Comparison of Soil-Pellet and Sieving-Floatation Methods for Enumeration of Sugarbeet-Root-Rot Fungus (*Rhizoctonia solani* AG 2-2)

Mitsuro HYAKUMACHI* and Tadao Uri*

百町満朗*・宇井格生* : 土壤塊法と篩別浮上法によるテンサイ根腐病菌
(*Rhizoctonia solani* AG 2-2) の菌量の比較

Key Words : *Rhizoctonia solani* AG 2-2, population, soil-pellet method, sieving-floatation method.

The dilution-plate method is widely used for determining populations of various microorganisms in soil. But this method has not been applied successfully to *Rhizoctonia solani* because of its relatively small numbers. Ways of detecting and enumerating *R. solani* in soil, are, the plant segment colonization method¹⁻⁴⁾, the immersion tube method^{1,5)}, the plant debris particle isolation method^{1,2)}, the seedling infection method^{2,3,5)} and the soil pellet method⁶⁾. All of these are indirect methods, making use of saprophytic or parasitic activity of the pathogen. The first four methods give approximate population density, while the last gives concrete numbers. Therefore, the last one is proper to determine the quantitative assay. On the other hand, the sieving-floatation method⁷⁾ is used as a direct method for enumeration. In this case, sclerotia are the only objects of the propagule unit of *R. solani*. After examining the viability of sclerotia extracted from soil, the propagule number (number of viable sclerotia) can be counted. This study was conducted in order to determine whether the soil pellet method (SPM) or the sieving floatation method (SFM) is more suitable for a quantitative assay of *R. solani* in a study on vicissitude of sugarbeet-root-rot in monoculture.

Thirteen soil samples were obtained from the soils around severely diseased roots (disease index 5)⁸⁾ in May 1981, after overwintering in the sugarbeet field where root-rot disease had occurred severely in the previous year.

SPM and SFM were compared to detect the enumeration of *R. solani* propagule in the soil. SPM was followed by Ko and Hora's method⁶⁾. Ten 50-200 mg soil pellets with 30% soil moisture were placed on a 9 cm Petri dish containing selective medium of *R. solani*. The percentage of appearance of *R. solani* hyphae from the soil pellet was sur-

* Faculty of Agriculture, Hokkaido University, Kitaku, Sapporo 060, Japan 北海道大学農学部

1) Davey, C. B. and Papavizas, G. C. (1962). Can. J. Microbiol. 8 : 847-853 2) Menzies, J. D. (1963). Ann. Rev. Phytopathol. 1 : 127-142. 3) Papavizas, G. C. and Davey, C. B. (1959). Plant Disease Repr. 43 : 404-410. 4) Ui, T. and Ogoshi, A. (1964). Mem. Fac. Agr. Hokkaido Univ. 5 : 5-16. 5) Martinson, C. A. (1963). Phytopathology 53 : 634-638. 6) Ko, W. H. and Hora, F. K. (1971). Ibid. 61 : 707-710. 7) Ui, T., Naiki, T. and Akimoto, M. (1976). Ann. Phytopathol. Soc. Japan 42 : 46-48.

veyed, and the propagule number of *R. solani* was calculated in 100 g dry soil. One hundred soil pellets (ten petri dishes) were used per one soil sample. SFM was followed by Ui *et al.*'s method⁷⁾. Thirty to fifty g of soil was stirred with 100 ml of 2 % aqueous solution of hydrogen peroxide. This soil suspension was poured through a 50 mesh sieve, and was washed under running tap water. Coarse particles remaining on the sieve, including sclerotia and plant debris, were then poured into a 500 ml separating funnel with a 2 % aqueous solution of hydrogen peroxide again, and were shaken vigorously. Floated sclerotia and plant debris were placed on a filter paper, and the number of sclerotia was counted under a dissecting microscope. Extracted sclerotia were placed on the acidified water agar (pH 4.5). After two days incubation at 25 C, number of germinated sclerotia was counted, and the propagule number of *R. solani* was calculated in 100 g dry soil. Viability of sclerotia was evaluated from the percentage of germinated sclerotia out of all the sclerotia extracted from the soil.

The propagule number of *R. solani* obtained from the two methods were compared (Table 1). There was a high correlation between them ($r=0.857$, $p<0.01$), and the propagule number obtained from SFM tended to be higher than that obtained from SPM. There were only two samples out of thirteen from which *R. solani* could not be detected by using SFM, and six samples by using SPM. There could also be seen a tendency that the propagule number of *R. solani* calculated by SPM was low in the soil of low sclerotial viability, while soils where *R. solani* was not detected were soils with less than 25 % of sclerotial viability. One of the reasons for the above was considered

Table 1. Comparison of the data of inoculum density of *Rhizoctonia solani* AG 2-2 obtained from sieving-floatation and soil-pellet methods

Soil sample number	Sieving-floatation method ^{a)}			Soil-pellet method ^{a)}
	Sclerotial number/100g dry soil	Viable sclerotial number/100g dry soil	Sclerotial viability (%)	Propagules/100g dry soil
1	1380	1173	85.0	580
2	1120	840	75.0	180
3	260	0	0.0	0
4	260	0	0.0	0
5	140	40	28.6	20
6	1420	106	7.5	40
7	6320	209	3.3	0
8	720	144	20.0	0
9	940	118	12.5	60
10	520	130	25.0	0
11	2940	735	25.0	100
12	4260	426	10.0	40
13	4560	228	5.0	0

a) by Ui *et al.* (1976)⁷⁾

b) by Ko and Hora (1971)⁶⁾

- 8) Naiki, T. and Ui, T. (1977). Soil Biol. Biochem. 9 : 377-381. 9) Hyakumachi, M. and Ui, T. (1982). Ann. Phytopathol. Soc. Jpn. 48 : 628-633.

to be the low saprophytic colonizing ability of *R. solani* from the soil pellet to the selective medium, especially during early spring when most of sclerotia lost their viability. On the other hand, in SFM, sclerotia were directly extracted from the soil, and if there were many sclerotia, the propagule number could be easily estimated even when the sclerotial viability was extremely low provided that it was not 0%.

From these results, SFM seems to be superior to SPM in estimating the propagule number of *R. solani* when saprophytic activity is low as it is in early spring. The viable sclerotia play an important role as the main inoculum of the sugarbeet root-rot fungus (*R. solani* AG 2-2)⁹⁾, therefore, SFM seems to be the most appropriate way to estimate the quantitative assay of this pathogen.

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