Vitis **38** (4), 185–186 (1999)

Research Note

Survival of sporangia from *Plasmopara viticola*, the downy mildew of grapevine

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S u m m a r y : The potential of aged sporangia from *Plasmopara viticola* to produce zoospores as a requirement to form new infections was determined. Sporangia were produced on potted grapevines and kept on the vines at 22 °C, 30 % r.h. or 22 °C, 60-80 % r.h., respectively. Under these conditions release of zoospores from sporangia could be observed microscopically until 7 d after outbreak of sporangia if liquid water was available. After suspending sporangia in water a first release of zoospores from fresh sporangia was observed after 30 min, from 3-7 d old sporangia not before 180 min. Sporangia kept under the above mentioned conditions were able to infect leaves for at least 9 d after outbreak.

K e y w o r d s : Plasmopara viticola, grapevine, downy mildew, sporangia, survival, epidemiology.

Introduction: *Plasmopara viticola* is a most destructive fungal disease of grapevines and is dependent on the presence of free water for propagation as the fungus infects stomata by motile biflagellate zoospores. In the vineyard, formation of sporangia only occurs in humid nights at temperatures >12-13 °C (BLAESER 1978). Germination of sporangia is dependent on the presence of liquid water and takes place also at daytime. To precast the risk of new infections, it is of great importance to know the amount of infectious sporangia at the onset of a period of leaf wetness caused by dew or rain. On detached leaves freshly produced sporangia were found to survive less than 24 h at 20 °C, 30 % r.h. and 48 h at 20 °C, 70 % r.h., respectively (BLAESER 1978). Based on these results, most *Plasmopara* disease risk predictions assume that *e.g.* a 24 h period with 40 % r.h. and 20 °C will kill all viable sporangia.

Spore trap experiments allowing to proof the presence of infectious sporangia (KAST and WALTER 1994, KAST 1997, KAST and BOROWKA 1998) were performed. They often revealed a close correlation to calculated survival rates of models relying on the epidemiological findings of BLAESER (1978). However, in some cases viable sporangia were found even after a couple of dry days when, according to the prediction of models, no viable sporangia ought to be present. Therefore, we investigated the survival of sporangia under semi-natural conditions on potted grapevines in the glasshouse.

Material and Methods: 20 greenhouse-grown 8-12-week-old potted grapevine cuttings, *V. vinifera* cv. Trollinger, were sprayed with a spore suspension (40,000 sporangia·ml^{-1}) using a hand sprayer. Then the inoculated plants were kept wet in plastic bags at 22 °C for 24 h in the dark. Following an incubation for 7 d at 20 °C, 30 % r.h. and a 12 h photoperiod, sporulation was induced by transferring the infected plants to saturated air humidity at 20 °C for 24 h. After sporulation, the plants were kept at 22 °C, 26-34 % r.h. (second set at 15-20 °C, 60-80 % r.h) until the end of the experiment. In intervals of 24 h sporangia from 2-3 leaves were brushed off, suspended in water and diluted to 40,000 sporangia·ml^{-1}. To assess viability of sporangia, detached leaves were placed with their adaxial surface on moist filter paper in Petri dishes; they were inoculated by pipetting 40 drops (5 µl each) of the sporangia suspension per leaf. Drops of the suspension were kept on the leaves alternatively for 4 or 24 h before drying with a cold air stream of a hairdryer. Additionally, 10 leaf discs per trial were inoculated according to KAST (1996). In all trials, disease severity was assessed as percentage of the infested leaf area 7 d after infection.

Simultaneously, 200 µl of the sporangia suspension were monitored microscopically (400x) every 20 min in wells of microtiter plates for the production of zoospores. When a release of zoospores was observed, the number of zoospores per ml formed from 40,000 sporangia·ml^{-1} was determined using a Thoma counting-chamber.

Results and Discussion: Release of zoospores was observed 30 min after fresh sporangia had been suspended in water (Figure). After 4-7 d of incubation of plants bearing sporulating lesions at 22 °C, 30 % r.h. the first zoospores appeared 180 min after suspending sporangia. ZACHOS (1959) also observed that under natural conditions aged sporangia need more time to release their zoospores.

