Effect of Soil Flooding and Paddy Rice Culture on the Survival of *Verticillium dahliae* and Incidence of Verticillium Wilt in Cotton

G. S. Pullman and J. E. DeVay

Postgraduate research plant pathologist, and professor, respectively, Department of Plant Pathology, University of California, Davis 95616.

The authors gratefully acknowledge the help of E. J. Butterfield, D. P. Matheron, G. E. Friebertshauser, and J. J. Stapleton. We also thank the California Planting Cotton Seed Distributors of Bakersfield, CA, for financial assistance throughout this study.

Accepted for publication 26 March 1981.

ABSTRACT

Pullman, G. S., and DeVay, J. E. 1982. Effect of soil flooding and paddy rice culture on the survival of *Verticillium dahliae* and incidence of Verticillium wilt in cotton. Phytopathology 72:1285-1289.

A single rotation with paddy rice controlled Verticillium wilt of cotton for 2–3 yr and increased lint yields by an average of 31% over 3 yr compared to areas in which cotton was grown continuously. Four years after treatment, soil population densities of *Verticillium dahliae* were still much lower than the initial populations. In field soil with *V. dahliae*, paddy rice culture compared to flooding alone reduced the time necessary to eradicate this fungus in both greenhouse and field experiments. Six weeks of soil flooding with or without rice was required before population densities began to decline. After 17 wk under paddy rice, *V. dahliae* was not detectable, while with flooding alone, 9% of the initial population was still present. Twelve weeks of flooding during the late fall and winter did not reduce soil population densities of *V. dahliae* or the incidence of Verticillium wilt in a subsequent cotton crop.

Additional key words: *Gossypium hirsutum*, soilborne pathogens, biological control, soil anaerobiosis.

Verticillium wilt, which is caused by *Verticillium dahliae* Kleb., is the major problem affecting cotton (*Gossypium hirsutum* L.) production in California. Estimates of losses caused by this pathogen were 7.6% in 1976 and 2.8% of the total California cotton crop in 1979 (2,5). Although wilt-tolerant Acala cultivars are commonly used in California to manage this disease, highly virulent strains of *V. dahliae* are widespread and cause significant losses in yield.

Flooded soils undergo physical, chemical, and biological transformations that greatly alter their microbial populations. These include decreases in aerobic and facultative anaerobic organisms and increases in anaerobic bacteria (9,14). Several reports on control of *V. dahliae* by soil flooding were made during the last decade (3,11,13). Menzies (11) found that 6 wk of soil flooding killed microsclerotia of *V. dahliae* and Nadakavukaren (13), using artificially infected soil, showed that *V. dahliae* was killed in flooded soil and also that the rate of decline is related to the soil temperature. In addition, grower observations in areas of California where cotton is normally rotated with paddy rice indicated that this rotation effectively reduces Verticillium wilt. Butterfield et al (4) confirmed this observation by following 15 wk of paddy rice culture with a crop of cotton.

The objectives of this study were: to monitor soil populations of *V. dahliae*, disease incidence, and lint yields in several successive cotton crops following a rotation of paddy rice; to determine how long soil must be flooded with or without rice plants to eradicate *V. dahliae*; to compare the control of Verticillium wilt in cotton following a single rotation of irrigated rice, paddy rice, or soil flooding alone; and to determine if long-term soil flooding during the winter months would control Verticillium wilt.

MATERIALS AND METHODS

Field experiments. Two field experiments were carried out from 1974 to 1979 within a 2.3-ha field on a deep deposit of Panoche clay loam located in the San Joaquin Valley near Five Points, CA, that had had a high percentage of cotton plants with symptoms of Verticillium wilt in previous years.

During 1974, Butterfield et al (4) conducted an experiment in which replicated field plots (8 × 40 m) were either continuously flooded for 15 wk with paddy rice (*Oryza sativa* L. 'Colusa 1600') or

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

0031-949X/81/12128505/$03.00/0

were planted with Acala SJ-2 cotton. In 1975, eight rows of cotton were planted 1 m apart in each plot and the incidence of Verticillium wilt, lint yields, and soil population densities of *V. dahliae* were reported (4). As part of this study, the plots of Butterfield et al (4) were continued and disease incidence, lint yields, and reinfestation of the soil by *V. dahliae* were monitored through 1978. Acala SJ-2 cotton was planted each growing season and standard cultural practices were followed. Subplots (2 × 6 m) were reestablished each year within the same area of each field plot, providing consistent areas for soil sampling and observations of disease expression.

