Appendix 2B: Conidial Production

The proposed seedling inoculation method reported by Mitter *et al.* (2006) required production of a suspension containing 10^6 conidia per mL. Four media types for culturing *F. pseudograminearum* were tested for the ability to stimulate adequate conidial production (Table 2B.1). Spezieller nährstoffarmer agar was not expected to produce great numbers of conidia as it is used to maintain the culture on a low nutrient medium, and therefore not allow profuse growth (Leslie & Summerell, 2006). Czapek Dox, a moderately high nutrient medium, has been used for *F. pseudograminearum* growth (Wildermuth & McNamara, 1994) but the potential for conidial production was unknown. Mungbean agar has previously been reported to allow profuse conidial production (Mitter *et al.*, 2006) while using combination white and black fluorescent lights. Starch nitrate agar had not been reported for *F. pseudograminearum* growth. It has mainly been used for the fungus *Bipolaris sorokiniana* but in initial tests with *F. pseudograminearum* it stimulated profuse growth of *F. pseudograminearum* conidia and hyphae.

Table 2B.1. Agar types tested for conidial production[a].

Agar Type
Starch nitrate (Dodman & Reinke, 1982)
Spezieller nährstoffarmer (Nirenberg, 1976)
Mungbean (Gale *et al.*, 2002)
Czapek dox (Thom & Raper, 1945)

[a]Details of media components are listed below.

Agar Media Components

Starch Nitrate Agar

Agar	36g
$NaNO_3$	7g
K_2HPO_4	2.33g
$MgSO_4.7H_2O$	1.17g
KCl	1.17g
Starch (soluble)	23.33g
H_2O	2100mL
$FeSO_4.7H_2O$/EDTA	23.33mL
(4.98g/L $FeSO_4$.7H2O + 5.96g/L EDTA (Na)	

1. Dissolve starch separately in the water while cold in glass beaker (crush lumps first)
2. Put solution on heater stirrer
3. Weigh dry ingredients
4. At near boiling add dry ingredients, while stirring

5. When all dissolved take off heat and only then add $FeSO_4 7H_2O$/EDTA
6. Pour mixture out into 250mL bottles
7. Autoclave the solution
8. Then pour into Petri dishes in the laminar flow cabinet.

Spezieller Nährstoffarmer Agar

KH_2PO_4	1g
KNO_3	1g
$MgSO_4.7H_2O$	0.5g
KCl	0.5g
Glucose	0.2g
Sucrose	0.2g
Agar	20g
H_2O	1L

1. Ensure all ingredients are thoroughly mixed.
2. Autoclave and pour into plates.

Mungbean Agar

Mungbeans	40g
Agar	15g
H_2O	1L

1. Boil 40g of mungbeans in 1L of water for 20 minutes.
2. Filter through cheesecloth.
3. Add 15g of agar.
4. Autoclave and pour into plates.

Czapek Dox Agar

Sucrose	30g
$NaNO_3$	2g
K_2HPO_4	1g
$MgSO_4$	0.5g
KCl	0.5g
FeSO4	0.01g
Agar	15g
H_2O	1L

1. Ensure all ingredients are thoroughly mixed.
2. Autoclave and pour into plates.

Agar blocks of *F. pseudograminearum* isolate A03#24 grown on spezieller nährstoffarmer agar were used for sub-culturing. Growth on each media type was tested at 22-24 °C with either no light (dark) or a combination of white and black fluorescent lights with a 12 hour photoperiod. A similar test was also performed using the same isolate passaged from infected wheat seedling tissues. Each set of conditions was replicated twice, except for starch nitrate agar (dark), which was replicated three times.

Conidia were harvested by flooding the plates with 5 mL of a 6% Tween20 solution, scraped with a fine paint brush and filtered through two layers of cheesecloth. The suspension was diluted to 25 mL with MilliQ water. Six separate conidial counts of solutions from each agar plate were performed using a counting chamber (Weber and Sons, Lancing, United Kingdom).

