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Light and Temperature Dependent Conidium and Sclerotium Formation in *Botrytis* spp.

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Botrytis spp. における光と温度に依存した分生胞子及び菌核の形成

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Abstract Four *Botrytis* species of *B. allii, B. cinerea, B. squamosa* and *B. fabae* were examined for their responses of conidium and sclerotium formation to continuous irradiation with near ultraviolet radiation (NUV) and continuous darkness at temperatures between 5 and 30℃ on the V-8 vegetable juice agar plates and on the host tissues. The number of conidia formed under darkness relative to that under NUV was greatest in *B. allii,* followed by *B. cinerea* and *B. squamosa. Botrytis fabae* did not form conidia under darkness. Dependency on the NUV for conidium formation was greater on the VJA plates than on the respective host tissues. Conidium formation of *B. fabae* was completely dependent on the NUV, even on its own host tissues. Favorable conditions of light and temperature for conidium formation and those for sclerotium formation were different. There was an antagonistic relationship between conidium formation and sclerotium formation. Sclerotia formed under conditions that were unfavorable for conidium formation of *Botrytis* species and for epidemic of the disease caused by the fungi. These results support the ecological role of sclerotia that are formed as a resting structure and serve as an organ for survival of the fungus under adverse conditions, while conidia are formed as dispersal propagules.

Introduction

Botrytis fungi have been known as a destructive group of fungi causing many economically important diseases. Geographically, *Botrytis* species occur wherever their host crops are grown, ranging from cool temperate zones to subtropical areas, but they are concentrated in the temperate zones (Trolinger and Strider, 1985). Conidia, mycelia, and ascospores are the three types of inocula of importance in *Botrytis* diseases. These three structures along with sclerotia are the four types of dispersal propagules (Trolinger and Strider, 1985). Production of these propagules has been recognized to be influenced by various environmental conditions. Honda et al. (1977) reported that five isolates of *B. cinerea* from cucumber, tomato, eggplant, and rose formed conidia under near ultraviolet radiation (NUV), but did not under darkness or under light filtered through plastic films with lower limits of transmission longer than 345 nm. Suzuki and Oda (1979) found that blue light inhibited sclerotial development of *B. cinerea. Botrytis squamosa* required light for conidium formation, and the effective wavelengths were restricted to ultraviolet radiation (UV) shorter than 340 nm (Sasaki et al., 1985). Sclerotium formation of *B. squamosa*

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was accelerated by the UV and suppressed by light of wavelengths between 340 nm and 520 nm. Later, most conidiophores formed directly on sclerotia. Generally, epidemics caused by *Botrytis* spp. through these propagules occur in cool, wet and humid weather, conditions that favor conidium formation, infection, and predisposition of the host (Trolinger and Strider, 1985; Yarwood, 1959).

The work described here reports in detail the results of experiments designed to investigate the influence of environmental conditions on conidium formation and sclerotium formation in *B. allii, B. cinerea, B. squamosa,* and *B. fabae,* with special reference to the effects of NUV and temperature, during growth on an agar medium and on tissues of each host plant.

Materials and Methods

Organisms: The isolate of *B. allii* Munn used in this study was isolated from a neck rot bulb of onion (*Allium cepa* L.). The isolate of *B. cinerea* Pers. ex Fr. was isolated from gray mold fruit of grape (*Vitis vinifera* L.). The isolate of *B. squamosa* Walker was isolated from leaf blight of Chinese chive (*Allium tuberosum* Rottler). The isolate of *B. fabae* Sardina was isolated from chocolate spots of broad bean leaves (*Vicia faba* L). Cultures of all isolates were initiated with single conidia. The cultures have been grown on vegetative juice agar (VJA: Campbell's V-8 juice 200ml, $CaCO_3$ 2 g, agar 17 g, distilled water 800ml; pH 5.8) slants.

