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APPENDIX 1

FUNGAL CULTURE MEDIA

1. Potato Dextrose Agar (PDA)

Potato	200 g
Dextrose	20 g
Agar	20 g
Water	1 L

Boil cleaned and chopped potatoes in water until soft (about 1 h).

Mash and squeeze as much of the pulp as possible through fine nylon gauze.

Add dextrose and agar.

Make up to 1 L with water.

Autoclave at 103 kPa (121 °C) for 20-30 min.

1/4 PDA is made using only 1/4 of the ingredients above made up to 1 L.

2. Czapek Dox Agar (CDA)

$NaNO_3$	2 g
$MgSO_4.7H_2O$	0.5 g
KH_2PO_4	1 g
KCl	0.5 g
$FeSO_4.7H_2O$	0.01 g
Sucrose	30 g
Agar	20 g
Distilled H_2O	1 L

Autoclave at 103 kPa (121 °C) for 20-30 min.

Czapek Dox broth is made using the same ingredients but omitting the agar.

3. Tap Water Agar (TWA)

Agar	15 g
Tap water	1 L

Autoclave at 103 kPa (121 °C) for 20-30 min.

4. Modified Alphacel Medium (MAM)

Oatmeal	10 g
$MgSO_4.7H_2O$	1 g
KH_2PO_4	1.5 g
$NaNO_3$	1 g
Coconut milk	50 mL
Agar	17 g
Distilled H_2O	1 L

Combine ingredients and autoclave at 103 kPa (121 °C) for 20-30 min.

Coconut milk NOT coconut cream should be used.

Modified alphacel broth is made using the same ingredients but omitting the agar.

5. Oatmeal Agar (OMA)

Oatmeal	20 g
Agar	20 g
Distilled H_2O	1 L

Combine ingredients and autoclave at 103 kPa (121 °C) for 20-30 min.

6. Malt Extract Agar (MEA)

Malt extract	20 g
Agar	20 g
Distilled H_2O	1 L

Boil malt extract with a little water until dissolved and make up to 1 L.

Autoclave at 103 kPa (121 °C) for 20-30 min.

Malt extract broth is made using the same ingredients but omitting the agar.

7. V8 Juice Agar (V8A)

V8 Juice	200 mL (Campbell's Soup Co.)
Agar	20 g
Distilled H_2O	800 mL

Combine ingredients and adjust to pH 6 by dropwise addition of 10 N KOH.

Autoclave at 103 kPa (121 °C) for 20-30 min.

Clarified V8 Agar (CV8A) is made as above but the V8 Juice is centrifuged for 30 min at 2800 rpm.

V8 Juice + benomyl Agar (V8 + b) is made as above for V8A with the inclusion of 16 mg benomyl (Grbavac, 1981).

V8 Juice broth is made using the same ingredients as V8A but omitting the agar.

8. *Bromus* Leaf Extract Agar (BLEA)

Senescent leaves of *B. diandrus*	15 g
Agar	20 g
Distilled H_2O	1 L

Leaf pieces are brought to the boil in 1 l of distilled H_2O and simmered for 15 min.

Resultant solution is filtered through several layers of nylon gauze (1 mm aperture) and made up to 1 l with distilled H_2O.

Autoclave at 103 kPa (121 °C) for 20-30 min.

Bromus leaf broth is made using the above ingredients but omitting the agar.

Wheat leaf extract agar (WLEA) and wild oat leaf extract agar (WOA) are made as for BLEA but substituting either wheat or wild oat leaf pieces respectively into the recipe.

9. Sucrose Water Broth (SWB)

Sucrose	30 g
Tap water	1 L

Combine ingredients and autoclave at 103 kPa (121 °C) for 20-30 min.

