Use of Selenate-Resistant Strains as Markers for the Spread and Survival of *Botrytis cinerea* Under Greenhouse Conditions

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ABSTRACT

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Botrytis cinerea marked strains combining traits of fungicide resistance or sensitivity (carbendazim, iprodione) with resistance to selenate were created and assessed for use in studying the dispersal of *B. cinerea* and its survival inside plant tissue under greenhouse conditions. Marked strains differed in their ability to cause lesions and to disperse in the greenhouse. A strain that was the most aggressive in infecting plants was also the most successful in spreading across the greenhouse. Following 7 to 14 days of exposure to marked inoculum, about 90% of plants showed quiescent *B. cinerea* infection with no significant difference between hosts or seasons. However, in a warm season, most of the plants were infected with wild-type *B. cinerea,* whereas most of the winter-recovered *B. cinerea* strains were of the marked phenotype, showing the importance of local inoculum from within the glasshouse in winter. The air of the greenhouse contained the same population of marked *B. cinerea* in warm and in cold periods, whereas the total population was significantly higher in summer. In the warm season, mycelium of *B. cinerea* inside plant debris lost viability within 3 to 4 months, whereas it stayed viable for 4 months in the winter (December to March) and started to lose viability in April.

Additional keywords: beans, gray mold, pepper, strawberry.

Botrytis cinerea, the anamorph of *Botryotinia fuckelina*, attacks a wide range of plant species, causing gray mold on many economically important crops such as vegetables, ornamentals, herbs, bulbs, and fruits (11). Most genetic and population studies of *B. cinerea* have used mating type and fungicide resistance as markers (3,14), because auxotrophic markers are difficult to obtain and morphological markers are frequently unstable (17). Resistance to fungicides is widely spread in *B. cinerea* populations in Israel (12), which renders resistance markers useless for many population and epidemiological studies. Mating type also has a limited application in population monitoring because sexual structures are rare in natural conditions and rather difficult to obtain in the laboratory (13). To cope with these limitations, the toxic analogs of common nutrients, such as nitrates or sulfates, were proposed for obtaining nutritional markers suitable for population, epidemiological, and ecological studies of *B. cinerea (37).* Chlorate, a nitrate analog, is reduced by nitrate reductase to hypochlorite, which is toxic to the fungal cell. Chlorate-resistant, nitrate-nonutilizing *(nit)* mutants of numerous fungi, including *Botrytis (4), Colletotrichum (22), Fusarium (7),* and *Verticillium (24),* have been reported and have been usually used for studying vegetative compatibility. Several previous studies have exploited *nit* mutants as markers in greenhouse and field experiments, mostly concerning the survival and pathogenicity of the *nit* mutants which were similar to or somewhat less than those of the wild-type parents (18,32,38). *nit* mutants were successfully used for testing survival of nonpathogenic strains of F. *fujikuroi* and *oxysporum* in rakkyo *(Allium chinense)* plants and their colonization of roots (21); for studying population dynamics of *F oxysporum* f. sp. *spinaciae* after different soil treatments (33); for quantification of root and stem colonization of watermelon by *F oxysporum* f. sp. *niveum (41);* and for characterization of spatial and temporal dispersal of *F oxysporum* f. sp. *radicis-lycopersici* through plants and soil (29). *nit* mutants of *B. cinerea* were used for studying the spread of internal latent infection through primula plants (2) and for testing the influence of flower-feeding thrips on Botrytis bunch rot in grapes (26).

Selenate, a toxic analog of inorganic sulfate, is inhibitory in many biological systems, and resistant mutants with altered sulfate transport and assimilation have been characterized in several fungal genera, including *Acremonium (34), Aspergillus (1,16), Botrytis (37), Fusarium (8), Magnaporthe (19),* and *Neurospora (27).* Information about the use of selenate-resistant mutants in ecological studies is limited. In greenhouse tests, selenate-resistant mutants of *M. grisea* were pathogenic on rice and indistinguishable from the corresponding wild type in their virulence (19), and the pathogenicity of selenate-resistant mutants of *B. cinerea* on bean was similar to that of the parent strains (37). To our knowledge, marked strains that combine the trait of resistance to selenate (or chlorate) with any other trait have not been constructed previously. Such double marked strains could widen the scope for exploiting marked strains in

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