only 11 inches below the bed surface on several occasions, little negative nutritional effects resulted because leaf-N concentration remained high.

In the Palm Beach study, the water table was maintained between 18 and 20 inches most of the season. The water table dropped to about 28 inches on 3 occasions and never rose above 14 inches below the bed surface.

At the Manatee, spring, 1989 location (same farm as fall, 1988), the water table was allowed to fluctuate greatly early in the season. Early in the season, during a drought period, the water table fluctuated between 14 and 36 inches and dropped below 30 inches on 4 occasions. Later in the season, the water table was maintained more uniformly between 15 and 18 inches below the bed surface.

Results of these studies show that current IFAS recommendations for N of 160 lb./acre are adequate for high yields of high quality fruits. These crop nutrient requirements were the same for crops in various seasons and locations. Results show that tomato growers could reduce N rates without sacrificing yield or fruit size. Similar results were obtained recently with pepper (9). Large-scale field demonstrations should be used to demonstrate results of small successive (10 to 20%) reductions in N rates to commercial tomato growers.

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RECOLONIZATION OF FUMIGATED TOMATO PRODUCTION SOIL IN DADE COUNTY BY *PYTHIUM* SPP.[1]

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Additional index words. pre-emergence damping-off, post-emergence damping-off, *Pythium aphanidermatum, Pythium catenulatum, Pythium oligandrum, Pythium ultimum*

Abstract. **Rockdale fine sandy loam soil in a field planted to tomato (*Lycopersicon esculentum* Mill.) in Dade County, FL was monitored at 20-day intervals over a 120-day season for the presence of *Pythium* spp. Points along transects across the bed were assayed with tomato-seedling baits and a medium selective for *Pythium* spp. *Pythium aphanidermatum* (Edson) Fitzp. and *P. ultimum* Trow accounted for up to 95% of all *Pythium* spp. recovered from fumigated or nonfumigated soil on a given sampling date. *Pythium oligandrum* Drechsler, *P. catenulatum* Matthews, and nonidentified species of *Pythium* were recovered less frequently. *Pythium aphanidermatum* was first recovered from fumigated soil on the interior edge of the bed 60 days after planting, and it was detected throughout the bed 80 days after planting. In contrast, *P. ultimum* was detected throughout the bed after 40 days, although it was never recovered as often as *P. aphanidermatum*. *Pythium aphanidermatum* and *P. ultimum* significantly reduced ($P < 0.05$) seedling emergence and caused post-emergence damping-off of 'Duke' tomato during pathogenicity tests in artificially infested potting mix. Treatment of soil in the field with metalaxyl prior to fumigation reduced recolonization of fumigated soil at some bed locations by both species of *Pythium*. Fruit yields were not increased by treatment with this fungicide.**

Tomato in Florida can be affected by soilborne factors which include nematodes, weeds, and pathogens. Within the last 25 years, fumigants such as methyl-bromide and chloropicrin have been used in conjunction with polyethylene mulches to reduce losses due to soilborne problems, such as "old-land" disease, fusarial wilt, and root-knot nematode in tomato-production areas throughout Florida (11).

That broad-spectrum fumigants reduce soilborne problems is widely recognized (10). The effectiveness of such

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fumigants derives from their extreme toxicity to most life forms. For example, 90% of the mycelial propagules of *Pythium ultimum* were killed after 14.7 hr exposure to 11.7 µl of methyl-bromide per liter of air (9).

The "biological vacuum" which follows such treatment facilitates a rapid colonization of treated soil by certain microorganisms including some soilborne plant pathogenic fungi (6,7). Cook and Baker (3) have used *Pythium* spp. as examples of soilborne pathogens that are capable of quickly recolonizing fumigated soil. It appears that soilborne pathogens that are capable of rapid, saprophytic growth are potential hazards in production systems which utilize soil fumigation.

Pythium-induced damping-off of tomato seedlings has been observed in recently fumigated soil in Dade County, Florida (Ploetz, unpublished). Since *Pythium* spp. may also cause disease on feeder roots of mature tomato plants produced in fumigated soil, a study of the activities of *Pythium* spp. in fumigated tomato production soil in Dade County was initiated. The objectives of the study were to: 1) identify *Pythium* spp. associated with tomato production in Dade County; 2) determine the extent to which fumigated soil is colonized by these fungi; and 3) determine the effect of metalaxyl on colonization of fumigated soil by *Pythium* spp. and on fruit yield.

