The Influence of Storage Temperature on Recovery of Pythium spp. and Meloidogyne incognita from Field Soils

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ABSTRACT

Soil was collected from Tifton, Fort Valley, and Blairsville, Ga., stored at 10 to 35 C in plastic bags for 4 or 8 weeks, and assayed periodically for *Pythium* spp. and root knot nematodes. The total *Pythium* population for the Tifton and Fort Valley samples increased above the initial population after storage at 10, 15, and 20 C, with the greatest recovery after storage at 15 C. Recovery was greatest at 10 C from the Blairsville sample. The population of *P. irregulare* determined the shape of the total curve in all samples. *Pythium splendens* was recovered from the Tifton sample more often at 15 C. *Pythium vexans* was the only species recovered at 35 C from the Fort Valley soil during the first 3 weeks of storage. *Pythium sylvaticum* was isolated more frequently when samples were stored at 25 C. Recovery of *Meloidogyne incognita* from the Tifton and Blairsville samples decreased with time regardless of storage temperature. However, recovery from the Fort Valley sample was greater than the initial population for the first 8-11 days at 10, 15, and 35 C. *Meloidogyne incognita* populations were most stable at 10 C in samples from all locations, but declined steadily after 16 days' storage regardless of temperature.

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Large numbers of soil samples are required to study population levels of plant-parasitic nematodes and other root pathogens such as *Pythium* spp. This means that soil samples must often be stored in the laboratory for some time prior to processing. The most satisfactory storage conditions for maintaining field population levels have not been clearly determined for certain nematodes, and information is almost completely lacking for species of *Pythium*. Our objective was to determine the effect of temperature and duration of storage on the recovery of certain *Pythium* spp. and *Meloidogyne incognita* from soil samples obtained in three geographical regions of Georgia.

MATERIALS AND METHODS.–Bulked soil samples were collected from three locations. One

sample was collected from tomato plots at the Coastal Plain Experiment Station at Tifton (southern Georgia) in August after the plants had died. A second sample was collected from tomato plots at the Mountain Experiment Station at Blairsville (northern Georgia) a few days before the plants were killed by frost in September. The third sample was collected from around actively growing peach trees at Fort Valley (central Georgia) in August. Samples were mixed in a portable cement mixer, divided into six equal portions, and within 24 hr of collection stored in polyethylene at 10, 15, 20, 25, 30, and 35 C. Tifton and Fort Valley samples were stored for 8 weeks, whereas the Blairsville sample was stored for 4 weeks. The experiment was designed so that factors such as moisture, pH, and aeration were as near constant as possible.

Pythium assays were made at weekly intervals for 4 or 8 weeks. Four-replicate 50-g samples were mixed with enough 0.3% water agar (WA) to make a total volume of 100 ml. One ml of this suspension was placed on a modified Kerr's medium (3, 6). Plates were incubated in the dark at room temperature for 2 days and washed, and individual colony counts were made. Colony counts were expressed as propagules per gram (ppg) air-dried soil. Two plates from each temperature at each sampling date were chosen at random, and every colony present was transferred to hempseed agar (HSA) and incubated at room temperature for 14 days prior to identification. Identification was based on Middleton's (7) treatment of the genus.

Four-replicate 100-g subsamples from each location stored at each temperature were assayed daily for nematodes by the sugar flotation method (5). *Meloidogyne incognita* larvae in each sample were counted using a stereomicroscope to determine the population levels at the constant temperatures. Counts were converted to per cent change from the initial population to permit comparisons between the samples.

All points from the figures mentioned specifically in the text are statistically significant. Other points may be statistically significant, but we doubt that they are biologically significant.

RESULTS.—*Pythium population.—Southern Georgia sample.—Pythium* decreased in the Tifton sample at 25, 30, and 35 C during the 1st week in storage, but no change occurred at 10, 15, and 20 C (Fig. 1). After 2 weeks at 10 and 20 C, there was a decrease from the initial population. At most temperatures, there were slight increases between the 1st and 2nd week followed by slight decreases or near stable conditions between the 2nd and 3rd weeks. After 8 weeks, populations approximated the initial population only at 10, 15, and 20 C. As storage temperature increased, there was a decline in population with time.

Pythium irregulare Buis. and *P. splendens* Braun composed the *Pythium* population of the southern Georgia sample, and were present in approximately a 5 to 1 ratio. This ratio was constant throughout the experiment except at 15 C. At this temperature, *P. splendens* became dominant and remained dominant from the 3rd through 6th week. The population of *P. irregulare* determined the shape of the total curve at all temperatures studied.