10. Medium Of Tomás & Bocku: (1987) (T&B)

$MgSO_4.7H_2O$	0.5 g
K_2HPO_4	1 g
NaCl	0.1 g
$CaCl_2$	0.13 g
Ammonium tartrate	5 g
Sucrose	9 g
Yeast extract	1 g
Distilled H_2O	1 L

Autoclave at 103 kPa (121 °C) for 20-30 min

11. Sucrose Asparagine Broth (SAB)

Sucrose	3 g
L-asparagine	0.675 g
K_2HPO_4	1.3 g
KH_2PO_4	1 g
KCl	0.5 g
Mg $SO_4.7H_2O$	0.5 g
$FeSO_4$	0.01 g
$ZnSO_4$	0.002 g
$MnCl_2.4H_2O$	0.001 g
Distilled H_2O	1 L

Combine ingredients and autoc lave at 103 kPa (121 °C) for 20-30 min.

12. Fries Medium (Fries)

Ammonium tartrate	5 g
NH_4NO_3	1 g
$MgSO_4.7H_2O$	0.5 g
KH_2PO_4	1.3 g
K_2HPO_4	2.6 g
Sucrose	30 g
Yeast extract	1 g

Trace element stock solution 2 mL

Trace element stock solution ingredients

LiCl	167 mg
$CuCl_2.H_2O$	107 mg
H_2MoO_4	34 mg
$MnCl_2.4H_2O$	72 mg
$CoCl_2.4H_2O$	80 mg
H_2O	1 L

Combine ingredients and autoclave at 103 kPa (121 °C) for 20-30 min.

APPENDIX 2

PREPARATION OF MATERIAL FOR MICROSCOPY

1. Light Microscopy

Leaf pieces were cleared and stained using a modification of the method of Keane, Limongiello & Warren (1988). The stain was prepared as follows:

ethanol 95 %	100 mL
chloroform	150 mL
lactic acid 90 %	125 mL
phenol	150 g
chloral hydrate	450 g
Chlorazol Black E	2 g (Sigma Chemical Co., Colour Index 30235)

The ingredients are mixed together and left stirring overnight with a magnetic stirrer.

The solution is filtered through several changes of Whatman No. 1 filter paper.

Leaf pieces are placed in the solution for 2 -7 days and agitated on a rotary shaker.

Leaf pieces are removed from the stain, rinsed with distilled water and placed in a saturated solution of chloral hydrate for 1 - 4 days until destained sufficiently for observation.

The stain should be prepared and used in a fume hood whilst wearing gloves. The bottle should be labelled "carcinogen".

2. Scanning Electron Microscopy

Primary fixation in 3 % glutaraldehyde in 0.1M Sorensens phosphate buffer, pH 7 for 12 h.

Buffer wash in 0.1M Sorensens phosphate buffer, pH 7 for 1 h (4 x 15 min changes).

Post-fixation in 1 % OsO_4 in 0.1M Sorensens phosphate buffer for 12 h.

Buffer wash in 0.1M Sorensens phosphate buffer, pH 7 for 1 h (4 x 15 min changes).

Dehydration in 50, 70, 80, 90, 95 and 100 % ethanol, x 2 changes of 10 minutes each.