The second experiment began in 1976 within the same field. A randomized complete block design contained six replicates of four treatments with individual plots measuring 8 × 40 m. During the 1976 growing season, plots were planted with cotton, dryland rice, or paddy rice with 17 wk of flooding, or were flooded for 17 wk. The dryland rice was irrigated twice monthly and in the flooded plots, fresh water was added continuously to maintain a depth of 15–30 cm. Rice seed was drilled in the soil before treatments began. Throughout the treatment period, soil samples were collected every 3 wk to detect changes in soil population densities of *V. dahliae*. All replications were sampled after 3, 9, and 17 wk of treatment and two replications were sampled after 6 and 13 wk. Flooding of the paddy rice began on 19 May 1976 and flooding of plots without rice was started 4 days later. Acala SJ-2 cotton was planted in all plots in 1977–1979.

A third field experiment was made at Davis, CA, in a field cropped to cotton since 1967. The soil type was Yolo clay loam and populations of *V. dahliae* exceeded 200 propagules per gram of soil in the upper 30 cm. The incidence of foliar symptoms of Verticillium wilt in previous cotton crops often exceeded 90%. Field plots 6 × 20 m were either flooded for 12 wk beginning 16 November 1975, or were left fallow. Treatments were replicated three times. Soil samples were collected every 2 wk from each replication. Acala SJ-2 cotton was planted during the 1976 growing season.

Measurement of soil and air temperatures. During 1976, soil temperatures were recorded continuously at a depth of 15 cm at Five Points in one plot of each treatment except those planted with cotton. Daily minimum and maximum air temperatures were obtained from a weather station located nearby.

During 1975, soil temperatures at Davis were recorded continuously at depths of 5 and 20 cm within one replication of each treatment. Daily minimum and maximum air temperatures were supplied by the U.S. Department of Commerce, National Weather Service, at Davis, CA (Location 05-2294-2).

Incidence of Verticillium wilt and cotton lint yields. Approximately 100 plants were examined for foliar symptoms of Verticillium wilt in mid-September in each plot. At Five Points, CA, seed cotton was harvested by machine from the center two rows (each 40 m long) of each eight-row plot. Gin turnout was approximated at 32% lint per seed, and lint yields were adjusted to a per hectare basis. Cotton bolls did not mature at Davis, therefore the number of bolls was used to estimate yields.

Soil population densities of *Verticillium dahliae*. Soil samples consisting of 10–12 random cores each 30 cm deep and 2.5 cm in diameter, were collected during May of each year within the plots. Soil cores were bulked, air-dried for 6–10 wk, and milled for 20 min in a 5-L revolving jar mill. The Anderson Sampler technique of Butterfield and DeVay (3) was used to spread five 100-mg samples of each soil sample onto a selective sodium polypectate medium. Microsclerotial colonies of *V. dahliae* were counted with the aid of a stereo dissecting microscope after incubation at 20–24 C for 14 days. As few as two propagules per gram of soil could be detected with this technique.

Greenhouse experiments. Field soil was collected in October, 1975, at Davis, CA, and air-dried for 2 wk in the greenhouse. Clods were broken up with a Lindig soil shredder (Lindig Manufacturing Corp., St. Paul, MN 55113), then the soil was passed through a 1.3-cm mesh screen and placed in sterilized (1-gallon) porcelain crocks. Soil assays indicated approximately 100 propagules of *V. dahliae* per gram. Six crocks were used for each of six treatments. The treatments, which began on 4 December 1975, were as follows: dry soil (not irrigated), irrigated soil, flooded soil, irrigated rice (Colusa 1600), flooded rice (Colusa 1600), and irrigated cotton (Acala SJ-2). Pots were watered daily and water levels in flooded treatments were maintained 2-4 cm above the soil.

Two soil cores 5 × 1 cm were collected from each pot with a cork borer at 2-wk intervals. Soil cores were bulked for each treatment and air-dried for 6–10 wk. After air-drying, the soil samples were milled in a 1.9 L revolving-jar mill for 6 min and assayed for *V. dahliae* as previously described.

Treatments were discontinued after 24 wk, when the rice plants headed and produced seed. The cotton and rice plants were removed from the soil and all pots were replanted with Acala SJ-2 cotton. Four months later plants were rated for vascular and foliar symptoms of Verticillium wilt.

