Neth. J. Pl. Path. 93 (1987) 91-93 SHORT COMMUNICATION

A method for detecting Polymyxa betae and beet necrotic yellow vein virus in soil using sugar-beet as a bait plant

A.B.R. BEEMSTER and A. DE HEIJ

Research Institute for Plant Protection (IPO), P.O. Box 9060, 6700 GW Wageningen, the Netherlands

Accepted 23 December 1986

Rhizomania of sugar-beet, caused by beet necrotic yellow vein virus (BNYVV), which is transmitted by *Polymyxa betae* Keskin, an obligate root parasite, is increasingly important in a number of European countries (Putz, 1985). In 1983 it was detected in two small areas in the Netherlands (Heijbroek, 1984). For the study of various aspects of the disease such as epidemiology and control, a reliable method to estimate quantitatively the level of infestation of soils by both *P. betae* and BNYVV is essential. Since there is no method available for testing soils directly, several workers have been using the method of growing beets for 4-8 weeks in the soil to be tested, after which the roots are tested for BNYVV (Putz, 1985; Häni, 1985). In this paper a method, also giving quantitative results, is described.

Bait plants. One week after sowing seeds of a sugar-beet cultivar susceptible to *P. betae* and BNYVV in sand, the seedlings are dug up and thoroughly rinsed in running tap water. If necessary the roots are cut back to 9-10 cm to promote growth of new rootlets before transplanting at a spacing of 3 × 3 cm to trays filled with coarse sand. One week later they are dug up again, thoroughly rinsed in running tap water and used as bait plants.

Soil sample. A sample of 50 g of thoroughly mixed, crumbled soil, to which 25 ml water is added, is heated for 30 min at 40 °C in a 100 ml beaker in a water bath. After cooling, the soil is spread evenly on the bottom of a Petri dish (diameter 20 cm). Alternatively, the Petri dish containing the soil-water mixture can be heated directly to the required temperature in an oven. After heating and cooling, a further 40 ml water is added to the soil.

Procedure. The beet seedlings, prepared as described, are carefully laid on the bottom of the Petri dish in such a way that the roots are immersed in the moistened soil, while the shoots are sustained by the edge of the dish. Depending on the experiment the number of plantlets arranged in a dish may vary (in our experiments 8-64). The seedlings are kept in the Petri dishes for about 4 days. To reduce evaporation, the soil in the Petri dish is covered by a metal disk (diameter 15 cm) supported by 2 cm high corks and water is added when needed. The dishes are incubated in a growth chamber at 21 °C with a photoperiod of 14 h (18 000 lux, fluoresent tubes). After this baiting period the seedlings are planted separately in pots of 200 ml filled with coarse sand

and to which a compound fertilizer (N-P-K: 12-10-18) at a rate of 0.25 g l^{1} is added. For further development of the plants, the pots are put in a greenhouse at 20–25 °C, each pot being placed in a Petri dish (diameter 9 cm) on a thick disk of filter paper. Water is added to the Petri dishes regularly so as to keep the soil most. Five weeks after transplanting *P. betae* has developed sufficiently to be detected in part of the rootlets visually, using a microscope, and in the remaining roots the presence of BNYVV is detected by ELISA (Clark and Adams, 1977). The number of plants found to be infected by *P. betae* and/or BNYVV is a measure of the level of infestation of the soil by each agent. When severe infestations occur, usually 100% of the bait plants become infected; in such instances the soil can be diluted with sterilized soil, in order to obtain a more accurate estimate of the level of infestation.

In the method described, heat treatment of the soil sample is included because preliminary experiments by G.J. Bollen, Laboratory of Phytopathology, Agricultural University, Wageningen, (personal communication) suggested that it stimulates germination of the resting spores. The degree of the activation of spore germination probably depends on the age of the spores.

To illustrate the potential of the method, Table 1 shows the results of testing soil samples from six sugar-beet fields. Observations of the crop during the growing season provided information on disease incidence in these fields. For two of the fields this was considered to be high, for two others it was low, while two fields situated adjacent to the others, were judged to be from rhizomania. Soil samples from these fields were obtained by collecting randomly cores of 40-50 g soil from each; the cores were bulked and from the composite sample a portion of 50 g was used for each test.

In general the two replicates show a satisfactory degree of agreement. These results are in agreement with what might be expected from the field observations, except for the fact that apparently healthy fields proved to harbour *P. betae* transmitting BNYVV, though at a low level. This indicates that the method can provide information which can not be obtained by visual observation of the field crop. In addition a

Table 1. Infestation of six fields by *Polymyxa betae* and beet necrotic yellow vein virus (BNYVV) expressed as percentage of infected sugar-beet bait plants.

Field number	Disease incidence based on field observations	Percentage bait plants infected			
		replicate 1[1]		replicate 2[2]	
		P. betae	BNYVV	*P. betae*	BNYVV
1	high	100	38	97	50
2	high	100	38	100	72
3	low	96	27	94	28
4	low	100	19	100	28
5	healthy	100	12	100	13
6	healthy	69	0	57	9

[1] 26 bait plants used.
[2] 32 bait plants used.

generally higher rate of infestation by *P. betae* than by BNYVV was found, indicating that a proportion of the resting spores of *P. betae* in these fields, including the severely infected ones, were virus free, or at least did not transmit the virus.

In these experiments the tests were performed without diluting the soil samples; in a dilution series quantitative data are likely to be much more accurate than in the test described here. It should be added that, for detecting BNYVV alone, the period of 5 weeks (in the greenhouse) can be shortened, perhaps to three weeks, provided that temperature conditions are favourable (minimum 22 °C). Also, for qualitative assays some of the steps can be simplified. For example, several seedlings can be planted together in one pot and ELISA can be performed on bulk samples of a number of root systems when only testing for BNYVV is aimed at.

Acknowledgement

Thanks are due to Dr M.J.C. Asher (Broom's Barn Experimental Station, Bury St. Edmunds, United Kingdom) for correcting the English text.

Samenvatting

Detectie van Polymyxa betae en rhizomanievirus in grond met behulp van biet als lokplant

Door in Petrischalen met grond bietezaailingen als lokplant voor de obligaat-parasitaire schimmel *Polymyxa betae*, een vector van het rhizomanievirus, te laten fungeren, kunnen zowel kwalitatieve als kwantitatieve gegevens over de besmetting van de grond met de schimmel en het virus worden verkregen. De methode en enkele ermee bereikte resultaten worden beschreven.

References

Clark, M.F. & Adams, A.N., 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. Journal of General Virology 34: 475-483.

Häni, A., Högger, C.H. & Merz, U., Vorkommen der Rizomaniakrankheit, ihres Vectors *Polymyxa betae*, sowie des Rübenzystennematoden, *Heterodera schachttii* an Zuckerrüben in der Schweiz. Mitteilungen für die Schweizerische Landwirtschaft 33: 52-58.

Heijbroek, W., 1984. Distribution of BNYVV in the Netherlands and initiated research programme. Proceedings Workshop Beet Necrotic Yellow Vein Virus, Colmar, France 95-96.

Putz, C., 1985. Connaissances actuelles sur le virus BNYVV de la rhizomanie et voies de la recherche. Compte rendu Symposium Rhizomanie Bruxelles, Institut Royal Belge pour l'Amélioration de la Betterave 53: 99-108.