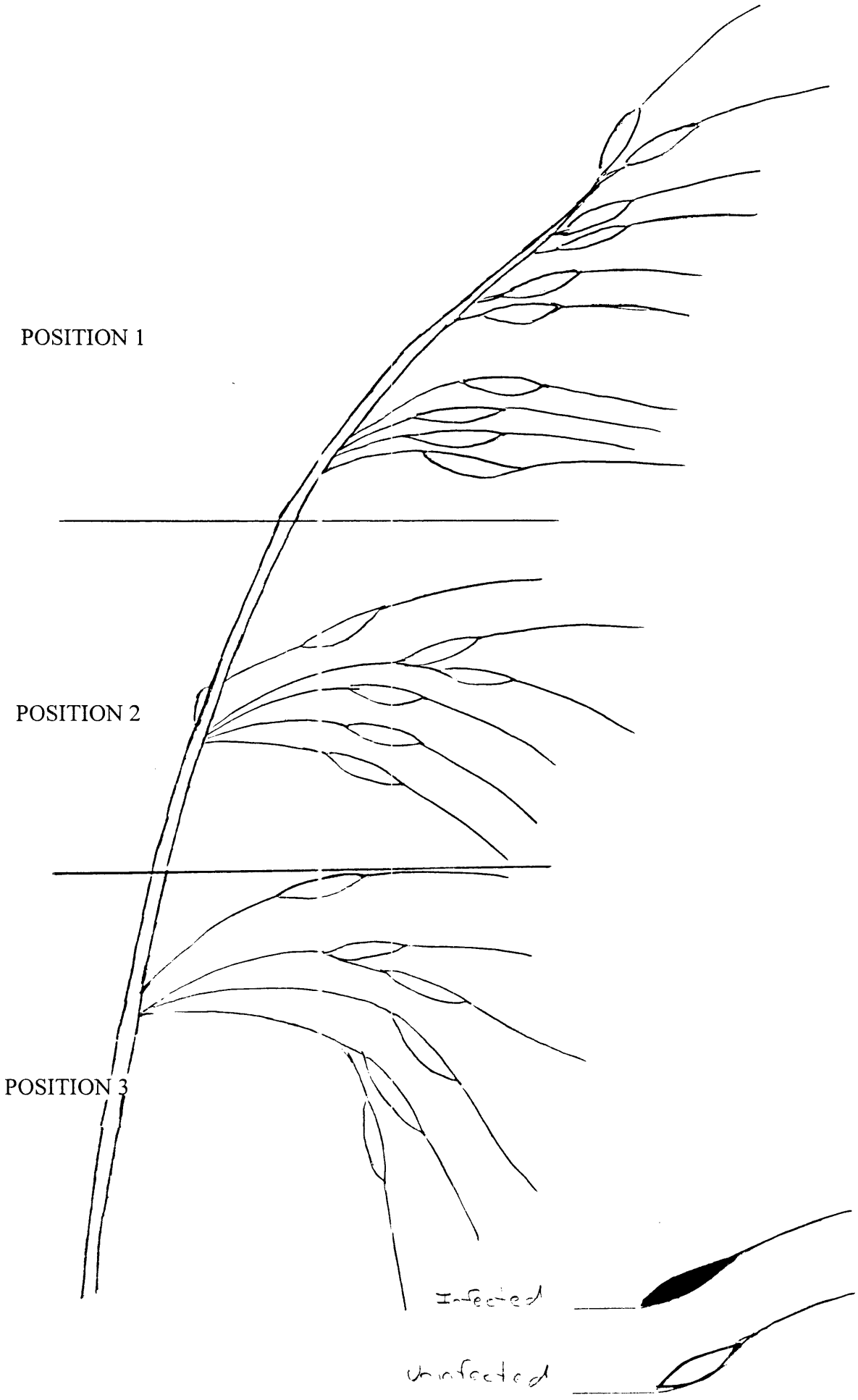
Specimens were placed in small wire baskets in a boat of 100 % ethanol and critical point dried in a Polaron Critical Point Dryer.

Specimens were mounted on brass stubs with "double-sided" adhesive tape and sputter coated with gold for 4 minutes at 2.2 kV.

APPENDIX 3

INFLORESCENCE MAPS

Inflorescence maps were used in one field study reported in Chapter 8 to determine the position on the inflorescences of *B. diandrus* that was most susceptible to infection by *P. semeniperda*. Seeds were removed from individual florets and examined for stromatal development of *P. semeniperda*. The position of the floret (either infected or not) was marked on a schematic inflorescence map in one of three inflorescence positions as shown on the next two pages. When an inflorescence contained more florets than the schematic, extras were appended. When an inflorescence contained fewer florets than the schematic, a cross was placed on the schematic floret to denote this.


POSITION 1
POSITION 2
POSITION 3
Infected
Uninfected

Rep No: 8 Treatment: RL1 68%

APPENDIX 4

STANDARD ERRORS OF DATA IN CHAPTER EIGHT

Table 8.1. The effect of inoculation of field grown wheat and *B. diandrus* with *P. semeniperda* on caryopses germination and development. when caryopses were harvested in January 1993. Data are standard errors of the mean.

