CHAPTER 11

DETECTION, QUANTIFICATION AND IMMUNOLOCALISATION OF *BOTRYTIS* SPECIES

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Abstract. Classical methods of detection of *Botrytis* species include plating-out of surface sterilized infected plant tissues, soils and airborne conidia on selective media and the identification, by microscopy, of the sclerotia, conidia and conidiophores, based on their characteristic shape, size and colour. Other methods are now available such as nucleic acid-based methods that can be used to track individual isolates or specific species. The determination of biomass levels in samples using these methods, however, is problematic because of the multinucleate nature of *Botrytis* conidia and thallus. Immunological methods employing genus-specific monoclonal antibodies, particularly quantitative laboratory-based plate-trapped antigen ELISAs, allow large numbers of samples to be processed easily within a few hours. These methods, combined with the modified plate spore trap, the Micro-Titre Immuno Spore Trap (MTIST), enable the quantification of conidia in microtitre wells. A rapid semi-quantitative immuno-chromatographic lateral flow device designed for use in the field or office promises to be a useful screening device for *Botrytis*. Development of species-specific monoclonal antibodies remains a challenge. The usefulness of Fourier transform infrared spectroscopy, nuclear magnetic resonance, liquid chromatography-mass spectroscopy and enzymic methods to detect and quantify specific secondary metabolites produced by *Botrytis* remains to be fully demonstrated.

1. Introduction

Detection and quantification of *Botrytis* infections in plants, seeds, air-borne conidia and sclerotia in soils has, until recently, depended on the plating out of infected material and the microscopic identification of sclerotia, conidia and conidiophores on the basis of their size, shape and colour. Although these methods yield valuable information, they are limited. Plating out is a time-consuming process in which surface sterilization is a general pre-requisite. Other methods are now becoming available that either yield more specific information, as is the case with molecular methods, or are faster and more easily replicated, as with immunological methods. These various methods will be addressed separately along with other quantitative methods.

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Sampling is a problem inherent to any detection assay. There are no sampling techniques unique to *Botrytis* spp., which has a generally rare (Poisson) spatial distribution and/or clumped (negative binomial) and sampling should be performed with the biology and epidemiology of the system being examined in mind (e.g. Marois et al., 1993). In the case of epidemiological studies, some stratification is often necessary to make meaningful inferences and different techniques are needed for different environments to address specific questions.

Assessing the extent of infection within a sample is also problematic. Care has to be taken when making comparisons that employ different methods. For example, the level of infection in a sample of grape berries assessed on a weight for weight basis will be different from that assessed on a number basis because rotted berries generally weigh less (Dewey et al., 2000). Estimates of fungal biomass of foliar infections are commonly determined by measuring lesion area (Elad et al., 1994), but these estimates are clearly different from levels based on percentage of leaf area that is sporulating (Köhl et al., 1995), the numbers of conidia derived from sporulating tissues by shaking at high speed in tap water with a detergent (Gerlagh et al., 2001) or CFU/cm² from macerated leaf samples plated out by limiting dilution (Lennox et al., 2003). The use of immunological methods to determine fungal biomass in individual and massed infections is promising (Sect. 3).

### 2. Classical plating out method

Common methods of surface sterilizing plant material prior to plating out include immersing excised tissues/seeds or fruits sequentially in sodium hypochlorite, ethanol and sterile distilled water (Meyer et al., 2000; Coertze and Holz, 2001). Where infected material is already sporulating identification can be confirmed by plating out single conidia picked up with a sterile needle. Special methods are employed for the detection of latent infections. Dipping berries in alcohol, freezing them at –20°C for a short time and then incubating in a moist chamber at room temperature for 7-10 days until the fungus has sporulated has proved to be an effective simple method (Mundy and Beresford, 2003). Others have induced sporulation by treatment of surface sterilized tissues or fruits with paraquat to reveal latent infections in grape berries (Gindrat and Pezet, 1994; Pezet et al., 2003), strawberries (Sutton et al., 1997), sweet cherry fruit (Adaskaveg et al., 2000) and fallen rose petals (Morandi et al., 2000).

Semi-selective and differential media are based on the selective inhibition of competing microbes, the encouragement of the target organism’s growth and/or the expression of a characteristic property of *Botrytis*. Viable conidia will germinate on many media, in the presence of free water, nitrogen and phosphate, and form colonies, but do not always sporulate. Confirmation of growth of any species of *Botrytis* requires microscopic examination. A medium based on Martin’s rose bengal agar amended with several fungicides and high concentrations of antibiotics was found satisfactory for assaying organic soils (Lorbeer and Tichelaar, 1970). Kritzman and Netzler (1978) developed a medium for isolation of *Botrytis* species from soil and onion seed based on the development of dark pigments in the medium.