To determine the effects of Protector and HML32 on *Botrytis cinerea* germination on microscope slides in the lab.

Methods

Wednesday 15 August 2012

Protector and HML32 concentrations

1. Make up stock solutions of **Protector** and HML32. **Mix well** and pipette 1 ml into a McCartney bottle
2. Add 9.0ml the ***Botrytis cinerea*** spore suspension to each tube, to a give a total volume of 10 ml (see table) then mix. Note - a total of about 60ml of the ***Botrytis cinerea*** spore suspension will be required.
3. Pipette two 75µl drops onto each slide and place a cover slip over each drop.
4. Place slides at high humidity at approx 20˚C on the lab bench.

Slides numbered	Number of slides	Volume and concentration of stock solution	Volume ***Botrytis*** spore suspension	Final concentration on slide
0,0,1,2,3,4,	6	1.0 ml H_2O	9.0 ml	0%
5,6,7,8,	4	1.0 ml of Soln 1 Soln 1 = 10 ml protector /100mL	9.0 ml	10ml Protector /litre
9,10,11,12	4	1.0 ml of Soln 2 Soln 2 = 13.5g HML32/100 ml	9.0 ml	13.50g HML32/litre
13,14,15,16	4	1.0 ml of Soln 3 Soln 3 = 13.5g HML32 + 3.0g Kumulus DF/100 ml	9.0 ml	13.50g HML32 + 3.00g Sulphur(Kumulus DF)/litre
17,18,19,20	4	1.0 ml of Soln 4 Soln 4 = 6.75g HML32/100 ml	9.0 ml	6.75g HML32/litre
21,22,23,24	4	1.0 ml of Soln 5 Soln 5 = 27.0g HML32/100 ml	9.0 ml	27.00g HML32/litre

Notes; The concentration of the ***Botrytis cinerea*** spore suspension was 2.1 $x10^5$ ***Botrytis cinerea*** conidia / ml. This means the final 10 ml mixture would have contained approximately 1.9 $x10^5$ ***Botrytis cinerea*** conidia / ml.

Thursday 16 August 2012

After 24 hours: place lacto aniline blue stain next to the cover slips on one of the slides numbered '0' (control), to check germination. If okay, check the other slide numbered '0'. If okay, stain all other slides and count the number of germinated ***Botrytis cinerea*** conidia out of 100 in four areas of each of the slides. Record averages in table below:

Results

Slide number	Treatment name	**% *Botrytis*** germination after 24 hrs					Max. germ tube length after 24h (µm)
		Rep 1	Rep 2	Rep 3	Rep 4	mean	
1,2,3,4,	0%	77	66	69	70	71	425
5,6,7,8,	10ml Protector /litre	0	0	0	0	0	-
9,10,11,12	13.5g HML32/litre	0	0	0	0	0	-
13,14,15,16	13.5g HML32 + 3.0g Sulphur/litre	0	0	0	0	0	-
17,18,19,20	6.75g HML32/litre	0	0	0	0	0	-
21,22,23,24	27.0g HML32/litre	0	0	0	0	0	-

Notes; Slides 1,5,9,13 & 17 were checked on 16th August but as there were no germinated ***Botrytis cinerea*** spores on any of the treatment slides all the other slides were examined at a later date. For the control slides the germinated ***Botrytis cinerea*** conidia appeared to be mostly confined to the outer edges of the coverslip so it could be assumed that oxygen was necessary for germination. For this reason only the ***Botrytis cinerea*** conidia around the outer edges of the coverslip were examined for possible germination.

