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FLOODING ORGANIC SOILS TO CONTROL SPECIES OF PYTHIUM WHICH ATTACK CARROTS AND OTHER VEGETABLES[1]

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Abstract. **Fallow flooding of organic soils was effective in reducing soil populations of** Pythium **sp. which attack carrot** (Daucus carota **L. var.** sativa **DC.) roots and other vegetable crops. Eight species were identified, 7 of these were pathogenic to carrot roots.** Pythium **populations increased following harvest and incorporation of crop residues and declined slowly thereafter. In fields which were flooded following harvest, populations rapidly declined and remained low throughout the flooded period. After fields were drained and crops were planted, populations increased slowly, but remained low for a period that allowed carrot roots to grow and develop in an environment with a reduced chance of root damage. Within a few days of flooding, oxygen was rapidly depleted and** Pythium **populations were reduced correspondingly. Populations were reduced, but not eradicated. Controlled environment studies verified field experiments and showed that the effect of flooding on** Pythium **species was highly temperature dependent; populations decreased very slowly below 20°C.**

In regions where it is feasible, flooding vegetable and crop production fields during fallow periods has been an important cultural method of pest control. In Florida (2, 4, 5, 9, 12, 19) and elsewhere (1, 3, 6, 7, 15, 20), fields (primarily organic soil) have been flooded with 5-30 cm or more of water for periods ranging from a few weeks to several months to help reduce numbers of pests (2, 4, 5, 9, 12, 19) and to reduce soil oxidation and subsidence (6, 19). Target pest species have included fungi (1, 2, 6, 9, 12, 13, 14, 20), insects (5, 6), nematodes (7, 9, 17) and weeds (3). Approaches to flooding for pest control have been largely emperical; specific effects of flooding on soilborne pests are not well known. It is known that oxygen is rapidly depleted when organic soils are flooded or saturated with water (10, 16, 19), and the notion that lack of oxygen is a primary cause of pest mortality, is a common one. However, other causes both physical and biological such as the toxicity of excess amounts of compounds such as CO_2 and ethylene or more direct activities of microorganisms which flourish in low oxygen environments are possible and more direct causes of pest mortality (8).

During its establishment and growth in Florida, the carrot production industry has often experienced severe yield reductions due to soilborne fungi especially *Pythium* sp. These losses are due to seedling mortality with resulting reduced and variable stand densities and to forked, stubbed and misshapen roots which must be culled. The culled roots are often a result of damage to young tap roots by *Pythium* sp. during early growth but there can be other causes as well (21, 23). No satisfactory fungicide treatments have been found which reliably reduce these losses due to *Pythium* sp. Flooding was tried and it worked. It has become a common cultural practice, but why or how it works to control *Pythium* sp. and other pests is not well known.

This paper reports initial results of a detailed study to determine the basic processes by which fallow flooding reduces populations of soilborne vegetable pests. Results of field experiments and supporting controlled environment tests are presented.

Materials and Methods

Pythium populations were estimated in composite soil samples (500 g per sampling site) within 24 hr of acquisition by the following method. Small subsamples (0.5, 1 or 2 g) were obtained from field samples which had been passed through a 10 mesh screen (flooded soils were not screened). Subsamples were added to 25 or 50 ml of 0.5% water agar and stirred for 1-2 min on a magnetic stirrer. One ml aliquots of water agar containing suspended soil particles were removed with a pipette while the sample was being stirred and distributed on the surface of modified PV agar plates. Plates were held for 24 hr at 20 C in the dark then the water agar was washed from the surface of the plate with a gentle stream of water. Colonies of *Pythium* species were counted at 30X with a binocular dissecting microscope. When the plates were illuminated through the bottom of the Petri dish, refracted light in conjunction with the selective medium enabled the identification and counting of *Pythium* colonies. A modified PV (22) medium was used. Seventeen g of dehydrated corn meal agar (Difco or BBL) were added to 1 liter of water and sterilized. Upon cooling to approximately 45°C the following were added before pouring the plates: 0.10 g of 75% PCNB, 0.01 g of pimaricin (50% active delvocid) and 0.01 g vancomycin.

At least 4 plates were evaluated for each soil sample. A portion of the remaining soil was dried for 24 hr at 110°C and the percent moisture determined. *Pythium* counts were expressed as propagules per g of dry soil.

