Continued on cover page 3

Eur. J. For. Path. 25 (1995) 185–190

ISSN 0300–1237

Division of Forest Protection, Taiwan Forestry Research Institute, Taipei, Taiwan

A selective medium for *Phellinus noxius*

By TUN-TSCHU CHANG

Summary

A selective medium was developed for isolation of *Phellinus noxius*. The medium consists of 20 g/l malt-extract, 20 g/l agar, 10 mg/l benomyl, 10 mg/l dicloran, 100 mg/l ampicillin and 500 mg/l gallic acid. For isolation of *P. noxius* from soils, 1000 mg/l tergitol NP-7 was added to restrict the size of individual colonies. Comparisons of this selective medium with other media selective for isolation of hymenomycetes showed that the former was more effective for isolation of *P. noxius*.

1 Introduction

Phellinus noxius (Corner) Cunningham is widely distributed in tropical regions (PEGLER and WATERSTON 1968). It causes brown root rot and decline of numerous agricultural and forest trees (PEGLER and WATERSTON 1968; HODGES and TENORIO 1984; NEIL 1986).

In recent years, the disease has become one of the most serious problems in fruit and forest trees at lower altitudes (<800 m) in central and southern Taiwan (ANN and KO 1992; CHANG 1992).

Although a stick-trapping technique (DECLERT 1986) has been used to detect *P. noxius* on the roots of wild trees, there is no selective medium available to isolate the fungus from wood or soils. In preliminary tests, several media selective for hymenomycetes (KUHLMAN 1966; VAARTAJA 1968; HUNT and COBB 1971; RISHBETH 1972; HUTCHINS et al. 1985; RIZZO and HARRINGTON 1988) have been used in attempts to isolate *P. noxius* from infected tissues and infested soils. None have been effective for isolation of *P. noxius*. Therefore, a medium selective for isolation of *P. noxius* from infected tissues and soils was developed.

2 Materials and methods

One isolate of *P. noxius* (isolate B8) obtained from a diseased root of *Cinnamomum camphora* (Linn.) Nees et Eberm. (CHANG 1992), an unidentified bacterium isolated from the same substrate, a *Trichoderma* sp., a *Penicillium* sp., an *Amblysporium* sp., and a *Cunnighamella* sp. were used as test organisms. Malt agar (MEA: 20 g/l Difco-malt-extract and 20 g/l Bacto-agar) was used as the basal medium. After autoclaving and cooling to 40–60 °C, different concentrations of ampicillin (Sigma, A-9393), dicloran (Schering, allisan 50% WP), benomyl (du Pont, benlate 50% WP) and gallic-acid (Sigma, T-0125) were added. Initially each compound was tested separately for growth inhibition of micro-organisms. Different combinations of compounds were then tested to find one that was optimal for isolation of *P. noxius*. Linear growth of *P. noxius* colonies were measured after 7-days' growth at 25 °C in the dark. The experiment was performed twice with four replicates for each medium–micro-organism combination.

For isolation from roots, naturally infected root sections (ca. 3–5 cm in diameter, 10 cm in length) collected from three diseased *C. camphora* trees and three *Delonix regia* (Boj.) Raf. were used to evaluate each test medium. Four root sections were collected from each

U. S. Copyright Clearance Center Code Statement: 0300–1237/95/2504–0185 $11.00/0

tree, washed with tap water, and blotted dry. A total of 25 root fragments (ca. 3 × 3 × 6 mm) cut from each root section were used for each test medium. A total of 300 root fragments for each tree species (3 trees × 4 sections × 25 fragments) were used for each of seven test media.

Five fragments were placed on each Petri plate containing a test medium and incubated at 25 °C for two weeks in the dark. Recovery rates were calculated as the percentage of root fragments from which *P. noxius* emerged after 2-weeks incubation. In addition, six other media selective for hymenomycetes were used for comparative studies. These were: MBC (HUTCHINS et al. 1985), BDP (HUNT and COBB 1971), BSMA (RIZZO and HARRINGTON 1988), OPP (RISHBETH 1972), PPP (KUHLMAN 1966) and PON (VAARTAJA 1968). Media were prepared using the modified methods and formulae described by WORRALL (1991), except for MBC, which was made as described by HUTCHINS et al. (1985).