Photos

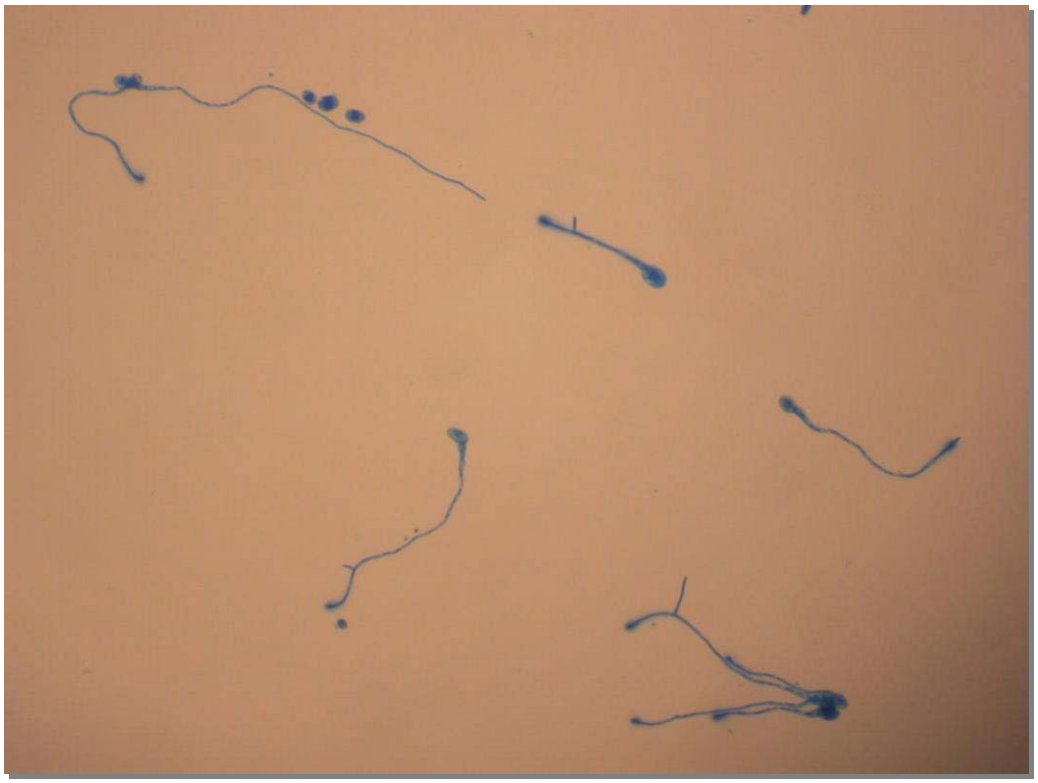

Figure 1. Control example. *Botrytis cinerea* spores (conidia) stained with aniline blue on treatment-free (water only) microscope slide at 200x magnification showing characteristic long Botrytis germination (germ) tubes. Photographed on 5th September 2012.

Figure 2. 1% Protector example. *Botrytis cinerea* stained with aniline blue on microscope slide at 200x magnification showing ungerminated spores (conidia). Photographed on 5th September 2012.

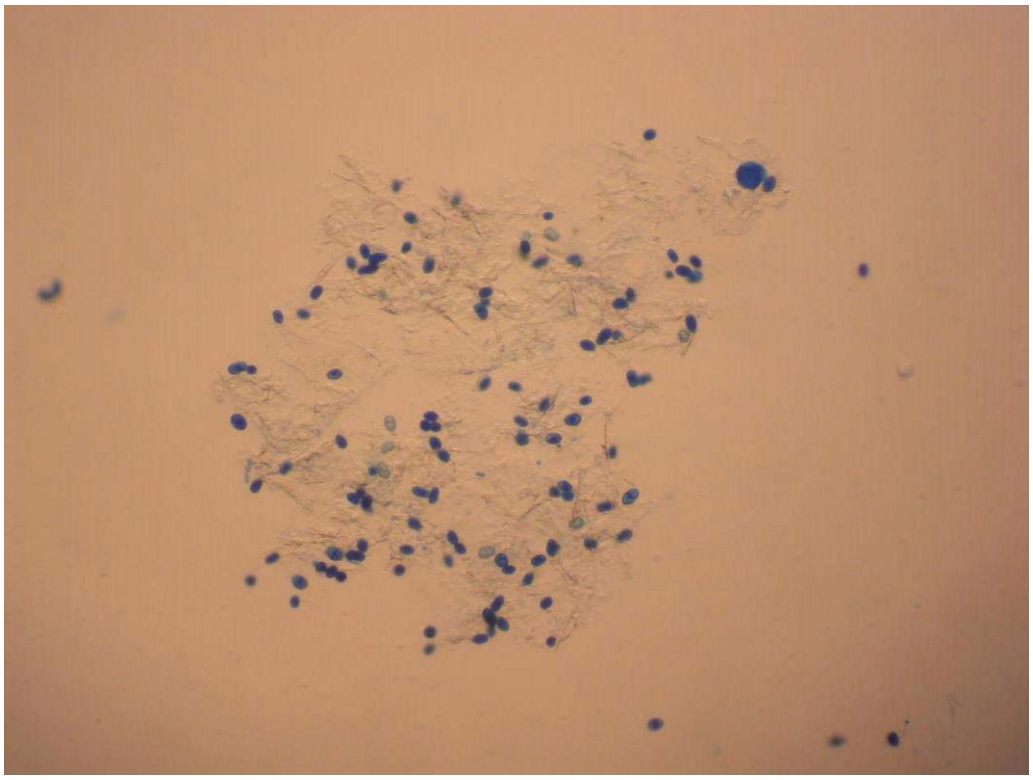

Figure 3. 13.5g HML32/litre example. *Botrytis cinerea* stained with aniline blue on microscope slide at 200x magnification showing ungerminated spores (conidia). Photographed on 5th September 2012.

