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Note

Laboratory Production of Oospores in *Pseudoperonospora humuli*

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*Pseudoperonospora humuli*의 실험실상의 난포자 형성

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ABSTRACT: In *Pseudoperonospora humuli*, the cause of hop downy mildew, environmental and host factors affecting laboratory production of oospore were examined. After 7 days incubation of leaf disk inoculated with sporangia on water, additional incubations were carried out under different conditions of temperature and moisture. Oospore production was also compared between very susceptible (Nugget) and resistant (Fuggle) hop cultivars. Oospores were not produced at 18°C regardless of other incubation conditions. Leaf disks failed to produce oospore when incubated on water for up to 18 days at 8°C. No oospores formed on infection sites without necrosis. However, abundant oospores were produced at necrotized infection sites when inoculated leaf disk incubated on dry filter paper for 5 days at 8°C. Both susceptible and resistant hop cultivars produced abundant oospores. In the measurement of optimal temperature for oospore production, oospores were produced at 6 to 12°C Most abundant oospores were produced at 8°C. We suggest that proper combination of low temperature, dryness and necrosis may be a critical environmental factors for oospore production of *P. humuli*.

***Key words*: downy mildew, hop, oospore, *Pseudoperonosra humuli*.**

Pseudoperonospora humuli, the cause of downy mildew of hop, is an oomycete fungus in which sexual reproduction involves fertilization of an oogonium, leading to the production of an oospore. The fungus is an obligate biotroph infecting primarily aerial parts of the hop plant. Oospores are formed in infected shoots and cones (3). Although the epidemiological role of oospore in downy mildew of hop has not been clearly studied, it has been suggested that oospores are at least partly responsible for the seasonal production of infected spikes and overwintering structure (5). Studies on sexual reproduction and breeding system in *P. humuli* is virtually scarce. Difficulty of artificial culture and minor crop status of hop have mitigated against studies of *P. humuli*. In oomycetes, it has been suggested that oospore production might be influenced by environmental and biological factors. In *Peronosclerospora heteropogonis*, oospores were produced in *Heteropogon*, but not in *Maize* (9). Drench *et al.* (4) demonstrated that oospores of *Phytophthora infestans* were produced in potato leaves at temperature ranging from 5 to 25°C, with an optimum of 10°C. Cohen *et al.* (2) showed that biological and environmental factors including freshness of leaf, low temperature and the continuous supply of moisture affect the oospore production of *P. infestans*. Michelmore (7) observed that no constraints were found in an extensive survey of environmental influences on oospore production in *Bremia lactucae*.

Oospore production could provide a long-lived source of inoculum and genetic diversity of pathogen population that may change the population structure and complicate disease control management. Therefore, a better understanding of biological and environmental factors in oospore production may provide insight into the disease control measures. Laboratory production of oospores of *P. humuli* could also provide critical tools to reveal whether sexual breeding system of the fungus is homothallic or heterothallic. In this study, we examined the influence of biological and environmental factors on laboratory production of oospores of *P. humuli* in hop. This is the first report to induce oospore production of *P. humuli* in the laboratory.

MATERIALA AND METHODS

Isolates of *P. humuli* were collected from Oregon and Washington hop yards in 1997. Heavily infected

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spikes were collected and returned to the laboratory. Sporulation was induced by enclosing spikes in a plastic bag and incubating at 18°C overnight in the dark. Infected leaves with dense sporulation were harvested from each spike. Sporangia were harvested from sporulating leaves by vigorously shaking the leaves in distilled water for 30 s. Spore suspension was centrifuged at 4,000 rpm for 3 min and the supernatant was discarded. The sporangial pellet was resuspended in distilled water. Inoculum concentration was adjusted to 1,000 sporangia per μl with the aid of a cytometer.

Fresh leaf disks were cut from fully expanded leaves using a no. 8 cork borer. Leaf disks were prepared from green house-grown hop cultivars Nugget (very susceptible) and Fuggle (resistant) (8). Four leaf disks were floated back surface up in a petri dish conatining 4 ml of distilled water. One 20 μl drop of sporanigial suspension (1,000/μl) was applied to the back surface of four leaf disks. Approximately 24h after inoculation, the remaining inoculum drops were removed by aspiration. Plates were incubated at 18°C for 7 days to ensure infection. After 7 days incubation at 18°C, additional incubations were carried out to examine oospore production at infection sites under the following conditions. These included; 1) 18 days incubation of infected leaf disks on water at 8°C, 2) 5 days incubation of infected leaf disks on dry filter paper at 8°C after 18 days incubation on water at 8°C, 3) 5 days incubation of infected leaf disks on dry filter paper at 8°C, 4) additional 8 days incubation of infected leaf disks on water at 18°C, and 5) 5 days incubation of infected leaf disks on dry filter paper at 18°C. To observe the occurrence of oospores in tissue samples, leaf disks were gently washed with excessive water to remove sporangia from their surface and then clarified in boiling ethanol for 5 min. After ethanol treatment, they were floated on water, and a glass slide was used to lift the tissue and mount it on their surface. The tissue was examined with a microscope at 40 or 100X. The number of oospores were counted at one 10 mm^2 sites per leaf disk.