For testing isolation from soil, an arthroconidial suspension (5 × 10^5 conidia/ml) was obtained from a 2-week-old culture of *P. noxius* growing on MEA medium and mixed with unsterile soil (about 10% soil moisture), resulting in 5 × 10^4 arthroconidia/g dried soil. After 5 days incubation at 25 °C in the dark, the infested soil was used to prepare serial dilutions (10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}; KAO and KO 1983) that were placed on the test medium and incubated at 25 °C for 2 weeks. For isolation of *P. noxius* from soil, an additional 1000 mg/l tergitol NP-7 (Sigma, T-7256) was added to the test medium. A total of 10 Petri plates were also prepared for each of the six other selective media for comparative purposes.

3 Results

At all tested concentrations of benomyl, growth of *Trichoderma* sp. and *Penicillium* sp. were inhibited, but not *P. noxius* (Table 1). Concentrations of 5 mg/l and 10 mg/l dicloran

Table 1. **Response of test micro-organisms to malt-extract agar (MEA) and MEA amended with individual antimicrobial compounds. Linear growth was measured after 7 days at 25 °C. Eight plates were used per medium/fungus combination. Growth rates on media amended with antimicrobial compounds were compared with those on MEA using Wilcoxon's two-sample test**

	Linear growth (mm/day)					
Medium	*Phellinus noxius*	*Trichoderma* sp.	*Penicillium* sp.	*Amblysporium* sp.	*Cunninghamella* sp.	Bacterium
MEA	5.2	6.7	1.4	6.5	7.1	1.2
Benomyl						
5 mg/l	5.3 ns	3.2*	0.8*	6.4 ns	7.3 ns	1.1 ns
10 mg/l	5.3 ns	1.2**	0**	4.7*	5.2 ns	1.3 ns
20 mg/l	5.1 ns	0.5**	0**	4.4*	5.3 ns	1.2 ns
Dicloran						
5 mg/l	5.4 ns	6.4 ns	1.3 ns	0.8**	1.0**	1.2 ns
10 mg/l	5.0 ns	6.5 ns	1.3 ns	0.4**	0.6**	1.0 ns
25 mg/l	3.2*	5.3 ns	0.7*	0**	0**	0.8 ns
Ampicillin						
100 mg/l	5.2 ns	6.8 ns	1.3 ns	6.2 ns	6.9 ns	0**
200 mg/l	5.5 ns	4.3*	1.1 ns	6.4 ns	6.8 ns	0**
Gallic acid						
500 mg/l	4.9 ns	3.5*	0.6*	6.4 ns	6.5 ns	0.8 ns

*, ** Significantly different at p = 0.05 and p = 0.01, respectively (ns = not significant)

did not inhibit *P. noxius* but did inhibit growth of *Amblysporium* sp. and *Cunninghamella* sp. (Mucorales). The bacterium associated with roots infected by *P. noxius* and soils was inhibited at 100 mg/l ampicillin. However, *P. noxius* still grew well at 400 mg/l ampicillin. Although gallic acid was not an effective inhibitor of fungi and bacteria, colonies of *P. noxius* turned dark brown when it was present in the medium. Based on these individual tests, and after testing four combinations of the various inhibitors (Table 2), the following medium was selected because growth of contaminant organisms was restricted, while that of *P. noxius* was not inhibited: 10 mg/l benomyl, 10 mg/l dicloran, 100 mg/l ampicillin, 500 mg/l gallic acid, 20 g/l Difco-malt-extract and 20 g/l Bacto-agar.

P. noxius grew from 92% of the root fragments that showed symptoms typical for an attack by this fungus (Table 3). On BDP medium, *P. noxius* grew from 75 and 73% of the root fragments from infected *C. camphora* and *D. regia*, respectively. The remaining root fragments were contaminated, mainly with bacteria. The other media were ineffective for isolating *P. noxius* from infected roots.