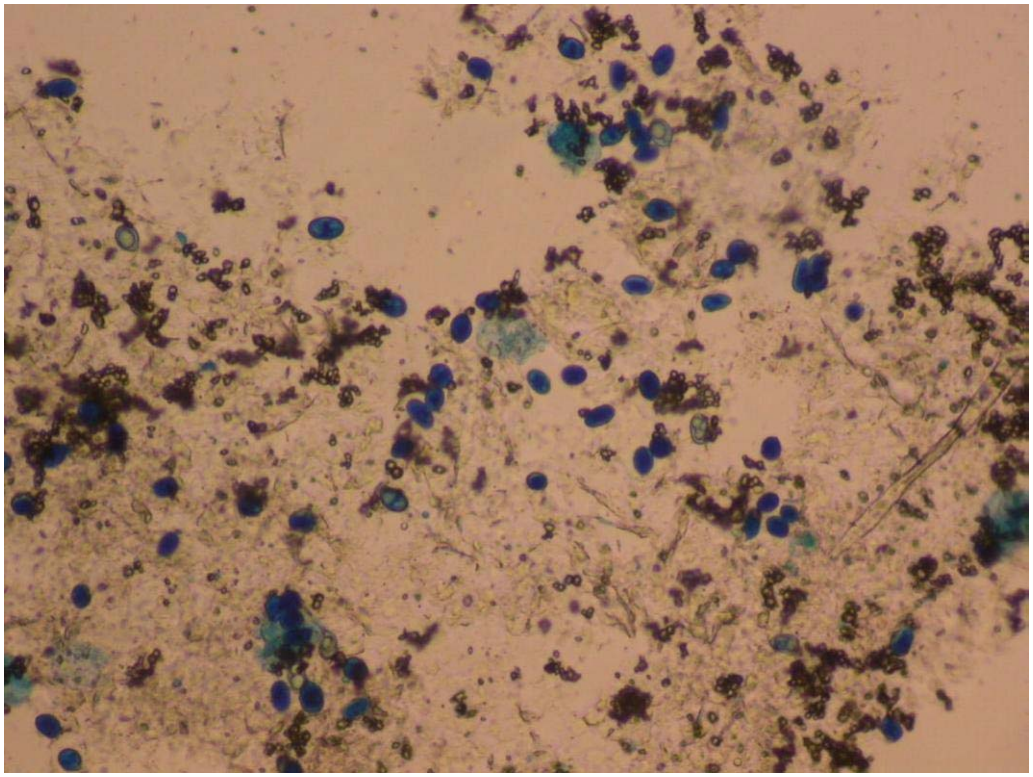

Figure 4. 13.5g HML32 + 3.0g Sulphur/litre example. *Botrytis cinerea* stained with aniline blue on microscope slide at 200x magnification showing ungerminated spores (conidia). Photographed on 5th September 2012.

