Pythium spp. Associated with Bell Pepper Production in Florida

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ABSTRACT

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Ten species of *Pythium* and a group of isolates that produced filamentous sporangia but did not form sexual structures (*Pythium* 'group F') were recovered from the root systems of fresh market bell pepper plants grown on polyethylene-mulched production systems in Florida. Pathogenicity tests using pasteurized field soil inoculated with infested wheat seed demonstrated that *P. aphanidermatum, P. myriotylum, P. helicoides,* and *P. splendens* can cause significant root rot and reductions in root growth of pepper. *P. aphanidermatum* and *P. myriotylum* caused the most severe root rot, the greatest reductions in plant weight, and 42 and 62% plant mortality, respectively. In pathogenicity tests with tomato plants, these four species produced similar plant weight losses and disease ratings to those observed in pepper, but little or no plant mortality. Low incidences of root tip necrosis in pepper plants were observed with P. *arrhenomanes, P. catenulatum, P. graminicola,* and *P. irregulare*, but none of these species caused losses in root weight and only *P. irregulare* reduced shoot weight. *P. periplocum, P. spinosum,* and *Pythium* sp. F colonized root tissue of pepper but caused no significant root rot and did not adversely affect growth. Similar trends were observed with tomato, except that *P. arrhenomanes* caused limited root tip necrosis without affecting plant growth and *P. catenulatum, P. graminicola, P. irregulare, P. spinosum,* and *Pythium* sp. F colonized at least some of the plants but did not cause root disease. A significant interaction between temperature and *P. aphanidermatum* or *P. myriotylum* was observed on pepper transplants. The greatest reductions in growth occurred at 28°C, whereas plant mortality only occurred at 34°C.

Additional keywords: *Capsicum annuum, Lycopersicon esculentum,* methyl bromide

Florida is the leading producer of fresh market pepper (*Capsicum annuum* L.) in the United States. In the 1997–98 production season, 7,600 ha produced pepper fruit valued at $272 million (2). Since the early 1970s, growers have used a polyethylene-mulched production system in which control of soilborne pests is achieved primarily through soil fumigation with methyl bromide (4,6). Methyl bromide has been implicated as a major ozone-depleting compound and a phase-out of its production and sale in the United States was initiated in 1999. The implementation of pest management strategies as alternatives to fumigation with methyl bromide will result in changes in disease development. Identification of pest complexes that impact crop production is essential to understanding the potential of new management systems.

In September 1996, symptoms typical of low fertility, including chlorosis, stunting, and reduced vigor of pepper plants, were observed in a commercial pepper farm (Green Cay Farm, Boynton Beach, FL) undergoing a transition from conventional pest management tactics, including fumigation with methyl bromide, to a biorational farm management system based on soil solarization and incorporation of compost (14). Similar symptoms observed previously in methyl bromide-fumigated plots were commonly attributed to soil compaction, low fertility, or salt damage. Preliminary isolations from root systems of symptomatic and asymptomatic plants in the field consistently produced colonies of several species of *Pythium* Pringsh. (D. O. Chellemi, *unpublished data*).

Pythium spp. are common inhabitants of cultivated soils and *P. aphanidermatum* (Edson) Fitzp., *P. myriotylum* Drechs., and *P. irregulare* Buisman have been isolated from pepper in Florida (1,5). However, information on the pathogenicity of these species and other *Pythium* spp. associated with cultivated peppers in Florida and their impact on plant health is lacking. Several of the *Pythium* spp. isolated from pepper, including *P. aphanidermatum* and *P. myriotylum*, also infect tomato (*Lycopersicon esculentum* Miller; 1,5,9–11), but comparative pathogenicity tests with the many species isolated from both pepper and tomato have not been conducted.

The objectives of this study were to (i) identify root-colonizing *Pythium* spp. associated with pepper roots in polyethylene-mulched production systems in Florida, (ii) determine the relative pathogenicity of *Pythium* spp. isolated from pepper roots to both pepper and tomato, and (iii) assess the impact of temperature on the capability of important pathogenic isolates to cause disease on pepper.