Central Georgia sample.—A decrease from the initial population occurred in the Fort Valley sample at 20, 25, 30, and 35 C during storage. The rate of population decrease was correlated directly with temperature, and the population was less than 10 ppg at 30 and 35 C throughout the 8-week storage period.

Pythium irregulare and five other *Pythium* spp., the major one being *P. vexans* d By., constituted the population of the Fort Valley sample. Other species involved were *P. ultimum* Trow, *P. pareocandrum* Drechs., *P. splendens, P. aphanidermatum* (Eds.) Fitzp., and *P. dissotocum* Drechs. The initial population showed a 10 to 1 ratio of *P. irregulare* to *P.* species, respectively. *P. irregulare* was the dominant species at all temperatures except 35 C, where *P. vexans* was the only species recovered during the first 3 weeks in storage.

Northern Georgia sample.—A decrease from the initial population occurred in the Blairsville sample at all temperatures during the 1st week of storage. The greatest decreases occurred at 10 and 35 C. However, an increase occurred at 10 after two weeks, and the population approached the initial population. The population decreased at all other temperatures.

Pythium irregulare and *P. sylvaticum* Campbell & Hendrix composed the population of the Blairsville sample, and were initially present in approximately equal numbers. This relationship was constant throughout the experiment except at 25 C, when *P. sylvaticum* became dominant between the 2nd and 3rd week, and remained dominant for the duration of the experiment. Isolates of *P. sylvaticum*, which is heterothallic, were mated with the type cultures to produce oospores for positive identification. Eighty-nine per cent of the isolates were the antheridial form.

The total population of *Pythium* spp. from the southern and central Georgia samples increased above the initial population at the lower temperatures (10, 15, and 20 C) during the test period. Recovery was greatest at 15 C from these; and at 10 C, from the northern Georgia sample. Generally, the population of *P. irregulare*, the predominant species in these samples, determined the shape of the total curve in all samples. Species recovery was influenced by storage temperature.

Nematode population.—Southern Georgia sample.—Decreases from the initial population occurred in the Tifton sample at all temperatures and duration of storage (Fig. 2). There were cyclic increases and decreases at all temperatures during the storage period, but the initial population level was never reached. The samples stored at 25 and 30 C decreased significantly from the initial populations at all durations studied. There was a gradual decline in populations after 16 days in storage, and this trend continued until the experiment was terminated.

Central Georgia sample.—Both increases and decreases from the initial populations occurred in the