Materials and Methods

Cultural practices. Standard bed preparation, fertilization, fumigation with methyl bromide plus chloropicrin, polyethylene mulch, direct seeding, and overhead irrigation were utilized in a field study in Homestead at the Tropical Research and Education Center of the University of Florida. Soil in the field was a Rockdale, fine sandy loam (Ruptic-Alfic Lithic Eutrochrepts clayey, mixed, hyperthermic) with particles $\leq$ 5 cm in diameter. Prior to fumigation, soil in some of the beds was drenched with metalaxyl (Ridomil 2 EC at 7 liters in 132 liters of water ha^{-1}); soil between rows (i.e., middles) was not treated. The metalaxyl and nontreated control treatments were replicated 4 times in a randomized, complete block design. 'Duke' was direct-seeded into the 105-cm-wide beds on a 30-cm spacing 5 days after fumigation. Seedlings were hand-thinned about 2 weeks after planting to a single seedling per hole.

Sampling protocol. Every 20 days after planting, nonfumigated soil between the beds and fumigated soil under polyethylene mulch in the bed were sampled for the presence of *Pythium* spp. in nontreated blocks; soil in blocks treated with metalaxyl was sampled every 40 days. Soil was recovered on sample dates along randomly chosen transects which ran perpendicular to the length of the bed and through a planting hole in the mulch. Transects were used only once and each was separated in the row by a buffer plant on either side which was not sampled.

On each sampling date, soil was removed from 9 points along a sampled transect. Two transects were sampled from each treatment replicate, and points along the transect were located at the planting hole and at distances of 5 and 30 cm on either side of the hole (inside the bed) and 5 and 20 cm outside and on either side of the shoulder of the bed. Hereafter, these locations will be referred to by their distance from the shoulder of the bed. Beginning with the planting hole, the respective interior locations were -45, -30, and -15 cm and exterior locations were +5, and +20 cm.

About 100 cc of soil were recovered from each point on the transect with spoons which were disinfested with 70% ethanol between each sample. Samples were placed individually in plastic bags and transferred to the laboratory in an insulated ice chest. Soil was processed within 4 hours of sampling.

Recovery and speciation of isolates of Pythium. Presence of *Pythium* spp. in each sample determined with baits of 'Duke' tomato seedlings and *Pythium*-selective media. Each soil sample was mixed and dispensed into 2 sterile, plastic 9-cm Petri plates; soil particles and rocks > 1.5 cm in diameter were discarded. Soil in Petri plates was flooded and 4 intact, 1- to 2-week-old 'Duke' tomato seedlings were placed in the flooded slurry in each plate such that bare roots of the seedlings were in contact with the soil and leaves were outside the edge of the plate. Lips of lids of the Petri plates were broken off before they covered the seedlings to avoid damaging stems of the plants. Seedlings were incubated on a laboratory bench at about 25C.

After 2 days, seedlings were recovered from flooded soil, washed under running tap water, and blotted dry with paper towels. Roots of seedlings were placed on Burr and Stanghellini's (2) medium on the first sample date (day 0), and thereafter (days 20 - 120) all roots were assayed with Mitchell's (8) modification of Tsao and Ocaña's (13) *Phytophthora*-selective medium. Roots were incubated without light at 35C, and were observed for growth of *Pythium* spp. within 2 days of plating.

Isolates of *Pythium* were speciated after eliminating contaminants by culturing each in van Tieghem cells embedded in modified Tsao and Ocaña's medium. Two- to three-day-old, axenic, V-8 agar cultures of each isolate were diced (2 - 3 mm^2) and added to boiled grass blades in sterile pond water. Isolates were observed for fruiting structures after 20 - 48 hours, and the key and species descriptions of van der Plaats-Niterink (14) were used to identify species of *Pythium*. For most of the study, *Pythium* spp. were identified on the basis of colony morphologies which were associated with the species on the selective medium. Speciation, as described above, was resorted to when "new" cultural forms of *Pythium* were observed on the selective medium or to periodically check the identification of commonly recovered forms.