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MATERIALS AND METHODS

Collection of *Pythium* spp. Root systems of pepper plants were obtained from commercial pepper farms located in Indian River, Martin, and Palm Beach Counties in December 1997 and December 1998. Roots were washed in tap water, cut into 1-cm segments, surface sterilized for 90 s in 0.1% sodium hypochlorite (2% Chlorox), rinsed in sterile deionized water, crushed and blotted dry in sterile paper towels, and placed onto agar plates containing PARP medium (8). Plates were incubated at 30°C in the dark for 1 to 2 days. Pure cultures were obtained from hyphal tips growing from cultures transferred to fresh plates of PARP.

Pythium spp. were identified using a grass leaf culture technique (13). Five-mm-diameter disks taken from 2-day-old colonies grown on V8 juice agar were placed into petri plates containing sterilized blades of St. Augustine grass (*Stenotaphrum secundatum* (Walter) Kuntze) in a 2:1 mixture of autoclaved distilled water plus pond water (13). Cultures were maintained at 20, 30, and 40°C under 12 h of light per day and observed after 1, 3, 7, and 14 days. Identification of species was determined using a key by Van der Plaats-Niterink (12).

Pathogenicity of *Pythium* spp. isolated from pepper plants. Isolates of *Pythium* spp. were evaluated for pathogenicity to pepper and tomato. Infested wheat seed (hard red winter wheat) was used as the inoculum source. A mixture of 25 ml of deionized water and 20 g of wheat seed was allowed to soak for 24 h in each of two 250-ml flasks for each isolate. The flasks were then autoclaved two times on each of two consecutive days. Each flask was inoculated with five 5-mm disks from a 2-day-old culture grown on V8 juice agar. Flasks were incubated for 2 to 4 weeks in the dark at 25°C and shaken periodically to ensure uniform growth of inoculum.

Field soil, a Myakka sand (sandy, siliceous, hyperthermic, Aeric, Haplaquad, pH 7.5), collected from methyl bromide-treated areas of Green Cay Farm (Boynton Beach, FL) was air dried, passed through a 2-mm sieve, adjusted to 6% soil moisture, and microwaved for 4 min on high power in 1-kg lots. Inoculum was added at the rate of 4 g of 2-week-old infested wheat seed per kilogram of soil. 'Jupiter' pepper seedlings were grown in Styrofoam seed flats containing potting mix (Metro-mix 300, Scotts Sierra, Marysville, OH) for 6 weeks. Individual seedlings were transplanted to 10-cm-diameter pots containing 400 g of soil infested with one of the 11 species of *Pythium* or noninfested wheat seed. Six replicate pots were prepared for each *Pythium* sp. and the noninfested wheat seed. The plants were maintained in greenhouses with temperatures ranging from 21 to 34°C. Because of the importance of *Pythium* spp. on tomato, the same procedures were employed for pathogenicity tests with 3-week-old 'Bonny Best' seedlings. Each experiment was repeated for pepper and tomato.

The incidence of plant mortality was recorded daily. After 4 weeks, surviving plants were harvested and fresh weights of roots and shoots were determined. Root disease was estimated based on an index where 0 = no root symptoms; 1 = 1 to 25% of root tips necrotic; 2 = 25 to 50% of root tips necrotic; 3 = 50 to 100% of root tips necrotic plus localized necrotic lesions on the tap root or crown; 4 = extensive root rot with few or no white roots, crown rot extensive; and 5 = root system completely necrotic and plant dead or moribund. Complete root systems were surface disinfested and plated on PARP medium as described above. Incidence of infection was determined and representative isolates from each species were subcultured and identified on grass-leaf cultures. Data were analyzed by analysis of variance (ANOVA). The data from repeated experiments were analyzed as repeated measures and combined if there was no interaction between experiment and treatment effects. Separation of means was determined by Duncan's multiple range test ($P = 0.01$).

Effect of temperature and *Pythium* spp. on growth of pepper transplants. A three-by-four factorial design was used to investigate interactions between temperature and two pathogenic *Pythium* spp. Constant temperature regimes of 20, 28, and 34°C were maintained in growth chambers illuminated with a combination of fluorescent and incandescent lamps which provided 400 $\mu E/m^2/s$ of photosynthetic flux density from 0600 to 1800 hours daily.

Inoculum treatments consisted of wheat seed infested with *P. aphanidermatum*, *P. myriotylum*, a combination of both *Pythium* spp., or uninoculated wheat seed. Each temperature–inoculum combination was replicated four times and the entire experiment was repeated once. Inoculum age was 30 days for experiment one and 13 days for experiment two.