RESULTS

Field experiments. Paddy rice increased lint yields of Acala SJ-2 cotton in successive crops by 70, 15, and 9% in the first, second, and third crops, respectively (Table 1). Soil population densities of *V. dahliae* were reduced by 96% and were still below the initial level 4 yr later (Table 2). The incidence of Verticillium wilt was significantly reduced in the first and second cotton crops (Table 3). Throughout the 3-yr period, a movement of inoculum probably occurred with cultivation of the plots which were eight rows wide. During the first year after a flooding treatment, most of the diseased plants were in the border rows; during the second year, in the outermost two rows, and in the third year only the center two rows had a reduced incidence of Verticillium wilt.

In the second experiment, paddy rice culture reduced the population of *V. dahliae* most effectively. Soil flooding without rice also reduced the population of *V. dahliae*. Propagule density was not changed in plots planted to irrigated rice or irrigated cotton (Table 2). Disease control and increased yields were still evident after the second cotton crop in both flooding treatments (Tables 1

TABLE 1. The effects of different crop, irrigation, and flooding treatments on the lint yield of Acala SJ-2 Cotton in two California locations

Treatments		Lint yield (kg/ha)[a]				
First year	Subsequent years	1975	1976	1977	1978	1979
Experiment 1 (Five Points, 1974)						
Cotton	Cotton	1,004 x	850 x	1,149 x	689 x	...
Paddy rice	Cotton	1,711 y	977 x	1,249 x	707 x	...
Experiment 2 (Five Points, 1976)						
Cotton	Cotton	...	...	1,149 x	689 x	721 x
Irrigated rice	Cotton	...	...	1,197 x	...	...
Flooded soil	Cotton	...	...	1,369 y	755 xy	836 x
Paddy rice	Cotton	...	...	1,508 y	843 y	860 x
Experiment 3 (Davis, 1975)[b]			(Bolls)			
Fallow	Cotton	...	8.7 x	...	...	...
Winter-flooded soil	Cotton	...	7.7 x	...	...	...

[a] Values followed by the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$).
[b] Lint yields were not available. Values represent average number of bolls per plant.

and 3). Dryland rice cropping did not effectively control the disease or reduce soil inoculum, therefore it was dropped from the experiment after the first year. Again, the pattern of diseased plants over 3 yr indicated that inoculum probably had spread from untreated soil into treated soil.

Soil population densities of *V. dahliae* in irrigated cotton and irrigated rice fluctuated throughout the season and generally increased slightly. Population densities in both flooding treatments showed little change for the first 6 wk, but declined rapidly thereafter (Fig. 1). Maximum soil temperatures at a depth of 15 cm during the experiment in irrigated rice, paddy rice, and soil flooding alone, were 37, 27, and 28 C, respectively. Additional soil and air temperature data are not reproduced here, but are available (15).

In the third experiment, winter flooding did not effectively reduce soil populations of *V. dahliae*, or Verticillium wilt in the following cotton crop (Tables 2 and 3). Soil assays indicated that population densities of *V. dahliae* decreased during the flooding but then increased beyond their initial concentrations. Soil temperatures rarely exceeded 15 C in either treatment. During late December and throughout January, the surface water in flooded plots often froze at night.

Greenhouse experiments. Long-term flooding coupled with rice plant growth in the greenhouse most effectively reduced soil populations of *V. dahliae*. Soil flooding without rice eventually eradicated this fungus, but an additional 6 wk of treatment was necessary. None of the other treatments controlled *V. dahliae* (Table 4). When cotton was planted in previously flooded soil, Verticillium wilt did not occur. In contrast, plants from the other treatments began to defoliate and show symptoms of Verticillium wilt 7–8 wk after planting (Table 4).

Viable propagules of *V. dahliae* began to decline in both flooding treatments after 6 wk (Fig. 2) and after 14 wk were no longer detectable in the flooded-rice soil, while viable propagules were still present after 17 wk in flooded soil without rice. Greenhouse temperatures during the period of the experiments (5.5 mo) varied between 18 and 26 C; however, temperatures generally remained varied between 22–24 C.