Pathogenicity of Pythium *spp.* Twelve, 5, and 6 isolates of *Pythium aphanidermatum, P. ultimum,* and *P. catenulatum* from the experimental field, respectively, were tested for pathogenicity in 2 experiments. Four and 8 seeds of 'Duke' were planted 1 cm below the surface of a peat-perlite potting mix in 10-cm-diameter pots during experiments 1 and 2, respectively. Isolates of *Pythium* spp. were grown on autoclaved sorghum seed and used for inoculation by placing 5 colonized seeds on the surface of the potting mix 2 days after seeds were planted. Noninfested seeds were used for a noninoculated control treatment. Each pot was considered a replicate and each treatment (isolate) was replicated twice in an experiment.

Emergence and post-emergence damping-off of tomato seedlings were evaluated 1 and 2 weeks after inoculation for each *Pythium* spp. by first dividing the number of seedlings which emerged by the number of seeds planted. This value was then divided by percent emergence for the noninfested treatment. Post-emergence damping-off was the percent of emerged plants that damped-off. Seedling survival was determined by dividing the number of emerged and healthy

seedlings 2 weeks after planting by the number of seeds which were planted; this value was then divided by the percentage of seeds that emerged and survived in the noninfested control treatment.

Yields. Fruit were harvested from metalaxyl-treated and nontreated plots 94 and 105 days after planting and counted, weighed, and sized as small (6 X 7), medium (6 X 6), or large (5 X 6).

Analyses. The presence of different species of *Pythium* at different bed locations was quantitated by dividing the number of bait root systems that were colonized by each species of *Pythium* by the total number of baits used to assay the location. Data for each location were combined such that 32 seedlings were assayed per treatment replicate and a total of 128 seedlings were assayed on a given sample date for each location. Thus,

8 (seedlings per point)
× 2 (points on a transect)
× 2 (transects per replicate)
× 4 (replicates)
= 128 seedlings.

A total of 64 seedlings were assayed for soil from the planting hole.

The significance ($P > 0.05$) of mean differences of *Pythium* recovery between treated and nontreated plots was assessed with the t-test procedure of SAS (12). Duncan's multiple range test was used to separate means for pathogenicity data, and the influence of metalaxyl on yields was analyzed with t-tests (12).

Results and Discussion

Pythium aphanidermatum and *P. ultimum* accounted for up to 95% of all species of *Pythium* recovered from fumigated or nonfumigated soil on a given sample date; *P. aphanidermatum* was the most frequently recovered of the two. *Pythium oligandrum, P. catenulatum,* and other nonidentified species of *Pythium* were recovered infrequently.

Although *P. aphanidermatum* was always recovered from nonfumigated soil (locations +5 and +20 cm), it was not recovered from fumigated soil until 60 days after planting and was recovered then only from the edge of the bed (the –15 cm location) (Fig. 1A). In fumigated soil, the pathogen was recovered relatively infrequently until 120 days after planting. *Pythium ultimum* was recovered from fumigated soil earlier than *P. aphanidermatum* (40 and 60 days after planting, respectively) and was the most commonly recovered *Pythium* spp. in fumigated soil until 80 days after planting (Figs. 1A and 1B).

Pythium aphanidermatum and *P. ultimum* were both pathogenic to 'Duke' tomato seedlings. Each significantly reduced emergence and seedling survival and caused post-emergence damping-off, but *P. aphanidermatum* was more virulent than *P. ultimum* in these studies (Table 1). *Pythium catenulatum* had no effect on seedling emergence or survival, nor did it cause post-emergence damping-off.

Recoveries of *P. aphanidermatum* were lower in fumigated, metalaxyl-treated soil than in fumigated, nontreated soil at the –15 and –45 cm locations 80 days after planting; recovery in metalaxyl-treated soil was lower for *P. ultimum* at –45 cm after 40 days (Table 2). Fruit weight and numbers of small, medium, large, and total marketable fruit were not affected by treatment with the fungicide ($P > 0.05$; Table 3).