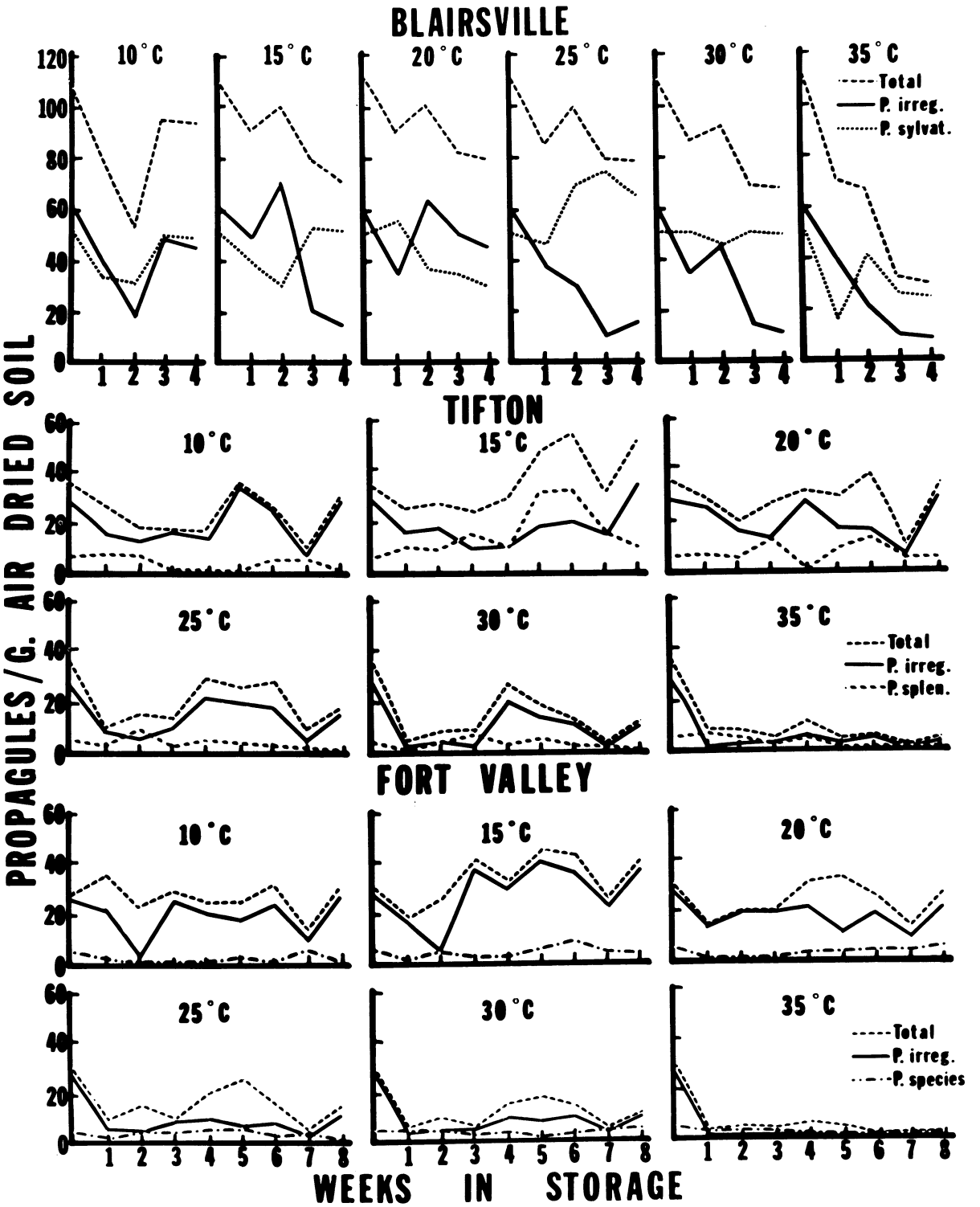

Fig. 1. Changes in *Pythium* populations and species composition in three soils stored at six temperatures. Blairsville and Tifton samples were tomato soils taken after harvest. The Fort Valley sample was from actively growing peach trees. Changes in total population of 15 propagules/g (ppg) from Blairsville, 10 ppg from Tifton, or 8 ppg from Fort Valley are significant at the 95% probability level.