Field soil collected from methyl bromide-treated plots at Green Cay Farm was heat pasteurized at 70°C for 4 h. Time release fertilizer (Osmocote 16-8-12, Scotts-Sierra Horticultural Products Co., Marysville, OH) was mixed into the soil at the rate of 10.5 g per kilogram of pasteurized soil (approximately 8% moisture).

Inoculum was incorporated into the soil at the rate of 4 g of infested wheat seed per kilogram of soil. For the treatment combining both *Pythium* spp., 2 g of seed with each species was added to each kilogram of soil. Immediately after incorporation of infested wheat seed, 2 kg of soil was placed into 3.8-liter pots, moistened, and one pepper (Jupiter) seedling was transplanted into each pot. Ages of transplants were 37 and 44 days for experiments 1 and 2, respectively. After transplanting, pots were watered by hand daily.

Measurements of growth and mortality were taken at 22 and 26 days after transplanting for experiments 1 and 2, respectively. Plant height was recorded in centimeters. Shoot and root dry weights were obtained. Using ANOVA procedures, data from repeated experiments were analyzed as repeated measures and combined if there was no interaction between experiment and treatment effects. Treatment means were compared using 95% confidence intervals.

RESULTS

Identification of *Pythium* spp. The following 10 species of *Pythium* were isolated from roots of pepper plants from various fields in Florida: *P. aphanidermatum, P. arrhenomanes* Drechs., *P. catenulatum* Matthews, *P. graminicola* Subramanian, *P. helicoides* Drechs., *P. irregulare, P. myriotylum, P. periplocum* Drechs., *P. spinosum* Sawada, and *P. splendens* H. Braun. An additional group of isolates with noninflated, filamentous sporangia obtained from roots of pepper plants did not produce oogonia in single or dual cultures used to test for heterothallism; this group of nonsexual isolates was thus identified as belonging to *Pythium* 'group F' (*Pythium* sp. F) according to Van der Plaats-Niterink (12).

Pathogenicity of *Pythium* spp. isolated from pepper plants. The results of the two repeated pathogenicity tests for both pepper and tomato were almost identical, except that plants were larger in the repeated experiment conducted later in the spring. Based on the lack of significant interaction between experiment and treatment effects, the results are presented as the means of the two experiments (Tables 1 and 2).

All 10 isolates of the 10 *Pythium* spp. and the isolate from *Pythium* group F infected pepper and tomato and were isolated from root systems of both hosts after 4 weeks (Table 1). Roots of 100% of the plants of both hosts grown in infested soil were infected by all of the species except *P arrhenomanes, P. irregulare, P. periplocum,* and *P. spinosum.*

Root disease ratings in pepper were highest for *P. aphanidermatum* and *P.*

Table 1. Root infection, plant mortality, root necrosis and growth of pepper plants after 4 weeks in soil infested with 11 species of *Pythium*

				Growth comparisons (%)[v]	
Pathogen[w]	Infection (%)	Mortality (%)[x]	Rating[y]	Roots	Shoots
Noninfested soil	0	0	0.0 a[z]	100 a	100 a
P. aphanidermatum	100	42	4.5 e	10 d	23 d
P. arrhenomances	50	0	0.6 b	100 a	93 ab
P. catenulatum	100	0	0.5 b	92 ab	92 ab
P. graminicola	100	0	0.5 b	90 ab	87 ab
P. helicoides	100	0	2.7 d	52 c	65 c
P. irregulare	75	0	0.8 b	86 ab	82 b
P. myriotylum	100	62	4.8 e	7 d	22 d
P. periplocum	42	0	0.4 ab	96 a	99 a
P. spinosum	8	0	0.1 a	86 ab	95 ab
P. splendens	100	0	1.3 c	80 b	84 b
Pythium sp. f	100	0	0.0 a	87 ab	92 a

[v] Growth measured as the fresh weights of shoots and roots of surviving plants in each treatment at final harvest and expressed as the percent of growth compared to plants in uninoculated soil.

[w] Six-week-old pepper seedlings were grown in soil infested with *Pythium* spp. grown on wheat seed or in soil with noninfested seed.

