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Preliminary study on the effect of benomyl on the fungal flora in a greenhouse soil

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Benomyl inhibits growth of a great deal of the fungal soil population (Bollen and Fuchs, 1970; Edgington et al., 1971). Too little information is available on the extent and duration of side effects on the soil microflora as a whole. Ponchet et Tramier (1971) found no influence on the number of species, while van den Berg and Bollen (1971) found quantitative changes in the mycoflora with 10 ppm benomyl in a freshly prepared potting mixture.

We studied the following model: A sandy greenhouse soil from Naaldwijk was selected which had been cultivated previously with various solanaceous plants and was known to contain a fair amount of *Fusarium* and *Pythium* propagules. Its content of organic matter (loss on ignition) is 6.9%, $CaCO_3$ 0.6%, pH (in water) 7.1, fine silt and clay (< 16 μm) 10%. Mitscherlich pots were filled with coarsely sieved soil in April 1971 and kept at about 30% moisture outdoors protected from rain, and covered with a perforated aluminium foil to prevent algal growth; any germinating seeds were removed. At fixed times two pots were treated by spraying with an aqueous suspension of Benlate followed by thorough mixing, resulting in a concentration of 10 ppm benomyl in the oven-dry soil. The other pots received a similar amount of water. The fungal spectrum was analysed semi-quantitatively by a soil-washing technique (Gams and Domsch, 1967). The *Pythium* density was roughly assessed by a soil crumb technique (Schmitthenner, 1962), using potato-carrot-agar with 5 ppm Endomycin and 50 ppm Streptomycin. The lay-out of the experiments is shown in Table 1.

Since very few fungi grew out from the mineral particles plated, percentages were calculated only for the numbers of isolates of each species growing on organic particles in proportion to the total number of isolates. Table 2 contains the most frequent species that occurred in almost all analyses, i.e. approximately 55% of the isolates, while altogether 117 species were isolated. The species of the genus *Pythium* were identified in two experiments as *P. ultimum* Trow, *P. intermedium* de Bary, *P. rostratum* Butler, *P. heterothallicum* Hendrix et Campbell, *P. vexans* de Bary, and unidentifiable species. Since no alteration due to benomyl was observed, no further effort in species analysis was made. After benomyl treatment no change in fungal density greater than in the two control samples could be demonstrated. Nevertheless, data are given in some detail as a mycological characterization of a soil which will serve for further experiments. The soil used shows particularly little adsorption of benomyl. The active substance could be eluted with water and was demonstrated bioautographically

Table 1. Lay-out of experiments and colonization ratios for 200 soil particles in each experiment.

Incubation period	Treatment	Analysis	Colonization ratios for		Number of organic particles
			organic particles	mineral particles	
Control A	–	5 Oct.	1.1	0.2	112
Control B	–	4 Jan.	1.4	0.2	160
1 week	28 Sept.	5 Oct.	0.9	0.2	112
3 months	28 Sept.	4 Jan.	1.1	0.1	160
6 months	28 Apr.	28 Oct.	1.4	0.3	160
3 + 6 months	28 Apr. 28 July	28 Oct.	1.3	0.3	160

Tabel 1. Proefopzet en kolonisatiequotiënten voor 200 gronddeeltjes per proef.

Table 2. Percentage of the most common species isolated from washed organic soil particles, and frequency of *Pythium* species on 100 soil crumbs. Information on sensitivity of the species taken over and extrapolated from Bollen and Fuchs (1970). (s = sensitive, ss = very sensitive).

	Control A	Control B	1 week	3 months	6 months	3 + 6 months
Fusarium oxysporum (s)	3.2	9.9	7.8	9.5	8.3	6.4
Cylindrocarpon destructans (s)	1.6	5.4	2.4	8.9	5.5	5.4
Mortierella elongata	2.4	1.5	7.8	7.1	4.6	4.4
Trichoderma pseudokoningii (ss)	4.0	2.5	2.0	3.0	7.4	4.9
Fusarium equiseti (s)	4.8	4.5	2.0	5.9	4.6	2.5
Monilia pruinosa (ss)	5.6	4.9	6.9	5.9	1.8	1.5
Penicillium janthinellum (ss)	2.4	4.9	2.0	1.8	3.7	5.9
Fusarium tabacinum (s)	3.2	3.5	2.0	2.4	2.3	4.9
Fusarium solani (s)	4.0	2.0	2.9	4.1	0.9	3.0
Mortierella candelabrum	4.8	4.5	1.0	2.4	0.9	1.0
Trichoderma viride (ss)	4.8	1.5	3.9	0.0	2.3	2.0
Volutella ciliata (ss)	1.6	1.5	1.0	3.6	1.8	1.5
Trichurus spiralis	2.4	3.0	2.9	1.2	1.8	0.5
Mortierella minutissima	1.6	2.0	2.9	1.2	1.4	2.0
Mortierella alpina	1.6	1.0	1.0	1.8	1.8	2.0
Acremonium furcatum (s)	0.8	1.5	0.0	2.4	1.4	2.5
Mortierella humilis	0.8	2.0	1.0	1.2	0.9	1.5
Chrysosporium merdarium (ss)	3.2	1.0	2.0	1.8	0.0	1.0
Pyrenochaeta acicola (s)	0.8	0.5	1.0	2.4	0.9	1.5
Pythium species	7	14	13	16	13	18

Tabel 2 Percentages van de meest voorkomende soorten, geïsoleerd van gewassen organische gronddeeltjes, en percentages Pythium soorten in 100 grondkorrels. Gegevens over de gevoeligheid van de soorten ontleend aan Bollen en Fuchs (1970). (s = gevoelig, ss = zeer gevoelig).

(Homans and Fuchs, 1970) after all treatments; after 6 months fungitoxic action could be demonstrated only when the soil was boiled during extraction.

The fact that even the most sensitive species were not inhibited may be explained by the particularly low metabolic activity in the soil. The colonization ratios (average number of species per particle) (Table 1) are 3 or 4 times lower than usually observed in agricultural soils (e.g. Gams and Domsch, 1967). Further experiments on effects of systemic fungicides on the saprophytic soil flora will have to be carried out with metabolically more active populations.

Samenvatting

Oriënterend onderzoek naar het effect van benomyl op de schimmelflora in kasgrond

Monsters van een humusarme zandgrond uit een kas in Naaldwijk werden gemengd met benomyl (10 ppm). Dit gebeurde 6 maanden, 3 maanden, 6 en 3 maanden en één week voor de schimmelanalyse. Saprofytische schimmels werden geïsoleerd van gewassen gronddeeltjes, *Pythium*-soorten van gezeefde grondkruimels. De isolatiepercentages van de op organische deeltjes meest voorkomende schimmels en van *Pythium* op grondkruimels zijn in Tabel 2 weergegeven. Een verschuiving in de schimmelpopulatie ten gunste van de benomyl-ongevoelige soorten kon niet worden aangetoond. Dit kan verklaard worden door de lage stofwisselingsaktiviteit van de bodemschimmelflora.

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