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Annual Report

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Project Title: Role of Weeds in the Survival of *Erwinia carotovora* in the Soil

On May 25, 1989, seventy permanent field plots, each 2m x 2m square, were established on soil previously fumigated by injecting Pichlor 60 (60% chlorpicrin and 40% Telone) at the rate of 350 lb/A and sealing by sprinkling with water. Each plot was delineated by surrounding it with metal lawn edging buried approximately 4 inches into the soil. This marked the location of the permanent plots and also served to retain Erwinia inoculum within the specified plot areas when applied as a soil drench. This effectively prevented cross contamination and facilitated repeated sampling within precise areas.

Seed of six species of weeds, hairy nightshade (Solanum sarrachoides Sendt), barnyard grass (Echinochloa crus-galli), green foxtail [Setaria viridis (L.) Beauv.], kochia [Kochia scoparia (L.) Schrad.], redroot pigweed (Amaranthus retroflexus), and common lambsquarters [Chenopodium album (L.)] was seeded into the plots on May 25, 1989. Each species was seeded into ten plots; ten plots were not seeded and served as fallow controls. Weeds were thinned to predetermined populations after emergence and fallow plots were kept weed free during the season by frequent inspection and hand weeding. A drip irrigation system was installed in each plot to apply irrigation water drawn from a well to the plots. Each plot received one acre inch of water per week applied in three equal applications.

Inoculum of Erwinia carotovora subsp. carotovora (EEC) and Erwinia carotovora subsp. atroseptica (ECA) was prepared in the laboratory and applied to each plot on July 12, 1989 when weed plants were 3 to 6 inches tall and had well established root systems. Water equivalent to one acre inch

containing sufficient Erwinia cells to establish initial populations of approximately 10^6 cfu/gram of oven dry soil was applied to each plot and allowed to soak into the soil for 24 hours before soil samples were taken to determine initial populations in each plot. ECC was applied to one half of the plots and ECA to the other half. The resulting design was a split plot with each Erwinia/weed combination replicated five times.

Soil samples were collected 24 hours after soil infestation and at 14 day intervals thereafter until the end of the growing season for assaying Erwinia populations. Six soil cores approximately 3 x 20 cm were collected from each plot and composited. Subsamples of 25 grams were taken from each thoroughly mixed sample, suspended in 50 ml sterile distilled water and either diluted and spread plated on Stewart's MacConkey Pectate medium or mixed with an equal volume of pectate based enrichment medium and incubated anaerobically before plating. These methods provided both quantitative and qualitative measures of the Erwinia population in the soil throughout the season.

Weeds were harvested at the end of the season and seed of each species was collected for replanting in 1990.

RESULTS

Data (Table 1) show that initial (24 hr) populations of ECC were reasonably uniform and near the target populations (10^6 cfu/g). ECA populations were reasonably consistent but considerably below the expected population of 10^6 cfu/g. This suggests that death of ECA cells occurs rapidly in the field soil. Quantifiable populations of ECC were present in soil for 34-44 days in plots with redroot pigweed, lambsquarters, hairy nightshade, and barnyard grass. Populations in fallow plots and those planted with kochia and green foxtail reached levels below the limits of quantitative detection in 21-34 days. Quantifiable populations of ECA were detected for only 6-21 days. Only the fallow control and green

foxtail plots had quantifiable numbers for more than 6 days.

Table 1. Populations of Erwinia carotovora in soil in relation to weed species planted in field plots.

Treatment (Weed Species)	Mean Erwinia Population (cfu/g oven dry soil)					
	Jul 13	Jul 18	Aug 2	Aug 15	Aug 24	Sep 16
ECC						
None (Fallow)	$6.54x10^4$	$1.5Ox10^2$	$4.57x10^2$	0	0	0
Redroot Pigweed	$3.84.10^4$	$1.88x10^3$	$2.O1x10^3$	$2.35x10^4$	0	0
Lambsquarters	$1.28x10^5$	$2.59x10^4$	0	$2.1Ox10^2$	0	0
Hairy Nightshade	$1.32x10^5$	$4.33x10^3$	$4.4Ox10^4$	$1.73x10^4$	0	0
Kochia	$6.34x10^4$	$1.53x10^3$	0	0	0	0
Green Foxtail	$4.65x10^5$	$3.56x10^5$	$6.34x10^2$	0	0	0
Barnyard Grass	$3.56x10^5$	$2.51x10^4$	$4.37x10^2$	$4.35x10^2$	0	0
ECA						
None (Fallow)	$1.66x10^3$	$2.12x10^2$	0	0	0	0
Redroot Pigweed	$1.38x10^4$	0	0	0	0	0
Lambsquarters	$1.28x10^5$	0	0	0	0	0
Hairy Nightshade	$5.42x10^3$	0	0	0	0	0
Kochia	$5.75x10^3$	0	0	0	0	0
Green Foxtail	$1.77x10^2$	$2.O7x10^2$	0	0	0	0