[x] Percentages of plants moribund (with wilted tops and necrotic roots) or dead.

[y] Root disease based on an index with 0 = no root symptoms; 1 = 25% of root tips necrotic; 2 = 25 to 50% of root tips necrotic; 3 = 50 to 100% of root tips necrotic plus localized necrotic lesions on the tap root or crown; 4 = extensive root rot with few or no white roots, crown rot extensive; 5 = root system completely necrotic and plant dead or moribund.

[z] Values followed by the same letters in a column are not different according to Duncan's multiple range test ($P \leq 0.01$).

myriotylum, intermediate with *P. helicoides* and *P. splendens,* and slightly but significantly higher than in the noninfested control for *P. arrhenomanes, P. catenulatum, P. graminicola,* and *P. irregulare* (Table 1). Root disease ratings were not significantly different than in the noninfested control with *P. periplocum* and *Pythium* sp. F.

Root and shoot weights of pepper plants in soil infested with *P. aphanidermatum* and *P. myriotylum* were greatly reduced in comparison to plants grown in the noninfested control in both experiments, and plant mortality ranged from 42 to 62% (Table 1). *P. helicoides* and *P. splendens* also caused significant losses in root and shoot weights in both tests. *P. irregulare* caused reduced shoot growth but did not reduce root weight.

Similar trends in root disease and plant weight responses to those observed with pepper occurred with the *Pythium* spp. on tomato, except that disease was generally less severe on tomato roots than on pepper roots (Table 2). *P. aphanidermatum* and *P. myriotylum* caused severe root and shoot weight losses, but generally did not kill tomato plants, and *P. helicoides* and *P. splendens* caused low but significant levels of root tip necrosis and intermediate losses in root and shoot weights. *P. arrhenomanes* caused limited root tip necrosis without affecting plant weights, and, although no root necrosis was detected with *P. periplocum,* root weights were lower than in the noninfested control. *P. catenulatum, P. graminicola, P. irregulare, P. spinosum,* and *Pythium* sp. F colonized at least some of the plants but did not cause significant levels of root tip necrosis or plant weight loss.

Additional pathogenicity tests were conducted as described above with *P. aphanidermatum* and *P. myriotylum* because they were found to be the major pathogens in experiments with both pepper and tomato. In repeated tests with three additional isolates of each pathogen, root and shoot weight losses in pepper were similar to those observed in the pathogenicity tests above and mortality averaged 50 and 89% with *P. aphanidermatum* and *P. myriotylum,* respectively. Although tomato roots were severely rotted by the pathogens, only an average of 17% and none, respectively, of the plants were killed with these two pathogens.

Effect of temperature and *Pythium* spp. on growth of pepper transplants. Test (repetitions of the experiment), temperature, and inoculum source all had a significant impact on plant height and root and shoot weights (Table 3). Differences in growth between the two tests were attributed to older transplants and the longer observation period in test 2. A significant interaction between temperature and inoculum was observed for root and shoot weights but not for plant height (Table 3).

In the absence of inoculum, plant height and root and shoot weights were greatest at 28°C and lowest at 34°C (Fig. 1). Inoculation of soil with *Pythium* spp. resulted in significant reductions in root weight of pepper at 20 and 28°C but not at 34°C. Significant reductions in shoot weight and plant height were also obtained in inoculated treatments at 20 and 28°C. *P. aphanidermatum, P. myriotylum,* and a combination of the two species were equally effective in reducing growth of pepper. Plant mortality occurred with *P. aphanidermatum, P. myriotylum,* and the combination of species at 34°C. No mortality occurred at 20 and 28°C.

DISCUSSION

P. myriotylum and *P. aphanidermatum* have been routinely isolated from rotted roots of pepper in several of our field studies, and they were the most common species colonizing pepper roots in the field. They were also the most destructive *Pythium* spp. identified in this study and the only species to cause plant mortality on pepper. Whereas *P. aphanidermatum* has been implicated in stem rot and wilt of pepper in North Carolina (3), potential damage from *P. myriotylum,* particularly under wet, hot conditions, has gone unrecognized. The present study confirms the destructive potential of both *P. myriotylum* and P. *aphanidermatum* on pepper in Florida and supports concern that *P. myriotylum* may be a major root-rotting pathogen of pepper in warm season polyethylene-mulched production systems in Florida.