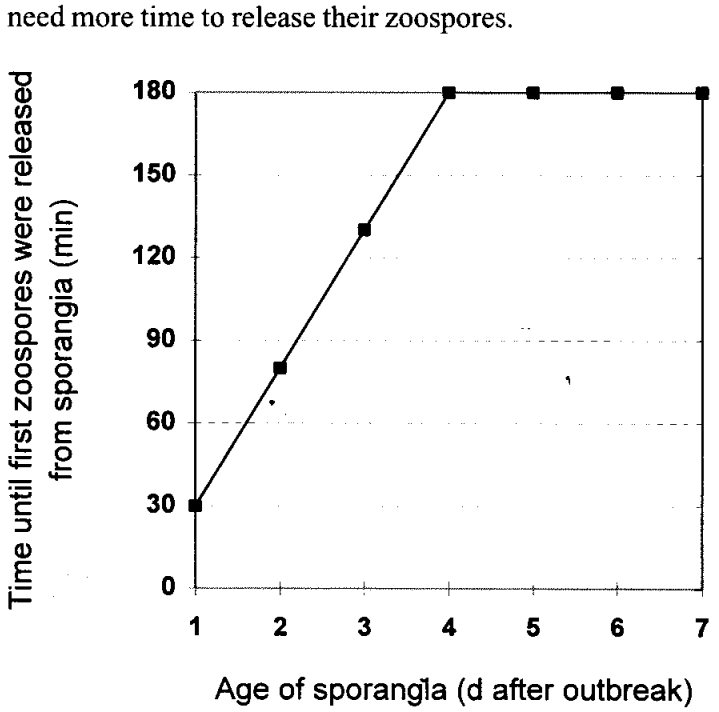

Figure: Germination of sporangia from *P. viticola* kept on sporulating lesions on leaves of potted grapevines at 22 °C, 30 % r.h. for various times.

Furthermore we investigated whether there was a difference in disease incidence between leaves that had been inoculated with a sporangial suspension for 4 and 24 h respectively. Within 6 d after sporangial outbreak no difference in the infection rate was observed. 7 d after sporangial outbreak disease incidence on leaves that had been kept wet for 24 h was still 100 % while it was only 1 % on leaves

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Table

Disease incidence on leaf discs inoculated with sporangia of *Plasmopara viticola* kept at different relative humidities r.h. for several days after outbreak

Days after outbreak	Sporangia kept at 22 °C, 26-34 % r.h.		Sporangia kept at 15-20 °C, 60-80 % r.h.	
	Disease frequency (leaf discs, %)	Disease severity (%)	Disease frequency (leaf discs, %)	Disease severity (%)
1	100	30	100	18
2	100	28	95	21
3	100	30	95	47
4	100	25	100	27
5	100	28	95	14
6	100	30	100	24
7	20	10	20	10
8	45	20	25	17
9	10	10	80	12
10	0	0	0	0

that had been wet for 4 h only (Table). Our results indicate that aged sporangia need longer to release their zoospores.

When a first release of zoospores was observed, the number of zoospores per ml formed from 40,000 sporangia per ml was determined. The number of zoospores released from 40,000 sporangia per ml decreased from 50,000 zoospores (fresh sporangia) to 1000 zoospores·ml^{-1} 7 d after the formation of sporangia. This indicates that 7 d after outbreak sporangia show a declining viability.

Sporangia on potted grapevines that had been kept either at 22 °C, 30 % r.h. or 15-20 °C, 60-80 % r.h., were able to infect new leaf discs for at least 9 d after outbreak of sporangia (see the Table). This contradicts results of BLAESER (1978) who found that sporangia kept at 20 °C, 30 % r.h. or 20 °C, 70 % r.h. were viable for only 24-72 h. An explanation for this discrepancy could be that BLAESER kept sporangia on detached leaves in Petri dishes containing different salt solutions or sulphuric acid to obtain different concentrations of air humidity. In contrast, our experiment was performed on leaves of potted grapevines in the greenhouse exhibiting a more favourable moisture regime for the sporangia due to stomatal transpiration. ZACHOS (1959) investigated the durability of sporangia under natural conditions in Greek vineyards. He found that sporangia on sporulated lesions were viable for 4-8 d provided they were produced on leaves in the shadow of the canopy and maximum temperatures did not exceed 22 °C. He assumed a lifespan of sporangia between 4-8 d at 22 °C dependent on air humidity. If temperatures ranged between 22 and 25 °C the lifespan of sporangia was only 2 d. Our data show that the survival of sporangia can be much longer than 3 d if temperatures are near 20 °C. Thus models calculating *P. viticola* infections should take into account that sporangia might be viable for 8-9 d, however, with declining viability. The survival of sporangia at different temperatures is to be investigated in the future.

The authors are grateful to Dr. G. HILL (LLVA Oppenheim) for many useful suggestions and to K. DIEDEL (LVWO Weinsberg) for technical assistance.

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