Culturing: Cultures on VJA slants formed conidia during an incubation for 20 days under continuous irradiation of NUV from a 20 W black light fluorescent lamp (FL20S BLB, Toshiba Corporation, Tokyo; 310-410 nm, max. 360 nm) suspended 18cm above the slant cultures. The average intensity of NUV was 245 $\mu W/cm^2$ at the colony surface. Intensities were measured by a thermopile having a quartz window (MIR-100Q, Mitsubishi Yuka Co., Ltd., Yokkaichi, Japan) coupled with a digital multimeter (TR6846, Advantest Co., Ltd., Tokyo, Japan) at several spots, and they were averaged.

Conidia were taken from a slant culture of each isolate by a nichrome wire inoculating loop and were inoculated to VJA (10ml/60mm diam. Pyrex Petri dish) plates. The inoculated plates were incubated for 15 days under the same light condition as described above. Conidia thus formed were collected by adding 10ml of distilled water with 0.05% Tween 20 to each plate and gently scraping the whole surface of the colony with a rubber spatula. Resulting conidium suspensions were filtered through two layers of gauze. Concentration of conidia was adjusted to 1×10^5 conidia/ml. The adjusted conidium suspension was inoculated to the host plant tissues by spraying it to the surface of each host tissue.

Irradiation at different temperatures: VJA plates were inoculated at the center with single conidia, and were incubated under continuous irradiation with NUV at the same intensity of 245 $\mu m/cm^2$ for 15 days under 6 different temperatures of 5, 10, 15, 20, 25, and 30℃. Control colonies in light-tight boxes were next to the test colonies for each temperature. Conidial counts from whole colonies (60mm diam. plates) were taken by adding 5ml of a 50% sucrose-0.05% Tween 20 solution to each plate and then gently scraping the surface of the colony with a rubber spatula. Conidial concentration was determined with a hemocytometer.

Conidium formation on the host tissue: *Botrytis allii*—Flat pieces of 3×3cm were cut from outer scales of onion bulbs and were surface-sterilized with 70%

ethanol for 1 min. Pieces of scales were dried after a rinse in sterilized distilled water. Conidium suspension (0.7ml/piece) of the fungus was sprayed to the inner surface of the scale. The inoculated scales were put on agar plates (agar 20g, distilled water 1000ml; 10ml/60mmPyrex Petri dish).

Botrytis cinerea — Leaves of grape (cv. Delaware) were stocked in a freezer at -20℃. Frozen leaves were cut into 3×3cm pieces. Then they were autoclaved at 121℃ for 20 min. Spore suspension (0.8ml/piece) of the fungus was sprayed to the upper side of the leaves. The inoculated leaves were put on the agar plates, one piece for each plate.

Botrytis squamosa — Leaves of Chinese chive were cut into pieces 3cm long and autoclaved at 121℃ for 20 min. Five to six pieces of the autoclaved leaves were put on each agar plate. Spore suspension (1.2ml/plate) of the fungus was sprayed to the leaves.

Botrytis fabae — Leaves of broad bean were cut into 3×3cm pieces and were surface-sterilized with 70% ethanol for 1 min. After a rinse in sterilized distilled water, conidium suspension (0.7ml/piece) was sprayed to the upper side of the leaves. The inoculated leaves were put on the agar plates, one piece for each plate.

The inoculated host tissues on the agar plate of Pyrex Petri dishes were subjected to continuous irradiation of NUV and continuous darkness for 15 days at 15℃ which was recognized as an optimum temperature for conidium formation of these *Botrytis* isolates on the VJA plates. The intensity of NUV was 245 $\mu W/cm^2$. The same host plant tissues without inoculation served as a control and adjacent to the inoculated ones. These tissues did not show any discernible changes after incubation.

