Edaphic Parameters Associated with Establishment of the Biocontrol Agent *Talaromyces flavus*

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ABSTRACT

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Talaromyces flavus populations were monitored for 13 wk in the greenhouse in 25 freshly collected field soils to which ascospores of *T. flavus* had been added. Five of 23 physical, chemical, and biological parameters measured in the 25 soils were related to survival and proliferation of *T. flavus* in a multivariate principal axis factor analysis. These parameters were cation exchange capacity; potassium, sodium, and zinc concentrations; and total soil bacterial population sizes. In subsequent experiments confirming the involvement of these parameters, potassium and zinc concentrations were positively correlated with *T. flavus* survival, whereas sodium concentration was inversely related. The possible relationships of cation exchange capacity and soil bacteria to *T. flavus* survival were not elucidated.

Additional key words: *Verticillium dahliae*

Talaromyces flavus (Klöcker) Stolk & Samson (anamorph *Penicillium vermiculatum* Dangeard) is an effective biocontrol of Verticillium wilt of eggplant under agronomic production conditions (22). All theoretical mechanisms that may mediate biological control of soilborne plant pathogens (competition, parasitism, and antibiosis) require an actively metabolizing biocontrol agent. Thus, the widespread adoption of any biocontrol agent for a soilborne disease will depend on the ability of that organism to establish itself in different soils.

Previous studies to determine the effects of edaphic parameters on microorganisms have initially involved systematic manipulation of a single parameter, even though it is generally agreed that it is rarely possible to relate accurately the behavior of soil microbes to one environmental parameter (12). Moreover, these studies commonly ignore interactions among parameters. Although the behavior of soil microorganisms can vary with soil type, previous studies were often conducted in a single soil type. This study was undertaken to identify biological, physical, and chemical edaphic parameters associated with survival of *T. flavus* and to determine the nature of these associations.

MATERIALS AND METHODS

Twenty-five field locations in Maryland and New Jersey, representing a broad range of soil types, were selected from soil maps. For example, soils ranged in organic matter from less than 1 to 3.2% and in pH from 3.9 to 6.9 (Table 1). About 7.5 kg of soil in the top 15 cm was collected at each site from a single area about 0.3 m^2. All soils were collected on the same day, transported in plastic bags, and stored overnight at room temperature. On the following day, soils were diluted in sterile distilled water and plated onto different semiselective media (reference for medium composition is indicated for each microorganism) to determine endemic population sizes of the following microorganisms: *T. flavus* (21), *Fusarium* spp. (18), *Penicillium* spp. (per liter: 6 g malt extract, 1 g peptone, 0.5 g yeast extract, 15 g agar, 1 ml Tergitol), *Trichoderma* spp. (27), total actinomycetes (9), and fluorescent pseudomonads and total soil bacteria (17). Soil was analyzed for 18 physical and chemical parameters by a regional soil-testing laboratory. *T. flavus* was added to the soils after the initial assays at the rate of 10^4 ascospores per gram dry weight of soil. Soil was placed in three replicate plastic pots (17 cm in diameter) in the greenhouse in a randomized complete block design. Pots were watered as if they contained plants and were weeded regularly. Population sizes of *T. flavus* were determined 24 hr after addition of *T. flavus* to the soils, after 1 wk, and biweekly up to 13 wk. Changes in population sizes of *T. flavus* at each sampling were calculated from the population sizes at 24 hr.

The population data were analyzed using a principal axis factor analysis (5,6,10). Factor analysis is a multivariate statistical technique in which variables are represented as a linear function of a small number of unobservable "common factors" and a single latent "unique factor." Because the analysis considers all variables simultaneously, it is possible to find interrelationships among several variables that are not apparent with univariate statistics. Hence, variables that are not linearly correlated may be interrelated in a factor analysis. The procedure standardizes data in all variables by setting $\mu = 0$ and $\delta = 1$. Variables included in the preliminary factor analysis were: pH, acidity, base saturation, cation exchange capacity (CEC), soluble salts, bulk density, percentage of sand, percentage of clay, percentage of silt, percentage of water at field capacity, nitrate, potassium, phosphorus, calcium, magnesium, manganese, copper, sodium, zinc, humic matter, and endemic population sizes of *T. flavus, Penicillium* spp., *Trichoderma* spp., *Fusarium* spp., actinomycetes, fluorescent pseudomonads, and total soil bacteria, and percentage of change after 13 wk in populations of *T. flavus*. Variables that were subsets of other variables (e.g., *T. flavus* and *Penicillium* spp., fluorescent pseudomonads, and bacteria), or variables that were or may be calculated from other variables (e.g., base saturation and CEC; % clay + % silt = 100% − % sand) were not simultaneously considered in any analysis. Hence, the maximum number of variables considered in any single analysis was 23. Variables not interrelated with survival of *T. flavus* in the preliminary factor analysis were not included in subsequent analyses. Relationships of variables that were interrelated with survival of *T. flavus* in the final factor solution were confirmed through the following additional experiments.