Field sampling sites were vegetable production fields near Zellwood, Florida. Site A had been flooded after a fall carrot crop followed by a spring sweet corn crop. Site B was similar and within 100 m of Site A but was across a road from A and was not flooded. During this study, both sites were planted with carrots (fall), sweet corn [*Zea mays* L. var. *saccharata* (Sturtev.) Bailey] (spring), fallow with weed growth and occasional mowing during the summer, and 3 crops of radish (*Raphanus sativus* L.) (fall of second year). Site C was planted with carrots (fall) then sweet corn (spring) then disked. Sampling began with the harvest and destruction of the corn crop and continued through the flooding period and subsequent carrot and radish crops. Site D was similar but not flooded. Site E was planted with sweet corn. Following harvest and disking, sampling was initiated before flooding. Soil oxygen probes were installed at 4 locations within the 5 ha field. Platinum electrodes were buried at depths of 5, 30 and 56 cm at the 4 locations before flooding. Soil samples were collected and evaluated weekly and soil oxygen was monitored by frequent measurements of platinum electrode potentials referenced to a calomel standard electrode. Millivolt readings were corrected for the reference electrode by adding +240 mv to the observed values and readings for the 4 locations were averaged for each observation date.

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Controlled environment experiments were conducted in a warm greenhouse with freshly collected field soil contained in 13-liter earthenware jars 24 cm diameter, 28 cm tall. Field soil was passed through a 0.5 cm mesh screen and jars were filled to within 5 cm of the top. *Pythium* populations in selected field soil samples were estimated by methods previously described and small aliquots of this soil were loosely packed into plastic tissue embedding capsules 35 mm diameter X 8 mm tall. The capsules had numerous perforations which allowed free exchange of soil gases and water. Capsules containing the soil were routinely buried 20 cm deep, but were sometimes placed at 10 cm (middle) and 2 cm (top) for one series of experiments. The soil in some jars was flooded by adding water over a 2-4 hr period until the water level was 5 cm above the soil (wet soil decreased in volume) and water was added as necessary. Soil in nonflooded treatments was watered as needed to maintain weed growth from naturally occurring weed seeds.

Small samples (25 g) of screened field soil containing previously estimated populations of *Pythium* sp. were also added to 150 ml serum or prescription bottles. One-hundred ml of water was added to simulate flooding; soil was immersed under 5-6 cm of water when the soil particles settled. Bottles were tightly capped and held in the dark for varying periods at specified temperatures. To estimate populations of *Pythium* sp. following these treatments, soil and water were removed from the bottles, separated by vacuum filtration through #1 filter paper and the soil tested using methods already described.

All experiments were carried out with or on Lauderhill muck (Histosol), pH 6.5. The sampling sites were intensively cultivated vegetable production fields about 20 yr old. Soil for controlled environment tests was collected from these fields.

Pythium species attacking young carrot roots were isolated by removing small root pieces bearing discolored areas or small lesions. The roots were washed for 30 min in running tap water, dried on paper towels, and plated on water agar. Fungal colonies emerging from the root pieces were isolated after 24-36 hr and transferred to corn meal agar slants for storage. To identify the isolates, pieces (1 cm) of grass blade were steam-sterilized and placed on CMA slants already colonized by the fungal isolates. After 24 hr, grass blades were transferred to tubes containing 10 ml of sterile pond water, then allowed to stand in ambient laboratory conditions for 3-4 days. Fungal structures on the colonized grass blades were examined and the isolates were identified by following the guidelines and taxonomic key of Waterhouse (24).

Isolates were also grown on corn meal agar plates for 3 days. The agar containing the fungus was placed in a blender with 250 ml of water and blended for one min. This suspension was mixed with 2 liters (volume) of steamed muck soil and placed in pots. Carrot seeds were placed in the pots and seedlings were grown in a warm greenhouse for 4 weeks. Indications of pathogenicity were checked by looking for damped-off or killed seedlings, or poor emergence compared with seeds planted in steamed soil. At 4 weeks, plants were washed free of soil and examined for disease symptoms.