During 15 days of incubation the inoculated host tissues were examined for conidium formation and sclerotium formation 5, 10, and 15 days after the beginning of incubation. *Botrytis cinerea*—inoculated leaves of grape were also examined for the number of conidiophores in a unit area and for the branching of the conidiophores at the apical region. Host tissues with conidia were brought into 5ml of a 50% sucrose-0.05% Tween 20 solution and were subjected to gentle shaking by hand for a minute. The concentration of resulting conidium suspension was determined with a hemocytometer. The experiment with two replicated plates for each treatment was repeated twice.

Results

Effect of light and temperature on conidium formation and sclerotium formation on VJA plates.

Botrytis allii formed conidia at all temperatures between 5 and 30°C with an optimum temperature at 15°C under both continuous NUV irradiation and continuous darkness (Fig. 1). The isolate formed conidia substantially even under continuous darkness, though NUV significantly enhanced the conidium formation. The ratio of conidium numbers under continuous irradiation of NUV to that under continuous darkness was 1000 to 100. Sclerotia did not form under any conditions.

Botrytis cinerea formed conidia abundantly under continuous irradiation of NUV at 15 and 20℃, followed by 25 and 10℃. Conidia did not form at temperatures of 5 and 30℃ even under NUV. Small number of conidia formed under darkness at temperatures between 10 and 25℃. The ratio of conidium numbers at 20℃ under

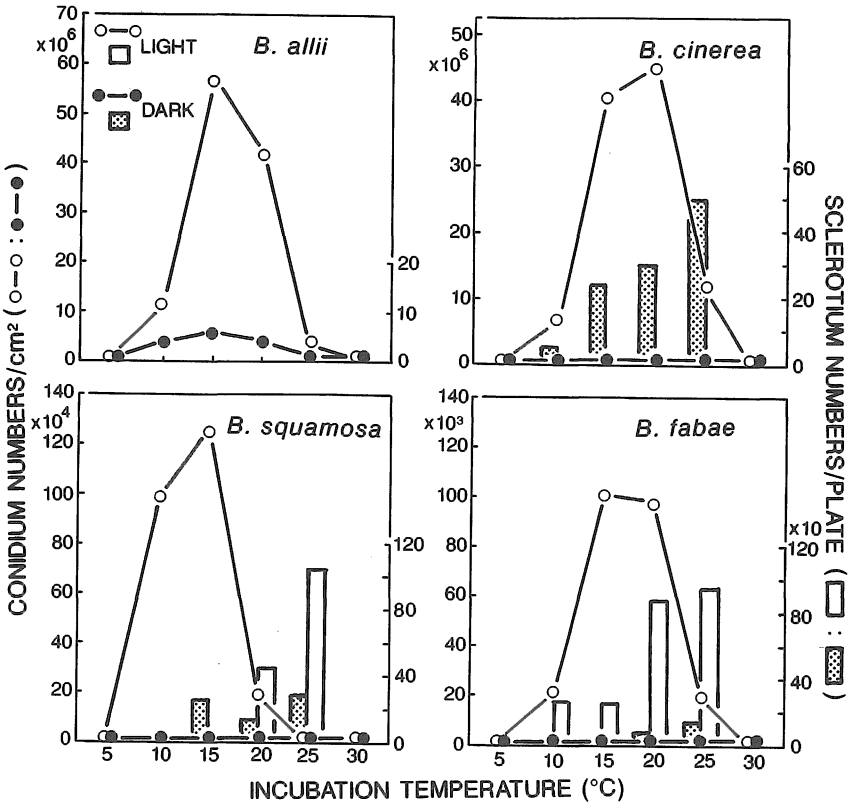

Fig. 1. Effects of light and temperature on sporulation and sclerotium formation on the V-8 vegetable juice agar plates in *Botrytis* spp. Spores and sclerotia were counted after 15 days of incubation.

continuous irradiation of NUV to that under continuous darkness was 1000 to 8. Sclerotia did not form under NUV, but formed abundantly under darkness with the optimum temperature at 25℃. The number of sclerotia decreased gently with a decrease of temperature from the optimum, while sclerotia did not form at a temperature higher than the optimum. The optimum temperature for sclerotium formation under continuous darkness was higher than the temperature optimum for conidium formation under continuous irradiation of NUV.