Effects of sodium, potassium, and zinc on survival of *T. flavus*. Ascospores (10^3/g of soil) of *T. flavus* were added to white sand washed with distilled water or freshly collected soil (Galestown gravely loamy sand). The sand and soil amended with the *T. flavus*

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were placed in 9-cm-diameter square plastic pots in the greenhouse and arranged in a randomized complete block design with five replicates. Pots were watered on alternate days with either distilled water; Hoagland's solution (15); Hoagland's solution minus sodium, potassium, or zinc; or Hoagland's solution with twice the specified amount of sodium, potassium, or zinc. Populations of *T. flavus* were determined at 2 and 4 wk by dilution plating onto a medium semiselective for *T. flavus* (21). Treatments were replicated five times, and the experiment was performed three times.

Effect of CEC on survival of *T. flavus*. Two soils (a loamy sand [soil A] and a Hatboro loamy sand [soil B]) were amended with either 50% of fine-textured white sand (w/w) or 10% vermiculite (w/w) passing through a 0.42-mm-opening screen or were not amended. The CEC values for the three treatments were 5.55, 11.1, and 16.1 for soil A, and 8.35, 16.7, and 21.7 for soil B, respectively. *T. flavus* was added to the soils at 10^3 ascospores per gram dry weight. The effect of water potential was separated from the effect of CEC by equilibrating three 30-g soil samples of each type for 24 hr in a pressure plate at −1 bar. Replicates were kept in airtight containers, which were opened daily for oxygen exchange. Water was added as needed to maintain a tension of −1 bar by weighing the containers of soil and adjusting accordingly. Populations were determined at 2 and 4 wk by dilution plating onto a semiselective medium (21). The experiment was performed four times.

RESULTS

Ranges and means of physical, chemical, and biological parameters measured in 25 field soils are listed in Table 1. There were no significant ($P \leq 0.05$) simple, linear relationships (r) between any of the 23 parameters and percentage changes in population size of *T. flavus* after 3 mo.

In each of the 25 soils, the population size of *T. flavus* declined during the first 2–4 wk, then increased until 6 wk, declined rapidly until 8 wk, and then slowly declined until the end of the test at 14 wk (Fig. 1).

Five of 23 physical, chemical, and biological parameters measured were related to *T. flavus* survival in a multivariate principal axis factor analysis (Table 2). These parameters were CEC, potassium, sodium, zinc, and total soil bacteria. Variables were considered to be interrelated within a factor if their factor loadings were greater than 0.35 (6,19,20). All parameters were interrelated in factor 1, which accounted for 63.6% of the overall variance. Factor 2 accounted for 13.7% of the overall variance and showed an interrelationship among bacterial populations, potassium, and zinc. In factor 3, only *T. flavus* and zinc were interrelated, and this relationship accounted for 10.6% of the overall variance. Estimates of communality were consistently greater than 0.83 and this three-factor solution accounted for 95.6% of the variance in *T. flavus* populations.

In greenhouse experiments, populations of *T. flavus* increased with increased zinc in both soil and sand ($P \leq 0.01$). For example, in soil at the 2-wk sampling, cfu $\times$ 10^3/g = (889.9 μg/L Zn^+ in solution) + 2.11. There was also a direct linear relationship between populations of *T. flavus* and potassium ($P \leq 0.10$). In soil at the 2-wk sampling, cfu $\times$ 10^3/g = (2.6 g/L K^+ in solution) + 4.6. Populations of *T. flavus* were inversely related to sodium ($P \leq 0.01$). At the 2-wk sampling, cfu $\times$ 10^3/g = (−743.2 μg/L Na in solution) + 21.6. Populations of *T. flavus* apparently were not related to increasing CEC ($P \leq 0.05$) in either soil.

