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Short communication

Long shelf life of *Talaromyces flavus* in coating material of pelleted seed

M.P.M. NAGTZAAM and G.J. BOLLEN
Department of Phytopathology, Wageningen Agricultural University, P.O. Box 8025, 6700 EE Wageningen, The Netherlands

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Abstract. Spores of the biocontrol agent *Talaromyces flavus* were recovered from coating material of chinese aster and tomato seeds in which they were incorporated 17 years before. The seeds had been stored at room temperature. About 20% of the ascospores had retained their heat resistance and survived treatment in aqueous suspension at 60 °C for 30 min. None of the chinese aster seeds and 90% of the tomato seeds germinated after the storage period. Presence of *T. flavus* during storage had not affected germinability of the seeds.

For commercialization of a biological control agent, a long shelf life is a strong point in favour [Bowers, 1982; Powell and Faull, 1989; Rodgers, 1993; Taylor and Harman, 1990]. According to Rodgers [1993], convential distribution chains for agrochemicals dictate that shelf lives of one to two years are requested for products stored under ambient conditions. Data on the shelf life of biocontrol agents applied to seeds in order to protect the seed and the young plant against seed- or soil-borne pathogens are scant. Gordon-Lennox et al. [1987] found that *Pseudomonas* sp. retained its viability for 120 days on sugar beet seeds that were treated with a suspension of the bacteria and that *Chaetomium globosum* survived for 2.5 years on seeds that were treated with ascospores in a methyl cellulose formulation. Suslow and Schroth [1982] obtained viable populations of rhizobacteria from sugar beet seeds treated with the bacteria in methyl cellulose and stored for one year. This paper presents an observation on the longevity of propagules of *Talaromyces flavus* (Kloecker) Stolk and Samson in the pellet material of chinese aster and tomato seeds.

T. flavus is a potential antagonist for the biocontrol of a range of soil-borne plant pathogens, e.g., *Rhizoctonia solani*, *Sclerotinia sclerotiorum* and *Verticillium dahliae* [Adams, 1990; Fravel et al., 1986; Marois et al., 1982]. Isolate F66 was used for pelleting chinese aster and tomato seeds. It was selected out of 20 isolates obtained from heat-treated greenhouse soils (70 °C, 30 min) and was shown to inhibit growth of fungi and bacteria in vitro [Bollen and Van der Pol-Luiten, 1975].

For production of ascospores, an autoclaved soil-oatmeal medium (5%

oatmeal, w/w) was inoculated with three 20-mm-discs from the edge of cultures on potato dextrose agar (PDA) plates. After two months, when cleistothecia were abundantly present, the soil-oatmeal culture was thoroughly dried and ground in a mill to particles smaller than 45 μm. In January 1976, the ground culture medium containing 10.4 (± 1.6) × 10^7 ascospores g^{-1} was incorporated in the pellet of seeds of chinese aster (*Aster chinensis,*, Super Choice Mixed) and tomato (*Solanum lycopersicum*, cv. Primset). The pellet material consisted of the *T. flavus* culture (40%), quartz flour and a polymer binder. Pelleting was performed by a commercial company. The procedure was by the split pill process as being used for commercial pelleting of seeds with clay products. In the control treatment the seeds were pelleted following the same procedure but using autoclaved soil-oatmeal cultures in stead of living ones. Originally, the seeds were used in a pilot experiment on the efficacy of *T. flavus* in controlling damping-off pathogens. Density of the ascospore population in the pellet material after the coating procedure was not assessed. It was only afterwards that it was decided to assess the stored seeds for survival of the ascospores. In June 1993, the pellet material of seeds that had been stored in the dark at room temperature since 1976 was assayed for *T. flavus* spores by plating it onto PDA plates. A heat treatment as being used for selective isolation of *T. flavus* from soil [Boosalis, 1956; Bollen and Van der Pol-Luiten, 1975] was included. From the pellets, the seeds were removed and the coating material was pulverized with pestle and mortar and subsequently suspended in sterile, distilled water and given a heat treatment for 30 min at 60 °C in a thermostatic stirring water bath. The temperature was recorded by a thermocouple placed in the test tubes. Dilution series up to 10^3 were made of heat-treated and untreated samples. Aliquots of 0.4 ml of the suspensions were spread on PDA plates with 50 ppm oxytetracyline to prevent bacterial growth (five plates for each dilution). The plates were incubated in the dark at 28 °C for 6–7 days and the number of *T. flavus* colonies recorded.

Part of the population of *T. flavus* had survived 17 years storage in the pellet material of chinese aster and tomato seeds (Table 1). The coating of aster and tomato seed contained approximately 1000 and 1200 spores per seed, respectively; this is about 0.5% of the amount initially applied to the coating material before the pelleting process. Since the assessment allow comparison of spore densities in the coating material before pelleting with those after the 17-years storage period, survival during the pelleting procedure and that during the subsequent storage period could not be distinguished separately. A long shelf life of the fungus is in line with the observation of Beuchat (1992) who found that in fruit powders, ascospores of *T. flavus* survived storage up to 30 months without loss of viability.
In dual cultures the re-isolate inhibited growth of a number of soil fungi and bacteria in the same way as the original isolated did, demonstrating that production of antibiotics was unaffected. The fungi were *Fusarium*

Table 1. Viable counts of *T. flavus* in coating material of pelleted seeds stored for 17 years and germination of the seeds

Treatment	Tomato	Chinese aster	#	
	Coated with viable spores	Coated with autoclaved spores	Coated with viable spores	Coated with autoclaved spores
	Number × 10^3 g^{-1} on PDA			
None	34.7 ± 2.3[1]a[2]	0	137.5 ± 14.9 a	0
60 °C, 30 min	8.5 ± 0.9 b	0	25.4 ± 0.9 b #	0
	Germination of seeds (%)			
None	92.7 ± 1.9	87.8 ± 1.1	0	0

[1] Mean and standard error of data of five replicate lots.
[2] Data followed by a different letter are different at P = 0.05 according to the Wilcoxon-Mann-Whitney test.

oxysporum f.sp. melongenae, *Phomopsis sclerotioides*, *Pythium sylvaticum*, *Rhizoctonia solani* and *Verticillium dahliae*; the bacteria were *Bacillus subtilis* and *Streptomyces sp.*

Part of the population had retained its heat resistance after the storage period (Table 1). The propagules in the substrate that was used for coating 17 years before were ascospores and had shown to endure the 60 °C treatment for 100%.

After removal of the coating material, seeds were incubated on wet filter paper for assessment of germinability. Germination was assessed in eight replicates with 50 seeds each, arranged as eight randomized blocks and placed in a climate room at 20 °C. None of the aster seeds and 90% of the tomato seeds germinated. Presence of *T. flavus* in the pelleting material during storage did not affect germination (Table 1).

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