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# Comparison of methods for detection of Rhizoctonia solani in soil

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**Abstract**

At a soil temperature that was favourable for the contaminating *Rhizoctonia* isolate, detection of the pathogen in soil by using Ko agar or fragments of *Juncus effusus* stems as bait or by plating soil samples in small clumps (Ko and Hora, 1971) gave comparable results. At unfavourable soil temperature the use of Ko agar baits was most successful. Water agar to which 0.5% inulin and a selective solution developed by G.M. Tichelaar (formerly: IPO, Wageningen, personal communication) was added was the best plating medium.

## Introduction

For studies on the persistence of *Rhizoctonia solani* Kühn in soils of bulb-growing areas, a suitable method to detect *R. solani* in soil was needed. For this purpose various methods of soil sampling, baiting, and plating were compared and combined.

## Materials and methods

*Soil samples.* Soil samples were taken at random from contaminated dune sand soil (Doornik, 1980) and either plated as soil clumps with a diameter of 5 mm (Ko and Hora, 1971) or passed through a 1-mm sieve onto a 0.4-mm sieve. The soil remaining on the 0.4-mm sieve was either washed in running tap-water for 3 min or placed in shallow water under vibration (Van Emden, 1971). Particles of washed or vibrated soil were collected randomly and plated on a selective medium.

*Baits.* Various baits were used. According to G.M. Tichelaar's (formerly: IPO, Wageningen) unpublished method, filter paper discs (6 mm in diameter) were dipped in a nutrient solution developed by Ko and Hora (1971) and then dried. These discs or 5-mm fragments of *Juncus effusus* (rush) stems were fixed with sellotape in perforations (diameter 5 mm) made 1 cm apart in 3-mm-thick strips of perspex. According to another method (Anderson and Huber, 1965), these holes were filled with an agar medium developed by Ko and Hora (1971), which will be referred to here as Ko, and were closed on both sides with sellotape, which was perforated once per hole with a thin needle passing through both upper and lower tapes (Fig. 1). The Ko baits, which were not prepared under sterile conditions, were

Fig.1. Ko agar baits in perspex strips.

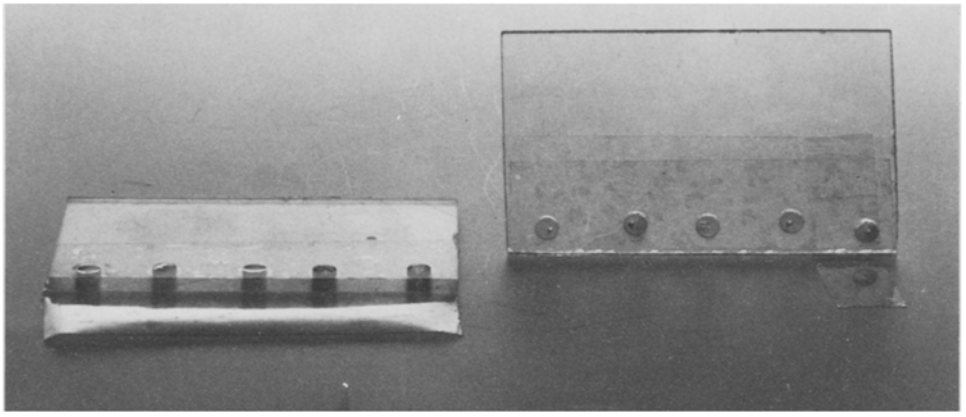

*Fig. 1. Ko-agar-loksubstraat in plaatjes van perspex.*

kept at 2 °C until use on the following day. All baits were pressed into contaminated soil to a depth of 4 cm. Following Papavizas and Davey (1961), Martinson (1963), Sneh et al. (1966), and Warren (1975), we left the baits in the soil for 3 days, after which they were plated on a selective medium and incubated at 20 °C.

*Selective growth media.* Three plating media were compared, i.e., Czapek Dox agar diluted to 1/3 of the concentration normally prescribed (CD); water agar (2% agar) to which 0.5% inulin was added to make the mycelium of *R. solani* more conspicuous, to be referred to as Wa; and the Ko and Hora (1971) medium, which consisted of an autoclaved nutrient agar (Ko) to which a non-autoclaved selective solution in sterile water (S) developed by Ko and Hora (1971) was added before use. Another non-autoclaved selective solution in sterile water (T), developed by G.M. Tichelaar (personal communication), was also tried; this medium contained 66 $\mu g \cdot g^{-1}$ benomyl (50% a.i.), 200 $\mu g \cdot g^{-1}$ aureomycin, and 210 $\mu g \cdot g^{-1}$ $CuSO_4 \cdot 5H_20$. Before use, 1 ml of this stock solution was added to 10 ml agar.