Species	Germ. %		Emerge. %		Col. length (mm)		Germ. seeds with stroma[a]		Ungerm. seeds with stroma[b]	
	U	I	U	I	U	I	U	I	U	I
B. diandrus	1.4	2.2	1.4	2.4	1.3	2.8	0	0	0	1.5
Wheat	1.2	3.5	1.5	3.2	1.2	2	0	0.5	0	1.1

[a]The proportion of seeds that germinated and showed stroma of *P. semeniperda.*

[b]The proportion of seeds that were ungerminated and showed stroma of *P. semeniperda.*

Table 8.2. The effect of inoculation of field grown *B. diandrus* with *P. semeniperda* on caryopses germination and development:

1. When caryopses were harvested in February 1993 and
2. When caryopses were harvested in January 1993 and incubated at 99 % relative humidity for 1 month.

Data are standard errors of the mean.

Harvest	% Germ.		% Emerge.		Col. length (mm)		% Stroma	
	U	I	U	I	U	I	U	I
Feb. 1993	0.7	1.4	3	1.4	1.2	0.6	0	2.3
Jan. 1993	0.5	2.4	2.4	2.7	1.7	0.8	0	1.4

Table 8.3. Effect of different inoculun types of *P. semeniperda* used to inoculate field grown *B. diandrus* and their effect on caryopses germination and development, when caryopses were harvested in January 1994 and 1995. Data were not pooled for separate trials because heterogeneity of variances was detected with Bartlett's tes . Data are standard errors of the mean.

Inoculum type	% Germ.		% Emerge.		Col. length (mm)		Germ. seeds with stroma[a]		Ungerm. seeds with stroma[b]	
	94	95	94	95	94	95	94	95	94	95
Myc[c]	3.1		3		1.3		0.4		1.3	
Myc + suc	2.7	4.1	2.6	1.3	1.5	1.8	1	0.3	2.1	1.7
Alginate[c]	1.6		3.1		1.2		0		1	
Conidia*	2.2	3.3	1.8	2.1	1.8	1.4	0.5	0.2	1.2	3.2
Con + toxin[d]		2.5		4.1		1.2		1		2.8
Toxic filtrate[d]		1.4		2.6		2.1		0.3		0.3
Uninoc	1.2	1.1	1.4	1.9	1.3	1.7	0	0	0.4	0.2

[a]The proportion of seeds that had germinated and showed stroma of *P. semeniperda*.

[b]The proportion of seeds that were ungerminated and showed stroma of *P. semeniperda*.

[c]These treatments were only trialed in the 1993/94 season.

[d]These treatments were only trialed in the 1994/95 season.

*Mean values of all post-anthesis conidia treatments.

Table 8.4. The effect of inoculation of field grown grass species with *P. semeniperda* on caryopses germination and development, when caryopses were harvested in December 1993. Data are standard errors of the mean.

Species	% Germ		% Emerge		Col length (mm)		Germinated seeds with stroma[a]		Ungerminated seeds with stroma[b]	
	U	I	U	I	U	I	U	I	U	I
Wheat	1.2	2.4	1.4	3.5	1.2	2.1	0	2.4	0.5	1.2
B. diandrus	1.6	2.3	1.6	2.8	1.3	1.8	0	3.4	1	5.3
A. fatua	1.1	1.8	1.4	2.4	1.2	1.7	0	1.1	0.2	0.6
L. rigidum	2.1	2.2	2.4	3.1	1.5	2.1	0	1.8	1	0.7
H. leporinum	1.4	1.5	1.5	2.2	1.5	1.9	0	4.1	0.6	1.3
V. bromoides	1.4	2.3	1.8	2.5	0.4	0.9	0	3.9	0.8	2.3

[a]The proportion of seeds that had germinated and showed stroma of *P. semeniperda*.

[b]The proportion of seeds that were ungerminated and showed stroma of *P. semeniperda*.