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Pollution of Surface Irrigation Waters by Plant Pathogenic Organisms

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Abstract

Systematic sampling of waterways and irrigation runoff from agricultural lands in the North Platte Project of Nebraska in July and August of 1972–1974 demonstrated that phytopathogenic organisms were disseminated. The organisms monitored included the bean common blight bacterium *Xanthomonas phaseoli*, the bean white mold fungus *Whetzelinia sclerotiorum* and various nematodes. Although many types of nematodes often were recovered from irrigation water, *Heterodera* sp. cysts which cause significant disease problems in the valley were found infrequently. Patterns of movement of the bacterial and fungal organisms were correlated with previous or current season infection of bean plants. The short-term survival of *X. phaseoli* in sterile deionized water may explain the detection of this organism only in runoff or ditches receiving runoff from common blight infected bean fields. Sclerotial bodies of *W. sclerotiorum* remained viable for at least 10–21 days in flowing water and were found throughout the irrigation waterways. Irrigation of beans with contaminated water can result in both common blight and white mold diseases. Dissemination of phytopathogenic organisms in irrigation reuse systems as well as agricultural land runoff should be considered in irrigation planning and system design.

Keywords: irrigation pollution, phytopathogenic bacteria, phytopathogenic fungi, nematodes, phytopathogen dissemination, epidemiology

The critical need for irrigation water has placed considerable pressure on expansion of irrigated acreage and on water reuse. The inevitable consequence of reuse is that water, after passing through fields with diseased plants or disease-infested soil and debris, may contain and thus disseminate phytopathogenic organisms. Some reports have indicated that root and stem rot fungi (Cooke, 1956) such as *Phytophthora* spp. (Klotz, *et al.*, 1959; Luce, 1953; McIntosh, 1966; McMurtrey, 1961) and *Pythium* spp. (Gill, 1970; Harvey, 1952; Lumsden and Haasis, 1964) and a soil-borne wilt fungus *Verticillium albo-atrum* (Easton, *et al.*, 1969) as well as phytopathogenic nematodes (Faulkner and Bolander, 1970) can be recovered from various parts of irrigation systems. In two instances reuse of drainage or runoff

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water resulted in increased losses due to diseases caused by the contaminating organisms (Faulkner and Bolander, 1970; Irwin and Vaughan, 1972). Previous studies on the spread of phytopathogenic organisms under Midwest irrigation conditions were inconclusive (Schuster, 1959; Schuster and Thorne, 1956). We present here methods for sampling irrigation water within an irrigation project and evidence of the dissemination of three different groups of phytopathogens: a bean foliage-infecting bacterium *Xanthomonas phaseoli*, a stem and foliage-infecting fungal pathogen *Whetzelinia sclerotiorum* [= *Sclerotinia sclerotiorum* (Korf and Dumont, 1972)] and various phytopathogenic nematodes in the surface-irrigated fields of the North Platte Valley in western Nebraska. A preliminary report of certain aspects of this study has been presented (Steadman, 1974).

Materials and Methods

Surveys were conducted to determine the extent and importance of certain plant pathogens in irrigation waterways of the North Platte Project in Nebraska (Figure 1). All sampling was systematically conducted during the months of July and August of 1972–1974 and included field runoff, drainage ditches, lateral canals, and main canals in the Nebraska portion of the project. During the initial season methods for obtaining and assaying water and debris samples were developed.

To determine the presence of the fungal pathogen *W. sclerotiorum* in various waterways, debris collected from a bottom benthic drag net of 1.41 or 0.595 mm (14 or 30 mesh) or 3.36 mm (6 mesh) set screen was examined for resting structures (sclerotia) of the fungus. Recovered sclerotia were tested for viability by plating on 1% water agar and for pathogenicity on bean plants. *X. phaseoli* initially was detected by passing 2 to 20 l (0.5 to 5.0 gal.) water samples through 0.25 mm (60 mesh) screen to remove debris, centrifuging at 4500 rpm for 15 min. and eluting in 0.1 of the original volume of water to effect a 10-fold concentration, and spraying the leaves of 18-day-old Red Kidney bean plants with the concentrated sample at 60 psi. A positive response consisted of water-soaked areas on leaves at 10 days, changing to necrotic lesions with yellow margins at 14–18 days after inoculation. A detection method used during 1973 and 1974 was direct irrigation of three replications of two 3 m (10 ft) rows of Great Northern (GN) #59 beans with 23 l (6 gal.) water samples, applying overhead irrigation for 4–8 hours and observing the plants 2 weeks later for typical symptoms. Controls consisted of irrigation of the plots with well water or well water plus known pathogen levels. Nematodes were collected in a small volume of water with a 0.125 mm (120 mesh) plankton tow net. In some cases, these samples were directly applied to pots planted with sugarbeets. After 6 weeks root-feeding nematodes were recovered by eluting roots and soil with water and examining the extract microscopically.