With the addition of 1000 mg/l tergitol NP-7 to the selective medium, colonies of *P.*

Table 2. **Response of test micro-organisms to malt-extract agar (MEA) amended with different combinations of antimicrobial compounds. Linear growth was measured after 7-days' growth at 25 °C. Eight replicates per medium/ fungus combination. Com 1: 10 mg/l benomyl, 10 mg/l dicloran, 100 mg/l ampicillin and 500 mg/l gallic acid; Com 2: 10 mg/l benomyl, 25 mg/l dicloran, 100 mg/l ampicillin and 500 mg/l gallic acid; Com 3: 20 mg/l benomyl, 10 mg/l dicloran, 100 mg/l ampicillin and 500 mg/l gallic acid; Com 4: 20 mg/l benomyl, 25 mg/l dicloran, 100 mg/l ampicillin and 500 mg/l gallic acid. Growth rates on Com 2, 3 and 4 were statistically compared with those on Com 1 using the Wilcoxon two-sample test**

Medium	Linear growth (mm/day)					
	Phellinus noxius	*Trichoderma* sp.	*Penicillium* sp.	*Amblysporium* sp.	*Cunninghamella* sp.	Bacterium
Com 1	5.3	0.3	0	0.2	0.2	0
Com 2	3.4*	0.3 ns	0	0.3 ns	0.2 ns	0
Com 3	4.5 ns	0.2 ns	0	0.3 ns	0.3 ns	0
Com 4	2.8*	0.1 ns	0	0.2 ns	0.1 ns	0

* Significantly different at p = 0.05 (ns = not significant)

Table 3. **Comparison between different selective media used for isolation of *P. noxius* from infected tissues. Growth rates on the newly developed selective medium were compared with those on the other media using the Wilcoxon two-sample test**

Selective medium	% of root fragments growing *P. noxius*	
	From *C. camphora*	From *D. regia*
Developed in the study	91	92
MBC	0**	0**
BDP	73*	71*
BSMA	25**	18**
OPP	0**	0**
PPP	4**	0**
PON	5**	0**

*, ** Significantly different at p = 0.05 and p = 0.01, respectively

noxius were restricted in size to less than 2 mm in diameter after 2 weeks of growth and were, thus, easy to count. With the other six selective media, it was not possible to recover *P. noxius* from soil (Table 4). On the selective medium amended with NP-7, 86% (4.3×10^4 out of 5×10^4 arthroconidia per g dried soil) of the arthroconidia were recovered from soil.

Table 4. **Comparisons between different selective media used for isolation of *P. noxius* from soil: MBC (Hutchins et al. 1985), BDP (Hunt and Cobb 1971), BSMA (Rizzo and Harrington 1988), OPP (Rishbeth 1972), PPP (Kuhlman 1966), PON (Vaartaja 1968)**

Selective medium	Number of colonies per g dried soil
Developed in the study	$4.3 \times 10^4 \pm 1.2 \times 10^2$
MBC	0
BDP	0
BSMA	0
OPP	0
PPP	0
PON	0

4 Discussion

Benomyl has been used for isolation of hymenomycetes, mostly to inhibit *Trichoderma* and *Penicillium*, while dicloran has been used to inhibit growth of Mucorales (Hutchins et al. 1984; Hunt and Cobb 1971; Maloy 1974; Worrall 1991). In those studies, *P. noxius* was not inhibited by either of these fungicides. Bacteria are generally present in the root samples and were occasionally troublesome. Ampicillin is, however, an effective antibiotic against these bacteria. Although gallic acid is not an effective inhibitor of fungi and bacteria, it causes colonies of *P. noxius* to turn dark brown (Davidson et al. 1938). This characteristic is a useful indicator of the presence of colonies of *P. noxius* unless there are other white rot fungi present which would give the same reaction.