Of the additional *Pythium* spp. isolated from pepper roots in the field, only *P. helicoides* and *P. splendens* were found to consistently cause significant root rot and reductions in growth. Several species, including *P. arrhenomanes, P. catenulatum,* and *P. graminicola,* caused low but significant levels of root necrosis without causing losses in root or shoot weight. These species can be considered subclinical pathogens of pepper. *P. periplocum, P. spinosum,* and *Pythium* sp. F infected root tissue but did cause disease in pepper. Because of the wide range of species isolated from this host, it is important to clarify both identification and pathogenicity for isolates obtained from the field before making conclusions on causes of damage.

Table 2. Root infection, plant mortality, root necrosis and growth of tomato plants after 4 weeks in soil infested with 11 species of *Pythium*

Pathogen[w]	Infection (%)	Mortality (%)[x]	Rating[y]	Growth comparisons (%)[v] Roots	Growth comparisons (%)[v] Shoots
Noninfested soil	0	0	0.0 a[z]	100 a	100 a
P. aphanidermatum	100	16	3.6 c	18 d	28 d
P. arrhenomances	67	0	0.6 b	81 abc	89 abc
P. catenulatum	100	0	0.2 a	81 abc	97 ab
P. graminicola	100	0	0.0 a	87 abc	92 abc
P. helicoides	100	0	1.0 b	66 c	82 c
P. irregulare	92	0	0.0 a	79 abc	99 ab
P. myriotylum	100	0	4.2 c	17 d	31 d
P. periplocum	33	0	0.0 a	73 bc	95 ab
P. spinosum	50	0	0.2 a	96 ab	95 ab
P. splendens	100	8	0.9 b	71 c	84 bc
Pythium sp. *f*	100	0	0.0 a	86 abc	100 a

[v] Growth measured as the fresh weights of shoots and roots of surviving plants in each treatment at final harvest and expressed as the percent of growth compared to plants in uninoculated soil.
[w] Three-week-old tomato seedlings were grown in soil infested with *Pythium* spp. grown on wheat seed or in soil with noninfested seed.
[x] Percentages of plants moribund (with wilted tops and necrotic roots) or dead.
[y] Root disease based on an index with 0 = no root symptoms; 1 = 25% of root tips necrotic; 2 = 25 to 50% of root tips necrotic; 3 = 50 to 100% of root tips necrotic plus localized necrotic lesions on the tap root or crown; 4 = extensive root rot with few or no white roots, crown rot extensive; 5 = root system completely necrotic and plant dead or moribund.
[z] Values followed by the same letters in a column are not different according to Duncan's multiple range test ($P \leq 0.01$).

Table 3. Mean squares for growth parameters of pepper as influenced by temperature and inoculum treatments

Source[z]	df	Mean squares[y] Root weight	Mean squares[y] Shoot weight	Mean squares[y] Plant height
Test	2	0.383***	2.265***	17.255*
Inoculum (I)	3	0.778***	5.508***	60.583***
Temperature (T)	2	1.208***	8.863***	134.850***
I × T	6	0.154***	0.737***	3.874
Residual	72	0.016	0.087	2.637

[y] *, **, and *** significant at the 0.05, 0.01, and 0.001 probability levels, respectively.
[z] Two tests were conducted, each using four different inoculum treatments and three temperatures.