Results and Discussion

Long-term population trends of *Pythium* sp. monitored at field sites A and B (flooded and nonflooded) showed that the populations were initially different just after flooding (approximately 1800 vs. 2700 propagules per g dry soil) and remained so during production of a carrot crop. However, by 30-40 weeks after flooding which included the production of carrot, then sweet corn crop, populations in the initially flooded field approached those of the nonflooded field. After 60-70 weeks which further included summer fallow (weed growth and intermittent mowing and disking) followed by 2 radish crops, the populations in the 2 fields were very similar (Fig. 1). These results indicated the beneficial effects of flooding (in terms of effects on *Pythium* sp.) were relatively short-term in nature, but appeared to be adequate to allow the production of a carrot crop under relatively low *Pythium* population levels after flooding. Shorter term population trends measured at sites C and D (flooded and nonflooded fields) showed that relatively high population levels (approximately 3100-3400 propagules per g dry soil) following the incorporation of sweet corn crop residue remained about the same for 2 weeks after site C was flooded, then dropped to very low levels (Fig. 2). Populations in a nonflooded field, site D, decreased during summer fallowing (weed growth with intermittent mowing), but not greatly so (Fig. 2). Following the flooded period, populations at site C increased steadily during land preparation, planting, and carrot production, but remained well below populations in a nonflooded field (site D) for about 10 weeks after flooding (week 15 after incorporation of sweet corn residue).

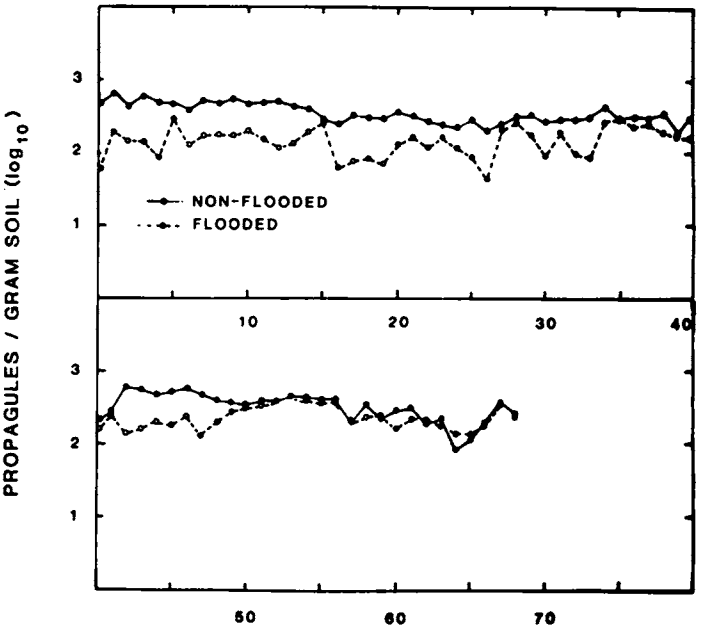

Fig. 1. Populations of *Pythium* species in vegetable fields at Zellwood, FL, over a 6-8 week period. Sampling initiated early in September. Crop sequence during observation: carrot, sweet corn, summer fallow (weeds), fall radish, radish. Site A was not flooded, Site B was flooded for 6 weeks prior to start of observation.

Results of population monitoring experiments in grower fields demonstrated that flooding may help reduce *Pythium* populations and thereby reduce the amount of damage caused to young carrot roots by pathogenic species. The period that *Pythium* sp. remained suppressed following flooding is sufficient to allow the early root growth and development of carrot to take place in an environment with relatively low populations of *Pythium* sp. Carrot roots are most susceptible to damage during this early development period (21, 23).

To better describe the effects of flooding on *Pythium* species, several fields were sampled more intensively during the flooding process. Representative results are presented in Fig. 2. Following complete inundation of the field, which often took 1-2 weeks after flooding was initiated,

