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Short communication

A new method for producing mycelium-free conidial suspensions from cultures of *Microdochium nivale*

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Abstract

A technique to improve the sporulation of *Microdochium nivale* in culture and to produce mycelium-free conidial suspensions was evaluated using cellophane-covered potato dextrose agar (PDA). Time to sporulation was significantly shorter on the cellophane-covered PDA ($P < 0.001$), yields of conidia were higher ($P < 0.01$) and conidial suspensions were produced virtually free of the mycelial fragments present in suspensions from PDA only. The conidial inoculum produced on cellophane had lower pathogenicity to wheat cv. Equinox in a detached leaf assay, showing significantly longer incubation periods ($P < 0.05$) and latent periods ($P < 0.01$), than conidia produced on PDA alone. However, the apparent decline in pathogenicity of conidial suspensions produced on cellophane compared to PDA alone was small.

Microdochium nivale is well documented as a causal agent of seedling blight, pink snow mould, stem rot and as a member of the *Fusarium* head blight (FHB) complex (Parry et al., 1995). Conidial suspensions have been used in the preliminary development of a detached leaf test to screen for whole plant resistance to FHB (Diamond and Cooke, 1999). However, some isolates of *M. nivale* show poor sporulation; thus scraping the colony and agar surface to yield a conidial suspension results in contamination of conidial suspensions with mycelial fragments. The use of cellophane-covered agar (Cooke and Brokenshire, 1975) in this case potato dextrose agar (PDA) and PDA alone was evaluated using eight single-spore isolates of *M. nivale* var. *majus* and four of var. *nivale*. Six isolates of *M. nivale* var. *majus* were isolated from wheat seed from the Irish 2001 harvest. A further two isolates of *M. nivale* var. *majus,* 2/2/M and 44/3/M and four isolates of *M. nivale* var. *nivale*, 44/S/N, SO20/1/N, SO28/2/N and SO48/1/N were obtained courtesy of Paul Nicholson, John Innes Centre, Norwich, UK, originally isolated from wheat seed by Simon Edwards, Harper Adams University College, Newport, UK. The cellophane membranes were cut to a 6-cm diameter from uncoated non-moisture proof cellophane cellulose film type PT300 (British Cellophane Ltd., Bridgewater, Somerset, UK), autoclaved in distilled water to remove plasticisers and to sterilise the cellophane, and each placed flat on the agar surface in separate 7 cm Petri dishes. Inverted plugs of mycelium, 6-mm diameter, taken from the edge of a growing colony were placed in the centre of the cellophane-covered PDA plates and incubated on coolplates (Cooke, 1980) for 7 days under a diurnal cycle of near-ultraviolet (NUV) and white light. Conidial suspensions were prepared by scraping the agar with 10 ml sterile distilled water (SDW) in the case of the PDA only plates. On the cellophane-covered PDA plates, the cellophane membrane was peeled off and inserted into universal bottles where conidia were gently removed by hand shaking for ~20 s in 10 ml of SDW with the exception of isolate SO48/1/N where the cellophane had markedly disintegrated making it impossible to peel the membrane off the agar surface. For this isolate the conidial suspension was prepared

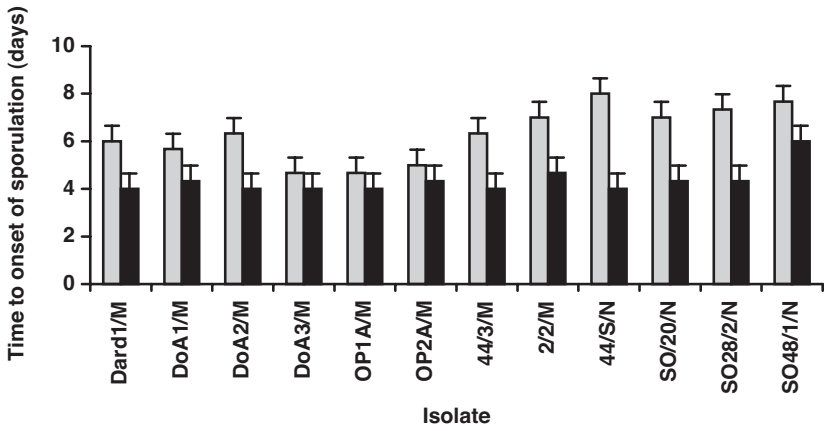

Figure 1. Time to onset of sporulation of isolates of *M. nivale* var. *majus* (M) and *M. nivale* var. *nivale* (N) on PDA only □ and cellophane-covered PDA ■. Bars represent standard errors of the mean and apply to all data.

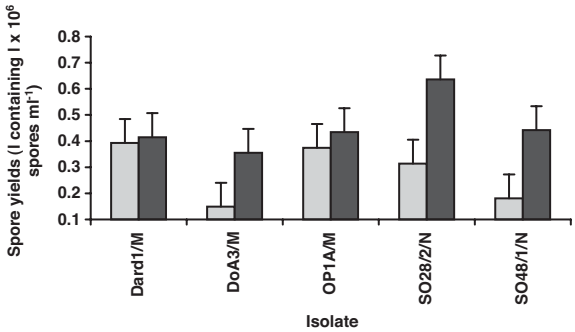

Figure 2. Volume of inoculum produced (l) containing 1×10^6 spores ml^{-1} of isolates of *M. nivale* var. *majus* (M) and *M. nivale* var. *nivale* (N) on PDA only □ and cellophane-covered PDA ■. Bars represent standard errors of the mean and apply to all data.

by scraping the surface as with the PDA only plates. The resulting spore suspensions were adjusted to 1×10^6 conidia ml^{-1}.