Results

From a total of 252 water sample collections during the 3 years of the study, 28% of the samples contained one or more viable sclerotia of *Whetzelinia sclerotiorum*. The average number of sclerotia per sample was 10, with a range of 1 to 84. The amount of debris from which sclerotia were separated varied from 5 to 500 g dry weight. There was no relationship between amount of debris and number of sclerotia per sample.

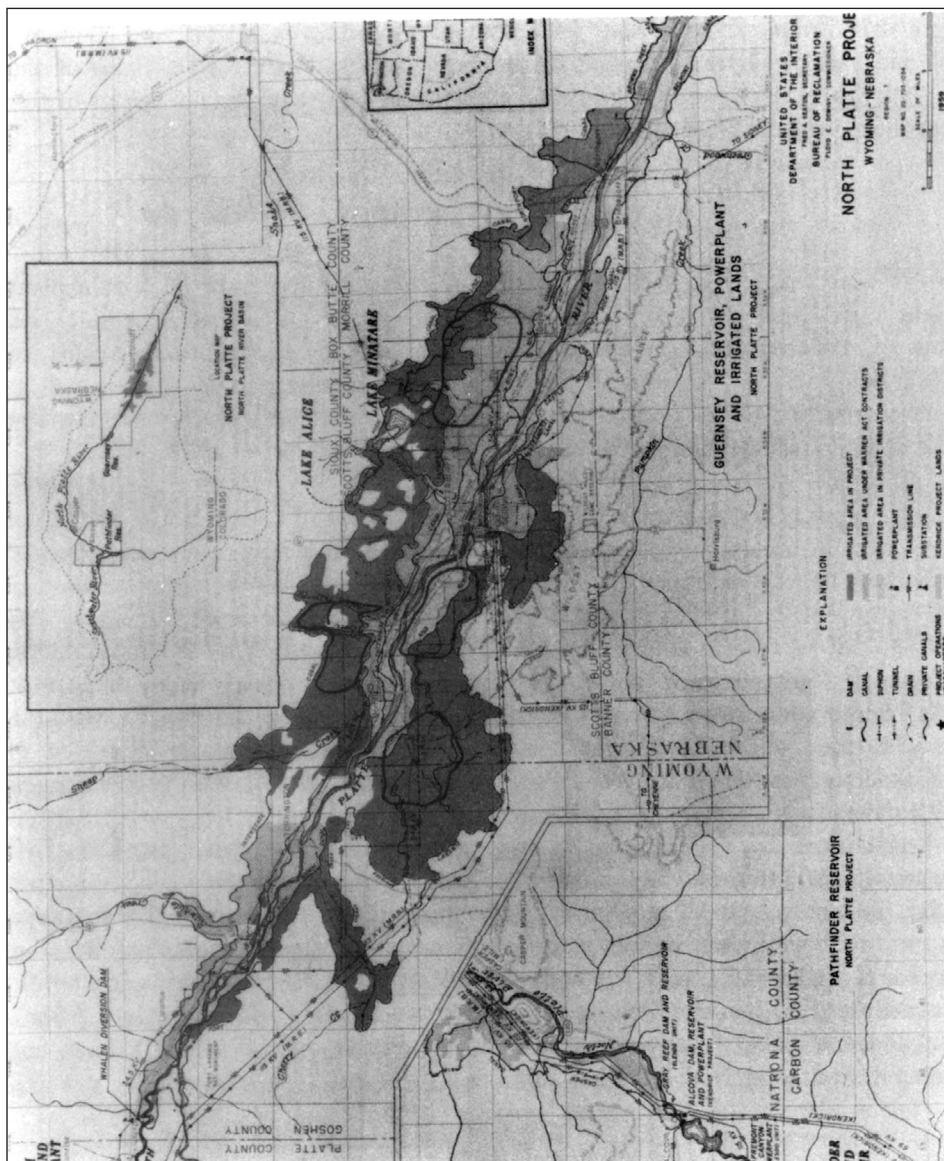


Figure 1. North Platte Project.