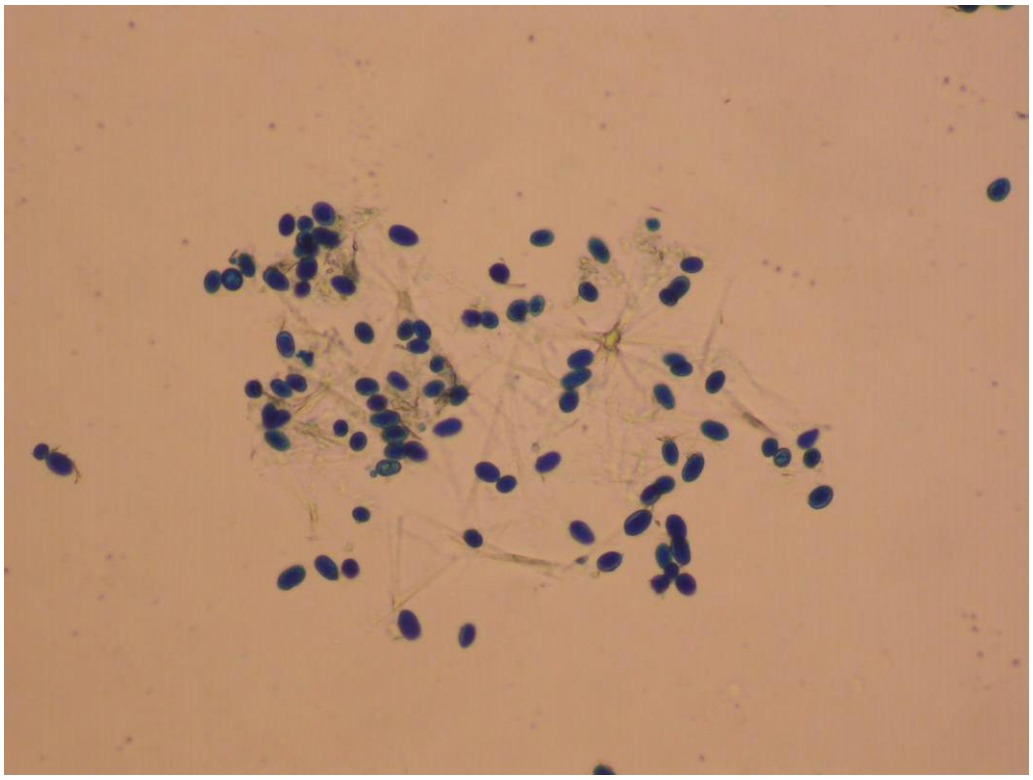

Figure 5. 6.75g HML32/litre example. *Botrytis cinerea* stained with aniline blue on microscope slide at 200x magnification showing ungerminated spores (conidia). Photographed on 5th September 2012.

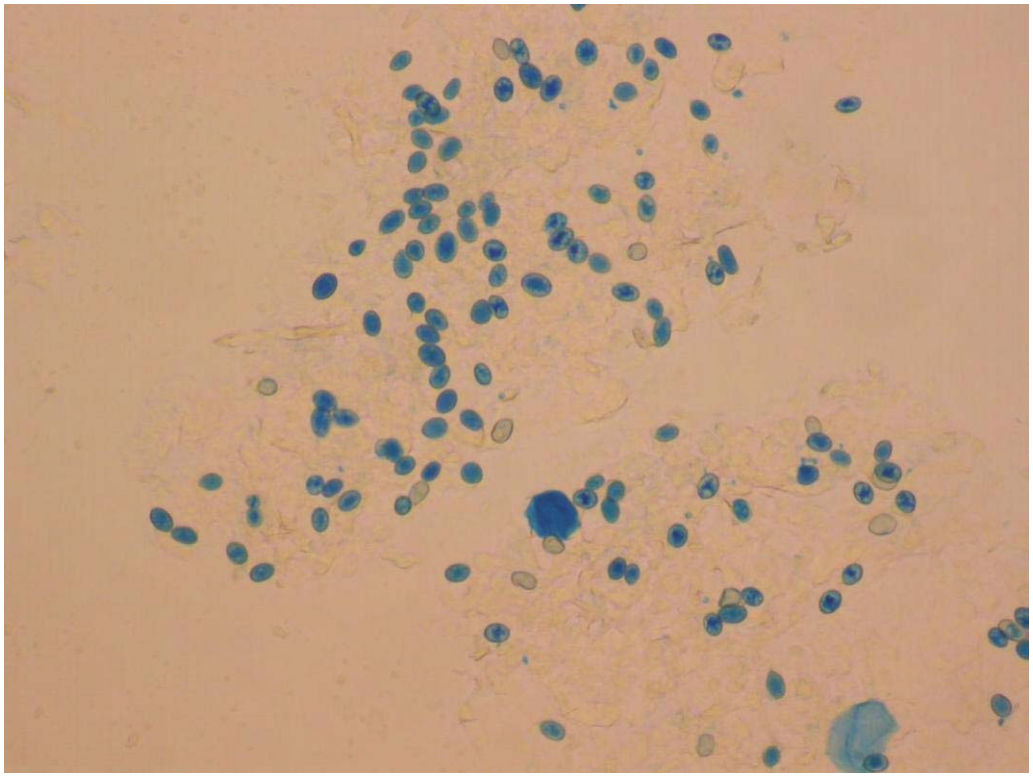

Figure 6. 27.0g HML32/litre example. *Botrytis cinerea* stained with aniline blue on microscope slide at 200x magnification showing ungerminated spores (conidia). Photographed on 5th September 2012.