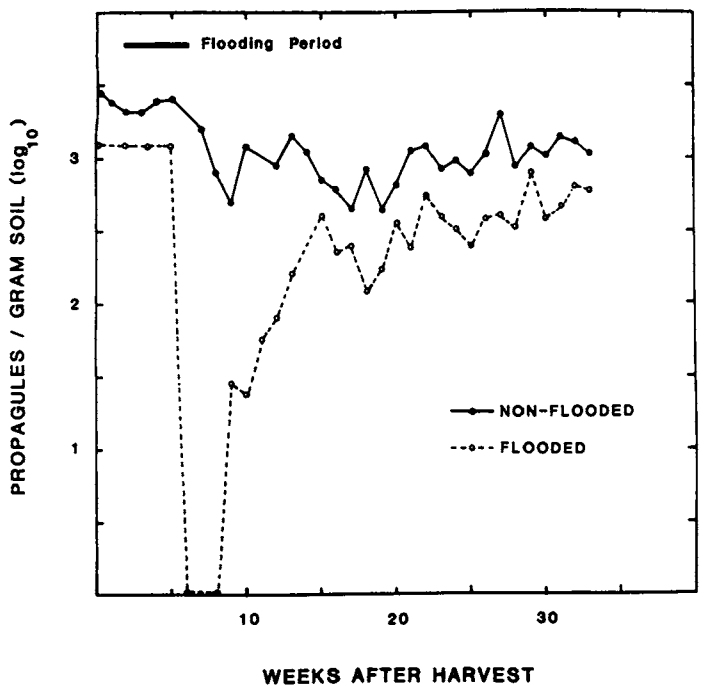

Fig. 2. Populations of *Pythium* species in flooded and nonflooded vegetable fields. Crop sequence: spring sweet corn; Site C nonflooded (weeds during summer); site D flooded following harvest of corn crop; both sites carrot then sweet corn.

Pythium populations decreased steadily for 3-6 weeks and remained low until the fields were drained and the land was prepared for planting. After land preparation, populations increased slowly, but occasionally they increased sharply. However, relative to unflooded fields, populations remained low for several weeks following the flooding treatment (Fig. 2).

Results from controlled environment studies were in good agreement with field observations. Populations of *Pythium* species in soil buried in plastic embedding capsules at depths of 2, 10, or 20 cm responded similarly to field populations (Fig. 3a and 3b). In unflooded soil, they decreased slowly over the 8-week sampling period (Fig. 3a). Depth in soil appeared to have some effect on survival, but counts were highly variable and the experimental methods used were not adequate to identify significant differences in survival at the 3 depths. Populations in flooded soil declined rapidly and decreased to very low levels after 4-5 weeks of flooding (Fig. 3b); depth in soil had no apparent effect in flooded soil. *Pythium* populations in small soil samples held in bottles in flooded or unflooded conditions responded similarly to populations buried in capsules in 13-liter containers. Thus, the small bottle methods were judged to be representative of field conditions and were used for further controlled environment studies.

When soil samples in small containers (bottles) were held at different temperatures in both flooded and unflooded conditions, populations in flooded soil responded markedly to temperature (Fig. 4). Rates of decline of *Pythium* populations were much slower at 15 and 20°C than at 25 and 30°C and were proportional to increasing temperature over the range tested (15-30°C). Thus, soil and water temperatures can be important in the length of flooding required and may impose geographical and seasonal constraints on this cultural control method.

At an intensively sampled field site (site E) which was rapidly flooded within 3-4 days, *Pythium* populations declined soon after flooding was initiated (Fig. 5a). Decreases in *Pythium* counts corresponded to relative decreases in