Although within each year distinct patterns of distribution occurred, there were two main periods of sclerotial movement in the irrigation waterways (Figure 2). In 1974 the highest frequency of sclerotial recovery was early July. In 1972 and 1973 sclerotial recovery was the greatest during the month of August. During the 3 years of this study in the valley white mold disease was initially detected in bean fields in the first week of August. In 1974 27% of the samples from main or lateral canals, 25% from corn field runoff, and 57% from bean field runoff were positive for *W. sclerotiorum*. Sclerotia were also recovered from an irrigation reuse settling pond. The sclerotia collected caused white mold disease when placed in proximity to Great Northern or Pinto beans under favorable environmental conditions.

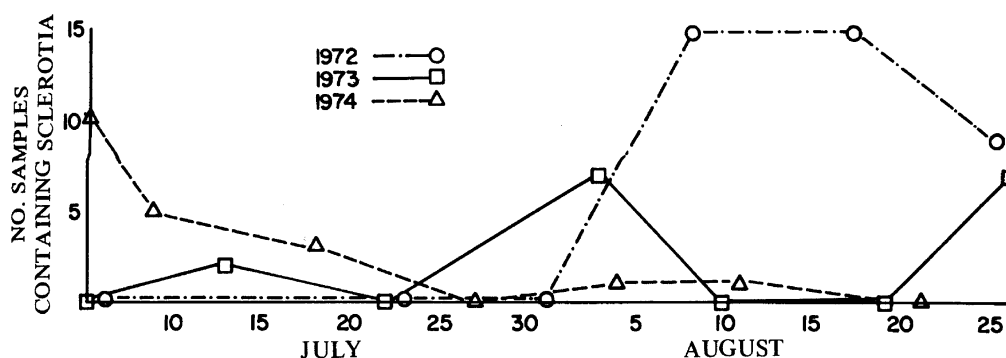


Figure 2. Occurrence of Sclerotia of *Whetzelinia sclerotiorum* during July and August of 1972-1974 in Irrigation Water Samples in the North Platte Valley, Nebraska.

Sclerotial samples from canals indicated that these structures were distributed throughout the depths of the waterways. When a sample of sclerotia collected from field-infected bean plants was placed in water, some sclerotia floated and some sank to the container bottom, while a smaller proportion remained suspended. The apparent wide range of buoyant densities of sclerotia also was demonstrated when some sclerotia remained on the bottom or suspended in the solution when sucrose was added to increase the density to 1.35.

Survival of sclerotia in water was tested in an irrigation canal and in small-scale simulated canals in the greenhouse. After 10 days in flowing or still canal water, 85–99% of sclerotia collected from white mold-infected bean plants and suspended in nylon sacks germinated to form hyphae on potato dextrose agar. After 21 to 51 days, however, few sclerotia were viable and most were completely decomposed. Debris collected in canals with dip nets and consisting primarily of seeds, stems, stalks, and other parts of plants was added to sclerotia in sacks but had no effect on sclerotial survival. In greenhouse tests 58, 60, and 50% of sclerotia collected from corn meal agar cultures, infected bean plants, and soil, respectively, remained viable after 21 days in flowing water. Sclerotia recovered from soil and placed on water agar germinated to form fruiting bodies termed apothecia. Ascospores produced and forcibly discharged from the apothecia play a major role in the epidemiology of the disease in Nebraska (Cook, *et al.*, 1975). Results from preliminary survival studies with ascospores demonstrated a longevity of at least 5 days in deionized water. Thus, although ascospore dissemination may occur in irrigation water, supportive data were not obtained under field conditions. Lack of distinctive morphological or physiological characteristics precluded detection of ascospores in irrigation water.