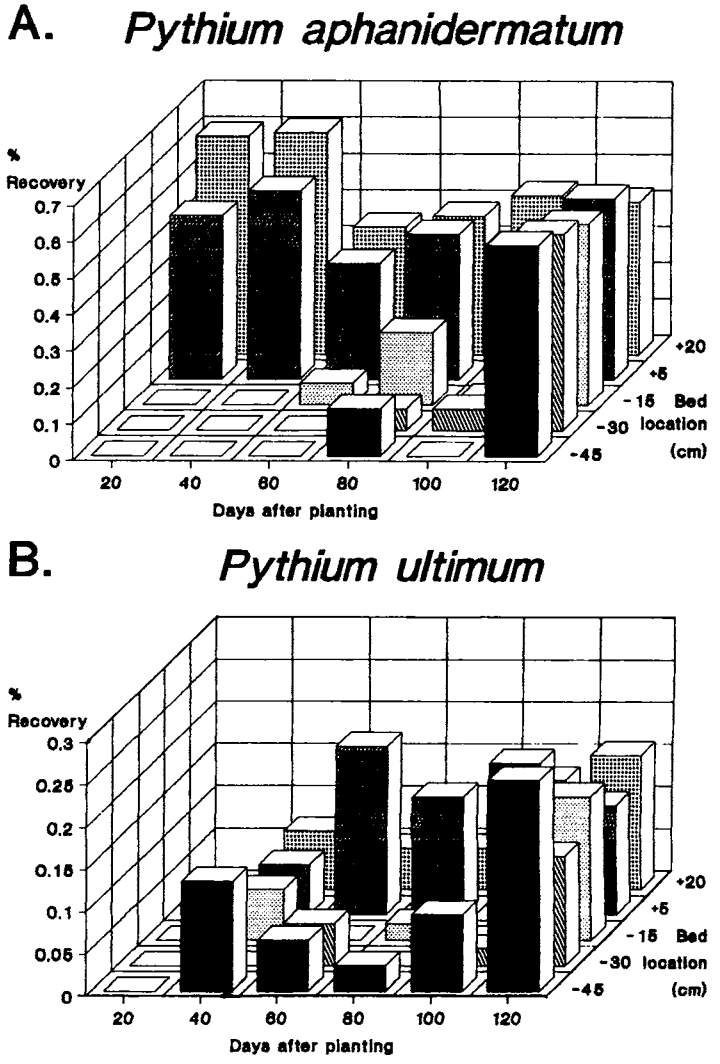

Fig. 1. Presence of A. *Pythium aphanidermatum* or B. *P. ultimum* in Rockdale fine sandy loam 20 to 120 days after direct seeding 'Duke' tomato. Soil was assayed for the pathogen with 'Duke' seedling baits and recovery is the percentage of baits from which the pathogen was recovered. Locations are relative to the edge of the bed and correspond to the planting hole 45 cm from the edges of the bed, 2 interior locations 15 and 30 cm from each edge of the bed (fumigated), and 2 exterior locations 5 and 20 cm outside each edge of the bed (nonfumigated).

TABLE 1. Influence of species of *Pythium* on seedling emergence, post-emergence damping-off, and seedling survival of 'Duke' tomato.[z]

Pythium spp.	Number of isolates tested	Emergence (%)[y]	Post-emergence damping-off (%)[x]	Seedling survival (%)[w]
Noninfested control	—	100.0 a	0 b	100.0 a
P. aphanidermatum	12	39.7 c	36.0 a	23.3 c
P. ultimum	5	66.1 b	2.7 b	64.0 b
P. catenulatum	6	91.8 a	0 b	91.8 a

[z]Seeds were planted in a peat-perlite potting mix. After 2 days, sorghum seeds infested with one of the *Pythium* spp. isolates was placed on the surface of the potting mix. Mean separation within columns by Duncan's multiple range test, $P > 0.05$.
[y]Mean emergence of seedlings for each treatment (*Pythium* spp.) is relative to the noninfested control and was recorded 1 week after inoculation.
[x]Post-emergence damping-off was recorded 2 weeks after inoculation.
[w]Mean seedling survival was the percentage of seedlings that survived 2 weeks after inoculation relative to the noninfested control.

TABLE 2. Effect of metalaxyl on net recovery of *Pythium aphanidermatum* and *P. ultimum* from fumigated soil.