Results

A conidial count of at least 200 conidia/0.2mm^3 was needed to make inoculum to the recommended concentration of 10^6 conidia/mL. Starch nitrate agar was the only medium which performed to this level, allowing consistently greater conidial production than any of the other three mediums tested (Fig. 2B.1). Conidia grown on starch nitrate agar showed no obvious deformities. Use of cultures recently isolated from infected plants (passaged) and use of combination white and black light conditions did not increase the conidial production on starch nitrate agar compared to growth in darkness. It is not known why the mungbean agar performed so poorly, even under a black light, as it has been reported to allow profuse conidial growth (Gale *et al.*, 2002; Mitter *et al.*, 2006). Maintaining *F. pseudograminearum* on spezieller nährstoffarmer agar and then sub-culturing onto starch nitrate agar, followed by growth for 14 days at 22-24 °C in the dark proved the most simple and effective method to produce sufficient conidia for inoculum production. The final method is reported in Chapter 2.

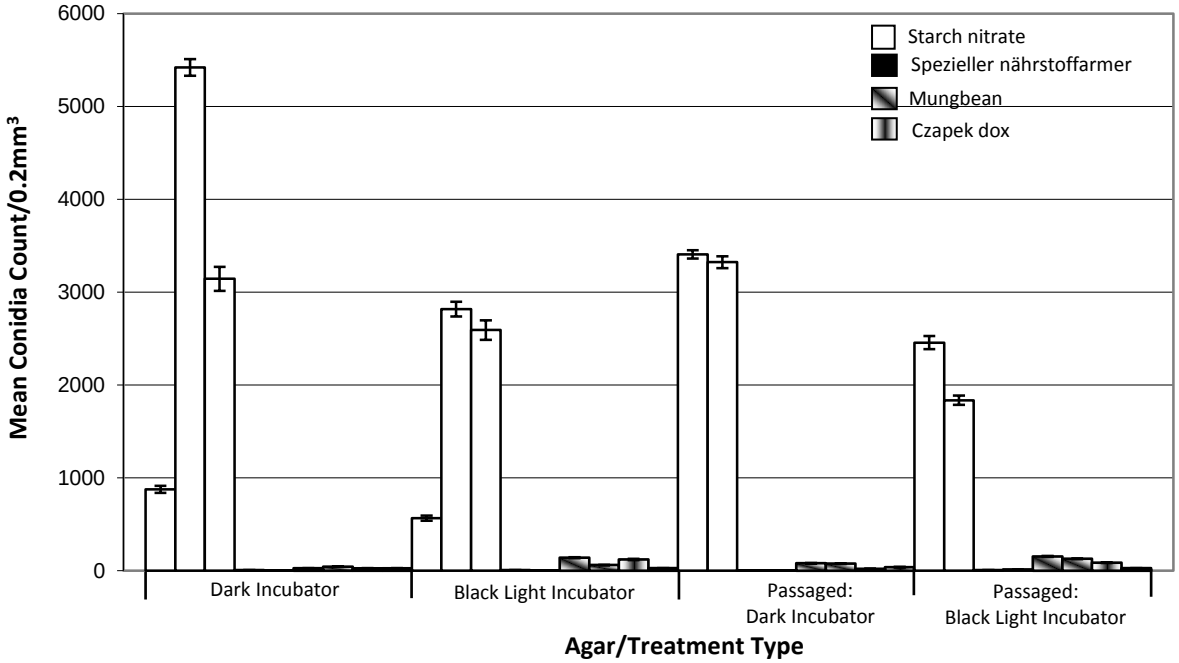

Figure 2B.1. Mean condial counts from four media types incubated under blacklight or in the dark using either cultured or passaged inoculum. Replicates of each media are shown separately. Bars represent the standard error of the mean of six conidial counts from a single agar plate.