In order to examine temperature ranges for the production of oospores, fully sporulated leaf disks of Nugget cultivar after 7 days incubation on water at 18°C were additionally incubated on dry filter paper at 4, 6, 8, 10, 12, and 14°C for 5 days, respectively. The number of oospores were counted as described above. Three leaf disks were counted for each temperature.

RESULT

All inoculation sites on leaf disks produced abundant asexual sporangia after 7 days incubation on water at 18°C. Until this time, necrosis has not been observed at inoculation sites. After additional incubation for 2 days, some inoculation sites became necrotic. We examined oospore production on leaf disks after additional 5 days incubation. Leaf disks inoculated with sporangia did not support oospore production when incubated on water for up to 18 days at 8 or 18°C. Such leaves, even though fully necrotized, showed no oospores. No oospores were observed at inoculation sites which remain intact green regardless of other incubation condition. In contrast, abundant oospores were produced at necrotized inoculation sites when leaf disks were incubated on dry filter paper for 5 days at 8°C following 7 days incubation on water at 18°C (Fig. 1). Oospores were not produced at 18°C regardless of incubation condition.

When we compare oospore production in Nugget and Fuggle hop cultivars, oospore production was observed

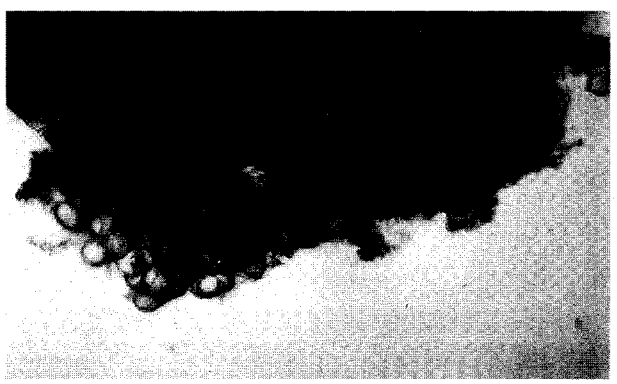

Fig. 1. Oospores of *Pseudoperonospora humuli* at infection site (40X).

Table 1. Effect of temperature and host cultivars on the number of oospores produced by *Pseudoperonospora humuli*

Factor	No. of oosporesa
Temperature (°C)	
4	0
6	36±8
8	52±10
10	46±8
12	20±8
14	0
Host cultivar	
Nugget	54±12[b]
Fuggle	52±10[b]

[a]values represent the mean number of oospores per 10 mm^2 of leaf disk of cultivar Nugget averaged over 4 leaf disks with standard deviation.

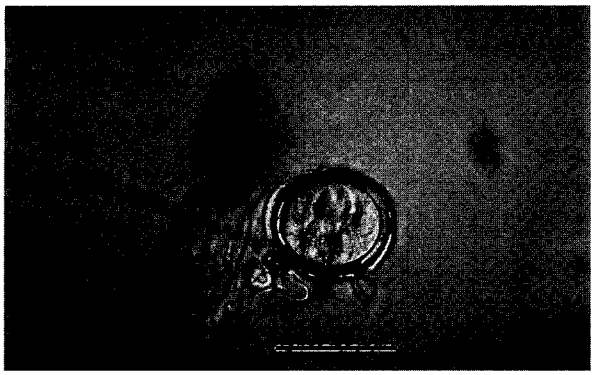

Fig. 2. An oospore of *Pseudoperospora humuli* attached with antheridium (100X). Bar represents 41.5 μm.

in both cultivar. The number of oospores observed was not significantly different between both cultivars. In the measurement of temperature range for oospore production, temperature ranging from 6 to 12°C provided oospore production while 4°C and above 12°C did not support oospore production (Table 1). Most abundant oospores were observed at 8°C. Oospores were spherical with smooth walls and a diameter of 41.45±0.5 μm. In some case, an antheridium attached to an oospore was observed (Fig. 2).