DISCUSSION

V. dahliae was effectively controlled by long-term summer soil flooding with or without paddy rice culture. The control of *V. dahliae* was directly associated with control of Verticillium wilt in subsequent cotton crops and with increased lint yields. In contrast,

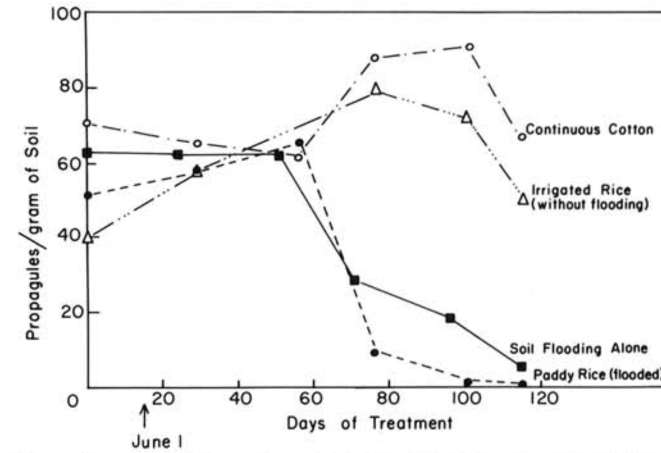

Fig. 1. Survival of *Verticillium dahliae* in field soil exposed to different crop, irrigation, and flooding treatments at Five Points, CA. Treatments began 19 May 1976, except for soil flooding alone (without rice), which began 4 days later.

TABLE 2. The effect of different crop, irrigation, and flooding treatments on soil populations of *Verticillium dahliae* in two California locations

Treatments		Propagules per gram of soil[a]					
First year	Subsequent years	1974	1975	1976	1977	1978	1979
Experiment 1 (Five Points, 1974)							
Cotton	Cotton	45 x	57 x	71 x	79 x	62 x	···
Paddy rice	Cotton	51 x	3 y	8 y	35 y	47 x	···
Experiment 2 (Five Points, 1976)							
Cotton	Cotton	···	···	71 x	79 x	62 x	55 x
Irrigated rice	Cotton	···	···	40 y	42 y	···	···
Flooded soil	Cotton	···	···	63 x	12 z	22 y	22 y
Paddy rice	Cotton	···	···	52 x	3 z	5 z	21 y
Experiment 3 (Davis, 1975)							
Fallow	Cotton	···	77 x	105 x	···	···	···
Winter-flooded soil	Cotton	···	69 x	115 x	···	···	···

[a]Field soil was collected during May of each year except for the Davis experiment where initial samples were collected in November 1975 and final samples in July, 1976. Values followed by the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$).

TABLE 3. The effect of different crop, irrigation, and flooding treatments for control of Verticillium wilt in Acala SJ-2 Cotton in two California locations

Treatments		Diseased plants[a] (%)				
First year	Subsequent years	1975	1976	1977	1978	1979
Experiment 1 (Five Points, 1974)						
Cotton	Cotton	95.5 x	54.0 x	92.9 x	91.4 x	···
Paddy rice	Cotton	2.3 y	8.0 y	80.5 x	88.0 x	···
Experiment 2 (Five Points, 1976)						
Cotton	Cotton	···	···	92.9 x	91.4 x	91.3 x
Irrigated rice	Cotton	···	···	84.6 x	···	···
Flooded soil	Cotton	···	···	7.1 y	51.7 y	87.7 x
Paddy rice	Cotton	···	···	4.3 y	33.1 z	93.0 x
Experiment 3 (Davis, 1975)						
Fallow	Cotton	···	95.6 x	···	···	···
Winter-flooded soil	Cotton	···	98.3 x	···	···	···

[a]Plants were rated for foliar symptoms of Verticillium wilt in mid-September except for experiment 1 during 1976 when plants were rated on 20 August. Values followed by the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$).

soil flooding during the winter months and irrigated rice without flooding were ineffective. These findings are consistent with those of previous work (7,11,13) and confirm the report of Butterfield et al (4) that a 1-yr rotation of paddy rice can eradicate *V. dahliae*.

The duration of soil saturation was important in both the greenhouse and field experiments where population densities of *V. dahliae* began to decline after 6–8 wk of flooding. This may explain why George et al (7) and Ioannou et al (8) were unsuccessful in controlling *V. dahliae* with short-term flooding treatments lasting 6 wk or less. Viable propagules of *V. dahliae* are usually multicellular, can germinate from many cells (1,6), and all individual cells must be killed to render a propagule dead. The 6-wk lag period before propagules began to die may have been necessary for the development of unfavorable soil conditions and for the successive death of individual microsclerotial cells.

Rice plants in flooded soil had a marked effect in decreasing the treatment time necessary to eradicate *V. dahliae*. Stoner et al (18) and Moore (12) also found the same trends when static soil flooding or paddy rice was used to control *Sclerotinia sclerotiorum*. In their studies, sclerotial decomposition of *S. sclerotiorum* occurred within 20 days under paddy rice, while a minimum of 35 days was necessary in soil with static flooding.