Distance from edge of bed (cm)[y]	Net recovery (%)[z]					
	P. aphanidermatum			*P. ultimum*		
	Days after planting			Days after planting		
	40	80	120	40	80	120
–15	nr[x]	–16*	+4	–5	–1	+8
–30	nr[x]	–1	–16	nr[x]	nr[x]	–9
–45	nr[x]	–13*	–16	–9*	–3	–25

[z]Net recovery is the percentage of tomato bait seedlings colonized by *P. aphanidermatum* or *P. ultimum* in metalaxyl-treated soil minus the percentage colonized in nontreated control soil. Differences marked (*) were significant at $P < 0.05$ according to t-tests.
[y]Locations were 15 and 30 cm inside the bed and at the planting hole (45 cm).
[x]Not recovered (nr) in either fumigated or nonfumigated soil.

Pythium aphanidermatum and *P. ultimum* have been reported frequently as pathogens of tomato in the U.S. and elsewhere (4,14), and their prevalence in the field examined in the present study was not surprising. The recovery of *P. ultimum* with a method which was designed to select *P. aphanidermatum*, however, was not expected. *Pythium ultimum* has optimum and maximum temperatures for growth of 25 - 30C and 35C, respectively (14). Its common recovery after only 2 days of incubation at 35C might suggest a tolerance of high temperature by Florida isolates of the pathogen, but temperatures higher than 35C were not tested for these isolates during our study. *Pythium aphanidermatum, P. catenulatum*, and *P. oligandrum* all have higher maximum temperatures for growth (40, 40, and 37C, respectively) or optimal ranges (30 - 35, 30 - 35, and 30C, respectively) than *P. ultimum*, and their recovery from soil at 35C would be expected (14).

This is the second report of *P. catenulatum* in association with tomato, although this species was apparently not pathogenic to tomato in our tests (see Table 1). In 1956, Frezzi (5) reported *P. catenulatum* on tomato in Argentina.

Low levels of *Pythium* were detected in fumigated soil during the first 2 to 3 months of the study. In general, levels of *Pythium* spp. did not reach those found in nonfumigated soil until the last sampling date 120 days after planting (Figs. 1A and 1B). It is possible that these fungi are incapable of rapidly recolonizing fumigated Rockdale soil. In the absence of sufficient organic matter for colonization (O.M. usually ranges from 0. 5 to 1.0% in this soil), these facultative parasites may rely primarily on host tissue when *recolonizing* fumigated Rockdale fine sandy loam.

Alternatively, conditions that are required for rapid recolonization of such soil may have not occurred during this study. *Pythium*-induced damping-off of tomato seedlings in Dade County has been observed in association with flooding of the bed after heavy rainfall which suggests a movement of inoculum with flood water. Although inoculum of *P. aphanidermatum* was detected in nonfumigated soil next to the bed 20 days after planting, movement of this inoculum to fumigated soil was not detected until 40 days later (or 60 days after planting) (Fig. 1). Splash-dispersal of other pythiaceous pathogens occurs and overhead irrigation used in the present study should have provided an effective mechanism for dispersal. Reasons for delayed movement of this pathogen to fumigated soil are not known.

In contrast to results from a previous study conducted in Florida (1), yields of tomato were not increased by a metalaxyl drench in the present study (Table 3). Because *P. aphanidermatum* and *P. ultimum* were generally not prevalent in nontreated or treated, fumigated soil until late in the season (Figs. 1A and 1B and data not shown), it is probable that disease caused by these species in fumigated soil also did not occur until late in the season. In older tomato plants, species of *Pythium* are pathogens of root tips. Hence, late-season disease caused by these pathogens may be expected to have much less of an effect on yield than early season disease. Early season colonization of fumigated soil and subsequent disease in the present study may have resulted in relatively higher yields in soil treated with metalaxyl.

Various factors, such as soil microbiota, soil water potential, and temperature, probably play important roles in the recolonization of fumigated soil by *Pythium* spp. Considering the tomato industry's reliance on soil fumigation and the potential for reduced stand establishment and disease on adult plants caused by these pathogens, the identification of such factors should assume greater importance.

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TABLE 3. Effect of metalaxyl fungicide on yields of 'Duke' tomato.