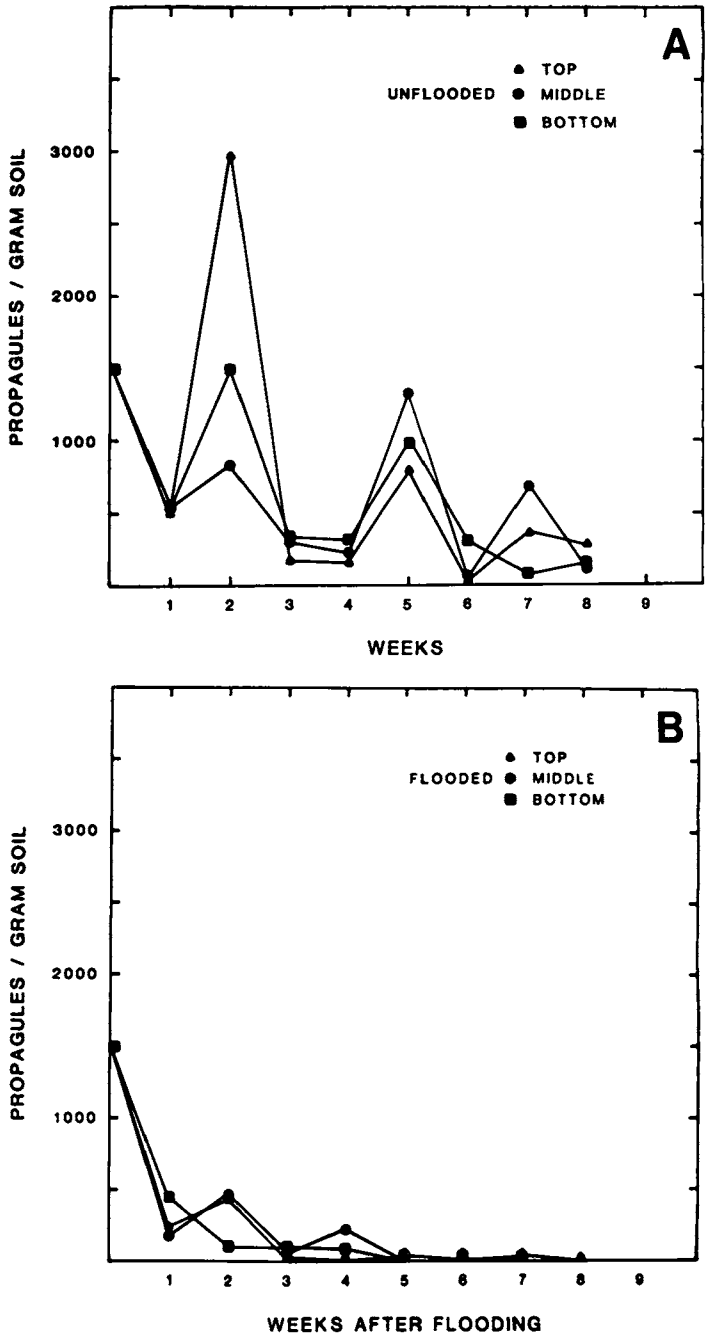

Fig. 3. Effect of flooding on *Pythium* populations at soil depths of 2, 10, and 20 cm in a greenhouse experiment. a) nonflooded treatment with weed growth; b) soil flooded, 5-8 cm water.

oxygen as indicated by the rapid drop in measured Eh values of platinum electrodes which had been buried in the soil prior to flooding (Fig. 5b). However, oxygen concentrations were very low in all 4 sampling sites at the 3 depths where Eh values were obtained. Values for all sampling sites decreased rapidly after flooding and remained well below +300 mv while the field was flooded. When the field was drained, Eh values increased greatly. The changes in Eh values corresponded in time with observed levels of *Pythium* populations and lend support to the concept that depleted oxygen is related to pest mortality in flooded fields. The use of platinum electrodes has limitations, but can provide adequate relative indication of oxygen levels in flooded soils (16). These data indicate that oxygen depletion and chemical changes were greatest in the upper 5 cm of soil. This is not unexpected because of microbial activity

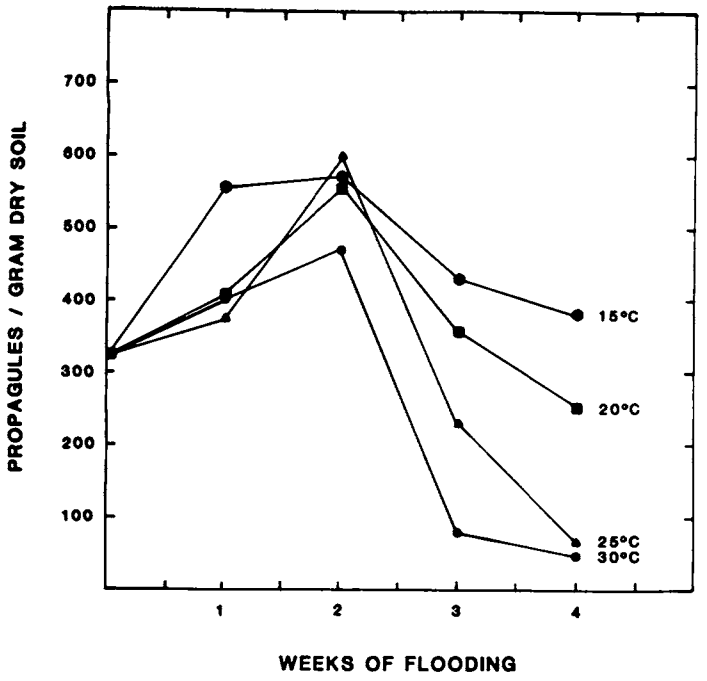

Fig. 4. Effect of temperature on survival of *Pythium* populations in flooded and non-flooded soil in a controlled environment.

and carbon and other nutrient levels in this zone (16). It is important because this is the zone from which soil samples were obtained.