DISCUSSION

Factor analysis has been used to relate shore juniper decline (10), Hypoxylon canker incidence (5), and nematode populations (26) to soil properties. Disease progress curves (6,19,20) and components of *Septoria* resistance in wheat (16) have been examined by this method also. In our study, factor analysis was used to identify edaphic parameters possibly related to survival of *T. flavus*. Rather than perform separate experiments to determine the effect of each of the 23 parameters considered, we were able to consider simultaneously the effects of 23 variables through a multivariate analysis. The analysis indicated that a relatively small number of variables (five) were strongly interrelated with *Talaromyces* survival. Three of the variables related to survival of *T. flavus* were cations: Na^+, K^+, and Zn^+.

In our study, increasing potassium levels in soil increased survival of *T. flavus*. Potassium is a macronutrient necessary for growth of fungi (7,11). It functions in enzyme activity, carbohydrate metabolism, and ionic balance. Potassium affects such diverse processes as oogonium formation (2) and protection from radiation damage (4). Absorption of K^+ is active and occurs even against a concentration gradient as high as 5,000:1 (13). When K^+ is transported into mycelium, H^+ is usually transported out,

TABLE 1. Ranges and means of edaphic parameters and endemic populations of selected microorganisms measured in 25 soils

Parameter	Minimum value	Maximum value	Mean	Standard deviation
pH	3.90	6.90	5.80	0.93
Cation exchange capacity (CEC) (meq/100 cm^3)	1.20	16.70	6.21	3.42
Acidity (meq/100 cm^3)	0.40	4.00	0.77	0.82
Base saturation (% of CEC)	15.00	98.00	83.20	20.62
Organic matter (%)	0.00	3.20	0.65	0.70
Water at field capacity (%)	25.60	72.40	39.20	10.45
Bulk density	1.00	1.59	1.28	0.14
Clay (%)	8.50	62.60	25.30	14.30
Sand (%)	6.90	85.30	60.70	23.40
Silt (%)	5.90	55.20	14.10	11.60
Soluble salts	5.00	62.00	21.00	16.00
Calcium (ppm)	0.10	6.57	2.00	1.42
Copper (ppm)	7.5×10^2	3.6×10^4	5.9×10^3	8.8×10^3
Magnesium (ppm)	0.06	1.99	0.89	0.51
Manganese (ppm)	1.4×10^2	1.4×10^3	1.2×10^3	3.4×10^2
Nitrate (ppm)	0.00	58.00	16.92	14.95
Phosphorus (ppm)	4.00	166.00	129.80	58.50
Potassium (ppm)	5.12	74.68	37.24	21.04
Sodium (ppm)	0.00	1.7×10^{-3}	1.4×10^{-4}	3.7×10^{-4}
Zinc (ppm)	8.0×10^2	1.4×10^4	4.7×10^3	3.5×10^3
Actinomycetes (cfu/g)	1.30	35.30	10.80	8.00
Bacteria (cfu/g)	4.00	5.0×10^3	8.4×10^2	1.4×10^3
Flourescent pseudomonads (cfu/g)	0.00	766.70	47.90	151.90
Fusaria (cfu/g)	0.00	9.00	2.90	2.50
Penicillia (cfu/g)	0.00	34.70	9.90	9.10
Talaromyces flavus (endemic) (cfu/g)	0.00	8.70	0.80	1.80
Trichoderma spp. (cfu/g)	0.00	34.70	1.80	6.90

TABLE 2. Factor analysis (principal axis method-unrotated) of physical, chemical, and biological parameters interrelated with *Talaromyces flavus* survival

Variable	Factor 1	Factor 2	Factor 3	Communality
T. flavus (% survival)	0.699*[a]	−0.209	0.652*	0.956
Cation exchange capacity	0.908*	0.021	−0.236	0.880
Sodium	0.872*	−0.301	0.049	0.853
Potassium	0.741*	0.522*	−0.110	0.834
Zinc	0.747*	−0.469*	−0.368*	0.914
Bacteria	0.797*	0.443*	0.092	0.840
Variance	0.636	0.137	0.106	···
Cumulative variance	0.636	0.773	0.880	···

[a] Values marked by an asterisk indicate interrelationship within a factor, because their loading is ≥0.35 (6,19,20).

although if the fungus has been previously loaded with Na^+, then Na^+ will be transported out. Furthermore, under appropriate conditions, K^+ can be exchanged for Na^+ (8). Thus, the effects of these two ions on populations of *T. flavus* may not be independent. Sodium is apparently not required by fungi as a micronutrient except for some marine fungi (3) and may be detrimental because in our study, increasing sodium decreased survival of *T. flavus*.