Botrytis squamosa formed conidia abundantly under continuous irradiation of NUV with the optimum temperature at 15℃, followed by 10℃. Conidium formation decreased greatly at 20℃, and almost no conidia formed at temperatures of 5, 25 and 30℃. Conidia barely formed under continuous darkness even at the optimum temperature of 15℃. The ratio of conidium numbers at 15℃ under continuous irradiation of NUV to that under continuous darkness was 1000 to 1. Sclerotia formed under NUV or under darkness. A temperature range for sclerotium formation was 15 to 25℃ and the number of sclerotia increased slightly with the increase of temperature under NUV. The optimum temperature for sclerotium formation was 25℃ and it was significantly higher than the temperature optimum for conidium formation under NUV. Although both of sclerotia and conidia formed under continuous irradiation of NUV at temperatures higher than 15℃, conidium formation decreased sharply with the temperature increase, while sclerotium formation

significantly increased with the temperature increase. The sclerotia was smaller under NUV compared with those formed under darkness, though the number of sclerotia was significantly greater under NUV than under darkness.

Botrytis fabae formed conidia abundantly under continuous irradiation of NUV at temperatures of 15 and 20℃, with the optimum at 15℃. Conidium formation decreased sharply at temperatures higher or lower than these temperatures. At temperatures of 5 and 30℃, no conidia formed. Conidia did not form under darkness, contrasting with the results of other *Botrytis* species examined in this study. Many sclerotia formed under NUV. The number of sclerotia increased gradually from 10 to 25℃ with the maximum at 25℃. At temperatures of 5 and 30℃, sclerotia did not form. Although both of sclerotia and conidia formed under NUV at temperatures higher than 15℃, conidium formation decreased significantly with the temperature increase, while sclerotium formation increased. Some of conidiophores developed directly from the sclerotia.

Effect of light on conidium and sclerotium formation on the host tissue.

As a result of the above experiment it was revealed that a favorable temperature for conidium formation of *Botrytis* spp. was 15℃. The effect of NUV on the conidium formation of *Botrytis* spp. infected on the respective host tissue at 15℃ was examined 5, 10 and 15 days after inoculation. Sclerotium formation was also observed.

Botrytis allii on the fresh outer scales of onion bulbs formed conidia under continuous irradiation of NUV and under continuous darkness. The number of conidia increased with incubation period, and was greater under NUV than under darkness (Fig. 2). The ratio of conidium numbers under NUV to that under darkness after 15 days of incubation was 1000 : 200. Relative sporulation under darkness to that under NUV was two times greater on the host tissue than on the VJA plate. Sclerotia did not form under NUV nor under darkness.

Botrytis cinerea on the autoclaved leaves of grape formed conidia abundantly under continuous irradiation of NUV, and moderately under continuous darkness. The number of conidia increased with the incubation period. The ratio of conidium numbers under NUV to that under darkness after 15 days of incubation was 1000 : 67. Relative sporulation under darkness to that under NUV was more than 8 times greater on the host tissue than on the VJA plate. An average number of conidia on a conidiophore under NUV after 15 days of incubation was about 6 times greater than that under darkness. The number of conidiophores in a unit area of the host tissue was 2 times greater under NUV than under darkness. These differences contributed to a greater conidium formation under NUV than under darkness. Sclerotia formed under darkness, but not at all under NUV. The maximum number of sclerotia was formed after 5 days of incubation, and it did not increase during the rest of incubation period of 15 days.