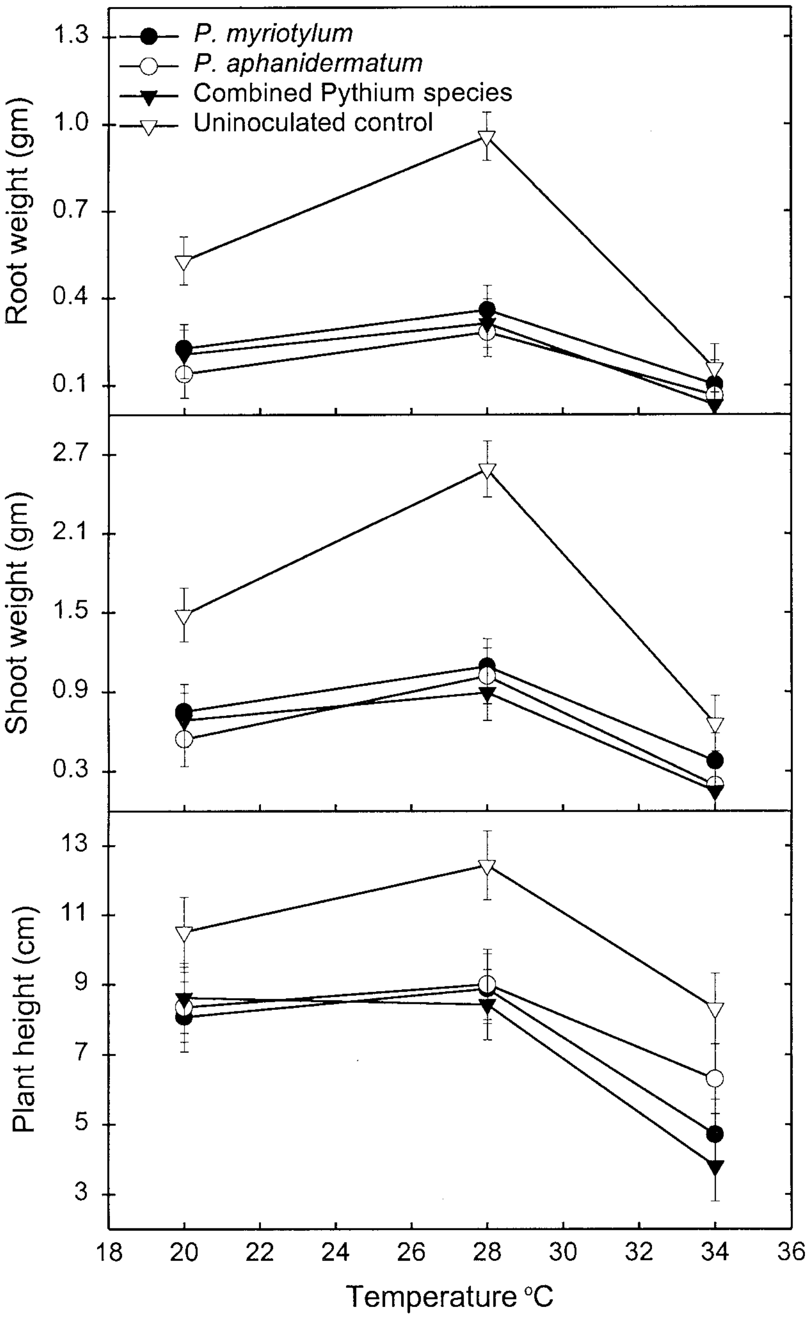

Fig. 1. Means and 95% comparison intervals for the effect of temperature, *Pythium aphanidermatum*, and *P. myriotylum* on the growth of pepper plants.

In the southeastern United States, *P. myriotylum*, *P. arrhenomances*, and *P. aphanidermatum* were shown to cause symptoms on tomato similar to those observed on pepper in this study (8,10). Recently, an aerial blight of tomato caused by *P. myriotylum* was reported from Florida (10). Both P. *myriotylum* and *P. aphanidermatum* have been associated commonly with rotted roots in our field studies with tomato, and their potential as root pathogens of tomato is supported by the pathogenicity tests in the present study. *P. arrhenomanes* infected tomato roots and caused a low incidence of root tip necrosis, but it did not affect plant weights. As with pepper, *P. helicoides* and *P. splendens* were also found to be pathogens of tomato, and, although they caused only limited root necrosis, plant weights were significantly reduced. *P. periplocum* did not cause root symptoms or shoot weight loss, but root weight was reduced in comparison to the noninfested control.

Mortality occurred only in plants held at 34°C in the growth chamber. Thus, disease caused by *P. aphanidermatum* and *P. myriotlyum* is more severe at higher temperatures. Maximum disease with various other crops, particularly in the tropics, occurs at temperatures of 30 and 35°C or higher (7). However, both *P. aphanidermatum* and *P. myriotylum* still caused dramatic reductions in growth at 20°C. Thus, these pathogens can still limit yield potential under cooler environmental conditions. At lower temperatures, their effects on yield may be deceiving due to the absence of aboveground symptoms. These effects may have been misdiagnosed in the past as responses to stress from fertility or cultural problems.

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