Anaerobic conditions and low oxidation-reduction potentials in flooded soils may be important in the killing of *V. dahliae*. The addition of readily decomposable organic matter such as root exudates to flooded soils low in oxygen generally stimulates microbial activity, which further decreases soil oxygen, increases CO_2, and decreases the soil oxidation-reduction potential (14). Menzies (11) reported that *V. dahliae* kept in moist soil under nitrogen for 3 wk was no longer viable. The addition of 1% alfalfa meal or 0.1% sucrose decreased the time necessary under anaerobic conditions to kill this fungus. In addition, soil microorganisms in the absence of oxygen often produce reduced metabolites which diffuse into the soil solution. These metabolites lower the oxidation-reduction potentials of soil and in some cases are directly toxic to plants (10,16).

Population density of *V. dahliae* declined during winter flooding; however, after treatment, population densities increased beyond their initial levels. Ioannou et al (8) observed similar effects in small plots at Davis, CA, which were flooded for 10–40 days during the late summer. In their longest flooding treatment, the production of new inoculum within infected tomato stem debris was halted, but later increased when the soil flooding was stopped. The lack of control of *V. dahliae* after 12 wk of winter flooding or 40 days of fall flooding (8) may be due in part to low soil temperatures. Nadakavukaren (13) demonstrated the importance of temperature in artificially infested soils where *V. dahliae* was eliminated within 3 mo in soils flooded and incubated at 15 C or higher. However, when soils were incubated at 10 and 5 C, propagules of *V. dahliae* were detectable for 6 and 8 mo, respectively.

Although little is known about the effects of microbial antagonists on fungal resting structures in anaerobic soils, some studies have shown the presence of organisms parasitic to fungal resting structures in aerobic soil solutions. Tolmsoff (19) isolated a phycomycete, *Hypochytrium catenoides*, from microsclerotia of *V. dahliae* incubated in soil solutions. This fungus produced zoospores and parasitized individual microsclerotial cells. Also Sneh et al (17) found in soil solutions several fungal and bacterial parasites on oospores of *Phytophthora megasperma*, *P. cactorum*, a *Phythium* sp., and *Aphanomyces euteiches*.

The effectiveness of soil flooding and the culture of paddy rice for controlling Verticillium wilt have been clearly demonstrated. At the present, however, the interaction between *V. dahliae* and environmental changes in flooded soils such as temperature, populations of antagonistic organisms, and physical-chemical conditions are not known. Further studies on the relative importance of these factors are necessary to shorten the soil flooding process and possibly apply it at other times of the year.

TABLE 4. The effect of different crop, irrigation, and flooding treatments in a greenhouse experiment on soil populations of *Verticillium dahliae* and control of Verticillium wilt in Acala SJ-2 Cotton in California

Treatments[a]	Propagules per gram of soil following 24 wk of treatment	Plants with: Foliar symptoms (%)	Plants with: Vascular discoloration (%)
Dry soil	106	65	65
Irrigated soil	100	83	94
Irrigated cotton	92	76	82
Irrigated rice	78	72	78
Flooded soil	0	0	0
Flooded rice	0	0	0

[a]Soil was treated for 24 wk in porcelain pots, Acala SJ-2 was planted and 4 mo later each plant was rated for vascular discoloration and foliar symptoms of Verticillium wilt.

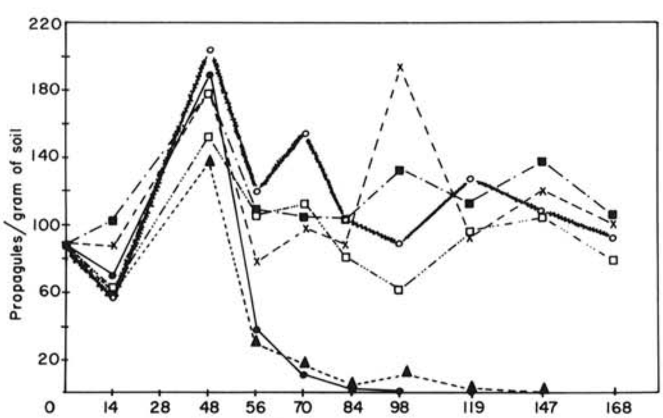

Fig. 2. Survival of *Verticillium dahliae* in field soil placed in porcelain crocks in the greenhouse and exposed to the following treatments: dry soil (■—·—■), irrigated soil (X - - - X), irrigated cotton (O——O), irrigated rice (□—· · —□), flooded soil (▲ - - - ▲), and flooded rice (●——●). Treatments began 4 December 1975.

