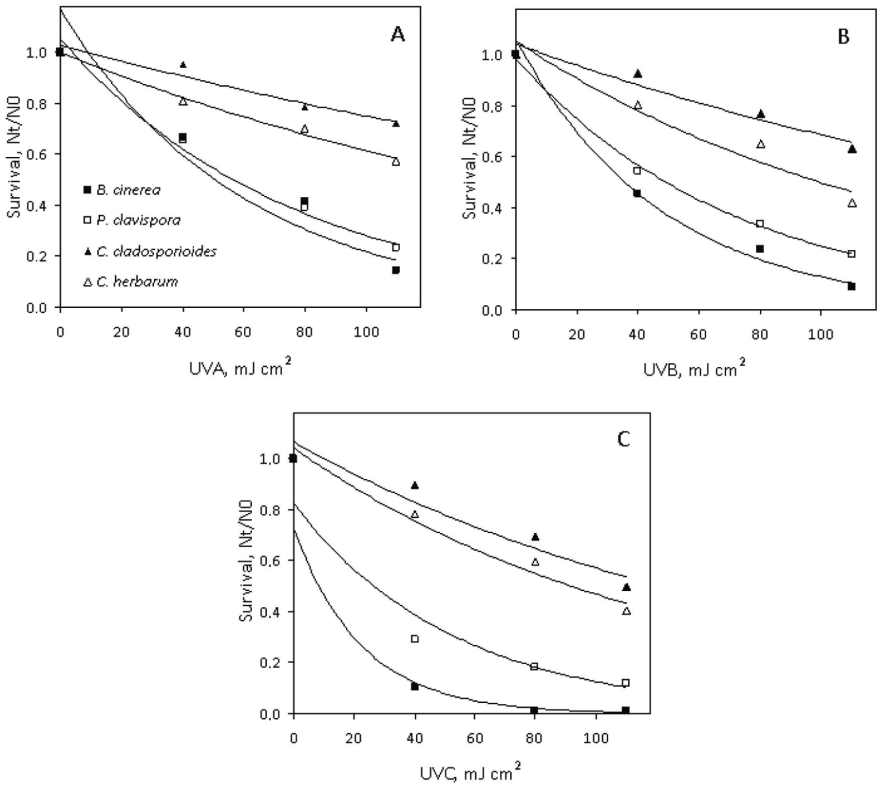

Figure. 1. Effects of ultraviolet light (UV) on the survival of conidia of *Botrytis cinerea, Cladosporium cladosporioides*, *C. herbarum* and *Pestalotiopsis clavispora* isolated from blueberries. A. UVA: $y_{Bc}=1.17e^{-0.0168x}$ ($R^2=0.91$, P=0.020), $y_{Cc}=1.03e^{-0.00316x}$ ($R^2=0.95$, P=0.032), $y_{Ch}=0.99e^{-0.00487x}$ ($R^2=0.99$, P=0.005), $y_{Pc}=1.05e^{-0.01324x}$ ($R^2=0.99$, P=0.004). UVB: $y_{Bc}=1.06e^{-0.021x}$ ($R^2=0.98$, P=0.002), $y_{Cc}=1.04e^{-0.004x}$ ($R^2=0.95$, P=0.030), $y_{Ch}=1.05e^{-0.007x}$ ($R^2=0.93$, P=0.025), $y_{Pc}=0.98e^{-0.0014x}$ ($R^2=0.99$, P=0.001). UVC: $y_{Bc}=0.73e^{-0.057x}$ ($R^2=0.93$, P<0.001), $y_{Cc}=1.07e^{-0.006x}$ ($R^2=0.93$, P=0.038), $y_{Ch}=1.05e^{-0.008x}$ ($R^2=0.97$, P=0.011), $y_{Pc}=0.83e^{-0.019x}$ ($R^2=0.95$, P=0.011).

Table 2. The fungal-dependent mortality rate constant (*k*) estimated for the conidia of *Botrytis cinerea, Cladosporium cladosporioides*, *C. herbarum* and *Pestalotiopsis clavispora* exposed to ultraviolet (UV) irradiance.

Pathogens	*k* values, $cm^2 \cdot (mWs)^{-1}$		
	UVA[1]	UVB[1]	UVC[1]
B. cinerea	16.83×10^{-3}	21.13×10^{-3}	57.28×10^{-3}
C. cladosporioides	3.16×10^{-3}	4.18×10^{-3}	6.25×10^{-3}
C. herbarum	4.87×10^{-3}	7.50×10^{-3}	7.99×10^{-3}
P. clavispora	13.24×10^{-3}	13.75×10^{-3}	18.83×10^{-3}

[1]Conidia were irradiated with either UVA (λ = 361 nm), UVB (λ = 302 nm) or UVC (λ = 254 nm).

