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Research Note

Status and probability of occurrence of grey mold on preharvest and postharvest grapes in Maharashtra, India

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Botrytis cinerea is the major postharvest pathogen of grapes causing grey mold disease in many countries and requires specific interventions to prevent losses (Couderchet 2003, Latorre *et al*. 2015, De Simone *et al*. 2020). It is a weak pathogen and thrives in warm, wet and humid weather. Latent infections occur at bloom stage and late season infections at or after veraison stage at specific weather conditions of 15-20 °C, minimum 4 hours of wetness and RH > 92 % (Broome *et al*. 1995, Keller *et al.* 2003). Sawant *et al*. (2008) and many others did not report *B. cinerea* among the fungi causing postharvest decay of grapes during ambient or low temperature storage, but overseas buyers frequently reported incidences of *Botrytis* decay in grapes from India. In view of this persisting skepticism regarding its possible presence in vineyards, a holistic study was undertaken to re-examine the status of *B. cinerea* infections in vineyards and/or during storage.

Seventy-five commercial and research vineyards of *Vitis vinifera* L. table and wine grape cultivars in Maharashtra (Sangli, Pune and Nasik) and five vineyards from Karnataka (Shirguppi) were surveyed in March 2012 for presence of gray mold symptoms on bunch and/or berries. The grapes in these vineyards were near the harvest stage (average TSS, 20.78 ± 2.80 °Brix) which is the growth stage with high *B. cinerea* sensitivity. Additional twenty-five vineyards were surveyed during fruiting seasons at Nasik, Sangli and Pune for eight consecutive years, 2012-2019. All bunches on 120 vines from each vineyard were closely observed for typical symptoms of *Botrytis* berry rot (Latorre *et al*. 2015). To assess for latent infections, twelve bunches from each vineyard were harvested, the laterals were separated, surface sterilized and incubated in growth chambers at 20 °C and RH ≈ 92 % with a photoperiod of 12 h for up to 15 d. Another set of twelve bunches was stored at 0 °C for 30 d. Grape bunches in all the eighty vineyards were visibly healthy and there was no sign or symptom on berries typically associated with *B. cinerea* infection, even in the tight clustered wine grape varieties. Even after incubation in growth chambers, none of the grapes sampled from the eighty vineyards, developed symptoms of *B. cinerea* infection. Furthermore, survey of the twenty-five vineyards conducted during 2012-2019 fruiting seasons also showed no signs or symptoms of *B. cinerea* infections.

Enumeration of *B. cinerea* inoculum from the top six inches soil under the drip circles of grapevines from six locations was done using *Botrytis* selective medium, and 500 L of air volume was sampled on spore trap medium plates using HiAir Petri Air sampling System Mark II (Hi-Media, LA637) (Edwards and Seddon 2001). To validate the enumeration methods, soil and air from eight strawberry plots at Mahabaleshwar-Panchgani, with history of regular *Botrytis* infection, were also sampled. Plates were incubated at 15 °C for up to 10 d. *B. cinerea* colonies developed in plates seeded with the soil (28.2 ± 11.6 thousand cfu·g^{-1} soil) and the air (2.9 ± 1.5 cfu·500 L^{-1}) samples collected from strawberry fields, thereby validating the methods. No colonies developed in plates seeded with the soil and air samples from the six vineyards which highlights the absence of the inoculum.

The weather data on rainfall, RH, leaf wetness and temperature from October 2011 to December 2011, recorded on METOS automatic weather station installed in the vineyard of ICAR-National Research Centre for Grapes, Pune were assessed. The weather data corresponding to the full bloom stages of fourteen vineyards pruned at different dates was fed into the forecasting model of *Botrytis* (Pessl Instruments, Austria). The output on disease forecast was generated on a scale of 0 to 100 where 0 stands for no chance of infection and 100 for maximum infection. The data were also analyzed manually for its suitability for infection of *B. cinerea.* The model predicted no chance of infection and the manual assessment of weather data confirmed that there was no rainfall and required leaf wetness during the susceptible full bloom period stretching from 20th October to 9th December 2011.

To check whether *B. cinerea* could infect inflorescences on artificial inoculation and survive in vineyard under the regular fungicide spray program, an isolate obtained from strawberry was used. Studies were conducted during the 2012-13 fruiting season at ICAR-National Research Centre for Grapes, Pune on field-grown 'Thompson Seedless' table grape. Thirty inflorescences at full bloom stage were inoculated twice at 24 h interval by dipping them in glass beakers containing 1×10^{6} spores·mL^{-1} and were then covered with moistened polythene bags for 48 h to maintain the wetness required for infection. Inflorescences treated with sterile distilled water and untreated inflorescences were kept as control. Vines were maintained with regular viticultural operations. Fifty flower and berry samples each were collected at 48 h and 96 h after inoculation and then at 1-2 mm, 3-4 mm, 5-6 mm, 7-8 mm, 9-10 mm

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berry size, veraison and at harvest for isolations on potato dextrose agar plates amended with 250 ppm rifampicin. At maturity, twenty-four bunches were harvested and twelve bunches were incubated in humid chambers at 20 °C and RH ≈ 92 %, while other twelve bunches were incubated at 0 ± 0.5 °C and RH > 95 % for 30 d and then kept on shelf at 15 °C for 4-5 d. *B. cinerea* could be isolated from inoculated bunches till 7-8 mm berry size, but not at 9-10 mm size, at veraison and at harvest. *B. cinerea* did not develop on bunches stored in humid chambers or at low temperatures. The isolation frequency was 75 % at 48 h post-inoculation but subsequently reduced to 46 % at 7-8 mm berry size. There was no *B. cinerea* growth in flowers and berries collected from sterile distilled water treatment or the untreated control. However, the berries developing from inoculated inflorescences remained smaller in size (10.8 ± 0.9 mm diameter) but with higher total soluble solids (24.7 ± 0.2 °Brix) as compared to berries developing from uninoculated inflorescences (14.3 ± 0.7 mm diameter and 22.2 ± 0.1 °Brix).

The study has clearly brought out that gray mold is not present in vineyards in the major grape growing areas of peninsular India, probably due to the absence of the pathogen, *B. cinerea*. Even if accidently introduced in vineyards during susceptible growth stages under favourable weather conditions, we presume that it will not survive till veraison.

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