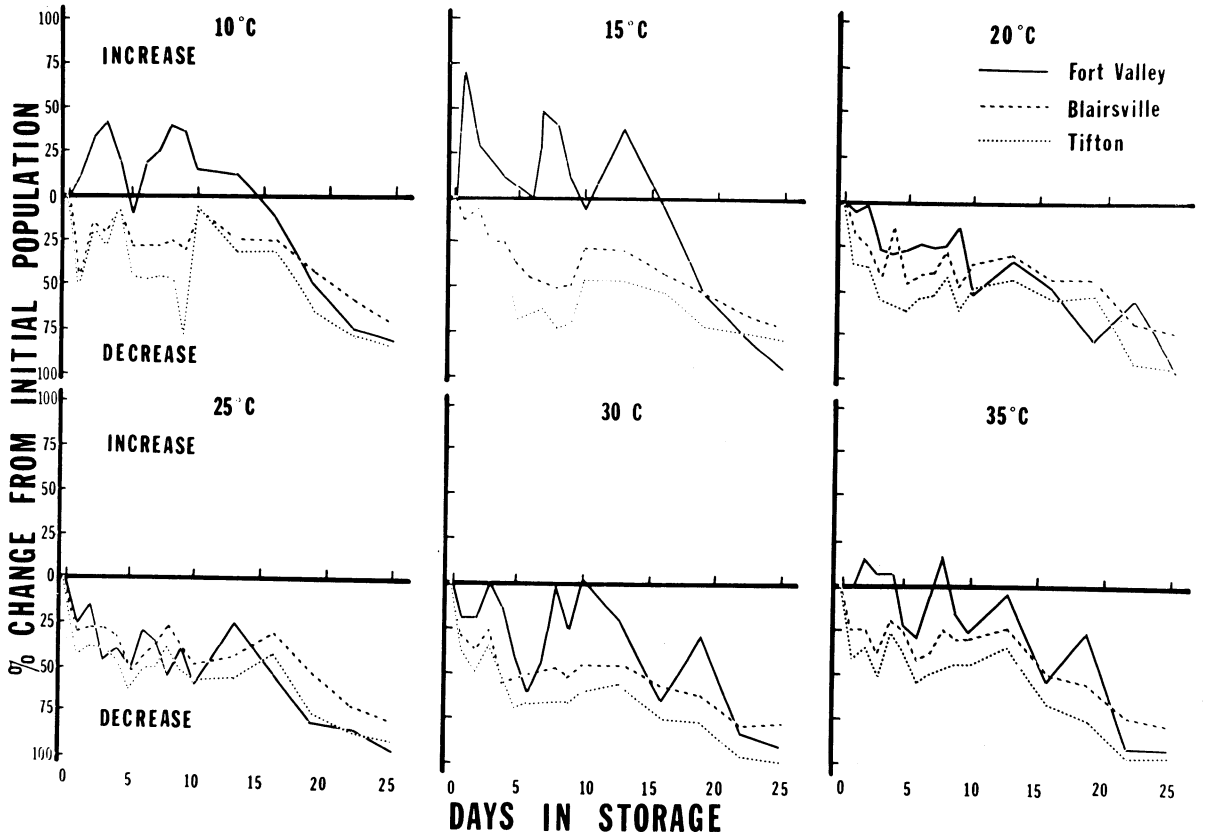

Fig. 2. Changes in population of *Meloidogyne incognita* from three soils stored at six temperatures. The Blairsville and Tifton samples were taken from tomato fields after harvest, and the Fort Valley sample from actively growing peach trees. Populations are different from the initial at the 95% probability level at the following points: Tifton: 10 C–1, 5-9, 16-25 days; 15 C–3, 5-25; 20 C–1-3, 5-25; 25C, 30 C–all points; 35 C–1-3, 6-7, 9-10, 16-25; Blairsville: 10 C–1-3, 5-25; 15 C-35 C, all points; Fort Valley: 10 C–19-25; 15 C–1, 22-25; 20 C–10, 16-25; 25 C–10, 19-25; 30 C, 35 C–22-25.

Fort Valley sample. However, the only significant increase occurred after 1 day in storage at 15 C. Generally, there were no significant changes in population levels at all temperatures studied until after the 16th day in storage.

Northern Georgia sample.—Like the Tifton sample, only decreases occurred from the initial populations at all temperatures and durations. Although the per cent decrease differed, fluctuation patterns of nematode populations from these locations were similar. Populations began to decline at approximately the 16th day, and this trend continued until the experiment was terminated.

The least amount of fluctuation in *M. incognita* populations occurred at 10 C in samples from all locations. A steady decline in populations was generally noted after 16 days' storage regardless of temperature.

DISCUSSION.—Hendrix et al. (4) showed that *P. irregulare* was isolated from the roots of diseased peach trees much more readily during cooler periods, whereas *P. vexans* was the predominant species isolated from the same trees during warm periods. In subsequent experiments, they found the optimum temperature for infection to be 10 C; and 30 C with *P. irregulare* and *P. vexans*, respectively (8). Results of this study lend support to their findings, since isolation of *P. irregulare* from field samples was greater at 10, 15, and 20 C, whereas *P. vexans* was isolated more readily at 35 C.

Results of our study indicate that populations of *M. incognita* fluctuated least at 10 C, agreeing closely with Bergeson's report (2) that 9.5 C was optimum for survival of *M. incognita* in soil as shown by galling of tomato roots. More recently, Barker et al. (1) found 13 C to be near optimum for storage of *M. incognita*. They support the suggestion of Van Gundy et al. (9) that lower storage temperatures reduce nematode activity and metabolism, thus keeping them physiologically young. Our study further supports this idea in that nematode population shifts in samples from the three locations were correlated with the condition of the host root system at the time of sampling. The southern and northern Georgia samples were collected from tomato plots in which the root systems were in a stage of decline. Eggs in the southern and northern Georgia samples were probably older, and a greater proportion were either

in advanced stages of maturity or nonviable. However, the central Georgia sample was collected from around roots of actively growing peach trees, and probably contained eggs in all stages of maturation, since the females were active.

Thus, optimum storage temperature and duration of storage for samples to be assayed for of both *M. incognita* and *Pythium* spp. may vary accordingly to the objectives of the study. For example, when presence or absence of one of the organisms is of primary interest, the samples should be stored at the temperature and for the length of time which permit the greatest recovery; e.g., 15 C for 6 weeks for *Pythium* species in Tifton soil. Conversely, if the investigator desires to closely approximate the actual field population of this organism from this location, he would have a choice of holding the sample at 10 C for 5 weeks or at 15, 20, or 25 C for 4 weeks. Similar procedures could be followed for *Pythium* species from other locations as well as for *M. incognita* from all locations.

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