DISCUSSION

Oospores produced by sexual reproduction can be found in infected hop tissue in most hop growing areas of the world (1). However, the role of oospores in the disease cycle is not well understood. It has been suggested that oospore production has to be considered with the knowledge of environmental factors. Cohen *et al.* (2) indicated that a constant supply of wetness is important for oospore production in *P. infestans*. We demonstrated that no oospore production occured in *P. humuli* under the condition of a constant supply of wetness and instead, reduced osmotic potential and dryness are needed for oospore production. It was reported that the temperature most favorable to oospore production in *P. infestans* was 8°C and temperature ranging from 8 to 23°C allowed oospore production (2). But unlike *P. infestans*, oospores of this fungus were not produced above 12°C. Temperature ranging from 6 to 12°C provided oospore production with the maximum production at 8°C. In *P. humuli*, low temperature appeared to be a crucial factor for oospore production. It has been reported that oospore production is induced by senescence or necrosis of the host tissue in *Peronospora parasitica* (6). In *P. infestans*, however, fully necrotized leaves failed to produce oospores and, instead, fresh leaf allowed oospore production. In our experiments, oospores of *P. humuli* were observed only in necrotized inoculation sites. Whether necrosis is the cause or the consequence of sexual reproduction is not known, but necrosis seems to be important factor for oospore production. We suspect that abundant asexual sporulation may cause necrosis associated with nutrient deficiency which result in oospore production under unfavorable environmental conditions. The host genotype had no significant effect on oospore production. A Fuggle hop cultivar (resistant) still allowed abundant oospores to be formed that was similar to a Nugget hop cultivar (very susceptible).

Although oospore production was readily induced under this experimental conditions, we do not know which environmental factors induce oospore production of *P. humuli* in nature. Our results demonstrated that oospore production is induced by a proper combination of low temperature, dryness and necrosis. These conditions most likely occur in the fall when oospores are observed in nature. In this study, laboratory induction of oospore production may provide valuable foundation to reveal the sexual breeding system of this fungus and to study on genetics and epidemiology.

요 약

Hop의 노균병을 일으키는 *Pseuoperonospora humuli*의 난포자 형성에 영향을 주는 환경 및 생물학적 요인들을 조사하였다. 무성생식포자를 유리 샤레 수면 위에 떠있는 hop 잎반에 접종하여 상온에서 7일간 배양하여 감염시킨 후, 온도 및 수분공급 상태를 변화시킨 조건에서 추가 배양한 후 난포자형성을 관찰하였다. 난포자 형성 최적 상태에서 hop의 감수성 품종(Nugget)과 저항성 품종(Fuggle) 간의 난포자 수를 또한 비교하였다. 실험 결과, 상온 배양 시에는 어떠한 다른 조건에서도 난포자가 형성되지 않았으며. 감염된 잎반을 수면 위에서 18일간 8°C에서 배양하여도 난포자는 형성되지 않았다. 괴사가 일어나지 않은 감염부위에서도 역시 난포자 형성은 일어나지 않았다. 하지만 감염된 잎반을 마른 여과지에서 5일간 8°C에서 배양 시에는 괴사된 감염부위에서 다량의 난포자가 형성되는 것이 관찰되었으며 Hop의 감수성 품종과 저항성 품종 모두 다량의 난포자를 형성하였다. 난포자 형성 온도 실험에서는 6°C에서 12°C 범위에서는 난 포자 형성이 되었으며 8°C에서 가장 많은 량의 난포자가 형성되었다. 따라서 *Pseudoperonospora humuli*의 난포자 형성에는 저온과 건조상태 및 기주 감염부위의 괴사 상태

의 적절한 조화가 중요한 환경 적 요인으로 작용한다고 사료된다.

REFERENCES

1. Bressman, E. N. and Nichols, D. A. 1933. Germination of oospores of *Pseudoperonospora humuli. Phytopathology* 23 : 485-486.
2. Cohen Y., Farkash S., Reshit, Z. and Baider, A. 1997. Oospore production of *Phytophthora infestans* in potato and tomato. *Phytopathology* 87 : 191-196.
3. Coley-Smith, J. R. 1962. Overwintering of hop downy mildew, *Pseudoperonospora humuli. Ann. Appl. Biol.* 50 : 235-242.
4. Drench, A., Turkensteen, L. J. and Gover, F. 1993. The occurrence of the A2 mating type of *Phytophthora infestans* in the Netherland. Significance and consequences. *Neth. J. Plant. Pathol.* 97 : 57-67.
5. Magie, R. O. 1942. The epidemiology and control of downy mildew on hops. *Tech. Bull. N.Y. State Agric. Exp. Stn.* 267 : 1-48.
6. McMeekin, D. 1960. The role of the oospores of *Peronospora parasitica* in downy mildew of crucifers. *Phytopathology* 50 : 93-97.
7. Michelmore, R. W. 1979. A study of sexual reproduction by *Bremia lactucae* Regel. Ph.D Thesis, University of Cambridge, U.K.
8. Royle, D. J. and Kremheller, H. TH. 1981. Downy mmildew of the hop. In : *the Downy Mildews*. Spencer, D. M. eds. Academic Press, Inc., New York, 413pp.
9. Williams, R. J. 1984. Downy mildew of tropical cereals. In : *Advance in Plant Pathology* Vol. 2 Ingram, D. S. and Williams, P. H. eds. Academic Press, Inc., London, 1-103.

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