IMPROVED SEMI-SELECTIVE MEDIUM FOR *HETEROBASIDION IRREGULARE*

ISOLATION AND SPORE TRAPING

MICHELLE CRAM

FOREST HEALTH PROTECTION, 320 GREEN ST, ATHENS, GA 30602

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An operational test of *Phlebiopsis gigantea* for biological control of Heterobasidion root disease required spore trapping to assess the presence of *Heterobasidion irregulare* and *P. gigantea* during treatment application. Growth rates of 4 isolates of *H. irregulare* and the *P. gigantea* stump treatment were tested on several different semi-selective media. The media tested included a peptone and PCNB medium (PP), the PP medium plus 4 ppm benomyl (PPB), and a malt extract medium with 30 ppm benomyl (MB), and a malt extract agar (MEA) control. After 12 days, the growth rate of *P. gigantea* was significantly greater on the MB medium (34 mm) than on either PP (10 mm) or PB (9 mm), but was not different than growth on MEA (34 mm). *H. irregulare* grew significantly better on the PPB medium (17 mm) than on MB (12 mm), but was not different than growth on PP (15 mm). All semi-selective media suppressed *H. irregulare* when compared to growth on MEA (34 mm).

Wood chips taken from a pine stump infected with *H. irregulare* were used to test 6 different media1. The media tested included the 4 media previously used for growth rates, plus 2 ortho-phenyl-phenol media at 60 ppm (OPP60) and at 18 ppm (OPP18). The percent isolation and growth rates of *H. irregulare* and *Trichoderma* species were assessed at 5 days. PPB was the only selective medium with significantly higher isolation rates of *H. irregulare* (68%) than found on MEA (21%). Both PPB and PP media had the highest *H. irregulare* growth rates (5 mm) than all other media (0 – 1 mm). *Trichoderma* isolation was suppressed by both PPB and PP (28%) media as compared to MEA (97%) and OPP18 (78%). *Trichoderma* growth was also lower on all of the semi-selective media than on MEA (32 mm) with the exception of growth on OPP18 (23 mm). The PPB medium had the lowest average *Trichoderma* growth at 1 mm. The average growth rate of *Trichoderma* on PP medium was 5 mm.

The presence of natural occurring *H. irregulare* was monitored by placing plates of PPB and MB media in 5 different areas of 3 treatment blocks at 24 hours and 72 hours after cutting (6 days total). The plates were exposed for 1.5-2.8 hours during the time stumps were treated. The resulting numbers of colonies were evaluated every 2-3 days for up to 3 weeks. Spore deposition on the PPB medium was higher for *H. irregulare* (0.65 dm2/hr) then for *P. gigantea* (0.16 dm2/hr). Spore deposition on the MB medium was higher for *P. gigantea* (0.57 dm2/hr) then for *H. irregulare* (0.36 dm2/hr). These results indicate that the PPB semi-selective medium was best suited for *H. irregulare* spore trapping and isolation from wood chips.

Parts per million of antibiotic or antifungal ingredient in media1

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| Ingredient | Media | | | | | |
| MB | PP | PPB | OPP18 | OPP60 | MEA |
| Streptomycin | 100 | 100 | 100 | 100 | 100 | - |
| PCNB | - | 190 | 190 | - | - | - |
| Benomyl | 30 | 0 | 4 | - | - | - |
| OPP | - | - | - | 18 | 60 | - |