Barnyard Grass	$2.97x10^4$	0	0	0	0	0

Qualitative anaerobic enrichment techniques (Table 2) revealed the presence of Erwinia in soil throughout the season. However, by September 28, 66 days after the bacteria were introduced into the soil, ECC was found only in samples collected from Redroot Pigweed, Lambsquarters, Green Foxtail, and Barnyard Grass plots.

Table 2. Presence of Erwinia carotovora in soil in relation to weed species planted in field plots.

Treatment (Weed Species)	% of Enriched Samples Yielding Erwinia													
	Jul 13		Jul 18		Aug 2		Aug 15		Aug 24		Sep 16		Sep 28	
	ECC	ECA	ECC	ECA	ECC	ECA	ECC	ECA	ECC	ECA	ECC	ECA	ECC	ECA
None (Fallow)	100	100	60	20	100	70	20	40	20	20	20	20	0	20
Redroot Pigweed	100	100	100	0	60	0	80	40	80	20	20	60	20	20
Lambsquarters	100	100	60	0	100	20	80	0	20	0	40	40	20	0
Hairy Nightshade	100	100	80	0	100	40	40	60	40	20	0	20	0	0
Kochia	100	100	100	20	80	20	40	40	0	20	40	0	0	0
Green Foxtail	100	100	100	60	100	0	60	60	40	0	60	60	40	20
Barnyard Grass	100	100	100	0	100	40	80	100	40	40	20	80	80	0

Eighty percent of soil samples from Barnyard Grass plots yielded ECC while only 40% of samples from Green Foxtail and 20% of samples from Redroot Pigweed and Lambsquarters plots yielded the organism. Erwinia was detected in small percentages of soil samples from fallow, Redroot Pigweed, and Green Foxtail plots infested with ECA after 66 days. However, serological tests showed that the organism found in the fallow and Green Foxtail plots were not ECA. It was not possible to purify the organisms isolated from the Redroot Pigweed plots sufficiently for identification.

Weed growth was excellent and target populations were achieved in nearly every plot. Few problems with volunteer plants were encountered on the fumigated soil. Fallow plots were easily maintained weed-free by minor hand weeding at the time each soil sample was collected.

DISCUSSION OF RESULTS AND FUTURE PLANS

The initial rapid decline in Erwinia populations was expected based upon previous experience with survival of this organism in soil and the fact that the 1989 growing season was unusually warm in the area where the plots were located. High soil temperatures are known to increase the rate of decline of Erwinia populations in the soil. ECA populations did not persist as well as ECC in the soil probably because of its weaker competitive ability.

Erwinia populations generally appeared to decline more slowly in plots planted with grassy weeds and some broadleaved species than in those planted with other broadleaved species or in fallow soil. It is also notable that viable Erwinia cells were still present in some plots at the end of the season and that the frequency of their detection was greater in plots planted with some weed species than with others. This suggests that, as hypothesized, some weed species may favor persistence of E. carotovora in soil. The critical test of the hypothesis will occur in 1990 when it will be determined if Erwinia persisted differentially over winter in plots planted with different weed species. Greenhouse studies

currently in progress will help to verify the field results and determine the effects of soil temperature and moisture on the survival of Erwinia in association with weeds.

All field plots will be replanted in the spring of 1990 with the same weed species as in 1989. Soil assays for the presence of E. carotovora will begin as soon as the soil thaws in the spring. Sampling will be carried out in the same manner as in 1989. Progressively more sensitive assay methods including anaerobic enrichment, water extraction of larger volumes of soil followed by filter concentration and anaerobic enrichment and sampling of weed roots and rhizosphere soil will be used to monitor the presence of Erwinia in the soil during the next two growing seasons.