The relative resistance to UV irradiance exhibited by *Cladosporium* spp. has been attributed to the presence of darkly pigmented conidia with high melanin contents (Margalith, 1992; Wang and Casadevall, 1994). Melanin pigmentation acts as UV-absorbing substance and has been shown to protect conidia against UV irradiance (Bjorn, 2007; Gao and Garcia-Pichel, 2011, Wang and Casadevall, 1994). *B. cinerea* presents hyaline, non-melanized conidia and is susceptible to UV irradiance, particularly UVB or UVC. These results are in agreement with previous findings (Rotem

and Aust, 1991) that found *B. cinerea* to be sensitive to UV irradiance and sunlight. The conidia produced by *P. clavispora* have two hyaline and two brown median cells, which are possibly due to melanin pigmentation (Espinoza *et al*., 2008). These conidia were also relatively sensitive to the UV irradiances in our study, although they were less sensitive than the conidia of *B. cinerea*.

As expected, the fungal-dependent rate (*k*) values were relatively low. This suggests that these filamentous fungal species are relatively resistant to UV irradiance in comparison with yeasts and other microorganisms (Menetrez *et al*., 2010). The *k* values for *C. cladosporioides* and *C. herbarum* were considerably lower than the *k* value for *B. cinerea,* which accounts for the considerable difference in the resistance to UV irradiance.

In this study, the conidia were irradiated with UVC that is currently absent from the solar spectrum reaching the Earth's surface (Gao and Garcia-Pichel, 2011); therefore, these results may be ecologically irrelevant. The germicidal effect, particularly with respect to UVC irradiance, has a potential application in the postharvest treatment of blueberries and has been proposed for other fruits (Marquenie *et al*., 2002a, 2002b). Further research is required for conclusive results.

For *C. cladosporioides, C. herbarum*, *B. cinerea* and *P. clavispora*, conidia are the most abundant type of inoculum produced on the aerial organs of blueberries and other hosts during the growing season. Within an orchard, these conidia are exposed to a wide variety of environmental conditions including desiccation, freezing, high temperature and UV irradiance. The large populations of *Cladosporium* spp. that are commonly found on blueberries (Latorre *et al*., 2011a) can be attributed to their intrinsic resistance to solar irradiance. Furthermore, the relative susceptibility to UV irradiance exhibited by the conidia of *B. cinerea* and *P. clavispora* may explain their relative infrequency on the aerial organs of blueberries.

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Resumen

B.A. Latorre, S. Rojas, G.A. Díaz y H. Chuaqui. 2012. Efecto germinicida de luz ultravioleta sobre hongos epifíticos aislados desde arándanos. Cien. Inv. Agr. 39(3): 473-480. En este trabajo se investigó el efecto de la luz ultravioleta (UV) sobre la inactivación de conidias de *Botrytis cinerea*, *Cladosporium cladosporioides, C. herbarum* and *Pestalotiopsis clavispora*, patógenos comunes en arándano (*Vaccinium corymbosum*) que frecuentemente son encontrados como epifitos. Estos hongos se cultivaron a 20°C en agar papa dextrosa acidificado (APDA) en oscuridad, y suspensiones de conidias (10^6 conidia·mL^{-1}) se colocaron en el fondo de placas de vidrio de Petri, con una altura máxima de 0.5 mm. Las placas de Petri no cubiertas fueron inmediatamente expuestas a UVA (λ = 361 nm), UVB (λ = 302 nm) or UVC (λ = 254 nm) a dosis que variaron de 40 a 110 mJ·cm^2. Los resultados obtenidos se expresaron como razón de sobrevivencia Nt/N0 (números de colonias obtenidas después de exponer las conidias a radiación UV/total de colonias fungosas en el control de no expuestas a la radiación UV), y la tasa constante dependiente de los hongos (k), que mide de la tasa de mortalidad, fueron estimadas con un modelo exponencial. *Cladosporium cladosporioides* fue la especie más resistente, seguido por *C. herbarum, P. clavispora* y *B. cinerea*. Las especies fungosas y el efecto de la

dosis de la radiación UV influenciaron significativamente (P≤0.001)la tasa Nt/N0, y se obtuvo una significativa (P≤0.004) interacción entre las especies fungosas y la dosis de radiación UV cuando las conidias se expusieron a UVB o UVC. La resistencia de *Cladosporium* spp. a la radiación UV puede explicar la abundancia de especies de *Cladosporium* en la naturaleza y por ende explicar las altas poblaciones de *Cladosporium* spp. a menudo encontradas en el follaje, flores y frutos de arándanos.

Palabras clave: *Botrytis cinerea, Cladosporium,* arándanos, hongos, *Pestalotiopsis*, radiación UV, *Vaccinium corymbosum.*

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