Recovery of *X. phaseoli* from irrigation water was successful in 22% of 137 water samples collected over 3 years. The distribution of positive samples during July and August of each year is summarized in Figure 3. No positive samples were collected earlier than August 1 in any year. Initial outbreaks of common blight in the North Platte Valley were observed in bean fields between July 26 and August 1 during these same years. The samples from bean field runoff or ditches receiving runoff from common blight-infected bean fields were the only positive samples detected. All blight-infected fields, however, did not result in blight-positive runoff samples. Runoff from pasture, corn, and sugar beet fields as well as larger canals, did not contain *X. phaseoli*. A reuse system, however, was found to contain the organism.

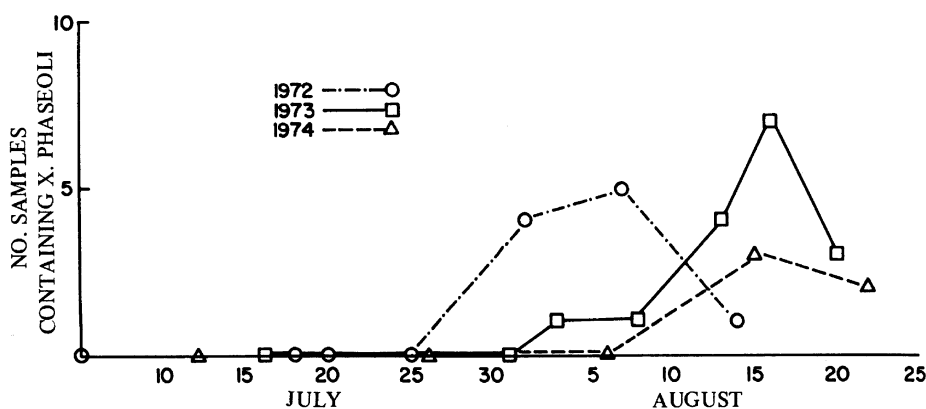


Figure 3. Occurrence of *Xanthomonas phaseoli* in Irrigation Water Samples Collected during July and August of 1972–1974 in the North Platte Valley, Nebraska.

The low background level of common blight in the assay plots is shown in Table I. Pre-plant fumigation with the biocide methyl bromide to remove residual *X. phaseoli* inoculum did not alter blight ratings significantly when aliquots of the same water sample were applied to fumigated or non-fumigated plots. Only populations of 1×10^5 cells/ml or higher of *X. phaseoli* were considered to produce significant blight (i.e., the majority of replicate samples were rated on the blight scale at 2.0 or greater). Except for experiment 2 shown in Table 1, the blight ratings generally were moderate (4.5 or less on the blight scale). Although more qualitative than quantitative the ratings indicate 1×10^5 to 1×10^6 cells/ml of *X. phaseoli* were the population levels in contaminated irrigation water samples. The 1972 data were based only on qualitative assays.

Survival of *X. phaseoli* in water was determined in the laboratory. *X. phaseoli* was grown on nutrient broth yeast extract agar (NBY) (Vidaver, 1967) for 3 days and placed in deionized water on a shaker. Samples of 0.1 ml were withdrawn periodically and plated on NBY agar for single colony counts. Following a sharp initial drop in number of viable cells/ml from 3×10^5 to 0.3×10^5 in the first 2 hr, the population decreased moderately to 1×10^3 cells/ml at 14 hr. After 20 hr in water, however, no viable cells were recovered.*

Analysis of water samples from 110 locations in 1972 demonstrated that nematodes were common in all waterways of the North Platte Project and at most depths of water. Canals

* Subsequent experiments have indicated that different water sources alter the survival of *X. phaseoli*.

Table 1. Effects of *Xanthomonas phaseoli* Populations in Surface Irrigation Water on Common Blight Disease Incidence and Severity in Great Northern Bean Assay Plots in Western Nebraska, 1973.