The cellophane method gave greater sporulation of all isolates of *M. nivale*, with a significantly shorter time to the onset of sporulation ($P < 0.001$) (Figure 1) and significantly higher yields of conidia ($P < 0.01$) (Figure 2), compared to the PDA alone. There was no significant method × isolate interaction although the increase in conidial inoculum was particularly high for isolates DoA3/M, SO28/2/N and SO48/1/N that showed poor sporulation on PDA alone, with smaller increases for isolates Dard1/M and OP1A/M which showed good sporulation using both techniques. The conidial suspension produced on cellophane was virtually free from mycelium whereas that produced on PDA

Figure 3. Sporulating colony of *M. nivale* var. *majus* isolate Dard1/M on cellophane membrane removed from the PDA surface after 7 days under a diurnal cycle of NUV and white light at 20 °C.

alone contained mycelial fragments. The cellophane membrane may have improved sporulation by acting as a permeable or semi-permeable membrane, enhancing photosporogenic effects of the diurnal cycle of NUV and white light by starvation stimulation of mycelium, but enabling access to sufficient nutrients to allow abundant sporodochia to form (Figure 3). The cellophane became progressively brittle with time suggests the

presence of fungal cellulases. The cellophane could therefore also have acted as an additional substrate sources of cellulose or its breakdown products, so stimulating sporulation.

The different methods of conidial spore production were evaluated for pathogenicity on the wheat cv. Equinox using 10 isolates of *M. nivale*, seven of var. *majus* and three of var. *nivale*, in a factorial experiment with three randomised blocks. Preparation of leaf material and inoculation of the detached leaves were carried out as described by Diamond and Cooke (1999). After inoculation, the leaves were incubated on a coolplate (Cooke, 1980) at 20 °C under a 24-h diurnal cycle of NUV and white light. The components of partial disease resistance measured were incubation period (days from inoculation to symptom development) and latent period (days from inoculation to sporulation). Assessments of symptom appearance and sporulation were carried out daily under a compound microscope (magnification 40×). The pathogenicity of the conidial suspensions produced on the cellophane-covered PDA were lower than those of the inoculum produced on PDA alone resulting in a significantly longer incubation period ($P < 0.05$) (Figure 4a) and latent period ($P < 0.01$) (Figure 4b). However the decline in pathogenicity was small with no significant method × isolate interactions. Cooke and Brokenshire (1975) found no significant difference in the pathogenicity of the inoculum of *Selenophoma donacis* (cause of halo spot disease of barley) produced in culture with or without

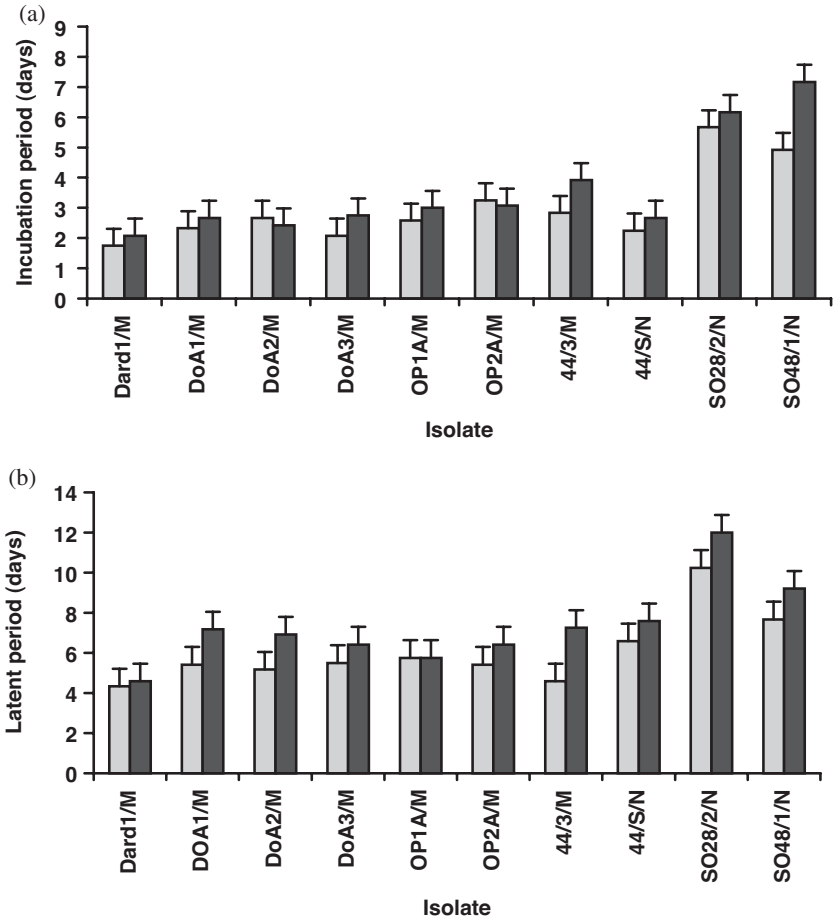

Figure 4. Incubation period (a) and latent period (b) on detached wheat leaves of cv. Equinox using conidial inoculum produced on PDA only □ and cellophane-covered PDA ■ of seven isolates of *M. nivale* var. *majus* (M) and three isolates of *M. nivale* var. *nivale* (N). Bars represent standard errors of the mean and apply to all data.

cellophane. Reduced pathogenicity of the conidial suspensions produced on cellophane may be due to the absence of mycelial inoculum and/or PDA residues compared to PDA alone, both of which could have enhanced the development of the pathogen.

The use of the cellophane technique to produce conidial spore suspensions of *M. nivale* has a number of advantages, namely the shorter period to the onset of sporulation and the increase in the yield of mycelium-free conidial inoculum produced in culture, particularly for isolates originally showing poor sporulation.

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