Botrytis squamosa on the autoclaved leaves of Chinese chive formed conidia abundantly under continuous irradiation of NUV, while conidia did not form during 10 days of incubation under continuous darkness. A few conidia formed after 15 days of incubation under darkness. The ratio of conidium numbers under NUV to that under darkness after 15 days of incubation was 1000 : 11. Relative sporulation under darkness to that under NUV was 11 times greater on the host tissue than on the VJA plate. Sclerotia did not form under NUV, and a few sclerotia formed after

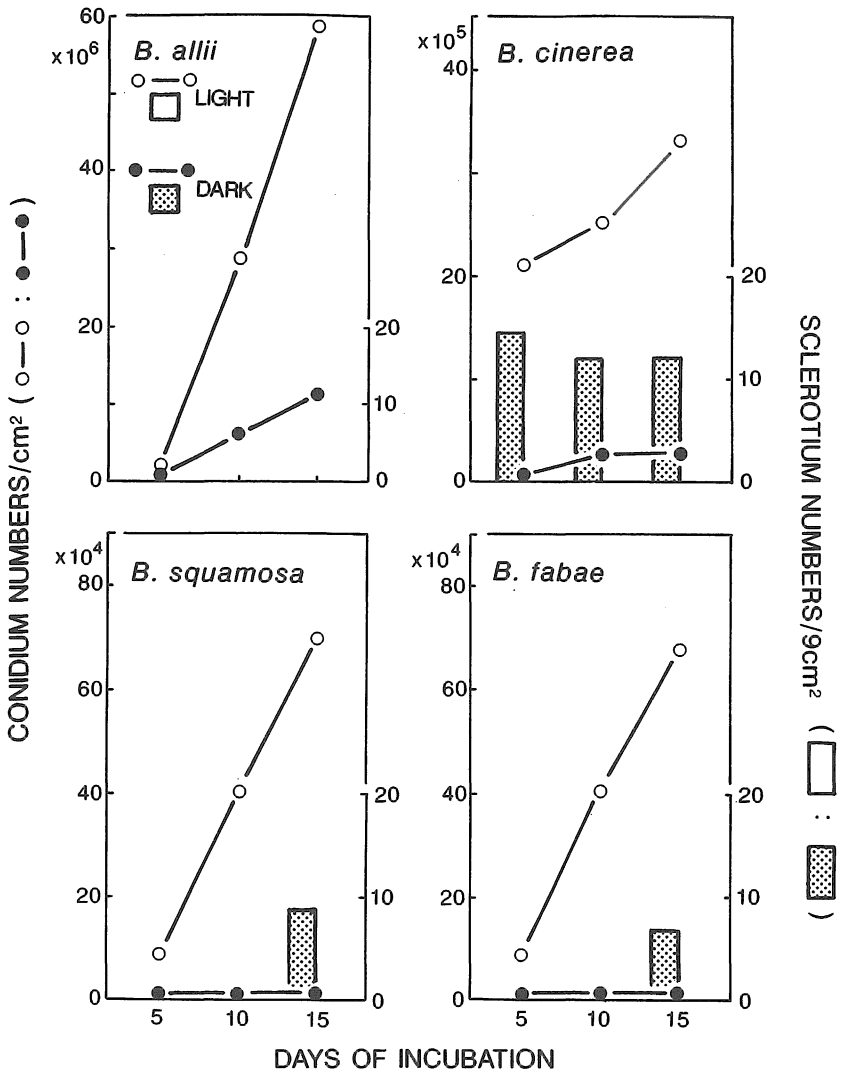

Fig. 2. Effect of light on sporulation and sclerotium formation on the host tissues in *Botrytis* spp. at 15℃.

15 days of incubation under darkness.

Botrytis fabae inoculated to the fresh leaves of broad bean formed conidia abundantly under continuous irradiation of NUV, but not at all under darkness. The number of conidia under NUV increased linearly with incubation period. Sclerotia did not form under NUV. Only a few sclerotia formed after 15 days of incubation under darkness.