Eight species of *Pythium* were isolated and identified from roots of young carrots and from carrot field soils as well as several unidentified isolates. Seven of the identified species *Pythium* caused root damage on carrots in greenhouse tests (Table 1). Six of the 7 identified species were also isolated from modified PV soil plates. *Pythium spinosum* Sawada ap. Swada & Chen was commonly isolated from soil and soil plates, but was not pathogenic to carrot roots. Identification of *Pythium* species is difficult; all identifications may not be correct. However, the results show that several species are involved and that almost all of them were represented on the modified PV soil plate estimations of populations. Thus, the method used for estimating populations were justified for the purpose of the study.

Mildenhall et al. (11) described damage to carrots caused by *Pythium* species in Wisconsin. Pratt and Mitchell (14) isolated some of the species listed in Table 1 from carrot field soils collected in Florida and Wisconsin and described *P. sulcatum* as a new species. There are numerous other reports of *Pythium* species attacking carrots, but there is little or no evidence that some species may survive flooded soil conditions better than others, but it is possible. The present study did not address this point nor were yield increases due to fallow flooding documented. These studies are now in progress.

Flooded or water-saturated soil conditions are conducive to reproduction and dispersal of *Pythium* and related fungi (4) and they have been isolated from irrigation sources such as ditches and ponds (18). The present study did not consider these points or how they may relate to carrot production, but they are probably important factors in the epidemiology of carrot root disease. The goal of this study was to begin to document and explain more easily observable effects of fallow flooding on a target pest such as the *Pythium* species involved in carrot root diseases.

Results of this study show that fallow flooding of vegetable production fields on organic soils greatly reduces populations of *Pythium* sp. and that populations remain low for periods which are sufficient to allow the early root