Treatment	Fruit size	Yield per hectare					
		Harvest 1[z]		Harvest 2[z]		Total[y]	
		kg	number	kg	number	kg	number
Nontreated	6 X 7	130	1544	7382	122623	7512	124168
	6 X 6	6222	58068	13877	136182	20098	194250
	5 X 6	16611	85867	2621	16370	19232	102238
Metalaxyl	6 X 7	162	1621	6643	114051	6805	115672
	6 X 6	7253	63781	12574	113513	19827	177294
	5 X 6	16243	76215	1953	12432	18197	88647
	Total	23658	141617	21170	239996	44829	381613

[z]Harvests 1 and 2 were conducted 94 and 105 days after planting, respectively.
[y]Treatment effects were not significant as determined with t-tests at $P < 0.05$.

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FUSARIUM WILT OF TOMATO IN FLORIDA BEFORE AND AFTER AN OVERSEASONING PERIOD

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Abstract. **During August 1987, a field of fine sand was artificially infested with races 1, 2, and 3 of the tomato *Lycopersicon esculentum* Mill.) wilt Fusarium (*Fusarium oxysporum* (Schlecht.) f. sp. *lycopersici* (Sacc.) Snyder and Hansen. The field then was planted to 2 consecutive tomato crops, a fall crop followed by a spring crop. The 3-month midwinter overseasoning period between crops greatly reduced the incidence of Fusarium wilt of race 1-resistance and tolerant 'Improved Rutgers' (caused by race 2 or 3), race 1-resistant 'Manapal' (caused by race 2 or 3), race 1 and 2-resistant 'Walter' (caused by race 3). Disease incidence caused by race 1 (but not race 2 or 3) was slightly decreased on susceptible 'Bonny Best.' Yield losses were sharply reduced by the overseasoning period on 'Rutgers' (caused by race 2 or 3), 'Manapal' (caused by race 2 or 3), 'Walter' (caused by race 3), and 'Bonny Best' (caused by race 1 or 2, but not race 3). Based on yields and disease incidence, race 3 survived better than race 2, and race 2 survived better than race 1. 'Manapal' was less susceptible to race 2 or 3 than 'Bonny Best' and no more susceptible than 'Improved Rutgers' with genes for tolerance to race 1 in addition to the 1 gene for resistance to race 1. 'Walter' was less susceptible to race 3 than 'Bonny Best' and no more susceptible than 'Improved Rutgers' or 'Manapal.'**

Fusarium wilt, caused by *Fusarium oxysporum* (Schlecht.) f. sp. *lycopersici* (Sacc.) Snyder and Hansen, remains one of the most destructive diseases of tomato. The most practical means of control is the use of resistant cultivars. With the appearance of race 3, however, there are no resistant cultivars available for the growers of fresh market tomatoes in the United States. Moreover, some question the wisdom of the use of monogenic resistance claiming that cultivars with monogenic resistance will be highly susceptible should a pathogenic race appear that is able to incite wilt of these heretofore resistant cultivars (1,4).

Rotation with other crops is another means of reducing the severity of Fusarium wilt. However, the literature indicates that years are necessary to rid the soil of race 1 (2,3). No information or estimates are available on the length of time needed for populations of races 2 and 3 to decline enough to permit a second crop to be grown successfully. It was stated that race 1 should survive better than race 2, and that race 2 should survive better than race 3 (4). However, no experimental evidence was presented to support this view.

A field experiment, therefore, was carried out to obtain information on the relative overseasoning ability of races 1, 2, and 3 of the tomato wilt Fusarium and to determine the effect of the 3 races on the incidence and severity of disease on 4 tomato cultivars with different wilt-resistant genotypes.

Materials and Methods

Raised beds of EuaGallie fine sand were formed, fertilized (using accepted commercial practices), and fumigated 11 August 1987. A 67% methyl bromide: 33% chloropicrin broad-spectrum fumigant was used at a 350 lb./acre. Beds were covered immediately after fumigation with 1.25 mil white-on-black polyethylene mulch. Two weeks later 2.25 inch diameter holes (40 holes per 50 foot long whole plot) were cut through the mulch in the drill row. The soil in 10 of these holes were infested with race 1, 10 with race 2, 10 with race 3, and 10 remained nonin-

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