Experiment 1 <i>X. phaseoli</i> (cells/ml) ²	Blight Rating Scale (0–10) ¹
1 × 10 ⁵	2.0 ± 1.0
5 × 10 ⁴	1.4 ± 1.0
1 × 10 ⁴	1.5 ± 1.0
0	0.8 ± 0.3
Experiment 2 <i>X. phaseoli</i> (cells/ml) ³	
1 × 10 ⁶	6.0
0	0.6 ± 0.2

1. Ratings represent an average of 3–5 replications ± the range of variation. Ratings used: 0.5 = 1 to 10 lesions/6m (20 ft) of row; 1.0 = 1–2 lesions/0.3 m (1 ft) of row; 2.0 = 1–2 lesions/plant and; 3–10 = increasing severity of infection.

2. Bacterial populations were prepared from 4-day-old potato dextrose agar cultures.

3. Population of *X. phaseoli* estimated from infected bean leaf tissue macerated in tap water.

near eastern Wyoming, however, had few stylet-bearing nematodes (includes known plant parasitic species) compared to the Nebraska portion of the project. Systematic sampling in 1974 confirmed the widespread nematode distribution, low frequency of stylet-bearing nematodes where the canals enter Nebraska, and frequency of certain types of nematodes (Table 2). *Heterodera* sp. (sugarbeet cyst nematode) has been the major disease problem on sugarbeets in Nebraska but was found infrequently in water samples. When soil from sugarbeet fields (irrigation runoff water samples also taken from these fields) was collected in July, planted to sugarbeets in pots in the greenhouse and then analyzed 1–3 months later, cysts of *Heterodera* sp. were found. An average of only 16 cysts/l soil (9/pt) were recovered from

Table 2. Incidence of Nematodes Identified in 43 Surface Irrigation Water Samples Collected in Western Nebraska in 1974.

Nematode Types	Incidence (% of Samples)
Known Plant Parasites (combined)	50
<i>Heterodera</i> sp.	5
<i>Pratylenchus</i> sp.	9
<i>Ditylenchus</i> sp.	26
<i>Aphelenchoides</i> sp.	14
Non-Plant Parasites (combined)	80
<i>Monochus</i> sp.	9
<i>Acrobeles</i> sp.	12
<i>Aerobeloides</i> sp.	28
<i>Rhabditis</i> sp.	21
<i>Aphelenchus</i> sp.	16
<i>Tylenchus</i> sp.	28
Dorylaims	65

soil collected from fields which had been fumigated with 1,3-dichloropropene earlier in the year as compared with 271 cysts/l soil (149/pt) recovered from non-fumigated fields. Similarly, lower nematode populations were observed in runoff water collected from fumigated fields than in runoff from non-fumigated fields (Table 3). In some instances water samples were kept in the laboratory for up to 90 days before discarding. Observations on nematodes in these samples indicated that many types remained viable.

Table 3. Number of Nematodes in 37.8 l (10 gal.) Irrigation Runoff Water Samples Collected from Fumigated and Non-fumigated Sugarbeet Fields in the North Platte Valley of Nebraska in 1974.

Treatment	No. Fields	Average No. Nematodes		
		Stylet	Non-stylet	Total
Fumigated	5	2	10	12
Non-fumigated	16	20	31	51
	21	16	26	12

Discussion

Movement of *X. phaseoli* and *W. sclerotiorum* in irrigation water was demonstrated in all 3 years of the study. The sclerotia of *W. sclerotiorum* were recovered from many kinds of waterways including main canals, lateral canals, ditches, and runoff from various fields. Sclerotial movement was also shown to occur in both July and August. *X. phaseoli*, however, was not detected until August and was only found in bean field runoff or ditches collecting this runoff. Since *X. phaseoli* can survive for at least one winter on the soil surface (Schuster, 1959), some movement could occur in May during initial irrigation of fields containing infested bean residue from the previous year. Although the present study did not indicate this type of early season dissemination, bacterial populations in the water may have been below detectable levels or may have lost viability during transport from the collection point to the assay plot. The explanation for the limited dissemination of *X. phaseoli* may be simply the poor survival of this bacterium in water. Careful consideration should be given to extrapolations from data determined *in vitro* to field situations. However, it would not be surprising that since phytopathogenic bacteria have no special protective structures, are poor saprophytic competitors, and are poorly adapted for survival away from host tissue, somewhat lower survival rates for bacteria such as *X. phaseoli* may occur in irrigation waterways than in sterile water. With a flow rate in the smaller ditches of 0.6–0.9 m/sec (3–4 ft/sec), however, viability even for a few hours would allow distribution of bacteria into neighboring fields using the contaminated runoff water for irrigation. Since *X. phaseoli* is primarily a foliar pathogen, inoculum carried by surface irrigation water must contact leaf tissue to cause infection. Lower leaves that are submerged by the irrigation water or subject to splashing can become diseased. Assay plot infection demonstrated the bacterial disease-producing potential of irrigation water particularly on lower leaves and where splashing occurred. Overhead irrigation however, may provide even greater disease-producing potential for bacterial pathogens. An alternate explanation of limited bacterial spread is dilution.