Since the selective medium did not inhibit growth of *P. noxius*, it was difficult to count individual colonies when arthroconidia were isolated from soils. However, when tergitol NP-7 was added, the size of *P. noxius* colonies was restricted and they were, thus, easily counted. Over 80% of the arthroconidia were recovered from soil on the selective medium amended with NP-7. This recovery rate is considerably higher than that of any of the other media tested. Tergitol NP-7 and NPX have been used to limit colony size in other fungi (Lee and Chuang 1992; Steiner and Watson 1965). Although the use of the BDP medium developed by Hunt and Cobb (1971) resulted in a relatively high recovery rate of *P. noxius* from infected tissues, it was not effective in isolating *P. noxius* from soil. The BDP medium was heavily contaminated with bacteria, probably due to the lack of a bacterial inhibitor.

In this study, a highly effective selective medium has been developed for isolation of *P. noxius* from infected tissues or soils. Management procedures for reducing the incidence of *P. noxius* in orchards and forests include mechanical, chemical and biological methods (Hashim 1990). However, effectiveness of these methods is difficult to assess due to an inability to detect and trace survival of *P. noxius* in woody residues and soil. Use of this selective medium will allow location of residual sources of inoculum, which will markedly improve our ability to evaluate control methods. The new selective medium will also be useful for epidemiological studies of *P. noxius*.

diameter after 2 weeks of growth and
e media, it was not possible to recover
n amended with NP-7, 86% (4.3×10^4
rthroconidia were recovered from soil.

**used for isolation of *P. noxius* from soil:
1), BSMA (Rizzo and Harrington 1988),
66), PON (Vaartaja 1968)**

Number of colonies per g dried soil
$4.3 \times 10^4 \pm 1.2 \times 10^2$
0
0
0
0
0
0

1

etes, mostly to inhibit *Trichoderma* and
growth of Mucorales (HUTCHINS et al.
ALL 1991). In those studies, *P. noxius*
cteria are generally present in the root
illin is, however, an effective antibiotic
effective inhibitor of fungi and bacteria,
AVIDSON et al. 1938). This characteristic
noxius unless there are other white rot

h of *P. noxius*, it was difficult to count
ed from soils. However, when tergitol
s restricted and they were, thus, easily
ered from soil on the selective medium
bly higher than that of any of the other
sed to limit colony size in other fungi
1965). Although the use of the BDP
sulted in a relatively high recovery rate
ive in isolating *P. noxius* from soil. The
ia, probably due to the lack of a bacterial

n has been developed for isolation of *P.*
procedures for reducing the incidence of
nical, chemical and biological methods
methods is difficult to assess due to an
n woody residues and soil. Use of this
urces of inoculum, which will markedly
The new selective medium will also be

Acknowledgements

The author thanks Dr W. H. KO of the University of Hawaii for advice. Appreciation goes also to Drs Alan F. WARNEKE and R. J. CHANG of the Taiwan Forestry Research Institute for critical review of the manuscript and making valuable suggestions. This study was supported in part by grant NSC 83–0409-B-054–012, from the National Science Council, ROC.

Résumé

Un milieu sélectif pour Phellinus noxius

Un milieu sélectif a été élaboré pour l'isolement de *P. noxius*: extrait de malt (20 g/l), agar (20 g/l), bénomyl (10 mg/1), dicloran (10 mg/1), ampicilline (100 mg/l) et acide gallique (500 mg/l). Pour l'isolement à partir du sol, 1000 mg/l de tergitol NP-7 sont additionnés pour réduire la taille des colonies individuelles. La comparaison de ce milieu avec d'autres milieux sélectifs pour hymenomycètes a montré qu'il est plus efficace pour l'isolement de *P. noxius*.