Zinc is essential for all fungi because it is a constituent or activator of many enzymes. Reduced growth and sporulation of fungi cultured in media with insufficient zinc is easily demonstrated (1). Yield of *Penicillium glaucum* Link increased with increasing zinc from 0 to about 90 μg/L, then declined with further increases of zinc (24). In our study, pots were watered on alternate days with solutions containing 0, 0.023, or 0.046 μg of zinc per liter and survival of *T. flavus* increased with higher zinc levels. Zinc deficiencies affect enzyme systems in *Neurospora crassa* Shear & Dodge (23) and *Ustilago sphaerogena* Burrill (14). In addition, zinc is very important in regulation of the production of secondary metabolites in several species of *Penicillium* (29,30).

The possible role of CEC in *T. flavus* survival was not clearly demonstrated. In our study, we altered CEC by soil amendments of sand or vermiculite. Because these amendments also would affect water relations in the soils, we adjusted all soils to −1 bar to distinguish the effects of the amendments on CEC from their effects on water potential. Perhaps, CEC was only apparently important in the factor analysis because of water potential, a variable that we subsequently removed. More information is needed on the importance of water potential on the survival of *T. flauvs*.

Total numbers of soil bacteria were also related to survival of *T. flavus* as shown by the multivariate analysis. The nature of this possible effect is not currently known. Soil bacteria may either enhance or suppress *T. flavus* populations and the relationship between *T. flavus* and the bacteria may be either positive or negative and either direct or indirect. For example, fluourescent pseudomonads produce siderophores, which are high-affinity Fe^{++} chelators, that enhance acquisition of iron in iron-deficient environments (25,28). In our study, neither iron nor fluorescent pseudomonads were interrelated with *T. flavus* survival. However, analogous complex interactions may be responsible for the possible involvement of bacteria in survival of *T. flavus*.

A greater understanding of the effects of ecological parameters on the survival and proliferation of biocontrol organisms should enhance their effective utilization under production conditions. The holistic approach employed in this study may be useful in identifying parameters involved in the survival and proliferation of other biocontrol organisms.

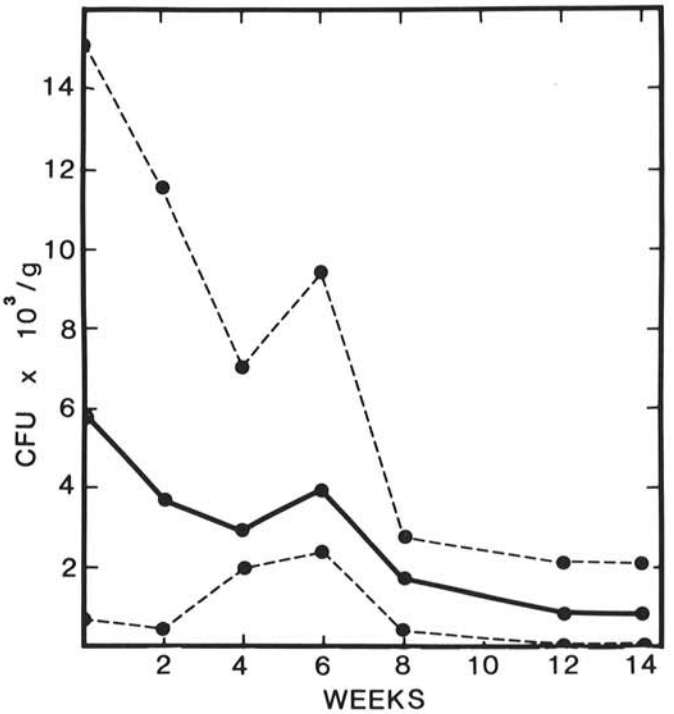

Fig. 1. Ranges and means of populations of *Talaromyces flavus* added to soils. The solid line represents the mean of the population in the 25 soils, and the broken lines indicate the extremes of the populations.

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