The fluctuation of recovery of sclerotia of *W. sclerotiorum* can be correlated with incidence and severity of the white mold disease in the North Platte Valley and to irrigation timing and frequency. Numerous sclerotia produced on each infected bean plant are returned to the soil as a result of harvesting procedures, and these sclerotia overwinter in or on soil in Nebraska (Cook, *et al.*, 1975). Thus, the amount of infection the previous year can be related to the extent of dissemination of these structures in irrigation water. Infection in bean fields was light in 1971, but 2 to 3 times greater in 1972. Consequently, sclerotia were not detected in irrigation waterways until August of 1972 and again throughout 1973. The severe white mold epidemic of 1973 correlated with frequent late August 1973 and early June 1974 sclerotial detection. Increased dissemination also could be related to increased irrigation due to a very dry June. Infrequent sclerotial movement in August 1974 may be attributed to very light white mold infection in the 1974 bean crop and to decreased irrigation because of white mold potential. Because recently formed sclerotia will not produce apothecia until they have been conditioned (i.e., for 2–3 months in soil) (Cook, *et al.*, 1975), germination on water agar will determine if sclerotia recovered from irrigation water were produced during the current season or a previous season. Survival experiments indicated that sclerotial conditioning was not altered by 21 days in flowing water. Viable sclerotia collected in June or early July formed apothecia while those collected in August formed mycelium. This confirmed the correlation of June–July dissemination with previous season inoculum and August dissemination with current inoculum production.

The wide distribution of viable sclerotia in waterways is primarily associated with sclerotial longevity. In a separate study, sclerotia retained viability for at least 3 years at various depths in soil (Cook, *et al.*, 1975). Results from the present study show that sclerotia can survive at least from 10 to 21 days in flowing water. In Florida (Moore, 1949) fields must be flooded with water for 23–45 days to destroy sclerotia of *W. sclerotiorum*. The role played by ascospores in dissemination of *W. sclerotiorum* by irrigation water may be as important as sclerotia. Any efforts to control white mold disease must consider the importance of dissemination of *W. sclerotiorum* in irrigation water.

Numerous nematode species, including some plant pathogens, were found throughout the waterways of the North Platte Project in Nebraska. However, *Heterodera* sp. cysts which have caused major disease problems on sugarbeets in the valley were found infrequently. Analysis of soil samples demonstrated that *Heterodera* sp. was present in the sugarbeet fields. If any significant distribution of this nematode does occur in irrigation water, it may be during the initial irrigation of beet fields in May or early June and apparently does not continue into the summer. Since *Heterodera* cysts normally can be found in the upper 30–60 cm (1–2 ft) of the soil, the opportunity for spread in runoff water would exist. In Washington (Faulkner and Bolander, 1970) nematode-infested irrigation water resulted in heavy infestation of parasitic nematodes in cropped land. Our results, however, indicated that soil fumigation can significantly reduce nematode levels in irrigation water.

The correlation of the problem of plant pathogen pollution of irrigation water and reuse of agricultural runoff water indicates the obvious control, the elimination or treatment of reuse water. Since reuse is a necessary component of irrigation strategy, treatment of the water to minimize the contamination of plant pathogenic organisms would be the most reasonable approach. The various patterns of dissemination of the three types of pathogens suggests that information on each type of organism involved must be known in order to formu-

late an effective control. Current reuse system designs are often ideal for dissemination of plant pathogens as shown in this study, and new systems may have to be designed to minimize this pollution problem.

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