Relative sporulation of *Botrytis* species under darkness to that under NUV on the VJA plate and on the respective host tissue under NUV or under darkness was summarized in Table 1. The number of conidia formed by each species under NUV was converted to 1000 to standardize the conidium formation for each species and a relative number of conidia formed on the VJA plate or on the respective host tissue under darkness was calculated for each *Botrytis* species. Conidium formation of

Botrytis species under darkness relative to that under NUV was greatest in *B. allii*, followed by *B. cinerea* and *B. squamosa*. *Botrytis fabae* did not form conidia under darkness.

Discussion

Ellis (1971) stated that sclerotia of *B. allii* are frequently found on natural substrata but less so in culture. Sclerotia form on surface-sterilized onion bulbs at 5℃ after 16 days of incubation, but not at temperatures higher than 15℃ (Matsuo, 1978). In accordance with these observations, *B. allii* did not form sclerotia on the VJA plate nor on the onion bulbs at 15℃ after 15 days of incubation, although conidia were formed under NUV or under darkness in this study.

Sclerotia of *B. cinerea* formed abundantly under darkness where conidia formed scarcely, while no sclerotia were formed under NUV where conidia formed abundantly. Sclerotia of *B. squamosa* and *B. fabae* formed under NUV as well as under darkness. However, the optimum temperature for sclerotium formation under NUV was 25°C, at which temperature conidia did not form. The optimum temperature for conidium formation was 15°C for these *Botrytis* species. These observations on *Botrytis* fungi in cultures on the VJA plate showed that favorable conditions are quite different between conidium formation and sclerotium formation. When conidium formation is suppressed by high temperature, sclerotia form. Lack of light also suppresses conidium formation and favors sclerotium formation. The results indicate an antagonistic relationship between conidium formation and sclerotium formation in *Botrytis* species. The same relationship was confirmed on the respective host tissue for each species. At the temperature optimum for conidium formation under NUV on the host tissue, sclerotia did not form. Sclerotia formed under darkness where conidia formed scarcely or did not form at all. An exception of this relationship was for *Botrytis fabae*. Both of conidia and sclerotia formed abundantly on the VJA plate at 20°C. In this species, however, many conidiophores developed directly from the sclerotia, and this temperature of 20°C was in the middle between the optimum temperatures of 15°C for conidium formation and 25°C for sclerotium formation.

Epidemics caused by *Botrytis* spp. occur in cool, wet and humid weather, conditions that favor conidium formation, infection, and also predisposition of the host (Trolinger and Strider, 1985). Matsuo (1978) reported that optimum temperatures for conidium formation were 17℃ for *B. allii*, 15℃ for *B. cinerea* and 15−16℃ for *B. squamosa*. We confirmed that relatively low temperatures are favorable for conidium formation in 4 species of *Botrytis*. On the contrary, the optimum temperature for sclerotium formation in *B. cinerea*, *B. squamosa* and *B. fabae* was 25℃, significantly higher than that for conidium formation. At this temperature conidium formation was strongly suppressed in all *Botrytis* spp. tested in this stuby. Disease incidence of *Macadamia* flower caused by *B. cinerea* has been reported to be depressed above 22℃ (Trolinger and Strider, 1985). Sclerotia, therefore, form under conditions that are unfavorable for conidium formation of the fungus and for epidemic of the disease. This observation supports an ecological role of the sclerotia that are formed as resting structures and serve as organs for survival of the fungus under adverse conditions.

Among 4 species of *Botrytis*, *B. fabae* and *B. squamosa* formed many sclerotia

Table 1. Light dependent conidium formation on VJA plate and on host tissues in *Botrytis* spp.