Zusammenfassung

Ein Selektivmedium für Phellinus noxius

Es wurde ein Selektivmedium für *P. noxius* entwickelt, das aus folgenden Komponenten besteht: 20 g/l Malzextrakt, 20 g/l Agar, 10 mg/l Benomyl, 10 mg/l Dicloran, 100 mg/l Ampicillin und 500 mg/l Tannin. Zur Isolierung von *P. noxius* aus dem Boden wurden 1000 mg/l Tergitol zugegeben, um die Größe der Kolonien zu reduzieren. Es konnte gezeigt werden, daß dieses Medium für die Isolierung von *P. noxius* effektiver als andere Selektivmedien für Hymenomyceten ist.

References

ANN, P. J.; KO, W. H., 1992: Decline of longan trees: association with brown root rot caused by *Phellinus noxius*. Plant Pathol. Bull. **1**, 19–25.

CHANG, T. T., 1992: Decline of some forest trees associated with brown root rot caused by *Phellinus noxius*. Plant Pathol. Bull. **1**, 90–95.

DAVIDSON, R. W.; CAMPBELL, W. A.; BLAISDELL, D. J., 1938: Differentiation of wood-decaying fungi by their reactions on gallic or tannic acid medium. J. Agr. Res. **57**, 683–695.

DECLERT, C., 1986: Une technique nouvelle de détection des agents de pourridié: la bonchette-piège. Son application à l'étude du *Leptoporus lignosus*. Rev. Mycol. **26**, 119–127.

HASHIM, I., 1990: A review on control of rubber root diseases. In: Proc. *Ganodema* Workshop, Bangi, Selangor, Malaysia, 11 September 1990. Ed. by DARUS, A.; SUKAIMI, J.

HODGES, C. S.; TENORIO, J. A., 1984: Root disease of *Delonix regia* and associated tree species in the Mariana Islands caused by *Phellinus noxius*. Plant Disease **68**, 334–336.

HUNT, R. S.; COBB, F. W. Jr., 1971: Selective medium for the isolation of wood-rotting basidiomycetes. Can. J. Bot. **49**, 2064–2065.

HUTCHINS, A. S.; FAY, H.; KNUTSON, D., 1985: A selective medium for *Phellinus weirii*. Can. J. For. Res. **15**, 746–748.

KAO, C. W.; KO, W. H., 1983: Nature of suppression of *Pythium splendens* in a pasture soil in South Kohala, Hawaii. Phytopathology 73, 1284–1289.

KUHLMAN, E. G., 1966: Recovery of *Fomes annosus* spores from soil. Phytopathology **56**, 885.

LEE, H. C. and CHUANG, T. Y., 1992: Distribution and seasonal variation of *Aspergillus flavus* in peanut fields at Penghu (in Chinese). Plant Pathol. Bull. **1**, 174–183.

MALOY, O. C., 1974: Benomyl-malt agar for the purification of cultures. Plant Dis. Reptr. **58**, 902–904.

NEIL, P. E., 1986: A preliminary note on *Phellinus noxius* root rot of *Cordia alliodora* plantings in Vanuatu. Eur. J. For. Path. **16**, 274–280.

PEGLER, D. N.; WATERSTON, J. M., 1968: *Phellinus noxius.* CMI Descriptions of Pathogenic Fungi and Bacteria No. 195.

RISHBETH, J., 1972: The production of rhizomorphs by *Armillaria mellea* from stumps. Eur. J. For. Path. **2**, 193–205.

RIZZO, D. M.; HARRINGTON, T. C., 1988: Root and butt rot fungi on balsam fir and red spruce in the White Mountains, New Hampshire. Pl. Dis. **72**, 329–331.

STEINER, G. W.; WATSON, R. D., 1965: Use of surfactant in the soil dilution and plate count method. Phytopathology **55**, 728–730.
VAARTAJA, O., 1968: Wood inhabiting fungi in a pine plantation in Australia. Mycopathol. Mycol. Appl. **34**, 81–89.
WORRALL, J. J., 1991: Media for selective isolation of hymenomycetes. Mycologia **83**, 296–302.

Author's address: Dr T.-T. CHANG, Division of Forest Protection, Taiwan Forestry Research Institute, 53 Nai-hai Road, Taipei, Taiwan

Received: 12.4.94; accepted: 13.4.95