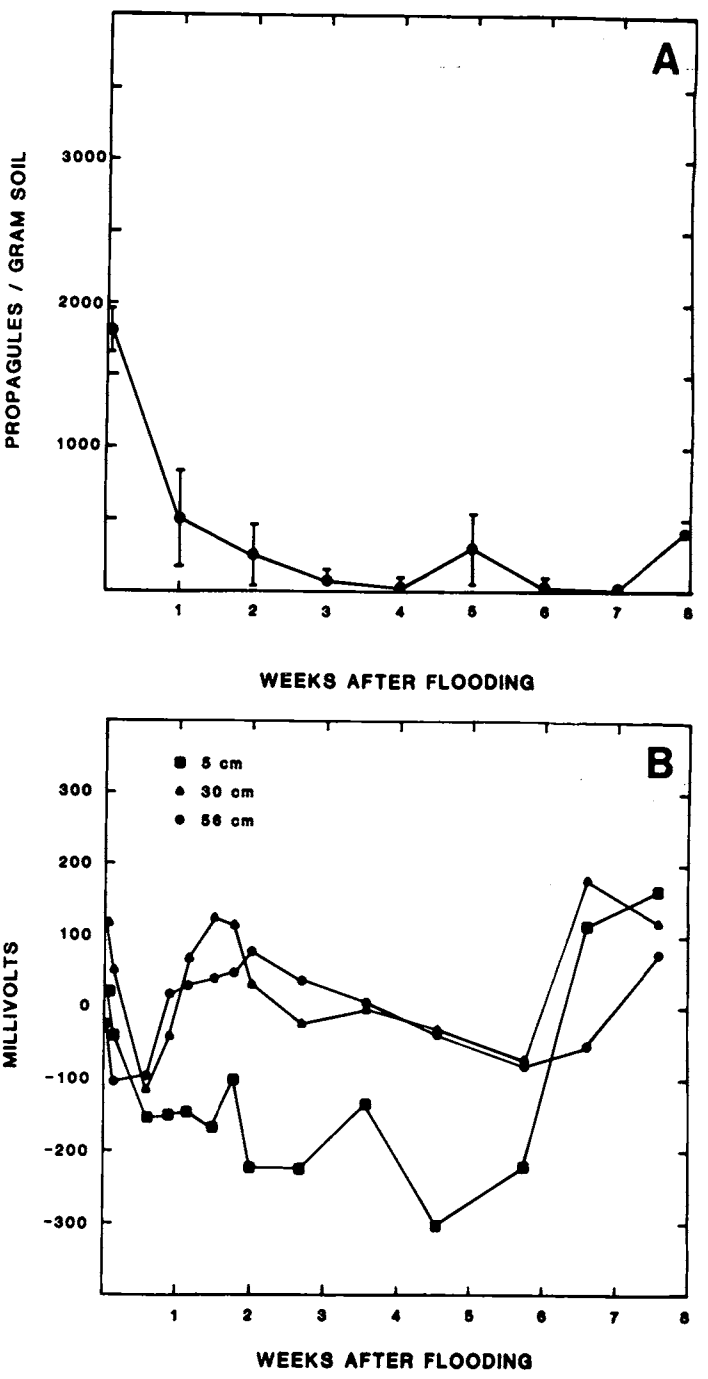

Fig. 5. Populations of *Pythium* species in a carrot field during flooding and draining (week 6). a) population estimates; b) Eh values measured at platinum electrodes buried at 5, 30, and 56 cm as relative indication of oxygen availability; values less than +300 mv indicate very low oxygen availability.

growth and development of carrot to occur with reduced risk of root damage from *Pythium* sp. *Pythium* populations are reduced to beneficially low levels within 4-6 weeks after flooding is initiated. Controlled environment studies confirmed field observations and showed that the rate of reduction of *Pythium* populations is temperature dependent; the rate is much lower at low temperatures. Low oxygen levels are associated with decreases of *Pythium* populations in a flooded soil, but may not be a primary cause of it. Although data on yield of carrots were not obtained, growers report that they are increased substantially when compared to similar fields which were not flooded. Widespread use of this cultural pest control practice by growers tends

Table 1. *Pythium* species isolated from small carrot roots at Zellwood, Florida, that were pathogenic to carrots in greenhouse tests.

Species	Pathogenic isolates (%)[z]	Isolated from PV plates
P. irregulare Buisman	30	yes
P. debaryanum Hesse	13	yes
P. ultimum Trow	13	yes
Unidentified	13	?
P. megalacanthicum deBary	10	rarely
P. sulcatum	7	rarely
P. vexans deBary	7	no
P. aphanidermatum (Edson) Fitzpatrick	3	yes

[z]Per cent of 36 total pathogenic isolates.

to confirm this notion. Thus, fallow flooding appears to be a valuable cultural practice where it is feasible to implement. A better understanding of the processes of flooding involved in reducing populations of *Pythium* species and other pests would contribute to a more efficient use of water resources required.

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WEED CONTROL IN ROOT CROPS GROWN ON ORGANIC SOILS[1]

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Abstract. **During a 3-yr period herbicides were evaluated for weed control efficacy and carrot** (Daucus carota **L.) and radish** (Raphanus sativus **L.) crop tolerance grown on organic soils. In radishes preemergence applications of CDEC (2.0 lb./acre), propachlor (4.0 lb./acre), metolachlor (1.5-3.0 lb./acre), thiobencarb (4.0 lb./acre), alachlor (2.0 lb./acre), pendimethalin (1.0-2.0 lb./acre) and diethatyl-ethyl (4.0 lb./acre) provided adequate control of goosegrass** (Eleusine indica **(L.) Gaertn), spiny amaranth** (Amaranthus spinosus **L.), lambsquarter** (Chenopodium album **L.), and purslane** (Portulaca oleracea **L.). Almost all herbicide treatments resulted in reduced yields. In carrots, preemergence applications of metolachlor (3.0 lb./acre), alachlor (3.0-6.0 lb./acre), thiobencarb (4.0-8.0 lb./acre), metribuzin (0.25-0.5 lb./acre), propachlor (2.0 lb./acre), linuron (1.0-2.0 lb./acre), and diethatyl-ethyl (4.0-6.0 lb./acre) provided acceptable control of spiny amaranth and purslane for 4 weeks. However, propachlor and metribuzin reduced crop vigor. Preemergence applications of metolachlor, propachlor, and diethatyl-ethyl provided acceptable control of goosegrass and broadleaf panicum** (Panicum adspersum **Trin.) 4 weeks after treatment. Postemergence applications of fluazifop-butyl (0.125-0.25 lb./acre), sethoxydim (0.15-0.3 lb./acre), haloxyfop-methyl (0.15-0.3 lb./acre) and DPX-Y6202 (DuPont Chemical Co.) (0.25 lb./acre) provided excellent control of the grass weed species. Combination postemergence applica-**

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