LITERATURE CITED

1. Ben-Yephet, Y., and Pinkas, Y. 1977. Germination of individual microsclerotia of *Verticillium dahliae*. Phytoparasitica 5:159-166.
2. Berry, R. W. 1980. Cotton disease loss estimate committee report 1979. Page 3 in: Proc. Beltwide Cotton Production Res. Conf., 6–10 January 1980, St. Louis, MO. National Cotton Council, Memphis, TN. 385 pp.
3. Butterfield, E. J., and DeVay, J. E. 1977. Reassessment of soil assays for *Verticillium dahliae*. Phytopathology 67:1073-1078.
4. Butterfield, E. J., DeVay, J. E., and Garber, R. H. 1978. The influence of several crop sequences on the incidence of Verticillium wilt of cotton and on the populations of *Verticillium dahliae* in field soil. Phytopathology 68:1217-1220.
5. Crawford, J. L. 1977. Cotton disease loss estimate committee report 1976. Page 3 in: Proc. Beltwide Cotton Production Res. Conf., 10–12 January 1977. Atlanta, GA. National Cotton Council, Memphis, TN. 258 pp.
6. DeVay, J. E., Forrester, L. L., Garber, R. H., and Butterfield, E. J. 1974. Characteristics and concentration of propagules of *Verticillium dahliae* in air-dried field soils in relation to the prevalence of Verticillium wilt in cotton. Phytopathology 64:22-29.
7. George, A. G., Pendery, W., and Hall, D. 1967. Summer flooding experiment, 1966. Page 75 in: Cotton Disease Research in the San Joaquin Valley, California. Research Report for 1966–67. Div. Agric. Sci., University of California, Berkeley. 178 pp.
8. Ioannou, N., Schneider, R. W., and Grogan, R. G. 1977. Effect of flooding on the soil gas composition and the production of microsclerotia by *Verticillium dahliae* in the field. Phytopathology 67:651-656.
9. International Rice Research Institute. 1964. Page 245 in: IRRI Annual Report. IRRI, Los Baños, Laguna, The Philippines.
10. Lynch, J. M. 1978. Production and phytotoxicity of acetic acid in anaerobic soils containing plant residues. Soil Biol. Biochem. 10:131-136.
11. Menzies, J. D. 1962. Effect of anaerobic fermentation in soil on survival of sclerotia of *Verticilllium dahliae*. (Abstr.) Phytopathology 52:743.

12. Moore, W. D. 1949. Flooding as a means of destroying the sclerotia of *Sclerotinia sclerotiorum*. Phytopathology 39:920-927.
13. Nadakavukaren, M. J. 1960. The effect of soil moisture and temperature on the survival of *Verticillium* microsclerotia. Ph.D. dissertation, Oregon State University, Corvallis. 64 pp.
14. Patrick, W. H., and Mikkelsen, D. S. 1971. Plant Nutrient Behavior in Flooded Soil. Pages 187–215 in: R. A. Olson, ed. Fertilizer Technology and Use. Soil Sci. Soc. of America, Inc., Madison, WI. 611 pp.
15. Pullman, G. S. 1979. Effectiveness of soil solarization and soil flooding for control of soil-borne diseases of *Gossypium hirsutum* L. in relation to population dynamics of pathogens and mechanisms of propagule death. Ph.D. dissertation, University of California, Davis. 95 pp.
16. Rao, D. N., and Mikkelsen, D. S. 1977. Effect of acetic-acid propionic-acid and butyric-acid on young rice seedlings growth. Agron. J. 69:923-928.
17. Sneh, B., Humble, S. J., and Lockwood, J. L. 1977. Parasitism of oospores of *Phytophthora megasperma* var. *sojae*, *P. cactorum*, *Pythium* sp., and *Aphanomyces euteiches* by oomycetes, chytridiomycetes, hyphomycetes, actinomycetes, and bacteria. Phytopathology 67:622-628.
18. Stoner, W. N., and Moore, W. D. 1953. Lowland rice farming, a possible cultural control for *Sclerotinia sclerotiorum* in the Everglades. Plant Dis. Rep. 37:181-186.
19. Tolmsoff, W., and Wheeler, M. H. 1977. Report of the Disease and Pathogen Physiology Committee—1976. Page 7 in: Proceedings Beltwide Cotton Production and Research Conferences, January 10–12, 1977, Atlanta, GA. National Cotton Council, Memphis, TN. 258 pp.