In these experiments, nematodes present in some of the soil samples or baits dispersed bacteria over the media in the dishes, which led to inhibition of the outgrowth of *R. solani*. The inhibition could not be overcome by a higher aureomycin concentration, because this also interfered with the outgrowth of *R. solani*. The problem was solved by the addition of 1.00 $\mu l \cdot l^{-1}$ parathion (100% a.i.) to the baiting medium, or, for soil samples, to the plating medium.

**Results and discussion**

*Outgrowth on growth media.* Isolation of *R. solani* was best on media containing solution T, but the outgrowth of *R. solani* was retarded as compared with growth on media without this solution, which meant that colony counting had to be postponed until 3 days after plating. Solution S gave less suppression of the outgrowth of

contaminating micro-organisms as compared with solution T. Isolation of *R. solani* was most successful on WA+T. Fewer *Rhizoctonia* and/or more contaminating bacteria and fungi grew out on CD+T and Ko+T. On the basis of the results, Wa+T was used in all further experiments.

*Bait catches.* Isolation of *R. solani* with Ko agar baits was at least twice as good as with filter paper discs or plating of washed or vibrated soil particles. Isolation from soil clumps and Ko agar baits was compared for soil contaminated with a cold preferring isolate (Doornik, 1980, 1981). The soil was kept at 9 °C, which is a favourable temperature for this isolate. The pathogen was found in 98 and 92% of these soil clumps and Ko baits, respectively. Both isolation methods were compared again in duplicate areas in a glasshouse at 12-14 °C, where the soil had been contaminated with a *Rhizoctonia* isolate with a preference for a temperature of 17 °C or higher. Less *Rhizoctonia* was isolated from clumps of soil than from Ko agar baits (Table 1). Isolation from Ko agar baits and from pieces of *Juncus effusus* stem was compared in the same glasshouse areas. In addition, similar baits were pressed into soil samples taken from these areas and kept at 20 °C during the 3-day baiting period. The results for Ko agar and *Juncus* baits in soil at 20 °C were comparable. However, isolation under the temperature conditions prevailing in the glasshouse was more successful via Ko agar baits (Table 1).

It can be concluded that under temperature conditions favourable for the pathogen detection of *R. solani* in soil clumps, Ko agar, or *Juncus* baits was equally successful. Under unfavourable conditions, however, Ko agar baits yielded more *R. solani.*

The recovery of *R. solani* from soil with any type of bait implies that the fungus had to grow actively into the bait, which means that a real saprophytic growth is measured. Moreover, observation can be done in situ.

Table 1. Isolation percentages for *R. solani* from soil clumps and *Juncus* or Ko agar baits from duplicate areas in a glasshouse contaminated with a warmth preferring isolate.

| | Soil temperature (°C) during the baiting periode | |
|---|---|---|
| | 12-14 | 20 |
| *Sampling 1* (n = 2 × 150) | | |
| soil clumps | 3[1] | |
| Ko agar baits | 30 | |
| *Sampling 2* (n = 2 × 100) | | |
| Ko agar baits | 24 | 92 |
| *Juncus* baits | 10 | 97 |

[1] Values are means of both areas.

*Tabel 1. De percentages R. solani geïsoleerd uit hoopjes grond en uit stukjes Juncus- en Ko-agar-loksubstraat van tweeling-veldjes in een kas besmet met een warmte-minnend isolaat.*

**Samenvatting**

*Vergelijking van methoden om Rhizoctonia solani in grond te bepalen*

De methode van Ko en Hora (1971), waarbij uit een willekeurig genomen grondmonster, 1 g in kleine hoopjes op een selectieve bodem werd uitgeplaat, is o.a. vergeleken met een methode om de schimmel in de grond aan te tonen door gebruik te maken van stukjes 'loksubstraat', die drie dagen op 4 cm diepte in de grond hadden gelegen. Gedroogde stengeldelen van *Juncus effusus* (pitrus) en druppeltjes van een voedingsagar ontwikkeld door Ko en Hora (1971) boden als loksubstraat toegepast de beste resultaten. Het voedingsmedium waarmee uit hoopjes grond en uit rus- en Ko-agar-lokaas de meeste *Rhizoctonia* werd geïsoleerd, was water-agar met 0,5% inuline waaraan een oplossing met selectieve werking, ontwikkeld door G.M. Tichelaar (voorheen: IPO, Wageningen, persoonlijke mededeling), vóór het gieten van de bodem werd toegevoegd.

Bij een bodemtemperatuur, die gunstig was voor het *Rhizoctonia*-isolaat waarmee de grond was besmet, waren de resultaten van beide soorten loksubstraat en de hoopjes grond vergelijkbaar. Bij ongunstige bodemtemperatuur werd met behulp van Ko-agar-loksubstraat de meeste *Rhizoctonia* geïsoleerd.

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