Botrytis spp.	Relative conidium formation [a]			
	VJA plate		Host tissue [b]	
	Light	Dark	Light	Dark
B. allii	1000	100	1000	200
B. cinerea	1000	8	1000	67
B. squamosa	1000	1	1000	11
B. fabae	1000	0	1000	0

a) The number of conidia formed under light was converted into 1000 and relative conidium formation under dark was calculated for each *Botrytis* sp.
b) Tissues of specific host for each *Botrytis* sp. were used.

under NUV, especially *B. fabae* formed more sclerotia under NUV than under darkness. Conidiophore formation on the sclerotia in *B. fabae* resulted in concomitant formation of many conidia and sclerotia under certain conditions. The same result has been obtained in *B. squamosa* at 20℃, where more sclerotia formed under NUV than under darkness, although the number of conidia was smaller under NUV. However, an optimum temperature range of 10-15℃ for conidium formation did not over up with that for sclerotium formation (20-25℃) in *B. squamosa*. Therefore, the simultaneous formation of many conidia and sclerotia was not observed, although it has been reported that sclerotia of *B. squamosa* on the soil surface can produce conidia repeatedly when the soil moisture is favorable (Sherf and MacNab, 1981).

Suzuki and Oda (1979) reported that blue light inhibited sclerotial development through induction of de-differentiation of sclerotial primordia in sterile hyphae, and NUV induced conidiophores on the sclerotial primordia, thus inhibited sclerotial development in *B. cinerea*. Light with a wide range of wavelengths from near ultraviolet to blue region inhibits sclerotial development through the induction of conidiophore formation on sclerotial primordia. In this experiment we observed abundant conidium formation under the NUV accompanied with the inhibition of sclerotium formation. Direct formation of conidiophores on the sclerotia has not been observed during the experiment period of 14-15 days in *B. cinerea* in this stuby. Desiccation of sclerotia before imbibition and near ultraviolet irradiation have been reported to be indispensable for sporogenic germination in *B. cinerea* (Suzuki, 1989) and *B. convoluta* (Jackson, 1972). In present study, sclerotia of *B. cinerea* formed on the VJA plate or on the host tissue, and were kept at a constant moisture content under an axenic condition. These conditions may not be favorable for sporogenic germination of sclerotia in *B. cinerea*, resulting in no conidium formation on the sclerotia, even under continuous irradiation of NUV. *Botrytis allii* did not form any sclerotia on the VJA plate, a distinct characteristic distinguishing this species from other *Botrytis* species tested in this study.

On the host tissue, the ratio of conidiophore numbers under NUV to that under darkness was 2.6 : 1, while the ratio of conidium numbers was 15.4 : 1 in *B. cinerea*. This results in 6 times greater number of conidia per conidiophore under NUV compared to that under darkness. Spores of *B. cinerea* are formed on conidiogenous ampullae at the apex of a conidiophore (Honda et al., 1991). From above result, NUV seems to stimulate dichotomous or trichotomous branching of the conidio-

phore apex. Thus, the NUV not only stimulates conidiophore formation, but also stimulates ampullae formation on the apical region of a conidiophore.

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摘　　要

近紫外線(NUV)連続照射及び暗黒処理に対する *Botrytis* 属菌4種, *B. allii, B. cinerea, B. squamosa* 及び *B. fabae* の分生胞子及び菌核形成反応をV-8野菜ジュース寒天培地(VJA)及び宿主植物組織上で調査した。培養温度は5～30℃の6段階に設定した。NUV連続照射に対する暗黒下での相対的な胞子形成量は *B. allii* で最も多く、ついで *B. cinerea* と *B. squamosa* が多かった。*Botrytis fabae* は暗黒下では胞子を全く形成しなかった。胞子形成のNUVに対する依存性は宿主植物組織上に比べVJA上で大きかった。*Botrytis fabae* の胞子形成にはVJA上だけでなく宿主植物上においてもNUV照射が必要であった。胞子形成と菌核形成の至適温度及び光条件は全く異なっていた。また、胞子形成と菌核形成は拮抗的であり、菌核は胞子形成や発病には不適当な条件下で良く形成された。これらの結果は、菌核は休眠器官として菌の永存に役立ち、分生胞子は伝搬体として病気の伝染に貢献するという各々の器官の生態的適応を示しているものと考えられる。